

ADVANCES IN SPACE BIOLOGY AND MEDICINE

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SETI Institute
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LIST OF CONTRIBUTORS

<i>Sjoerd L. Bonting</i>	SETI Institute NASA-Ames Research Center Moffett Field, California
<i>André Brack</i>	Centre de Biophysique Moléculaire, CNRS Orléans, France
<i>Anatolyi D. Egorov</i>	Institute of Biomedical Problems Moscow
<i>Claude Gharib</i>	Laboratoire de Physiologie de l'Environnement Faculté de Médecine Université Claude Bernard Lyon, France
<i>Anatolyi I. Grigoriev</i>	Institute of Biomedical Problems Moscow
<i>Helmut G. Hinghofer-Szalkay</i>	Department of Physiology School of Medicine University of Graz Graz, Austria
<i>Gerda Horneck</i>	Institut für Flugmedizin, DLR Köln, Germany
<i>Richard L. Hughson</i>	Department of Kinesiology Faculty of Applied Health Sciences University of Waterloo Waterloo, Ontario, Canada

- Robert P. Kann* Department of Biochemistry and
Cell Biology
State University of New York
Stony Brook, New York
- Katharine Kato* NASA-Ames Research Center
Moffett Field, California
- Eva M. König* Department of Physiology
School of Medicine
University of Graz
Graz, Austria
- "*
- Abraham D. Krikorian* Department of Biochemistry and
Cell Biology
State University of New York
Stony Brook, New York
- Howard G. Levine* Department of Biochemistry and
Cell Biology
State University of New York
Stony Brook, New York
- D.A.M. Mesland* European Space Research and
Technology Center
Noordwijk, The Netherlands
- Jaime Miquel* Department of Neurochemistry
School of Medicine
University of Alicante
Alicante, Spain
- Stefania A. O'Connor* Department of Biochemistry and
Cell Biology
State University of New York
Stony Brook, New York
- Delbert E. Philpott* NASA-Ames Research Center
Moffett Field, California

INTRODUCTION TO VOLUME 2

In the Introduction to Volume 1, it was stated that an annual "Advances" series on space biology and medicine is both a needed and an ambitious undertaking. Needed, because the findings and accomplishments in this field need to be brought to a wider group of scientists than the relatively small group of biologists and physiologists currently involved in space experimentation. Ambitious, because the contributions must cover the entire spectrum of biology: humans, animals, plants, cells, and biomolecules. Moreover, they cannot be directed to a narrow group of specialists but must appeal to a wider circle of readers, including those scientists who currently are not active in space experimentation. Hence, not only the problems investigated and the results obtained must be discussed, but also some of the technical aspects peculiar to this field are to be treated. The editor hopes that the first volume has fulfilled these requirements, and that this second volume will be a suitable successor.

Space biology and medicine is a relatively young field concerned with the study of the effects of the space environment (low gravity and radiation) on living organisms. While the more obvious effects of the space environment on humans and animals have been well documented in the last 30 years, much remains to be learned about the mechanisms of these effects and about adaptation of the organism and possible countermeasures to these effects.

In addition to this applied side of space biology and medicine, there are the fundamental questions of the role of gravity in the evolution, development, and reproduction of life on Earth and beyond; questions that can virtually only be studied with the aid of space experiments.

This volume has contributions from the United States (3), Russia (2), and Europe (4). These contributions include investigations of biological problems encountered in spaceflight by humans, animals, plants, and single cells, as well as studies of a fundamental biological problem aided by space experiments. Two extensive chapters attempt to determine the mechanisms of the effects of long-term space missions on the human body (Grigoriev and Egorov, Russia), and the adaptative mechanisms operative in the human body under these conditions (same authors). Other chapters deal with ultrastructural observations of myocardial deconditioning (Philpott et al., United States), fluid and electrolyte regulation (Gharib and Hughson, France/Canada), human nutrition in space (Hinghofer-Szalkay and König, Austria), growth and cell division in plants (Krikorian et al., United States), mechanisms of the effects of gravity on single cells (Mesland, The Netherlands), orbital exobiology studies of the origin of life (Horneck and Brack, Germany/France), and my own contribution on the use of chemical sensors for space biomedical research and monitoring of water recycling.

The editor hopes that Volume 2 constitutes another useful educational tool to bringing the findings of space biology and medicine to a wider scientific audience; the very intention of this series.

Sjoerd L. Bonting
Editor

GENERAL MECHANISMS OF THE EFFECT OF WEIGHTLESSNESS ON THE HUMAN BODY

Anatolyi I. Grigoriev and Anatolyi D. Egorov

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I. INTRODUCTION

Based on the results of spaceflights, as well as simulation studies on Earth, the view is now accepted that during missions on board modern manned spacecraft and space stations with large pressurized habitable modules and an Earth-like atmosphere, the most noticeable effects upon the human body are induced by weightlessness.¹⁻⁴

The results of spaceflight studies, when ordered and generalized, have produced abundant phenomenological information about the effects of weightlessness on the main body functions. Yet, the mechanisms of these effects are still not fully identified and understood. At the same time, a theoretical analysis of these mechanisms is of great scientific importance for purposeful planning of future space life sciences research.

This chapter, based on generalization and physiological analysis of the results obtained during spaceflight studies and simulation studies on the ground, presents a general scheme—still largely hypothetical—of the effects of weightlessness on the human body.

II. GENERAL EFFECTS OF GRAVITY AND WEIGHTLESSNESS

A. Gravity

In the course of evolution, the entire process of the development of living organisms, the evolution of humans, and their adaptation to the environmental conditions, took place under the effect of the Earth's gravitational field. Gravity is defined here, in the sense of Newton's attraction theory, as the interaction (attraction) between any two material bodies (with linear dimensions much smaller than the distance between them), which is directly proportional to their masses and inversely proportional to the squared distance between them.

On Earth, humans experience support with each lower segment of the human body supporting the segment above it. This results in an uneven distribution of the effect of weight loading, created by the force of gravity. The force of gravity is the vector difference between the force of attraction of the body to the Earth and the efferent inertial force resulting from participation of the body in the daily rotation of the Earth. If the body is supported by a horizontal plane near the surface of the Earth, the force of gravity acting on it is compensated by the force of the response of the supporting plane. The magnitude of pressure exerted as a result of the force of gravity by the body on the horizontal plane, which prevents its free fall, is termed the weight of the body. This effect increases from the upper segments to the lower ones (Fig. 1).⁵

The force of gravity leads to the development of elastic deformations in the structure of the human body: a change in the position of material elements relative to each other, resulting in a change in shape and dimensions of the body or its

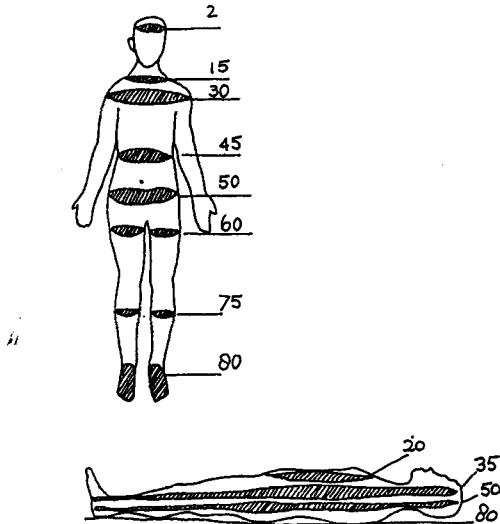


Figure 1. Distribution of forces with which upper body is acting on lower body in upright (a) and supine (b) positions. Numbers denote kilograms (ref. 5).

segments. This is accompanied by a change in the forces of interaction between the elements, that is the development of mechanical tension of the structures of the human body. An obvious manifestation of this deformation induced by the force of gravity is a decrease in height when the body is in the vertical position. This effect is most pronounced in young people.

A direct consequence of this deformation may be the compression of the lower tissues by the higher ones. This results in several effects:

- increased tension of ligaments and joint capsules, and compression of cartilage in the joint region;
- increased tension of the ligament apparatus fixing the viscera, and deformation of the visceral parenchyma;
- distension of the hollow internal organs, lined by smooth muscle, by the weight of their content; and
- distension of blood vessels (mainly small veins and capillaries), lymphatic vessels, intercellular spaces, and glandular ducts by the hydrostatic pressure exerted by the blood and other body fluids, increasing in the direction of the gravity vector.

The force of gravity also exerts an indirect pressure upon the body in inducing a reflex tonic contraction of the antigravity muscles. This contraction is aimed at

maintaining a certain posture, primarily vertical, during exposure to the Earth's gravitational field.

These gravity-induced deformations (compression, displacement, distension) of various tissues and organs, as well as the tonic tension of antigravity muscles, create a continuous load upon the human body. This load is associated with an excitation of gravireceptors responsible for the perception of gravity by means of the vestibular system, the muscle proprioceptors, and the mechanoreceptors of skin, bones, and viscera.

Hydrostatic pressure in the vertical position results in a specific distribution of body fluids. They become pooled in parts of the body below the hydrostatically indifferent point (HIP). This point is characterized by a stable level of the hydrostatic blood pressure, independent of posture and situated in a horizontal plane 5 to 10 cm below the diaphragm.⁶

In addition, there is an increasing vascular pressure in the caudal direction. Due to the distensibility of the vascular walls (primarily of the veins), the hydrostatic pressure causes a distension and a volume increase of the veins below the HIP, mainly in the lower limbs. Blood pools in this region are proportional to the distensibility of the venous bed. These passive shifts are compensated to a certain extent by the activation of neurohumoral responses from arterial baroreceptors, and from mechanoreceptors of the cardiopulmonary region.

B. Weightlessness

In weightlessness, the external gravity field affecting a material body or mechanical system does not cause a reciprocal pressure of parts of the body or system upon each other, and thus no deformation occurs. Here we describe the principal element and main factors of the effect of weightlessness on the human body.

With the transition from the Earth's gravity field to the condition of weightlessness, the effect of gravity ceases. This results in the disappearance of the deformations and the hydrostatic fluid pressure induced by gravity in the human body. Also disappearing are the reflex tonic contraction of the antigravity muscles. These are the primary changes manifested during spaceflight. They result in the following changes observed in cosmonauts:

- increase of body height;
- loss of otolith mass;
- decrease of friction force during eyeball movements;
- decrease of muscular effort during motion;
- decrease of distension of vessels and hollow organs; and
- disappearance of the deformation of parenchymal organs and the structures fixing them, caused by the effect of gravity.

Elimination of the deformation and the mechanical tension of the body structures

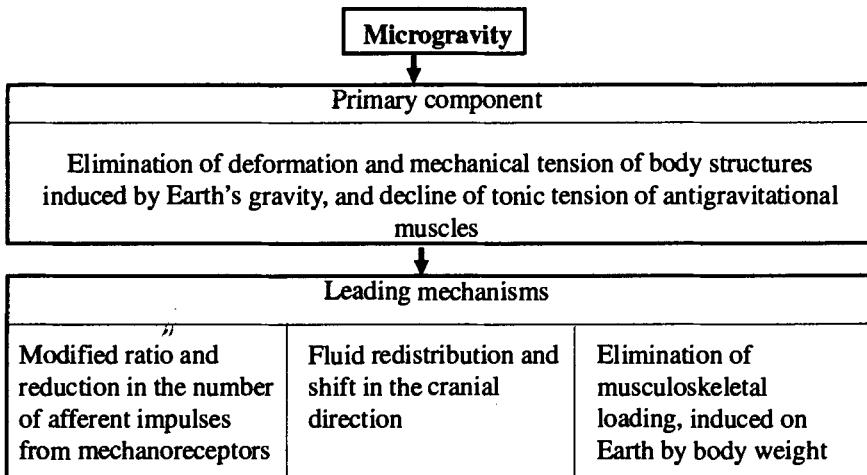


Figure 2. Primary mechanisms of physiological changes in microgravity.

existing on the Earth due to gravity, the disappearance of reflex tonic contraction of antigravity muscles, and the resulting decrease of functional loading of a number of body systems, may be viewed as the principal effect which precedes and causes the activation of numerous secondary changes in physiological functions during weightlessness (Fig. 2).^{4,5,7}

These secondary functional changes include:

- changes of the sensory input seen as the establishment of a different ratio and a decrease in the total volume of afferent impulse flow from gravireceptors. Gravireceptors are mechanoreceptors whose function is related to the effect of gravity during stay on Earth;
- changes in distribution of body fluids with a headward fluid shift; and
- elimination of weight loading and lowering of functional loading upon the musculo-skeletal system. This is associated with the maintenance of posture and the performance of motions since there is no necessity to counteract the force of gravity.

III. CHANGES OF SENSORY INPUT DURING WEIGHTLESSNESS

A. Afferent Impulse Flow

In this section, weightlessness-induced changes in afferent impulse flow from gravireceptors are analyzed, and possible mechanisms of these changes are dis-

cussed on the basis of general physiological concepts.^{2,4} The relevant receptors considered are: proprioceptors, vestibular apparatus, visceral mechanoreceptors, cutaneous mechanoreceptors, and osteoceptors.

Proprioceptors

Adequate stimulators for muscular proprioceptors are:⁸

- change in length and velocity during muscular distension and relaxation (muscle spindles parallel to muscle fibers);
- muscular tension during contraction and passive distension (tendon receptors or Golgi bodies); and
- spatial orientation of separate body segments and their orientation relative to each other, and the velocity of movements in joints (receptors in a joint bursa).

In weightlessness there is probably a decrease in the impulse flow from muscular proprioceptors, resulting from an assumed disappearance or decrease of the reflex tonic contraction of antigravity muscles; a decrease of the force of tonic muscular contraction when a certain posture is assumed; a decrease of muscular tension during motor acts; a decrease of tension in ligaments, joint capsules; and a decreased compression of cartilage in the joint region.

These assumptions are confirmed to a certain extent by the following observations made during spaceflight studies:

- at rest there is a tendency to assume an embryonic posture with curved spine, head bent forward, and upper and lower extremities shifted to a position similar to the quadrupedal posture, which indicates a lowered tone of the antigravity muscles;⁹
- a disturbance in proprioception is displayed in two ways: (1) a difficulty in perceiving orientation of body segments, manifested in a marked increase in number of errors in evaluating elbow and knee angles during the first three days in weightlessness, and (2) a diminished ability to determine target positions with closed eyes, as compared to terrestrial conditions;¹⁰
- a progressive decrease in muscle bioelectric activity of crew members during the *Spacelab D-1* mission;¹¹ and
- a redistribution of the tonic electromyographic activity level of the leg muscles, with a marked decrease in extensor activity and predominance (in contrast to terrestrial conditions) of flexor activity. This is accompanied by an increased forward inclination of the cosmonaut's body with rigid fixation of the feet on a special platform.¹²

Vestibular Receptors

The peripheral sensory vestibular organ consists of an otolith (macular) apparatus positioned in the horizontal (macula utriculi) and sagittal (macula sacculi) planes, and three semicircular canals (anterior, posterior vertical, and posterior horizontal). In the macular region and semicircular canals, a sensory ciliary epithelium is immersed in a gelatinous mass consisting of mucopolysaccharides. In the maculae, this mass, which contains calcium carbonate crystals, forms the otolith membrane. The widened segments of the semicircular canals (ampullae) contain hairs of ciliary receptor cells, which stick together and are known as the ampullar cupula. Under natural conditions, accelerations cause a bending of the ciliae. Linear accelerations cause ciliar bending in the maculae; angular accelerations in the semicircular canals.

It is known that only a stimulus tangentially directed to the otoliths is effective. This would lead one to believe that in weightlessness the otoliths in the horizontally positioned utriculus should give a baseline impulse flow in any position of the head.¹³ However, in reality the head position during routine activities is practically never entirely vertical. Receptor sacculus cells, at rest on the ground, generate a continuous impulse flow due to the tangential shift of the otoliths. In weightlessness there is no shift of the sacculus otoliths, and thus the impulse flow will correspond to the baseline level. Hence, it is likely that in weightlessness there is a decrease of the afferent impulse flow from the receptors of the otolith apparatus to the central nervous system. It follows that the otolith apparatus during exposure to weightlessness will not signal the spatial orientation of the head to the central nervous system, so any movement is perceived as linear. Such a reinterpretation of the otolith signal by the brain during weightlessness may cause the development of space motion sickness in susceptible subjects.¹⁴

The sensitivity of the otolith system during spaceflight is not markedly different from the preflight level. This was shown by experiments with the "sled" linear acceleration facility during the *Spacelab D-1* mission.¹⁵ In a study of the oculomotor interaction, an increased dynamic excitability of the visual and vestibular inputs was found during the initial period of exposure to weightlessness.¹⁶ There was a lowered threshold of optokinetic vestibular nystagmus upon optokinetic and vestibular stimulation. In the same study a decreased static vestibular excitability was observed during the tracking of a moving target while the head was fixed, which was ascribed to a prolapse of the saccades.

During the *Spacelab-1* mission, the effect of caudal acceleration as in a sudden fall was studied by suddenly moving the body with the help of elastic cords. This resulted in a progressive decline of the regulatory effect of otolith stimulation after inducing Hoffman's monosynaptic spinal distension reflex by electric stimulation of motor nervous fibers.¹⁷ This phenomenon may be related to the loss of the adaptive significance of this regulatory effect of otolith function for the astronauts during flight. Irrespective of Hoffman's reflex, during "fall" there was also a

progressive lowering of the EMG activity of the gastrocnemius-soleus muscle as a manifestation of a weightlessness-induced change of the otolith-spinal reflex.¹⁰

Visceral Mechanoreceptors

Visceral mechanoreceptors are stimulated by a direct mechanical effect upon their surface. They are also stimulated by distension of the walls of vessels and hollow smooth-muscle organs such as bladder, stomach, or lungs by the weight of their contents.¹⁸

In weightlessness there will be less distension of hollow organs and vessels because of the absence of weight of their contents, a diminished deformation of tissues and parenchyma, and a decreased tension of ligaments fixing the viscera, which under normal conditions counteract the effect of gravity. This will lead to a decrease of the afferent impulse flow from the visceral mechanoreceptors to the central nervous system.

Cutaneous Mechanoreceptors

Under terrestrial conditions, mechanical stimulation of the skin, particularly during continuous contact and with support, is perceived by three types of receptors:⁸

- pressure receptors, which react to intensity and shifts, and adapt slowly;
- contact receptors, which react to velocity, and adapt quickly; and
- vibration receptors, which react to acceleration, and adapt very quickly.

In weightlessness, there is support only if there is contact with objects or fixation in a chair (as at the work station). Nevertheless, the stimulation of the skin will probably be less marked than on the ground, due to the absence of body weight. Studies performed during the *Spacelab D-1* mission indicate that information from the cutaneous mechanoreceptors plays an important role in stabilizing some body functions in weightlessness. When an astronaut unfastened himself from a chair and began to float freely, thus losing support, the amplitude of the optokinetic nystagmus increased. This effect could be related to alteration of the somatosensory and sensory interactions.¹⁹

Osteoceptors

The bone tissue (periosteum, endosteum, bone marrow, and vessels) contains polymodal receptor systems, which provide various types of reception, including perception of mechanical effects related to deformation of the bone tissue. Bone deformation can be caused by the direct action of the body weight on the skeleton, and indirectly by the tension of the antigravity musculature on the skeleton. It can

also be caused by muscle contraction during active movement and the resulting shifts of individual body segments.

In weightlessness, bone deformation is decreased because of the lessened mechanical loading of the bone system, particularly spine and tubular bones. This means that the afferent impulse flow from the osteoceptors to the central nervous system will probably be diminished. In addition, the bone demineralization occurring during prolonged spaceflights may lead to a decreased supply of nutrients and oxygen to the bone tissue, and thus alter the chemomodal impulse flow from the osteoceptors.

The foregoing theoretical analysis, based on general physiological concepts of mechanoreceptor functioning, leads to the hypothesis that there will not only be a weightlessness-induced change in impulse flow from varying receptor groups, but also a decrease in the total volume of impulse flow (Fig. 3).

B. Responses to Changed Afferentation

A change of afferent impulse flow during weightlessness may result in a disturbed interaction between different systems; altered cortical/subcortical ratios and an altered functional status of the central mechanisms regulating afferent and vegetative functions (Fig. 4). These responses will be discussed in more detail below.

Interaction between Afferent Systems

Altered afferentation during weightlessness will inevitably result in a disturbance of the interaction between sensory systems, which transform excitation energy into neural impulses and conduct these to the central nervous system. These disturbances specifically manifest themselves as sensory conflicts. Such conflicts occur when there is a discordance between information received from various sensory systems and the pre-existing image that is integrally-perceived and that is based on previous experience. They reflect a certain position and shifting of the body in space. Such sensory conflicts are probably the leading cause of the development of space motion sickness.²⁰

Theoretically, sensory conflicts may develop between various groups in the vestibular system when there is a disturbed interaction between the visual and the canalo-otolith systems, and between the visual system and non-labyrinthine mechanoreceptors. They may also be manifested as an altered ratio of impulse flow from mechanoreceptors of vessels situated above and below the hydrostatically indifferent point (Fig. 5).^{4,13}

For the development of space motion sickness, the greatest importance is attached to the oculo-vestibular and canalo-otolith conflicts.²⁰ In this respect, it is important to note that studies performed during a short-term space flight have revealed alterations in vestibulo-ocular and canalo-otolith interaction.¹⁶

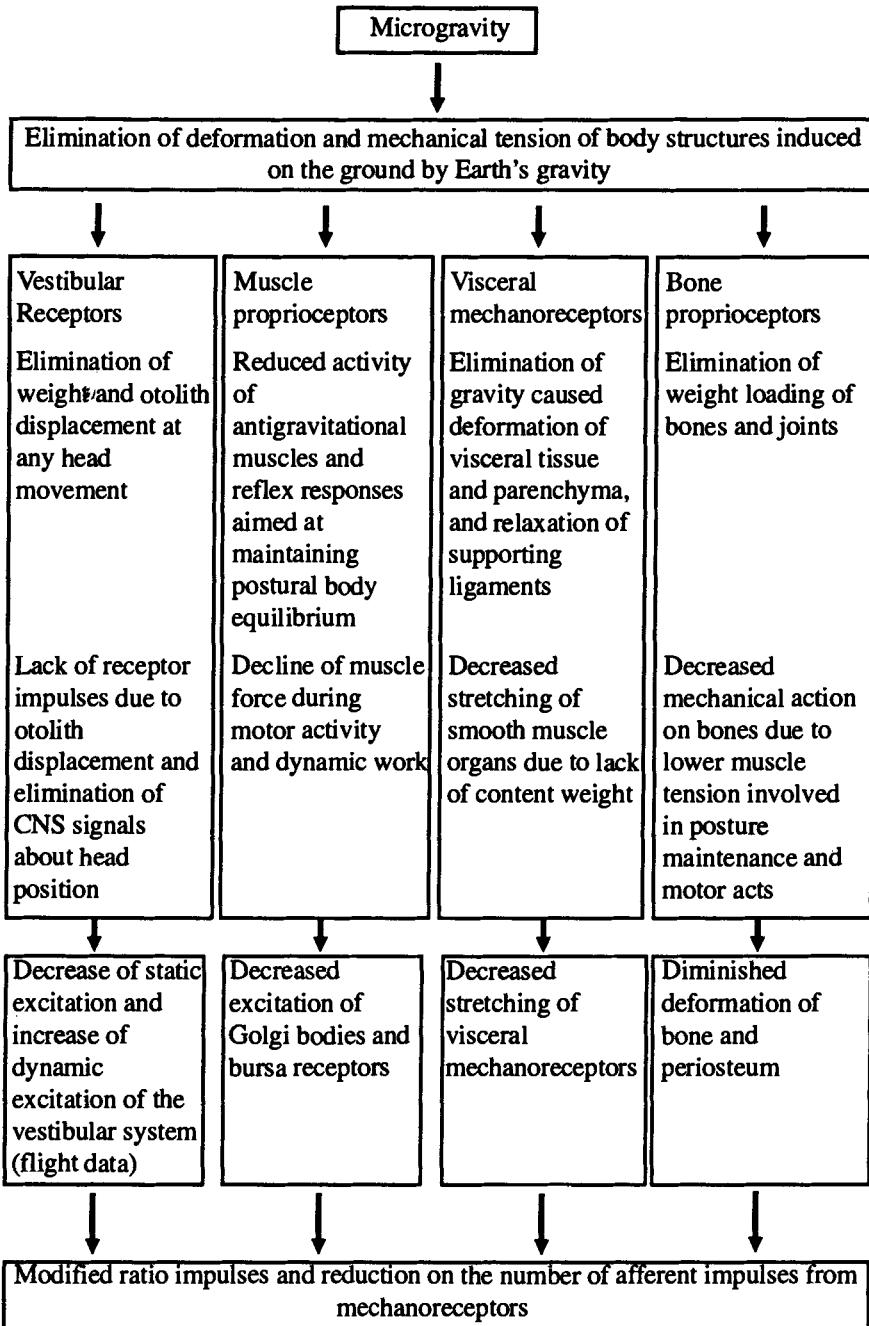


Figure 3. Hypothetical model of changes in afferent impulses from mechanoreceptors in microgravity.

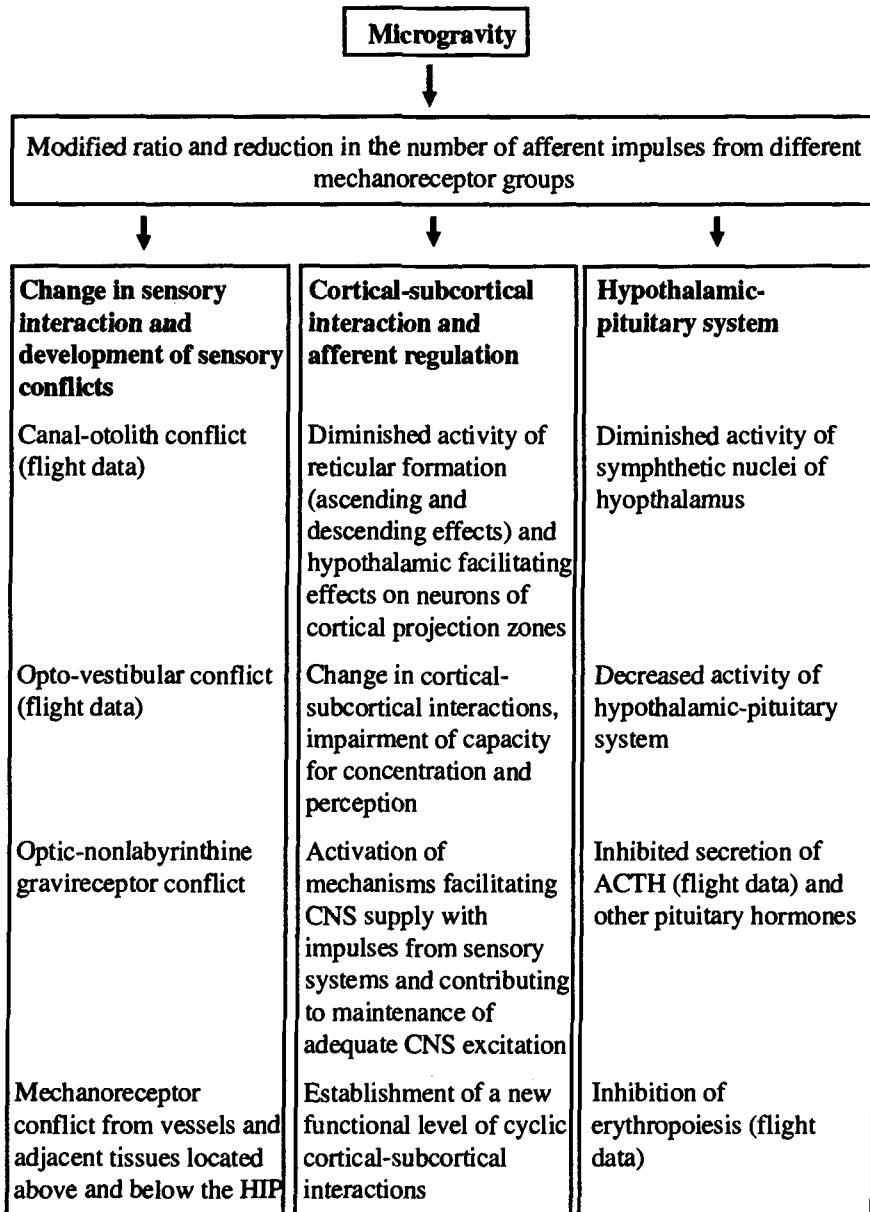


Figure 4. Hypothetical model of responses associated with changes in afferent input in microgravity.

On Earth (normal)	In Microgravity (sensory conflict)
1. Canal-otolith interaction	
Head rotatory movements elicit signals in canal receptors against background of continuous impulses from otoliths	Head rotatory movements elicit signals in canal receptors against background of drastically reduced (to the spontaneous level) impulses from otoliths
2. Interaction of visual system and canal-otolith system	
Both systems send signals about body position as related to space coordinates	Visual system sends adequate signals about spatial body position whereas canal-otolith system sends modified signals that are discrepant with those from visual system
3. Interaction of visual system and nonlabyrinthine gravitational mechanoreceptors	
Visual system, proprioceptors, visceral and skin mechanoreceptors send correct signals about body position	Visual system sends accurate signals about body position while mechanoreceptors send signals conflicting with body position
4. Asymmetry of vestibular system	
Congenital asymmetries of vestibular system (incl. those associated with weight differences between left and right otoliths) are compensated by stable interaction of sensory systems developed during ontogenesis	Changed interaction of sensory systems causes decompensation and manifestation of asymmetry of vestibular system
5. Relationship between impulses from receptors in different vascular regions	
Prevalence of afferent impulses from vessels and adjacent tissues located below HIP due to blood hydrostatic pressure.	Prevalence of afferent impulses from the vessels and adjacent tissues located above HIP due to fluid shifts in cranial direction.

Figure 5. Hypothetical sensory conflicts occurring in microgravity.

Cortical/Subcortical Interactions and Control of Afferent Functions

A deficit of the impulse flow from mechanoreceptors during weightlessness may be accompanied by a lowered activity of the dorsal portion of the hypothalamus and the reticular structure, with a decrease in its ascending and descending effects.²¹⁻²³ From an analysis of the cortical effects on underlying brain structures,²³ it seems quite possible that there is a decrease in tone and that the inhibitory effect of the cortex on subcortical structures diminishes (Fig. 6).

During the initial period of weightlessness, the expected alteration of reticulo-hypothalamo-cortical interactions may be accompanied by a decreased ability to focus attention¹ and to perceive due to the following two effects:

- a decreased blockage of the afferent pathways by descending inhibitory effects of the reticular structure. This blockage will lead to an increased reactivity of the brain cortex in areas not related to focusing attention on a given sensory stimulus, and thus to a decreased ability to concentrate on this stimulus; and
- a decrease of the mitigating influence of the hypothalamus on the cortical neurons of projection zones and of cortical segments activated by sensory stimuli. This may be accompanied by a difficulty in establishing links between the momentarily active stimulus and preceding sensory experience. As a result, there will be a diminished ability to recognize and perceive an object.

Based on general physiological concepts of sensory system regulation, bearing in mind the possible alteration of cortical / subcortical interactions in weightlessness, one can expect an attenuation of the inhibitory influence of the cortex of the large cerebral hemispheres and brain stem on the following parameters:

- the synaptic activity and the threshold of synaptic transfer;
- the dimension of the receptive field; and
- the conduction of afferent impulses.

These mechanisms probably facilitate the impulse flow from a number of sensory systems to the central nervous system; help maintain adequate excitation in the central nervous system; and establish a new level of cyclic cortical/subcortical interactions.

Hypothalamo-Pituitary System

A lowered activity of the dorsal (sympathetic) section of the hypothalamus is probably associated with a diminished activity of the hypothalamo-pituitary system participating in the control of adrenal, thyroid, and other endocrine functions (Fig. 7).^{22,24}

Hence, in weightlessness one should expect the following endocrine changes:

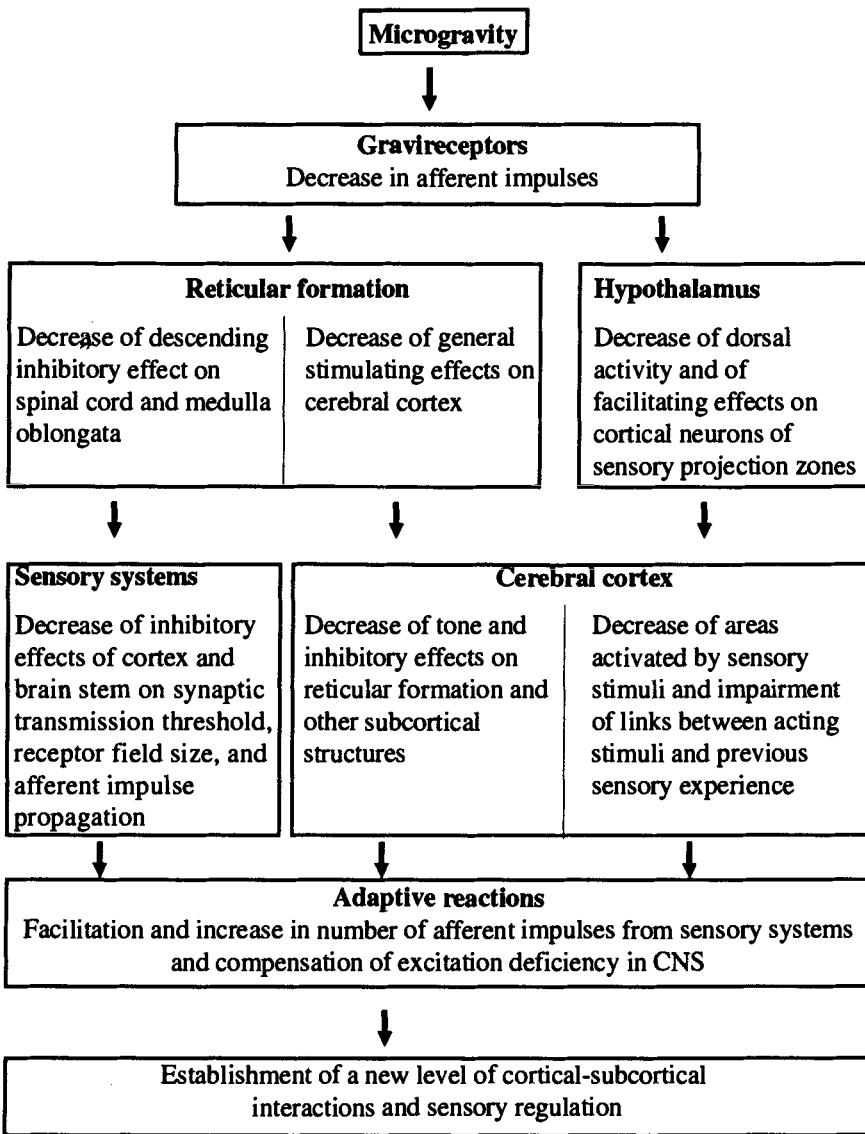


Figure 6. Hypothetical mechanisms for the effects of afferent impulses on cortical/subcortical interaction and sensory regulation in microgravity.

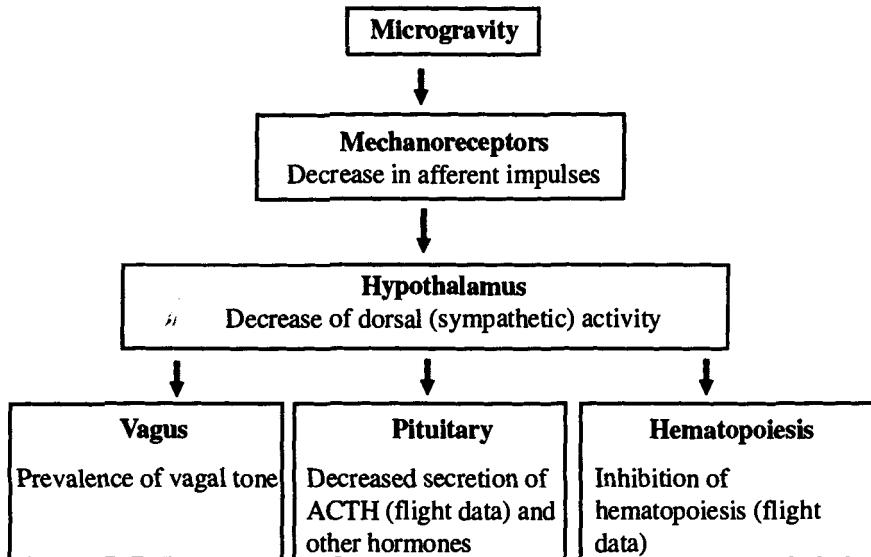


Figure 7. Hypothetical mechanisms for the effects of afferent impulses on autonomic regulation in microgravity.

- a decreased adrenal secretion of corticosteroids which participate in the regulation of metabolism and in the development of adaptive responses;
- a decreased secretion of catecholamines;
- a decreased secretion of thyroid hormones, particularly thyroxin which increases the intensity of oxidative reactions in the body;
- a decreased secretion of calcitonin which regulates the blood calcium level;
- an attenuation of sympathetic influences leading to a decreased tone and of pressor responses of smaller vessels leading to neurohumoral stimuli; and
- an inhibition of the hematopoietic activity of the bone marrow, which has been demonstrated by Chernigovsky et al.²⁵

These theoretical considerations have been confirmed by some of the results of studies during prolonged spaceflights^{4,26,27} which revealed: (1) lowered plasma level of pituitary ACTH; (2) normal blood catecholamine level, with a decreased or preflight (initial) level of their urinary excretion; (3) the absence of noticeable changes in urinary 17-oxy corticosteroid excretion; (4) a tendency to a decrease of some blood pressure parameters; and (5) an inhibition of hematopoiesis. At the same time, there was a tendency to an increased cortisol level in blood (with unchanged deoxycortisol levels and urinary excretion of these hormones), which is in disagreement with the decreased plasma ACTH content.

IV. BODY RESPONSES RELATED TO FLUID SHIFTS

Redistribution of body fluid upon exposure to weightlessness has been amply validated, both theoretically and experimentally (Fig. 8). The phenomenon has been discussed extensively in numerous publications.^{2,3,4,7,28,29}

A. Mechanisms of Fluid Redistribution

Blood

Transition to weightlessness is accompanied by elimination of hydrostatic pressure, and this alters the distribution of the deformation of blood vessels and surrounding tissues. On Earth the walls of the veins of the legs are distended by the blood that has accumulated there. The tension in the walls of these veins is higher than that in the walls of the veins in head and neck, which are in a collapsed state or have a negative pressure. Upon entering the weightless state, this terrestrial

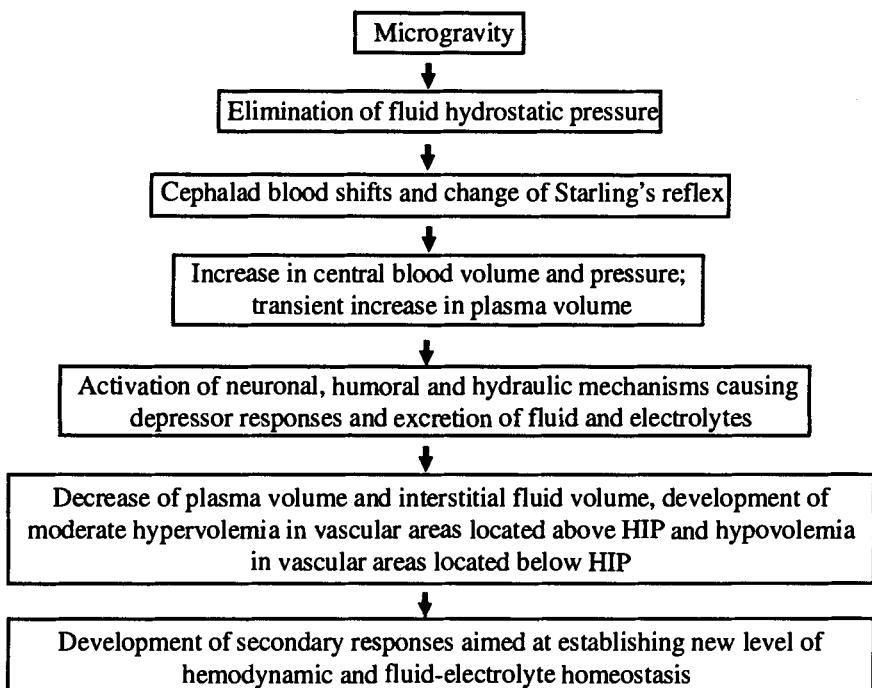


Figure 8. Main stages of physiological changes due to headward fluid shifts in microgravity.

forced pressure gradient between venous walls above and below the HIP (hydrostatically indifferent point) leads to a headward shift of venous blood from the lower extremities (Fig. 9).

The question of the localization of the blood that shifts from the lower extremities in cranial direction can be discussed on the basis of our knowledge of normal circulatory physiology and the results of space investigations. In principle, the shifted blood could be pooled in the veins of the head and neck, the cardiopulmonary compartment, and some of the visceral organs, which are natural blood depots. In view of their small volumes, the head and neck veins can accommodate only a small fraction of the blood.⁹

The major part of the blood apparently enters the cardiopulmonary compartment shortly after orbital insertion. This shift is facilitated by an increase of the cardiac volume due to the increased blood supply (Frank-Starling mechanism), the high distensibility of the pulmonary vessels and the relatively low pressure in them, and the assumed elimination of the inhomogeneity of the gravity-dependent pulmonary blood flow in weightlessness. Supporting evidence is provided by the transient increase of stroke volume and end-diastolic volume during the first day of space-flight, by the ultrasonic observation of increased pulmonary blood filling⁴⁰ and by rheographic studies.

Subsequently, there is an onset of a mechanism restricting excessive inflow of blood in the pulmonary circulation and of one precluding a pressure increase in this region. The first mechanism involves the alteration of the filtration/absorption ratio in the pulmonary capillaries. The second mechanism involves reflex reactions from cardiopulmonary mechanoreceptors responsible for visceral blood pooling.

The increased size of the visceral organs like liver, spleen, and pancreas, observed during extended spaceflights and shown by echocardiography to be the result of venous congestion,⁴⁰ could be caused by blood pooling in these organs due to the effect of unloading reflexes from low-pressure receptors. This limits the blood flow into the cardiopulmonary region and predicts the occurrence of hypertension in the pulmonary circulation.

Tissue Fluid

Under normal circumstances there is a dynamic balance between the volumes of fluid filtered from the capillaries to the tissue and absorbed from the tissue to the capillaries. A shift in this balance is accompanied by a redistribution of intravascular and intercellular fluid volume.

According to the Starling equation, the fluid volume change per minute (V -ml/min) is determined by: the filtration coefficient (C ; ml/min/mm Hg), the hydrostatic pressure in the capillary (P_c ; mm Hg) and in the interstitial fluid in the tissue (P_i), the oncotic pressure of the plasma in the capillary (P_{oc} ; mm Hg), and of the interstitial fluid in the tissue (P_{ot}):

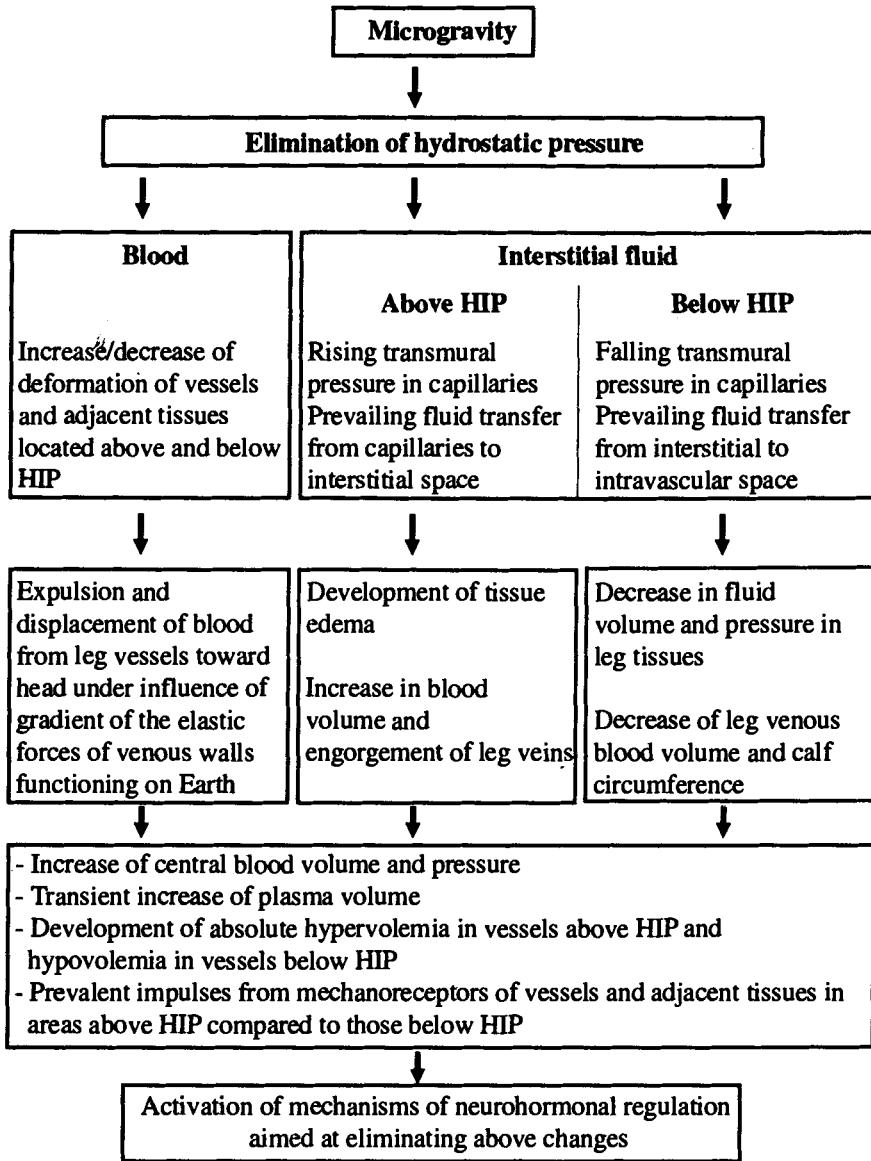


Figure 9. Hypothetical mechanisms for fluid shifts in microgravity.

$$V = C(P_c + P_{\text{ot}} - P_t - P_{\text{oc}}).$$

The most important component in this equation is the hydrostatic capillary pressure (P_c) which represents the force of filtration. The P_c value is dependent on the arterial (P_a) and venous (P_v) pressures, and on the resistance of the pre-capillary (R_a) and post-capillary (R_v) vessels. It can be represented by the Pappenheimer-Soto-Rivera equation:

$$P_c = [P_a + (R_a/R_v)P_v] / [1 + (R_a/R_v)].$$

Based on this analysis of the factors which determine the ratio of filtration and absorption in the capillaries,³⁰ we may expect an initial decrease of the transmural pressure in the capillaries of the leg upon exposure to weightlessness. This is due to the elimination of the hydrostatic factor, leading to a decrease of filtration and an increase of transmural absorption. It leads to a net fluid transition from the tissues to the intravascular space, a lowered fluid content and pressure in the tissues of the leg. In capillaries above the HIP level, an increase of the transmural pressure causes an increase of fluid filtration into the interstitial space and a decrease in fluid absorption. All this may be accompanied by an initial increase of intravascular volume, a higher leg vein distensibility (due to the lowered tissue pressure in this region), a diminished leg volume, and the development of tissue edema above the HIP level.

Subsequently, a new equilibrium level between capillary filtration and absorption is established. In regions above the HIP there is an increase of the hydrostatic pressure and a lowering of the oncotic pressure of the tissue fluid, as it accumulates in the interstitial space. There is a simultaneous increase of the oncotic pressure in blood plasma due to a decrease of the plasma volume. These changes are likely to limit the transfer of intravascular fluid to the tissues. In regions below the HIP, a decrease of the interstitial fluid volume will lead to a decrease of the hydrostatic pressure and an increase of the oncotic pressure. This will prevent a decrease of the interstitial fluid volume below a certain limit, corresponding to the new equilibrium level for the capillary filtration/absorption process.

B. Direct Effects of Body Fluid Shifts

Change in Leg Volume

Leg volume during spaceflights diminishes most rapidly during day 1, reaching a relative steady state after 3 to 5 days.⁹ During the 84-day *Skylab* mission, the leg volume decreased by about 2 liters, equivalent to 13% of the total thigh and shin volume, after several days. The arm volume remained unchanged, as might be expected.

Soft Tissues and Veins

In regions below the HIP, the following changes take place:

- a decreased distension and blood filling of cutaneous veins of the lower leg and anterior thigh, as compared to the vertical position on the ground (as shown by photographs);⁹ and
- a lowered venous pressure (as shown by occlusion plethysmography and rheography) in the lower leg region (on the average by 50%) in all six cosmonauts studied, decreased arterial blood influx and vascular pulse blood filling (by 29%);^{31,32}

In regions above the HIP, the reverse changes occur:

- overfilling and distension of the jugular veins, the cutaneous veins of the face and forehead, and the veins of arm and hand;⁹ and
- venous pressure in the arm region became equal to that in the lower leg region (two cosmonauts studied), and increased arterial influx and vascular pulse blood filling by 14 to 40%.^{31,32}

Plasma Volume and Distribution

A number of studies indicate an initial increase of plasma volume during head down tilt³³ and water immersion.³⁴ After 25 minutes of immersion the plasma volume had increased by 9%, while after 6 to 8 hours of immersion the plasma volume was diminished by 10 to 15% and did not further go down.³⁵ During the early hours of a head down tilt of 5°, an isotope technique showed an increase of the plasma volume in the neck and head region by 6% and in the thoracic region by 9%, while there was a simultaneous fall in the abdominal region by 11%, and in the legs by 8%. After 24 hours and on day 7, the plasma volume in the upper body was virtually identical to the initial values, while in the lower body it had fallen by 11 to 23%.³⁶ This was associated with a fall in the circulating blood volume of 3% after 8 hours, of 10% after 24 hours, and of 8% on day 7.

Although plasma volume has so far not been measured during spaceflights, postflight measurements after flights of 4 to 84 days showed decreases of 4 to 13%, while the erythrocyte mass decreased by 7 to 17%.²⁷ After 96- to 175-day missions on *Soyuz* and *Salyut*, there was a 12 to 20% fall in hemoglobin mass.³⁷

Central Blood Volume and Pressure: Simulation Studies

Since no direct measurements of central blood volume and pressure have been carried out during spaceflight, the results of simulation studies are discussed first.

During immersion the thoracic blood volume increases by about 700 ml, 25% of

this volume being in the cardiac chambers.³⁴ During antiorthostasis (head down tilt) the increase of the blood volume in the central vascular regions amounted to only 400 to 600 ml.³⁶ There were also increases in other cardiac volume parameters in most studies: end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), cardiac output (CO), and left ventricular ejection fraction (LVEF).³⁸⁻⁴⁰

During head down tilt, there was increased right ventricular blood filling during diastole at tilt angles of 70° and 20°, while prolonged hypokinesia at a tilt angle of 10° led to an increase in the left atrial diameter.⁴⁰ The central venous pressure (CVP) already increased after 15 to 30 minutes,⁴¹ and by 40 to 50% after 2 hours.⁴² The systemic arterial pressure increased by 3 to 9 mm Hg during the first 3 hours of head down tilt at 20°, while after 4 to 5 hours it had decreased by 5 mm Hg.⁴⁰ The pulmonary tissue volume increased by 10% after 7 hours and returned to normal after 7 days.⁴³ Pulmonary artery pressure increased after 2 to 3 hours of exposure, then decreased from day 1 to day 7 to levels below the initial values.⁴⁴ During long-term head down tilt (50 and 120 days) there was also a regional redistribution of blood filling among various pulmonary compartments with continued increased organ blood filling.⁴⁰

During water immersion the SV increased by 35%, and the CO by 32% (at unchanged heart rate) within 25 minutes.⁴⁵ There were also increases in the EDV and SV in the left ventricle on the first day, followed by a decrease on the third day.⁴⁰ Right ventricular blood filling was increased throughout the 3-day exposure.⁴⁰ Right atrial pressure increased by 18 to 20 mm Hg, and the transmural pressure by 13 mm Hg.^{34,45} The arterial pressure increased on the average by 10 mm Hg and the pulmonary artery pressure by 12 mm Hg during the first hour of immersion.⁴⁵ In 7-day "dry" immersion studies (subject loosely wrapped in water-impermeable material, and immersed up to the neck in the water), the CVP was lowered from day 2 or 3 to the end of exposure, at which time the arterial pressure and the pulmonary artery pressure had significantly decreased.⁴⁶

Thus, during the initial stages of head down tilt and water immersion, there was usually an increase of cardiac chamber volume, pulmonary blood filling, CVP, right atrial and pulmonary artery pressures, and systemic arterial pressure. As a rule, these parameters returned to normal or even decreased within 2 to 3 days. An increase of right ventricular and pulmonary blood filling and of left atrial diameter was noted from time to time during 50-, 120-, and 360-day head down tilt studies.⁴⁰

Central Blood Volume and Pressure: Spaceflight Studies

Inflight studies have provided a variety of indirect data suggesting a possible increase of the central blood volume:

- echocardiographic and rheographic evidence for an increase of the stroke volume on the first day of flight and its subsequent reduction during a 7-day mission,⁵³ confirmed by rheographic data obtained in the early flight stage;³²

- an increase, in the majority of cases, of jugular vein blood filling, concluded from the increase of the presystolic and diastolic phlebogram waves;^{31,49}
- an elevation of jugular vein pressure, deduced from phlebographic data during exposure to lower body negative pressure (determination of the pressure values at which no venous pulse wave 'a' is registered) and from the application of another non-invasive technique during prolonged missions;^{31,47-51} and
- an increase of blood filling and diameter of the vena cava inferior in long-term flights, seen in ultrasonic observations.⁴⁰

Nevertheless, there are inflight data which are not in agreement with the foregoing, namely a decrease of the pressure of the peripheral arm vein after 20 to 30 minutes of flight and 22 hours after launch by 1–4 cm H₂O.⁵² The main discrepancy refers to data obtained by indirect measurements of CVP (pressure in cubital and jugular veins) and SV. The reasons for this discrepancy may be:

- differences in method for CVP measurements by different investigators;
- different timing of measurements: in the first minutes and hours of short-term flights, or after a week of an extended flight;
- use of indirect techniques which are not adequate for the hemodynamic situation induced by weightlessness.

Hence, the indirect measurements need to be verified in the future. In this respect, a useful contribution can be expected from American investigations of the CVP by means of catheterization during the *SLS-1* mission.

As shown by Leach,²⁸ the shift of 1.8 liters of fluid from the legs during the initial days of spaceflight is considerably in excess of the fluid volume shifted during postural exposures on the ground. However, the concomitant loss of body fluid, an average of 1.5 liters in 3 days, does not allow to assess clearly the extent of additional fluid volume in the upper body during prolonged exposure to weightlessness.

However, the hypothesis of body fluid redistribution during weightlessness, associated with the headward fluid shift, appears to be confirmed by: (1) the observations of a decreased leg volume during the first day of flight; (2) the increased distension of veins above the HIP and decreased distension below this level; (3) the increased jugular venous and pulmonary arterial pressures; (4) the occurrence of tissue edema in the neck and head region; (5) the increase of the central blood volume; and (6) the short-term increase of plasma volume observed in simulation studies.

Location of Additional Blood Volume in the Thoracic Region

Concerning the location of the blood moving from the legs to the thorax, the following suggestions can be made. The increases of EDV and cardiac stroke

volume, observed sometimes in the beginning of spaceflight^{32,53} and during the initial stage of simulation studies, suggest an increase in cardiac blood content.

However, there is also evidence suggesting that additional blood is pooled in the lungs. This is indicated by a fall in the SV, usually seen on days 2 or 3 of orbital flight and in simulation experiments (after a brief initial rise). Blood would move from the leg region to systemic veins, and from there into the pulmonary vascular bed. It is not clear whether the fluid remains in the vascular bed, or is filtered into the interstitial space. However, a rise in blood filling and a decrease in oncotic pressure due to an initial increase of plasma volume in pulmonary vessels, should lead to increased filtration of fluid into the interstitial space of the pulmonary tissue.

C. Mechanisms of Neurohumoral Regulation

Neurohumoral responses, aimed at correcting fluid shifts, are triggered by the following four mechanisms:

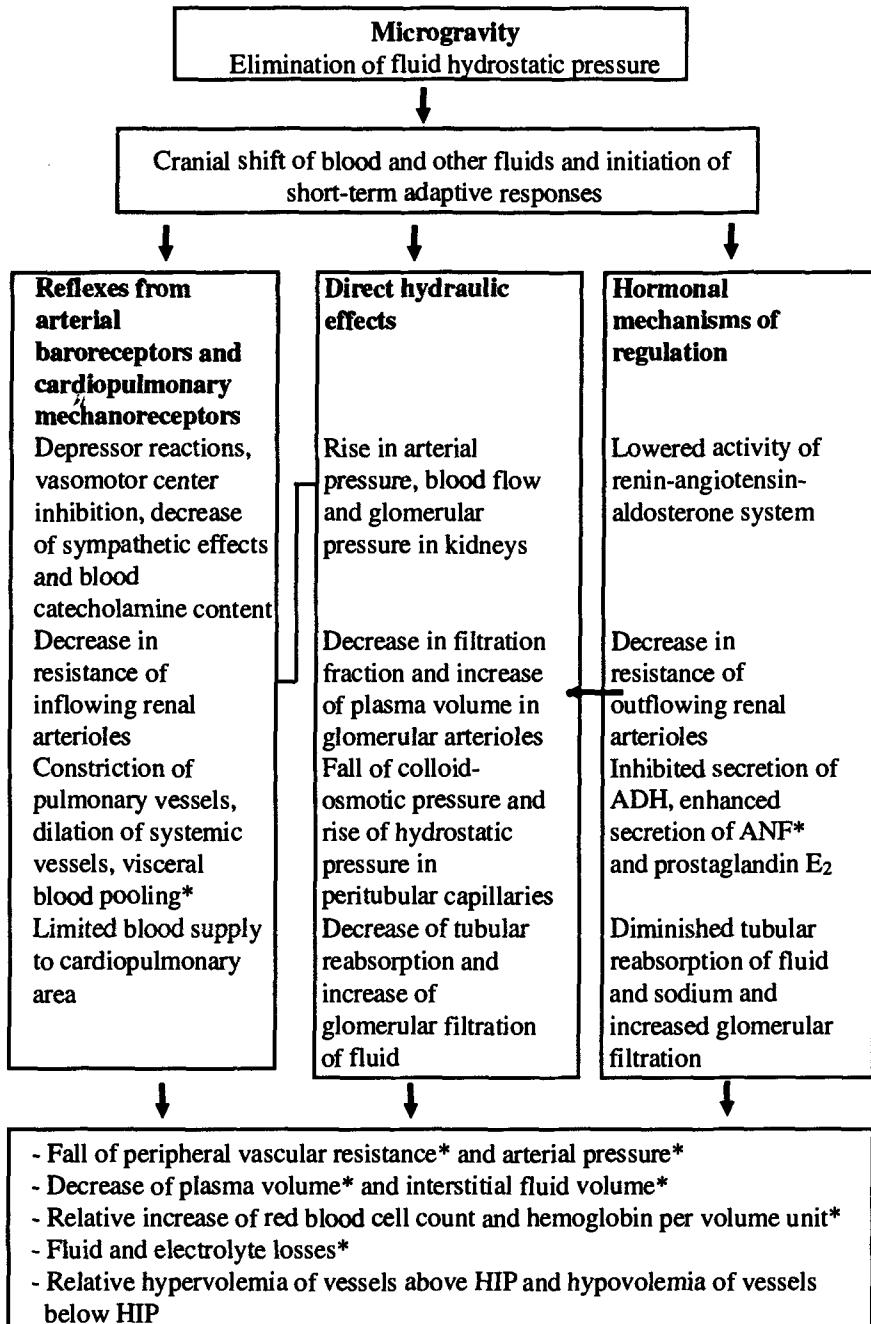
1. an increase of central blood volume, and consequently of pressure in this region, perceived by the body as an increase of effective blood volume;
2. a short-term increase of intravascular fluid volume;
3. direct hydraulic effects (related to blood redistribution) involving intrarenal mechanisms; and
4. asymmetry of the impulse flow from mechanoreceptors of vascular beds situated above and below the HIP, due to the increase of central blood volume and the decrease of the blood volume in the legs.

Role of Mechanoreceptors

The initial postulated increase of intravascular fluid and central blood volumes has two effects. First, it causes a deformation of the arterial baroreceptors of the high pressure system, consisting of the arteries of the systemic circulation and the left ventricle during systole. Second, it causes a distension of the mechanoreceptors of the vessels of the low pressure system, consisting of the veins of the systemic circulation, the right heart, the entire pulmonary circulation, the left atrium, and the left ventricle during diastole.

This will result in a number of reflex responses, which may cause changes in cardiovascular control, pulmonary ventilation, and hormonal mechanisms for the control of water/electrolyte metabolism (Fig. 10).

Normally, baroreceptor reflexes develop most quickly. This is followed in 10 to 20 minutes by activation of mechanisms associated with relaxation of the vascular wall and alteration of the functioning of the renin-angiotensin system. Finally, in 20 to 60 minutes, responses aimed at regulating renal water and electrolyte excretion take place, including mechanisms of both renal and hormonal control.^{54,55}



* Flight data

Figure 10. Hypothetical mechanisms for initial responses to the elimination of hydrostatic pressure in microgravity.

The action of baroreceptor reflexes leading to blood pooling and of reflexes affecting vascular capacity and resistance is a complex process. Stimulation of the arterial and cardiopulmonary mechanoreceptors may be accompanied by three types of responses:^{16,54-56} (1) development of depressor reflex responses; (2) inhibition of the vasomotor center with attenuated adrenergic effects and decreased adrenal catecholamine secretion; and (3) activation of unloading reflex responses from receptors of the low-pressure system (constriction of pulmonary vessels, dilation of systemic vessels, pooling of blood in the viscera, and decreased circulating blood volume). This limits excess blood supply to the cardiovascular region and establishes a balanced blood flow to the heart.

On Earth there are gravity-dependent inhomogeneities in blood flow, ventilation, gas exchange, alveolar dimension, intrapleural pressure, and parenchymal tension caused by hydrostatic pressure in the pulmonary circulation and deformation of elastic tissue due to the weight of the lungs.⁵⁷ These inhomogeneities are eliminated in weightlessness, and may lead to increased perfusion and changed perfusion-to-pulmonary ventilation ratio. This may be associated, during the initial period of weightlessness, with a tendency to some reflex increase of pulmonary ventilation resulting from congestion of pulmonary circulation and the necessity of oxygenation of a greater (compared to Earth) volume of venous blood flowing into the pulmonary vessels.

A number of other changes may be due to the effect of reflex responses from mechanoreceptors of the systemic and pulmonary circulation:^{4,31,40,41}

- decreases in total peripheral resistance;
- small decreases in arterial pressure parameters;
- maintenance of cardiac stroke volume at the preflight levels;
- increased blood filling of liver and lungs, shown by rheography and ultrasonic studies; and
- increased sizes of liver, kidney, and spleen.

Stabilization of the new functional level of circulation is probably brought about by reflex mechanisms from the carotid sinus, which are known to remain active throughout the exposure to weightlessness and to prevail over other reflexogenic responses.

Renin-Angiotensin System

The cranial blood shift during weightlessness, which causes an increase of the central blood volume, a short-term hypervolemia, and an increase of the renal blood flow (due to a direct hydraulic effect and cardiorenal reflex, see below), may also lower the activity of the renin-angiotensin system. This is confirmed by head down tilt and water immersion studies, which reveal a marked lowering of blood renin level within 2 hours of exposure.²⁹

The inhibition of the activity of the renin-angiotensin system in the early stage of weightlessness and simulation studies may have a number of consequences: a decrease of sympathetic influences; a potentiation of depressor responses developing when baroreceptors are activated; a lowering of renal efferent arteriolar resistance; aldosterone secretion; and thirst.

Regulation of Extracellular Fluid Volume and Electrolytes

The kidneys play a primary role in the regulation of fluid volume, ion composition, and levels of osmotically active substances during weightlessness. In turn, renal activity is controlled by efferent nerves and several hormones.

The greatest contribution is made by the following hormones:

- pituitary antidiuretic hormone, which increases water reabsorption in the distal segments of the nephron and decreases diuresis;
- atrial natriuretic factor (ANF), which increases sodium and fluid excretion;
- aldosterone, which increases tubular sodium reabsorption and potassium secretion; and
- E₂ prostaglandins (PGE₂), which inhibit sodium and water reabsorption in the proximal tubules.

During weightlessness the regulatory function of the kidney may be altered through direct hydraulic effects stimulating intrarenal mechanisms,²⁸ as well as through reflex responses from volume receptors.^{4,7,28,29,41} Direct hydraulic effects are caused by cranial blood shifting, and by growth of the central blood volume and pressure. This results in an increase of renal arterial pressure and blood flow, as well as a raised glomerular pressure in the capillary net of the glomerulus.²⁸ A similar effect is brought about by a decreased renal afferent arteriolar resistance, which is the result of baroreceptor reflexes leading to attenuated sympathetic effects and decreased blood catecholamine levels (see above).

The renal blood flow increases simultaneously with the diminished renal efferent arteriolar resistance that is induced by a lowered activity of the renin-angiotensin-aldosterone system. The increased renal blood flow is probably accompanied by a decrease of the filtration fraction, the percentage of fluid filtered into the blood plasma flowing through the glomerular capillaries (normally 19%). This leads to a greater amount of blood plasma flowing from the efferent glomerular arterioles into the peritubular capillary net, causing a decrease of the peritubular capillary colloidal osmotic pressure. The increased glomerular pressure and decreased resistance of the efferent renal arterioles, branching into a peritubular capillary net, probably increases peritubular capillary hydrostatic pressure. In addition, the increased glomerular pressure results in an increased glomerular filtration rate.

Thus, a higher renal arterial pressure is associated with a diminished colloidal osmotic pressure and a higher hydrostatic pressure in the peritubular capillaries.

This leads, together with the hormonal mechanisms reviewed below, to a decreased tubular fluid reabsorption, increased glomerular filtration and loss of body fluid, which is accompanied by the decrease of the plasma and interstitial fluid volumes observed after spaceflights of varying duration.

The increased central blood volume and pressure lead to an inhibition of ADH and aldosterone secretion. The ADH secretion is probably decreased through a volume receptor reflex mechanism from receptors of the low pressure region (Henry-Gauer reflex). The decreased aldosterone secretion is due to the lowered activity of the renin-angiotensin system. The secretion of ANF is stimulated. All this results in a decreased fluid and sodium tubular reabsorption and renal excretion.

These theoretical considerations have, to a great extent, been confirmed experimentally. Head down tilt and water immersion studies have revealed the following early changes.^{29,35,36,41,58}

- increase of renal blood and plasma flow, as well as of peritubular blood flow during the initial stage of simulation, followed by a return to the initial level on day 2 of the exposure;
- increased rates of excretion of fluid, sodium and osmotically active substances;
- increased diuresis with diminished water consumption, reaching a maximum at 4 to 6 hours after the start of immersion. On the average during day 1, diuresis increased by more than 50%; sodium excretion rate was tripled; and there was a negative water balance of 600–800 ml per day, while plasma volume decreased by 10 to 15%;
- potassium excretion increased, though less than that of sodium (60% increase of the Na/K ratio), during the first hours of immersion. The urinary potassium level was diminished;
- during day 1, renal potassium and magnesium excretion increased to the same extent as diuresis; and
- during the early hours of exposure, osmotic urine concentration fell by half, and the osmotic index from 3.04 ± 0.1 to 2.21 ± 0.09 at the same time as diuresis increased.

During the acute period of simulation exposure there was also a diminished ADH secretion,^{29,59} higher blood ANF level,⁶⁰ lower blood renin level (after 2 hours), lower aldosterone level (after 4 to 6 hours),²⁹ and an increased PGE₂ level.⁶¹ Since these responses appear during the initial period of simulation without changes in the concentration of osmotically-active substances and the blood ion composition, this confirms the dominant role of a volume-regulating reflex from low pressure receptors.

Thus, an analysis of the data presented shows that the alterations seen during the early period of simulation conform with the hypothesis that the loss of body fluid

and electrolytes is due to the activation of renal and hormonal regulatory mechanisms.

Studies performed during the first days of spaceflight revealed the following changes:^{27,62}

- increase in urinary excretion of ADH during the first 24 hours;
- the total fluid volume, measured by means of $^{18}\text{O}_2$ -labeled water in three crew members, decreased by 3% within the first 2 days of the flight. The plasma volume was diminished by 10.5% after 10 days;
- an increase in the plasma ADH level during the early days of the flight, which does not lead to fluid retention;
- a rise of plasma ANF level in four *Spacelab-2* crew members 30 hours after the launch and a decrease on flight day 7;
- a decline in plasma osmolarity on day 2 of the *Spacelab-2* flight and its increase on day 7; and
- a decrease of the plasma angiotensin-1 level in the first 2 days and an increase after 3 days in the crew members of three orbital *Spacelab* missions. The increase on day 3 was accompanied by a fall in the plasma sodium level and an unchanged aldosterone level .

The observed increase in ADH secretion may be due to the fact that by the time of collection of inflight blood and urine samples an initial suppression of ADH secretion, as seen in the first few hours of simulation studies, had already passed. It is also possible that the secretion of ADH and other hormones in the first hours and days of a flight are substantially influenced by emotional tension, after-effects of orbit insertion accelerations, changed work-rest regimen, preflight hydration status, and other factors. The increased secretion of ADH may also result from stimulation of an emetic reflex due to space motion sickness.⁶³

According to Cintron et al.,⁶² the rise of the ADH level in the blood may prevent a decrease of the body fluid volume or one of its compartments below a fixed level. The fall in the blood level of ANF on flight day 7 after its initial rise on flight day 2 indicates that this factor may contribute to the development of hyponatremia at the beginning of flight, but that in a later period a decline of the blood sodium level is governed by other mechanisms, in particular prostaglandins.

Summarizing, the microgravity-induced cranial blood shift, which results in short-term increases in the volumes of plasma and circulating blood, and in an increment of the central blood volume, is perceived by the baroreceptors and volume receptors as a rise of an effective blood volume. This triggers some neuronal, humoral, and direct hydraulic mechanisms, which lead to the loss of apparently excess fluid and some electrolytes from the body. As a result, the extracellular fluid volume decreases, mainly through such key mechanisms as a suppressed renin-angiotensin-aldosterone activity, an inhibited ADH secretion, and

an increased ANF secretion.^{4,7,27-29} Development of a hypohydration status may be the result of a diminished fluid intake due to a decreased thirstiness. The latter may be due to the inhibited renin-angiotensin activity, and also to developing space motion sickness. In addition to the fluid loss, there is also an increased sodium excretion, induced by a rise in the secretion of ANF and prostaglandin E₂, an inhibition of aldosterone secretion, and an elevation of the glomerular filtration rate.

Other Changes in Long-Term Space Missions

As mentioned above, much of the fluid shifted from the lower extremities to the upper body in response to reflex reactions is excreted from the body. This is associated with the following three phenomena:

1. a decrease in plasma volume, which is supported by the experimental data on hemoglobin concentration from the *Skylab* flights, and a simultaneous rise in plasma colloids;²⁸
2. a gradual decrease in the extra fluid volume in the upper body, even though the veins of face and forehead and the jugular veins remain distended and completely filled;⁹ and
3. normalization or decline of the systemic arterial pressure.

All this leads to the development of some long-term adaptational responses (Fig. 11). The reflex responses of the low pressure receptors⁵⁶ contribute to the gradual establishment of a balanced cardiac blood supply, and to the normalization of the cardiac output and cycle phase structure.

The persistent elevation of blood filling of the areas located above the HIP and its decline below the HIP can cause deconditioning of the mechanisms for vascular control in the lower extremities and a fall of the vessel tone. This occurs particularly against a decreased activity of the adaptive mechanisms counteracting the hydrostatic pressure effect in a 1-G environment, and in the absence of adequate vascular bed training.

Plasma volume decrease, followed by an increase in the erythrocyte mass, suppresses red blood cell formation. This results in a gradual decrease in total erythrocyte volume and hemoglobin content, and promotes a further fall of the circulating blood volume.

Typical for a prolonged stay in space, besides a decrease in plasma volume by 4 to 13% as observed after *Skylab* flights,²⁷ are the diminished volumes of total body water (by 820 ml) and extracellular fluid (by 330 ml), pointing to a substantial loss of intracellular fluid.²⁸ A decrease in intracellular fluid can be associated with a change of fluid osmolarity, degradation of tissues as a result of disuse, and with an increase of blood cortisol. The latter contributes to a shift of fluid from the intracellular to the interstitial space.²⁸

Prolonged Exposure to Microgravity

- ↓
- Stimulating effects occurring in initial stage of exposure**
- Decrease of plasma volume and interstitial fluid volume
 - Relative increase of red blood cells* and hemoglobin* per volume unit
 - Water and electrolyte losses*
 - Relative hypervolemia of vessels above HIP and hypovolemia of vessels below HIP
- ↓

Blood

Inhibited hematopoiesis: loss of red blood cell mass* and hemoglobin

Further decrease of circulating blood volume

Arrest of red blood cell mass decrease after 60 days in space (24)

Renal and hormonal mechanisms

Increased activity of renin-angiotensin-aldosterone system* and negative potassium balance*

Inhibited ADH secretion due to predominant impulses from the vessels located above the HIP and enhanced ADH sensitivity of renal tubule

Increased Na excretion* and decreased blood osmolarity* due to involvement of regulatory mechanisms preventing further reduction of plasma volume

Blood circulation

Balanced blood supply to heart, normalization of stroke volume*, and tendency toward normalization of STIs*

Deconditioning of mechanoreceptors of vascular regulation

Increased distensibility* and decreased compliance* of leg veins

↓

↓

↓

Formation and stabilization of the system of homeostasis regulation in microgravity

- Central compensation of asymmetry of impulses from mechanoreceptors of vessels above and below HIP
- Normalization of renal excretion of ADH** and Na**
- Establishment of new level of blood circulation with decreased resistance to gravity effects*
- Stabilization of the system of homeostasis regulation in microgravity based on formation of systemic structural track

* Flight data; ** Data obtained during 8th month in space

Figure 11. Mechanisms for physiological changes in humans during long-term spaceflight.

The early changes during spaceflight consist of decreased total and extracellular fluid volumes and the loss of some electrolytes. Hypervolemia of vascular areas above the HIP and hypovolemia in the vessels located below this point appear to result in the activation of some neurohumoral mechanisms. These factors gradually lead to a new hemodynamic and fluid-electrolyte homeostasis.

Inflight studies have, in general, supported the hypothetical model presented here. In particular, the observed increase in aldosterone secretion is a factor in establishing a negative potassium balance, a partial loss of body fluid, a decrease in plasma and total erythrocyte volumes, an increased compliance and decreased contractility of the vascular bed of the leg, and the formation of zones of free venous compliance in the leg.^{4,29,31,41,64}

This model of the changes in some physiological functions due to removal of hydrostatic pressure, like any model, naturally cannot fully reflect the actual processes occurring in the body. Among the paradoxical phenomena observed in the *Skylab* flights are an elevated natriuresis, hyponatremia, hypoosmolarity, and diminished urinary excretion of ADH.²⁶ These changes develop simultaneously with the absence of a negative water balance and a decrease in plasma volume. The discrepancies mentioned here may be caused by the following four phenomena:

1. an inhibited secretion of ADH as a result of microgravity-induced absolute and relative hypervolemia in the cardiorespiratory area with a dominant input of the low pressure receptors;
2. an increased tubular sensitivity to ADH, which in the *Skylab* flights was manifested by a slight decrease in free water clearance reflecting a reactivity of the renal collecting duct epithelium;²⁷
3. an involvement of control mechanisms preventing a further decline in blood plasma volume at the expense of a decreased sodium level in the blood, which leads to hyponatremia and hypoosmolarity; and
4. an involvement of other neurohumoral mechanisms for the regulation of the fluid and electrolyte metabolism.

With time there occurs a partial normalization of a number of initially developing changes related to the redistribution of body fluids. One to 2 months postflight, there is a tendency to normalize some parameters of circulation, red blood mass, and other values. Studies performed during the eighth month of flight suggest that there may also develop changes in the neurohumoral response pattern. All this leads to the establishment of a novel, relatively steady-state level of blood system functioning and fluid/electrolyte balance, a decreased plasma volume, a changed response to stress, and a decreased tolerance to gravity and exercise effects. A detailed analysis of the mechanisms responsible for this stabilization and adaptation of the various body systems to a prolonged stay in a microgravity environment, developed by means of long-term adaptive responses,⁶⁵ is provided later.

Long-term adaptation in response to repeated or continuous effects is based on

the formation of a systemic structural track. This process uses an existing relationship between function and genetic apparatus of cells, controlling the synthesis of nucleic acids and proteins. It involves a selective increase in the capacity of the structures responsible for control, ionic transport, and energy supply in all organs and cells that constitute a single functional system responsible for adaptation.

V. BODY RESPONSES TO MUSCULOSKELETAL UNLOADING

In microgravity, the lack of weight load upon the musculoskeletal system, as well as a sharp reduction in the muscular effort for static and dynamic work required in the Earth's 1-G environment, is responsible for muscle underloading and a deficit of muscular activity which can cause broad changes in the body functions (Fig. 12).

Theoretical analysis suggests that in this case there probably occurs a decrease in activity of postural muscles and in muscle tone, development of subatrophy or atrophy and deconditioning of antigravitational muscles, a decline in strength properties of some muscle groups, a reduced involvement of muscular activity in blood circulation, as well as a fall in the intensity of energy metabolism and plastic maintenance of functioning.

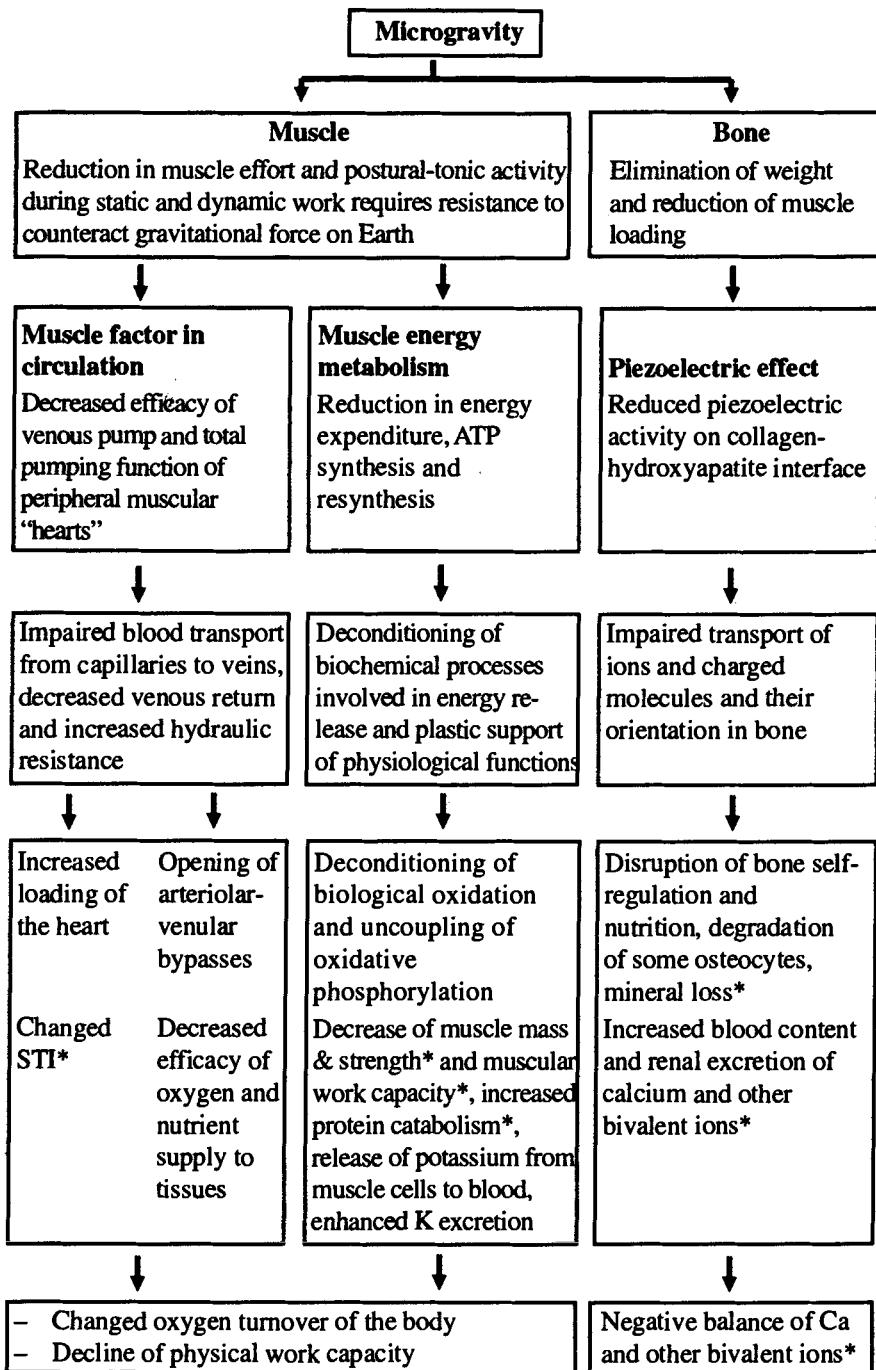
A. Postural Activity

In order to perform any movement, it is necessary to maintain a normal body posture. In other words, under conditions of gravity exposure the head and lower and upper extremities of the human body must be fixed in space by means of tonic (long-term) tension of the muscles which maintain this posture. This is achieved through an interaction of sensory input (an integrative response of the visual, vestibular, somatosensory, and proprioceptive systems) and motor output.

Normally there always exists a particular tension of the muscles, which provides, even in a passive position, the maintenance of a coordinated distribution of the body parts resulting from a reaction to the muscle receptor impulses in response to a passive or active stretch (stretch reflex).

The gravity force causing the stretch of the skeletal muscles (the extensors, first of all) is a natural stimulus of the muscular receptors responding to stretching (muscular spindles). Any exposures disturbing the assumed posture lead to a reflex compensatory movement.

In microgravity, due to the lack of a necessity to withstand the effect of Earth's gravity, the tension of the muscles and their contraction force is at a minimum, directed only at maintaining mutual positioning of the parts of the body as required for operating a spacecraft or carrying out any other element of a spaceflight program. Muscular effort required for a change in posture or carrying a heavy object is significantly less in microgravity than in the Earth's 1-G environment. This



*Flight data, STI = systolic time intervals

Figure 12. Hypothetical mechanisms for physiological changes due to elimination of weight loading of the musculoskeletal system in microgravity.

results in a decreased muscle tone of the antigravitational musculature and in the absence of adequate physical exercise, leads to functional atrophy and a declined force of some muscular groups, as has been observed after long-term space missions.

Investigations performed in a short-term (7 days) spaceflight indicate that in microgravity, unlike on Earth, an erect posture is maintained due to a prevailing activity of the leg flexors.¹² The changes in postural activity caused by moving the arm or tiptoeing were similar to the shifts noted during the analogous exposures on Earth. In this case, there exists a well-arranged and reproducible sequence of activation of the flexors and extensors of the ankle joint, thigh, and knee.

A more pronounced tilt of the body at the beginning of a stay in microgravity is related to the disappearance under these conditions of the static moment effect of the terrestrial gravitational forces on the ankle joint.¹² In order to prevent a backward tilt of the upper body induced by elastic extensor forces, and to maintain body position similar to that in the 1-G environment (with some forward tilt), an activation of the flexors occurs. This is reflected by a redistribution of the EMG-activity between the flexors and extensors with the prevalence of the EMG-activity of the tibial muscle. Relative to preflight levels, the postural EMG-activity of *m. soleus* and *m. biceps femoris* was significantly decreased, while that of *m. tibialis* and *m. quadriceps* increased. In a cosmonaut, who had repeatedly participated in spaceflights, there was also a decrease in the EMG-activity of *m. quadriceps*.

B. Muscular Factors Affecting the Circulation

According to current concepts, the blood circulation in the human body is maintained by three muscular pumps: the heart, the venous pump, and the peripheral muscle "heart" (PMH).

Venous Pump

The function of the venous pump is to compress the veins passing between the muscles during their contraction. The blood is squeezed out only in the direction of the heart since the venous valves prevent backward flow. In microgravity the pump effectiveness is diminished due to a decrease in the activity and tone of the muscles of the lower extremities, as well as by a lack of venous dilatation. This was observed in the *Skylab* flights.⁶⁴

Peripheral Muscular "Heart"

New data on the role of muscle function in blood circulation have been obtained in studies by Arinchin et al.⁶⁶ Their findings indicate: (1) that obstruction of the blood outflow of an active muscle results in an increase of venous pressure to the levels exceeding the maximum arterial pressure, and (2) an isolated skeletal muscle

is able to maintain blood flow through an artificial closed-loop circulation, which is excluded from the central heart system. The muscle thus appears to have an intramuscular peripheral "heart".

These findings have been used as the basis for considering the visceral pumping function as an intramuscular peripheral "heart" which pumps the blood from the muscle arteries via capillaries to the veins in the systemic circulation. The PMH operates not only as a delivery system but also as an active suction device. Its pumping function is manifested both when the muscles are contracted, relaxed, and at rest. This is because in the resting state asynchronous contractions of muscular fibers or their groups are developed, which support the antigravity muscle tone.

In microgravity, as a result of an anticipated decrease in the activity and function of peripheral "hearts", a central heart-developed "vis a targo" ("pushing-through force"), as well as a suction function of the heart ("vis a fronte"), will probably be increased; therefore load on the central heart will be enhanced. This assumption is supported by the findings of simulation studies disclosing that under hypokinesia the total delivery function of the peripheral "heart" is decreased.⁶⁶

The inflight development of muscle deconditioning, which results in a decreased effectiveness of the muscular factor in circulation, apparently leads to increased systolic work of the heart. The suction function of the heart (active diastole) in moving blood becomes apparent in shorter phases of isometric contraction and relaxation, and longer ejection time and phase of rapid filling of the left ventricle of the heart.³¹

In weightlessness, a decreased peripheral "heart" function may also hinder blood flow from the arteries via the muscular capillaries to the veins. This will lead to an increased regional pressure in the arterial capillaries and to opening of arterial-venous shunts through which part of the blood shifts to a venous compartment bypassing the capillaries. This may decrease the effectiveness of the supply of oxygen and nutrients to the tissue, which appears as an inflight decline in oxygen intake of the skin of the lower arm.⁶⁷

C. Plastic Support of Function

It is known that plastic maintenance of the functions, aimed at preventing the wear of cellular structures during human body functioning, is stimulated by a certain activity level of a particular organ or of the whole body. The phenomenon is based on the interaction between the genetic apparatus responsible for protein synthesis and the level of physiological activity of the cells (energy deficit, DNA, RNA, protein) which prevents wear of the cell structures. This suggests that the rate and degree of renewal of any structure is exclusively determined by the extent to which its functional load acts as a stimulus of the cellular genetic apparatus responsible for protein synthesis.⁶⁵ In other words, the structure and mass of the organelles, cells, tissues, and systems of the body are controlled by the magnitude of the functional load acting upon them.

Normal functioning of muscle fibers and the entire muscle requires the continuous and adequate resynthesis of the adenosine triphosphate (ATP) that is broken down by the contractile substance myosin to adenosine diphosphate (ADP) and inorganic phosphate during muscle contraction.

In microgravity, the decrease in the functional load of the skeletal muscles leads to an impairment of the biochemical processes related to energy production and plastic maintenance of the cell functions. This causes an inflight increase of protein catabolism (negative nitrogen balance, elevated excretion of creatine, sarcosine, 3-methylhistidine, and hydroxyproline), and a decrease in muscle mass.^{4,26,27,29} During a prolonged stay in microgravity, the coupling between oxidation and phosphorylation may be decreased. As evidence for this, a decline of tissue respiration, oxidative phosphorylation, and the coupling between respiration and phosphorylation has been observed in the muscles of rats after 18 to 22-day flights on biosatellites.⁶⁷ Based on the results of hypokinesia simulation studies, these changes may lead to decreases in the number of mitochondria and their total surface area in ATP synthesis and energy production for muscle contraction, resulting in diminished muscular performance.⁶⁷ Development of muscular deconditioning, increased protein catabolism, and loss of muscle mass appear to produce potassium transfer from the muscle cells to the blood with an increased urinary excretion of this cation, leading to a negative potassium balance during spaceflight.^{26,27,29}

D. Energy Metabolism

The gravity force is known to affect energy metabolism. Thus, a change of posture from supine to upright is followed by an increase of basal metabolism by 15 to 19%. Comparative physiological studies indicate that this factor also increased the metabolic cost during phylogenesis by the development of certain adaptive mechanisms; for example, increased bone marrow mass and hemoglobin concentration in terrestrial animals as compared to aquatic ones.

In microgravity a decreased gas exchange and metabolic cost could be expected to occur. However, in space missions the anticipated decrease of metabolic cost may be offset to some extent by other factors, such as:

- daily physical exercise;
- emotional stress during important flight stages;
- development of space motion sickness symptoms;
- establishment of different motoric behavior in an early stage of the flight;
- change in the ventilation/perfusion ratio resulting in a reflex increase of the pulmonary ventilation;
- decreased effectiveness of oxygen and nutrient supply to the tissues, associated with the opening of arterial-venous shunts due to the decreased role of the muscular factor in circulation; and

- development of uncoupling of oxidative phosphorylation during prolonged stay in microgravity.

E. Piezoelectric Effect in Bone

Bone deformation by exerting tension on the bone surface at the interface between collagen and hydroxylapatite causes a piezoelectric effect, which is proportional to the magnitude of the deformation.⁶⁸ The concave areas of the deformed bone are negatively charged and the convex areas are positively charged. Electric stimulation of the medullar cavity of the femur apparently causes bone formation in the negatively charged region of the bone. These *in vitro* experiments suggest that piezoelectric effects may act as a pump for the transfer of ions and charged molecules in bone. This may facilitate the orientation and delivery of nutrients and oxygen to the bone tissue. The data further suggest that an increased load on the bone may cause bone remodeling as a result of adequate supply of nutrients and a decrease in the catabolic processes in the bone.

Decreased loading of the skeleton in microgravity can be associated with a partial loss of osteocytes, loss of bone mineral, and change in bone crystal orientation and bone structure. The microgravity-induced decline of bone mineral density underlies the changes in the metabolism of calcium and other divalent ions, which is manifested by their negative balance.^{27,29}

VI. CONCLUSION AND SUMMARY

A comparative analysis of the effects of various space flight factors on the human body has been presented. This analysis indicates that microgravity plays a significant role in the functional changes occurring in a variety of physiological systems. The basis for the microgravity effects is the elimination of the deformation and the mechanical stress of the body structures caused by body weight, as well as a decrease of reflex tonic contraction of antigravity muscles in the 1-G environment. This results in changes in mechanoreceptor impulse output, shifts of body fluid, and a decreased functional load on the musculoskeletal system.

Theoretical analysis based on our insight in mechanoreceptor function suggests not only a change in the afferents from various receptor groups but also a decrease in their general input from graviceptors. The latter point is supported by a tension decrease of the postural muscles, a decrease in muscular effort during movements of the body and its individual parts, a change in otoliths system functioning, a decreased tension of hollow smooth muscle organs and vessels, a decreased deformation of parenchymatous organs, and a decreased load of the osteoarticular system.

The change in the input ratio and the diminished total input from graviceptors can induce four effects: (1) a disturbance of the interaction of the afferent systems,

leading to sensory conflicts which are the main cause of occurring space motion sickness, (2) a decreased activity of the dorsal compartment of the hypothalamus and the reticular formation with a weakening of the ascending and descending effects, (3) a change in the cortical/subcortical interactions and the control of the afferent functions, and (4) a fall in the hypothalamic and hypophyseal activity.

The removal of the hydrostatic pressure in microgravity causes a cranial shift of body fluid and alterations in the Starling equilibrium. This may lead to an increase of the central blood volume and a transient increment of the intravascular fluid volume. The latter is interpreted by the receptors as an increase of the effective blood volume, and is followed by an involvement of various neurohumoral mechanisms and the development of direct hydraulic effects. All these factors cause the development of depressory reflex reactions, a change in the relationship between perfusion and pulmonary ventilation, a decrease in renin-angiotensin-aldosterone activity, a suppression of the hypophyseal ADH secretion, an increased secretion of atrial natriuretic factor, and possibly, prostaglandin E₂, as well as hypohydration and elevated excretion of some ions.

In a later stage, there is a decrease in plasma volume and extracellular fluid volume, hypovolemia below HIP level, and hypervolemia in the vessels above this point. This leads to secondary reflex reactions, which activate the renin-angiotensin-aldosterone and other regulating systems, suppress erythrocyte production, and stabilize the circulatory and fluid/electrolyte homeostasis at a new level.

In long-term space missions there is a decreased renal ADH excretion, increased natriuresis, hyponatremia, and hypoosmolarity with a simultaneous decrease of the plasma volume. These effects are probably a consequence of the following changes:

- suppression of ADH secretion due to hypervolemia and predominance of inputs from vascular areas located above the HIP (mainly from the low pressure receptors);
- an increased renal tubular sensitivity to ADH; and
- an involvement of control mechanisms which prevent a further decrease of the plasma volume, possibly at the expense of a decline in the blood sodium level.

The body also responds in various ways to the removal of the gravitational load on the skeleto-muscular system and the resultant decreased muscular efforts upon performing static and dynamic exercise in microgravity. There is a decrease in postural muscle activity and in muscle tone, a deconditioning of antigravity muscles, a decreased role of the muscular factor in circulation, and a diminished intensity of plastic function maintenance. The decreased loading of the musculoskeletal system is also considered to play a role in bone demineralization with attendant loss of mineral components, manifested by negative balance of calcium and other divalent ions.

This chapter analyzes the mechanisms of the adaptation of the human body to microgravity, a process which leads to a redistribution of the functional load on the

various systems and regulatory mechanisms, and thus to the establishment of a new level of functioning and homeostatic regulation of the body's primary physiological systems. Stabilization of this level is achieved through the mobilization of immediate and delayed adaptive responses, and the formation of new or reinforcement of existing functional systems at the expense of increased power and hyperfunction of the structures involved in these systems. Decrease of the functional load, as on the musculoskeletal system, during prolonged stay in microgravity leads to deadaptation processes, like the reversible bone demineralization followed by an impairment of some functions and an activation of regulatory mechanisms. However, the use of countermeasures in long-term space missions can reduce or stop a number of these microgravity-induced changes (see next chapter).

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PHYSIOLOGICAL ASPECTS OF ADAPTATION OF MAIN HUMAN BODY SYSTEMS DURING AND AFTER SPACEFLIGHTS

Anatolyi I. Grigoriev and Anatolyi D. Egorov

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I. INTRODUCTION

The duration of manned spaceflight is limited by the human ability to adapt to microgravity and to readapt to 1-G upon return to Earth. From our experience to date it can be concluded that humans can adequately adapt to spaceflights of up to 12 months duration and readapt upon return to the Earth thereafter.

Since astronauts during prolonged missions are subjected to a variety of measures to counteract the unfavorable effects of weightlessness, it is virtually impossible to

Table 1. Countermeasures Against the Unfavorable Effects of Weightlessness

-
1. Wearing of special suits to create loading along the longitudinal body axis, simulate weight, model a certain deformation and stimulate mechanoreceptors in the musculoskeletal system.
 2. Physical exercise to maintain fitness of important systems and work capacity of the body, to activate venous pumps and peripheral muscular "hearts," to stimulate some receptor groups, and to preserve motor skills necessary for maintaining upright posture and performing locomotion after return to Earth.
 3. Simulation of the effect of hydrostatic blood pressure to diminish blood redistribution and to stimulate neuroreflexory mechanisms which regulate circulation during vertical position of the body: lower body negative pressure, occlusion cuffs, electrical muscle stimulation.
 4. Fluid retention in the body by lower body negative pressure and water-electrolyte supplements, and prevention of pooling of fluid in the lower extremities by elastic and anti-G suits, both aimed at increasing acceleration tolerance and orthostatic stability.
 5. Balanced diet and correction of nutrient uptake: supplementing food with salts, amino acids, and vitamins.
 6. Effective medication to decrease unfavorable symptoms or to correct undesirable responses.
-

Table 2. Symptom Complexes Developing during Spaceflight**1. Early Phase (One Week)**

- Subjective symptoms associated with the redistribution of body fluids
- Space motion sickness
- Changes in general pattern and coordination of motor activity

2. Extended Duration

- Changes in motor apparatus and muscle system
- Establishment of negative calcium balance and reduction of bone density
- Changes in metabolism and its regulation (prevalence of catabolic processes, changes in hormone secretion, electrolyte losses, etc.)
- Deconditioning of cardiovascular system and development of orthostatic and exercise intolerance
- Development of functional transient erythrocytopenia
- Decline of immunological reactivity

fully evaluate the physiological alterations and adaptational processes that occur in astronauts. Actually, the main purpose of the countermeasures currently used in spaceflight (Table 1) is to counteract the adaptation to weightlessness. This is achieved by providing an axial loading upon the body to create a certain deformation in the musculoskeletal system and to stimulate mechanoreceptors in this system, and by modeling some effects of hydrostatic pressure to decrease the redistribution of blood.

Physiological responses upon return to Earth gravity are influenced not only by flight duration but also by the quality of life in space. The latter aspect depends on such matters as the use of countermeasures, the proper arrangement of work, rest and sleep, and the environment provided. In this respect, the 11- and 12-month missions of three cosmonauts aboard the space station *Mir* were very satisfactory; the health condition of these cosmonauts was comparable to that of members of earlier shorter missions, and for certain parameters, was even better.

During and after spaceflight, various body systems show functional changes which are adaptive in nature. They are manifested in a number of symptom-complexes,¹ which are summarized in Table 2.

It is currently assumed that adaptation to microgravity obeys general biological laws. Exploration of the mechanisms of human adaptation to the space environment is very important because it may help us to understand how to control this process. The purpose of this chapter is to summarize available medical observations and to discuss possible mechanisms of adaptation of physiological systems to microgravity and their readaptation to Earth's gravity after flight.

II. GENERAL MECHANISM OF ADAPTATION

Phenotypical adaptation is a process developing in the course of the life of an individual, as a result of which he or she acquires resistance to a specific environmental factor that was not present before. As a result, the individual can live in an environment that was previously incompatible with life and can resolve problems that were formerly insoluble. The mechanism of phenotypical adaptation proposed by Meerson² is outlined in Figure 1.

Homeostatic changes, occurring under the influence of biologically important environmental factors, stimulate specific systems which induce the development of a specific component of adaptation. They also stimulate nonspecific systems (adrenergic and pituitary-adrenal systems) associated with the development of a nonspecific component of adaptation. The specific systems develop short-term adaptive reactions that are realized immediately after the stimulus has appeared via available physiological mechanisms and later they develop long-term adaptation in response to repeated or extended effects.

An important mechanism of adaptation to changing environments is the formation of a functional system. According to Anokhin,³ such a functional system is a dynamic self-controlling organization consisting of nervous devices, executive organs, and physiological processes that help the body to attain useful adaptive results which are evaluated by means of back afferentation.

It should be noted that the existence of a functional system or the formation of a new one is not sufficient for effective adaptation. Stable adaptation is achieved only when structural alterations develop in the cells and organs of the system, increasing its power to the level required by the environment. This is achieved by the formation of a "structural track," which is the basis for long-term specific adaptation.²

A. Immediate and Long-Term Adaptation

Immediate adaptation includes activation and hyperfunction of existing functional systems (that are predominant in specific adaptation), or relatively rapid emergence of new and closely interrelated functional systems. These mechanisms provide an early but often imperfect adaptive reaction. Transition from immediate to long-term adaptation can take place when the cells and organs constituting the functional systems develop structural changes that ensure the fixation of the system and increase its physiological capacity to the required level. It has been demonstrated that an enhancement of the functional load induced by environmental effects leads to an increased synthesis of nucleic acids and proteins which is actually the key event in adaptation. In other words, long-term adaptation is based on the formation of a systemic structural track that selectively increases the capacity of structures responsible for the control, ion transport, and energy supply in all organs and cells that constitute a single functional system responsible for adaptation. The cells of the system that plays a predominant role in adaptation display accelerated

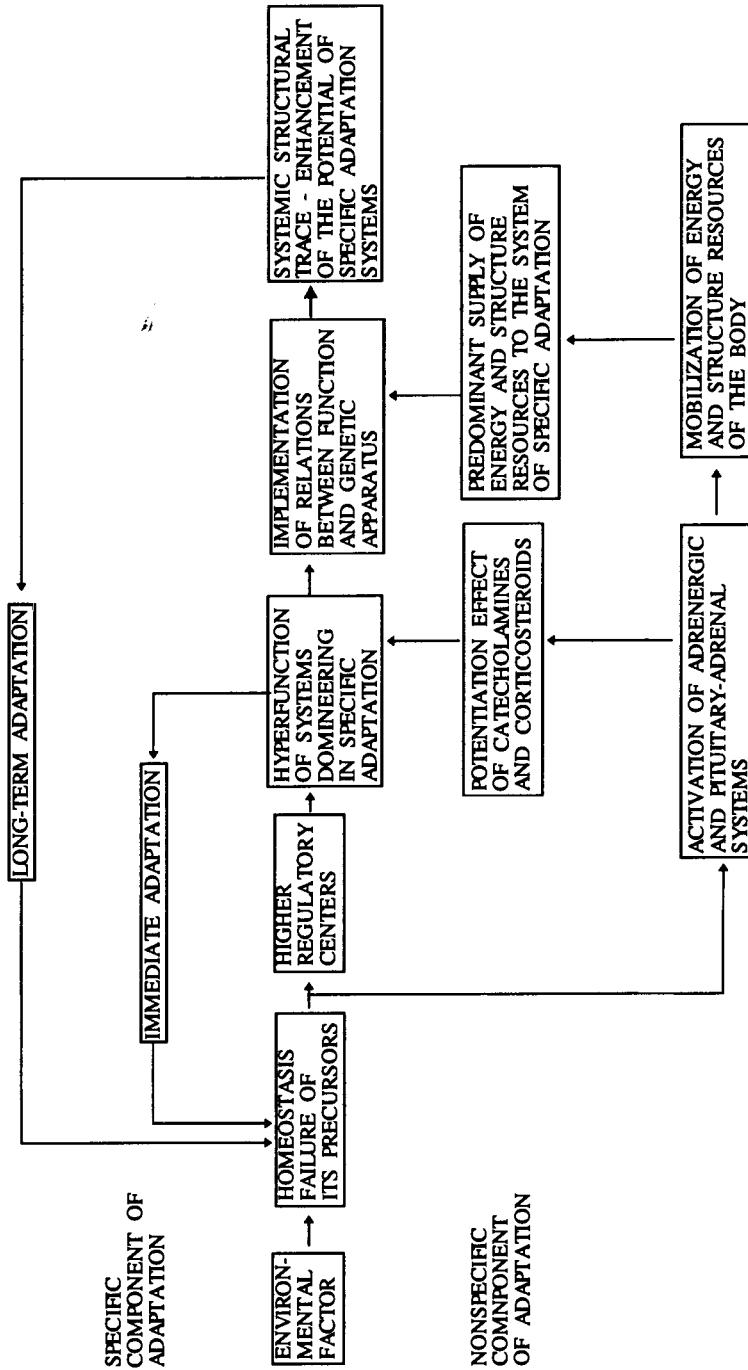


Figure 1. Schematic presentation of phenotypic adaptation according to Meerson (ref. 2).

synthesis of nucleic acids and proteins, while those of other systems show an inhibited synthesis. This implies that there is a relationship between a functional system and a genetic system, and that dominant systems are better supplied than other systems of the body.

The relationship between functional and genetic systems works faster and more extensively in neurons than in other cell systems. The neurons may grow in size and weight, owing to a stimulated biosynthesis in these cells as well as in neuronal processes and synaptic structures. Ultimately, interneuronal synaptic bonds develop and multineuronal systems form the structural foundation for the environmental adaptation. In a similar manner, the formation of structurally supported dominant systems occurs at the level of executive organs. In other words, a branched structural track emerges in the entire functional system.

Activation of nonspecific adaptive mechanisms (adrenergic and pituitary-adrenal systems) forms the foundation of the adaptation syndrome which mobilizes energy and structural reserves by shifting them from systems not involved in specific adaptation to the dominant system responsible for this adaptation. The latter system can perform its adaptive hyperfunction by a regular supply of oxygen, substrates, and amino acids and nucleotides for the synthesis of proteins and nucleic acids. This ensures the formation of a structural track. Upon repeated or extended stimulation, this process terminates and the adaptation syndrome attenuates.

According to existing concepts, microgravity is the most important factor in causing adaptive changes to establish adequate relationships between the body and the space environment. It is responsible for changes in afferent input, elimination of the hydrostatic pressure of blood, and removal of weight loads upon the musculoskeletal system. These changes are the major causes of functional changes and adaptive responses of the physiological systems of the human body. In spaceflight, the effects of microgravity are to a large extent compensated by the various countermeasures used by the astronauts. In fact, the physiological changes seen are residual deviations that cannot be eliminated with the aid of the countermeasures available today.

During the most challenging flight stages, particularly during and immediately after orbital insertion, there are changes in several physiological parameters such as heart rate and respiratory rate. There are also hormonal changes during orbital flight. Plasma ACTH decreases, probably because the activity of the dorsal compartment of the hypothalamus diminishes due to reduced impulsation from gravireceptors. Cortisol increases, and renal excretion of 17-hydroxycorticosteroids and catecholamines decreases or remains unchanged.^{1,4,5} These findings suggest that extended exposure to microgravity is not accompanied by typical signs of the stress syndrome. However, this syndrome becomes very distinct during postflight readaptation. It can therefore be concluded that nonspecific adaptive reactions develop to a greater extent at Earth's gravity after return from a spaceflight.

B. Deadadaptation

Deadadaptation, which is the inverse development of long-term adaptation, occurs upon return to the Earth after a spaceflight. When the spaceflight conditions stop acting, the systemic structural track disappears leading to reduction of the synthesis of nucleic acids and proteins, to activation of molecular mechanisms of disintegration of the adaptation track and to genetically-determined instability of structures that have developed in the course of adaptation.² This process of deadadaptation leads to a series of new adaptive reactions and changes in the entire phenotype that satisfy the specific requirements of the Earth environment.

Physiological deadadaptation is characterized by the elimination of the structural track and of adaptation itself, and a return of the body to its normal preflight status. If deadadaptation continues to develop, as may be the case after prolonged underloading of the vital systems in hypokinesia or microgravity, key cell structures may lose mass and strength and vital systems may change function and lose efficiency.

Deadadaptation after an extended period of microgravity is equivalent to adaptation to Earth's gravity and vice versa. The decrease in the functional load during stay in microgravity leads to a decline of the activity of the genetic apparatus and structural changes in some systems (e.g., decrease of muscle mass or loss of bone mineral) to satisfy the environmental conditions of spaceflight. Thus structural changes in the musculoskeletal system produced by functional underloading may be regarded as adaptation to microgravity or deadadaptation to Earth's gravity.

C. Functional Systems

According to Anokhin's concepts,³ any functional system includes various universal peripheral and central mechanisms. These include:

- the useful adaptational result as the leading component of the functional system;
- the resultant receptors;
- back afferentation from the receptors to the central elements of the functional system;
- a central structure representing a selective unification by the functional system of nervous elements of varying levels; and
- executive somatic, vegetative, and hormonal components leading to an organized purposeful behavior.

The formation of any functional system obeys general laws and develops through several stages, as shown in Figure 2.

The stage of afferent synthesis takes place in the central nervous system (CNS) and consists of comparison, selection, and synthesis of numerous afferentations, which differ in functional significance and are caused by various effects upon the

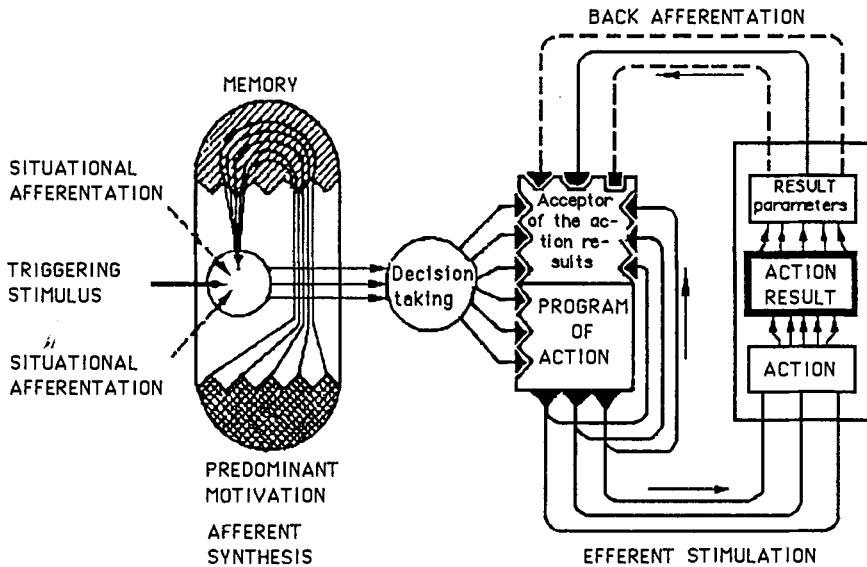


Figure 2. General scheme of a functional system according to Anokhin (ref. 3).

body. This forms the basis for the initial phase of the development of the functional system and ensures the formation of the purpose of action. In the process of afferent synthesis, four components of afferentation are integrated in the nervous centers:

1. the dominant afferentation, which is due to metabolic, hormonal and social factors and determines the need of the body in its current situation. It facilitates processing, evaluation, and active selection of sensory information sufficient for initial motivational excitations, and plays the leading role in the formation of action;
2. situational afferentation, which signals to the body the effects of all environmental factors accompanying adaptational activity, ensures pre-start integration of excitations, and generates specific responses of adaptational significance in a given environment;
3. triggering afferentation transforms an already formed pre-start integration of excitations into action; and
4. the memory apparatus, which mobilizes fragments of past experience and ensures maximal accuracy of the adaptive response.

All this information is received by the brain and is continuously supplemented by an active orientation-research reaction.

The basis of afferent synthesis is formed by the convergence of stimuli from

various receptor structures on the same neurons in the CNS. This ensures interaction, comparison, and synthesis of excitations in the axoplasm of the nervous cells. Though various CNS compartments are involved in this process, the most complete synthetic interaction of afferent ascending excitations occurs at the level of cerebral cortex and gives rise to the formation of purpose and the production of satisfactory adaptational results.

The decision-making stage is characterized by the choice of a certain behavioral response pattern from many possibilities the body has at its disposal at each given moment. In other words, at this stage there is a quick liberation from excessive degrees of freedom and organization of a complex of afferent excitations for a certain action. Significant signs of making a decision are:

- active afferent choice of a maximal number of parameters of a given situation with the help of an orientation-research reaction, which process results in an adequate action; and
- at the stage of afferent synthesis, a large number of afferent impulses with a strictly limited number of effective excitations is utilized after a decision in action formation is made.

From a neurophysiological point of view, there is at the decision-making stage a selective excitation of a certain neuronal complex which may produce the only peripherally behavioral action (reaction) corresponding to the results of afferent synthesis. Efferent excitation is transmitted by the nerves to the executive organs. In the end, efferent synthesis provides dynamic integration of somatic and vegetative functions and results in a purposeful action.

The acceptor of these results of action is a physiological apparatus for prediction and assessment. It determines the process of establishing the purpose of action and higher motivation. Until the action takes place, it is based on previous experience as a consequence of afferent synthesis and decision making. This acceptor is a preventive afferent model of the main parameters of the results of action corresponding to the afferent excitations which will appear in the future as a back afferentation from the parameters of real results. Thus, the model of the action results acceptor reflects the dominant need of the body in the form of preventive excitation of the CNS.

The stage of efferent synthesis is characterized by the integration of efferent excitations in CNS and peripheral apparatus, and is completed by an action. Efferent synthesis includes a program of the action, which is a central integration of efferent excitations in various CNS compartments and which generates a certain sequence of nervous commands to executive organs. Efferent excitation is transmitted by the nerves to the acting organs. In the end, the efferent synthesis preceding the action provides a dynamic integration of somatic and vegetative functions, resulting in purposeful action.

The stage of purposeful action includes dynamic integration of somatic, vegeta-

tive, and hormonal reactions, providing the interaction of the body with its environment. At this stage, back afferentation of the phases of action provides a continuous comparison in the acceptor-of-action results and correction of afferent synthesis with the help of an orientation-research reaction. Thus, the dominant need is satisfied in the form of the end-adaptive result. The main useful adaptive results are: homeostatic (body constants), behavioral (satisfying the main biological needs of the body); social and psychological.

Parameters of the ultimate results of action are evaluated with the help of back afferentation, which is transmitted to the CNS. There the acceptor of the results of action compares results and prediction. If they disagree, the functional system changes so that ultimately a result is achieved which is useful to the body. Anokhin³ postulates that the useful result is a system-forming factor transforming a random multitude of components into a system. According to his concept, the body is a hierarchy of functional systems acting on the basis of a multirelated interaction principle. There is always one dominant functional system and other systems arranged in a certain hierachal order in relation to the former. Alteration of the activity of any functional system always affects all other functional systems.

During weightlessness, the activation of the main mechanisms are already altered and the first shifts appear at the stage of afferent synthesis. This means that the CNS receives unusual afferentation characteristics for the real external and internal environment of the body, which is not imprinted in the memory as the body has never encountered this situation during phylogenesis and ontogenesis. As a result, difficulties in the development of an action program may appear at the decision making stage. Such a program could have ensured adaptation of the main systems of the body to weightlessness. In this unique situation the body may not have the optimal degree of freedom, which may lead to both adequate and inadequate adaptive responses, or to a chain of consecutive responses; i.e., to a search for an optimal solution. If the result obtained is inadequate in the given situation, the body will need some time to gain experience; to search for and select an optimal form of adaptive response which will ensure the formation of a new functional system compensating for the encountered disturbances; and to establish a new level of homeostatic regulation.

III. VESTIBULAR SYSTEM AND SENSORY INTERACTIONS

Numerous studies during and after spaceflights have shown that vestibular functions and sensory interactions change markedly in the process of adaptation to weightlessness and subsequent readaptation to terrestrial conditions. These changes are accompanied by a disturbance of spatial orientation and appearance of clinical symptoms of space motion sickness (SMS) during the initial period of the mission. SMS occurs in more than 50% of all cosmonauts flying for the first time. Vestibular

disfunction occurs more after prolonged missions, with vegetative symptoms noted in 49% of cases, and illusions in 50% of cases.

Objective studies have revealed a disturbance of vestibular function and sensory interactions, an increased dynamic excitability, and a decreased static excitability of the semicircular canal system; a disturbed vestibulo-oculomotor interaction with a trend to normalization of these functions on the fifth day of flight.^{6,7}

The most widely accepted hypothesis of SMS development assumes that this phenomenon may be associated with a modification of the excitability of the labyrinthine receptors, and an impairment of the correlation in the function of different sensory systems responsible for spatial orientation, which is manifested as a sensory conflict.⁸ Some significance is attributed to the asymmetry of the vestibular system and a number of other functions.^{9,10} The mechanisms thought to underlie SMS are shown schematically in Table 3.

A. Vestibular Functioning in Microgravity

Otolith receptors are known to be stimulated by gravity and by linear head movements. Under terrestrial conditions, alterations of the position of the head change the impulsion from the otoliths due to the effect of gravity. In the weightless state, inclination of the head is not associated with a change in afferentation from the otoliths, as their mass is virtually zero; thus no information about the head position in space is transmitted to the CNS.^{11,12} Under these conditions only linear translations induce responses from the otolith receptors.

The result, theoretically expected and later confirmed by studies during orbital flights, is that during adaptation to weightlessness any head movement is perceived as linear. The changed cerebral perception of otolith signals in susceptible subjects is associated with the emergence of SMS symptoms.¹¹ This concept can also be viewed as a complex sensory conflict involving changes in interaction between otoliths as well as between otolith affermentation and signals from proprioceptors of the cervical muscles and the ocular apparatus during a change of head position.

During the *Spacelab-1* mission, studies were performed to validate the hypothesis of otolithic reinterpretation. During the early period of the mission, a sudden exposure of the astronaut to a footward acceleration induced by moving the body with elastic cords, was perceived (like before the flight) as a fall. Also, Hoffman's reflex (H-reflex) was identical to that seen in preflight. Subsequently, there was a progressive decrease of the modulating effect of otolith stimulation upon the H-reflex, and on day 6 of the flight the sudden fall was accompanied by a sensation of a quick and hard linear motion (but not of a fall) without intensification of the H-reflex.¹¹ This phenomenon may be related to the loss of adaptive significance of otolithic modulation in flight since the force of gravity is balanced by the force of inertia. Hence, the fall is perceived as a linear movement. The spinal reflex and perceptive responses of the fall are absent, and the adapted brain interprets all signals from the otoliths as a linear movement. However, the sensitivity of the

Table 3. Mechanisms Underlying Space Motion Sickness

1. Disorders in the Interaction of Afferent Systems	
Development of Sensory Conflicts	
Earth Gravity	Microgravity
Spatial orientation is based on afferent signals from the vestibular system, eyes, proprioceptors and interoceptors, etc. Information from these systems is complementary and consistent with the acquired experience stored in memory (neuronal model).	Information from various vestibular receptors and other sensory systems is inconsistent with the neuronal model formed on the basis of the previous experience. This causes sensory conflicts, the most important of which are vestibulo-visual and canal-otolith conflicts.
Interlabyrinthine Asymmetry	
Earth Gravity	Microgravity
Vestibular function is normally asymmetric (asymmetry: otolith, canal, blood and cerebrospinal fluid flow, hemispheres, extralabyrinthine signals to right and left labyrinths). Differences in impulses from right and left labyrinths are compensated by the CNS.	Weightless otolith membranes, disorder in canal-otolith interaction and development of interhemispheric asymmetry of circulation disturb Earth compensation system, resulting in secondary vestibular asymmetry of impulsion of opposite sign. This leads to space motion sickness.
Cerebral Reinterpretation of Otolith Signals	
Earth Gravity	Microgravity
Otoliths send to the CNS signals about both linear acceleration and position relative to the gravity vector.	Otoliths do not send signals about head position and any head movement is perceived as linear. Susceptible subjects develop space motion sickness.
2. Factors Facilitating Development of Space Motion Sickness	
Hemodynamic Model. Blood and cerebrospinal fluid flow changes produced in microgravity by fluid displacement in the cranial direction are accompanied by microcirculatory changes at the tissue and interstitial levels, changes in fluid-electrolyte metabolism, asymmetry in the blood filling of cerebral vessels and, probably, increase of intracranial pressure - all this facilitates the development of space motion sickness.	
Endocrine Changes. Changes in the hormonal status occasionally enhance susceptibility to space motion sickness.	

otolith system in flight, as shown by tests during the *Spacelab D-1* mission using a sled to produce a linear acceleration, does not differ much from the preflight level.¹³

B. Conceptual Model of Adaptation of Sensory Systems

Manifestations of adaptation of the vestibular apparatus and other sensory systems to microgravity are the following:

- disappearance of SMS symptoms after 3 to 8 days of spaceflight;
- normalization on day 5 of spontaneous and induced oculomotor activity;⁶
- decrease of the sensitivity of the semicircular canals to adequate excitation on mission day 8 (as compared to preflight);¹⁴ and
- recovery of otolith afferentation in separate vestibular fibers of the frog by day 5 which was considerably decreased at the beginning of the flight.¹⁵

Immediate Adaptation

The immediate adaptation reactions are realized through behavioral and neurophysiological mechanisms. The behavioral mechanism in humans and primates manifests itself as a sharp decrease in the rate and amplitude of head movements in weightlessness. Registration by accelerometers of motor activity in four astronauts during the *Spacelab-1* and *Spacelab D-1* missions revealed a limitation of head movements with a gradual growth (with progressing adaptation) of accelerations and variance of these movements.¹⁶ Although all movements provoked SMS symptoms, pitching and rotational movements, particularly with eyes opened, were the more significant. Another characteristic is the refusal or selective restriction of food and water intake.

Monkeys also demonstrated drastic restrictions of motor activity early in the flight. Two hours after launch there was an increase in the time (to 1.5 hours) needed for the performance of 256 conditional reflex reactions, developed preflight and related to quick gaze fixation on targets presented to them.¹⁷ By mission day 7 the time of performance had returned to preflight levels.

Neurophysiological adaptation in microgravity aims at restoring an adequate balance between the afferent impulsation entering the CNS from the vestibular system and from other sensory systems. It is known that information from the vestibular system reaches the cerebellum via primary vestibular fibers and via fibers from the vestibular nuclei. Both pathways from the vestibular system have their terminals on the granular cells in the flocculo-nodular lobe of the vestibulo-cerebellum. These cells stimulate the Purkinje inhibitory efferent cells whose axons end directly on vestibular nuclei (or by switching in cerebellar nuclei), or on labyrinthine receptor cells.^{18,19} In the region of the vestibular nuclei there are neurons that send centrifugal axons to the vestibular receptors. These axons exert tonic descend-

ing inhibitory effects on the input of the vestibular system which increases with growing vestibular, proprioceptive and optic afferentation.¹⁹

An increase in the afferentation flow from the vestibular apparatus during performance of movements in microgravity (due to a greater dynamic excitation of the canals) will enhance the inhibitory effects of the Purkinje cells and of specific cells of the vestibular nuclei on the labyrinthine receptor apparatus. This may limit, to a certain extent, the vestibular impulsation to the CNS. The lower coefficient of the vestibular-oculomotor reaction is an indirect indication of increased inhibitory effects of the Purkinje cells on the transmission function in the arch of the reflex.¹⁷

During weightlessness there is thought to be a decrease of the ascending and descending influence of the reticular formation, resulting from the decrease of afferent impulsation from the gravireceptors. This may lead to a change of cyclic cortical-subcortical interactions. Taking this into account and considering general physiological concepts of sensory system regulation,^{20,21} one can expect a fall of inhibitory influences of the cortex of the large hemispheres and the brain stem (because of decreased reticulo-spinal effects) on synaptic activity and the synaptic transmission threshold. The same would be true for the receptive field size and transmittance of afferent impulsation, which may facilitate conduction of the impulsation to the CNS.

Long-Term Adaptation

The mechanisms described here may, to some extent, promote recovery of the disturbed interaction of the sensory systems, but they are not adequate to arrest the developing shifts. The adaptive effect which is useful for the body may be achieved by the triggering of long-term adaptation mechanisms, leading to the formation of a corresponding functional system of compensation of the sensory conflicts that arise, and of the manifestations of SMS.

Based on the concepts here described, which have been developed and experimentally confirmed by Anokhin,³ a conceptual model of adaptation of the vestibular apparatus and the sensory systems interacting with it during spaceflight has been theoretically validated and developed.^{1,12} This is summarized in Table 4.

In microgravity, due to the changes in the pattern of afferentation and development of sensory conflicts, the CNS is supplied with unusual environmental afferentation already at the stage of afferent synthesis. This afferentation describes the real external and internal environment in which the organism exists, but which has not been imprinted in memory because the organism has never before encountered this unique situation in the course of its phylogenesis or ontogenesis. Hence, at the stage of decision-making, there may be problems in the development of a program of action which could provide for adaptation of the vestibular apparatus and other afferent systems to the weightless state.

Thus at the stage of efferent synthesis, the organism may not have only one obligatory optimal degree of freedom with selective blockade of neuronal func-

Table 4. Conceptual Model of Adaptation of Vestibular and Related Sensory Systems to Microgravity and Arrest of Space Motion Sickness

General patterns of formation of functional systems on the Earth	Adaptation of vestibular and related sensory systems in μG
<i>Stage of Afferent Synthesis</i>	
Comparison, selection and synthesis in CNS of numerous afferentations from external and internal environments (predominant motivation, situational afferentation about real body situation triggering afferentation, memory mechanisms).	Disordered interaction of afferent systems and emergence of sensory conflicts lead to CNS receiving situational afferentation characterizing an unusual body environment, not imprinted in memory during phylo/ontogenesis.
<i>Stage of Decision Making</i>	
Blockade of neuronal links involved in central apparatus of the functional system that are not aimed at specific goal to be achieved. Development of program of action and system of evaluation of the results (acceptor).	Impeded formation of purposeful program of action (unique situation signalled by situational afferentation, lack of model of this situation in memory). Purpose of which is to ensure adaptation of vestibular and related afferent systems to μG .
<i>Stage of Efferent Synthesis</i>	
Efferent impulses are integrated providing peripheral action, involvement of locomotor and autonomic components under the influence of efferent commands determined by program of action.	Unique body situation may have non-optimal degree of freedom which may lead to realization of adequate or inadequate adaptive results.
<i>Results of Action (Back Afferentation)</i>	
Back afferentation of results of action enters CNS, reaches results acceptor and is compared with the predicted level. If real and predicted results coincide, action completed. If mismatch, the functional system changes and useful result is achieved (self-controlling system).	If inadequate results are obtained, body needs time to acquire individual experience, seek and select optimal pattern of useful adaptive result responsible for formation of new system compensating manifestations of space motion sickness.

tional linkages that are not involved in the integration of efferent excitations. This may bring about both adequate responses and a more or less extensive sequence of inadequate responses. In the latter case, the organism will need some time to acquire experience and to seek and choose the optimal decision or response which could ensure—through back afferentation of the results of an action—the formation of a new functional system for the compensation of sensory conflicts and the elimination of SMS symptoms. During this period, susceptible people exhibit SMS symptoms of varying severity. The lower susceptibility to SMS seen in crew

members during repeated spaceflights indicates that in weightlessness long-term adaptation develops through the formation of a systemic structural track.

IV. MUSCULOSKELETAL SYSTEM

A. Muscular System

Investigations of the muscular system in the early days of manned spaceflight demonstrated temporal shifts in accuracy and coordination, as well as changes in posture at rest and in the control of the upright posture.²²⁻²⁴ Postflight changes were observed in sensory inputs and spinal automatisms, parameters and metabolism of the muscular system, and in the motor control system. The level of these changes is not strictly correlated with the flight duration, but is largely determined by the specificity, intensity, and scope of physical exercise during flight.^{25,26}

The major causes for muscle changes in spaceflight seem to be the following:

- removal of weight load on the musculoskeletal system;
- elimination of foot support which results in functional changes of proprioceptive and other sensory systems and their interaction;
- weightlessness-induced restructuring of stereotyped motoric patterns; and
- deconditioning of the postural musculature as a result of disuse due to decreased loading.^{1,25,26}

Rearrangement of the Biomechanical Structure of Movement

One of the main factors responsible for coordination disorders is a rearrangement of the biomechanical structure of movements in weightlessness. Removal of weight load on the musculoskeletal system is likely to induce:

- changes in the relation between body mass and the amount of effort required for its motion;
- elimination of static work, like holding a load or a part of the body;
- decrease of dynamic work, like shifting loads or moving inside a spacecraft;
- reduced activity of postural-tonic musculature;
- inadequate fulfillment of certain “Earthly” movements; and
- formation of new coordination patterns and alteration of the motor activity as a whole (cross-synergy as “swimming” or “soaring” in a spacecraft).

These characteristics of motor activity during a prolonged stay in weightlessness appear to be accompanied by profound structural, functional, and coordinational adaptive processes in different elements of the muscular system.²⁵ This rearrangement leads to a need to recalibrate the effort scale towards lower values, to

deprivation of the phase-tone interaction including alpha- and gamma-coactivation, and to a modification of the stereotypic motoric patterns.

Changes in Cortex-Subcortex Relations and Sensorimotor Control

As already mentioned, reduction of the total flux of afferent impulsion and a change in its ratio from various groups of mechanoreceptors may cause a decrease in the activity of the hypothalamic dorsal section and reticular formation with alleviation of its ascending and descending influences, shifts in cortex-subcortex relations, and interaction of sensory systems leading to the development of sensory conflicts. All this strongly affects the processes of motoric control. A decrease of the hypothalamic dorsal activity induces diminution of the inhibiting influences on the cortex neurons of sensory projection zones.²⁷ As a result, there occurs a narrowing of the areas activated by sensory irritants. This hinders the interaction between a present irritant and a previous sensory experience, which may extend the time interval between intention realization and a motor act as observed in the initial period of spaceflight.

Changes in the Afferent Input

The absence in weightlessness of deformation and mechanical tension of body structures, and of support stimuli for the unfixed body, will lead to a diminution of the stimulating influences of mechanoreceptors, mainly from muscle and vestibular and skin proprioceptors. This must be the main cause of the loss of muscle tone in the antigravity muscles. Based on investigations of the impact of support on the development of muscle atony in model experiments, Kozlovskaya²⁵ concludes that a deprivation of foot support is responsible for the observed changes. A decrease of muscle tone in the antigravity muscles of the leg was measured after completion of spaceflights as well as after water immersion and hypokinesia.^{25,26}

As is known, skeletal muscle tone is supported by the pool of tonic alpha-motoneurons. Their impulsion depends on the level of stimulation of the primary endings of muscle spindles which are being stretched upon excitation of gamma-motoneurons. The latter are controlled by the reticular formation²⁸ and other structures of the CNS. Normally the gravity force stretches skeletal muscles (extensors in particular), and stimulates the receptors of muscle spindles. In the absence of gravity, the activity of muscle spindle receptors decreases, and muscle tone is reduced correspondingly. In turn, a decrease of afferent impulsion from mechanoreceptors induces a reduction of descending reticular spinal influences. This may lead to inhibition of gamma-motoneuron activity, thus promoting maintenance of alpha and gamma-motoneuron coactivation (coupling) on a new, lower functional level.²⁵

According to current knowledge, reduced muscle tone may result from changes in afferent innervation of muscles and motoneuron pools, and in mechanisms of

reflexory interactions and the trophic apparatus.²⁵ Deprivation effects can be accompanied by:

- deterioration of the accuracy of movements, since muscle atony or atrophy causes a discrepancy between central commands and the current state of executive organs;
- lower activity of motor control systems due to insufficient tonic stimulation; and
- decreased efficiency of the supraspinal motor centers.

According to experimental data and theoretical assumptions, the elimination of support load and the resulting reflexory changes in tone and sensory systems are playing the main role in the development of motion disorders during the initial period of exposure to weightlessness. In a later period of weightlessness, structural reorganizations, such as functional atrophy, arise as the consequence of insufficient loading of the muscular system.

Atrophy of Disuse

Atrophy of disuse, or functional atrophy, is a reduction in size and function of cells, tissues, and organs as a result of decreased loading. Insufficiency of cell function produces a weakening, or even an absence, of stimuli which provide definite levels of assimilation and dissimilation processes in the cells of an idle or unloaded organ. A decrease of static and dynamic loads during physical activity in weightlessness, together with some measure of hypokinesia and hypodynamics, and a diminution of the total scope of physical loads in long-term spaceflight, provokes the development of atrophic and sub-atrophic processes in the muscular system. These processes are predominantly in antigravity muscles, and apparently in all other elements of the muscular system.

Extensive data about the state of the muscular system during and after spaceflight show the need of gravity action for the preservation of normal muscle structure and function, especially with regard to the antigravity muscles which maintain upright posture on Earth. In weightlessness these muscles undergo atrophic changes with a decrease in mass, volume, strength, tone, and elasticity. Animal studies have shown proliferation of connective tissue, disintegration of certain proteins and some other changes in the metabolism and fine structure of muscle fibers.

Acuteness and duration of muscular disorders depend less on flight duration than on the type, scope, and regimen of physical exercise, and the physical state of the astronaut. It has been observed that accurate execution by crewmembers of the prescribed program of physical exercise generally decreases the disorders in their muscular system. Thus, in cosmonauts who were orbiting for 11 to 12 months and diligently executed two sessions of physical exercise per day, motoric changes were less conspicuous than in two members of two other spacecrews who, for various

reasons, did not perform the entire exercise program during flights of several months. However, all cosmonauts fully restored their motor functions in the postflight period.

B. Skeletal System

Changes in the skeletal system include bone demineralization and metabolic changes with mobilization of calcium and other bivalent ions from depots and their elevated excretion, along with loss of collagen matrix shown by an increased urinary excretion of hydroxyproline.^{29,30} The mechanism of bone demineralization induced by weightlessness is not well known. Factors such as removal of gravitational load on the skeleton and changes in hormonal and immune status seem to play an important role in this process.

Unloading of the Skeleton and Endocrine Changes

Changes in bone status during exposure to weightlessness can be associated with the diminished load on the skeleton and the modification of the deformation forces, as well as with changes in blood flow and metabolism in bones. Removal of weight and a decrease of muscle load on the bone system yield reduced bone deformation. This seems to be accompanied by a decrease in the piezoelectric effect at the interface of the collagen-hydroxylapatite systems and consequent changes in the movements of ions and charged molecules and in nutrient supply.³¹ These changes, combined with the increased parathormone levels in blood, the high cortisol levels in plasma and urine,⁵ and the decreased calcitonin levels observed after prolonged flights, can activate osteoclasts and reduce osteoblast activity, inhibit bone formation, and intensify bone resorption.

Immune System and Bone Homeostasis

The lymphocyte-produced osteoclast activating factor (OAF) plays an important role in the maintenance of bone homeostasis. Like parathormone, OAF *in vitro* induces bone resorption and is blocked by calcitonin, phosphate and, though less than parathormone, by cortisol.³² We may assume that OAF participates in bone resorption as a short-distance local factor.

As is seen from the data about various bone diseases, bone resorption occurs only in the simultaneous presence of OAF and also short-distance local factors as prostaglandins Gs and E, whose endogenous synthesis is necessary for OAF production.³³ Locally produced, Gs prostaglandins are the necessary mediators of cell-mediated bone resorption.³⁴ This process also appears to call up interleukine-1 which, in physiological conditions, gives rise to mediated osteoclast stimulation by initially affecting osteoblasts.³⁵

Based on this information, Konstantinova has proposed an OAF-mediated

mechanism for bone resorption in weightlessness and during hypokinesia. It was also suggested that OAF was synthesized by delayed type hypersensitivity (DTH) T-cells, which are the major "inflammation lymphokine" producers. The increased activity of these cells in spaceflight and bed rest studies is probably due to a decreased activity of specific T-suppressors.³⁶ OAF, generated by immunocompetent cells, might provide a feedback control of the spongyous bone. This may affect the dimension of the hemopoietic zones, as well as the production of formed elements from precursor cells.

Based on literature data, Konstantinova³⁶ has also suggested that OAF, together with interleukine-I and some other factors, maintains a balance between osteoblast and osteoclast function. In addition to increases in the levels of DTH effector cells and lymphokine and monokine producers, weightlessness might raise OAF production, leading to activation of osteoclasts and loss of calcium from the bone tissue. This appears to be confirmed by long-term bed rest studies. During 120- and 240-day bed rest studies, OAF production increased with increasing length of hypokinesia. There was a concurrent activation of the DTH-effectors and a raised calcium loss of the organism. Hence, there appears to be an intimate relationship between the state of the immune and hematopoietic systems on the one hand, and bone metabolism on the other hand, with OAF being one of the controlling factors.

During a prolonged stay in weightlessness, reduced piezoelectric effects, hormonal disorders, and elevated OAF production may thus cause a loss of calcium and phosphate from bone tissue, leading to increased blood levels and excretion of these ions. Ultimately, mineral density declines and destructive processes in bone tissue begin to develop. Characteristically, the lumbar vertebrae and other elements of the skeleton undergo changes in various parts of the spongyous tissue; there are changes in mineral density of posterior elements and vertebrae as a whole; and in the volume of spinal muscles. However, there is no distinct correlation between these changes and the flight duration. The occurrence of specific vertebral segmental demineralization, which is considered to be unfavorable to the weight-bearing function of the skeleton, is believed to be largely a result of inadequate exercise. Nevertheless, available countermeasures sometimes fail to prevent vertebral mineral loss. However, even during the longest manned flights to date, calcium loss and consequent deterioration of the skeletal strength have not been beyond normal limits.

Hence, long-term adaptive rearrangement of the musculoskeletal system can be described as a partial loss of the properties and qualities existing under the effect of Earth's gravity. In other words, weightlessness causes the partial elimination of a systemic structural track with respect to the musculoskeletal system. This appears to be confirmed by morphological studies on rats flown in biosatellites. There were changes in the structure and metabolism of muscle fibers, mainly the slow-twisted type, and transformation of slow-twisted fibers into fast-twisted fibers in the calf muscles. There was also reduced osteogenic activity, inhibited longitudinal growth of bones, intensive bone resorption, development of osteoporosis, and decreased mechanical strength of the bones. In addition, decreases in respiration, oxidative

phosphorylation, and respiration-phosphorylation coupling were observed in the femoral muscles, suggesting increased oxygen intake and oxygen debt without adequate increase of energy expenditure.^{37,38}

V. CARDIOVASCULAR SYSTEM

During extended space missions the main functions of the cardiovascular system are characterized by:

- maintenance of its primary integrative parameters, stroke volume, and cardiac output at the preflight level;
- changes in the ratios of the main cardiac cycle phases of the left ventricle related to an enhancement of the systolic work of the heart and its sucking function;
- slight changes in arterial pressure, a trend towards reduced total peripheral resistance and increased distensibility and decreased compliance of the leg veins;
- redistribution of pulse blood filling and changes in vessel tone in different areas followed by an increase in some visceral dimensions; and
- a more distinct inflight change of some parameters in response to provocative exercise and lower body negative pressure, and a marked postflight decrease in physical and orthostatic tolerance.^{1,39,40}

A. General Mechanisms of Inflight Changes

The major factors playing a role in the circulatory and fluid electrolyte balance during exposure to microgravity are blood shifts and reduced muscle function. Blood shifts in the cranial direction are due to the elimination of the hydrostatic pressure of the blood, which triggers nervous-reflex and humoral mechanisms to provide adequate conditions for cerebral and thoracic circulation. Reduced function of the muscular system, due to its underloading and deconditioning, affects blood propagation and venous return.^{1,39-41}

Short-term adaptive reactions develop in response to the cranial blood shift and the increase of intravascular and central volume. They are realized through the activation and hyperfunction of physiological mechanisms that occur in the body. At an early stage, these reactions are manifested by an increase in the heart rate (Bainbridge reflex) and a rise in arterial pressure (Cushing reflex). At later stages, the following two processes are probably sequentially triggered: depressor reflexes from arterial mechanoreceptors, and unloading reflexes from mechanoreceptors of the cardiopulmonary area.⁴² These reactions are expected to cause a decrease of the vascular tone, an increase of blood pooling in the viscera, a reduction of plasma volume, and an increase of electrolyte excretion.^{5,41,43,44} The displacement of the

center of body mass in the cranial direction, the redistribution of blood, and the blood reduction of the blood volume in the legs by 1.5 to 2.0 liters²⁴ are accompanied by, (1) the development of relative hypervolemia and the prevalence of impulses from mechanoreceptors of vessels above the hydrostatically indifferent point (HIP), and (2) hypovolemia of vessels below the HIP.

Long-term adaptive responses occur under the influence of the above changes induced by short-term adaptive responses. They consist of activation and tension of a neuroreflex and hormonal mechanisms that complete the formation and maintenance of a new level of hemodynamic and fluid-electrolyte homeostasis adequate for the weightless environment. Some systems that become underloaded develop deadaptation with respect to the Earth,² thus representing adaptation to microgravity.

B. Specific Mechanisms

Maintenance of the main integrative parameters of cardiac function, particularly the stroke volume at or near the preflight level, probably results from the development of adaptive responses during a prolonged mission. There is some tendency to an increase of the heart rate, and consequently of the cardiac output. This may be due to intensive activity of the crew members and the possibly associated development of work tension.

Muscle deconditioning in microgravity may aggravate the loss of venous "pump" efficiency that has been observed during spaceflight⁴⁵ and of the total pumping function of peripheral muscle "hearts" as observed in bed rest studies.⁴⁶ These factors are probably responsible for the enhancement of the systolic work of the heart (*vis a tergo*) and its sucking function (*vis a fronte*, active diastole) in blood propagation, which is manifested as a shortening of the hemodynamically ineffective isometric phase and an elongation of the left ventricle fast-filling phase.^{1,39,40}

During extended spaceflights, a decrease of the total peripheral resistance and minimal arterial pressure has been noticed. Another observation is a decline of the tone of small vessels in the area of the internal carotid arteries, which enhances blood outflow and prevents further increase of venous engorgement. These two observations seem to result from depressor reflex reactions of the mechanoreceptors of the systemic and pulmonary circulation.^{1,39,41} As is known from circulatory physiology, reflexes from stretch mechanoreceptors of the cardiopulmonary area lead, when stimulated, to reduction of adrenergic effects, enhancement of vagal tone, decline of vessel tone and arterial pressure, contraction of lung arterioles, blood pooling in the visceral organs and, finally, to the restriction of blood inflow to the cardiopulmonary area.⁴² Upon insertion into orbit, these reflexes are induced by an increase of the central blood volume. They may then be maintained by prevailing afferent influences from mechanoreceptors of vessels above the HIP, particularly in the cardiopulmonary area, because of the relative hypervolemia in this area.

Increased distensibility and decreased compliance of the leg veins, and a reduced

blood flow rate in these veins, indicate a lower blood inflow into the legs.³⁹ This can be accounted for by a reduction of the load upon vessels below the HIP, which diminishes the activity of adaptive mechanisms that counteract the hydrostatic pressure effect on Earth, and may enhance deconditioning of the mechanisms regulating vessel tone. Among other factors, a decreased soft tissue turgor and diminished leg volume should be noted, leading to a partial venous collapse with the formation of zones of free distensibility, as well as the growth of depressor reflex effects, the lowering of sympatho-adrenal activity, and a change of local control mechanisms resulting from an increased central blood volume and its pressure. Opposite changes in the distensibility, compliance, and blood filling of forearm vessels can be attributed to an extended increase of blood filling in the vessels above the HIP.

During flight, an increase in the size of the parenchymatous organs may be induced by an activation of unloading reflexes from mechanoreceptors of the low pressure system, described by Parin,⁴² which follows the dilation of capacity vessels of liver, spleen, and pancreas with blood pooling in them.

The role of various factors in the growth of blood filling and increased visceral dimensions has been analyzed in detail.^{47,48} The increased blood filling of the liver and hepatic veins can, to a certain extent, be due to any of the following:

- the ability of this organ to dampen quick oscillations of the blood volume in the cardiopulmonary region;
- a functional role of the liver as a vascular buffer for the right atrium and as a reservoir of congestive blood; and
- a change in lipid metabolism during flight and the possible shifts in the bile acid formation with alterations in cholesterol transformation into bile acids.

The increased size of the pancreas may be related to the existence of a dense vascular net within this organ, which allows an increase of the pancreatic blood volume by normal vasodilation. The increased dimensions of the spleen and its veins is probably caused by the physiological role of this organ as the regional pool of the portal system. Another factor could be the possible plasmotization of the spleen due to an autoimmune process resulting from the sensitization of the body by the products of muscle disintegration due to the functional underloading of the musculoskeletal system during weightlessness.⁴⁷ Changes in pulmonary blood volume may be induced by the dumping properties of the pulmonary vessels and the possible triggering of unloading reflexes in microgravity.

In the genesis of increased kidney size, a particular role may be played by a change in fluid-electrolyte homeostasis in microgravity, which appears to result in an increased renal loading due to elevated excretion of monovalent and divalent ions. Another possibility is the occurrence of congestion in renal capillaries, constriction of outflowing renal vessels and fluid accumulation in the interstitia. This possibility is supported by findings in rats flown on *Cosmos* biosatellites.

There was a consistent increase (20 to 28%) of the kidney mass, accompanied by blood congestion in vessels of the medulla and juxtaglomerular apparatus as well as a stimulation of the renin-angiotensin system.⁴⁹ A dilation of the renal cavity may be the result of a changed urine flow in the renal pelvis and upper urinary tract.

In summary, it can be said that an increase in the size of the internal organs during flight may be induced by:

- unloading reflexes leading to the pooling of blood in the viscera; and
- changes in metabolism (liver), immunity (spleen), and fluid-electrolyte homeostasis (kidney).^{47,48,50,51}

The visceral blood pooling, particularly in liver, spleen and lungs, may be regarded as an adaptive response, which limits the amount of blood entering the cardiopulmonary area and thereby prevents hypertension in the pulmonary circulation. From the foregoing, it follows that circulatory adaptation to weightlessness occurs at the expense of immediate adaptive responses (reflexes from the vascular mechanoreceptors of the systemic circulation and the cardiopulmonary region), and long-term adaptive responses (tension of regulatory mechanisms maintaining a new level of functioning of the cardiovascular system). At the same time there is the development of the deadaptation responses of vascular tone and muscle deconditioning.

C. Hemodynamic Changes during Provocative Tests

Analysis of the changed hemodynamic responses during inflight provocative tests and the lowering of postflight physical and orthostatic tolerance shows that the leading role in this process is played by the establishment of a new level of circulatory functions during weightlessness.^{1,39,40,52} This level is characterized by the following:

- fluid redistribution with a diminished fluid content in the legs, a postulated increase (at least relative) of the central blood volume and a changed tissue fluid/blood volume ratio;^{1,43–45,53}
- decreased plasma and interstitial fluid volume;^{44,54,55}
- decreased vascular tone, increased distensibility capacitance of the leg veins with a simultaneous decrease of their compliance,^{39,40,56}
- formation in the leg region of zones of free vein distensibility;⁴⁵ and
- lowering of muscular tone and a decreased contribution of the muscle-related factor (venous pumps and peripheral muscular hearts) to circulation, due to inadequate muscle loading and to muscle deconditioning during weightlessness.^{1,39,40,56}

The changed tolerance to physical and orthostatic effects during the flight may

largely be due to a diminished activity of the sympatho-adrenal system and various pressor hormones, expressed as a decreased peripheral resistance and usually a decrease in the blood level and renal excretion of catecholamines and antidiuretic hormone.^{1,4,5,57}

During the early postflight period the blood venous return is diminished, which may be due to the gradual recovery of blood distribution typical of terrestrial conditions. This consists of a shift of blood from the thoracic, cervical, and head vessels to the lower extremities, where there existed during flight hypovolemia, decreased fluid content in tissues surrounding the vessels and a decreased muscular tone. Under these conditions, even short-term orthostasis induces a rise of venous pressure in the legs and creates "a sudden hemorrhage" effect⁴⁵ in the presence of a diminished blood volume.

A compensating role in the postflight hemodynamic changes may be played by an increased sensitivity of baroreceptor mechanisms to a lowered blood pressure due to orthostatic effects, as compared to the situation in weightlessness.⁴⁵ In the latter situation, these mechanisms are adapted to the increased blood volume (possibly this is relative) in areas above the HIP. During the postflight period there may be more marked compensatory responses observed as higher vascular tone, arterial pressure, and heart rate.

Due to the altered circulatory status in weightlessness, exposure to inflight provocative tests is accompanied by a greater blood displacement from the cardio-pulmonary area to the leg vessels and a more pronounced decrease in blood volume followed by enhanced adrenergic and pressor reflex effects, as compared to the situation on Earth.

In summary, it can be said that the adaptation and deadaptation responses of the cardiovascular and hemodynamic systems and the fluid-electrolyte balance developed during flight lead to a new level of functioning of these body systems with a decreased tolerance to gravity and exercise effects.

VI. FLUID-ELECTROLYTE METABOLISM

The inflight status of the fluid-electrolyte balance is characterized by a decreased blood level and an increased urinary excretion of some ions, leading to a negative balance for these ions.^{5,44} After extended flights, increased blood levels and decreased renal excretion of monovalent ions with decreased diuresis is accompanied by a simultaneous elevation of divalent ions.^{4,43,57} Water-salt provocative tests have revealed a mismatch of the ion-regulatory system as a multidirectional change in the excretion of fluid and some ions.

A. Microgravity Responses

Changes in the fluid-electrolyte balance are a response to the cranial blood shift

and the increase in intravascular and central blood volumes. The changes are the result of the immediate compensatory mechanisms (neuronal, humoral, and hydraulic) which lead to the removal of an apparent excess of fluid from the body, a decrease of the blood plasma volume, and an increase in the renal excretion of some electrolytes.^{43,44}

Long-term adaptive responses develop under the effect of the diminished blood plasma volume, and consist of an activation of the renin-angiotensin-aldosterone system (RAAS). This is manifested by higher plasma activities of renin (during the eighth flight month) and angiotensin (during the first two weeks of flight), and an increased renal excretion of aldosterone.^{4,5,44}

The inflight *Skylab* studies revealed changes in the parameters of fluid-electrolyte balance and its regulation, which at first sight seem paradoxical and inconsistent. On the one hand, there were an increased renal excretion of sodium and antidiuretic hormone (ADH), hyponatremia and a decline in blood osmolarity. On the other hand, after the seventh inflight day there was no net loss of water, while the blood plasma volume declined by 8.4 to 15.9% and the renal excretion of aldosterone was elevated.^{5,44,54}

However, these contradictory changes can be interpreted from the standpoint of renal physiology. In weightlessness, the cranial fluid shift to the vascular bed above the HIP may cause a relative hypervolemia in this area compared to the leg vessels. Afferent impulsion from mechanoreceptors in the cardiopulmonary area can inhibit ADH secretion. A decrease in the free water clearance (relative to preflight levels) was also observed in the *Skylab* missions, suggesting an increased sensitivity of the renal collecting ducts to ADH,⁴⁴ which probably also contributes to the decrease of ADH secretion.

Hyponatremia and a decline of blood osmolarity against a decrease in blood plasma volume can be attributed to the mechanism of homeostatic regulation. This mechanism comes into play during a severe loss of extracellular fluid, which is of crucial significance for the body. It will then prevent a further decrease of the plasma volume in spite of the occurrence of a low sodium level in the blood. The absence of a negative water balance in the *Skylab* crew members, despite an inhibition of ADH secretion, suggests that for the body which has already lost a large amount of extracellular fluid (8.4 to 15.9% of plasma and 1.9% of interstitial fluid), the highest priority is to prevent a further reduction of the plasma volume because this may lead to more deleterious effects than a sodium loss.⁵⁸ Other neurohumoral mechanisms, including changes in the secretion of different groups of prostaglandins, may also play a role in these processes.

An increase of the aldosterone level may be compensated to a certain extent by enhancement of the renal blood flow.⁴⁴ However, there is no direct evidence for this, and it is more likely that the renal blood content is increased, as suggested by the postflight enlargement of the kidneys in cosmonauts.⁴⁷ Rats flown on *Cosmos* biosatellites showed a 20 to 28% increase in kidney mass, vascular congestion in

the renal medulla and juxtaglomerular apparatus, and an activation of the renin-angiotensin system.⁴⁹

During the last (eighth) month of the 237-day *Salyut-7* flight, there was a decreased renal sodium excretion and an increased ADH excretion, while the aldosterone excretion remained elevated.⁴ This can be accounted for by central compensation of unusual impulses (sensory conflict) from baroreceptors above and below the HIP due to the formation or reinforcement of the regulatory functional system of fluid-electrolyte homeostasis in microgravity on the basis of a systemic structural track. This system, characterized by tension of the hormonal mechanisms, may become stabilized before the eighth month, although there are no data on fluid-electrolyte balance between the third and eighth month of this flight.

Observed changes in potassium and calcium metabolism during flight may be due to further deadaptation of the musculoskeletal system, which leads to negative calcium and nitrogen balances, potassium loss, excretion of creatinine, sarcosine, and 3-methyl histidine and a postflight increase in hydroxyproline excretion.^{5,29,30,44,55,59,60} The above processes are stimulated not only by the elimination of weight loading on the musculoskeletal apparatus, but also by the endocrine changes observed during flight. Muscle atrophy and bone demineralization are facilitated by increased levels of cortisol in plasma and urine, short-term increase of growth hormone in plasma, reduced insulin, and increased thyroxin (postflight measurements).⁴⁴ The catabolic effect of cortisol may inhibit protein synthesis in muscle and bone, and enhance the mineral loss in bone leading to pronounced negative balance of calcium.

The persistent negative potassium balance, combined with the increased aldosterone excretion, can be associated with a decline of potassium pooling capability of the muscles due to their deconditioning and loss of mass during prolonged flights.⁴³

Bone mineral loss in microgravity is reflected in changes in the metabolism of calcium and some other bivalent cations, shown by their negative balance and increased renal excretion after flight.^{5,43,60}

B. Postflight Responses

During the postflight period, there is a decreased excretion of water, sodium, and chloride. This is primarily caused by the necessity to compensate their loss during flight, but in part also by the reverse blood shift after return to Earth as well as by a change of afferentation from low pressure receptor zones, including zones of antidiuretic and natriuretic reflexes. Hence, there are, during the postflight period, an increased ADH secretion and a raised renin-angiotensin-aldosterone system activity. These are a manifestation of an adaptive response aimed at increasing the intravascular fluid volume which was diminished inflight.

An increased secretion of ADH and aldosterone facilitates fluid and sodium retention in the body, while the increased angiotensin activity raises the vascular

tone and ensures a concordance of vascular capacity and blood volume. During the first day postflight, fluid and electrolyte retention have been observed directly as well as in load tests. The results indicate that the decreased blood volume, which has arisen during weightlessness, is insufficient for the maintenance of an adequate circulatory homeostatic functioning under terrestrial gravity.

The fall in diuresis during water load testing is probably related to an increased tubular reabsorption of fluid, since the glomerular filtration rate measured by creatinine clearance was equal to the preflight value. This response may be due to a number of causes: (1) inadequate osmoreceptor function; (2) diminished ability of the neurohypophysis to decrease ADH secretion with a fall in plasma osmolarity; (3) delayed ADH inactivation; (4) extrahypophyseal ADH secretion; (5) decreased volume and redistribution of extracellular fluid in the body; (6) disturbed renal function due to a decreased glomerular filtration rate or altered tubular function; (7) slower intestinal water absorption; (8) hormonal changes; and other factors.^{43,57}

Decreased sodium reabsorption during the postflight water loading test may be due to an imbalance between the systems which regulate water and salt metabolism. Water loading, given on an as yet unstabilized hemodynamic system, may be perceived by the body as a signal to excrete not only the excessive intravascular fluid volume, but also sodium and chloride. The body has difficulty in differentiating between information from osmoreceptors and from specific sodium receptors, leading either to volume increase by water only, or of water with sodium, which leads to an imbalance of the regulatory systems. From the data presented, it follows that the shifts in blood ion composition and in concentration of biologically active substances may alter the renal response to ADH and probably to other hormones. Low potassium and calcium levels in the blood may cause decreased absorption of water as well as of sodium and chloride.

Changes in osmoregulatory renal functions were seen immediately upon completion of spaceflight, particularly in the form of a decreased diuresis. This could be caused by the increased ADH production observed after flights, which leads to an increased water permeability of the walls of renal tubules and collecting ducts.⁴³ Hence, an increased reabsorption of osmotically free water would be expected during the postflight period. However, cosmonauts after landing, showed a fall in reabsorption of osmotically free water and in osmolaric clearance. Postflight urine concentrations of osmotically active substances were lower than preflight values at any level of diuresis.

Such alterations may be interpreted as a defect of osmotic concentration, since during a water deprivation test after the flight urine osmolarity was markedly lower than preflight, even though diuresis was unchanged or decreased. After prolonged flights osmotic concentration in the urine fell primarily because of a lowering of the urea content, while after flights of up to 18 days, duration of this fall was related to a decrease in urinary electrolyte content.⁴³

VII. RESPIRATION AND ENERGY METABOLISM

Respiratory functioning during extended spaceflight has not yet been adequately investigated. Studies have been performed only during a small number of flights, and no unified methodologic approach adequate for the flight environment was used.

Oxygen consumption and carbon dioxide production were measured during short- and long-term missions on *Salyut stations 3, 4 and 6*. Both parameters were increased in most cosmonauts, at rest as well as during graded exercise tests.⁶¹ The more detailed examinations of the *Skylab-3* crew revealed increased oxygen consumption and carbon dioxide production at rest, and decreased oxygen intake during the third grade of exercise.⁶²

The mechanism of these changes still remains unclear, since the findings are not entirely in agreement with theoretical expectations. Blood pooling in the pulmonary vasculature due to the cranial bloodshift in microgravity will cause pressure elevation in the pulmonary vessels. In addition, there is the need to oxygenate an increased volume of venous blood entering the lungs. Both effects will lead to a reflexive rise in the lung ventilation. An early inflight increase of oxygen intake can thus be related to this change of the pulmonary ventilation-perfusion ratio. In addition, during the first days of the mission some other factors may play an important role: development of a new stereotype of movements, emotional stress, occurrence of space motion sickness symptoms, and others.

An increase of oxygen intake at rest observed during extended missions may be related to the opening of arterio-venous shunts as a result of a decreased role of the muscular factor in blood circulation. Other factors may be the decline in the pumping function of peripheral muscle "hearts" during hypokinesia,⁴⁶ and the decreased effectiveness of the venous pumps in a weightless environment.⁵⁶

A decrease in oxidative phosphorylation coupling could also contribute to the increased oxygen consumption in microgravity. This assumption is supported by the decreases of tissue respiration, oxidative phosphorylation and a decline of the coupling of respiration and phosphorylation noted in rat muscles after 18 to 22 day biosatellite flights.³⁸ This could theoretically lead to an elevated oxygen intake and oxygen debt without a corresponding rise in energy expenditure. However, this assumption is not in agreement with the lack of an increase in respiratory metabolism (gas exchange) during inflight physical exercise. Such a reaction however can be regarded as a systematic training effect on a limited group of muscles during exercise.

VIII. BLOOD SYSTEM

The main hematologic change observed during spaceflight concerns the red blood cells which consists of functional erythrocytopenia, a decrease of the number of

red blood cells, the amount of hemoglobin per unit volume, and the total red cell mass and hemoglobin mass.^{44,55,63,64} There is also an increased fraction of red blood cells with an abnormal shape, and a change of erythrocyte metabolism were also noted.

A. Erythrocytopenia

Several hypotheses to explain the mechanism of spaceflight erythrocytopenia have been proposed:

- the decline of the functional load upon the muscular system may lead to a reduced metabolic activity and lower oxygen requirement of muscle, which would diminish erythropoiesis;^{1,44}
- a decline in afferent inputs from mechanoreceptors in weightlessness may decrease the activity of the hypothalamic dorsal compartment, which normally stimulates the formation of red blood cells both directly and by enhanced production of erythropoietin, as well as by a changed activity of the hypophysis;⁶⁵
- the microgravity-induced redistribution of blood and decrease of plasma volume would trigger, on a feedback basis, the compensatory mechanisms tending to maintain the major parameters of the circulating blood. This would result in a decrease of red blood cell mass (but not of the number of red cells per unit volume); and
- the destructive, osteoporosis-like process with disturbance of metabolic processes in bone during weightlessness might lead to a erythrocytopenia because of the close relationship between the bone marrow and red cell formation;
- an increased lysis of erythrocytes induced by the reticuloendothelial system. A nearly threefold increase of red blood cell lysis was noted in the rats returning from *Cosmos* biosatellite flights.⁶⁶ The inflight and postflight alterations in erythrocyte shape and the enlargement of the spleen observed in some cosmonauts⁴⁷ could mean that erythrocytes are destroyed by their selective retention and subsequent breakdown in spleen, bone marrow, and other organs. In the *Spacelab-1* astronauts, an elevated plasma content of ferritin was noted, which might point to splenic erythrocyte destruction.⁴⁴ However, a haptoglobin decline, which is typical of hemolysis, was not found in these astronauts;
- direct effect of microgravity on red cells. This hypothesis, however, seems unlikely because there is no relationship between the duration of spaceflight and the extent of erythrocyte loss. However, changes in erythrocyte interaction and agglutination, observed in an *in vitro* experiment during the *Discovery* flight, point to the possibility of a direct microgravity effect on the structure of the cell membrane;⁶⁷ and
- the possible role of the bone marrow, which deserves to be studied further with

respect to its relevance for human erythropoiesis during spaceflight. This is based upon data from rats flown on the *Cosmos 936* flight, which, after return to Earth, showed an increase of granulocytic colonies of 73% (28% in controls) and a decrease of erythroid colonies of 13.6% (52% in controls), with a simultaneous decrease in erythropoietic activity and increase in granulocytopoiesis.

Postflight recovery of red blood cell mass is severely delayed because erythropoiesis is a slow process in contrast to the rapid recovery of the plasma volume. As a result, there is an apparent decline of erythrocyte content immediately postflight, which recovers within 6 to 8 weeks postflight.

B. Change of Erythrocyte Shape

An increased proportion of spherical red blood cells and echinocytes has been observed in the blood of astronauts.⁶⁸ Usually there is rapid postflight return to normal proportions. An exception is the *Spacelab-1* crew, which displayed a doubled echinocyte count for a week after return. The increased occurrence of spherical forms of erythrocytes may be the result of chemical changes in the blood plasma. Increasing the levels of lecithin and free fatty acids in blood plasma has been shown to lead to echinocyte formation.⁶⁹ This effect might be mediated through changes in the erythrocyte membrane, as was the case in the addition to the plasma of lysolethicin, which accumulates in the cell membrane. However, the contents of these substances have not been determined in the flight blood samples.

The altered ratio of red blood cells of different shape does not appear to affect the health status or physical work capacity of the astronauts. Although in sick persons the appearance of ellipsoid and drop-like erythrocytes is viewed as a poor prognostic clinical sign, the rapid disappearance of modified erythrocytes from the circulating blood of returning astronauts indicates that we are dealing here with a harmless and adaptive response to weightlessness.

IX. IMMUNE SYSTEM

After prolonged spaceflights, the state of the immune system was characterized by a decreased activity of T-cell immunity and negligible changes in the activity of the humoral B-cell immunity system.³⁶ The available data on immunity changes during space missions, and possible mechanisms for these changes, have been reviewed by Konstantinova.³⁶

A. T-Killer Cells

Diminished postflight phytohemagglutinin (PHA)-reactivity of T-lymphocytes

has been ascribed to the effect of increased blood levels of corticosteroids due to a stress reaction.⁶⁸ Some support for this hypothesis is provided by the finding of a decrease in PHA-reactivity and increases in immunoglobulin A and G concentrations without decrease of the T-cell content after a 2-day flight in which an emergency situation caused distinct stress.³⁶ In contrast, after 7-day missions there were no changes in PHA-reactivity, but a T-cell decrease in 50% of the cosmonauts and a decreased activity of natural helper cells and T-killer cells occurred in some cosmonauts. Thus, there is some evidence for a specific role of microgravity in immune system activity through its neuroendocrine regulation.

A decreased T-lymphocyte content could, to some extent, be due to their selective migration into bone marrow, induced by an elevated corticosteroid (cortisol) level in blood. This effect has been observed in animals exposed to acute stress or corticosteroid administration.^{36,70}

An increased blood corticosteroid level may affect the immune system in another way, namely by inhibiting the production of interleukine-2,⁷¹ which is required for the generation of T-killer cells and natural killer cells. The increased postflight blood levels of catecholamines, and in some cases of insulin and other biologically active substances in cosmonauts, may also play a role.³⁶

Similar changes of hormonal status can have a negative, adenylate cyclase-mediated effect on lymphocyte metabolism, leading to suppression of RNA and DNA synthesis.⁷² The RNA and DNA synthesis rates in lecithin-activated lymphocytes are found to be decreased.⁷³

From these findings it appears that hormonal changes, such as increased cortisol and insulin levels and activation of the sympatho-adrenal system, may explain the decreased T-killer cell content and activity observed after extended space missions. There are several ways in which the hormonal changes could cause the decrease in activity of the T-killer cells.

B. Natural Killer Cells

The action of natural killer cells is a complex phenomenon, which involves at least two key functions, namely "recognition" of target cells and lysis of these cells. Studies of these two functions, conducted at the single cell level during spaceflight, have revealed a significant decline (twofold and more) in the capacity of peripheral blood lymphocytes to recognize target cells and to form strong conjugates with them.³⁶ The lytic function, although it depends on the intracellular localization of Golgi apparatus, granules and cytoskeleton and is quite labile, was not significantly affected.

The recognition function involves the expression of the lectin receptor molecules on the cell surface, and is quite resistant. It depends on the activity of cyclo-oxygenase—an intracellular enzyme catalyzing the oxidation of arachidonic acid to the eicosanoates A and B. These substances increase the intracellular cAMP level, which leads to the inhibition of the activity of natural killer cells at the "recognition" stage.⁷⁴

The decrease in natural killer cell activity could also be due to a deficiency in the release of mature natural killer cells from the bone marrow to the blood. This process may, for instance, lead to a decreased antiviral resistance.

Lymphokines, in particular interleukine-2, are known to stimulate the immunological responses. It has been demonstrated that the granules of large granular lymphocytes contain a large amount of lymphokines and lymphotoxins,⁷⁵ which can be quickly mobilized by exocytosis. A diminished secretion of lymphokines can thus be due to a decrease in the number of large granular lymphocytes in the blood, as indicated by an observed decline of natural killer cells in the blood.³⁶

X. CONCLUSION AND SUMMARY

A. Adaptation to Microgravity

Analysis of a number of physiological responses to microgravity has shown that the changes seen are accompanied by initiation of immediate as well as long-term adaptive responses. It is very likely that the latter are beginning already in the first few days of flight. Long-term adaptive responses are aimed at the formation of new functional systems of regulation of the basic homeostatic functions of the human body; the formation of a systemic structural track.

At the same time, a reduction of the functional load on some physiological systems leads to deadaptation processes and loss of properties acquired by man in the course of his individual development and life in the Earth's gravitational field. As a result, long-term exposure to microgravity produces changes in structure and metabolism of myofibers, atrophy of certain groups of skeletal muscles, bone osteoporosis, deconditioning of mechanisms regulating maintenance of the vertical posture and performance of locomotion on Earth, and deconditioning of mechanisms of vascular tone regulation. Three stages can be distinguished in the process of human adaptation to microgravity.

Stage 1: Activation of Immediate Adaptive Responses

These responses develop upon transition into orbit as a result of adverse reactions caused by the changed interaction of afferent systems and blood redistribution. Mechanisms of immediate adaptation utilize available functional systems in stimulating neurohormonal mechanisms for the regulation of fluid-electrolyte and hemodynamic homeostasis consistent with the specific environmental conditions, and in restoring an adequate correlation between afferent impulsation from the vestibular and other sensory systems.

In spite of the initiation of these immediate adaptive responses, the adverse effects (illusionary sensations, space motion sickness, blood rush to the head, etc.) usually persist during this period. This means that mobilization of short-term

adaptive responses is not sufficient for the establishment of an adequate relationship between the human body and the modified environment.

Stage 2: Formation of Structural Tracks

This stage is characterized by the gradual formation of a branched structural track in the functional systems which experience the elevated load (existing or new systems). It consists of two phases: one involves the musculoskeletal system, the other hemodynamic and fluid-electrolyte homeostasis.

In the first phase the musculoskeletal and other systems that are underloaded in comparison to the situation on the ground, show signs of deadaptation. At this stage various functions and their regulatory systems are restructured, with earlier changes from the basic phase of adaptation (which lasts about a week) leveling off and adaptive reactions being produced. During the second phase of 4 to 6 weeks, these reactions lead to the establishment of a new level of hemodynamic and fluid-electrolyte homeostasis and other adaptive changes.

Stage 3: Stabilization of Physiological Reactions

This stage is characterized by a stabilization of the vital physiological systems. Their stability, which may persist for a long period of time in the absence of extreme conditions, is ensured by the mechanisms of long-term adaptation.

Thus, spaceflight gives rise to both immediate and long-term adaptive reactions. Increase of the load on regulatory mechanisms responsible for the maintenance of a new level of hemodynamic and fluid-electrolyte homeostasis, as well as formation of new or enhanced existing functional systems, is evidently accompanied by reinforcement of systems responsible for adaptation, formation of sufficiently stable new temporal links, and hyperfunction of the corresponding structures (including cortical neurons) based on the formation of a systemic structural track. Subsequent activation of immediate and long-term processes of adaptation ensures the attainment of a new level of cortical-subcortical interactions, compensation for deficiencies in CNS excitation, formation of new stable temporal links in the brain, and development of new working skills and motor activity stereotypes, as well as formation of adequate homeostasis of the vital systems of the human body.

B. Readaptation to Earth's Gravity

Readaptation is man's habituation to Earth's gravity after spaceflight. This process is also characterized by the emergence of immediate and long-term adaptive responses, the goal of which is to reach an initial (preflight) level of performance. The immediate responses include nonspecific reactions related to the development of the stress-syndrome upon return to the Earth after a long exposure to microgravity. The structural systemic tracks, deadaptation processes and functional

rearrangements of vital physiological systems, which develop in this situation, act in general as the mirror image of the adaptive responses that occurred in microgravity.

After extended spaceflights, the human adaptive potential declines to a lesser or greater extent, which makes it more difficult to readapt upon return to Earth's gravity. Responses during the readaptation period are influenced not only by the flight duration but also by a regular and adequate use of countermeasures, adherence to an optimal work-rest cycle, and rational arrangement of the life activities during flight. Three stages can again be distinguished.

Stage 1: Activation of Immediate Adaptive Responses

Primary reactions consist of the development of a nonspecific stress-syndrome in response to re-encountering Earth's gravity after prolonged exposure to microgravity, and of phenomena associated with specific gravitational effects.

Postflight stress-reactions involve stimulation of the adrenal glucocorticoid function, the sympathico-adrenal system and the thyroid function.

The specific changes induced by gravitational effects include:

- subjective disorders: such as apparent increase of the weight of the body and of various objects manipulated by the cosmonauts, mildly-painful sensations in leg and back muscles, vertigo, and sometimes-vestibular discomfort;
- decreased coordination of vertical posture regulation and locomotion;
- fluid-electrolyte balance disorders; for example, a negative balance of calcium and potassium; and
- diminished orthostatic and exercise tolerance.

These primary reactions produce immediate adaptive responses, consisting of stimulation of functional systems and other mechanisms aimed at eliminating the disorders in the postflight period and making the physiological responses adequate to the requirements of Earth's gravity.

Stage 2: Formation of Structural Tracks

The second stage of readaptation is characterized by the formation of a branched structural systemic track due to the increased load on some functional systems, in particular the musculoskeletal system. In functional terms this stage may be regarded as consisting of two phases:

- The first and major phase of readadaptation is characterized by the gradual adaptation to Earth's gravity. It involves sensations and shifts in the physiological systems with a leveling off of the nonspecific stress-syndrome developed during the first stage;

- The second phase—the phase of readaptation completion—involves the final formation of long-term adaptive responses to the gravitational field of the Earth and replacement of wave-like variations of the physiological functions typical of the first phase with normal stereotypes of regulation.

Stage 3: Stabilization of Physiological Reactions

The third stage is characterized by stable, long-term adaption and the existence of a normal level of functioning of all systems of the body, comparable to that before the flight.

In summary, in the course of adaptation to microgravity and readaptation to Earth's gravity immediate and long-term adaptive responses are consecutively triggered and developed. This is accompanied by the formation of structural tracks in some systems and by the development of deadaptation in other systems. However, until now there is no reliable information about the point at which the process of adaptation to the space environment has been completed.

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ULTRASTRUCTURAL AND CELLULAR MECHANISMS IN MYOCARDIAL DECONDITIONING IN WEIGHTLESSNESS

Delbert E. Philpott, Katharine Kato, and Jaime Miquel

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I. INTRODUCTION

Cardiovascular deconditioning has been shown to occur during immobilization on Earth as well as in the microgravity environment during spaceflight.¹ It may lead to degenerative changes in the heart muscle. This is due to the altered functional demand on the heart, which causes changes in the mitochondria and the energy metabolism of the myocardium. For future long-term space missions, like on the *Space Station Freedom* or on a mission to Mars, it is very important to have more information on the phenomenon of myocardial deconditioning in microgravity.

Important questions to be answered are:

- which factors are causing myocardial deconditioning: the reduced work load or the headward fluid shift, or both?
- do these changes eventually become irreversible?
- can they be prevented or decreased by physical exercise or medical intervention?

The first factor, hypokinesia (reduced activity), is an important problem in modern medicine and has also become one of the major areas of concern in the space program. The reduced effort required for movement in weightlessness, compounded by the restricted area in the spacecraft, will result in a significantly decreased motor activity of the body unless deliberate steps are taken to exercise. Associated with this are reduced energy requirements and changes in metabolism which affect both skeletal and cardiac muscle.

In addition, there is a second important factor; the changed fluid distribution in microgravity. As a result of the upward fluid shift, space travelers feel congestion in the head and their face puffs up. The redistribution of body fluids is believed to be the primary biological reaction of the body to the reduction in the force of gravity.² On Earth the gravity force displaces fluid (primarily blood) downward to the lower half of the body. There is a loss of periodic distension of blood vessel walls and body fluid compartments in the lower half of the body. In the absence of gravity, changes may occur in the vessel walls themselves or in the central nervous system mechanisms that are responsible for their control.³

The effects of hypokinesia have been studied in simulation experiments on Earth, both in humans and in animals, and mostly rats. In one study, six men were subjected to 100 days of bed rest, three of whom were allowed to exercise.⁴ Changes in heart size (13 to 18% decrease), and in contractile force were observed in both groups, but they were smaller in the exercise group. Complete recovery took 3 to 6 months.

Hypokinesia in animals has been studied mainly in rats by means of the tail or hindlimb suspension method. In rats tail-suspended for 2 months, the rate of contraction and relaxation of papillary muscle under isotonic conditions was increased 1.5 times.⁵ The volume and surface of the longitudinal canalliculi of the

sarcoplasmic reticulum and the T-system were reduced by 57% and 60%, respectively. However, hindlimb suspension studies of rats showed that no hemodynamic response patterns generally associated with cardiovascular deconditioning were elicited after 9 days.⁶

Although many observations on the cardiovascular effects of spaceflight in humans have been collected,¹ our present understanding of the causes and basic mechanisms of cardiovascular deconditioning is still quite inadequate. For example, it has not been possible to make a clear distinction between the effects of hypokinesia and fluid shift.^{7,8} Spaceflight experiments are necessary to contribute to a better understanding of these phenomena and the underlying mechanisms, the extent and time course of these changes, and their reversibility. This will provide information essential to assure the well-being of individuals involved in prolonged spaceflight under conditions of microgravity and significantly diminished physical activity.

Since observations on humans are mostly limited to non-invasive techniques, there is a need for well-designed animal experiments permitting histological and biochemical studies of myocardial tissue. In order to distinguish between the effects of hypokinesia and fluid shift, suspended animals on the ground should be studied under "hypokinetic" (restricted activity), as well as under "normokinetic" conditions (activity held at the same level as controls).

Cardiovascular tissue from the animals will have to be studied for changes in mitochondria (e.g., accumulation of fluorescent lipopigment resulting from the autophagic breakdown of peroxidized mitochondrial membranes), glycogen, lipid, connective tissue infiltration, and muscle degradation. Calcium channel blockers such as nifedipine need to be used to determine whether these will ameliorate the effects of restricted exercise and tail suspension. Finally, there is the question whether the aging process acts synergistically with hypokinesia and/or fluid shifts in the production of myocardial changes.

II. EARLY ANIMAL STUDIES

A. Ground-Based Studies

Myocardial deterioration has been observed in the heart of Rhesus monkeys placed in a horizontal bodycast for up to 6 months.⁹⁻¹² After 3 months of immobilization the myocytes were infiltrated with lipid droplets, and some fibers were excessively thinned. There was vascular degeneration and intercellular infiltration of connective tissue which increased with time, as well as fibrotic infiltration. Elevated levels of hydroxyproline in both the right and left ventricles confirmed the increase of connective tissue in the heart. An increase in total free lysosomal enzyme activities in the ventricles provided additional evidence for active degradation.

Ultrastructural changes have also been found in the myocardium of restrained rabbits. After 19 to 30 days of exercise restriction there were areas of increased fatty deposits, protein dystrophy, necrobiosis, and necrosis of small groups of fibers with evidence of fine-focal fibrosis.¹³ After immobilization for 7 months, the resulting myocardial deconditioning was accompanied by spotty protofibrillar destruction near the Z-disk, damage to the capillary bed, and mitochondrial swelling. Capillary damage suggests that these mitochondrial changes might be the result of cellular anoxia due to immobilization-induced damage to the capillary bed. There was also increased lysosomal activity and the appearance of extremely pleomorphic mitochondria.¹⁴ In rabbits forced to run to exhaustion after 4 months of immobilization, there was an extensive lysis of the actin and a granular disorganization of the myosin contractile filaments. This was considered to indicate a "striking" loss of the functional reserve of the myocardium.¹⁴

In suspended rats, heart mass decreased 4.3% and oxygen consumption decreased from 62 mm³ to 38 mm³/100 mg/hr after 120 days of restraint.^{15,16} Electron microscopical observation revealed non-uniform swelling and a decrease in the mitochondrial cristae and changes in their patterns of orientation.^{17,18} There was also swelling of the endothelium of the capillaries, and impairment of the structure of the cell membrane.

Hypokinesia in rats, induced by exercise restriction, resulted in reduced heart function accompanied by a clear decrease in mass and changes in the ultrastructural elements involved in biological oxidation processes.¹⁹ In rats restrained for 30 days there was a decrease in the number of true capillaries, the appearance of nonfunctioning empty vessels, and an opening of arteriovenular shunts.²⁰ Changes were also found in the rheological properties of the blood, namely increased viscosity, adhesiveness, hematocrit, and erythrocyte sedimentation rate. The results indicate a reduction in blood flow and a decrease in the nutrition of the tissues.

In another study, male rats were restrained for up to 120 days. Electron microscopic analysis revealed an increase in the number and a decrease in the size of the mitochondria by day 14.²¹ By day 30 there was some reversal of this effect, the number slightly smaller with a concomitant increase in size. At days 45 to 60, the size and number of mitochondria appeared normal, but by day 120, both size and number were greater than in the controls. The changes in the left ventricle were greater than in the right ventricle. It was suggested that adaptation to restraint is responsible. When rats were restrained for up to 60 days,²² the contractile function was reduced during the first five days, returned to normal by day 15, while from day 45 to 60 the rate and strength of cardiac contractions increased and the functional reserve of the heart decreased. The authors concluded that if restraint was carried on long enough it would lead to cardiac failure. Hypokinesia in the tail-suspended rat model^{16,23} was found to reproduce accurately the circulatory changes seen in astronauts exposed to microgravity.²⁴ This reveals, again, the confusion between the effects of hypokinesia and fluid shift, both of which occur in the tail-suspension model.

Stereological methods were used to study skeletal and heart muscle changes after 30 days of hypokinesia.²⁵ Forty-eight male rats were confined in cages allowing virtually no movement. The ratio of muscle to connective tissue in the myocardium decreased, indicating connective tissue proliferation. The relative volume of intracellular material increased, indicating edema. The microcirculatory bed was altered, suggesting that there must have been a degrading of metabolic processes. Both morphological and stereological analysis revealed myocardial atrophy. It was concluded that the disruption of synthetic processes leads to a decrease in the reproduction of the basic organelles in the cardiac muscle cell, indicating that only the structures of greatest functional importance to the tissue were being retained.

In monkeys, body-casted in an upright position, there is breakdown of myocardial mitochondria after 2 weeks of immobilization with autophagic transformation of the organelles into multilamellar dense bodies.^{26,27} The myocardium of these animals also showed an increased accumulation of glycogen and lipid droplets, suggesting that the decline in metabolic rate during immobilization results in a surplus of the compounds normally used for the production of energy. This concept agrees with the finding that the activity of adenylate cyclase was strikingly lower in the myocardium of the body-casted monkeys than in that of the controls. Because there were no fluid shifts in the animals, it is clear that profound hypokinesia causes highly significant changes in the heart.

In rats that were tail-suspended for 14 days, changes in fine structure and biochemistry of the heart were observed.²⁸ Glycogen was increased, and there was a significant increase in lipid droplets in the left ventricle tissue. Small areas of myofibrillar degeneration and loosely packed protofibrils were seen, and there seemed to be atrophy of the intercalated disks in the myocytes. An increase in swollen, darkly stained mitochondria was observed in myocytes of the left ventricle.

It is known that a significant portion of protein phosphorylation in heart muscle cells occurs via the action of cAMP-dependent protein kinase in response to various stimuli. Some of the morphological alterations seen in this study may be connected with a decrease in protein kinase activity observed in other simulated weightlessness studies.

B. Space Experiments

Early Soviet spaceflight observations suggested that the rat heart is insensitive to weightlessness.^{29,30} However, in later studies ultrastructural changes have been observed in the rat heart after exposure to space microgravity during the *Cosmos 936* flight.^{31,32} The volume fraction occupied by mitochondria and smooth endoplasmic reticulum was significantly decreased compared to that in the synchronous controls and the vivarium controls. The average number of mitochondria was decreased and the glycogen content was increased. Mitochondrial damage and myofilament atrophy were observed. Similar changes were observed in the heart

muscle of rats that were kept on 1-G control centrifuge, so centrifuging the animals during the flight did not prevent the structural changes.

Examination of rats from a 22-day flight, sacrificed 4.5 to 9 hours postflight, revealed consistent changes in the capillaries and venules. These changes consisted of edema of endothelial cytoplasm, appearance of myelin bodies, and swelling of mitochondria. There were also breaks in the endothelium with accumulation of plasma, erythrocytes, and vacuoles between the muscle fibers. In the cardiomyocytes, exposure to microgravity resulted in an increase in the number of lipid droplets and a decrease in the amount of extramitochondrial glycogen. Glycogen granules were occasionally seen in the intramitochondrial matrix, which suggests that exposure to microgravity results in a derangement of energy metabolism. Many mitochondria showed signs of autophagic breakdown with accumulation of membranous lipid debris inside the organelles or in their immediate vicinity.

The mitochondrial changes seen in space-flown animals are very similar to those encountered in the presence of various forms of myocardial hypoxia.³³ This may be due to the fact that the heart experiences a drastic increase in load when the rats change from the weightless environment to Earth's gravity. Because exposure to microgravity results in changes in the microvasculature,³² compatible with impaired tissue oxygenation, the increased metabolism associated with readaptation to Earth's gravity may lead to tissue anoxia and concomitant mitochondrial disorganization.

The norepinephrine (NE) and epinephrine (E) contents in the four compartments of the heart were measured in the rats flown on the *Cosmos-782*, -936 and -1129 missions.³⁴ The right atrium had the maximum concentration of norepinephrine, there was less in the left atrium and right ventricle, and the least amount occurred in the left ventricle. This suggests that there occur functional changes in the sympathetic nervous system leading to an elevation of catecholamines in the heart after spaceflight. However, the left atrium, which is considered to be the most sensitive to weightlessness, showed a drop in norepinephrine. Maximum changes in norepinephrine occurred in the right side of the heart.

The heart of male rats, flown for 7 days on the *Spacelab-3* mission and sacrificed 12 hours after return to Earth, showed a decrease in microtubules.³⁵ Microtubules are a component of the cytoskeletal structure of cells, providing structural integrity to the cell. They are believed to orient the myofibrils of the muscle cell because breakdown of microtubules by colchicine disrupts the orderly arrangement of the myofilament bundles in regenerating frog skeletal muscle.³⁶ The number of microtubules in the rat heart increases during the first 9 postnatal days and then decreases to a steady state.³⁷ After the establishment of well-oriented, densely-packed myofilament bundles, the number of microtubules decreases, reflecting a decreased necessity for scaffolding, and only the microtubules required for growth remain. Stress-induced hypertrophy leads to an increase in the number of microtubules,³⁸ and these microtubules temporarily reorganize into bundled arrays.

In the rats flown on the *Spacelab-3* mission, the ultrastructural changes in the

heart included an increase in glycogen and small lipid droplets, compared to ground controls. Cyclic AMP-mediated hormonal regulatory responses were evaluated by measurement of the activation and inhibition of cAMP-dependent protein kinase by specific hormones and inhibitors. Photoaffinity labeling of cAMP-dependent protein kinase regulatory subunits was determined in cell fractions previously labeled with an azido analog of cAMP. Cyclic AMP protein kinase activities were decreased by 20 to 30% ($p < 0.01$) in flight animal cells. Similar observations were made for the inhibition of the enzymatic activity by a heat-stable inhibitor of azido-cAMP binding to the protein kinase regulatory subunits. A significant decline in azido-cAMP binding to the regulatory subunits of the particulate cell fractions was observed by autoradiographic technique in the muscle of flight animals. Similar changes were seen in the secretory cells from the salivary glands of SL-3 flight animals.³⁹ Phosphorylation studies showed that a salt extract of the particulate fraction in the flight animals has a different phospho-acceptor banding pattern than that in the ground-based controls. The specific activities of adenylate cyclase (basal, F-stimulated) and low-K_m cAMP phosphodiesterase were unchanged, but a loss of high-K_m cAMP phosphodiesterase activity ($p < 0.01$) occurred in the flight animals.

III. EXPERIMENTAL DESIGN

It will be clear from the foregoing review of earlier experiments concerning the effects of microgravity on the myocardium that there is a need for a more precise understanding of the structural and biochemical mechanisms leading to myocardial deconditioning during spaceflight and to the subsequent readaptation to Earth's gravity. There is also a great need to separate the effects of hypokinesia and fluid shift on these mechanisms. This means that an experiment must be planned which will satisfy these two needs. The design of such an experiment is discussed below.

A. Parameters to be Studied

Cytoskeleton

The cytoskeleton supports and maintains the subcellular structures of the cell, and its preservation is therefore essential for maintenance of cell function. Many cytopathological processes are associated with alterations of the cytoskeleton. Exposure of rats to space weightlessness decreases the number of cytoskeletal microtubules in the myocardium.³⁵ This means that the ultrastructural study of the myocardial tissue must include an evaluation of the state of the cytoskeleton.

Mitochondria

Experimental evidence indicates that an optimum work load imposed on the heart, that is moderate physical exercise at Earth's gravity, will maintain mitochondrial structure and function. This is in agreement with the concepts presented by Miquel.⁴⁰⁻⁴⁷ Under microgravity conditions, it appears that either hypokinesia, fluid shift, or both, will reduce the work load on the heart. This decreased functional demand, under conditions of spaceflight microgravity or immobilization on the ground, will cause disuse atrophy, accompanied by mitochondrial changes.

Under conditions of decreased functional load, the myocardium will not need its full complement of ATP-synthesizing organelles. This teleological explanation does not clarify the specific mechanisms involved in controlling the number and size of mitochondria present in the myocardial cells under normal or decreased functional loads, but it is probable that decreased activity triggers a process of subcellular degradation. This concept is supported by increased proteolytic activity in the myocardium of rats exposed to microgravity.^{29,48} It is likely that lysosomal enzymes play a key role in the digestion of the unneeded mitochondria.⁴⁹⁻⁵²

Cyclic AMP-Dependent Protein Kinase

Cyclic AMP (cAMP) accumulation is significantly elevated within 30 seconds following the onset of ischemia or hypoxia in the left heart ventricle.⁵³ The elevation of cAMP may be mediated by increased beta-adrenergic action, which stimulates the adenylate cyclase activity. Similar mechanisms may be responsible for an increase in cAMP (which in turn mediates protein kinase activity) in the myocardium of animals exposed to hypokinesia. During beta-adrenergic stimulation the sarcoplasmic reticulum is phosphorylated and this stimulates calcium transport. The general mechanism, by which agents that elevate cAMP levels modify cellular function, is by phosphorylation of protein catalyzed by cAMP-dependent protein kinase. In cardiac cells this occurs with phosphorylase kinase, the inhibitory subunit of troponin, myosin, and with the membrane associated protein phospholamban, localized in the sarcolemma (the sarcoplasmic reticulum membrane).

Phosphorylated phospholamban stimulates the calcium ATPase of the sarcoplasmic reticulum in ventricular tissue⁵⁴ and it may be associated with the regulation of calcium transport by sarcoplasmic reticulum. Evidence obtained from our studies on male rats flown on the SL-3 mission, as well as in simulated weightlessness studies, revealed marked changes in cAMP protein kinase specific activities, and in the distribution patterns of the R-I and R-II subunits after exposure to either regime.⁵⁵ This indicates that the cAMP protein kinases in the left ventricle are compromised, suggesting that the phosphorylation of cAMP-dependent proteins, such as phosphorylase kinase, myosin, and phospholamban, may be altered.

It has been established that rat heart cytosol contains mainly the R-I subunit of protein kinase, and that neither the concentration nor the distribution of R-I are

markedly influenced by spaceflight.⁵⁵ The R-II subunit, however, is markedly recompartmentalized in the particulate cardiac cell fractions of animals exposed to various altered gravity conditions. Morphometric analysis of gold labeling intensity can be carried out to quantitate the cellular distribution of cAMP-dependent protein kinase R-II subunits.

Our studies on simulated weightlessness²⁸ and true microgravity⁵⁵ have shown decreases in cAMP binding capacity by the regulatory subunits of cAMP-dependent protein kinase. By comparing the morphological alterations seen in the ultrathin sections with the immunogold technique and the protein kinase measurements, these studies can reinforce each other and help give a clearer picture of the rate and amount of morphological change.

Calcium Homeostasis

Hypoxia induced changes in the intracellular levels of calcium play a key role in the disorganization of the myocardial mitochondria.⁵⁶ During an ischemic or anoxic episode, the mammalian myocardium loses its capacity to maintain homeostasis. This leads to serious electrophysiological disorders, such as slow electrical responses, uncoupling, and depolarization. Initially, mitochondrial calcium uptake activity increases, and calcium uncouples oxidative phosphorylation with a resulting decrease in energy production. This decrease in energy production may underly the physiological symptoms. It can lead to cellular edema, muscle contraction, and to necrosis. The raised calcium levels present in the anoxic myocardium can also contribute to the mitochondrial disorganization by activating the enzyme phospholipase, which breaks down the structural lipids of the subcellular organelles. An accelerated destruction of membrane lipids in cells which have sustained a loss in their ability to produce energy, and therefore to resynthesize the membrane components, leads to irreversible damage of the mitochondria.

The calcium antagonists (slow channel blockers) are a relatively new group of pharmacological agents, which are clinically used for the control of angina. They have shown great effectiveness in counteracting many of the injurious effects of myocardial hypoxia and the resulting derangement of calcium homeostasis, including the pathological changes occurring in mitochondria.⁵⁷ The widely used calcium antagonist nifedipine preserves the respiration and the energy production of the myocardial mitochondria during the anoxic episode as well as the subsequent reoxygenation phase. Treatment with nifedipine results in a decreased accumulation of calcium in the mitochondria and improved maintenance of the high-energy phosphate levels.⁵⁷ This provides a better preservation of the organelles and of the physiological functions of the heart. Nifedipine is effective in preventing mitochondrial pathology in the hypoxic condition induced by coronary artery occlusion. Since it appears that some degree of hypoxia is also involved in the pathogenesis of disuse atrophy, this drug may be effective in preventing mitochondrial changes which result from prolonged exposure of organisms to space weightlessness.

Table 1. Experimental Design to Test Effects of Nifedipine

	No Drug/No Pump	Drug/Pump	Saline Pump
	Number of animals		
Hypokinetic Suspended	6	6	6
Normokinetic Suspended	6	6	6
Hypokinetic Horizontal	6	6	6
Normokinetic Horizontal Controls	6	—	—

Nifedipine was administered to rats by means of osmotic pumps in test dosages based on the work of Hall and Hungerford.⁵⁸ The concentrations used were 4 mg/ml (2.85 mg/kg) and 8 mg/ml (5.7 mg/kg). Four groups of rats were tested: 8 mg/ml nifedipine/tail suspended, 4 mg/ml nifedipine/tail suspended, saline/tail suspended, and controls. The saline/tail suspended group showed an increase of lipid droplets and glycogen and some morphological degradation. The 4 mg/ml group showed less change in morphology and only a slight increase in glycogen and lipid droplets. The 8 mg/ml tissue showed no morphological difference from the control group.⁵⁹ There was a partial or complete reversal of other changes seen under conditions of simulated or true microgravity (*SL-3* mission), such as decreases in the cyclic-AMP binding capacity of the regulatory subunits of cyclic-AMP dependent protein kinase, disruption of microtubules, and the accumulation of glycogen and lipid.⁶⁰

In a study of 21 healthy men (age 35 to 40 years) during 120-day head down tilt of -4.5°, marked changes in the ECG were observed.⁶¹ There were changes in the phase of ECG regularization; that is a decrease, flattening, and often deformation of T-waves of the bimodel type. After completion of the period of bed rest, there was development of relative tachycardia, especially during the period of readaptation. Twelve of the men were given prophylactic treatment. Changes in the electrocardiographic and blood electrolyte parameters were reduced by exercise and the administration of nifedipine.

On the basis of these findings, further studies of the use of nifedipine (Table 1) for counteracting the cardiac effects of spaceflight appear to be desirable.

Aging

Aging causes a decline in cardiac output and in the ability of the heart to adapt to increased functional demands in healthy human beings and animals.⁶²⁻⁶⁴ This decline in cardiac output is accompanied by an increase in the number of degener-

ating mitochondria, lipid droplets, and lipofuscin at the submicroscopic level.^{41,46,65-67}

Age related changes respond to beta stimulation with decreases in contractility.⁶⁸ In the aged human being, responses that elevate serum catecholamines produce smaller increases in the heart rate than in young adults.⁶⁹ The molecular mechanism of this change in physiological response appears to involve decreases in specific protein phosphorylation by cAMP-dependent protein kinase.⁷⁰

These cardiac changes during the normal aging process may enhance the effects of heart deconditioning induced by immobility or spaceflight, particularly the dystrophic changes in the myocardial mitochondria. A regime of physical exercise in young men results in a striking increase in the number of myocardial mitochondria. However, in men over 55 years of age, there is no increase in the number of mitochondria, but a moderate increase in mitochondrial mass due to enlargement of the individual organelles is noted.⁷¹ This finding is in agreement with the concept that aging is associated with a loss in the regenerative ability of mitochondria through the process of mitochondrial replication.^{46,72} Aging causes myocardial changes involving mitochondrial reactions and loss of adaptation responses of the bioenergetic processes which are similar to those occurring in young hypokinetic animals and humans. On the basis of these observations, an hypothesis concerning the synergistic effects of age and hypokinesia deserves to be further investigated in space experiments and ground based simulation experiments.

B. The Rat as a Model for Man

In designing an animal experiment aimed at increasing our understanding of the cardiac effects of simulated or actual microgravity in man, a crucial question is, of course, which animal species can best serve as a model for man. Primates offer the advantage of being much closer anatomically and physiologically to man than rodents, but it is much more difficult and costly to use primates in spaceflight than rodents.

An ad hoc panel composed of NASA and university scientists,⁷³ well versed in both rat and monkey cardiovascular physiology, was asked to study this problem. The panel's conclusion was that the rat is an excellent model for the study of cardiovascular deconditioning associated with actual and simulated microgravity.

In an attempt to answer some basic questions concerning the conduct of rodent experiments in *Spacelab*, the following considerations generally represent investigator attitudes about several important issues.

Appropriate Animal Model

Questions about the appropriate animal model for man in life-sciences space experimentation were discussed. In cardiovascular studies in space, would an

animal such as a rabbit be more suitable than a rat because of its longer hydrostatic column?

Are the endocrine, skeletomuscular, and other metabolic and biochemical changes observed in rodents flown in space applicable or comparable to the changes occurring in man? Studies of the cardiovascular changes in rats subjected to 5° or 15° head down tilt to simulate microgravity indicate a similarity in the effects of man's response to microgravity. This has been confirmed by studies of the rats on the *Cosmos* flights.

Because the rat grows rapidly, it shows a quick metabolic and cardiovascular response to microgravity. In particular, rats are more homogeneous in their cardiovascular responses than man. This means that accurate cardiovascular data can be obtained without having to use a large sample size. In weightlessness, the rat has an increased right atrial pressure and a small but significant change in venous pressure.

Regardless of the length of the hydrostatic column, the rat responds to weightlessness like a human. Fluid and electrolyte shifts due to microgravity are also similar in rats and humans. For example, rats stressed or fed a salt-deficient diet have similar alterations in the ADH-renin-angiotensin axis as in humans. Other endocrine changes are also similar. Studies of bone and muscle physiology in rats have shown a significant muscle (soleus) mass decrease within 7 days of entering weightlessness, and a decrease in muscle protein within 3 days. These changes in skeletomuscular physiology are similar to those seen in astronauts with the advantage that in the rapidly growing rat these alterations in bone show up rapidly.

Based on the known effects in both species and the fast growth, rapid metabolic turnover and homogeneous cardiovascular responses in rats, the consensus of the panel was that the rat appears to be the best model for man in studies of the cardiovascular, fluid-shift, endocrine, and bone-muscle responses to hypokinesia and to microgravity.

Rat Strain

There would be no problem adapting experimental protocols to any specific strain of laboratory rat, so long as each investigator can begin collecting baseline information about the selected strain as far in advance as possible. Sprague-Dawley rats are currently used by most of the investigators, with the Wistar rat being the second most utilized strain. On this basis, the Sprague-Dawley rat was the first choice as the rodent strain to be used in space experiments.

Tail suspension is not especially stressful to the rat. This is shown by the observation that, although suspended rats decrease their food intake and lose weight during the first day, thereafter they return to their normal eating behavior and their pre-suspended weight.

Table 2. Experimental Design to Distinguish between Effects of Hypokinesia and Fluid Shift

	<i>Activity Level</i>	
	<i>Hypokinetic</i>	<i>Normokinetic</i>
Tail suspended	Cell 1 +F +H	Cell 2 +F oH
Horizontal position	Cell 3 oF +H	Cell 4 oF oH

Key: +F = Fluid Shift; H = Hypokinesia; + = Produces Effect; o = No Effect

C. Isolation of the Causative Factors

Hypokinesia and Fluid Shift

It is necessary to separate the effects of fluid shift from reduced activity in order to determine the importance and extent of the contribution from each perturbation. As pointed out earlier, pathological alterations have been reported after restricted activity, after tail suspension and after spaceflight. The fluid shift that occurs during tail suspension and spaceflight is generally accompanied by hypokinesia. Knowing the separate effects of hypokinesia and fluid shift in causing myocardial deconditioning will aid in the development of effective countermeasures to prevent this undesirable effect of spaceflight.

This means that an experiment must be planned which will allow to separate these two factors and their effects. Table 2 illustrates an approach to solving this problem. The controls are in cell 4 and serve as a comparison for the experimental animals in the other three cells. Cell 2 (normokinetic), compared to cell 4, will show the effect of fluid shift. Cell 3 (hypokinetic), compared to cell 4, will show the effect of hypokinesia. Cell 1 (fluid shift, hypokinetic), compared to cell 4, will determine whether there is an enhanced effect resulting from the combination of fluid shift and hypokinesia.

Cell 1. Hypokinetic suspended group: Tail suspension²³ combines the fluid shift and hypokinesia allowing observation and quantitation of these two factors.

Cell 2. Normokinetic exercised suspended group: Rats should be exercised on an exercise wheel (modified to allow the tail suspension to continue and therefore for the fluid shift to remain) with a counter attached to the wheel assembly.²³ The method of exercise on the wheels should follow the basic technique described by Samorajski et al.⁷⁴

Cell 3. Hypokinetic horizontal group: Rats maintained in small size cages (11 in. x 4 in. x 4½ in.) in the normal horizontal position are exercise restricted without fluid shift.⁷⁵ The cages have a smooth top in order to prevent any climbing.

Cell 4. Normokinetic horizontal group: Control animals maintained in the same size cages as the tail-suspended animals.

Old rats (22 to 24 months) should be compared with young 180-gram rats to study the effects of age, using the same protocol for the fluid shift and exercise restricted experiments.

Drug Intervention

The possible beneficial effects of a calcium channel blocker such as nifedipine should be followed up⁷⁶ and assessed for prevention of myocardial changes. Delivery of the drug to the rats can be accomplished by an osmotic pump (Alzet, Alza Corp., Model 2001) implanted between the shoulder blades. Initially, nifedipine could be administered in a dosage of 8 mg/ml (5.7 mg/kg per day)⁵⁸ as indicated by the initial success obtained by using this dosage. Exposure periods should be similar to those in previous suspension and spaceflight experiments.

IV. RESULTS OF THE *COSMOS 2044* MISSION

A. Experimental Details

The flight of *Cosmos 2044* provided a rare opportunity to repeat the experiments carried out on the *Cosmos 1877* flight.⁷⁷ Since recovery of the animals after the *Cosmos 1877* flight took an unintended 48 hours instead of the 12 hours elapsing after the *Cosmos 2044* flight, there was also an opportunity to look for differences that might be due to different recovery times.

Five male rats of the Czechoslovakian Wistar strain were used in each of the four categories in the *Cosmos 2044* mission: flight (FL), synchronous control (SYN), vivarium control (VIV), and tail suspension (TS). A tail suspension experiment on Earth was included in order to see whether this technique would yield effects comparable to those in the flight animals. The *Cosmos 2044* flight lasted 14 days and tissue preparation took place 12 hours after recovery. Selection of animals and all procedures for handling the animals were kept as close as possible for the two flights.

The rat hearts were collected by the Soviet scientists and immediately placed in ice cold saline to reduce metabolic activity. The hearts were then placed on cold dental wax for the dissection of the left ventricle.⁷⁸ The dissected samples were immediately placed in plastic screw top vials containing cold Triple Fix,⁷⁹ labeled and shipped to the NASA-Ames Research Center in California. The assistance of

the Institute of Biomedical Problems in Moscow (particularly of Dr. I.A. Popova) in flying this experiment, and in preparing the tissues after flight, is gratefully acknowledged.

Upon arrival at NASA-Ames Research Center the tissue was immersed in 1% osmic acid and 1% potassium ferricyanide for 1 hour,⁸⁰ dehydrated by ascending concentrations of acetone, infiltrated with Epon-Araldite, embedded, sectioned, and stained for electron microscopy. Electron micrographs of the samples were taken with a Philips 300 electron microscope.

Volume density (VD), therefore the fraction of a component in a thin tissue section, was determined by point counting as described by Weibel,⁸¹ using 240 micrographs (8 in. × 10 in.) at a magnification of 27,500 \times . A statistical analysis of the counting results was carried out by means of two-way analysis of variance (ANOVA), using an Epistat software program. Capillary counts, using randomly selected open-grid squares in the electron microscope, were converted to counts per 600 μm^2 . All histological and evaluation procedures were kept as close as possible to those used for the *Cosmos 1887* flight.

B. Comparison with Results of *Cosmos 1887* Mission

Flight and Recovery

Cosmos 2044 was flown to compare the results with those from the *Cosmos 1887* flight and to gain additional insight into the effects of microgravity. Recovery from *Cosmos 2044* went smoothly and the tissues were obtained 12 hours after landing, a significant reduction in time compared to the 48 hours elapsing before sacrifice of the *Cosmos 1887* rats. This was important because there was some concern that the longer recovery period after the *Cosmos 1887* flight might have permitted some tissue recovery, thus decreasing the observable changes. However, the results obtained for the left ventricles of the *Cosmos 2044* animals compared to those for the *Cosmos 1887* animals do not support this assumption.

General Findings

Figure 1 shows the postflight comparison of mitochondrial and glycogen volume densities in the *Cosmos 2044* animals (#1) and the *Cosmos 1887* animals (#2) in the flight (FL), synchronous (SYN), and vivarium (VIV) groups. No tail-suspension studies were carried out during the *Cosmos 1887* mission. The results are very similar for the two missions. In both cases, the mitochondrial volume density was lowest in the flight group, highest in the vivarium control group, and intermediate in the synchronous group. Glycogen volume-density was highest in both flight groups, while the vivarium and synchronous groups showed small differences between the two missions.

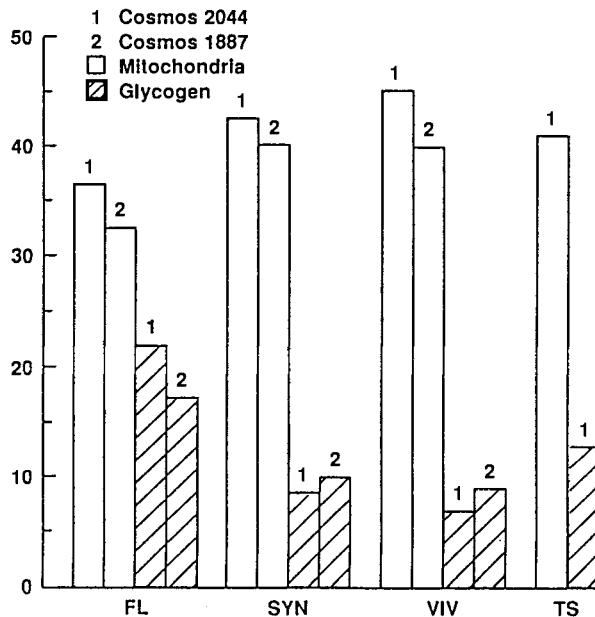


Figure 1. Effects of spaceflight on mitochondria (M) and glycogen (G) in the left ventricle of rats flown on *Cosmos* missions. FL flight animals, VIV vivarium controls, SYN synchronous controls. TS represents the tail suspended animals used with *Cosmos 2044*. Bars marked 1 refer to *Cosmos 2044*, and marked 2 to *Cosmos 1887*.

C. Effects on Mitochondria and Myofibrils

Some electron micrographs of myocardial muscle cells are shown in Figures 2–9. The first of these, Figure 2, shows the excellent preservation obtained in a vivarium control. An increase in glycogen can be seen in Figure 3 of a synchronous control, which simulated the conditions of flight except for the microgravity. An increase in glycogen can be seen in flight tissue (Fig. 4); the edematous nature of the cell is also apparent. It is possible that the edema may be linked to tissue breakdown, with a concomitant increase in osmotic pressure and fluid entry into the cells. An increase in glycogen is often visible around the nucleus including lipid and residual/dense bodies in flight cells (Fig. 5). Tail suspension results appear similar and can be seen in Figure 6, where an increase in glycogen, a loose appearance of the tissue and residual bodies are noticeable.

Affected myofibrils may exhibit super contraction, filament disorganization, increased glycogen, and lipid in flight cells (Fig. 7). Cross-sections of the altered myofibrils exhibit myofilament loss from the flight cells (Fig. 8); this figure can be compared to the cross-section area of an unaffected myofibril from flight tissue in Figure 9.

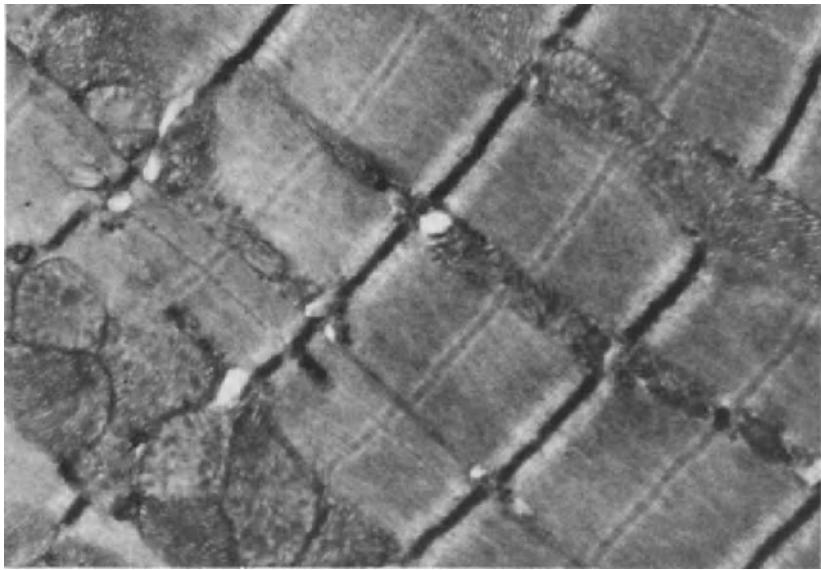


Figure 2. Electron micrograph of left ventricle from vivarium control. All micrographs in Figures 2–10 are from left ventricle of rats in the *Cosmos 2044* experiment. Note excellent preservation. Magnification 16,500 \times .

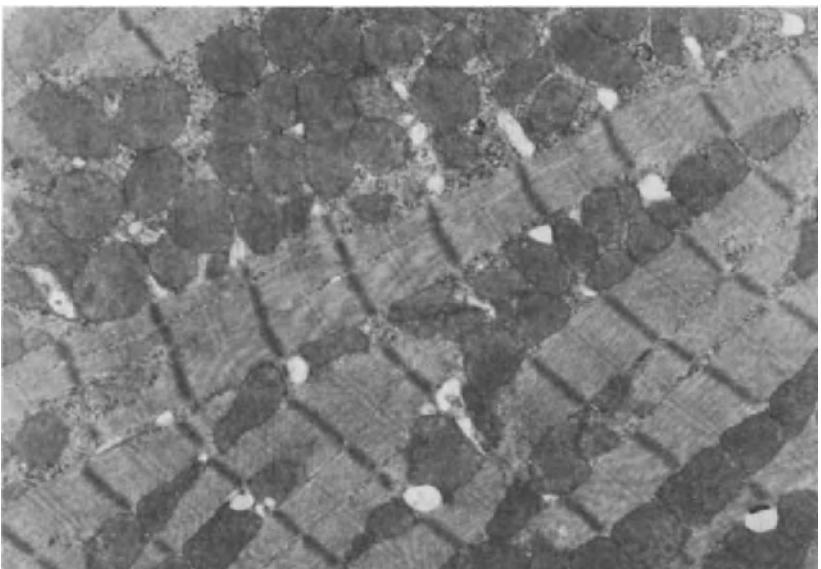


Figure 3. Electron micrograph of left ventricle from synchronous control. Similar to vivarium control shown in Figure 2; however some increase in glycogen is visible. Magnification 11,700 \times .

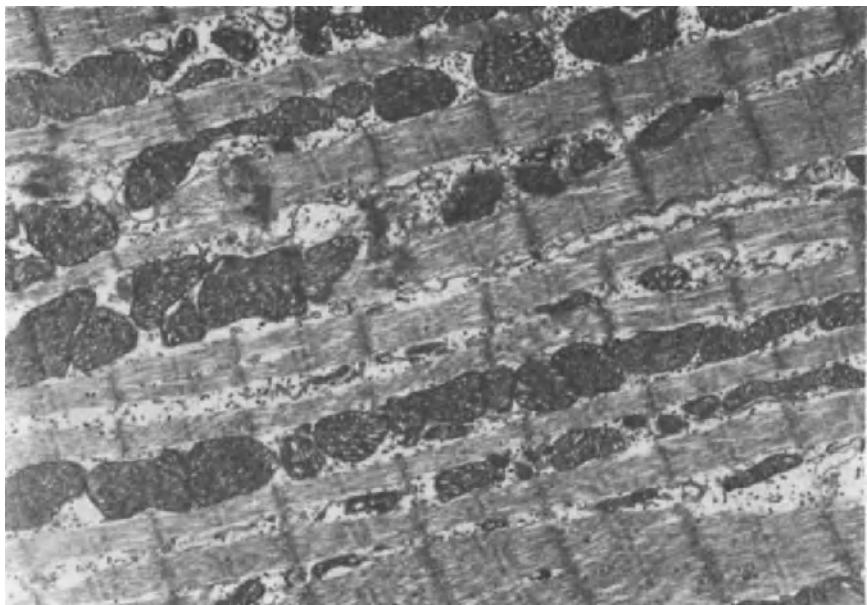


Figure 4. Electron micrograph of left ventricle from flight rat. Note the increase in glycogen and edematous appearance. Magnification 16,500x.

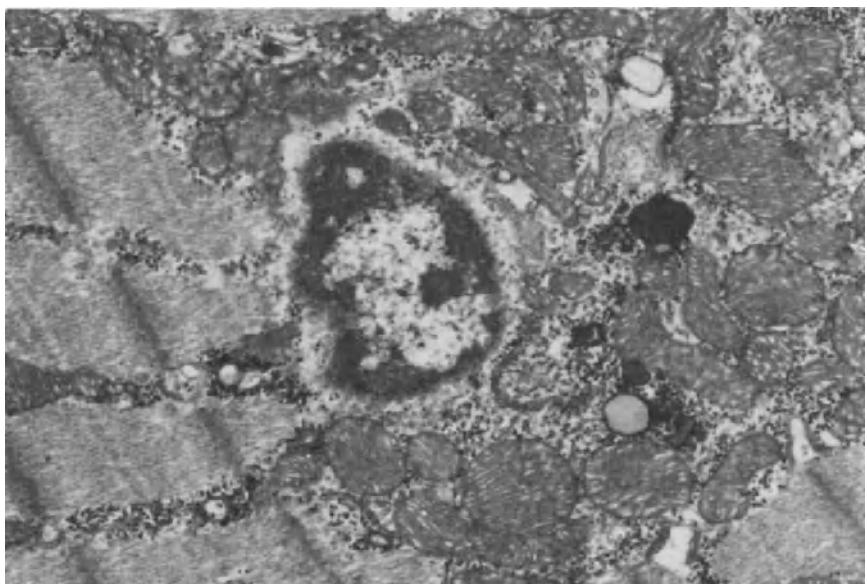


Figure 5. Electron micrograph of left ventricle from flight rat. Note the increase in glycogen, especially around the nucleus, and the presence of lipid and residual/dense bodies. Magnification 15,750x.

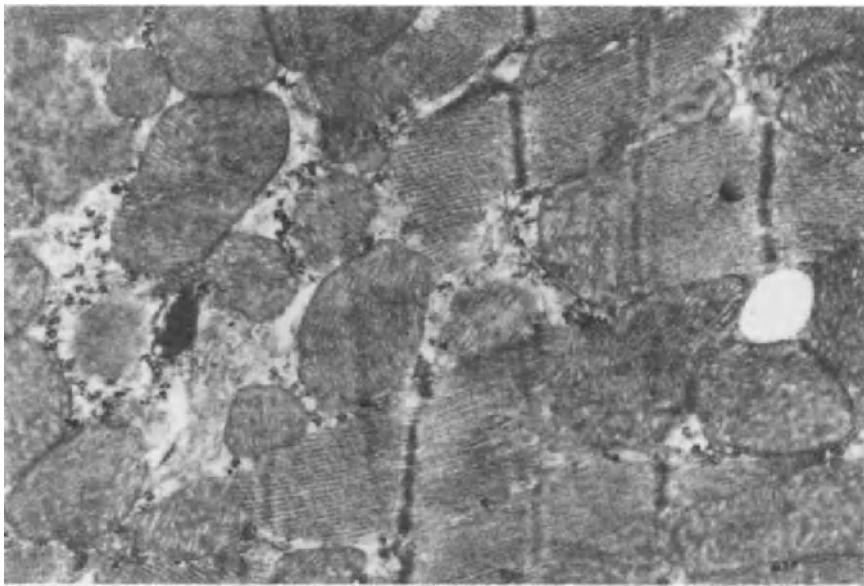


Figure 6. Electron micrograph of left ventricle from tail suspended rat. Note glycogen accumulation, loose appearance of the tissue and residual bodies. Magnification 27,500 \times .

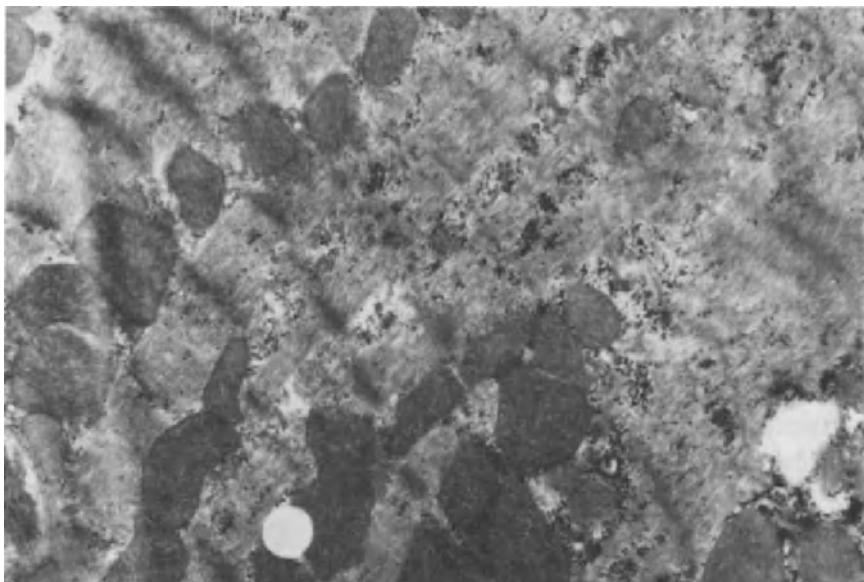


Figure 7. Electron micrograph of left ventricle from flight rat. Supercontraction, disorganization of filaments, glycogen and lipid visible in this affected myofibril. Magnification 27,500 \times .

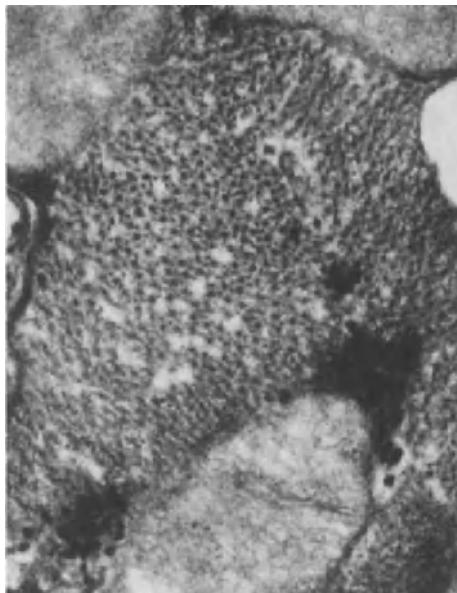


Figure 8. Cross-section of left ventricle from flight rat. Note the missing myofilaments. Magnification 78,500 \times .

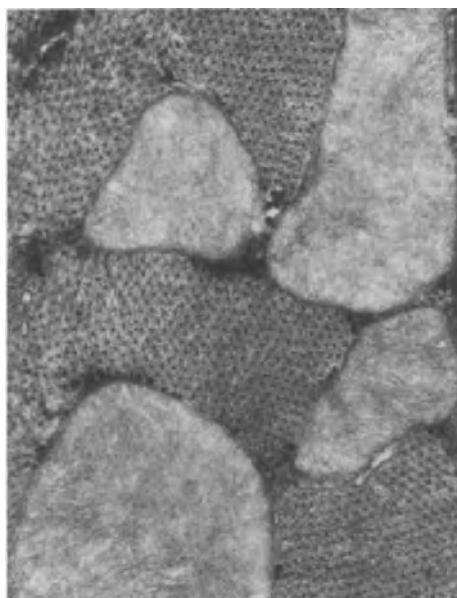


Figure 9. Cross-section of left ventricle from flight rat. Note the normal appearance of an unaffected flight specimen. Magnification 50,000 \times .



Figure 10. Electron micrograph of left ventricle from flight rat. Note light staining mitochondria in the center of the micrograph. Magnification 42,500 \times .

Some lighter-staining mitochondria appear among the darker mitochondria in a flight cell (Fig. 10). The normal appearing myofibrils generally contain the darker mitochondria, suggesting that the decrease in volume density of the mitochondria may be preceded by some morphological alteration. Several authors have reported that changes in the supply of oxygen and nutrients to the organelles result in fine structural and biochemical changes in the mitochondria of ischemic or anoxic myocardium.⁸²⁻⁸⁶ A decline in sympathetic nervous activity may also influence mitochondria through an effect on blood perfusion.²⁴ Hence, both sympathetic and oxygen-mediated mechanisms may underly the changes. It has also been reported that the sympathetic nervous system exerts a trophic influence on the biosynthesis of myofibrils and mitochondria.⁸⁷ Therefore, a decreased sympathetic activity during microgravity or immobilization could play a role in their degeneration.

Alterations in the capillaries may also play a role in the observed mitochondrial changes. Capillary changes were observed in the *Cosmos 1887* mission.⁷⁷ Similar capillary alterations were observed in immobilized rabbits, indicating a possibly impaired delivery of oxygen and nutrients to the cells.⁸⁸ A decrease in the blood supply would decrease the synthesis of ATP which is needed for maintenance and repair of mitochondria.⁸⁹ The present data support the view that an optimum work

load imposed on the heart, at normal Earth gravity, is essential to preserve mitochondrial function and structure.^{46,47}

D. Effects on Capillaries

Since an ultrastructural change was seen in the capillaries of the rats on the *Cosmos 1887* mission,⁷⁷ it was decided to make a count per unit area of the capillary bed in the *Cosmos 2044* left ventricles. The capillary counts in the flight group and the tail-suspended group were significantly increased relative to the control groups (see next section). These results indicate that there appears to be a need for an increased number of capillaries in the flight and tail-suspension animals. Atrophy may also play a role in bringing the capillaries closer together. It was not possible to determine if all the capillaries were functional. However, there was a visual increase in the number of platelets in the flight- and tail-suspended capillaries and not in the synchronous and vivarium controls.

The flight tissue in *Cosmos 2044* appeared to have a small increase in collagen comparable to the observation in the *Cosmos 1887* rats. The increase was not considered significant, but there may well be a progressive increase during long-term flights. This could present a problem for crew members in such flights. In order to obtain more information on this point, collagen accumulation should be monitored in any prolonged animal flight experiments. Connective tissue proliferation was also detected in rats during one month of exercise restriction.²⁵ In studies of monkeys maintained for up to 6 months in a horizontal bodycast there was also a collagen increase, as measured by hydroxyproline.¹⁰ This was accompanied by elevated lipid levels, some muscle thinning, and an increase in total free lysosomal enzyme activities in the ventricles; findings that are all indicative of active tissue degradation.

E. Statistical Analysis

Group averages for five myocardial volume density parameters, mitochondrial glycogen, lipid, residual/dense bodies, and capillaries, are shown in Table 3. The four groups are: flight group (FL), tail-suspended group (TS), synchronous group (SYN), and vivarium group (VIV).

Two-way analysis of variance applied to the data for the five animals in each group showed no significant differences for each of these five parameters. This indicates that the groups were homogeneous. Three sets of P-values for the intergroup comparisons are presented: for the flight group versus the control groups (SYN plus VIV); for the tail-suspended group versus the control groups (SYN plus VIV); and for the tail-suspended group versus the flight group. The synchronous and vivarium groups were combined as control groups, since there were no significant differences between them. The flight animals differed significantly from the control groups in all parameters. Mitochondrial volume density was signifi-

Table 3. Comparison of Five Myocardial Volume Density Parameters in Rats on Cosmos 2044 between Flight Animals, Tail-Suspended Animals, Synchronous Controls, and Vivarium Controls

	FL	TS	SYN	VIV
Mitochondria	36.5 $P < 10^{-6}$	41.7 $P = 7 \times 10^{-3}$ $P = 3 \times 10^{-4}$	42.2	44.8
Glycogen	21.2 $P < 10^{-6}$	13.6 $P < 10^{-6}$ $P < 10^{-6}$	8.2	6.8
Lipids	1.18 $P < 10^{-6}$	0.86 $P = 2 \times 10^{-4}$ $P = 0.12$	0.44	0.48
Dense bodies	1.17 $P = 4 \times 10^{-5}$	0.87 $P = 3 \times 10^{-2}$ $P = 6 \times 10^{-2}$	0.59	0.55
Capillaries	18.7 $P < 10^{-6}$	16.5 $P < 10^{-6}$ $P = 0.31$	11.1	9.4

Group averages (5 animals per group) are given.

P values in FL column are from comparison of flight group vs. control groups (SYN and VIV).

P values in TS column are from comparison of tail suspended group vs. control groups (SYN and VIV).

P values between FL and TS columns are from comparison of tail suspended group vs. flight group.

FL flight group; TS tail suspended group; SYN synchronous control group; VIV vivarium control group.

cantly reduced ($P < 10^{-6}$). Glycogen, lipids, dense bodies, and capillary density were all significantly increased ($P < 10^{-6}$, $P < 10^{-6}$, $P < 10^{-6}$, $P = 4 \times 10^{-5}$, and $P < 10^{-6}$, respectively).

A lipid increase has generally been observed in previous spaceflights, and also in tail suspension and exercise restriction. It has been suggested that lipid peroxidation can be blocked in the early stages of hypokinesia.⁹⁰ The increased residual/dense body count is indicative of increased lysosomal activity; other evidence for atrophy of the myocardial muscle cells.

The tail suspended group behaved similar to the flight group in all five parameters. In all five parameters this group differed significantly from the control groups. The values for all parameters were intermediate between those for the flight group and the control groups, leading to significant differences with the flight group in mitochondrial, glycogen, and dense body densities. This indicates that tail suspension is a useful, but not perfect model for the effects of weightlessness.

F. Effects of Reentry and Readaptation

The role of reentry and readaptation to Earth's gravity may be an important factor in the observed tissue changes. When pocket mice were returned from the *Apollo 17* lunar mission, they had trouble standing in their cages, and when moved around they generally dragged their bodies (Philpott, personal observation). In Soviet spaceflights it was observed that the return to Earth's gravity produces a drastic increase in cardiac load, and that the resulting excessive exercise causes myocardial degeneration.³³ It has been suggested that the postflight events of reentry and readaptation may be responsible for some of the observed changes.⁹¹ This may help explain why the longer recovery period after the *Cosmos 1887* flight did not appear to have a significant effect on the myocardium.

V. CONCLUSIONS AND SUMMARY

Our findings for the tail-suspended animals in the context of the *Cosmos 2044* project indicate that for all five myocardial parameters studied, the tail-suspended animals showed similar, but much smaller changes than the flight animals. This indicates that while ground-based model experiments are valuable, flight experiments must be carried out to verify the results obtained from the former and to aid in planning future experiments.

The opportunity to obtain heart tissue from the *Cosmos 2044* rats 12 hours after recovery of the capsule, and to compare it to tissue obtained 48 hours after recovery of the *Cosmos 1887* capsule, provided an opportunity to look for reversal of flight-induced changes during the first 2 days after landing. Since the changes observed in the left ventricles of the rats on the *Cosmos 2044* mission were not significantly different from those seen in the animals on the *Cosmos 1887* flight,⁷⁷ there does not appear to have been any significant recovery of the heart tissue during the first 2 days after landing. It cannot, however, be excluded that the tissue recovery process was retarded due to stress caused by the difficult rescue and transportation of the *Cosmos 1887* animals. On the other hand, there is some evidence that longer periods are needed for tissue normalization; only partial recovery of muscle cathepsin activity was found after 25 to 26 days.⁴⁸ It would, however, be very helpful to be able to dissect the animals in space and return the fixed specimens to Earth for analysis. Until this is possible, the effects of reentry and recovery before sacrifice will remain difficult to assess.

At present the basic mechanisms of cardiovascular deconditioning are still not fully understood. The data, reviewed and presented here, suggest that further experiments involving both flight- and tail-suspended animals will be needed. Some confusion currently exists in the literature regarding the role of hypokinesia in myocardial deconditioning. Sometimes, and particularly in the Soviet literature, the word hypokinesia has been used to describe the cause of the myocardial

deconditioning taking place in weightlessness or in ground-based simulation experiments. The word, hypokinesia, should be used in its literal sense, as describing a state of greatly reduced movement and activity of the body, as compared with the controls. The problem is that one can have weightlessness or simulated weightlessness with activity levels which are less than, equal to, or even higher than the activity level of normal ground controls. In fact, we do not know what the real activity levels were in many spaceflight experiments. The same holds true for many ground-based simulation studies reported in the literature. If the changes in the heart in weightlessness are due to real hypokinesia, then it should be possible to reproduce these changes on Earth in the absence of weightlessness by reducing the activity level of the animals.

The other candidate for the causative agent of heart changes—namely the fluid volume and pressure shifts that are known to occur in weightlessness—can be simulated by changing the position of primates and human beings from upright to supine, or by inducing a fluid shift in rats by tail suspension. If the structural changes in the heart can be demonstrated in situations where body muscular activity is held at the same level as in the controls (“normokinesia”) then it can be assumed that the fluid pressure and volume changes in the cardiovascular system are likely to be the causative agent. The word “hypokinesia” is then used in its literal sense. There is, of course, another possibility; namely that both hypokinesia and fluid shift contribute to the cardiac changes. By quantitating the results of an experiment as illustrated in Table 1, it should be possible to determine the relative contribution from each hypothesized causative agent, if both are contributing. Since in this experiment the tail-suspended animals were not running on a treadmill, the present results do not permit this distinction.

Heart deconditioning is accompanied by morphological changes; for example changes in mitochondrial number and/or size, cytoskeletal loss, increase in collagen, and cAMP dependent protein kinase changes. There is an increase in collagen after body-casting and tail suspension. It is possible that once connective tissue has been formed as in the lung, it is permanent and irreversible. This would mean that if connective tissue has increased in the myocardium after exposure to weightlessness, we will be dealing with a “scarred” heart which will have permanently lost some of its elasticity.

On the other hand, if the heart returns to its normal state after a spaceflight or after simulated weightlessness exposure—that is, the connective tissue disappears and structural and biochemical parameters return to normal—then the changes are adaptive. The review of earlier weightlessness studies was intended to show the experimental data, how these were interpreted by the investigators, and that some of the changes appear to be degenerative and irreversible. We believe that additional research is needed to determine more exactly the time course and the possible reversibility of the myocardial changes, and to ascertain unequivocally whether these are irreversible changes. Reversibility studies will permit us to determine whether there are any irreversible changes.

Heart deconditioning and the resulting difficulties in readaptation to Earth's gravity may pose a limit to the duration of space missions, especially in aged subjects who are past their physiological prime. Hence, in addition to studies on young rats, the effects of hypokinesia and fluid shifts should be investigated in aged rats. This will test the hypothesis that aging potentiates cardiac deconditioning.

Another important topic for future experiments is the study of countermeasures against cardiac deconditioning. In this respect, our finding in tail-suspension experiments that application of the calcium antagonist, nifedipine (2.85 and 5.7 mg/kg body weight), can prevent edema, lipid, and glycogen accumulation and myofibrillar granulation deserves further study in flight experiments.

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FLUID AND ELECTROLYTE REGULATION IN SPACE

Claude Gharib and Richard L. Hughson

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I. INTRODUCTION

The problem of the fluid and electrolyte shifts occurring during the weightlessness of human space travel has been investigated for more than 30 years. In spite of this, our knowledge concerning the role of the volume regulating hormones, renin, angiotensin, aldosterone, vasopressin (antidiuretic hormone), and atrial natriuretic factor, is still limited.

A number of factors have prevented the application of strict scientific principles to these studies. These factors include: (1) the voluntary water restriction by the

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astronauts immediately prior to launch; (2) the recumbent launch position;¹ (3) the limited opportunities for sampling, especially during ascent and the early orbital phase; (4) the diurnal effects on the physiological variables; (5) the difficulty of recording all required variables simultaneously; and (6) the often inadequate facilities for acquiring and storing samples.

In order to gain further insight into fluid, electrolyte, and volume regulating hormone modifications induced by spaceflight, many investigators have used ground-based simulation of weightlessness²⁻⁴ by means of bed rest, head down tilt (HDT), immersion, or lower body positive pressure (antigravity suit). Yet, in these studies the questions are complex. On one hand the results of different simulation studies are often contradictory; on the other hand too few spaceflight data are available to permit making valid comparisons. Basically, the problem is how to simulate a state (weightlessness) about which only hypotheses can be advanced.

II. FLUID VOLUME

It is well known that during weightlessness there is a fluid shift from the legs to the upper part of the body.^{1,5-8} The reduction of the total leg volume amounts to about 2 liters after 1 week of weightlessness (Fig. 1). This shift occurs in both the intravascular and extravascular volumes.

A reduction in body fluid volume has been consistently demonstrated during both

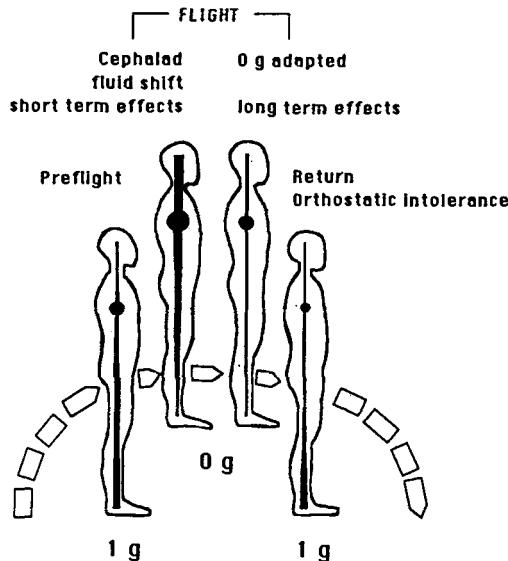


Figure 1. Schematic representation of fluid shift and adaptation during a spaceflight.

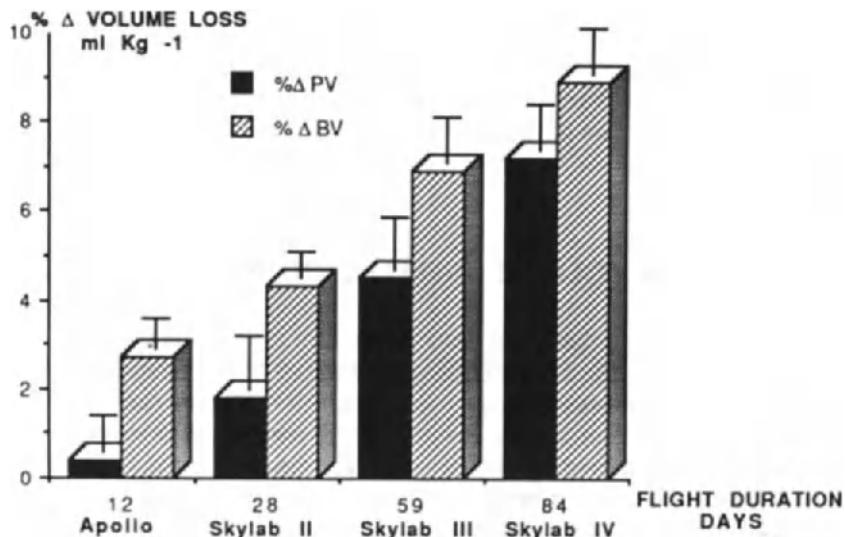


Figure 2. Plasma (PV) and blood volume (BV) loss during *Apollo* and *Skylab* missions (ref. 5).

American and Soviet spaceflights.⁸ The first complete data were obtained from the astronauts of *Apollo* and *Skylab II, III and IV*.⁵ The fluid volume changes were studied for space missions lasting from 11 to 84 days. The data of Johnson⁵ are summarized in Figure 2. It is interesting to note that the loss of plasma volume was linearly related with the flight duration, at least up to 84 days.⁹ During bed rest, the plasma volume was also reduced linearly with time and to a similar extent as in space travel.⁴

Red blood cell mass decreases linearly during bed rest for up to 30 days duration.^{4,10} The evidence from spaceflight suggests that while the absolute decline in mass may be greater than in bed rest, the point of maximum decline may be reached after 30 days in space. During the *Skylab* flights, the greatest postflight reduction was seen after the shortest 28-day *Skylab II* mission.⁹ Thus, the ratio of red blood cell mass loss to plasma volume loss appears to decline with increasing flight duration. The mechanism responsible for the reduction in red cell mass may be related to the decline of the plasma erythropoietin concentration by approximately 20% found on mission days 1 and 7.¹¹

The conflicting results concerning the decline in extracellular fluid volume for bed rest and spaceflight studies have recently been summarized.¹⁰ Johnson⁵ has indicated that the inherent inaccuracy of the method for the measurement of the extracellular fluid volume probably means that the changes in plasma volume and extracellular fluid volume were approximately equal. Some studies have indicated

that the extracellular fluid volume may be restored after a longer duration of bed rest,⁴ although the recent data of Fortney¹⁰ argue against this. One problem that must be considered with respect to the extracellular fluid volume is how to express the volume. Recognition of the loss of tissue mass (especially muscle) makes it necessary to consider the variation in extracellular fluid volume per kg of body mass.

III. URINE VOLUME AND ELECTROLYTE EXCRETION

Table 1 shows urine volume and water intake data for the first four days of the *Skylab III* mission.¹² The data for urine volume and all related parameters obtained during spaceflight are often difficult to interpret. This is not only due to the limited number of subjects, but also to methodological concerns such as grouping of 24-hour urine data according to 6-day dietary cycles, and providing mean data across several missions.¹² Complications due to the space adaptation syndrome with potential nausea, emesis, and voluntary reduction in water consumption, or environmental factors, such as elevated space vehicle temperature, can further confound the interpretation of the data.

Some interesting data for the early phase of a spaceflight are those of C. Leach¹¹ who described an unchanged urine excretion for the first 36 hours of flight in spite of a 10-fold increase in vasopressin (Fig. 3). Sodium excretion was initially unchanged, but then decreased while aldosterone was unchanged.

During *Skylab* missions, urine volume tended to be low during the first days of flight (Fig. 4a), with variable, but near normal volumes after that.¹³ Antidiuretic hormone excretion went down (Fig. 4b). Sodium excretion was quite variable,

Table 1. Urine Volume and Water Intake During the First 4 Days of *Skylab III*

Mission Day	1	2	3	4
Total Urine Volume, ml/24 hrs				
CDR	2123	1024	993	924
SPT	552	924	950	878
PLT	1507	858	748	965
Total Water Intake, ml/24 hrs				
CDR	1560	1942	1539	2016
SPT	1551	1128	1607	2045
PLT	1794	1474	1977	1740

Data from Leach, Johnson and Rambaut, ref. 12.

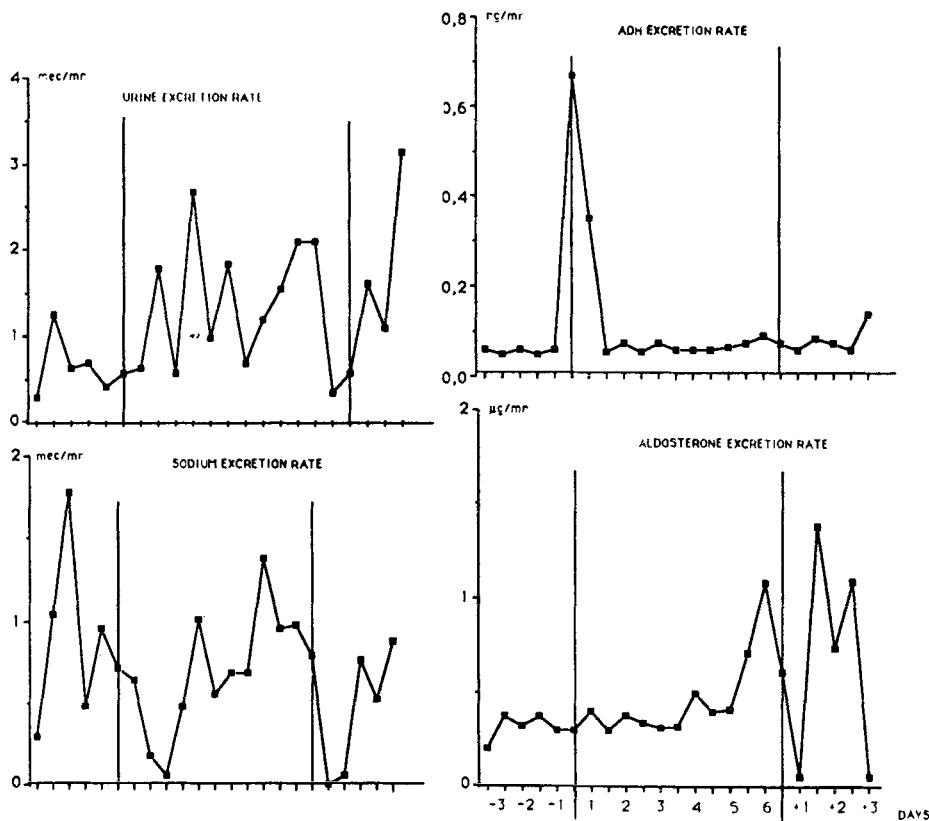


Figure 3. Urine, sodium, antidiuretic hormone (ADH) and aldosterone excretion rate before, during and after spaceflight for one mission specialist (ref. 11).

especially between missions (Fig. 4c). These data highlight the difficulty in describing a clear pattern of response.¹⁴

In bed rest studies, there is generally a clear cut increase in diuresis and natriuresis on the first day. The differences between bed rest and spaceflight are probably explained by some of the complicating factors mentioned in Section I.

During a 25-day flight, we have found a 20% decrease in both urine output and sodium excretion on days 5 and 19.¹⁵ However, these data are questionable, because the environmental temperature was elevated (25 to 30°C) and the atmosphere in the *MIR* space station contained 1% CO₂. The careful analysis by Leonard⁶ of the data from the three *Skylab* missions demonstrates a clearly negative water balance during the first days of spaceflight (Fig. 5). This is the result of reduced water intake, whether due to the effects of space adaptation syndrome, to reduced thirst or to voluntary water restriction. The urine volume declined during this same period.

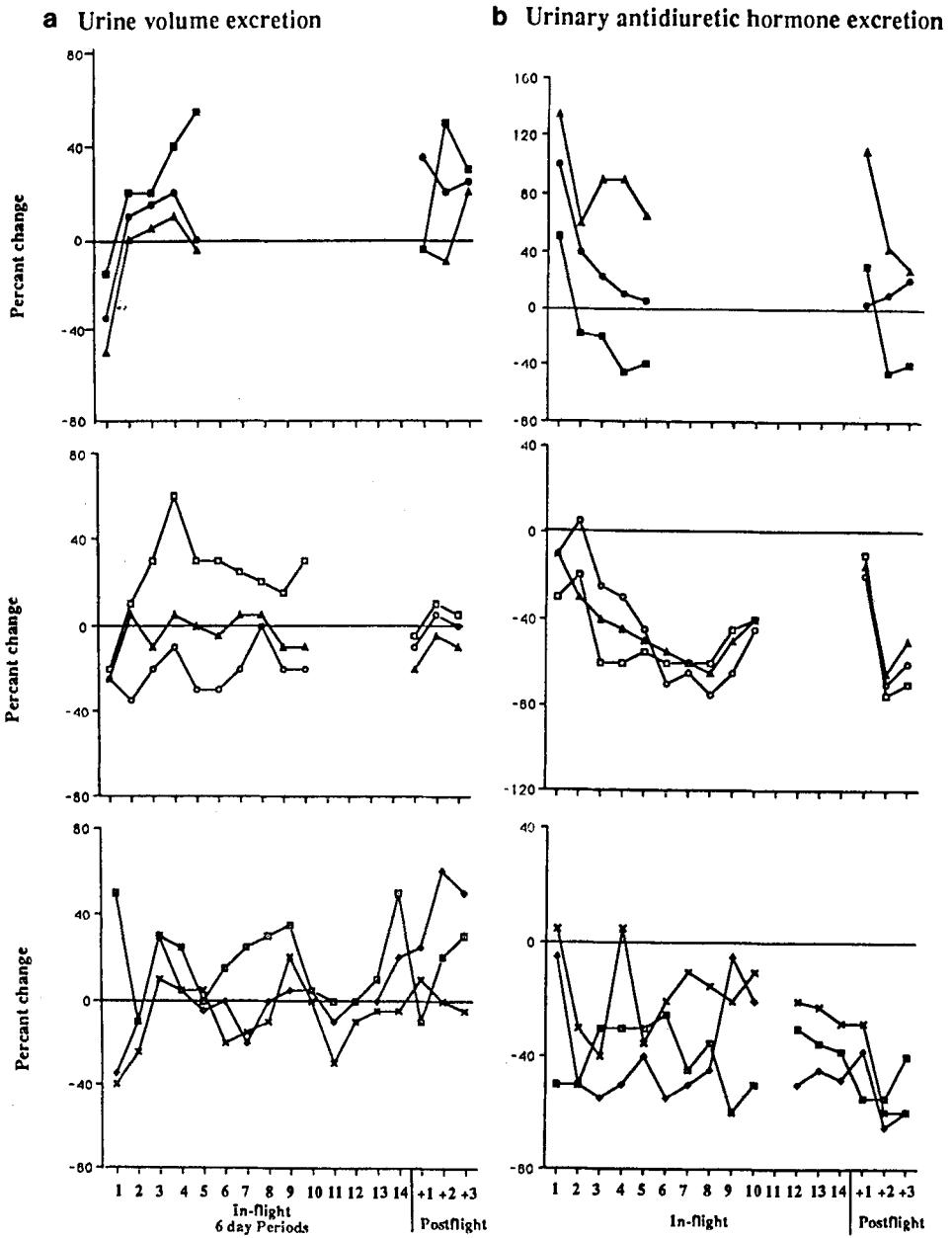


Figure 4. Urine (a), ADH (b) and sodium (c) excretion rate during the three *Skylab* missions (ref. 12).

C Urinary sodium excretion

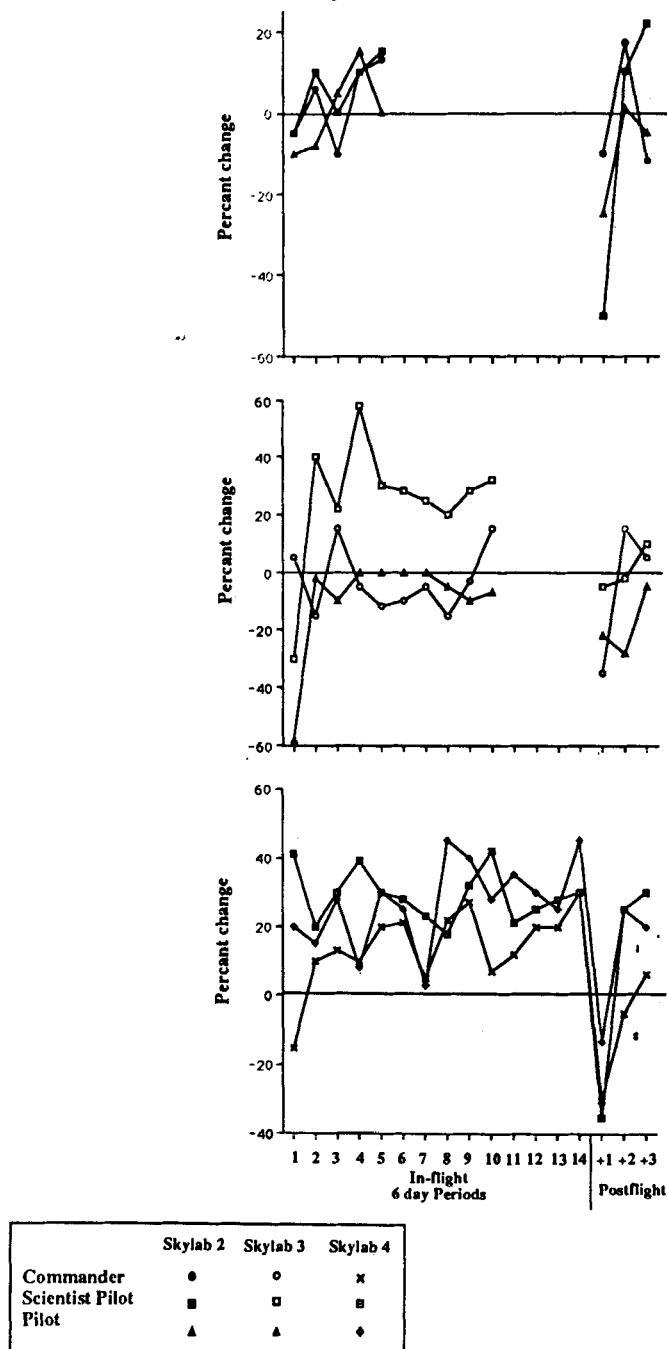


Figure 4. (continued)

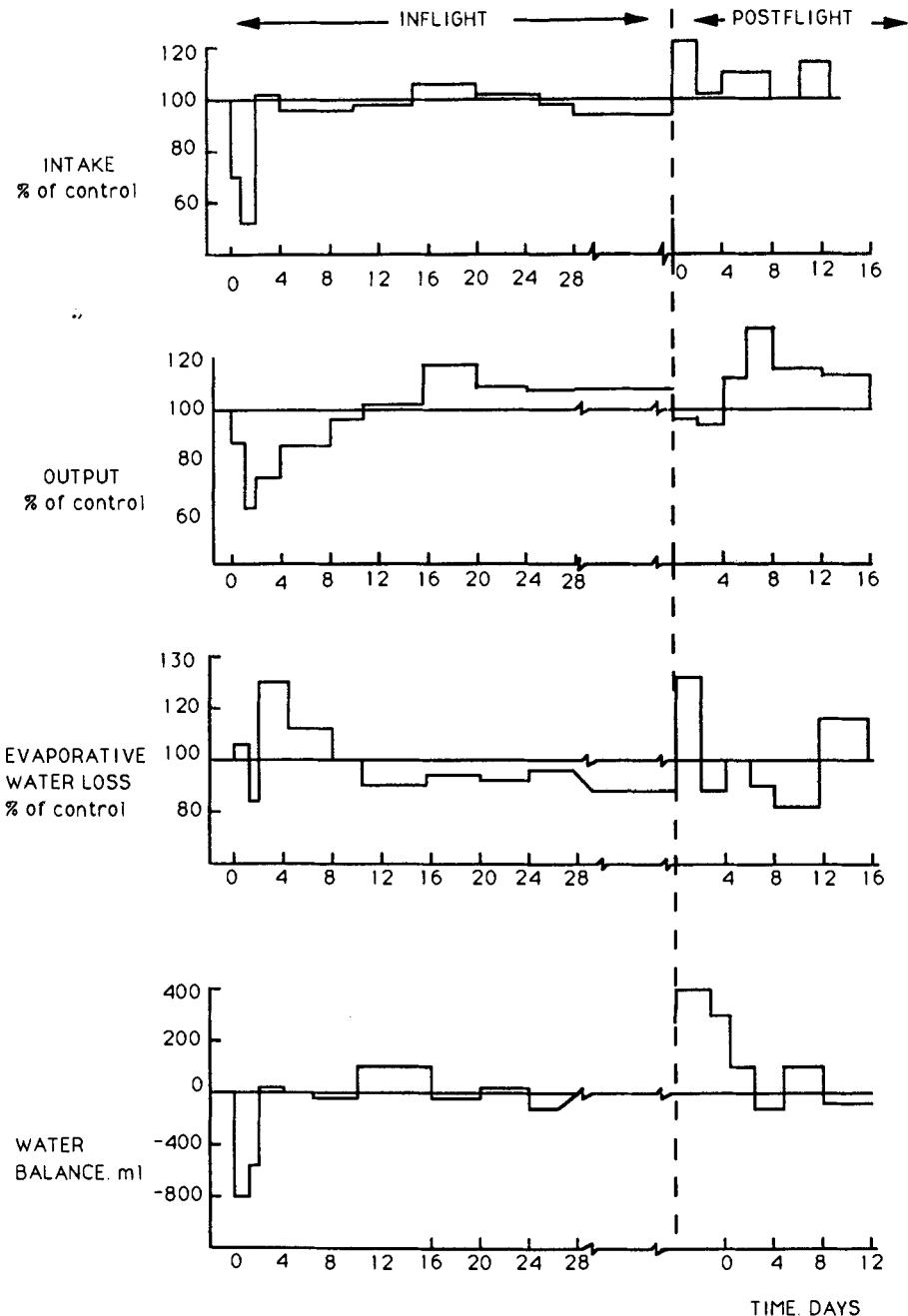


Figure 5. Skylab water balance—nine subjects (ref. 6).

IV. VOLUME REGULATING HORMONES

In spaceflight, as in bed rest, two distinct phases in the modification of the volume regulating hormones might be anticipated. The first phase consists of the first 2 days of spaceflight and bed rest, while the second phase refers to the subsequent several days.

In the first phase, a relative central hypervolemia caused by the fluid shift from the lower to the upper parts of the body will induce water and electrolyte excretion.⁴ A major early consequence of the fluid shift is a short-term increase in central venous pressure.¹⁶⁻¹⁸

In bed rest, a transient increase in atrial natriuretic factor, with a decline in the renin-angiotensin-aldosterone system (Fig. 6), is associated with a marked diuresis. The time course of the atrial natriuretic factor change seems to parallel that of the central venous pressure.¹⁹

In spaceflight, the vertical launch position of the *Shuttle*¹ will initiate a fluid volume shift with a resultant transient increase in central venous pressure. It is not known whether during launch and the early orbital period the central venous pressure is again elevated. If such an increase occurs, it will probably be transient.¹⁷ One might expect the atrial natriuretic factor to follow, as it does in ground-based simulation studies. However, the atrial natriuretic factor has been reported to be elevated on flight days 1 and 2 by 36 to 82%,¹⁸ as well as on day 5 of flight.¹⁵ The latter data were found at an elevated environmental temperature. Because the atrial natriuretic factor inhibits plasma renin activity, the observed decline in this hormone during the period of elevated atrial natriuretic factor was not unexpected.¹⁹

In the early phase of bed rest studies, a reduction in vasopressin is commonly found. In contrast, plasma vasopressin was elevated during the course of *Spacelab* missions,¹⁷⁻¹⁹ as shown for one mission in Figure 7. This finding appears to be in contrast to the anticipated responses based on the Gauer-Henry reflex, the decrease in arginine-vasopressin secretion induced by central venous pressure increase. Thus, the hormonal profile in the period preceding launch and during the early phase of spaceflight is only partly similar to that in bed rest. This phenomenon may be related to the finding that, unlike in bed rest, there is no increased diuresis in the early phase of spaceflight.⁶

The second phase of modification of volume regulating hormones develops later during the first week of bed rest or spaceflight. Figure 8 shows that plasma atrial natriuretic factor concentrations declined to 48 to 59% of preflight values by day 7 of the *Spacelab* mission SL-1.¹⁹ They return to normal levels after a few weeks in space, since on day 19 during a mission on the *MIR* space station they were not different from preflight.¹⁵

Plasma renin activity, angiotensin II, and aldosterone concentrations increased with time during bed rest, while norepinephrine remained depressed.^{2,3} In spaceflight, results of plasma hormonal concentrations have been variable. Excretion of vasopressin was reduced during the *Skylab* missions, but plasma vasopressin was

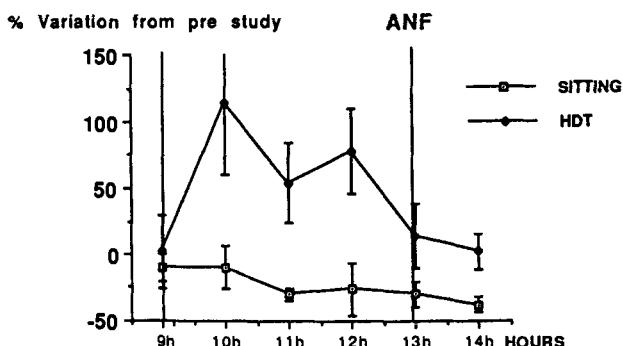
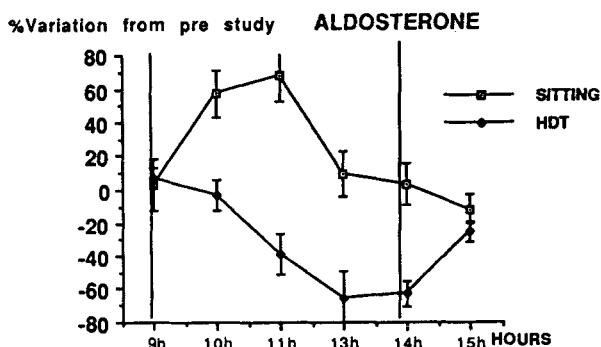
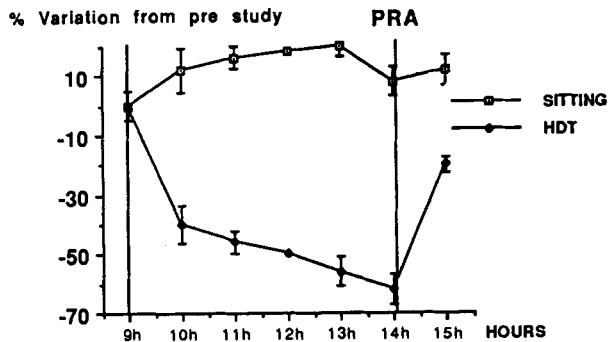


Figure 6. Effect of 5-hour head down tilt (HDT) on plasma renin activity (PRA) and aldosterone, and 4-hour tilt on atrial natriuretic factor (ANF) compared to a seated control. Data from two protocols (ref. 2).

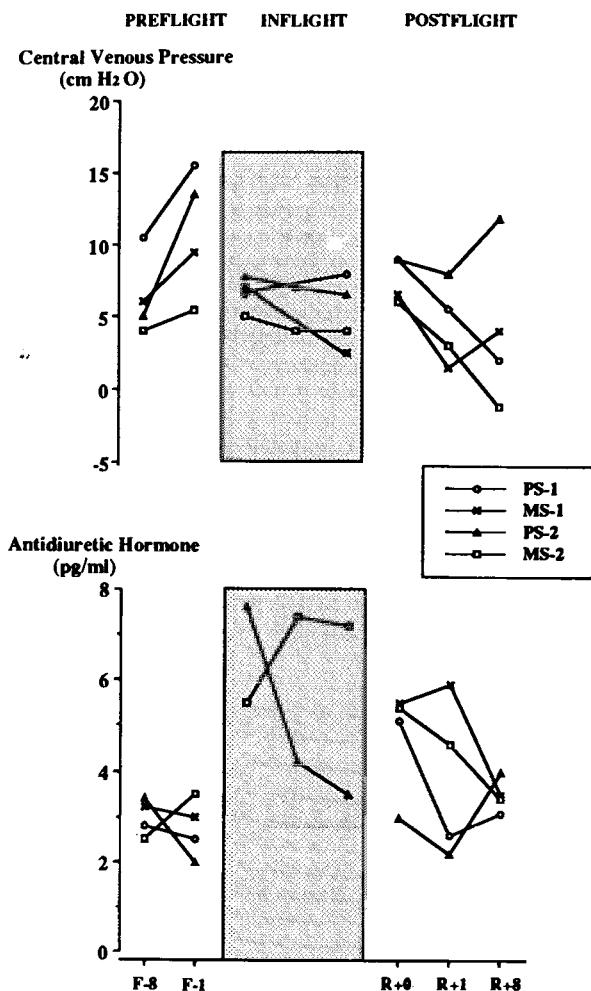


Figure 7. Central venous pressure and antidiuretic hormone values before, during and after flight. Samples taken day 8 and day 1 before flight (F-8, F-1); 22 hours after launch, and on mission days 2 and 7; 1 hour (R + 0), 2 hours (R + 1), and 8 days (R + 8) after flight (ref. 17).

elevated during a *Spacelab* mission.¹¹ Plasma renin activity was elevated during a longer duration spaceflight, as it was in bed rest. However, inconsistent changes in angiotensin and aldosterone indicate that there may be a dissociation of these hormones from plasma renin activity.¹⁹

In addition to an effect of spaceflight on hormonal levels, one should also consider the possibility that the number and binding affinity of hormone receptors might be affected in weightlessness. Studies of hindlimb suspension in rats have indicated that such an effect may be involved in the altered renal response to atrial natriuretic factor.²⁰

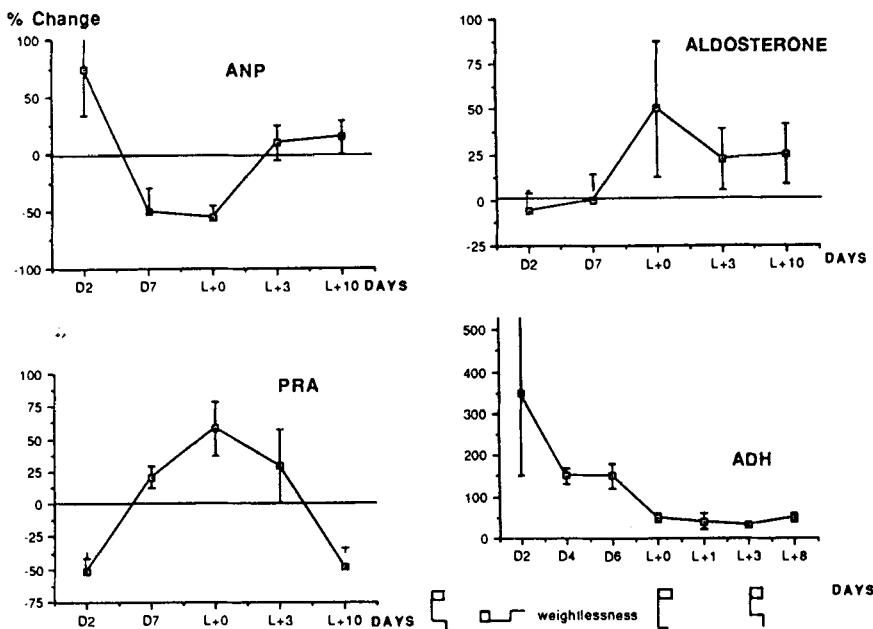


Figure 8. Percentage changes in atrial natriuretic factor (ANP), plasma renin activity (PRA) aldosterone and antidiuretic hormone (ADH) from preflight levels (ref. 19).

V. QUESTIONS RAISED BY SIMULATION STUDIES

In this paper we have made several references to simulation studies in man or animal. For the cardiovascular system all types of simulation have the same goal: to produce a cephalad fluid shift which is thought to be the first step of the adaptation of the human body to microgravity.

Animal studies have mostly been done with hindlimb-suspended rats. The question is whether it is valid to apply results obtained with a quadruped to man. In humans, 70% of the blood volume is below the heart level, while in quadrupeds, 70% of the blood volume is at or above heart level.²¹ Nevertheless, all currently available data indicate that the responses observed in an animal placed in a head down position are similar to those described in humans: early increases in central venous pressure²² and atrial natriuretic factor,²³ a long-term increase in aldosterone,²⁴ or an altered baroreflex function.²⁵ Thus it is very useful to have such a model, where long-term studies are possible in which also muscle atrophy and bone demineralization have been demonstrated.

Another question is whether the hormonal changes observed in humans during bed rest are entirely due to the hemodynamic effects, or that confinement and inactivity may also play a role. The same question arises for the animal-simulation

experiments. Our group had the opportunity to compare a 28-day confinement study (ISEMSI), sponsored by the European Space Agency,²⁶ to a head-down bed rest study at the French space research center CNES of the same duration.²⁷ One of the important conclusions of this comparison was that many of the changes observed during the head-down bed rest study also appeared in the confinement study (Fig. 9). So it could be interesting for our understanding of the changes induced by spaceflight to take into account that confinement and reduced activity go along with microgravity. This makes it worthwhile to consider the study of "analogous situations" like polar expeditions and stay on a nuclear submarine. For example, Arita et al.²⁸ reported cardiovascular deconditioning after a 7-day saturation dive at 31 ATA, which they ascribed to inactivity.

VI. ORTHOSTATIC HYPOTENSION

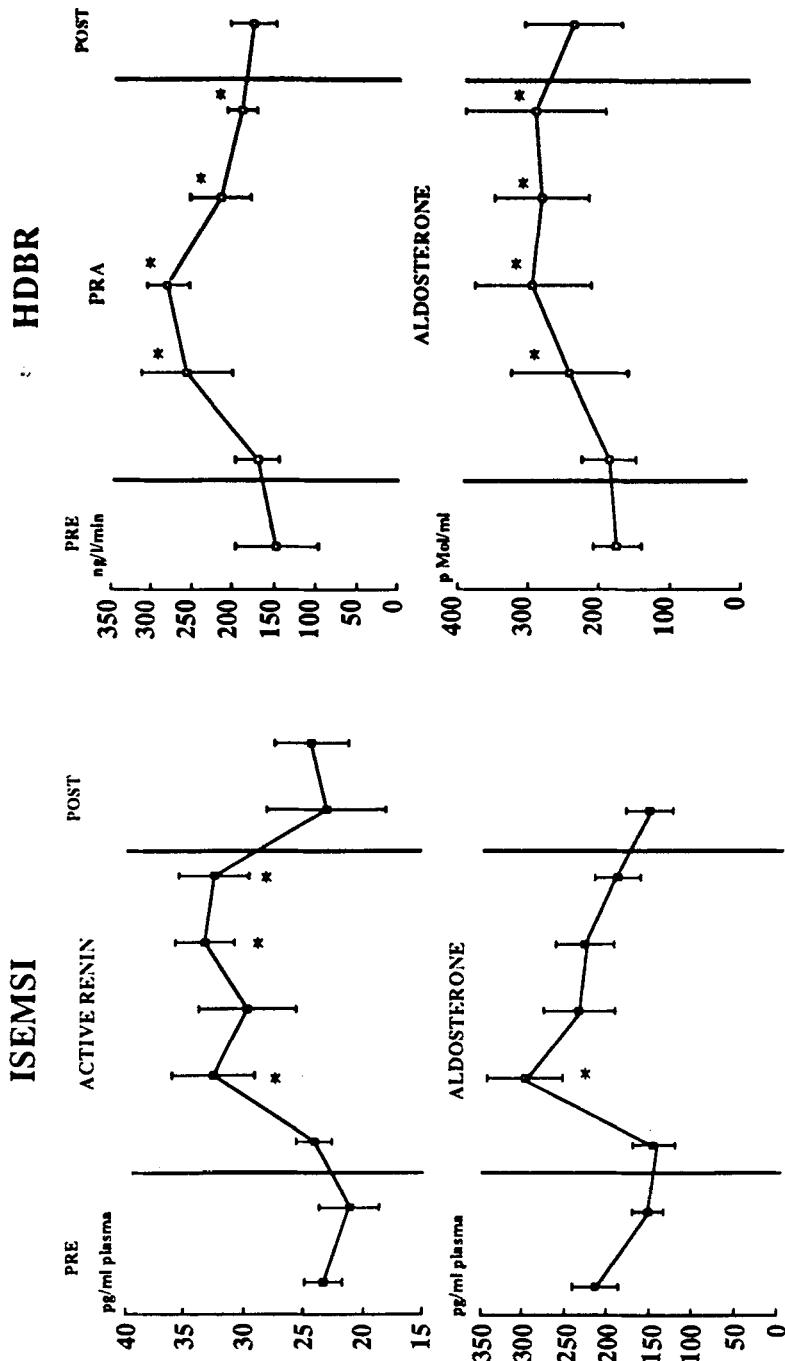
The decline in plasma volume during spaceflight seems to be an appropriate effect of the weightlessness conditions of space. Namely, the removal of the head-to-foot gravity vector leads to an increase in the central blood volume. The negative water balance during the first days of spaceflight²⁹ and the subsequent changes in volume regulating hormones are adaptive changes that reduce the central blood volume to an equilibrium value for the weightless environment.

Upon return to Earth the reduction in plasma volume contributes to orthostatic hypotension, but this is probably only one of the factors playing a role in this phenomenon. Several other mechanisms are involved in the pathophysiology of orthostatic hypotension, such as muscular atrophy, changes in the properties of venous walls and attenuation of the activity of the baroreflex.²⁹⁻³² The baroreflex occurs when baroreceptors located in the carotid arteries and aorta are stimulated by the stretching of the vessel upon increasing pressure. These receptors then send signals to the brain, which in turn sends signals to the circulatory system, leading to a reduction of the arterial pressure.

A more complete understanding of the mechanisms responsible for the cardiovascular stability in space will provide a framework for understanding the cardiovascular inadequacies upon return to the 1-G environment.^{16,32-34}

VII. CONCLUSION

The results discussed above leave us with the conclusion that fluid and electrolyte regulation during spaceflight is at least a biphasic process, the first phase taking place during the first 24 hours in space and the second phase during the subsequent days. This is summarized in Figure 10. The results of the experiments conducted during the first *Space Shuttle* mission dedicated to life sciences research (SLS-1) will be of great help to increase our understanding of the mechanisms of the adaptation of the cardiovascular, renal and endocrine systems to microgravity.



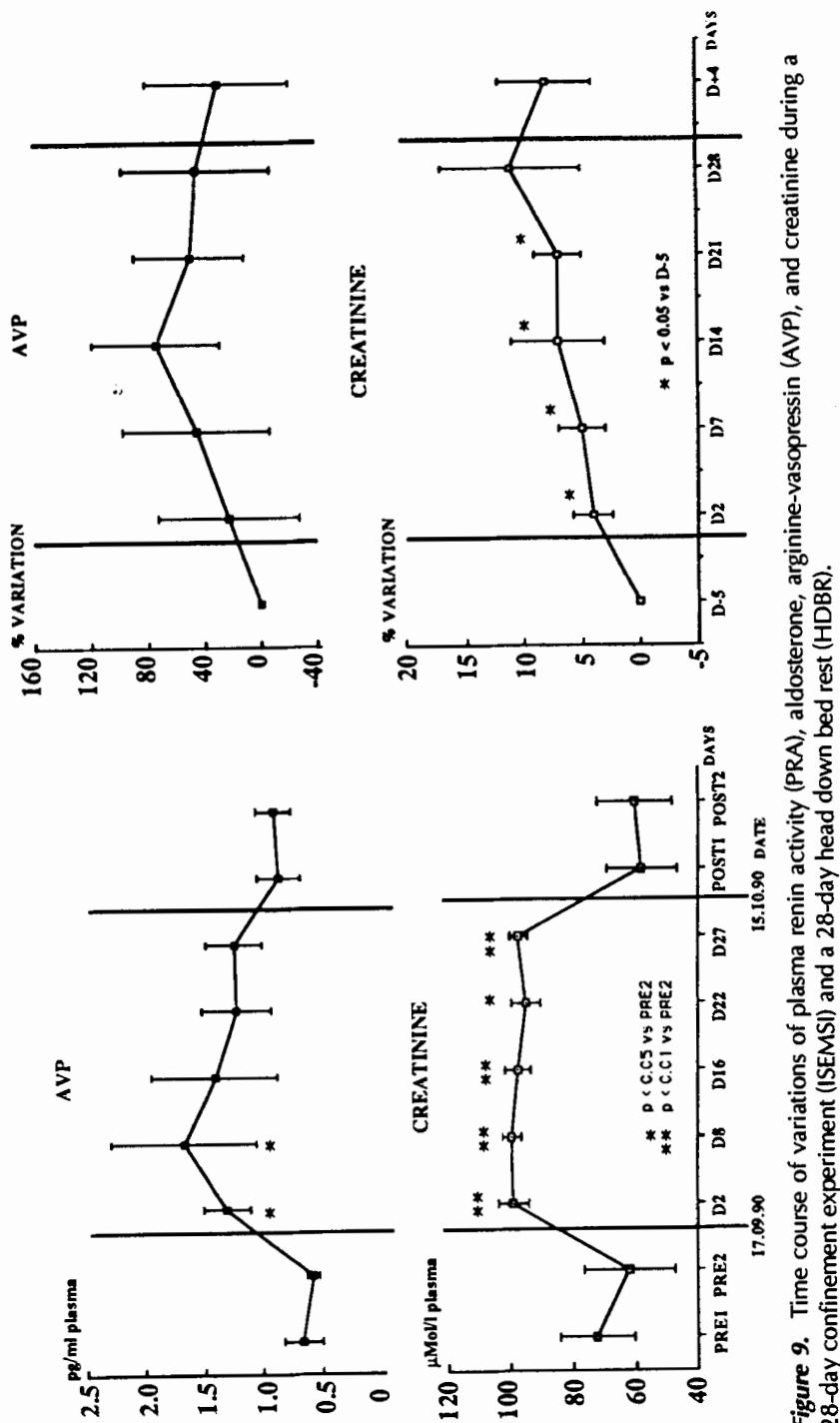


Figure 9. Time course of variations of plasma renin activity (PRA), aldosterone, arginine-vasopressin (AVP), and creatinine during a 28-day confinement experiment (ISEMSI) and a 28-day head down bed rest (HDBR).

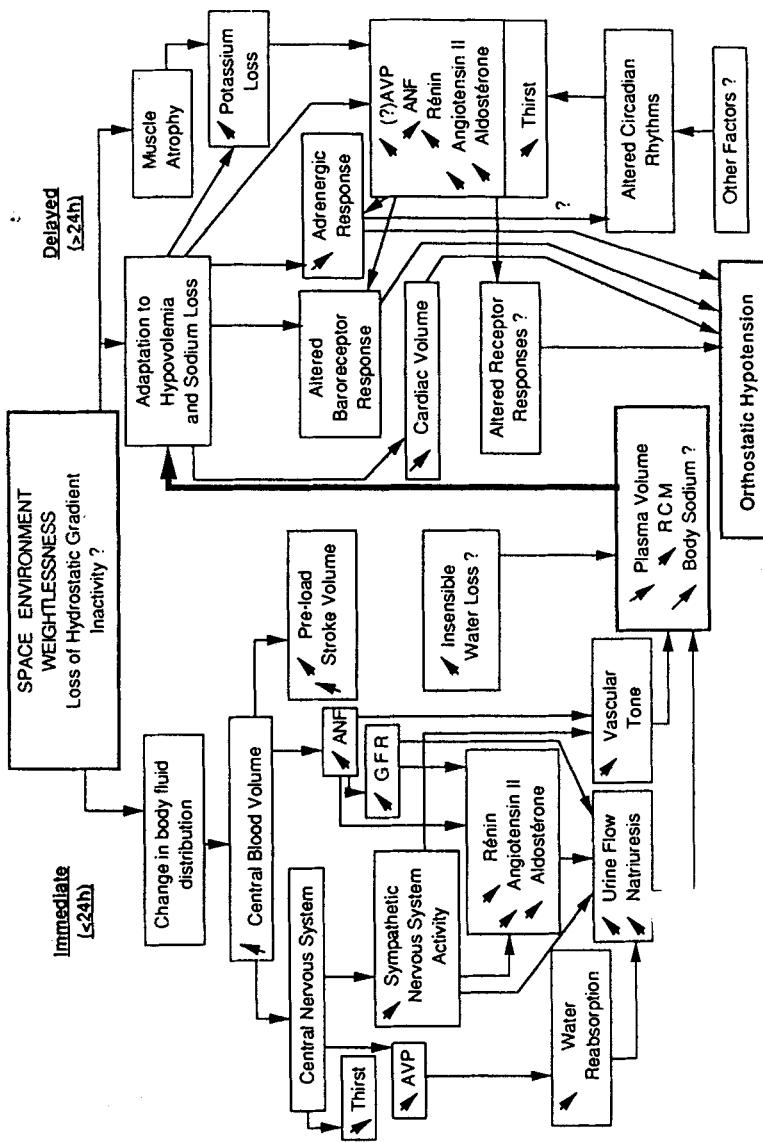


Figure 10. Suggested responses of volume-regulating hormones to real and simulated weightlessness.

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HUMAN NUTRITION UNDER EXTRATERRESTRIAL CONDITIONS

Helmut G. Hinghofer-Szalkay and Eva M. König

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I. INTRODUCTION

Life in a weightless environment, or with a gravitational force different from Earth's gravity, brings about certain physiological and psychological changes. The major biologic effects include changes in body shape and composition, overall bone and muscle loss, and neurological dysfunctions. It has been tried to prevent or limit such changes by means of various nutrients, but the results obtained so far are difficult to interpret because nutrition affects the function of any subsystem of the organism, on different biochemical and physiological levels, in an extremely complex fashion.

Although specific nutrients are known to be particularly important for certain physiological functions, they are dependent upon the presence of other nutrients for optimal effect. Thus, no single substance by itself can maintain the proper functioning of any biological subsystem; rather, a balanced intake of all necessary nutrients is needed for optimal nutrition of astronauts.

Short-term periods of spaceflight (weeks) present no serious problems in terms of nutritional surveillance and planning; eventual body weight losses and energy deficits can easily be sustained on short missions. Originally it was assumed that less energy would be required in spaceflight than on Earth because of the low muscular load in microgravity. However, the metabolic cost of physical work in space is higher than anticipated, and the energy content of the space diet has been progressively increased, particularly on the longer Soviet spaceflights. The proportion of fat versus carbohydrate and protein was also readjusted to provide sufficient energy, yet at the same time, high nutritional value.

However, even an increased food supply does not entirely prevent inflight weight loss. It is not known which part is played by the elements involved in the physiological change in body mass and body composition occurring during spaceflight. Stress factors probably play a role, as evidenced by hormonal changes and biochemical indices. But the "weightless" condition is unique by virtue of its very nature, and cannot be fully simulated on ground. Few data are at hand to date which would enable us to assess, on solid scientific grounds, the underlying physiological systems readjustments in the human organism when subjected to spaceflight. To arrive at more convincing information, studies performed in actual flight are necessary, since the astronaut's metabolic changes can only partly be understood by means of simulation techniques.

With increasing mission duration, the scientific and logistic problems grow significantly. Long-duration space missions call for new research efforts, an extended data base, in-depth systems analysis approaches, and physiological investigations providing the basis for decisions on the technology to be employed and scenarios to be adopted. Nutrition for long-duration space missions raises questions of nutrient supply, nutrient utilization, immunological and physiological adaptation mechanisms, waste management, and biomass recycling under the particular cir-

cumstances of orbital flight, outer space missions, and variable G-load on a Moon-base or a Mars mission.

Proper nutrition of space travellers must guarantee that all essential nutrients are supplied in adequate balance to maintain the physiological range of life functions and the well-being of astronauts under the conditions of spaceflight and working and living in a Moon or Mars habitat. Whenever inadequate amounts of nutrients are provided to the tissues, nutritional deficiencies will occur. In the following sections, the published findings from recent spaceflights will be reviewed, and a concise overview on the present knowledge of nutritional principles will be presented, with emphasis on aspects which are, in our opinion, likely to become the main tasks of a future field of investigation—a field which might be termed “human nutrition under extraterrestrial conditions.”

II. TYPE AND AVAILABILITY OF NUTRIENTS

The major part of the human diet is composed of macronutrients—water, proteins, carbohydrates, fat, and nucleic acids. Other substances, which are present in small quantities, are also vital. These micronutrients, mainly trace elements and vitamins, regulate metabolic processes in many different ways. The majority act as coenzymes or as essential elemental constituents of enzyme complexes, which regulate the utilization of proteins, carbohydrates and fats.

The dietary input of a nutrient does not necessarily equal the amount actually assimilated by the organism. As an example, from a total of 15 mg iron in daily nutrition, only 1 mg may be taken up and transferred to the blood by the intestine. This fraction available to the body is therefore “bioavailable.” Special mechanisms are employed to transfer a nutrient from the intestinal lumen to the blood. These mechanisms are functionally modified by the body’s actual metabolic status, so the degree to which a nutrient is available for absorption and assimilation depends on the physiological condition of the body. Further, mutual matrix effects influence the degree to which certain nutrients (mostly minerals and trace elements) are taken up by the intestinal mucosa.¹

Some examples can be given. A high energy intake increases the need for vitamin B₁; fiber intake decreases the availability of calcium and magnesium; high protein levels can increase calcium excretion and increase the need for vitamin B₆ and zinc; high calcium and phosphate levels may cause zinc deficiency; and high zinc intake can exacerbate copper deficiency. Weightlessness results in a substantial loss of electrolytes that are linked to nerve and muscle function, and the digestive processes may be changed due to physical confinement and altered stress patterns.²

The problem of bioavailability will become significant in long-term space missions. During life on Earth many interdependencies between different trace nutrients, on both biochemical and physiological grounds, have evolved. This has created an intricate biological balance, which may be hazardously imbalanced

during stay-on extraterrestrial bases with different kinds of soil, crops, and food. Therefore, much space is devoted to our current understanding of micronutrient mass balance in this chapter, since an optimal approach to mineral and trace element supply to plants, animals, and humans will present a major challenge to future long-term spaceflight nutrition scenarios.

A. Macronutrients

Protein

During spaceflight, a loss of protein occurs through muscle atrophy.³ Physical activity is not only the major variable affecting caloric expenditure but also determines the nutritional need for amino acids. From the 20 amino acids which are supplied by dietary protein, about half can be readily formed from common intermediates in metabolism and do not need to be provided by food. These are referred to as non-essential amino acids. However, eight are essential in the human diet because their carbon skeletons cannot be synthesized at adequate rates, two others are essential for children, and two are produced from essential amino acids (methionine and phenylalanine) and if present in the diet, they spare their parent amino acids.

The requirements for essential amino acids range from 3 to 15 mg/kg per day in adults. Proven combinations of protein sources are cereals with milk, meat, eggs, or fish; potatoes with milk, dairy products, eggs, and meat or fish. Animal protein generally provides good patterns of essential amino acids.⁴

The recommended daily allowance (RDA) for protein, assuming a mixture of animal and vegetable protein, ranges between 0.8 and 2.0 g/kg body weight per day, depending on age, gender, muscular activity, and energy intake. Western humans have a customary intake of some 100 g of protein per day. Dietary protein intake influences albumin metabolism⁵ and hence, among others, blood volume. Fluid balance, cardiovascular functioning, and body composition are influenced in this way. Protein malnutrition results in immune deficiencies, edema, and a catabolic state.

In the *Apollo* and *Shuttle* missions, protein consumption was about 80 g per day, based on ingredients used and on amino acid analyses. Large individual differences were observed (up to 160 g per day). The Soviets have prescribed higher amounts of protein, in an effort to counteract muscle atrophy. For U.S. astronauts, protein provided 18.5% of nutrient energy,^{2,6-7} and 22.7% for Soviet cosmonauts.² Besides to the total protein content of the diet, the extent to which each amino acid contributes to the total amount of protein provided. Further, if the proportions of the major energy sources—fat, carbohydrates, and protein—are greatly changed, the dietary pattern needs to be readjusted. It needs to be tested how astronauts can adjust to such changes. Based on present experience, it can be assumed that the tolerance to such changes will vary greatly between individuals.

The kinetics of protein and amino acids in the body⁸ need investigation in future flights in order to fully understand the mechanisms of metabolic adaptation to weightlessness. After the *Salyut* missions, a decrease of cosmonaut blood plasma levels of most amino acids, particularly the essential amino acids, was found. It was concluded that astronauts should be supplemented with certain amino acids pre-flight, inflight, and postflight.⁹

Mechanical devices, as the Soviet penguin suit, can be used to apply compressional and elastic stress on the musculoskeletal system. This seems to be effective in preventing protein catabolism and muscle atrophy.¹⁰ A loss of 1000 g muscle tissue would constitute a 200 g loss of protein, since muscle is 20% protein. There is a need to relate the demands of physical performance in space with preservation of muscle mass. Dietary strategies can assist in preventing amino acid and protein loss from the body of astronauts.

Optimizing the amino acid pattern of astronaut diets may also have benefits for anabolic processes in muscle which utilize major amounts of branched chain amino acids.¹¹

Fat

Ingested fat acts as a vehicle for food flavors and fat-soluble vitamins (A, D, E, K), gives texture, delays gastric emptying, and cushions body organs.^{12,13} Dietary fat is a mixture of lipids which consists more than 90% of triglycerides. Also present in small amounts are phospholipids, sphingolipids, glycolipids, cholesterol, and phytosterols. Fat and cholesterol consumption influences not only the total level of caloric consumption, but also plasma cholesterol level, blood viscosity, fibrinogen level, and blood pressure.^{14,15}

Fat has the highest caloric value (39 kJ per gram) of all nutrients. In addition, fat consumption furnishes essential fatty acids. This is the only known specific requirement for fat in the diet. The needs can be met by a daily intake of 15 to 25 g of dietary fat. Fat is of primary importance for buffering not only long-term but also short-term energetic needs. Day-to-day fluctuations in the energy balance seem to be almost exclusively met by body fat rather than by carbohydrate or protein.¹⁶

The contents of saturated, mono- and polyunsaturated fatty acids in the total dietary fat should be about equal, but in Western diets the contents are approximately 35, 40, and 15%, respectively. Humans require a dietary source for certain polyunsaturated fatty acids, namely linoleic acid, alpha-linoleic acid, and arachidonic acid. The latter is not a dietary essential because it can be biosynthesized from linoleic acid. The amount of linoleic acid required by humans is about 1 to 2% of dietary energy. This is easily met by the usual diets, because linoleic acid is a prominent component of the dietary fats. Essential fatty acids and their derivatives are precursors of prostaglandins, thromboxanes, and prostacyclins. They are involved in membrane structures, chiefly as phospholipids, and they play a beneficial role in cardiovascular and blood pressure regulation.¹⁷

Fat intake of U.S. astronauts (*Gemini*, *Apollo*, *Shuttle*) decreased inflight, compared to the consumption on the ground.¹⁸ Data from *Apollo*, *Skylab*, and *STS* suggest that the energy costs of vigorous exercise, such as in extravehicular activities (EVA), are consistent with those associated with comparable physical activity on Earth. An assessment of fat mass by different methods indicated that in three *Skylab* flights, fat loss contributed to 45% of total body weight loss, and lean mass up to 55%.¹⁹

Carbohydrates

The main advantage of a high dietary carbohydrate content is that it permits the limitation of (saturated) fat intake.²⁰ Carbohydrates are not indispensable in the sense that essential amino acids or fatty acids are. However, digestible carbohydrates are the most important source of food energy, supplying 17 kJ/gram, while indigestible polysaccharides contribute to dietary fiber intake. Brain and red blood cells, which depend almost entirely on carbohydrates for energy metabolism, may consume 180 g of glucose per day. Most other tissues (e.g., muscle cells) also use glucose as an energy fuel. Typically, 50 to 55% of dietary energy is provided by carbohydrates. This corresponds to 5 to 6 MJ metabolizable energy from 300 to 350 g of digestible carbohydrate. A minimum of about 2 g/kg body weight is required to prevent ketosis.

The metabolizable carbohydrates can easily be converted into glucose, whereas gluconeogenesis from fats or proteins is limited to the glycerol parts of fats and the glucogenic amino acids. Vegetable food is the main source of carbohydrate energy and the only source of dietary fiber. Foods which are rich in starch also contain dietary fiber and a variety of essential nutrients. Consumption of fiber is desirable from a long-term preventive medical point of view, because it increases stool mass, decreases turnover time of bowel contents,²¹ and binds potential carcinogenic compounds. On the other hand, it binds essential nutrients and hence decreases their bioavailability. Further, fiber is subject to bacterial decomposition in the large intestine and gives rise to flatulence. Therefore, dietary fiber in space food requires careful consideration.

Dietary fiber²² is composed of cell wall constituents, cellulose, hemicellulose, pectin, and lignin. Cellulose gives a dehydrated stool, while pectin may increase the lubricant properties of stool. The effect of fiber on stool weight may be due to its water-holding capacity, as fibers resist mucosal absorption of water, or it may be an indirect due to bacterial growth or increase in metabolites (e.g., volatile fatty acids).

In astronauts, carbohydrate consumption was higher inflight (averaging 400 g per day) than under control conditions (350 g per day), whereas crude fiber intake was the same inflight as on the ground (5 to 10 g per day). During the *Gemini*, *Apollo*, and *Skylab* missions, an inflight decrease in glucose tolerance was observed.²³ During the first *Shuttle* missions, postflight blood glucose increased more

than 20%, with great differences between individuals. Data should, therefore, be considered on an individual basis rather than as mean values in pertinent future investigations.

B. Minerals

Sodium

The estimated safe and adequate daily dietary intake of sodium is 1.1 to 3.3 g in adults.²⁴ Even a several times higher daily intake is well-tolerated, since excess sodium is secreted by the kidneys. Intake of sodium may be assessed by urinary sodium output. As an average figure in nine *Skylab* crew members, there was a net loss of approximately 100 mM of sodium (corresponding to 0.4% of the overall body stores) from the extracellular space, stabilizing at a lower level after the initial inflight disturbance.^{25,26} It was concluded from these data that homeostatic adjustments, probably mediated by the atrial natriuretic peptide of the heart, establish a new adapted steady state in the fluid-electrolyte and cardiovascular regulatory systems after transition to weightlessness. It remains to be determined whether salt supplementation, alone or in combination with other procedures, is a suitable countermeasure against cardiovascular deconditioning after reentry.

Potassium

Daily potassium intake ranges between 3 and 11 g per person. A daily minimum need of 1 g can be reasonably assumed, with estimates ranging from 0.8 to 3 g per day. With the usual diet, this amount is exceeded in any case, and there is no danger of an insufficient supply of potassium. Evidence from *Gemini 7*,²⁷ *Apollo 15-17*,²⁵ and *Skylab*,²³ shows that body potassium stores steadily decrease during space-flights for periods exceeding one month in duration. A loss of approximately 240 mM in 30 days, representing a 0.7% decrease of the body stores was found,¹⁹ despite adequate potassium ingestion throughout these missions.

Calcium

In the human body, calcium constitutes 1.5 to 2% of the total body mass. More than 99% of it is present in bones, where the ratio to phosphorus is nearly constant and somewhat greater than 2:1. The calcium content of an average adult male amounts to some 1200 grams, while the total phosphorus content is about 700 grams. About 85% of body phosphorus is in bone in the form of calcium phosphate and hydroxyapatite. The remainder is in cells and extracellular fluid in the form of organic phosphoric acid esters, phosphoproteins, phospholipids, and phosphate ions.

In the usual diet, the phosphorus content is much higher than that of calcium

because phosphorus is naturally more abundant in most of the major foodstuffs. Meats, poultry, and fish supply 15 to 20 times more phosphorus than calcium, whereas eggs, grains, and nuts provide at least twice as much. Only milk, most unprocessed cheeses, and many green leafy vegetables contain more calcium than phosphorus. The recommended dietary Ca/P ratio of 1:1 is almost impossible to achieve, especially in diets high in protein.

Purified proteins and amino acid mixtures, as they are used in experimental diets, lead to calcium loss in humans.²⁸ This could theoretically exaggerate the problem of the negative calcium balance during long periods of microgravity. There is a large body of evidence indicating calcium loss induced by a high protein diet²⁹⁻³³ as may be desirable for space menus. Commonly used complex dietary proteins do not show these effects in strictly controlled long-term human studies,³⁴ in which large amounts of dietary protein with a high phosphorus content are used. A balanced intake of protein, calcium, and phosphorus³⁵ may therefore be desirable for space travellers. There is clearly an urgent need for further investigation in this area.

There is controversy how much calcium is needed for maintaining body stores, particularly bone mass. On the basis of calcium balance studies conducted with groups of individuals accustomed to adequate intakes of foods high in calcium, the daily allowance is 800 mg for individuals 18 years of age and older.³⁶ Absorbility of calcium from food sources is not influenced by solubility, but is mainly determined by other food components³⁷ and hormonal influences, and is decreased by stress and immobilization (Fig. 1).

Vitamin D increases the gastrointestinal calcium absorption,³⁸ underlining the significance of sufficient vitamin D intake in astronauts. The 0.8 g per day given to the *Shuttle* astronauts may be insufficient. It could be increased to 1.2 to 1.5 g per day by means of supplements or calcium-rich foods; this is a level beyond which additional calcium is usually not absorbed. However, this may raise concerns about hypercalciuria and possible renal stone formation. Experimental studies on the requirements of calcium, phosphorus, and vitamin D in relation to hormonal reactions (e.g., calcitonin) and bone metabolism could be useful; in such studies phosphate intake would have to be standardized in order to avoid possible effects on the sodium and zinc stores. If calcium supplementation were provided on a continuous basis (e.g. starting 2 weeks prior to flight) urinary and fecal excretion studies would show which percentage of dietary calcium is retained by the body under circumstances of spaceflight.

Because of the complex control of phosphate homeostasis, hyper- or hypophosphatemia does not occur in healthy humans.³⁹ The phosphorus intake of most diets is considerably in excess of requirements. There are data which indicate that phosphorus supplements, which significantly lower the Ca/P ratio, have beneficial effects on calcium retention and bone health.^{32,33,40} Phosphorus enhances calcium retention by reducing urinary calcium excretion. There is also an influence of protein consumption on calcium (and zinc) excretion: Elevating the dietary protein

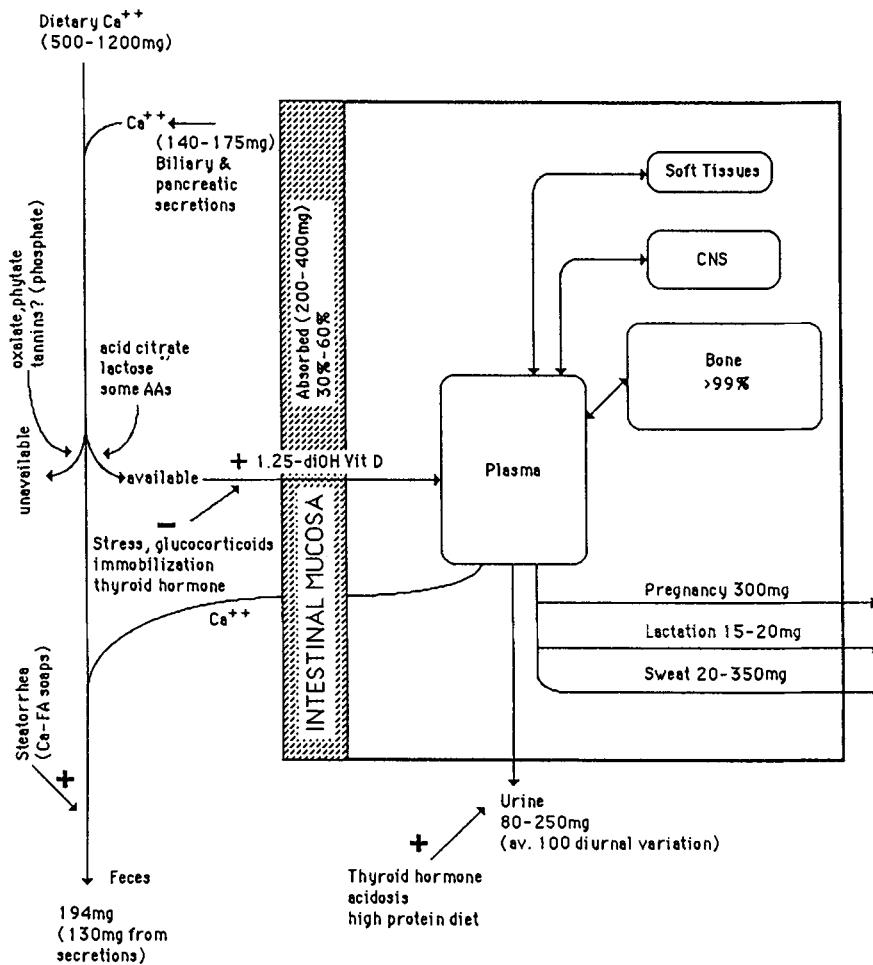


Figure 1. Calcium turnover. More than 99% of body stores are in bone, which is the major source for regulatory shifts due to homeostasis of extracellular free calcium concentration.

supply from 65 to 94 g per day results in a slight but significant increase of urinary Ca and Zn excretion.⁴¹

In weightlessness, or with a reduced gravitational load, there is decreased mechanical stress in the trabeculae of the lower body's bones. This is not balanced by an increased mechanical demand in the upper parts of the body. Despite some net shift from the legs to the bones of the skull, neck, and arms, the calcium content in an astronaut's body decreases continually even after several months inflight. There is a negative calcium balance, due to an increased urinary output which is

not balanced by an equivalent calcium resorption from the intestine. This negative calcium balance cannot be compensated by increasing the dietary calcium supply. The blood plasma calcium concentration is significantly elevated inflight, from 9.5 to 10.2 mg per day.¹⁹ The urinary calcium excretion is increased about twofold after several weeks mission duration. The renal excretion of phosphate was found increased in astronauts by some 20%, due to the continuing loss of bone mass.

Both in simulation studies and during prolonged spaceflight, the renal loss of calcium and potassium reaches a maximum by the second month, most probably caused by a reduced capacity of tissues to retain electrolytes. This phenomenon of a diminished mineral pool can be reduced but not completely prevented by means of countermeasures, like exercise, LBNP, and salt-water supplement.^{42,43} It is an open question whether the gravity levels on Mars (0.4 G) and Moon (0.16 G) are sufficient to prevent bone loss and whether supplements in calcium, phosphorus, vitamin D, fluoride, or hormones, in addition to exercise countermeasures, will be needed during long-term stay on an extraterrestrial base.

Magnesium

Sixty percent of the magnesium in the body is located in the skeleton. The rest is mainly within the cells of soft tissues, where more than 300 enzymatic systems are magnesium-dependent, such as ATP-dependent enzyme reactions.^{44,45} Magnesium plays a key role in neuromuscular transmission and activity. It is essential for the normal metabolism of calcium and potassium in adult humans, and for the mobilization of calcium from bone.⁴⁶ There is a complex relationship between magnesium, calcium, and parathyroid hormone.

The intake of magnesium varies widely, as its content in different foods does. The average intake in healthy Western individuals probably ranges between 180 to 480 mg per day, while the RDA is 300 to 350 mg per day for adults.⁴⁷ Two-thirds of the ingested magnesium is excreted in the stool. Absorption is influenced by intestinal transit time, rate of water absorption, and the amounts of calcium, phosphate, and lactose in the diet.

Magnesium is widely distributed in foods. It is the mineral ion of chlorophyll; hence, green vegetables are an important source. Rich in magnesium are cereals and breads, chocolate, potatoes, and potato products. Magnesium deficiency can occur with malabsorptive gastrointestinal disorders, renal tubular dysfunction, and inadequate intake as in protein-calorie malnutrition.⁴⁸ A diminished extracellular magnesium concentration may lead to dysfunction of excitable tissues within the neuromuscular and cardiovascular systems such as tetany and neurological disorders.⁴⁹ After profuse sweat loss, hypomagnesemia and a tendency to develop muscular cramps may occur.

The following is an assessment of the magnesium status: (1) serum magnesium indicates the extracellular magnesium status, but not total body magnesium; (2) peripheral mononuclear blood cell magnesium seems to be a good indicator for

total body stores.⁵⁰; (3) when the magnesium intake is either excessively high or deficient, parallel changes occur in the urinary magnesium output⁴⁹, and (4) some decrease in serum magnesium has been reported postflight, but evidence of significant inflight magnesium changes seems to be lacking.

C. Trace Elements

Many trace elements are known to be essential for life, health, and reproduction and have well established functions, serving as cofactors in enzyme reactions, components in body fluids (electrolytes), sites for binding of oxygen (in transport), and structural components of nonenzymatic macromolecules.⁴⁴ There is evidence that some trace elements formerly not considered essential are also needed for normal metabolism.

Identification of inadequate trace element nutrition requires accurate methods of assessment. Trace element status usually cannot be reliably determined by means of a single parameter, and biochemical indices must frequently be combined with functional tests. The number of indices that are presently available to assess accurately the status of most trace elements is limited.⁵¹

Absorbed trace elements usually circulate in the bloodstream as protein-bound complexes which are not always in free equilibrium with tissue stores. Circulating levels may not reflect the status of the element available for nutritional needs. Tissue stores of a trace element may not be available to meet the needs during deficient supply because the element may be bound to enzyme proteins from which it cannot be mobilized. Thus, a relative deficiency may occur in anabolic or growing tissues; e.g., a positive zinc balance may exist during increased protein synthesis, but the plasma level falls and deficiency results in spite of nutritional support.⁵² On the other hand, even with a negative zinc balance, deficiency does not occur in catabolic states because of a net outflow from the cells.⁵³

The action of trace elements depends on the metabolic status (anabolic vs. catabolic state, age, gender) and nutritional factors (availability of agonists and antagonists). The existence of a deficiency cannot be deduced simply from the plasma level of the element. Subclinical functional changes must therefore be taken into consideration as a way of defining needs. Trace elements play an essential role in immunity⁵⁴ and therefore need attention also from the point of view of a possible decrease in defense capabilities in conditions of relative immobilization, isolation, weightlessness, or altered gravity levels.

Iron

Iron deficiency is one of the most common deficiency diseases in the world. The most obvious result is hypochromic, normocytic anemia, which is more common in women because of iron losses in menstruation, pregnancy, partuition, and

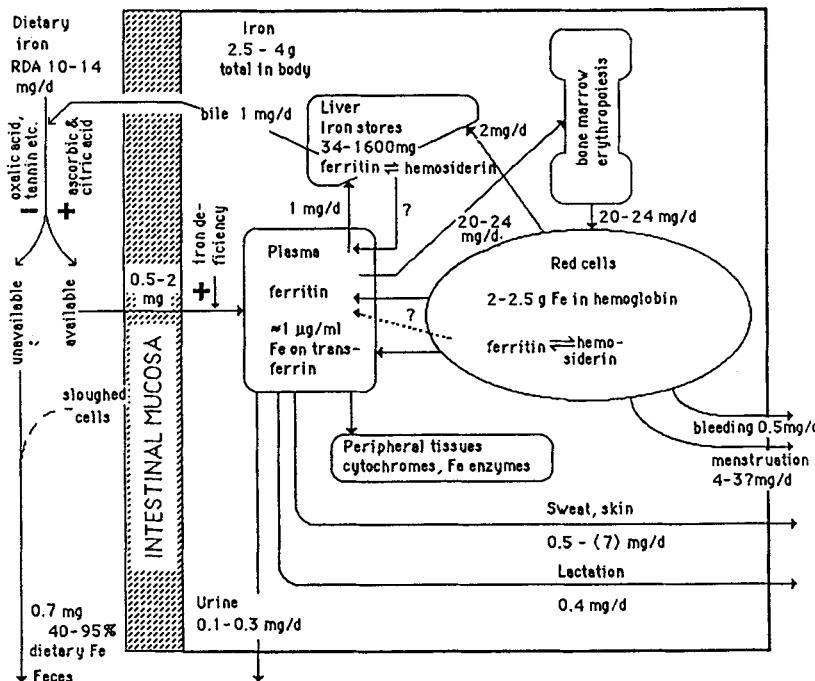


Figure 2. Iron turnover. Most body iron is in hemoglobin of red cells. From nutritional sources, 1 to 20% iron is absorbed by the gut, depending on the chemical environment of dietary iron.

lactation.⁵⁵ Iron is an essential trace element for human beings because of its function in oxygen transport (hemoglobin) and in catalytic processes.⁵⁶

The normal iron content of the human body amounts to about 2 to 5 g. Most of the body iron exists in bound form, to hemoglobin, myoglobin, and the nonheme protein transferrin for transport, ferritin for storage, and hemosiderin.⁵⁵ The body normally maintains an iron balance, with the development of iron deficiency prevented by continuous reutilization within the body, storage in the form of ferritin, and regulation of the absorption of iron affected by actual needs (Fig. 2).

The basal physiologic loss of iron from the body is ≤ 1 mg per day in men. In females, menstrual losses, amounting to 0.5 to 0.8 mg per day, must be added to the basal loss. Up to 0.7 mg per day are excreted in feces,⁵⁷ 0.2 mg in urine,⁵⁸ and significant amounts via the skin during profuse sweating⁵⁹ (Fig. 2). By modifying the rate of gastrointestinal absorption, the needs of the body for iron can be adapted in a wide range.⁶⁰ Iron deficiency, hypoxia, and anemia increase iron absorption.

Two kinds of iron in the diet must be distinguished with respect to the mechanism of absorption: heme iron and non-heme iron. Heme iron (from red meat) is absorbed

very easily,^{61,62} the availability of this form of iron cannot be judged from studies with elemental iron.⁶³ Non-heme iron is probably taken up in ionic form by receptors or by a transport protein on the luminal surface of intestinal cells. The absorption of non-heme iron is markedly affected by the iron status of the subject, e.g., more is absorbed in iron deficiency. It is also affected by many dietary factors: meat, fish, and ascorbic acid enhance iron uptake, while phytates, fiber, polyphenols, and tannins inhibit it⁶⁴ (Fig. 2). Thus, tea and coffee can inhibit iron absorption. High intakes of cobalt, zinc, cadmium, copper, and manganese also interfere with iron absorption, presumably through competition for binding sites. Iron deficiency reduces copper absorption, just as copper deficiency reduces iron absorption.⁶⁵

Healthy males require about 1 mg of absorbed iron per day; menstruating females about 2 mg per day. The usual diet contains a daily total of 10 to 20 mg iron (approximately 15 mg/10 MJ); the absorption of heme iron from meat is about 20%. However, when large amounts of heme iron are given in a meal, absorption decreases to about 3%. Despite the high meat consumption, heme iron contributes only 1 to 2 mg per day in the average Western diet. A mixed diet, providing 11 MJ energy per day, provides about 2 to 3 mg bioavailable iron—roughly 2 to 3 times the required minimum for males. In other words, if 10 mg total iron are provided in an average daily combination, there is still a safety margin to meet a 1-mg need for bioavailable iron.

Assessment of the iron status is possible by determining ferritin levels. Ferritin is produced by cells in proportion to the amount of body iron to be stored, so the plasma ferritin level is the best indicator of bodily iron stores.^{66,67} Ferritin levels, however, are unreliably high during inflammation, infection, malignancy, and liver disease.⁶⁸ A serum ferritin concentration of 1 µg/l corresponds to 140 µg/kg body weight of iron stores.⁶³ The average ferritin plasma level in men ranges from 20 to 200 µg/l.⁶⁹

Zinc

Zinc is an ubiquitous essential trace element, of which the human body contains about 1.5 to 2 grams. It is part of about 120 enzymes, such as carbonic anhydrase and superoxide dismutase in red cells.⁷⁰ Zinc is involved in numerous cellular reactions such as carbohydrate and energy metabolism, protein degradation and synthesis, nucleic acid synthesis, heme biosynthesis, and carbon dioxide transport,⁴⁴ especially supporting skin, male reproductive organs, and pancreas. Zinc is necessary for the retinol binding protein and therefore plays an important role in vitamin A metabolism.

A daily intake of 15 mg dietary zinc is recommended. Zinc absorption is between 18 to 30% of the total amount of zinc in the food. Bioavailability is lowered by high fiber and phytate content, calcium, and phosphate (Fig. 3). Mucosal uptake is influenced by other metal ions, e.g., iron and copper compete for binding sites.

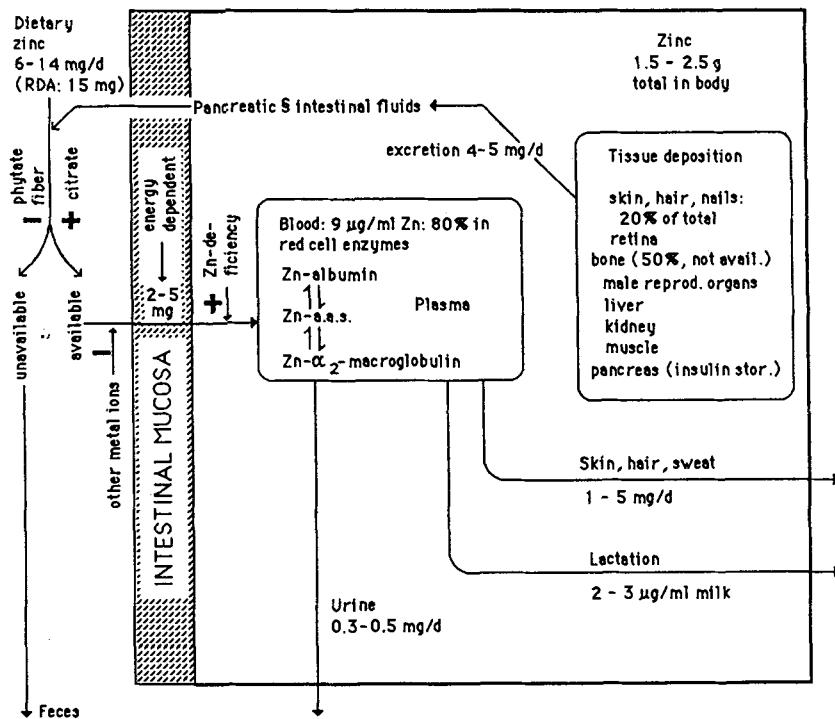


Figure 3. Zinc turnover. Urinary losses are minimal, "zinc status" is not evident from single measurements, the compartmental distribution of zinc is inhomogeneous.

More influence on zinc absorption is probably exerted by another intestinal iron-zinc interaction due to the formation of complex ligands in the food.⁷¹ Animal protein (particularly red meat, liver, and seafood) is rich in zinc, whereas fruits and fats are low in zinc. Whole cereal grains are rich in zinc; nuts and legumes are also good plant sources, but phytate lowers its availability.⁷² Zinc requirements (total and daily) depend on dietary protein intake and phosphorus supply, but this interaction is inconsistent.^{73,74}

The major organ involved in zinc metabolism is the liver. Metallothionein binds several metal ions and has an integral role in the hepatic pathway of zinc utilization.⁷⁵ Fecal zinc losses rise with increased intake due to unabsorbed zinc. Urinary losses are not influenced by intake. Significant losses may occur with profuse sweating, but with growing deficiency such losses diminish.⁷⁶ Zinc deficiency, at least at a marginal level, is not uncommon and may occur because of inadequate intake (malnutrition) or availability, malabsorption, chronic diseases, or increased rates of loss from the body. Zinc deficiency can result in impaired immune function, such as lymphopenia, depressed T-cell responses, and decreased natural killer-cell

activity.^{54,77,78} Other symptoms are delayed wound healing, mood lability, impaired taste and smell, and night blindness.^{44,52,72,79-83}

The assessment of the zinc status, and particularly the detection of a mild zinc deficiency, is very difficult. The significance of zinc concentration in hair or salivary sediment is still unclear.^{84,85} Leukocyte zinc levels may more accurately reflect body zinc stores.^{67,86} Other useful parameters are the activities of erythrocyte copper, zinc superoxide dismutase, and serum alkaline phosphatase.⁷² However, the best way to assess zinc requirements and status is by multiple clinical assessments.

Copper

Copper is part of several intra- and extracellular enzyme systems, such as cytochrome oxidase and superoxide dismutase, which is an important antioxidative protection factor. Copper is bound to albumin and to a higher degree to ceruloplasmin.⁴⁴ Interactions exist between copper and other metals (zinc, iron, cadmium, molybdenum, and manganese) at the level of absorption, transport in the blood, and cellular uptake.⁸⁷

The copper content of food underlies wide variations, because it is influenced by many factors, such as the amount of metals in the soil, climate, and food processing.⁸⁸ Depending upon dietary source and amount, about 1/3 of the copper consumed is absorbed.⁸⁹ Safe and adequate daily dietary intakes of copper for adults are 2 to 3 mg.⁹⁰ The normal diet supplies 2 to 4 mg per day. Copper content in the adult human body seems to be less than 100 mg,⁹¹ and the daily turnover rate is 2% of body stores (Fig. 4). The major route of excretion is the bile by which about 0.5 to 1.3 mg per day is excreted.⁸⁹

Diets high in zinc depress copper availability, and a high zinc-copper dietary ratio is a risk factor for coronary heart disease via the cholesterol metabolism.^{44,88} Copper is needed for the development and function of the central nervous system where its deficiency or excess can cause functional changes.⁸⁷ While clinical copper deficiency is very rare,⁸⁸ experimentally induced copper deficiency decreases organ levels, and causes hypercholesterolemia, hypertriglyceridemia, glucose intolerance, and changes in amine metabolism.⁹²

The assessment of copper status is not simple. The plasma copper level is reduced in deficiency, but is also affected by factors altering the ceruloplasmin level. A more sensitive indicator seems to be the specific activity of ceruloplasmin, as estimated by the ratio of enzymatic to immunoreactive ceruloplasmin.⁹³ Erythrocyte copper, zinc superoxide dismutase activity also may be a good copper status index,⁹⁰ but copper content in hair is not a reliable index of deficiency.^{93a} The significance of saliva copper concentration needs to be further tested.⁹⁴

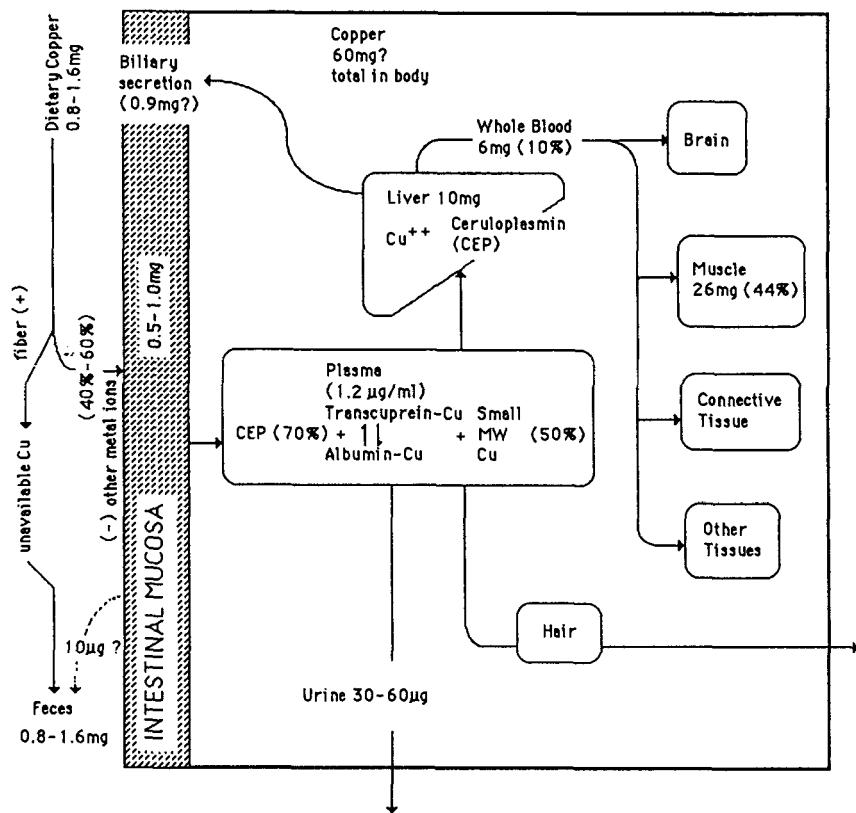


Figure 4. Copper turnover. About half of dietary copper is absorbed. Biliary secretion is the major route of copper loss from the body.

Selenium

Selenium is an essential element for humans.⁹⁵⁻⁹⁷ Its highest concentration is in liver, kidney, pancreas, and cardiac muscle.⁹⁸ The levels are responsive to dietary intake. The selenium level in food depends on the availability of selenium in soil where crops are grown.⁹⁹

The recommended daily allowance for selenium is 50 to 200 µg for adults. Dietary selenium intake in Western countries is in the range of about 60 to 220 µg. Seafood, organ, and muscle meats are generally good sources of selenium; grains and cereals have various contents; and fruits and vegetables have very low levels.¹⁰⁰ Intestinal absorption is quite efficient¹⁰¹ (Fig. 5). Selenium is excreted to about 40% in urine and to about 60% in the feces.¹⁰²

Selenium is part of glutathione peroxidase (GPx) which destroys potentially harmful oxygen radicals (peroxides) before they can damage cell membranes. GPx

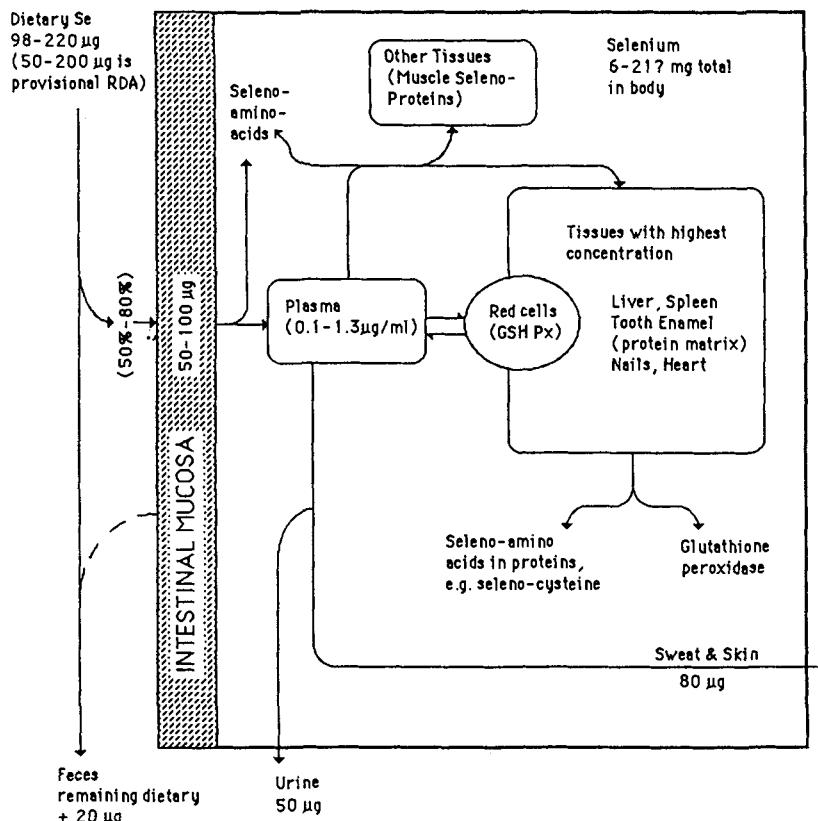


Figure 5. Selenium turnover. Selenium provides membrane protection since it is required for function of glutathione peroxidase (GSH Px).

complements vitamin E, which is another antioxidant membrane protection factor. Selenium and vitamin E are interrelated in their actions, and the deficiency of one can be partially corrected by the other. Severe selenium deficiency causes diseases of the cardiovascular, neuromuscular, immunological, and other systems in humans,^{96,97} whereas selenium supplementation stimulates the immune system on a broad basis.¹⁰³

Assessment of the selenium status is possible in several ways. Selenium concentration in whole blood and plasma,¹⁰⁴ or in erythrocytes, platelets and leukocytes,¹⁰⁵ can be measured with atomic absorption spectrometry. Plasma and platelet GPx is sensitive to selenium intake and can be used to assess selenium needs.¹⁰⁶ A good correlation is also found between the GPx activity in red blood cells and serum selenium values.¹⁰⁷ Selenium intake can be assessed by measuring urinary excre-

tion.¹⁰⁸ Turnover studies will certainly be needed to assess the selenium status during future long-term space missions.

Cobalt

Cobalt is part of the vitamin B₁₂ molecule, and cobalt homeostasis can be achieved in humans by a sufficient supply of this vitamin. Vitamin B₁₂ has important stimulating effects on erythropoiesis.⁴⁴ Inorganic cobalt is probably released from vitamin B₁₂ during its metabolism. Most of the cobalt absorbed is usually not in the form of vitamin B₁₂.¹⁰⁹ The average intake in Western diets is about 5 to 10 µg per day, mainly from vegetables and whole grains.⁴⁴ The daily intake usually exceeds this value considerably. Average whole body content is about 1.1 mg. Cobalt intake levels of 20 to 30 mg per day can produce toxic manifestations such as thyroid hyperplasia, myxedema and congestive heart failure.¹¹⁰ No information appears to exist on cobalt metabolism in spaceflight.

Fluoride

Fluoride is widely distributed in human diets. Intake ranges between 0.3 and 4 mg per day, and 50 to 80% of fluoride in human diets is absorbed¹¹¹ (Fig. 6). Fluoride is required for proper functioning of bone and may be a candidate for possible countermeasures against decalcification during spaceflight.

There are recent studies dealing with the effects of long-term ingestion of therapeutic fluoride doses on bone metabolism. The net result shows increased skeletal mass and decreased negative calcium balance.¹¹² There are conflicting results on dosage and duration of the supplementation with or without combination with calcium administration.¹¹³ Further investigations are needed to define optimal fluoridation in astronauts because no countermeasure yet applied was sufficient to prevent bone loss induced by weightlessness. Careful dosage is essential because a chronic fluoride load exceeding the demand by a factor of 10 or more results in toxicity symptoms.

Manganese

Manganese is part of many enzymes involved in carbohydrate and lipid metabolism, and in brain function. The human body contains 12 to 20 mg of manganese, mainly concentrated in the mitochondria.¹¹⁴ The dietary requirements are unknown, and there is no clear evidence for the existence of manganese deficiency in humans. However, a "safe and adequate" intake level of 2.5 to 5 mg per day has been estimated.¹¹⁵ Only 3 to 4% of an oral dose of manganese is absorbed,¹¹⁶ so that the amount retained is about 50 to 400 µg per day.¹¹⁷ Absorption of manganese is enhanced with low intakes and depressed when intake is high. Sources of dietary manganese are mainly plant foods (particularly tea), while animal tissues contain

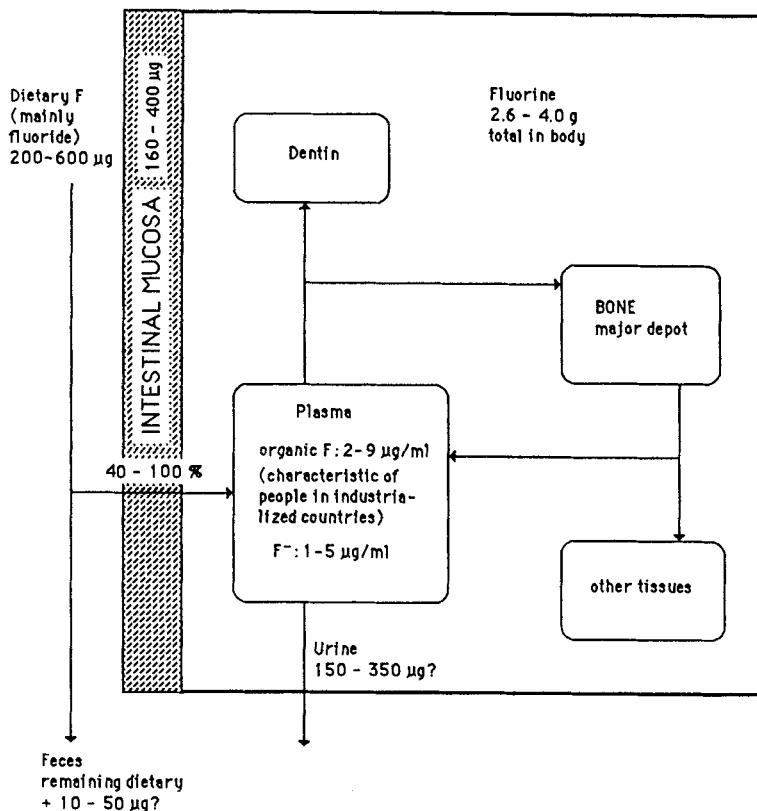


Figure 6. Fluoride turnover. Important for bone mineralization and dentin integrity, fluoride is readily absorbed by the gut, stored in bone and other tissue, and mainly excreted by the kidneys.

very low amounts.¹¹⁸ A diet consisting of green vegetables, nuts, cereals, seeds, tea, and coffee can provide 20 to 25 mg/kg dry diet.¹¹⁵

There is as yet no straightforward assessment of the manganese status. Serum and urinary manganese concentration are not sensitive to dietary alterations,¹¹⁹ but probably reflect the manganese status. The measurement of the activity of manganese-containing superoxide dismutase might provide a more accurate assessment method for the nutritional status of manganese.¹²⁰

Since manganese is linked to central nervous system function, it is conceivable that investigation of the manganese balance in astronauts may be necessary for extended missions. No information on inflight manganese metabolism is available to date.

Chromium

Chromium is an essential ultra-trace element. Its need by humans is apparently influenced by nutritional or physiological stress. The dietary need for chromium is elevated with increased insulin metabolism.¹²¹ Various kinds of stress alter hormone secretion and thus glucose metabolism. Chromium is likely to contribute to glucose tolerance and may enhance insulin-stimulated tissue uptake of leucine.¹²² It is probably absorbed in organic form since inorganic chromium is absorbed to less than 3%,¹²³ whereas organic sources (e.g., from yeast) may yield an absorption of 10 to 25% of an oral dose. In natural sources, chromium exists in a complex form called the glucose tolerance factor.¹²⁴ This organic complex appears to be the form in which chromium becomes available in food.

The recommended daily dietary intake is 50 to 200 µg.¹²⁵ Meat and inner organs, whole grains, nuts, and cheeses supply significant amounts of chromium. Conversely, poultry, fish, and refined cereals contribute little chromium. Existing food tables do not provide sufficient quantitative information on chromium contents.

The assessment of the chromium status is rather complex. Plasma chromium levels are reduced in deficiency but also by acute illness.¹²⁶ Hair chromium declines in situations likely to be associated with deficiency.¹²² At present, the best way to verify chromium deficiency is to demonstrate improved glucose clearance after chromium supplementation.

Iodine

Iodine is essential for the biosynthesis of the thyroid hormones. The nutritional need for iodine depends on growth, body weight, age, and gender. The recommended daily allowance is 2 µg/kg body weight in adults, or about 0.15 mg/day. An intake between a minimum of 0.05 mg and a maximum of 1 mg is considered to be safe. Iodine is concentrated in milk and eggs, which are second only to seafood as the richest sources of iodine. Vegetables and fruits supply little iodine. Meat contains more iodine and its content depends on the food.¹²⁷ Iodine may be supplemented with iodinated sodium chloride if the dietary supply is insufficient.

Other Trace Elements

Silicon,¹²⁸ nickel, tin, and vanadium are "new" essential trace elements, as possibly are the so-called ultra-trace elements lithium, cadmium, lead, arsenic, boron, and bromine.¹²⁹ There are no indications of primary deficiencies of these elements in Europe and the U.S. However, an increasing number of studies have been reported that examined the relationship of specific ultra-trace elements to various forms of nutritional, metabolic, hormonal, or physiological stress in animals.¹³⁰

These studies indicate that there are situations in which some of the ultra-trace

elements are of nutritional significance. Also, a secondary deficiency under the influence of antagonists cannot be excluded. With the advent of closed biological life support systems, it is quite possible that even these ultra-trace elements will gain significant importance¹³¹ and need to be monitored in the supply to edible plants, livestock, and ultimately also humans participating in the "biospheric" loops of such extraterrestrial systems.

D. Vitamins

In contrast to numerous trace elements where there is a significant lack of quantitative knowledge on nutrient needs and metabolism, the role of most vitamins for human health and well-being has been thoroughly investigated. We therefore present only a short overview on significant facts which may have important implications for astronauts.

Vitamin A

Vitamin A (retinol) is essential for vision, growth, epithelial cell differentiation, reproduction, and the integrity of the immune system.^{35,103} Retinol also influences calcium metabolism and calcification.¹³² Various carotenoids are biologically active as vitamin A; the main sources in the diet are provitamin A carotenoids from vegetables and retinyl esters from animal tissues. Retinyl esters are converted to retinol in the intestinal lumen; carotenoids are partially converted to retinol in the enterocytes.

Vitamin A is best supplied by carrots, cabbage, milk and dairy products, eggs, and liver (which is the main site for vitamin A storage). Deficiency is heralded by night blindness, and may lead to an abnormal perception of color. It also leads to increased susceptibility to infections, metaplasia, and keratinization of epithelial cells.¹³³

The recommended daily allowance is 1 mg of retinol equivalent in adults.¹³³⁻¹³⁵ Vitamin A toxicity can occur at 10-fold higher doses taken for months. The syndrome usually presents itself as a pseudo brain tumor. In U.S. space missions, vitamin A intake exceeded the recommended daily allowance by up to 200%.^{18,136} For the *STS Orbital Flight Tests (OFT)* a standard menu was provided which contains 5000 IU (3 mg) vitamin A per day.⁷

Vitamin D

Vitamin D and its various metabolites have a central position in the regulation of calcium and phosphorus metabolism and, hence, bone metabolism. Vitamin D stimulates active transport of calcium across mucosal cells of the small intestine. It is formed in the skin by means of ultraviolet radiation, which path may provide an adequate supply for the body's needs. It is also absorbed through the gastroin-

testinal tract. The bioactive form of vitamin D, 1,25-(OH₂)-D₃, is produced in the kidney under the influence of parathyroid hormone.

Milk is usually fortified since average food is poor in vitamin D. Cod liver, fish oils, and egg yolk are very high in vitamin D. The recommended daily allowance is 5 to 10 µg per day in adults.¹³⁷ This figure is disputed and may be considerably lower, if the person receives significant amounts of UV radiation. Doses greater than 10 times the recommended daily allowance can be toxic and lead to hypercalcemia. Hypovitaminosis, resulting either from insufficient vitamin production and dietary lack or from malabsorption of fats, leads to osteomalacia.

Vitamin D₂ was provided at the full recommended daily allowance in U.S. space foods by multivitamin supplements. This was considered advisable considering the inflight absence of UV light. It is a matter of discussion which lighting system will be optimally suited in future space habitats in order to meet all biological needs. Unless there is full-spectrum light or quartz windows to permit penetration of solar UV rays, it is likely that there is insufficient endogenous vitamin D production.

Vitamin D is of special significance in the space environment because it is a primary regulator of calcium absorption. The amount of vitamin D required to alleviate insufficiency needs to be determined. At the same time, toxic levels should be considered. A safe range of exogenous vitamin D intake on long-term missions has not yet been established.

Vitamin E

Vitamin E (tocopherols) serves as an antioxidant, preventing (per)oxidation of polyunsaturated fatty acids in cell membranes. It also may play a role in female and male reproductive function; as an anticarcinogenic factor,^{44,138} and as an erythropoietic factor.¹³⁹ It is also thought to slow down aging processes.⁴⁴ There are unclear interactions of vitamin E with vitamin C, vitamin B₁₂, and zinc which may have important consequences for a balanced action on the antioxidant level of cell metabolism.¹⁴⁰

Deficiency causes organ degenerations and cataract. The recommended daily allowance of vitamin E is 10 to 20 mg tocopherol equivalent, but this depends also on the ratio of vitamin E to polyunsaturated fatty acids. A ratio of 0.4 or more is considered desirable.¹⁴¹ Requirements vary with the selenium supply.¹⁴² Tocopherols are found in plant oils, especially those with polyunsaturated fatty acids, and grains. Processing of wheat to ordinary flour removes most of the vitamin. With the exception of liver, animal products are poor sources of vitamin E.

Vitamin E status can be assessed by the levels in circulation and functionally by peroxide-induced red cell hemolysis¹⁴³ which is increased in deficiency. Due to the protective action of this vitamin on cell membranes, an adequate supply in space foods may be particularly important. In the *Shuttle* OFT program, 15 IU vitamin E (15 mg tocopherol equivalent) per day is provided by the standard menu.⁷

Vitamin K

Vitamin K (phyllochinone) is necessary for hepatic synthesis of several blood clotting factors. It is not readily stored within the body, and therefore must continually be provided. Vitamin K is widely distributed in green leafy vegetables (spinach) and animal foods. An appreciable quantity of the human requirement is probably obtained from its synthesis by intestinal bacteria. The requirement for vitamin K is about 1 mg/kg body weight or 70 mg per day.^{144,145} Like other fat-soluble vitamins (A, D, E), the uptake of phyllochinone is jeopardized by impaired fat absorption.

Vitamin C

Vitamin C (ascorbic acid) is a general antioxidant that affects the body's "redox potential". It is involved in hydroxylation reactions, procollagen synthesis, and transport/absorption of iron.^{146,147} Vitamin C enhances the response of neutrophils to chemotactic stimuli,⁴⁴ and also may exert other positive effects on the immune system.¹⁰³

"Wild" plants are rich in vitamin C: citrus fruits, collard greens, blackcurrant, broccoli, spinach, horseradish, pickled cabbage, tomatoes, and potatoes.¹⁴⁸ Ascorbate is very rapidly and efficiently absorbed from the diet. However, the actual dietary intake may be considerably lower than the amount originally present in the ingested food because of its destruction by heat and oxygen, and its loss in cooking water.¹⁴⁹ Vitamin C deficiency may occur upon steady consumption of an extremely unbalanced diet. This results in scurvy, which is characterized by weakness, depression, impaired wound healing, and spontaneous hemorrhages. Scurvy can be prevented by 10 mg ascorbic acid per day. However, in order to attain better tissue saturation, the recommended daily allowance is 60 mg per day in adults.¹⁴⁹ Noninvasive screening of vitamin C status may be done by measuring ascorbic acid in buccal cells.^{149a}

Ascorbic acid is nontoxic, even in very high dosage. Significantly higher intake, up to 100 times the recommended daily allowance, provides increased immunological defense capacity and therefore may be useful as a countermeasure to possibly decreased immunological capability in astronauts during flight. This option needs further discussion. Vitamin C has been present in U.S. space menus at almost 10 times the recommended daily allowance, in view of findings of low blood concentrations of vitamin C after various types of stress.

Vitamin B₁

Vitamin B₁ (thiamin) is involved in enzymatic energy production. It is found in cereals and cereal brans, green vegetables, fish, fruit, and milk. Meats and legumes contain limited amounts of thiamin. Refined foods (polished rice, sugar) and fat are

poor sources of thiamin. The current recommended daily allowance is 0.5 mg per 1000 kcal, or about 1.3 mg per day.¹⁵⁰

There is concern about persisting borderline thiamin nutritional status even in the developed countries. It is unclear whether psychological and neurological manifestations may accompany thiamin deficiency in the subclinical range.¹⁵¹ It is obvious that an adequate thiamin supply is essential in any space diet. This is even true for short-term missions since the half-life of vitamin B₁ is relatively short. The STS OFT standard menu provides 1.4 mg thiamin per day.⁷ Thiamin status is assessed by measuring renal vitamin excretion and the transketolase activity in erythrocytes. Thiamin metabolism will require close monitoring during any long-term missions.

Vitamin B₂

Vitamin B₂ (riboflavin) is an enzyme cofactor that is involved in many metabolic reactions in the organism, such as oxidation of glucose and fatty acid for ATP production. Major sources of riboflavin are meat and meat products, beer, milk, and dairy products. Additional sources are some vegetables (asparagus, broccoli, spinach) and fish. Riboflavin is heat stable, but exposure to light decreases riboflavin content.

The recommended daily allowance for riboflavin ranges from 1.2 to 1.6 mg per day.¹⁵² Daily intake should possibly be related to energy expenditure. Various physiological factors influence riboflavin needs. Bile salts enhance riboflavin absorption. Dietary deficiency of riboflavin occurs with daily intakes below 0.6 mg¹⁵³ and is generally observed in association with deficiencies of other vitamins. Riboflavin content in the standard meals provided for OFT astronauts was 1.6 mg per day.⁷

Niacin (Nicotinic Acid)

The term "niacin" is used as the generic name for pyridine-3-carboxylic acid and its derivatives exhibiting qualitatively the same biological activity as nicotinamide. Niacin is a coenzyme of electron carrier enzymes, and is involved in many biochemical processes. Nicotinic acid and its amide are readily absorbed in the intestine. Niacin is found in whole grains (germs), seeds, nuts, and high protein foods. The amino acid tryptophan can be used as a source for metabolic conversion to niacin. As a nutritional surrogate, 60 mg of tryptophan is equivalent to 1 mg of niacin, since about 1/60 of the tryptophan in the diet is converted to nicotinic acid and nicotinamide.⁴⁴ An imbalance in the intake of essential amino acids can result in niacin deficiency. The requirement for nicotinic acid seems to be about 4 niacin equivalents per 1000 kcal; the recommended daily allowance is 6.6 niacin equivalents per 1000 kcal, amounting to about 18 mg per day.¹⁵⁴

Niacin deficiency symptoms are weakness, indigestion, and loss of appetite. The

classical symptoms of pellagra (dermatitis, diarrhea, and dementia) develop with a lasting niacin deficit. We are not aware of published data on the niacin balance in astronauts. U.S. standard space meals contain exactly the suggested recommended daily allowance of 18 mg per day.⁷

Vitamin B₆

Vitamin B₆ (pyridoxine) is a major cofactor for amino acid metabolism. Its bioavailability is affected by food processing, fiber type, and content.^{155,156} The best sources of vitamin B₆ are liver, whole grain, potatoes, and nuts.¹⁵⁷ The western diet often does not meet the need because the high protein content and wide use of refined cereals may result in a marginal B₆ deficiency.

Daily requirement is 2.0 to 2.2 mg vitamin B₆ for basal and also for stress conditions.^{44,158} Deficiency is indicated by an increased urinary excretion of metabolites of tryptophan, methionine, and glycine. Vitamin B₆ deficiency is also associated with impaired collagen maturation,¹⁵⁹ atherosclerosis,¹⁶⁰ glucose intolerance, and glucose/glycogen metabolism.⁴⁴ The OFT standard menu provides 2 mg per day of vitamin B₆ to the *Shuttle* astronauts.⁷

Vitamin B₁₂

Vitamin B₁₂ (cobalamin) is required for normal hematopoiesis and functioning of the brain. It is not found in plant and vegetable foods, and is lacking in the diets of vegetarians. Cobalamin is derived ultimately from bacteria.^{161,162} It is absorbed in the small intestine by B₁₂ receptors. Human colon bacteria make large amounts of vitamin B₁₂ which, however, is not absorbed by the mucosa of the large intestine. Cobalamin absorption depends on the presence of the intrinsic factor and R-proteins in gastric juice. The average adult requirement for vitamin B₁₂ is about 1 µg per day; the recommended daily allowance for adults is 3 µg per day.¹⁶³ The hepatic body stores, if adequately filled, have a supply capacity for up to 3 months.

In cases of inadequate B₁₂ nutrition, deficiency symptoms can occur in the long run. A typical symptom is macrocytic anemia, initially indicated by pallor, weakness, dyspnea upon exertion, neuropathy, paraesthesia, and a sore tongue. Severe deficiency causes diffuse and progressive loss of myelin from the nerve sheath, and consequently severe neurological disorders.¹⁶⁴ Unless otherwise fortified, space diets lacking adequate amounts of animal products like meat, liver, milk, fish, and eggs would therefore provide dangerously low vitamin B₁₂.

The *Shuttle* OFT standard menu provides 3 µg cobalamin per day,⁷ in agreement with the recommended daily allowance guidelines. In long-duration missions, cobalamin will have to be supplied by self-contained biological life support systems in order to avoid symptoms of deficiency.

Folacin

Folacin is a generic term used to describe folic acid and related compounds which exhibit the biological activity of folic acid. The group of compounds having a specific chemical structure are called "folates." Folate is needed for a variety of biological reactions, including DNA synthesis.¹⁶⁵ There is a cooperative activity of vitamin B₁₂ and the folates in terms of erythropoiesis. This means that the vitamin B₁₂ status must be assessed in combination with the folate status. The bioavailability of folacin in foods varies from 25 to 95% (mean: 50%). The minimum daily requirement for folacin is 3 µg/kg body weight.¹⁶⁶ There is an inherent variability in the folate content of foods, because it is sensitive to cooking and storage. Orange juice, whole grains, dried beans, raw plant food, liver, and yeast are good sources of folacin.

Macrocytic anemia is one of the early consequences of folate deficiency. Interrelationships of the B-complex vitamins are essential in the performance of metabolic and catabolic reactions in the body; thus B-vitamins have generally been supplied at a high level in U.S. space menus.¹⁸ The *Shuttle* OFT standard menu supplies 400 µg per day of folate⁷ which is identical to the recommended daily allowance.

The 10 to 15% decrease in the red cell mass of astronauts prompted the recommendation of a folic acid supply of double this amount. It has not been settled whether any higher folate level in astronaut diets would be effective in boosting red cell synthesis, and if so, whether an increased erythropoiesis would be beneficial in terms of cardiovascular adaptation to weightlessness. It might well be that on the level of blood viscosity and microcirculatory exchange, the adapted state of "space anemia" provides more advantage than a "normal" red cell mass vis-a-vis reduced extracellular fluid volume.

E. Availability and Utilization

Even with relatively great mass shifts within the body, regulatory mechanisms are designed in a way that only minor amounts have to be exchanged with the environment. For example, more than 100 mg per day iron are retrieved within the body for de novo synthesis of hemoglobin after heme destruction, but only 1% of this amount needs to be supplied by the diet. However, although spaceflight diets meet the criteria of conventional food composition requirements, marginal deficiency states might still arise as a result of inadequate intake, or because the conditions of spaceflight place higher demands on certain nutrients.^{136,167}

Micronutrients are absorbed by the intestine to a very different extent (1–100%) of what is present in the diet. Dietary input of any nutrient may considerably exceed the fraction actually "bioavailable" to the body. The efficiency of absorption mechanisms is fine-tuned in accordance to the needs of the body and its metabolic status. Bioavailability also depends on the presence of other substances in the food.

Physiological stress factors, such as motion sickness, nervousness, and anxiety may interfere with the mechanisms of digestion, absorption, and metabolism. Eating while agitated, fatigued, or worried may give rise to gastrointestinal disturbances. Nutrient utilization depends upon a great number of psychological and neuro-hormonal control mechanisms. These adjust and correlate the activities of the various systems within the body, adapting them to the changing demands of the external and internal environment.

Digestion is a series of physical and chemical events by which nutrients are altered in preparation for absorption. This takes place in the mouth, pharynx, esophagus, stomach, and small and large intestines. Bile salts emulsify fat, and the pancreatic juice contains most of the enzymes needed to break down chemically complex nutrients to absorbable compounds. The main site of absorption is the upper part of the small intestine.

Absorption takes place at the mucous lining of the intestinal tract. The absorbed nutrients enter the portal circulation, the transport route from intestine to liver, or into the systemic bloodstream via the lymphatic vessels. In the liver, various molecules are converted by enzymatic reactions. Some are held in storage by the hepatic cells to be released into the bloodstream as needed; others are used by the liver itself. The remainder is directly released into the circulation where the nutrients are taken up by their target cells and put to work.

Metabolism involves all chemical changes that nutrients undergo from the time they are absorbed until they are lost from the body. There are two general phases that occur simultaneously; anabolism and catabolism. Anabolism allows for construction of the biochemical components and specific products of the cells and tissues of the body, such as hormones, enzymes, and intracellular and extracellular building elements. Catabolism is the sum of reactions in which these compounds are broken down to be excreted. Catabolism also liberates energy in small, usable amounts. During adaptation to weightlessness, catabolic processes occur in the legs, whereas anabolism is stimulated at different locations of the body, for example, in the upper arms. The result is a net mass transfer of minerals and organic compounds within the body.

Similar to exercise or lower body negative pressure, food can be regarded as a countermeasure that deserves consideration in the maintenance of muscular fitness, cardiovascular stability, good health, and high performance.

III. NUTRITIONAL STATUS AND BODY COMPOSITION

Space diets may be designed according to commonly recommended nutrient intake values which were developed for terrestrial use. The actual needs of astronauts may, however, significantly deviate from these general guidelines. The major differences between weightlessness and a 1-G environment, as they influence metabolic needs, are in the cardiovascular, fluid/electrolyte, and musculoskeletal systems.

A. Fluid and Electrolyte Balance

To maintain optimal fluid and electrolyte balance is of particular importance for astronauts and space workers. A large part of space food today comes in dehydrated form, since dehydration cuts down on mass and transportation costs. Dehydration of solid food results in a mass/volume reduction by a factor 3 (meat) to a factor 10 (components extremely rich in water). Eggs may be compacted to about 30% of initial volume, potatoes to about 25% of initial volume (potato chips contain almost 3 times as much protein and carbohydrates as potatoes, and are rich in fat), and fruit from 20 to 25% of initial volume. In future autonomous food systems of the CELSS type, water will be recycled and dehydration will probably not be employed.

The need for potable water depends on the net fluid balance of the body. This balance differs during spaceflight from that on Earth. Relative water loss occurs early inflight and stabilizes within a few days. This may be due to an adaptive response which eliminates part of the fluid present in the body tissues on Earth but which would put an unbalanced volume load on the cardiovascular system in microgravity.¹⁶⁸

The physiology of fluid volumes and electrolyte concentrations during spaceflight is closely connected to the regulation of energy metabolism, biomechanics, bone remodeling, endocrinology, kidney physiology, and cardiovascular functioning. There is still a lack of understanding how the body subsystems interact quantitatively along the time-line of 0-G adaptation and 1-G readaptation processes. States of water loss or gain primarily involve alterations in extracellular fluid volume.¹⁶⁹⁻¹⁷¹ About 60% of the body weight is water. Lean body mass is defined as whole body mass minus adipose tissue in the living organism.¹⁷² Lean body mass contains 75% water, 5% "essential lipids" (e.g., in the brain), and about 20% cell solids.¹⁷³

Fluid exchange between the intravascular and the extravascular parts of the extracellular compartment occurs with a half-time of 10 to 15 minutes; the corresponding value for water exchange across the cell membranes is 10 times as long. The interstitial volume is relatively stable, which is due to several factors.⁹⁴ These are: microvascular self-adjustment of capillary fluid filtration; structural resistance against interstitial volume changes; automatic accommodation of lymph flow; and additional local feedback mechanisms. The interstitial compartment is a major functional link between changes in the physical environment of a free floating body and fluid volume shifts.

Glycoproteins are a basic component of the interstitial ground substance that contains more than 10 liters of water in an adult. The glycoprotein metabolism is subject to diet-induced modifications.¹⁷⁴ The mechanisms of this process are only beginning to be partially understood. The physiological significance of such modifications, particularly under the circumstances of spaceflight, are unknown but presently an exciting field for research in space medicine.

There are basically three factors influencing capillary fluid shift, lymph flow, and the hydration of connective tissue:¹⁷⁵

1. hydrostatic effects due to the lack of gravitational pull on the cardiovascular contents, including reflex, metabolic, and hormonal counterregulations;
2. the absence of gravitational deformation effects acting normally on the tissues; and
3. the altered state of reflexly sustained muscle tone and movement patterns.

It is still an open question whether, or to what extent, interstitial composition is influenced by weightlessness or chronically altered G-levels. Computer model analyses of fluid-electrolyte indices suggest that there is no overall change in interstitial fluid volume. This would indicate that, on the average, tissue gel and lymph flow tend to return the interstitium to the preflight state after initial unloading.

Experimental findings suggest that a combination of low extracellular fluid volume with a reduced sodium concentration appears to promote an appetite for salt. This is the pattern occurring in astronauts, who were provided with salt tablets and fluid intake as a countermeasure to oppose their orthostatic intolerance due to cardiovascular deconditioning upon return to Earth.

Sodium ions are excluded from cells, and therefore, changes in sodium concentration will produce shifts in cellular volume until there are secondary changes in the cell solutes.^{173,176} An important missing link in the regulation of body sodium was found with the discovery of the atrial natriuretic peptides (ANP). Their implementation into mathematical models of extracellular volume control made these models work much more satisfactorily (*Skylab* biomedical analyses).

It came as a surprise that body fluid osmolality is significantly decreased inflight. This is caused by a 3% hyponatremia which remains unresolved and puzzling.²⁶ Extracellular sodium decreases by about 100 mequ during the first 2 days and then remains stable. Natriuretic peptides may contribute to this phenomenon. The adaptive value of this process may be a protective role of extracellular volume at the expense of extracellular osmolality. However, since ANP act on a rather short-term basis, this hypothesis remains to be proven.

Control of vasopressin seems to change during early inflight adaptation.²⁶ A current hypothesis holds that after a transient state where ADH is primarily pressure sensitive, there would be a shift favoring osmo-control as pressures decline below control level. During acute hypogravitic stress, the headward shifts lead to an initial suppression of volume-guarding hormone levels.²⁶

Thus, weightlessness causes volume depletion which is not counteracted by thirst. Volume-depleted subjects may be reluctant to drink enough fluid to obtain sufficient sodium to compensate this deficit. As they absorb fluid, the extracellular sodium concentration will be reduced. This, in turn, suppresses thirst, and the subjects need to be advised to drink sufficient amounts of water. It can generally

be said that thirst is mostly dependent on elevated sodium concentration with little contribution from the extracellular fluid volume. This again raises the question how the changes observed during spaceflight can best be interpreted, and how dietary interventions may be designed to arrive at the desired hydration status.

B. Energy Balance

The cells perform most of the metabolic work, and their total volume therefore determines the magnitude of energy transfer and oxygen consumption in the body. Energy is derived from the radiant energy of the sun, which drives photosynthesis in the chloroplasts of the plant cells. Other sources of energy in the biosphere, such as gravitation, geothermic energy, or electric potentials, are of negligible importance in the total energy flow, but may determine some conditions of the ecosystem.¹⁷⁷

Energy is stored in the human body in the form of fats, proteins, and complex carbohydrates synthesized from simpler molecules. Formation of these substances is part of the anabolic processes. Food intake and energy consumption is linked to appetite and satiety. There are many physiological connections between the type of food ingested, peripheral and central nervous receptor mechanisms responding to the nutritional and metabolic stimuli, and related effects on energy metabolism.¹⁷⁸

Factors modifying energy requirements are physical activity, body size, gender, climate, and environment.¹⁷⁹ If energy expenditure cannot be easily measured with reliable methods (calorimetry; doubly labeled water technique), the linear relationship between heart rate, work load, oxygen consumption, and heat output may be employed. Heart rate monitoring was used for predictions of energy expenditure in astronauts. Reliable results can only be obtained on the basis of individual calibration,^{180,181} and as a worst case, errors may still exceed 50%.¹⁸² If oxygen and carbon dioxide flows can be measured, energy expenditure calculation is relatively precise. When urinary nitrogen excretion is taken into account, precision is further enhanced.¹⁸³

As a general guide for defining space diets, NASA has used the recommended daily allowance values which were published by the Food and Nutrition Board of the National Research Council. Actual needs of astronauts may differ, at least in terms of catabolic shifts due to bone/muscle degradation in microgravity. Energy and protein supply have been gradually increased in space diets, the highest figure being reached with the *Voskhod* cosmonaut diet providing 3600 kcal per day.¹⁸ Table 1 shows the composition of the diet provided for the crew on the *Salyut-6* mission.

Short-lived essential nutrients, or those which show a rather swift exchange within the body pools and short-time constants, must be supplied even on a short-term basis.

Nutritional factors also play a role in blood pressure control¹⁸⁴ and therefore need close consideration in situations where cardiovascular stress situations might occur

Table 1. Space Diet of Salyut-6 Crew

<i>First Breakfast</i>	<i>Lunch</i>
100 g cheese	100 g canned ham
24 g coffee with sugar	165 g russian soup in tube
45 g bread with honey	52 g beef and mashed potatoes
40 g pork with green pepper	45 g rye bread
50 g dried plums	25 g biscuits with cheese
	30 g apple juice
<i>Second Breakfast</i>	<i>Dinner</i>
100 g beef tongue in jelly	50 g nuts
50 g candy	53 g mixed canned meat
40 g cherry compote	30 g wheat bread
	50 g dried plums and cherries
	23 g tea with sugar

Diets consisted of 65% freeze-dried foodstuffs.

Energy content 3300 kCal per day.

Composition: 159 g protein, 131 g fat, 396 g carbohydrate, 0.9 g phosphate, 0.5 g magnesium, 3.8 g potassium, 6.1 g sodium.
(from ref. 220).

during space missions. Estimation of energy expended on various muscular activities in space is required in order to be able to plan a diet for long-term space missions. The question of metabolic expenditure over relatively long periods in space has not been fully resolved.

C. Adaptation and Long-Term Balance

Movement in a weightless environment entails higher metabolic cost than originally predicted. Tasks that ordinarily depend on friction for their reactive force require muscular work to supply that force.¹⁸⁵ Therefore, the extent and pattern of muscular activity depends on the G-load acting on the body, as the respective energy requirements do. This has important bearing on body composition balance and on average nutrient needs.

Loss of body mass is one of the best documented early physiological changes associated with spaceflight. The detailed physiological background of this phenomenon is not very well understood. Both lean and adipose tissues are responsible for the decrease in body mass of astronauts.^{26,186} Only a few experiments and model calculations have been performed and they are of limited predictive value. One major link is between water and electrolyte balance on the one hand, and nutrient

and energy balance on the other. New concepts have emerged in this context which have partly resolved the contradictions between inflight results and what was initially expected by experts. However, experimental data are still lacking, particularly those conveying information related to acute changes during transitional phases, and there is a need for suitable mathematical models for analyzing complex biological interactions. Future long-term exposure of humans to reduced gravity will reveal the end-points of nutritional and metabolic adaptation under these unique circumstances.

IV. SELECTED ORGANS AND TISSUE SYSTEMS

A. Central Nervous System

The brain is the organ where sensory, metabolic, cardiovascular, and nutritional factors are linked to behavioral and motor functioning. All these factors influence human physiology during space missions. Recently there have been numerous studies on the influence of nutrition on the function of nerve cells and brain.^{187,188} Minerals, vitamins, trace elements, and amino acids are integrally involved in the synthesis of various neurotransmitters,¹⁸⁹ and links exist to carbohydrate and protein intake. Therefore, deficiencies are likely to be a causal factor in changes of mood and central drive. For example, a decreased concentration of brain serotonin causes depression, and feeding the serotonin precursor tryptophan may alleviate the symptoms.

The availability of dietary precursors influences at least five neurotransmitters: (1) tryptophan to serotonin; (2) tyrosine to catecholamines (dopamine, norepinephrin); (3) histidine to histamine; (4) threonine to glycine; and (5) choline to acetylcholine. These precursors cannot be synthesized by the brain, so diet and plasma concentration determine their availability to the nerve cells. Therefore, the rate at which the neurons produce and release serotonin and catecholamines depends on the availability of tryptophan and tyrosine, respectively.

It is conceivable that nutrient supplements might be used pharmacologically to produce changes in neuronal output as a compensation for any undesirable effects of spaceflight or stay in another extraterrestrial habitat.¹⁹⁰ For example, brain serotonin is increased upon ingestion of a meal rich in carbohydrates; as a result, pain sensitivity is diminished, but the individual is less effective and responsive to its environment.^{191,191a}

The effects on brain function of dietary variations and plasma fluctuations of vitamins and minerals have received little study. Most investigations have dealt with neurological consequences of severe deficiency states, but evidence for brain response to marginal undernutrition is relatively scarce. However, evidence is accumulating that marginal deficiencies of minerals and vitamins have behavioral consequences, and therefore may jeopardize operational stability of astronauts.

Circadian rhythms play an additional modifying role in the effect of food intake on brain function; and vice versa, the utilization of nutrients underlies diurnal changes which are ultimately synchronized by the brain. Circadian rhythms have important effects on metabolism and performance in space. Astronauts are urged to eat at regular times to avoid perturbation of biological rhythms. Saliva samples can be collected to measure the levels of melatonin, a hormone which is involved in circadian rhythms and is quite responsive to environmental stimuli.^{191b} It is possible that manipulation of light-dark cycles and food schedules could have behavioral consequences in astronauts. Studies on interactions of nutrient intake, metabolism, and biorhythms under the special circumstances of long-term space missions should receive increased attention.

Although it is difficult to establish causal links between normal variations in diet, changes in brain neurochemistry, and changes in central nervous system output, there is evidence that dietary strategies influence brain precursor uptake and function. The influence of food constituents on humans living in space and on extraterrestrial bases in terms of brain function, mood, behavior, and performance require careful consideration.^{121,191-195} Concern about suboptimal nutrient supply also seem to be justified.

B. Musculoskeletal System

The muscles adapt to the removal of gravity by internal resettings, commensurate with the reduced mean mechanical load.²⁶ Muscle atrophy leads to losses in potassium, magnesium, and nitrogen with corresponding increases in their concentrations in the extracellular compartment.

The skeleton, which consists largely of extracellular solids, comprises approximately 15% of total body mass or 20% of the lean fraction. This is a compartment of notably little change in the short-term range but subject to ongoing long-term shrinkage if gravitational load is chronically absent. This is accompanied by chronic increases in extracellular calcium concentration and by a progressive calcium loss from the body.²⁶

The balance of catabolic and anabolic pathways in bone and muscle depends on the amount and time-profile of mechanical load. This load can be considered to be composed of basically two factors: (1) acceleration forces acting on the body as a whole (G-load); and (2) resistant forces to be counteracted (movement, isometric exercise). The combination of these two factors determines the metabolic balance and nutrient demand of the musculoskeletal system. A reduction of the average forces to which this system is subjected leads to atrophy which cannot be counteracted by nutritional measures alone. Metabolic changes, decreased oxygen utilization, and fluid shifts are interrelated events in this situation.¹⁹⁶ Commensurate with the mean load, the myocyte muscle cells and the osteoblast/osteoclast system of bone remodeling cells adapt by internal changes and by accommodating their blood

supply by autoregulatory means. In the long term, this may lead to devascularization of the musculoskeletal system in astronauts.¹⁹⁷

It is still not known how to fully prevent the disuse osteoporosis of spaceflight, and what the steady-state level of bone calcium will be after long-duration flights or in environments of low gravitational loads. Immobilization leads to adaptive metabolic responses in the bones which seem to be transient. It is difficult to predict the duration of the transient phases that lead to a new steady state or the degree of bone loss involved.¹⁹⁸ It may be useful to perform extravehicular activities during flight in order to maintain fitness inflight.¹⁹⁹

A particularly intriguing, yet unanswered question, is what gravity level is sufficient to maintain "normal" musculoskeletal metabolism. Is it the amount of gravity existing on Mars (0.4 G)? What is the quantitative relationship between G-load and bone/muscle metabolic balance, and is there a critical G-level required for an acceptable long-term steady state in the musculoskeletal system? These important questions cannot be answered to date. Dietary countermeasures, like oral calcium supplementation, are limited for several reasons. First, calcium absorption is regulated according to the actual needs of the body. Second, calcium overloading may result in the formation of kidney stones. Third, calcium supplementation interferes with the absorption of other important minerals and trace elements as discussed in Section II.

C. Cardiovascular System

There are a multitude of mechanisms whereby nutrients may influence the cardiovascular system.²⁰⁰

- caloric intake via diet composition, energy generation, and metabolic requirements;
- carbohydrates via energy metabolism, membrane synthesis, insulin regulation and sodium excretion, catecholamine regulation, and vascular tone;
- proteins via peptide synthesis, control of cellular function, membrane transport systems, and plasma colloid osmotic capacity;
- lipids via energy source, cell membrane components, prostaglandin synthesis;
- sodium via intravascular volume, hormone regulation, and membrane potential;
- potassium via vascular tone, hormone regulation, and cation transport;
- calcium via receptor-ligand binding, hormone synthesis and release, and vascular contractile proteins;
- magnesium via regulation of calcium channels, energy production, and contractile protein interaction;
- phosphorus via membrane structure, energy metabolism, and second messenger;
- copper via vascular integrity, manganese via energy metabolism, chromium

- via carbohydrate and lipid metabolism, and vanadium via membrane cation pump;²⁰¹ and
- tocopherols via prostaglandin synthesis, vitamin D via calcium balance, and vitamin B₆ via enzyme cofactor mechanisms.

Taken together, function and adaptability of the cardiovascular system is linked to proper nutrient supply in many ways. Astronauts can help to keep full functional ability of their cardiovascular systems by ingesting food which is tailored to specific needs of life on extraterrestrial habitats.

D. Immune System

Adequate nutrition is a prerequisite for proper functioning of the immune system. An excellent immune status is mandatory to maintain health and resilience of astronauts. Protein-calorie malnutrition, vitamin, and trace element deficiency all affect the functions of the immune system.^{103,202} Low energy and protein intake has negative consequences, mainly for cell-mediated immunity. It affects the specific defense reactions of lymphocytes, action of natural killer (NK) cells and phagocytes, and it may cause lessening of lymphoid organs. Immune function is generally suppressed by a deficiency of iron, zinc, copper, manganese, or selenium (see Section IIC).

The effects of vitamin C deficiency on the immune system usually are relatively mild and reversible, and lymphocyte functions are not significantly diminished. The protective capacity of vitamin C fortification is questionable. Low vitamin A intake may result in a high infection rate and reduced antibody secretion. Serious effects may result from deficiencies in vitamin B₆ and vitamin B₁₂, since numerous symptoms of reduced immune capacity have been documented in humans. Tocopherol assists the protective mechanisms of cell membranes, and tocopherol deficiency decreases antibody production and lymphocyte responses to mitogens. This effect is exacerbated by selenium deficiency and inadequate vitamin C supply.

Dietary fat plays a modifying role in immune response.²⁰³ Diets high in polyunsaturated fatty acids have been shown to be immunosuppressive, possibly because polyunsaturated fatty acids influence cell receptor distribution and function via altered membrane fluidity. Another explanation is that excess production of prostaglandins may suppress NK- and T-lymphocyte function, since some polyunsaturated fatty acids are precursors of these immunoregulatory compounds. Marked differences in the effects on inflammatory and immune cell functions have been demonstrated between different kinds of polyunsaturated fatty acids (n-3 versus n-6). The n-3 type of polyunsaturated fatty acid can decrease the production of eicosanoids, which can be synthesized in various amounts by all immune cells, especially macrophages and monocytes which are responsible for antigen presentation to lymphatic cells. On the other hand, hyperlipidemia results in impaired lymphocyte and phagocyte functions. High cholesterol levels may suppress im-

mune function by inhibiting cholesterol synthesis needed for the functioning of immunocytes.

The immune status is significantly influenced by spaceflight. Microgravity may alter biological processes at the cell level. Activation of human lymphocytes by the mitogenic lectin concanavalin A is decreased by 90%, while their production of interferon is increased by 500% in microgravity.²⁰⁴ In addition, microbiological safety always presents a potential problem in closed systems. It is known that the water supply on spacecraft is not sterile and may contaminate food and drinks. Space menus must therefore be carefully arranged to guarantee the best nutrient patterns possible which are sufficient to meet the needs for optimal immunological protection, particularly under circumstances of increased mental and physical stress.

V. FUTURE REQUIREMENTS AND RESEARCH

A. Ecological Life Support Systems

As spacecraft and space habitats get farther away from Earth, resupply of food and nutrients will become increasingly difficult and costly. Hence, it will be necessary to regenerate life support materials completely. Bioregeneration is an efficient way of recycling waste products. Photosynthesis could be a central part of processes supplying oxygen, potable water, and food to the astronauts. Several systems studies for bioregenerative recycling, waste management, and food production scenarios have been elaborated; the so-called CELSS and BLSS concepts.²⁰⁵

The formulation of any such system has to be based on a very wide knowledge of food science and technology. In order to minimize the production load and waste treatments, a maximum "index of nutritional quality" needs to be achieved. Nutritional quality and consistency may be optimized with respect to any stress conditions or crew requirements which have to be specified on an individual basis.

Future results from long-term, broad-based projects, like the *Biosphere 2* project in Oracle, Arizona,²⁰⁶ will provide a wealth of new information on subsystems interdependencies and (in)stabilities in highly complex biological and ecological networks. To date, no database exists on the features of such systems on sufficiently reliable quantitative grounds. Biosystems of such an order of magnitude will be required to arrive at reliable and stable recycling and food production. Food production will involve grains, vegetables, fruits, potato plants, spices, fish, small animals, eggs, and lactating animals to provide milk for the production of dairy products. Expert knowledge concerning the long-term behavior of such complicated biosystems will be needed to design an efficient and reliable system for the extraterrestrial production of balanced, health-supporting, and palatable diets.

The aspect of food acceptance is of major importance for any mission where

humans are, for an extended period, in a remote and isolated, challenging situation. Mood and motivation heavily depend on the hedonistic and rewarding character of eating.²⁰⁷ The attitude towards a meal, and the many different physiological and psychological effects of food intake and nutrient supply, will have a great consequence for the ability to take stress and challenges. This will require careful design and operation of future life support systems.

B. Assessment of Nutritional Status

Nutritional status is a complex entity, encompassing a great number of different functional and clinical parameters and variables. The significance of nutrient deficiency or excess is not the level of the nutrient per se, but rather its impairment of physiological function. For most of these functional variables, however, specificities and sensitivities are not well known,¹⁹ and many require invasive procedures or unconventional laboratory equipment for their determination. Suitable compartment analyses and quantitative approaches to study the kinetics involved are needed to obtain information which is not directly available from measurements.

Amino acid metabolism determines protein pools and the integrity of cells and organs, and can be studied by different approaches. The daily turnover of the total body protein pool in humans amounts to 2 to 3%. The mass of different tissues and organs is directly linked to nutrition and metabolism. The interpretation of body mass changes in astronauts depends on precise knowledge of the alterations of the physiological steady-states, and which compartments are subject to change to what extent. Changes in body mass generally reflect a change in water, protein, fat, and minerals. Therefore, in addition to nutritional surveillance and metabolic assessment, it is necessary to monitor the body composition.

Most methods for the determination of body composition are based on a two-compartment model of the body (fat and fat-free). Traditional methods comprise: total body water measurement; total body potassium measurement (potassium is concentrated in the cells but not present in stored fat); urinary creatinine excretion (98% of the precursor creatine is located in skeletal muscle); whole body densitometry (the fat-free body has an average density of 1.1 g/ml at 37°C, fat 0.9 g/ml); and anthropometric measurements (e.g., bone measures, and skinfold thickness).

New methods are: neutron activation analysis for assessment of body calcium or body nitrogen; measurement of muscle metabolites (endogenous 3-methylhistidine excretion); photon absorptiometry (bone mineral content is assumed to be directly proportional to the amount of photon energy absorbed by the bone); total body electrical conductivity methods;²⁰⁸ computerized tomography; and subcutaneous adipose tissue thickness via ultrasound, infrared interactance, and magnetic resonance imaging. With modifications for use at different G-levels and in weightlessness, the electrical impedance method,^{209–211} which is relatively easy to use, will

probably render frequent routine body composition assessments amenable for use in spacecraft and extraterrestrial bases.

C. Identification of Optimal Nutrient Patterns

Enormous research needs are to be met before all questions of nutritional support under extraterrestrial conditions are defined and can clearly be answered. As an example, the issue of "newer" trace elements is open and partly unsettled, and awaits many important new results (Section II-C). After definition of the respective circumstances and prevalences, balanced diets can be identified which will supply sufficient amounts of all essential nutrients.

It seems reasonable to assume that vegetable food will be the major source in any long-duration flight scenario where a closed-loop biological life support system is used. On an extraterrestrial base, foods of plant origins will probably contribute a major part of the diet. Cereals, vegetables, fruits, peas, beans, lentils, nuts, and seeds will likely be the cornerstones of a space diet. The advantages and disadvantages of such a vegetarian diet will need to be considered. If no animal food is provided for months or years, nutrient deficiencies are likely to occur. A balanced pattern of essential amino acids^{212,213} can be achieved by adequate mixing of plant proteins. Data are available on amino acid content of various edible plant proteins and can be used for the planning of a balanced protein supply with vegetarian diets.

However, deficiencies of electrolytes and trace elements can hardly be avoided with such a prolonged, strictly vegetarian diet. This applies to calcium as well as to iron, zinc, and other trace elements. The latter cannot be easily supplied in inorganic form because of their low absorption efficiency (see Section II-C). Careful planning of balanced diets can prevent long-term nutrition problems even with minimal sources of animal food added to the diet. Total abstinence of animal food results in low serum vitamin levels, particularly vitamin B₁₂ and possibly vitamin D for which body reserves are small.

Considerable amounts of essential fatty acids (linoleic and alpha-linoleic acid) occur in plants. Seeds in general have relatively high proportions of linoleic acid in their oils; seeds and nuts have a vegetable oil content of 5 to 40% of dry weight. Essential lipids provide building units for the vascular system, and precursors of prostaglandins which influence vascular tone and thrombus formation. A vegetarian diet helps to prevent cardiac disease for many reasons; the favorable lipid composition probably being the major factor.^{214,215}

Any combination of food, resulting in certain diets, should be continuously monitored for all essential nutrients. This can be done by computer applications which not only analyze meal composition, but also allow for automatic checking whether individual needs are met by certain intake patterns. However, this type of supply monitoring needs to be supplemented by regular nutritional assessments. As stated earlier, functional parameters are ultimately decisive in judging whether a given nutritional pattern is adequate or needs improvement in terms of acceptance and physiological suitability.

D. Compartmental Analysis

One of the major tools in modern biological systems analysis is mathematical modeling. Compartmental analysis for quantitative understanding of the dynamics of metabolic events, with nutritional substrates as one important input among others, are currently being developed and are gaining sophistication. An important question is which variables shall be measured, and which of them can be determined frequently during a given spaceflight. From a scientific standpoint, invasive measurements are desired to obtain more insight in highly complex regulatory processes within the body. This has important bearings on the design of possible future tests of nutrition experimentation.

However, invasive techniques for scientific, diagnostic, and prognostic purposes are greatly restricted in order to safeguard health and well-being of the crew. Thus, many variables will thus not be amenable to direct measurement, and several types of modeling will have to be employed for the study of the dynamic behavior of an astronaut's metabolic systems. With the right model, the experimental data would fit the simulation results in such a way that differences between the two are minimized.

Systems analysis approaches allow for parameter identification and quantification.^{26,197,216,217} This is used in many fields of nutritional science; e.g., digestion of foods, absorption of nutrients, and whole-body metabolism of nutrients.²¹⁷ Choosing an optimal modeling approach depends on: previous experience and knowledge; the system under investigation; and availability of hardware, software, and measurable variables.^{8,218} The level of appropriate model complexity and available knowledge must be chosen in such a way that the simulation procedure is not based on too many assumptions, and the remaining uncertainty will not eventually jeopardize the validity of the model.²¹⁶

The question whether the oral intake of a single nutrient, or a set of nutrients, does or does not meet the individual needs is particularly difficult to answer. Compartmental modeling will supplement biochemical, functional, and clinical indices of deficiency²¹⁹ which can occur even with "normal" levels in a single compartment, usually the blood. With abnormal losses from the body, deficiencies may occur even with normal supply and intact absorption of the respective nutrient(s). In order to define actual needs more accurately, it will be advisable to monitor subclinical functional and morphologic changes.

VI. CONCLUSION

Nutritional support under extraterrestrial conditions is a very complex question, and a great number of investigations will have to be carried out to find an optimal answer. These problems are not of concern to short-term space travellers, but with increasing mission duration, the scientific and logistic problems grow significantly. Nutrition for long-duration space missions embodies aspects of nutrient supply,

nutrient utilization, physiological and immunological adaptation mechanisms, waste management, and biomass recycling under the particular circumstances of orbital flight, outer space missions, and variable G-load on a Moon base or a Mars mission.

Future investigations on spaceflight nutrition and metabolism should be performed by scientific teams who have a strong commitment to scientific thinking, study design, data acquisition, hypothesis testing, and concept reevaluation. Conceptual modeling is to be supplemented by mathematical modeling, with compartmental conceptualization and systems simulation being the leading approach. Long-duration space missions call for new research efforts, an extended data base, in-depth systems analysis approaches, and physiological investigations providing the basis for decisions on the technology to be employed and scenarios to be adopted. Spaceflight can serve as a unique model for studying long-term metabolic regulation in humans, and may prove an exceptionally worthwhile field for human nutritional research.

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EFFECTS OF SPACEFLIGHT ON GROWTH AND CELL DIVISION IN HIGHER PLANTS

Abraham D. Krikorian, Howard G. Levine,
Robert P. Kann and Stefania A. O'Connor

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I. INTRODUCTION

Critical experimentation using higher plants under spaceflight conditions is in its infancy. Opportunities to carry out experiments of significant duration and with adequate controls in the space environment have thus far been relatively few.^{1,2,3,4} Moreover, because this is a new area of research, plant scientists have not yet

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accumulated the required experience to reliably plan and perform well-controlled experiments in the same way they have been able to do on Earth for a very long time. It is also important to recognize that there are frequently constraints to the carrying out of sound space experiments which are beyond the control of even the most diligent investigator.⁵ In short, there is a "learning curve" to every new type of investigative activity, and there may be inadequate appreciation of this state of affairs when it comes to doing experiments in space.

Nevertheless, it has been appreciated that no long-term space biology program should neglect plants, because:

- plants have the ability to regenerate the atmosphere;
- they will surely play the ultimate and key role in controlled ecological life support systems and "space agriculture;" and
- they have considerable interest as organisms which, by virtue of their fully autotrophic nutrition, not only exist independently of animals but are, in fact, the ultimate support of the animal kingdom.

In addition, there are the obvious and important questions that surround the ability of higher plants which have evolved in a gravity-oriented environment, not only to merely survive in microgravity, but also to develop and pass repetitively through all phases of tissue differentiation, growth, and reproduction. Moreover, all nutrition and metabolism support and are mediated by highly asymmetric systems that range from the fine structure of the plant protoplasm to enzymes, membranes, and organelles,⁶ and up to organs such as root and shoot⁷ (and references there cited). Thus, the important question arises whether any or all of these events can be established and continued in an environment in which one major source of asymmetry, namely gravity, has virtually been abolished.

For some time, we have been concerned with the development and evaluation of systems suitable for use in a microgravity environment. The basic questions to be answered, at the outset, are general and direct:

- can isolated plant cells in a space environment divide in ways that permit the establishment of the patterns that characterize early embryogenesis on Earth, especially those involving polarity?;⁸
- are there gravity thresholds below which these patterns do not or cannot be adequately established or maintained?;
- can the features of metabolism of demonstrably or theoretically developmentally competent cells be simulated, sustained and manipulated in the microgravity environment of space in ways identical to what can be done on Earth?; and
- can features of the space environment provide us with tools to test the role of environmental parameters such as electromagnetism in plant development?⁹⁻¹⁰

To this end, our specific research efforts have fallen into three problem areas as follows:

1. study of nutrition, physiology, and metabolism of quiescent and actively growing plant cells;
2. characterization of morphogenetic (especially embryogenic) competence in cell and protoplast cultures; and
3. study of the environmental factors and their interactions controlling the growth of higher plants and the development of various cell, tissue, and organ culture systems.

Our research has been aimed not only at the description, but also at the understanding of the expression and modulation of the genetic information which is present in developing and growing systems. This information has been shown to persist in virtually all living cells, and at least in theory, can still be expressed by them. At the cellular level, genetic information involves an understanding of the external and internal growth regulatory controls which operate in resting or quiescent cells as they are activated to grow again at rapid rates. Because of the known responsiveness of plant growth and development to a variety of external factors, the space environment would introduce into these considerations an entirely new set of conditions, the consequences of which are for all practical purposes still largely unexplored. In this chapter, an attempt is made to provide the rationale for our approach. Considerable effort will be made to integrate our own findings, limited as they may be, into a broader picture.

II. ASEPTICALLY CULTURED TEST SYSTEMS FOR SPACE EXPERIMENTS

We have made significant progress in terms of developing testable systems and acquiring baseline data against the time that serious experimentation can be carried out in space. Even so, the more we have worked on these systems, the more apparent it has become that growth and development is incredibly complicated. While this may seem a platitude, it has instilled in us an increasing concern for the need to hone the systems very sharply and to seek ever simpler "models."

Our general working hypothesis is that it is likely that higher plants, which unlike most animals are sessile and do not move about, have through evolution developed various adaptive mechanisms to respond to a variety of signals and environmental cues that at first sight reflect a measure of being "overbuilt" to deal with various levels of "slack" or "imperfection" in signal processing. Some regard this ability to react or compensate as "stress tolerance."¹¹ These mechanisms include the ability to deal with subtle (and some not-so-subtle) signals¹² which are imposed, triggered, processed, and modulated by uptake and canalization of nutrients at the cellular

level. They range from the influence of gravity and magnetic fields on the establishment of a developmental pattern to the adaptation to low temperature and drought. Cultured somatic cells that can develop into embryos, and partially developed or incomplete organ systems (propagules) which can generate the "missing" or incompletely formed organ, provide by their nature ideal systems to disclose and probe these kinds of controlling mechanisms. By using systems at different levels of initial organization, yet capable of attaining or achieving the most advanced degrees or levels of development under closely controlled conditions, we should be able to learn whether highly asymmetric growing regions of root and shoot are crucial to responsiveness.¹³

Stated another way, what is the minimum graviresponsive unit in terms of morphology? To what extent can free cells or cultured higher plant parts detect and respond to gravity?^{14,15} Such systems must be grown aseptically, and hence do not suffer influences of contaminating microorganisms. They do not take up much volume and thus allow appropriate numbers of replicates to be tested. Because they are developmentally "plastic," they provide unique means to study early pattern formation and organogenesis from far less morphologically developed progenitor units. Another advantage that aseptically cultured systems afford is that they are a key link in the development and utilization of modern plant biotechnologies.^{16,17} Any data gained from experimentation with *in vitro* systems in the space environment will provide useful information that can be used in operations aimed at future space biotechnologies and genetic engineering efforts geared specifically to a long-term presence of man in space.

III. USE OF CULTURED CELLS AND PROTOPLASTS IN SPACE STUDIES

Experiments on the *Kosmos 782* (1975) and *Kosmos 1129* (1979) biosatellites with embryogenically competent cells of wild carrot (*Daucus carota* var. *carota*) showed that while the broad events of asexual development took place, the transition of one stage to another was slowed down significantly.⁸ In comparison to ground controls, a greater proportion of embryonic plantlets exposed to microgravity conditions were still at stage 2, and fewer embryos had progressed to stages 3 or 4 ($P < 0.001$). It is important to note that stages 1 and 2 are those where no distinct polarity is evident, whereas stages 3 and 4 are those developmental stages where distinct organ formation occurs.

The embryogenic cell system used for the *Kosmos* experiments involved the generation of competent cells, their induction on Earth to produce embryogenic units called preglobular stage proembryos, and their subsequent exposure to spaceflight so as to evaluate their capability and capacity to form later stage embryos. The normalcy of the developmental pathway of cells to proembryos, and to later stages of embryogeny could thus be scored.¹⁸ Similarly, the temporal aspects

could be traced. However, an ideal experimental design could not be implemented insofar as the temporal aspects were concerned. Efforts were made by cooling to near-freezing temperature to arrest cell division and thus to eliminate chances for progression of growth on Earth prior to flight. But even a simple engineering device as a long-duration temperature recorder can have its shortcomings. Ground controls can be carried out, but it is difficult to simulate the lift-off and recovery environment both in terms of vibration, G-levels, and radiation. Another shortcoming of the experiments was that fixation or termination of the experiment could not be done in space, but only after return to Earth; examination of the flight samples occurred a few days after landing. A 1-G control centrifuge was available on *Kosmos* 782, but not on *Kosmos* 1129.^{8,13} These points indicate that it is difficult to carry out a well-controlled experiment in space, and that one should be careful not to draw unwarranted conclusions.

Using an embryogenic system of an organism similar to carrot (anise, *Pimpinella anisum*), Theimer et al.¹⁹ reported an increased biomass of embryonic structures generated in space in liquid cultures. However, the results of that experiment, like the *Kosmos* carrot cell culture experiments, remain equivocal, since no 1-G centrifuge controls were carried out. Of greater concern is the likelihood that the cells, which were already embryogenically induced on Earth, continued their development in subtle ways on Earth,¹⁸ and then continued them still further in space. Because different procedures were used to activate the embryogenesis, and because the anise work was carried out in a liquid medium in contrast to the semi-solid agar substrate used for the carrot, the two systems are not directly comparable. For the present, the results are irreconcilable¹³ unless one proposes the not unreasonable hypothesis that the space environment has some selectivity for cells of a particular hierarchical status. Indeed, in carrot there seems to be a developmental stage-related sensitivity.⁸

To some it might be reassuring for the short term to establish that several systems can seemingly function without major catastrophic consequences, or in some cases even seem to grow "better" in microgravity.²⁰ Yet, from the scientific perspective, it is still an open question whether consecutive generations can be established through as many cycles as desirable. This is true for model flowering plant systems as in *Arabidopsis* with "seed to seed"-type experiments, as well as for model cell culture systems comprised of embryogenic cells, somatic embryos, or protoplasts from morphogenetically competent systems or units. An answer to this question is absolutely necessary if one is to eliminate the real possibility of retention of what may be termed, a "memory"¹⁴ of contact or a residual responsiveness to gravity or polarized fields inherent in the genetics of the system, be it protoplast, cell, embryo, or regenerative unit.¹³

In our laboratory, protoplast experimentation was geared initially towards developing a system which would permit us to work with wall-less counterparts of embryogenic or totipotent free cells as a model system for a fertilized egg cell. The plan was to establish totipotent cells and permit them to develop in space as

organized somatic embryos after the start of a determined course of cell division. The cells could thus give rise to plantlets, and these in turn, could be reduced to protoplasts by means of cellulose-degrading enzymes. The next step would be the regeneration of cell walls on these units, which would then reorganize into totipotent structures and go on to form embryos and plantlets.²¹ In this way, one would be able to create an "artificial life cycle" without an intervening sexual phase. Everything would be simulated, or occur asexually or vegetatively: embryogenically competent or totipotent cells → protoplasts → embryonic plantlets → protoplasts → cells, etc. The great advantage of this aseptic culture system would be the large numbers of units that could be dealt with, and the availability of a range of levels or hierarchies of developmental complexity that one could subject to a sustained space or microgravity environment.

We use embryogenically competent cells as the source of protoplasts.^{21,22} These cells have a built-in responsiveness, and have already been rendered capable, as it were, by means of an elaborate culture process to undergo substantial cell division and express their morphogenetic competence. The methods have been successfully used with protoplasts derived from suspension cultures.²¹ But the same methods, applied to cells which are approaching the critical level of differentiation, are only partially successful. Namely, as long as the totipotent cells are maintained in the mode of continuous cell division and proliferation, the cells can be reduced to protoplasts and made to divide, all the way to plants. As the sequential treatments devised to trigger differentiation of cultured cells in suspension onto a developmental pathway to make organized structures are entrained, the ability to yield protoplasts does not seem to be drastically altered. However, their ability to regenerate cell walls and to achieve cell division is greatly reduced.²²

Results of an experiment flown in September 1989 on *Kosmos 2044* (*Biokosmos 9*) by a group of Scandinavian and Soviet scientists, using fresh protoplasts prepared from suspensions of embryogenic carrot cells and from hypocotyls of rape (*Brassica napus*), are of real interest in this context. In this experiment, a significant number of protoplasts did not regenerate cell walls. In carrot 56%, and in rape 82%, regenerated walls and grew compared to ground controls. Moreover, chemical analysis showed a decreased amount of cellulose. Similarly, peroxidase and total protein levels were also much reduced in flight samples. Significant negative impact on regeneration was observed as well.^{23,24} Unfortunately, 1-G centrifuge controls were not carried out, so again we are unable to positively attribute the results to microgravity. It could well be that indirect factors, or spaceflight conditions that could not be accurately simulated on Earth, were responsible. Certainly it cannot be ruled out that indirect effects of factors associated with the space environment had no influence on the expression of developmental competence normally encountered on Earth. At the same time, it is appreciated, of course, that the protoplast findings are consistent with the *Kosmos 782* and *Kosmos 1129* cell culture results. More will be said in the next section on their being in accord with other data as well.

IV. CHROMOSOMES AND PLANT CELL DIVISION

It is critical for our understanding of cell division capability in space to have a thorough knowledge of chromosome behavior of cells of higher plants grown in orbit or exposed to extended spaceflight conditions.

An occasion to look into this aspect of growth²⁵ arose in November 1981 when we studied root materials grown in space on STS-2 as part of a bioengineering test (named HEFLEX, HBT) carried out by Brown and Chapman, Department of Biology, University of Pennsylvania.²⁶ It seemed an excellent opportunity to examine whether the processes of cell division in an organ such as a root would be affected by growth in microgravity.²⁷ The chance to do this on roots of a plant like sunflower (*Helianthus annuus*), however few specimens might be accessible, was all the more attractive since experience on its cytology existed in our laboratory.²⁸ We decided to carry out karyotype analyses on cells arrested in metaphase, since the available sample size would be small and it was felt that the gains could be great relative to the efforts expended in analysis.²⁹ The results are summarized below.

A second opportunity arose with a repetition and extension of the HEFLEX test (HEFLEX, HBT-II) on STS-3 (March, 1982). We also had access to material grown on this flight as part of an experiment entitled "Gravity-Influenced Lignification in Higher Plants" (principal investigator Dr. Joe Cowles, then at the University of Houston).²⁸ With this material, we attempted to confirm and extend the results obtained in the first experiment. Roots of sunflower seedlings grown in space in the HEFLEX, HBT-II test were used, as well as roots of oats (*Avena sativa* cv. Garry) and mung bean (*Vigna radiata*) obtained from Dr. Cowles. This broadened the base of the material on which observations were to be made. In addition to the monocotyledon of oats, there would be the dicotyledons of mung bean and sunflower. The lignification experiment was repeated on the SL-2 mission (August, 1985), and again we had access to some roots of oats. Finally, access to a few seedlings of maize, also grown on the SL-2 mission but in the absence of light, was made possible through the student experimenters program (Dr. R. S. Bandurski, experiment advisor).

Despite some reservations, which will be addressed below, the results showed:

1. In each of the three species examined for chromosome damage (karyology of corn was not attempted), all test specimens showed a substantial reduction in the number of cells in division after they went through their first cell division on Earth.
2. In oat and sunflower roots there were severe chromosomal aberrations ranging from aneuploidy, breakage, bridge formation, etc.
3. In mung bean, no chromosomal aberrations could be detected.²⁹

Figure 1 shows representative damaged metaphase division figures from oats grown on the SL-2 mission. The chromosomal damage is fairly apparent, but in

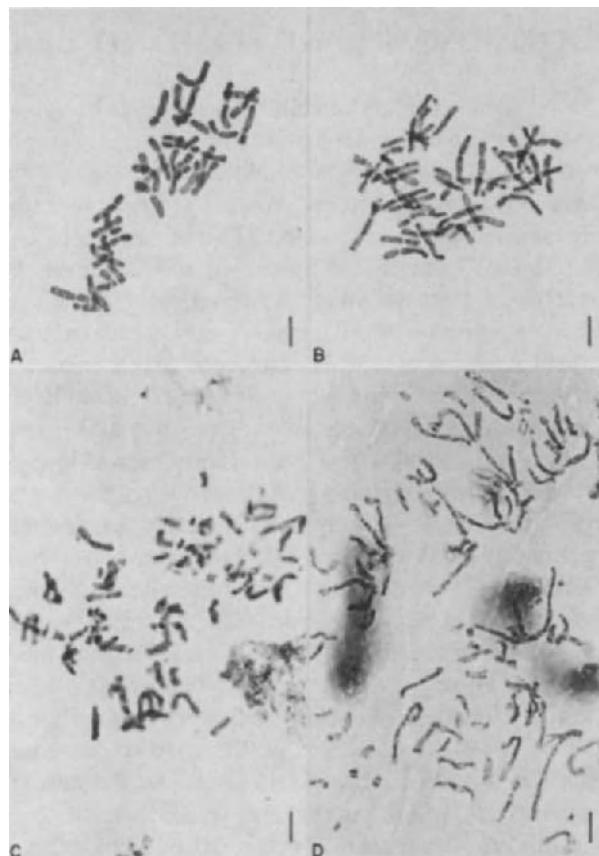


Figure 1. Division figures in oat root tip squashes of seedlings germinated and grown in space. Representative range of division figures encountered in oat root tip squashes of seedlings germinated and grown in space and treated on recovery with 0.1% colchicine for 3.5 hours to permit metaphase analysis. A. a metaphase figure showing very contracted but apparently normal chromosomes. B. another metaphase figure from the same root showing less contracted and apparently normal chromosomes. C. a metaphase figure showing very contracted and fragmented chromosomes. D. a metaphase figure showing much less contracted but severely fragmented chromosomes. Note the Feulgen-staining (nuclear) components that look like smudged "blobs." Scale bar equals 5 μm .

addition to the clear-cut damage encountered, one has also to account for the situation in which exposure to colchicine brings about irregular contraction patterns. Chromosomes generally contract in predictable ways depending on the amount of colchicine or other pre-fixative used.²⁸ Flight materials examined by us have generally revealed irregular contraction patterns. Note in Figure 1 that the

"blobs" of Feulgen-staining material were also found in sunflower on the STS-2 mission (p. 53 of ref. 29).

In evaluating the validity of the findings listed above, Krikorian and O'Connor²⁹ emphasized that the paucity of cells in division encountered could be a true reflection of reduced cell division activity in the space environment. The near-normal root histology of the oat and mung bean material examined by Slocum et al.³⁰ would seem, initially at least, to argue against that interpretation. But it should be emphasized that experimental circumstances preforce did not permit random collection of flight roots for microscopic examination. The root samples examined by Slocum et al.³⁰ were a tiny proportion of the small sum available as part of a pioneer trial "parts program;" the larger number of samples were utilized by the principle investigator, and by Krikorian and O'Connor. Certainly the response or situation encountered in roots at the karyological level using metaphase arrest techniques would not have been detected at the light microscopic level using regular histology fixation procedures.

The karyological results of Krikorian and O'Connor²⁹ could also mean that upon return to Earth, the root cells had been so traumatized or perturbed by the conditions of flight, re-entry, etc., that they were unable to rapidly re-establish their normal cell cycle. Stated differently, the cells had adapted to spaceflight conditions, but could not readapt quickly enough to give a "normal" karyological profile when processed promptly on Earth. In this view, nominal but observable chromosomal damage could reflect an artifact of the otherwise adequate examination technique, since the integrity of chromosomes at metaphase could be sensitive to cellular factors which themselves had been affected in unusual ways by exposure to spaceflight. Certainly, cell division had occurred during spaceflight, since organs had formed and a recognizable seedling had emerged. This indeed indicates that nothing "catastrophic" had occurred insofar as organogenesis was concerned. The fact remains, however, that the first cell division cycle on Earth after recovery was adversely affected. Table 1 shows that the ground control data of the oat root tip were fairly consistent in two flights, and that the range of cell division depression encountered in flight samples was roughly comparable.

These data, however incomplete, strongly suggested that the space environment (be it microgravity or other factors) caused a reduction in the number of cells in metaphase arrest when treated with colchicine in a timeframe consistent with the occurrence of the first cell division cycle on Earth.²⁹ Not only were there fewer cells in division, there were severe chromosomal aberrations in many of the flight samples. Tests made in our laboratory on "control" material failed to disclose similar aberrations. It is also noteworthy that in none of the cases examined by us, including the ground controls, were the high number of cell divisions routinely encountered in so-called "bench-top" controls duplicated in the flight modules. All this suggests that either the flight conditions, or the growing conditions in space for the roots provided by the growth medium, the Plant Growth Unit (PGU)⁵ (and literature there cited), or Plant Carry-on Container hardware, were less than optimal

Table 1. Effect of Spaceflight on Cell Division in Oat Root Tips

Mission	Controls		Flight	
	STS-3	SL-2	STS-3	SL-2
Mean Percentage* of Cells in Division	2.6	2.7	0.04-1.3	0.13

Controls represent ground control plants

Flight represents preparations made promptly on recovery after spaceflight from missions STS-3 (April 1982) and SL-2 (August 1985)

*Percentages derived from samples containing from 1000 to 20,000 cells.

..

for the kind of root studies and chromosomal investigation attempted. The former interpretation, flight conditions at fault, is more likely because we have since been able to obtain actively growing roots with numerous cell divisions in plant growth chambers. The use of "soil" in the case of *Helianthus*,^{26,31} a bleached white urethane foam³² and Miracloth "sandwich" support system which served as the growth envelope for the seedling roots,²⁷ and finally, the use of mung bean which has very small chromosomes generally unsuited to karyotype analysis, were less than ideal for obtaining definitive karyological data. However, as guest investigators who sought to use available material not needed by the principal investigators, we had few options.

In addition to the chromosome data, attention may be drawn to other observations which are indicative of subtle conditions that can be imposed in the space environment, the consequences of which may at this time be merely surmised.³³ Figure 2 shows some representative root tips of oats from seedlings germinated and grown under "ground control" conditions (upper panels) and in the SL-2 space environment (lower panels). The control roots showed abundant root hairs and a healthy aspect characterized by a densely Feulgen-stained tip. When squashed, the root cells displayed well-rounded nuclei that suggested good metabolic activity and vigor. Flight roots, on the other hand, showed fewer hairs and a less healthy aspect insofar as they stained poorly with the Feulgen DNA stain (not easy to see in the black and white photograph).

Another abnormality is that flight roots showed some branching, which is unusual in oats, whereas controls showed none. One interpretation of this phenomenon may be that the hormone balance was upset in the main root tip and thus other, normally suppressed, primordia grew. Another explanation may be that there was an influence of ethylene. Finally, squashes from space grown roots disclosed smaller nuclei and fewer division figures. The one presented here reflects poor metabolic activity. The presence of xylem elements in the flight squashes indicates that the zone of maturation is abnormally close to the apex. All this suggests senescence and premature aging.

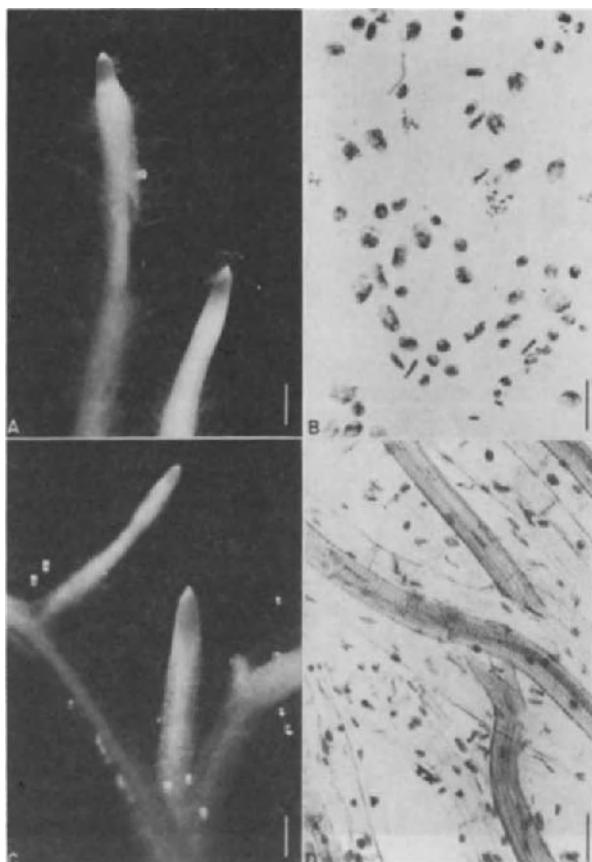


Figure 2. Root tips of oats from seedlings germinated and grown in space and on ground. Comparison of representative root tips of oats from seedlings germinated and grown under ground control conditions (*upper panels*) and from the space environment (*lower panels*). See text for details. Scale bar on **A** and **C**, 1 mm., on **B** and **D**, 5 μ m.

By any of several measures, the roots from flight specimens did not look as healthy as those from ground controls. Figure 3 shows scanning electron microscope (SEM) pictures of representative oat root tips from seedlings germinated and grown under ground control conditions (upper panels) and under spaceflight conditions (lower panels) from the *SL-2* experiment.

Additional interesting aspects were disclosed on examining maize roots grown in darkness. Figure 4 shows some sections of etiolated shoot (upper panels) and roots (lower panels) of *Zea mays* L. grown in space on the *SL-2* mission in darkness from seeds. Figure 4A shows a longitudinal section of the shoot tip region and

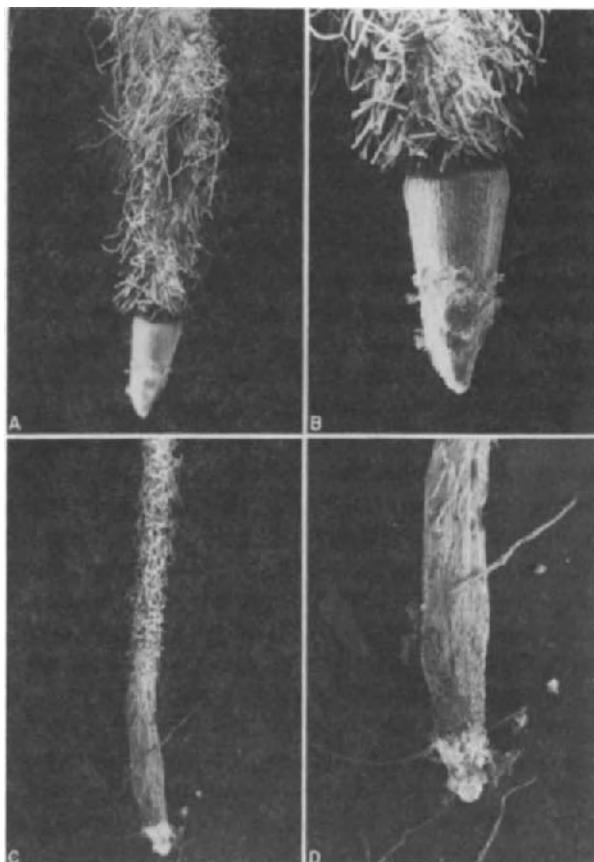


Figure 3. Scanning electron micrographs of root tips of oat seedlings grown in space and on ground. Compared are ground controls (A and B) and space flight specimens (C and D). A shows well developed root cap and hairs; B, higher magnification of the same root. Individual root cap cells are clearly visible C, flight root showing poor root cap and paucity of root hairs. A and C, 30x; B and D, 60x. All preparations were fixed in aqueous 3% glutaraldehyde buffered with 0.03 M PIPES at pH 7.5, dehydrated in ethanol series, critical point dried, sputter coated with gold and viewed with a JEOL JEM-35 SEM.

Figures 4B and 4C show the apical growing zone at increasing magnification. There is a pronounced bulging of some of the cells in the so-called tunica layer (see Fig. 4C in particular). Figures 4D and 4E show root tip sections, but the cells here do not reveal the pronounced rounding encountered in the stem tip. The root cap is well-formed but some cells of the protostele show poor staining of the cytoplasm, and indeed, the cells look almost devoid of cytoplasm. This may be a preliminary indication of the premature differentiation of the vasculature encountered in oats

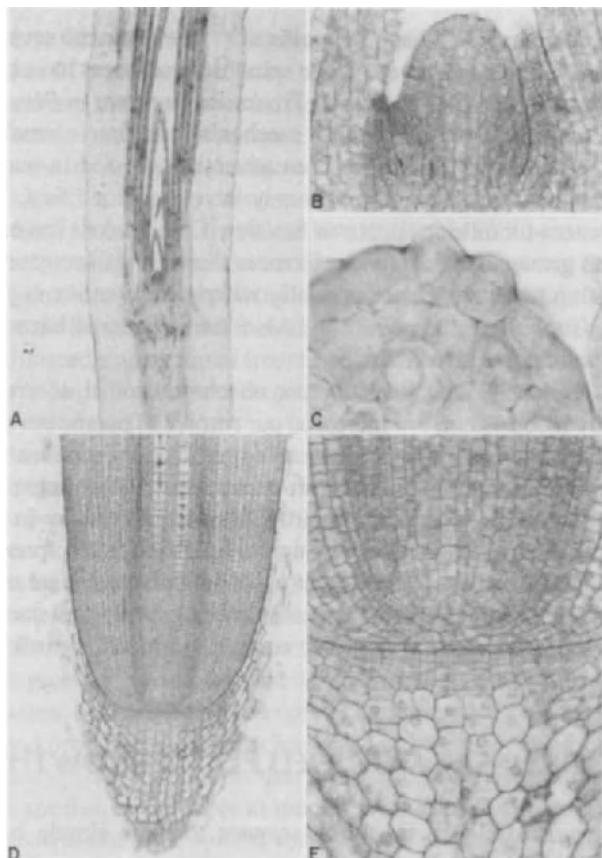


Figure 4. Sections of shoot and root of *Zea mays* grown in space from seeds in darkness. Representative sections of etiolated shoot (upper panels) and root (lower panels) of *Zea mays* grown from seeds in darkness. A, longitudinal section of shoot tip region (16 \times); B and C, higher magnifications of same. Note bulged tunica layer cells (193 \times and 1055 \times , respectively); D and E, longitudinal section of root tip region (80 \times and 307 \times). No pronounced rounding is detectable in the root meristem.

(cf. Fig. 2D), but rounding of cells has also been reported by Soviet investigators.³⁴⁻³⁶ The rounding encountered here was totally unexpected, was not seen in the other systems examined histologically, and was not thought to be an artifact of processing.³⁷ If it is indeed a space-related phenomenon, it may be explainable on the basis of reduced surface tension and poor wall deposition at the stem tip cells in microgravity. Alternatively, it may reflect decreased microtubule assembly resulting in a disruption of the cytoskeletal structure. Moore et al.³⁸ have drawn attention to the very interesting observation that root caps of maize removed prior to spaceflight are not regenerated in microgravity.

Various speculations^{33,39-41} have been offered¹⁻³ to explain the several noteworthy observations referred to above. Mitotic spindle disturbances have been encountered before in space samples such as in *Tradescantia* flown in *Biosatellite II* by Sparrow and co-workers.^{42,43} DNA repair mechanisms that are normally operative in unstressed systems may well have been adversely affected in our flight samples.^{44,45} Intracellular calcium ion levels may have been modified, which could have consequences for mitotic apparatus function. Calcium loss has been reported in roots of peas grown in space³ (and references there cited). Increased sensitivity to space radiation levels which are normally without substantive negative consequences was also viewed as a possibility. Also, the vibrational hazards of lift-off and recovery were not overlooked.⁴⁶

Reports by various Soviet investigators on chromosomal aberrations,^{20,47-49} although highly inconsistent,^{50,51} increased our resolve to pursue our findings (see Section V). There was some suggestion that the growing environment was responsible, but we were unable to identify with certainty any obvious problem areas. For example, Platonova et al.⁴⁸ attributed a substantial increase in meiotic cell division irregularities to the abnormally high temperature in the spacecraft. However, crucial details that would make that paper interpretable⁵² are not provided. Krikorian and O'Connor²⁹ provide other examples of cytological damage that are not directly related to space conditions, such as hormone disturbances due to water-logging of the root environment.⁵³

V. USE OF NASA's MODIFIED PLANT GROWTH UNIT

A test system was designed to obtain answers to three simple but important questions:

1. Can roots of sunflower (*Helianthus annuus*), oats (*Avena sativa*) and daylily (*Hemerocallis*) be initiated and undergo subsequent growth in microgravity (on the order of 10^{-4} G) at a level at least equivalent to that which occurs at 1 G on Earth?
2. Can the fidelity and mode of partitioning of the chromosomes in the root tip meristems be maintained during and after exposure to flight conditions?
3. Can the normal rate and patterning of cell division in the root tip be sustained during and after exposure to microgravity?

Criteria for comparison could include the number of roots formed; determination of length, weight and assessment of "quality" based on subjective appraisal, as well as quantifiable morphological and histological measurements, such as cell size or wall thickness. Chromosome analyses of root tip cells could be carried out by study of cells arrested in metaphase by use of a cytostatic like colchicine, but other stages in the mitotic cycle would be amenable to study as well.^{25,28}

NASA's Plant Growth Unit (PGU) is currently (and appropriately) viewed as a prototype piece of hardware that must be evaluated and upgraded in a step-wise fashion as more and more experience is gained with it. To that extent, the PGU will evolve as information on its performance increases. For the present, it is best viewed as useful for the growing of a range of small plants in space, although it was originally designed as an experiment-specific instrument.²⁷ Availability and access to the PGU dictated that an experiment like "CHROMEX" be used to assess its performance after being upgraded with a new air exchange system (AES).^{54,55} The PGU was originally designed to accommodate a closed system growing environment without air flow.²⁷ The AES modification enables middeck cabin air to be filtered, conditioned, and rendered free of contaminants such as microbes, ethylene, and a variety of organic compounds prior to being passed through the individual plant growth chambers (PGCs).⁵⁴

Oats and sunflower were abandoned because performance of seedlings in the period anticipated for an experiment would be compromised.⁵⁵ The dicotyledon *Haplopappus gracilis* (Nutt.) Gray (Family Asteraceae) was adopted as a substitute for sunflower. It is a flowering plant which is unique in that it has only 4 chromosomes in its diploid state ($2n=4$). As such, it is a very useful plant for studies on chromosome morphology and behavior. Moreover, we have developed an in vitro system for culturing *Haplopappus* that permits several distinct phenotypes of plantlets to be grown.⁵⁶ In addition, the monocotyledon daylily (*Hemerocallis*) ($2n=22$) was chosen, because it also has special karyotype features, and considerable tissue, cell, and protoplast culture technology has been developed for this species in our laboratory.²¹ The two plants thus represent a dicotyledonous and a monocotyledonous species, which serve as models for the development of experimental systems for growth and development studies under microgravity conditions.

The CHROMEX experiment employed, for the first time in a space-based experiment, tissue culture-derived plantlets (for both species). In addition, for *H. gracilis*, comparably sized seed-derived plants, described below, were included in the experimental protocol. In all cases the experimental plants began as fully differentiated individuals, complete with leaves, shoots, and roots, which had been maintained under aseptic conditions from their initiation. The use of fully developed plants was a critical aspect of this work which necessitated a procedural protocol more complex than that required for investigations employing seed germination and the subsequent early development of seedlings.

Figure 5 provides a partial sequence involving the use of daylily. Morphogenetically competent cells are induced to generate plantlets by means of a three-step procedure. Cells maintained with auxin and cytokinin are selected and maintained by serial transfer every 21 days in a maintenance medium.²¹ In this medium, no organized development ensues. When the auxin level is reduced or eliminated by repeated washing, and an aliquot of the suspension is transferred to a fresh auxin-free medium, a gradual transition is achieved during which the units become determined; i.e., they embark upon a route of morphological organization. The first

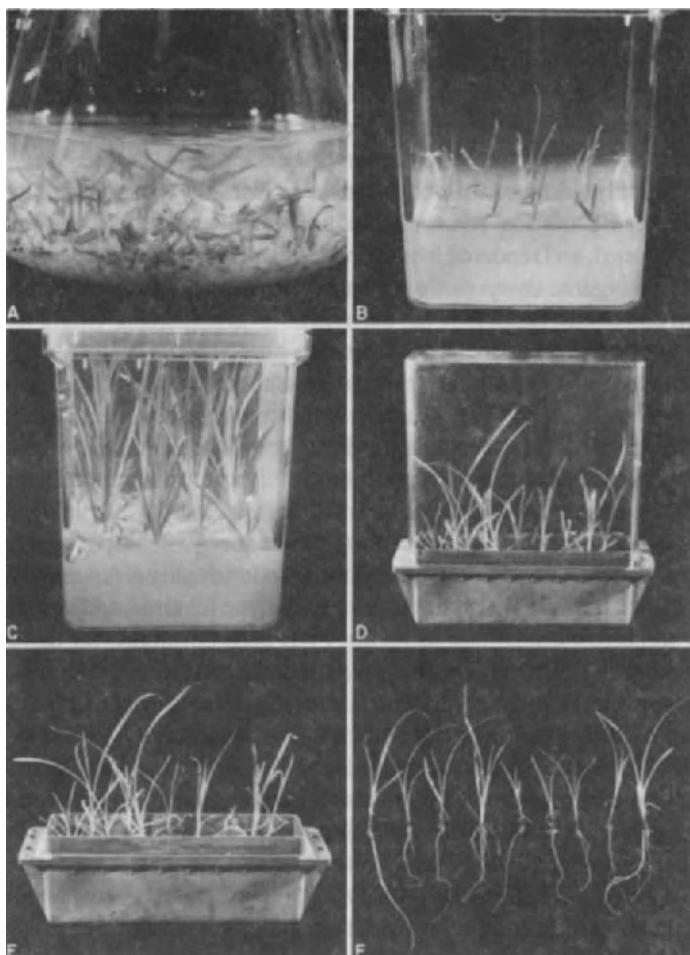


Figure 5. Use of daylily plantlets for the study of growth in space. Plantlets derived from cell suspensions and their mode of use for the study of growth (esp. roots) in plant growth chambers (PGCs). **A**, close-up of liquid medium-grown shoots in erlenmeyer flask; **B**, shoots transferred from environment of **A** and placed on semi-solid agar nutrient medium to foster further growth; **C**, shoots on semi-solid agar medium after 3 to 4 weeks of growth; **D**, plantlets whose roots had initially been severed grown in a plant growth chamber. Note the lexan lid. (The PGC is approximately 260 mm high x 195 mm wide and 55 mm deep.) Here no air exchange system "tubes" are in place, i.e. the system is sealed; **E**, PGC with lid removed; **F**, rooted plantlets removed from PGC base and lined up.

visible signs of such organization entails the formation of so-called pre-shoots. The continued development of shoots without concurrent formation of roots provides a means of handling rootless propagules (cf. Figs. 5A and 5B). If larger plantlets are wanted, however, they must be allowed to grow, and at this point roots form (Fig. 5C). For the "CHROMEX" experiment, roots are carefully severed and "shoots" are "planted" in special nylon inserts,⁵⁷ which are in turn introduced into a foam substrate that is "watered" once with a nutrient medium. Root formation ensues in a few days depending on clone, degree of root severing or trimming, nutrition, etc. The same holds true for *Haplopappus*.⁵⁷ Figure 6 provides a cutaway view of a plant growth chamber with special emphasis on the air exchange tubes, the foam insert/support medium (labelled "root cube" in the diagram), and the nylon (Nitex) netting which precludes growth of the roots into the foam substrate during the mission.⁵⁷

It will be appreciated from the above that "CHROMEX"-type experiments can have both broad and more narrowly defined objectives. It will also be clear that the execution of a test utilizing two species, both derived and maintained by aseptic plant tissue or cell culture procedures, requires a substantial amount of work.

While it is more difficult to cultivate plants aseptically, the required effort is justified based on the following considerations. First, it is potentially detrimental to release species of microbes into a space habitat without fully understanding their control systems. Mutated forms could devastate attempts to cultivate plants, whether the objective is experimentation, food production, or recycling. Even the crew could be adversely affected. Second, with asepsis, experimental results are much more assessable and reliable. The complication introduced by the presence of organisms other than those of interest is not a trivial one. An added advantage of the use of aseptic propagules is that data and experience for implementing modern cell culture and genetic engineering-based biotechnologies in space can be acquired.

For *Haplopappus*, three distinct subpopulation types were employed: (1) *Haplopappus gracilis* capitulum-derived plantlets (HGCP), (2) *Haplopappus gracilis* apex-derived plantlets (HGAP; strain KH-1) which were derived from an undifferentiated callus stock initially supplied by Dr. R. Tanaka, Laboratory of Plant Chromosome and Gene Stock, Hiroshima University, and (3) two seedling clone lines initiated from pre-sterilized germinated seeds (HGSC). In all cases, these different populations, once initiated, were maintained and multiplied by the severing and subsequent planting of apices (at 3 to 4 week intervals) in Magenta culture vessels containing full strength Murashige and Skoog medium (3% sucrose).

We have published our procedure for initiating shoot cultures *in vitro* from *H. gracilis* capitulum tissue.⁵⁶ All plant growth chambers (PGCs) had one planting slit which received five individuals from this HGCP population. Each PGC had a second planting slit which received five individuals from the apex-derived plantlet population (HGAP), and a third which received five individuals from the sterile seedling clone populations (HGSC) discussed below. The fourth planting slit from each PGC received five individuals from the daylily plantlet population (DLP).

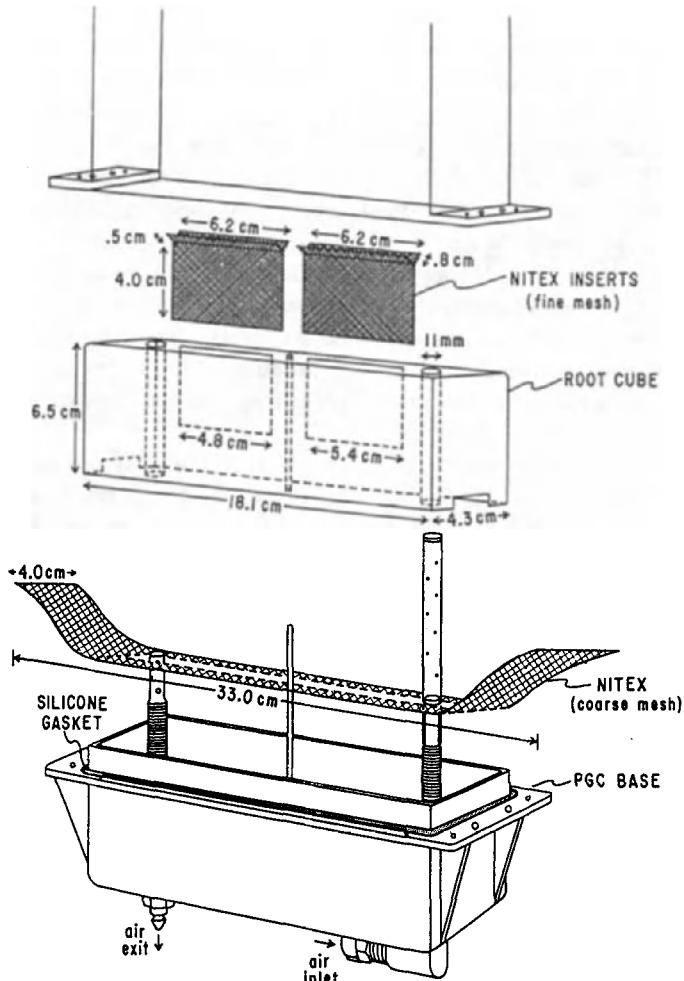


Figure 6. Plant growth chamber assembly. The figure shows how plantlets are supported in a substrate which permits their growth under aseptic conditions. The nylon inserts permit easy introduction of plantlets and prevent roots from growing into the substrate. A coarser mesh nylon "liner" permits the block and plantlets contained therein to be lifted out without damage. See text for details.

It is quite interesting to compare the tissue culture-derived plantlets and their seed-derived counterparts. This is of particular importance in the CHROMEX project because of the phenomenon of somaclonal variation, in which tissue culture strains experience what are presumed to be increased rates of DNA alteration in culture¹⁶ (and references there cited). We therefore established sterile seedling clones from the aseptically germinated and maintained seeds of *H. gracilis*.

Technically, the germination units for *Haplopappus* are achenes, but for the sake of simplicity we shall refer to them as seeds. Two lines of seedling clones were employed, each being derived from a single seed. One line (HGSC-C) went into PGCs 2, 3, 4, and the second (HGSC-D) into PGCs 5 and 6.

CHROMEX was launched on March 3, 1989 on the STS-29 mission (Discovery) as a middeck locker experiment. The details of the flight environment have likewise been presented.⁵⁴ Observations on root formation and growth in *Haplopappus* have been published.⁵⁷

Although a detailed account is not possible here, the following points may be made. CHROMEX was designed to determine whether cytologically "normal" roots of daylily and *Haplopappus* could be initiated from rooted shoots which had been trimmed on Earth. Prior to insertion into the PGCs, DLP shoots were trimmed to a uniform length of 4 cm and the roots severed at about 1 cm from the shoot base. Shoots were not trimmed for the *Haplopappus* subpopulations, but the roots of the HGCP and HGSC populations were severed to a length of about 1 cm. Individuals from the HGAP population were not subjected to trimming of any kind. They therefore functioned as non-wounded controls, and also guaranteed the presence of at least some root tips containing cells which would have undergone mitotic divisions under microgravity conditions if the mission would have been terminated prematurely during the first three days. The method of trimming the pre-formed roots resulted in the production of numerous roots (up to 50 per plant) from the *Haplopappus* test populations within the brief flight period (5 days) allotted. Since daylily does not grow roots as vigorously in that time span, fewer roots were generated.

Asepsis was maintained throughout both the STS-29 spaceflight and the ground control experiments. New roots were obtained from both pre-existing roots (severed at the time of insertion) and from the lower portions of the shoots of the *Haplopappus* populations. Root growth occurred randomly in all directions under flight conditions.³⁴ By contrast, root growth was uniformly positively gravitropic in ground controls.

A 20% subset of the HGCP and HGSC populations was used to generate data on the number and length of roots obtained during the five-day mission.⁵⁷ Several individuals initiated flower bud development during the mission. As is the case on Earth, this resulted in both fewer and shorter roots in comparison with their vegetative cohorts. Interestingly, both the HGCP and HGSC populations produced equivalent quantities of root tissue when compared to each other during both the flight and ground control experiment; that is both populations were equally affected by conditions existing in the flight- and ground-control experiments. Yet this result was clearly achieved by two different strategies. The HGSC individuals produced more roots per plant than the HGCP plantlets, but the individual roots produced by the HGCP population were longer.⁵⁷ These root production characteristics have consistently been observed for these two population types, both in space and on Earth. As a result of this "trade-off" between root number and root length, both

populations produce equivalent quantities of root tissues. This indicates that biomaterials with specific clone characteristics can be selected for use in space experiments according to perceived needs. It also provides an explanation for some of the differences in observations reported in the literature.

An unexpected result was that for both populations (HGCP and HGSC), overall root production was 40 to 50% greater under flight conditions than in the ground control tests. We speculate that within the foam-based growing environment and experimental set-up used, the condition of microgravity resulted in a more "moist" (yet still aerobic) environment in the vicinity of the roots under flight conditions. This results in a more favorable environment for root formation,⁵⁷ yielding enhanced root production.

It is not surprising that in the absence of a gravitational force, which pulls moisture downward within the cultivation substratum, the upper portions would experience higher moisture levels than their ground-control counterparts. Indeed, others have also commented on this space-related phenomenon,²⁶ (also refer to Samilov cited in ref. 2). The paucity of root hairs from oat seedlings subjected to flight conditions (see Section IV) may conceivably have resulted from such an elevated moisture artifact during that experiment.

The short duration of the STS-29 mission necessitated our primary reliance upon pre-formed root primordia to give rise to roots during the five-day mission. These pre-formed, but quiescent, primordia lie within the interior of the roots severed at the time of plant insertion into the PGCs. The severing process released them from a root tip-based apical dominance relationship, and they proceeded to develop into lateral roots sprouting forth from the severed parental root. Although we obtained new primordia within roots formed by these pre-existing primordia, this did not occur until the end of the mission, and only sparingly so. Consequently, the population of roots formed during the mission was largely a function of the population of root primordia existing at the start of the experiment. The tendency for the capitulum-derived *Haplopappus* population to form fewer roots may in fact reflect a clonal disposition toward producing fewer root primordia in comparison with the seedling clone populations employed.

A related phenomenon is the occurrence of very few roots from the flight material in the small 1 to 5 mm-long size class. This size category represents the newest or most recently produced roots. Yet, in the ground control experiment, a more normal complement of roots of this size class was present.

To explain this result we refer to our speculation that the altered moisture distribution pattern within the foam substratum results in a more favorable rooting environment under spaceflight conditions, plus the fact that the population of roots produced is primarily a function of the number of pre-existing primordia. The population of root primordia would therefore burst forth as a pulse with something approaching a normal distribution pattern; that is few in the early phase of the experiment, most during the middle part of the flight, and few in the final stage of the mission. In fact, this was the pattern obtained if root length can be taken as an

indication of when the roots sprouted. Earth-based experiments have confirmed that this assumption holds for experiments of this duration, with the early formed roots attaining the longest lengths by day 5, and the newest roots being the shortest present. We therefore submit that the paucity of smaller roots in the flight experiment was a result of the fact that all pre-existing root primordia had already burst forth earlier in the mission. And the slightly less favorable rooting environment within the ground control experiment (due to a lower moisture level in the vicinity of the roots) delayed the pulse of primordia-originated roots sufficiently to give more roots in the 1 to 5 mm-long size class at the time of mission termination.

This phasic production of roots could be used to advantage in a longer duration mission. Not only would roots derived from pre-existing primordia be obtained, but an additional pulse of roots derived from totally space-originated primordia would become available. Thus, the objection to any Earth-based origin of the roots could be eliminated.

At the end of the experiment the percentage of cells in division in the roots of the flight materials and their ground control counterparts was determined for each of the species and clonal populations used: daylily (DLP), *Haplopappus* seedling clone (HGSC), *Haplopappus* apex-derived plantlets (HGAP), and *Haplopappus* capitulum-derived (HGCP) plantlets. Recall that cytological fixation was carried out in such a way that the cells were caught in their first division cycle on Earth after the period in space. In every case, there was a smaller percentage of root tip cells dividing in the flight material than in the ground controls (Table 2). This was true regardless of the degree of shoot growth achieved.

There was a correlation between increasing levels of chromosome damage (both in prophase and metaphase), and a decreased number of cells found dividing at the time of fixation on Earth. Also, in all three *Haplopappus* populations, roots from flight samples showed an increase in the number of cells in prophase relative to those in metaphase. The apparent accumulation in space of root cells in prophase was reflected by a ratio of cells in division of some 40% in prophase to 60% in

Table 2. Effect of Spaceflight on Cell Division in the CHROMEX Experiment

	<i>Percentage of Cells Dividing</i>	
	<i>Flight Specimens</i>	<i>Ground Controls</i>
Daylily plantlets	3.6	5.6
<i>Haplopappus</i> seedling clones	4.1	6.7
<i>Haplopappus</i> apex-derived plantlets	3.3	5.4
<i>Haplopappus</i> capitulum-derived plantlets	3.6	6.1

*CHROMEX was launched on March 3, 1989 on the STS-29 mission.

metaphase, whereas in ground controls, the comparable ratio was 20% in prophase to 80% in metaphase. This indicates that some factor in the space environment prevented or slowed down the progression of dividing cells into metaphase. In daylily, the increase in cells in prophase in the flight samples was not seen.

In all three *Haplopappus* populations and in daylily, the plantlets in the third plant growth chamber (PGC 3) showed a greatly diminished (about 90%) amount of both metaphase and prophase damage. To date, we cannot explain this finding, but it suggests that there was a subtle difference in the environment of PGC 3 and PGC 2, 4, 5, and 6, which might provide a clue to our understanding of the observed chromosomal changes. It also suggests that countermeasures might eliminate or at least minimize the adverse chromosomal changes.

VI. CONCLUSION AND SUMMARY

From the foregoing, it will be apparent that no clear answers can be provided at this time as to what is happening in these systems. Flight opportunities have been very limited and the means for on-board fixation have not yet been provided. Clearly, this is needed to resolve the outstanding questions. One can cite many references which either confirm or contradict our findings^{4,20,36,51} (also references cited in ref. 51). However, we hope that our attempts to call attention to the opportunities for significant and reliable experimentation in the space environment will be appreciated.

Some discussion of the two major factors—radiation and gravity—that may explain the observed chromosomal changes resulting from spaceflight is in order. In addition, some other factors that might play a role will be considered.

A radiation measurement package, developed by Dr. E. Benton (Eril Research, Corte Madera, CA), was placed inside the plant growth unit (PGU). Figure 7A presents data obtained from two thermoluminescent detectors (TLDs) contained in this package, and from two TLDs contained in Ground Movement Units (GMU). The doses recorded for the flight samples have been adjusted for background. The backgrounds are given for the Ground Movement Units. Dr. Benton has interpreted these results as being typical of the level expected for a shuttle flight of the duration and orbital characteristics of the STS-29 mission.⁵⁸ In addition, data from Passive Radiation Dosimeters located at six locations in the orbiter were averaged and compared with similar data collected from three other shuttle flights to yield the data presented in Figure 7B. Of the four missions, the STS-29 mission had the lowest daily dose level. These levels would not seem to be sufficient to account for the degree of chromosomal damage observed.³⁹⁻⁴¹ Therefore, unless there is some major synergistic interaction between the microgravity environment and low levels of radiation,^{50,59} we may conclude that it appears unlikely that the chromosome effects are due to radiation damage alone.⁶⁰

Next we discuss the possibility of a role of gravity effects. An attempt was made

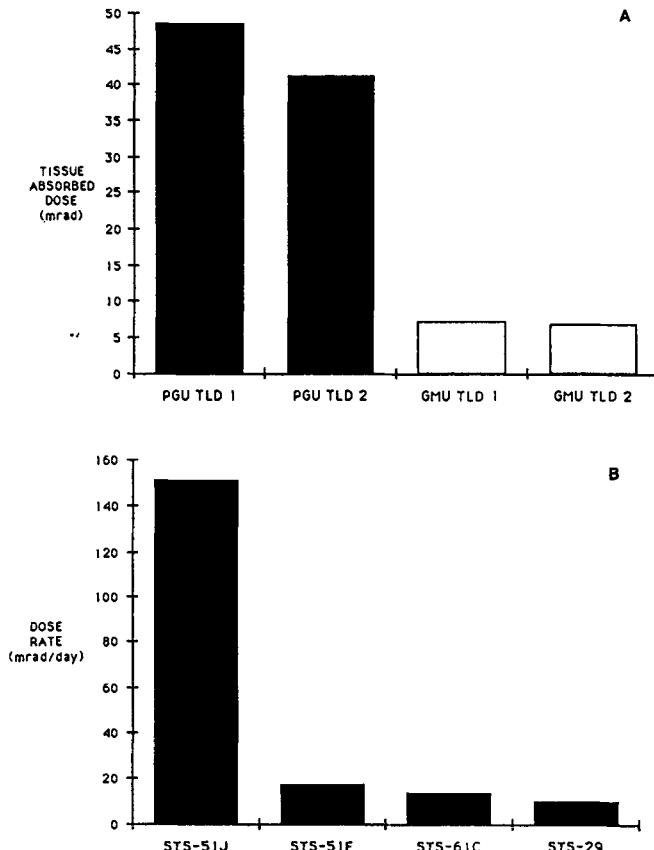


Figure 7. Graphic representation of radiation encountered in various Shuttle flights. **A**, STS-29 radiation as measured by thermoluminescent detectors (TLD) contained in the air exchange system package of the plant growth unit (PGU) during flight and within the so-called Ground Movement Unit, GMU. (The GMU is a near-synchronous ground control package that travels with the "experimental" radiation detectors). The TLD doses have been adjusted for background levels (see text); **B**, mission radiation data comparisons from *Shuttle* flights STS-51J, STS-51F, STS-61C, and STS-29 (the mission that carried CHROMEX). Numbers are means obtained by averaging values from passive radiation dosimeters at 6 locations within the orbiter. Data graphed from information presented in CHROMEX Radiation Report by Dr. Eugene Benton.

to measure the microgravity levels in the middeck area of the shuttle during the STS-29 mission. Unfortunately, the deployment of the microgravity measuring device was not successful, so we do not have any measure of the G environment experienced by our samples. The shuttle is generally rated as having a 10^{-4} or 10^{-3} G environment in orbit.^{1,5} It is regrettable that measuring and recording G levels

for individual flight packages in the shuttle is not yet a routine procedure. Far too little attention has been given by any experimenters to measuring the "real" G levels experienced by their flight samples. After all, the capability of achieving sustained microgravity is *the* major attraction for experimentation in space. Even a quick perusal of the literature indicates that this important area is overlooked more often than not. As a consequence, we cannot eliminate the possibility that our samples underwent periodic and significant G "shock" as a result of crew activity⁵ (and references there cited).

In this connection, it is regrettable that there has not been the possibility to have 1-G controls during the spaceflight, because appropriate 1-G control centrifuges have not yet been developed. Thus, we can only speculate that it is microgravity, and not any other feature of the space environment, that is responsible for a given set of observations and measurements.

Another limitation is that fixation in space was not possible. All data referred to in this paper relate to counts and observations performed on flight samples that were processed or fixed on Earth after return, and not in space during the flight. This major shortcoming cannot be ignored, even though the cells scored represent progeny of mitotic divisions which occurred in root tips of plants grown aseptically in space. However, we cannot exclude the possibility that the observed cytological aberrations reflect a phenomenon related to a re-entry disturbance or to the re-adaptation to Earth gravity.⁶¹ The alternative explanation that the observed changes are due to an enhanced sensitivity response of microgravity-exposed cells to colchicine treatment after return to Earth can be eliminated, since similar nuclear aberrations were found in some samples fixed immediately after recovery in acetic acid/ethyl alcohol, a procedure that does not utilize colchicine to achieve metaphase arrest.

The preponderance of the data thus far available suggests that space can cause significant chromosome damage in plant cells. For the time being, we prefer to interpret these effects as being the result of a common cell disturbance, perhaps with a sharp threshold, and that this disturbance is capable of differentiating between genetically equivalent but somehow different mitotic and pre-mitotic cells. While several explanations are possible, it is not unreasonable to suggest that chromosomes and the spindle apparatus can sense gravity,^{14,15} and that certain cytoplasmic components are also capable of this.^{62,63} However, it is too early to be certain about what can or cannot happen at the single cell level.⁶⁰

There is also evidence that certain perturbations in hormone levels can lead to chromosome aberrations. These hormone interactions may be dependent on the genotype of the experimental specimen. It has even occurred to us that utilization of aseptically cultured propagules may somehow yield data that might be different from what might happen in intact systems. The use of an unsevered control population (HGAP) permits us to address the question of whether the wounding component of the sample preparation procedure may have contributed to: (1) the lower level of cells in division, (2) the shift in percentage of cells in prophase versus

metaphase, and (3) the increased levels of chromosomal aberrations. In addition, the inclusion of two seedling clone lines permits us to address the question of whether tissue culture-derived systems are more susceptible to these disturbances than their seed-derived counterparts. All three of these phenomena were observed within each of the three *Haplopappus* populations regardless of whether they originated from tissue culture procedures instead of seeds, or whether they were wounded or not.

Only future space experiments under carefully controlled conditions will allow us to resolve these interesting and important questions. We cannot be confident that even the simplest questions about assuring satisfactory plant growth in space have been adequately addressed. Even so seemingly simple a task as providing a means of reliable water and nutrient delivery has not yet been resolved.^{5,64} Successful completion in space of a life cycle of a higher plant (*Arabidopsis thaliana*) has, in fact, been reported, but critical events in the cycle seem to be slower than in ground control plants, and certainly the vigor of the plants raised in space leaves much to be desired.^{4,65-67} Even this level of success has not been achieved without substantial difficulties.^{4,66-70} All this should have heuristic value and serve as an encouragement to those seeking to utilize the unique characteristics of the space environment to probe the secrets of plant life. Hopefully, there will be sufficient resolve to rise up to the challenge.

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MECHANISMS OF GRAVITY EFFECTS ON CELLS: ARE THERE GRAVITY-SENSITIVE WINDOWS?

D.A.M. Mesland

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I. INTRODUCTION

The ESA *Biorack* experiments performed during the German *Spacelab D-1* flight in 1985 were the first biological experiments flown under extremely well controlled conditions during all phases of a spaceflight mission. Most important were the on-board 1×G control centrifuges, located inside the *Biorack* incubators, and the synchronously performed ground control experiments in an identical flight-qualified *Biorack* unit. These guaranteed that scientific results would stand up against criticism on methodology, which most previous experiments had been sensitive to. Although many more cell biological experiments have been performed in Russian and American spacecraft, and in European sounding rockets, none of these were designed to provide unambiguous proof that weightlessness directly affects cellular functions.

Therefore, the striking effects found on the functioning of both prokaryotic and eukaryotic cell systems, reported in ESA SP 1091,¹ had a serious impact on the current thinking about the significance of gravity for nature's basic living processes. Also earlier results in space biology, which had been puzzling, but were open to a variety of explanations due to lack of proper controls, now seemed to be of more interest. The message, evolving from the total of results, appeared to be that gravity plays a role in the biological functioning of single, eukaryotic and prokaryotic cells.

II. GRAVITY EFFECTS ON SINGLE CELLS

The effects of spaceflight observed in various types of microorganisms and single plant and animal cells have been reviewed extensively by Cogoli and Gmünder.² The main effects are summarized in Table 1. No satisfactory explanation for these effects has been offered so far. It was realized that gravity could act: (1) directly by means of cellular organelles or macromolecules serving as gravity receptors; or (2) indirectly by inducing an adaptation provoked through physicochemical changes in the cellular environment. These two approaches will be discussed in the next two sections.

A. Gravity Receptors

Nace³ considered whether the cytoskeleton might act as a gravity receptor in cells. He calculated the torque imparted on a cell with a diameter of 6 μm by a sedimenting starch granule and a buoyant oil vacuole suspended in the cytoskeleton. The torque L is defined as:

$$L = F d \sin Q,$$

where F is the force (weight), $d \sin Q$ the length of the lever arm, and Q is the angle between F and d . He calculated a value of $L = 2.5 \times 10^{-13}$ dyne cm. On the other

Table 1. Summary of Effects of Spaceflight on Single Cells

<i>Effect</i>	<i>Organism</i>	<i>Mission</i>
Decreased glucose consumption	WI 38 cells	<i>Skylab-3</i>
Increased proliferation	<i>Proteus vulgaris</i>	<i>Soyuz-12</i>
	<i>Bacillus subtilis</i>	<i>Spacelab D-1</i>
	<i>Chlamydomonas</i>	<i>Spacelab D-1</i>
	anise plant cells	<i>Spacelab D-1</i>
	<i>Paramecium</i>	<i>Spacelab D-1</i>
Enhanced conjugation	<i>E. coli</i>	<i>Spacelab D-1</i>
Increased interferon production	lymphocytes	<i>Spacelab Salyut-6</i>
Reduced mitogenic activation	lymphocytes	<i>Spacelab-1</i>
Increased resistance to antibiotics	<i>E. coli</i>	<i>Spacelab D-1</i>
	<i>Staphylococcus aureus</i>	<i>Salyut-7</i>
Reduced cell aggregation	lymphocytes	<i>Spacelab-1</i>
	erythrocytes	<i>STS-26</i>
Reduced receptor-mediated gene expression	lymphocytes A431 cells	<i>Biokosmos-9 Maser-3, -4</i>

hand, the force developed by a bundle of 6 microtubules was found to be 10^{-6} to 10^{-5} dyne, which applied to a lever arm of 6 μm yields a torque of 5×10^{-9} dyne cm. Thus, the torque imparted by the starch granule and the oil vacuole appears to be small compared to that which a few microtubules can produce.

Lorenzi and Perbal⁴ have provided experimental evidence that in plant statocytes in the root cap of lentil seedlings the cytoskeleton does contribute to gravity perception. At $1 \times G$, the position of the nucleus is near the proximal cell wall (proximal means further away with respect to the root tip). Upon centrifugation at $19 \times G$ to $41 \times G$, the nucleus is displaced towards the distal cell wall, indicating that the nucleus is sustained by a network of actin filaments. When the polymerization of actin filaments is inhibited by means of cytochalasin B, the nuclei sediment already at 1 G on the distal cell wall. In a control experiment in the slowly rotating clinostat, using cytochalasin treated seedlings, there was a bimodal distribution; about half of the nuclei remained near the proximal cell wall, the other half was close to the distal cell wall. This means that in plant cells the nuclei and the cytoskeleton appear to act as gravireceptors in addition to the amyloplasts.

However, these observations have been made in cells in intact tissues of whole plants which are specialized to perceive the direction of gravity. Yet, in single cells such as bacteria, fungi, and animal cells, such capabilities have never been reported. Nevertheless, this does not prevent such cells from responding to changes in the direction and magnitude of the gravity vector. It indicates that direct effects through intracellular gravity receptors cannot satisfactorily explain the effects of gravity on most single cells.

B. Physicochemical Effects

Lack of sedimentation and thermal convection in microgravity may lead to the formation of stationary films (boundary layers) around the cells, resulting in concentration gradients for nutrients, oxygen, and waste products. When the diffusion rate for oxygen and nutrients is much smaller than their normal uptake rate, cell metabolism may be markedly changed. For example, the yeast, *Saccharomyces cerevisiae*, maintains oxidative breakdown of glucose at low growth rates, but with increasing growth rate it switches from oxidative to oxido-reductive glucose breakdown with production of ethanol. The growth rate at which this switch-over occurs is extremely sensitive to oxygen limitation.⁵

On the other hand, non-motile cells will be free-floating in microgravity, whereas at normal 1-G gravity they tend to sediment and aggregate in pellets. In the latter case, the supply of oxygen and nutrients will be inadequate and waste products may accumulate near the cells. This might reverse the changes of cell metabolism and behavior mentioned in the preceding paragraph.

Lack of sedimentation and convection in microgravity may reduce cell aggregation in primary, diploid cell lines and human lymphocytes, as has indeed been observed (Table 1). This might explain the reduction in lymphocyte activation found in microgravity, since there is evidence that activated lymphocytes need to anchor and spread in order to achieve an optimal proliferation.² However, virtually normal cell-cell interactions were observed in electron micrographs of lymphocyte cultures fixed during spaceflight, which implies that other factors than cell-cell contacts prevail in affecting lymphocyte responsiveness.²

The above considerations suggest that it seems unlikely that indirect effects through physicochemical changes in the cell environment can explain the effects of gravity on single cells.

III. DIRECT OR INDIRECT EFFECTS

Several arguments can be adduced against the proposition that gravity directly affects the cellular machinery. The first argument is based on the relative importance of forces inside a typical biological cell, as shown by Nace³ and others. It appears that gravity is orders of magnitude weaker than the forces that govern the macromolecular interactions.^{6,7} Obviously, this argument does not explain why effects have been found nevertheless. Such explanation is provided by at least two arguments. One view rightfully notices that in almost all cases studied so far, influences attributed to microgravity in reality are influences of at least two space-related factors: microgravity and space radiation.^{8,9} Cell changes would be caused primarily by radiation effects, which then somehow would be enhanced, or made manifest by microgravity in a two-step process. Only space experiments in

a completely shielded environment (around 10^3 g/cm²), which would have radiation comparable to that on Earth, could verify that proposition.

The other argument, already discussed in the previous section, assumes that gravity does not directly influence the cellular machinery, but exerts its effect on the bulk volume of the surrounding medium. Gravity-induced convection flow at the cellular boundary would normally support the proper influx of nutrients and oxygen, and the proper efflux of cell metabolites. When in microgravity such a flow is abolished, this could indirectly affect cell membrane properties, and lead by a sequence of amplifying effects to changes in cellular function.⁷

Obviously, it is of considerable scientific importance to know whether the cellular machinery itself is directly sensitive to gravity and is not indirectly reacting to a gravitationally changed chemical environment. In the case of a direct effect, gravity would be a fundamental factor in the physiology of the cell, at least at a particular moment of cellular function. Microgravity would then be an important tool for the study of the mechanism of this gravitational effect. Apart from the immediate consequences for manned spaceflight, new insights in the function and evolution of molecular systems in the cell could be expected. In the discussion that follows, it is shown that the gravity vector may provide signals to the cell that cause it to assume certain meaningful states. In addition, these signals may only be required during certain limited periods in the life of the cell; windows in the state-transition period of the cell. Research methods are proposed that could reveal these gravity-sensitive windows in investigations on the ground.

IV. ACTIONS OF GRAVITY

As has been discussed elsewhere^{10,11} and is shown in Figure 1, actions of gravity on cells that have taken place during evolution will have become manifest in the genes of living systems. Genes code for the basic form and function of the organism, "expecting" gravity to be present: up-down asymmetry, structural strength, size of force-producing elements, and sensory systems to determine the vector of gravity. Although environmental influences may eventually shape the ultimate detailed form of an organism during ontogenesis and morphogenesis, its basic form is established by the execution of the genetic program. Consequently, it cannot be expected that the basic three-dimensional morphology of any organism would be different whether it developed in normal gravity or in weightlessness, provided that the execution of the program is not gravity dependent. This expectation seems to be confirmed by experiments on developmental biology and plant morphogenesis,¹²⁻¹⁶ although differences at the level of single cells in a tissue do occur that cannot be easily explained.¹⁷

Real time actions of gravity on living organisms may become manifest via sensors in that organism, which either exist specifically to determine the gravity

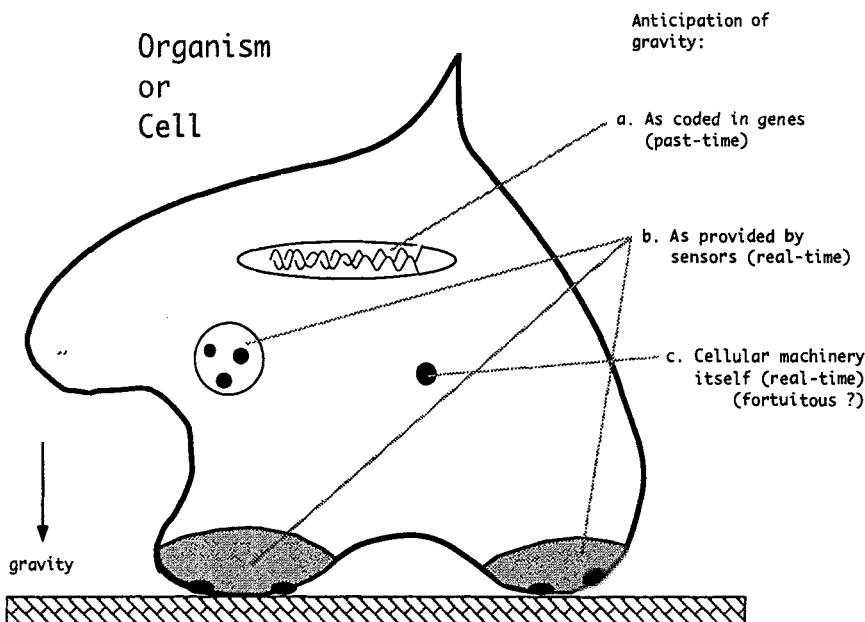


Figure 1. Actions of gravity on organisms. Schematic organism to illustrate its anticipation of gravity: (a) Level of genes, coding for the organism's morphology and structural strength. Genes represent the evolutionary past actions of gravity; (b) Level of sensors, coded for by genes, but providing real-time data on gravity; (c) Level of cellular machinery, also coded for by genes, but utilizing real-time gravity actions for the proper functioning of cellular systems.

vector (otolith system, statocyte system), or which are non-specifically contributing to the organism's information on gravity (muscle tension sensors, pressure sensors).

In single cell organisms no organelles or molecular complexes for the specific sensing of gravity seem to exist. Manifestations of geotactic behavior by these organisms may in all probability be caused by a combination of cellular mass distribution, swimming mechanism, and community effects (bioconvection phenomena^{18,19}). However, the possible existence of a gravity sensor in single cells cannot be excluded on the basis that mass differences at the subcellular level present forces that are negligible compared to other forces inside the cell. Gravity-induced movements of amyloplasts in plant root statocytes apparently provide, at least in community, sufficient information for the root to react to a changed gravity vector (for a review, see ref. 20). Thus, even if the force produced by gravity inside a single cell is orders of magnitude weaker than those that control biomolecular interactions, specialized mechanisms appear to be able to amplify this weak energy to above the level of random thermal energy. If this is the case in the statocyte, may this also

happen in cells that are not specialized in gravity detection? Could there be "fortunate" gravity sensing by cells, a term introduced by Todd⁶ and further elaborated by Albrecht-Buell in his stimulating article on the subject,⁷ which are direct rather than indirect? Indeed, could certain cellular processes anticipate the vector of gravity needed for the proper functioning of the cellular machinery?

V. AMPLIFICATION AND BIFURCATION

The answer to these questions depends on the probability that systems exist in the cell which are able to specifically amplify the relatively weak gravity signals to above the level of thermal noise. Such amplification could be achieved in a number of ways, some of which will be discussed here.

A. Amplification through Focusing

Amplification may take place through the accumulation of the same type of signals in a small space, like in the focusing of light. Such focusing occurs on many levels in biological systems. An example is the mammalian ear, where the structure comprising the tympanic membrane, the malleus, the incus, and the stapes, focuses sound waves into the inner ear. On the cellular level focusing may for instance occur through a leverage function of the cytoskeleton. For example, approximately 40 kcal/mol of energy can be generated by the translocation of a spherical nucleus with a relative density of 0.2 g/cm³ and a diameter of 7.5 μm over a distance of only 0.5 μm. This energy can be focused onto a few molecules in the plasma membrane (see Fig. 2). It can provide a signal per macromolecule which is approximately 60 times stronger than the random thermal energy. This situation can be achieved in any cell, provided that the cytoskeleton of the cell has the requisite dynamic properties and that the direction of the gravity vector is constant for a certain length of time.

Another possibility for focusing seems to be provided by cellular networks, in this case microtubules, which are in a state of continuous ATPase-mediated vibration.²¹ This state could be sensitive to minor changes in the spatial distribution of particles embedded in the network. A change in the gravity vector may then induce a different vibration pattern, which could become focused in a certain cell domain. Such molecular motor-driven oscillations of the cytoskeleton could explain the rapid response of the microfilament organization to microgravity observed in Chara rhizoids,²² as well as in cultured A431 epidermoid carcinoma cells.²³

Such systems would operate due to a unique circumstance which distinguishes the effect of gravity from other intracellular effects; namely its spatial unidirectionality over the entire volume of the cell, in contrast to the multidirectionality of other forces. This means that a certain time of acceleration in a constant direction would be required in order to manifest the effect of gravity as a signal inside the cell.

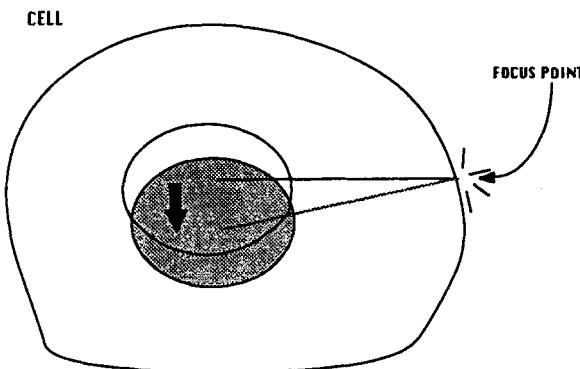


Figure 2. Focusing of gravitational energy. Presumed leverage action of the cytoskeleton. Energy gained by the translocation of a cell nucleus (or any other organelle in the cell) becomes significant only because of: (1) unidirectional action of gravity during a certain minimal time, generating a certain minimal displacement into one direction; (2) focusing, probably via the cytoskeleton, of this energy onto one or a few molecules of the biomolecular machinery (e.g., of the plasma membrane).

B. Amplification through Non-Linear Translation

Energy thus focused may then, for example, overcome the threshold for local polymerization of network proteins, or cause the opening or closing of ion channels in the membrane. Subsequently, this could lead to another type of amplification, one that involves translation steps. Here the amplification process leads to signals of a different kind than in the case of focusing. Generally it is brought about by a non-linear dynamic system that consumes energy to produce the amplification. A typical example is the receptor-mediated signal transduction in cells (see Fig. 3). The signal presented by a growth factor molecule is of an entirely different nature and a much lesser magnitude than the signals channelled into the mechanism responsible for mitosis and cell division. Binding of the growth factor initiates a cascade of molecular interactions and feedback loops resulting in the highly complex process of cell division. There is no simple linear relation between the phenomena of receptor binding and cell division. The low energy involved in receptor binding is thus being translated into the relatively high-energy requiring process of cell division and growth, but the process depends on many factors that influence its occurrence.

Such non-linear amplification can also be triggered by non-specific signals. Thus, the local opening and closing of membrane ion channels or the onset of network filament assembly may, through a sequence of non-linear amplifications, become manifest at the cellular level as the "accelerated cell state". This state may be different at various gravity levels. Weightlessness, being an extreme condition, may

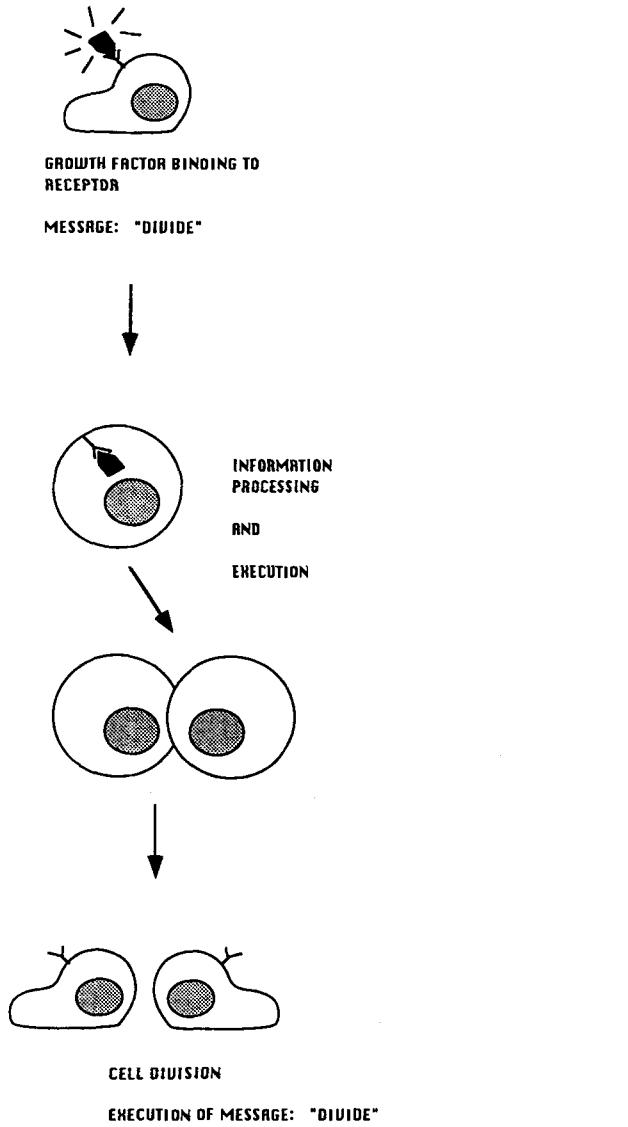


Figure 3. Amplification of signals in non-linear systems. Low-energy signals are translated and amplified into high-energy requiring effects. The low-energy binding of a growth factor molecule to its membrane receptor (signal) initiates a cascade of molecular interactions and feedback loops that result in the highly complicated and energy-consuming process of cell division (effect). This is information-processing and execution of the biological message "divide". There is no simple linear correlation between the two phenomena; it depends on many factors whether or not the process will occur and how it will occur. Non-specific, low-energy signals may trigger similar amplification mechanisms, causing cellular effects which have no apparent meaning.

therefore lead to states that could differ profoundly from those which are normal for Earth-bound conditions.

C. Bifurcation

Non-linear dynamic systems are the rule rather than the exception in biology. Recently it has been shown that such systems may themselves exist in a state of transition that renders them extremely sensitive to external forces. By their very nature they would be able to integrate a weak signal over time and make it decisive for the future evolution of the system.²⁴ During such a state of transition (e.g., in population growth or in weather development) the system may reach a bifurcation point, at which the signal determines which of two possible states the system will adopt. As has been argued earlier,¹⁰ it is not impossible that in certain state transitions of the cell gravity plays the role of a signal that drives the system into one of the two states. In normal gravity conditions, the other state would never be entered; it would be biologically meaningless. Obviously, the evolution of molecular mechanisms that produce meaningless biological states would have had no survival potential. It is presumed here that gravity always prevented these states from occurring. Thus, under normal Earth gravity conditions the state transition mechanisms that evolved were successful and met with a positive feedback since the gravity vector was always present. However, in the artificial condition of microgravity, the same, evolutionary-successful biological system may now reveal its intrinsic bifurcating character.

Figure 4 presents a hypothetical example of a membrane-associated microtubulin that polymerizes in response to the binding of a specific factor to its membrane receptors. Under conditions of Earth gravity, a certain level of binding would always lead to the required polymerization due to the effect of gravity at the presumed bifurcation point. In conditions of microgravity, however, no such effect would be sensed, and both branches of the bifurcating system may be entered with equal probability. Polymerization may or may not occur. Absence of polymerization, or even depolymerization, would be an erroneous response of the cell.

Should such systems indeed exist, then microgravity would have the effect of reducing a necessary signal and the cell could enter a meaningless state. Entry into a meaningless state could produce cells that would be totally disturbed in their normal biological functioning. It needs to be stressed that, per bifurcating event, this is just a chance, but the chance is equal to the chance that the event proceeds normally, as under normal gravity conditions. Hence, in the same sample of a space experiment cells could be found that are perfectly normal as well as those that are malfunctioning.

The result of the above discussion shows that, even if the force of gravity at the subcellular level is extremely weak by comparison with other forces acting on the cell, the spatial unidirectionality of gravity, the fine structure of the cell, and the non-linearity of most of its molecular systems may provide the amplification required to allow cells to sense gravity in a fortuitous, but direct way. It is even

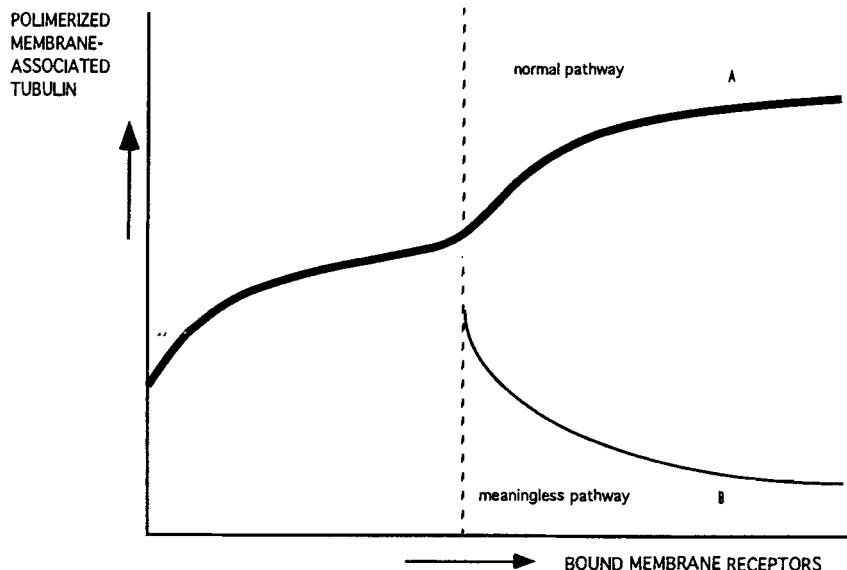


Figure 4. Hypothetical case of gravity-assisted non-linear state transition through suppression of bifurcation. After reaching a certain threshold number of bound membrane receptors, the system will result in polymerizing membrane-associated tubulin (pathway A), but only if the cell is in an accelerated state. Gravity provides a signal that results in pathway A to be followed, and no bifurcation can occur. Under similar conditions, but in microgravity, no signal will be available and the system can bifurcate. That means that either normal polymerization will occur (pathway A), or depolymerization may occur (pathway B).

possible that, rather than on fortuitous sensing, cellular functions may depend on the vector of gravity for proper execution. Key mechanisms would be focusing, non-linear amplification, and suppressed bifurcation.

VI. EXPERIMENTAL TOOLS

How could direct sensing of gravity by cells be discriminated from indirect effects, such as different local chemical composition of the medium, caused by the presence (Earth's gravity) or absence (microgravity) of convection flows, as mentioned in the Introduction?

A. Experimental Methods

There are a number of possibilities to test the hypotheses advanced above. In microgravity, a forced medium flow could be generated to adequately overcome

the absence of convection flows. The acceleration of cells due to the forced medium flow may be kept low and could be calculated for each system. If performed with the proper controls, such an experiment may be conclusive. Ideal for this purpose would be the use of a test system of adherent cells showing well-described effects in weightlessness. The A431 epidermoid carcinoma cells, which have been flown in the ESA CIS-1 module on the *Maser-3* and *Maser-4* sounding rocket mission, might provide the system of choice. EGF-induced *c-fos* gene expression in these cells has been found to be suppressed by about 50% in microgravity.^{25,26} Medium flows could be forced in this system without causing the cells to experience accelerations. It would provide an excellent test case to prove a direct action of gravity on cells.

Another approach to obtain a flow of medium along the cell boundary is the application of unicellular motile cells. This has already been accomplished in several experiments. Especially in the case of *Paramecium tetraurelia*, which swims by means of the coordinated beating of hundreds of cilia on its surface, it is most probable that an efficient exchange of nutrients and metabolites will occur at the cell surface. Nevertheless a pronounced increase in cell proliferation was found in weightlessness, but not in the inflight 1 × G control cultures.²⁷ This result provides rather strong evidence for a direct gravity effect on cells, although these large cells (approx. 100 × 50 µm) cannot be considered as typical examples of a biological cell. Indirect evidence for a similar effect of increased proliferation in weightlessness was found in the much smaller (approx. 10 × 8 µm) unicellular green algae, *Chlamydomonas reinhardtii*, an organism swimming with two flagella.³⁰

As discussed in the previous section, the action of gravity could be particularly important during state transitions of cells. Unicellular organisms, in comparison with multicellular organisms, normally exhibit a relatively small repertoire of cellular state transitions. Cell division, gametogenesis, and sexual interaction may appear to be the processes in which studies should be concentrated.

B. Gravity-Sensitive Windows and Appropriate Research Tools

Are there experiments that can be carried out on the ground? Interestingly, in proposing gravity sensing by cells via gravity-induced convection flows in the surrounding medium, or, to phrase it differently, microgravity sensing of cells through the absence of such flows, Albrecht-Buehler concludes that the rapid-rotating clinostat²⁹ does not provide an adequate simulation of microgravity;⁷ that is because clinorotation would induce even stronger medium flows. Occurrence of such flows have not been observed in video recordings obtained with the clinostat microscope (see also Todd³⁰ on this subject). However, if such flows would occur in the clinostat, then results obtained with this instrument that are comparable to those of real microgravity effects, should be considered even more seriously. The results may have to be explained by direct gravity sensing rather than medium-mediated sensing. It is premature to disregard the clinostat as a device for simulation

of microgravity just on the presumption that gravity does not act directly on cells. On the contrary, experimental evidence seems to support a positive relation between clinostat results (if they are different from $1 \times G$ controls) and effects observed in microgravity.^{11,26}

In Section V, it was concluded that candidate mechanisms of gravity sensing were focusing, via the structural networks of the cell, and suppression of bifurcation in non-linear state transitions. Both mechanisms would require a minimal time of unidirectional gravity. If this time were milliseconds, the clinostat may not cancel out the signal. If it were seconds to minutes, however, the fast rotating clinostat could potentially eliminate the gravity signal and hence provide a good simulation of microgravity. Interestingly, if minutes (or more) of unidirectional gravity would be required by the system, then slow to very slow rotating clinostats (rotational speeds equal or less than 1 rpm) would already be able to essentially cause effects that resemble those of microgravity (Fig. 5). In the so-called "tumbling table", described by Demets and Tomson,³¹ aggregates of mating *Chlamydomonas* cells were turned upside down once every 20 seconds, the effect of which appeared to be similar to the effect obtained in the fast (60 rpm) rotating clinostat. Another most interesting study by Gruener and Hoeger³² shows synapse formation to be inhibited in the clinostat both at slow and fast rotating speeds. Todd³⁰ has shown that these results can hardly be attributed to any other cause than so-called "non-vectorial gravity".

The approach discussed above, that gravity provides a vector signal of certain duration during particular transitions in non-linear dynamic cell processes, suggests the possible existence of short-duration windows, during which the gravity signal is required for the cell function to proceed (Fig. 5). These gravity-sensitive windows may have durations from milliseconds (molecular interactions) to hours (multicellular interactions). Indications for such windows have already been obtained in the first flight of *Biorack* on *Spacelab D1*: hatching of *Drosophila* larvae appeared to be affected only if the process of oogenesis occurred in microgravity.¹² Comparably, in the insect, *Carausius*, only exposure to microgravity of embryos in a well-defined early stage of development caused a 50% decrease in hatching rate of these animals, roughly 50 days after recovery.⁸ The length of these windows is not known but in this respect the 7 minutes of microgravity achieved in the sounding rocket flights are extremely useful and interesting. Biological data have already been obtained, showing that gravity-sensitive periods may indeed be as short as minutes.^{26,33} A pilot experiment on *Xenopus* development, performed in a sounding rocket by Ubbels, seems to suggest that such a short sensitive period may immediately follow *in vitro* fertilization of the eggs. Further development on Earth of recovered specimens resulted in aberrant larvae having badly-developed tail sections.³⁴

The above studies further reveal that cell biological or developmental biological experiments could be performed in a device that would only generate short periods of free-fall conditions. An existing example of such a device is the so-called

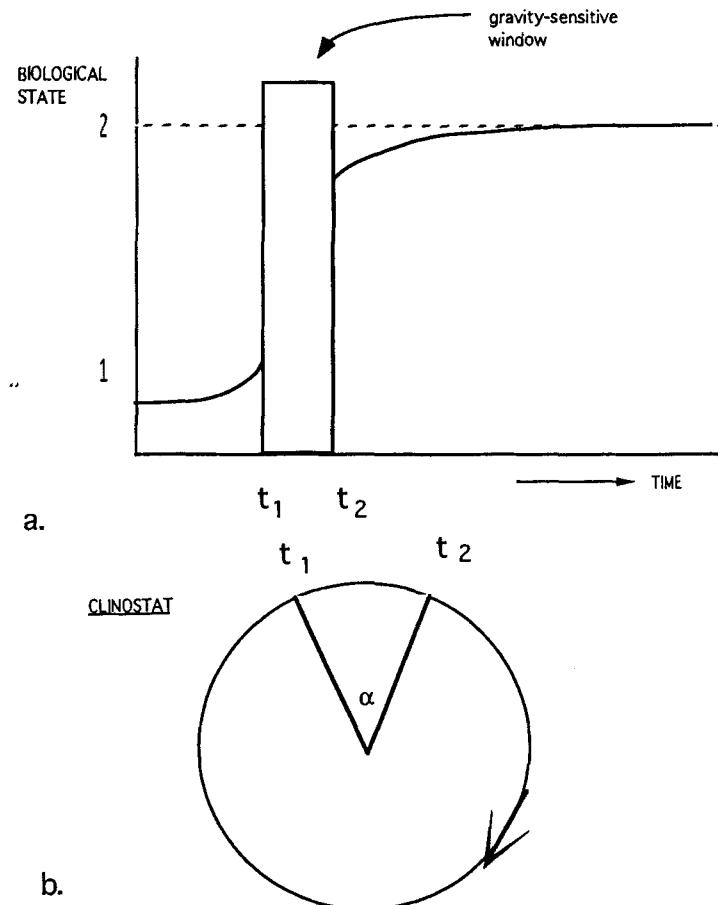


Figure 5. Gravity-sensitive windows in biological state transitions and their revelation by means of a clinostat. A gravity sensitive window is supposed to exist during transition from state 1 to state 2. In the sensitive window unidirectional accelerations of certain minimal duration would be required. Dependent on the length of the window presumed, different clinostat rotational speeds may provide adequate simulations of microgravity in the system studied. For example, one could assume a maximal angle of gravity vector rotation α , that would still be sufficiently "uni-directional" to generate the gravity signal required by the biological system (a reasonable assumption could be $\leq 45^\circ$). In other words, at a rotational speed ω of the clinostat, the angle covered during $t_2 - t_1$ (see a.) shall be larger than α to eliminate the signal (a reasonable assumption could be 2α). Hence, the clinostat can simulate absence of gravity for those systems having a gravity-sensitive window $t_2 - t_1$, by selecting a rotational speed ω where: $\omega(t_2 - t_1) > \alpha$ (see b). A reasonable assumption could be: $\omega(t_2 - t_1) = 90^\circ$. Application of speed variation, from low to high, may thus reveal the existence of gravity-sensitive windows in a certain biological system. In principle, a threshold speed could be found above which an effect occurs that will not change anymore at higher speeds.

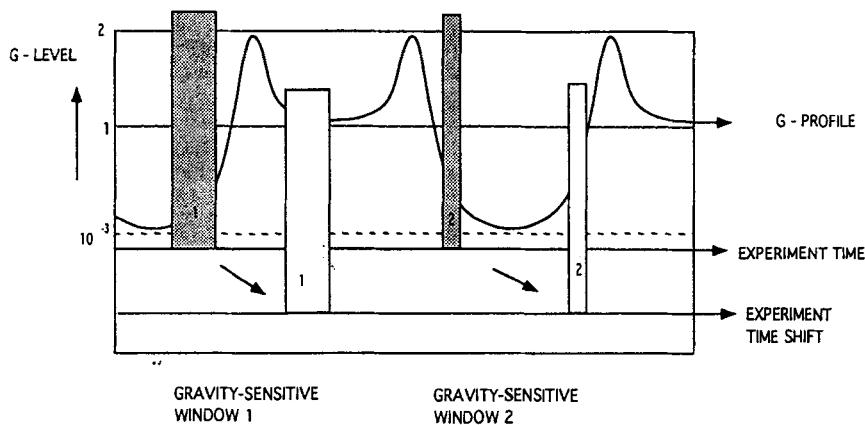


Figure 6. Parabolic flights: a useful tool for biological research. The duration of a biological experiment may span one whole parabolic flight mission, where one or more recurring microgravity periods may just coincide with (unknown) sensitive periods in the biological system. If properly synchronized, shifting times in the experiment may reveal different windows.

parabolic flight facility, which is available at NASA³⁵ and in France.³⁶ An airplane is flown through a series of parabola, which produce some 20 to 25 seconds of microgravity preceded and succeeded by short periods of approximately $2 \times G$. The duration of a biological experiment may span one whole mission, where one or more recurring microgravity periods may just coincide with (unknown) sensitive periods in the biological system. In other words, an array of short-duration microgravity windows are thus laid over the experimental period of interest. Therefore, this facility, which is being used by ESA on a frequent basis, could be much more important as a biological research facility than presently anticipated (see Fig. 6). Essential in the search for gravity-sensitive windows is the ability to synchronize the cells under study.

Table 2 summarizes the experimental facilities that could be used for the study of gravitational effects on cells or cellular systems, assuming the existence of gravity-sensitive windows of increasing durations.

A final remark on the facilities discussed above may be made. In Section III, it was mentioned that results obtained with biological cells in spaceflight and in sounding rocket flights could have been caused by synergistic action of space radiation and microgravity, a possibility that can not be cancelled out by using an in-flight $1 \times G$ control centrifuge. The clinostat and the parabolic flights, operated on or very near to Earth, are shielded from space radiation by the Earth's atmosphere, and therefore experiments performed in these facilities are not susceptible to such interpretations.

Table 2. Applicable Experiment Facilities for Increasing Lengths of Presumed Gravity-Sensitive Windows in a Biological System

Window length	Ground facility	Space facility
< 0.1 sec	PF	SR
0.1 – 30 sec	PF, CS-fr	SR
30 – 60 sec	CS-sr, TT	SR
7 min – hr	CS-vs _r , TT	OF

PF = parabolic flight; SR = sounding rocket; CS-fr = clinostat, fast rotating (typical 60 – 0.5 rpm); CS-sr = clinostat, slow rotating (typical 0.5 – 0.05 rpm); CS-vs_r = clinostat, very slow rotating (typical < 0.05 rpm); TT = tumbling table; OF = orbital facility.

VII. CONCLUSIONS AND SUMMARY

Based on theoretical considerations, direct action of gravity is possible. The key mechanisms could be:

1. Focusing via cell networks.
2. Amplification via non-linear dynamic systems.
3. Suppression of bifurcation in non-linear dynamic systems.

In particular, biological state transitions may have windows of gravity dependence. In these windows, a minimal time of uni-directional gravity would be required for an effect. The existence of windows for gravity dependence suggests the usefulness of experimental facilities such as the sounding rocket, parabolic flight, and of the fast and slow rotating clinostat.

Since the first flight of the ESA *Biorack* on the German *Spacelab Mission D-1* in 1985, evidence has been obtained that biological cells and small unicellular organisms function differently under conditions of microgravity. However, there is still insufficient scientific proof that these effects are caused by a direct influence on cells in the weightless condition. The question how normal gravity may play a role in cellular activity is being addressed, and the results show that gravity may provide important signals during certain state transitions in the cell. These would be gravity-sensitive windows in the biological process. Also, by amplification mechanisms inside the cell, the cell may assume a state that is typical for normal gravity conditions and would change in microgravity. Experimental tools are discussed that would provide the conditions to obtain evidence for direct action of gravity, and for the possible existence of gravity-sensitive windows.

An earlier outline of the discussion presented above has been published elsewhere.³⁷

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STUDY OF THE ORIGIN, EVOLUTION AND DISTRIBUTION OF LIFE WITH EMPHASIS ON EXOBIOLOGY EXPERIMENTS IN EARTH ORBIT

Gerda Horneck and André Brack

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I. INTRODUCTION

Throughout its history, mankind has wondered about the origins and the distribution of life in the universe. Until the development of space technologies, extraterrestrial biology was mainly restricted to the domain of metaphysics. Since the exploration of the Moon and of our neighbor planets by means of space vehicles, extraterrestrial biology has been established as a scientific discipline. It was introduced by J. Lederberg under the term "exobiology."¹ Exobiological research has moved from the mere goal of searching for extraterrestrial life towards the much broader endeavor of identifying those pathways that may be connected with the appearance, evolution, and distribution of life.

Today, the principal goal of exobiology is to reach a better understanding of the principles leading to the emergence of life from inanimate matter, its evolution and its distribution on Earth, the solar system, or beyond. To reach this goal, research has focused on the different steps of the pathways that may be taken by the "biogenic" light elements—these are hydrogen, carbon, nitrogen, oxygen, sulphur, and phosphorus which make up the bulk of living matter—from their primary chemistry to their assembly in advanced life forms.²

This chain of evolutionary steps includes:

- the cosmic chemistry of the biogenic elements;
- the prebiotic chemical evolution of organic molecules in water;
- the evolution of early forms of life; and
- the evolution of advanced life including the search for life elsewhere in the Galaxy.

Whereas the different steps of this evolutionary pathway have been discussed elsewhere,^{2–8} this chapter will mainly cover those aspects of exobiology which can be investigated by experiments in Earth orbit. We will elaborate on the processes of organic chemical evolution in different bodies of our solar system and beyond, and will discuss the relevance of these organic compounds to the emergence of life on Earth or elsewhere. We will also discuss the possibility of interplanetary distribution of life, and present experimental data testing this hypothesis.

II. SUPPLY OF ORGANIC MOLECULES AS BUILDING BLOCKS FOR LIFE

A. Extraplanetary Organic Molecules

Interstellar Medium

Primary organic chemistry first occurred in interstellar space. So far, more than fifty different organic molecules have been identified by radioastronomy (Table 1).

Table 1. Organic Molecules, Observed by Radioastronomy in the Interstellar Medium (Status 1988)

Table 2. Carbonaceous Species
Detected in Comet Coma or Tail

<i>Coma</i>	<i>Tail</i>
HCN	CH^+
CH_3CN	CO^+
CN	CO_2^\ddagger
C_3	
C_2	
CH	
CO	
C	

Because of the very low temperatures, from 10 to 30K, and the extremely low concentrations of molecules in the gas phase, the synthesis of these molecules is thought to occur in dense clouds in which reactions are initiated by collisions of high energy cosmic ray particles with hydrogen and helium, or by photochemical processes.⁹

The ultraviolet radiation, which exists abundantly even in the space between the stars, is believed to provide the major energy source for the chemical evolution in the interstellar medium.¹⁰ The ultimate mantle material of interstellar grains will be the result of photochemical processes which have occurred over some billions of years. In laboratory experiments, the accretion of an organic layer onto interstellar dust grains by photolysis has been simulated. From the photon flux, it was estimated that 1 hour of irradiation in the laboratory was equivalent to 1000 years in the interstellar medium. Experiments in Earth orbit, using the high influx of solar UV-radiation, will easily be equivalent to a time span in the interstellar medium of several billions of years.

The most important compounds for prebiotic chemistry probably are HCN, H_2CO , C_2H_2 , HC_2CN , and $\text{CH}_3\text{C}_2\text{H}$. Although there is little basis for assuming that interstellar organic molecules arrived intact on the prebiotic Earth's surface, there is growing interest in the possibility that interstellar molecules may be preserved intact in comets and, in altered form, in carbonaceous meteorites.

Comets

Comets also contain organic material (Table 2). The *Vega* and *Giotto* spaceprobes found that Halley's comet is richer in organic material than was predicted.¹¹⁻¹³ About 30% of particles are dominated by the light elements C, H, O, and N, and about 35% are close in composition to carbonaceous chondrites. The remaining 35% are mixtures of these two components. Among the identified organic molecules is hydrogen cyanide (HCN) which may have been a precursor of important

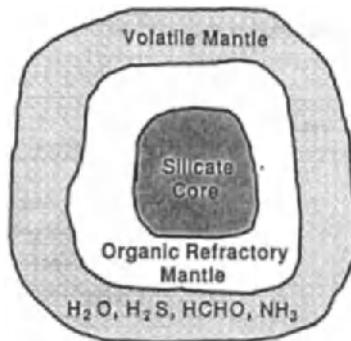


Figure 1. Chemical model of interstellar grains in diffuse and molecular clouds (from ref. 17)

biological building blocks, as suggested by primitive Earth simulating experiments. Polymers of formaldehyde may also be present.^{14,15} This finding is of special importance because formaldehyde is thought to have played a central role in prebiotic chemistry. However, at the present time, the chemical nature of the organic compounds detected in comet Halley is not known.

Some particles have a small, or perhaps no, mineral core suggesting that they may have been ice-processed by ionizing radiation as proposed by Brown et al.¹⁶ The composition of other particles may support the formation model proposed by Greenberg¹⁷ in which comets are aggregates of interstellar dust particles. Each particle contains a silicate core of about 0.05 µm radius, an inner mantle of complex organic molecules or polymers, and an outer mantle rich in water (Fig. 1).

The basic processes governing the evolution of the core-mantle grains will be: (1) accretion of the condensibles which include O, C, N, and S; (2) UV photo-processing of the accreted ices at 10 to 15K; and (3) grain explosions which replenish molecules in the gas-phases.

Gas-phase ion molecule reactions plus grain surface interactions and explosions lead to a predominance of water-ice in the outer grain mantle. Photo-processing of the water-ice outer mantle makes complex organic material sufficiently tough and refractory to survive.

Comets, witnesses of the beginnings of our solar system, give evidence of a dynamic organic chemistry during the condensation of the solar nebula, when UV-radiation of the young sun and of surrounding stars have driven the photochemical reactions. The organic compounds, so far detected in cometary coma and tail, point to much more complex "parent" molecules in the comet nucleus from which they originated. Future *in situ* investigations of comets, as planned for the *CRAF* and the *Rosetta* missions,¹⁸ are expected to answer the questions of the complexity and condensation processes of these organics. Such ambitious projects

Table 3. Relative Abundances of Selected Elements

Element	Relative Abundance (mass %)		
	Cosmos	Earth	Biomass
Hydrogen	70.0	0.003	10.5
Helium	28.0	0	0
Carbon	0.34	0.005	17.9
Oxygen	0.92	29.9	71.0
Nitrogen	0.12	0.00001	0.23

require careful preparation, which has to be done in laboratory facilities on simulated comet material,¹⁹ or directly in space.

B. Organic Molecules on the Primitive Earth and the Emergence of Life

The main elements constituting the biomass on Earth are carbon, hydrogen, oxygen, and nitrogen. However, relative to the cosmic matter, the Earth is severely depleted in the volatile elements carbon, hydrogen, and nitrogen that are essential for life (Table 3).

Life is also characterized by the dominant use of liquid water. Living organisms spent 90% of their evolutionary time in water before populating the land. Life can thus be considered as the encounter of organic molecules with liquid water:



Liquid water is a fleeting substance which can persist only within a limited range of temperatures and under relatively high pressure. Earth's size and orbit in the solar system are such that neither runaway glaciation, as on Mars, nor runaway greenhouse effect, as on Venus, have taken place.²⁰ As a source of organic molecules on Earth, two potential pathways are considered; terrestrial production of organic molecules, and import of extraterrestrial organic compounds to the Earth.

Terrestrial Production of Organics

Charles Darwin, in 1871, first formulated the classical scenario for an *in situ* production of complex organic compounds. He wrote: "If we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc. present, that a protein compound was chemically formed....."²¹

This idea was tested in an experiment by Stanley Miller. In 1952, he exposed a mixture of methane, ammonia, hydrogen, and water to electric discharges. Among the compounds formed, he identified four of those 20 naturally occurring amino

Table 4. Products of Organic Synthesis Under Simulated Primitive Earth Conditions

<i>Gas mixture</i>	<i>Compounds Produced By</i>	
	<i>Electric Discharge</i>	<i>Ultraviolet Photochemistry</i>
CH ₄ , NH ₃ , H ₂ O (H ₂)	amino acids, hydrocarbons, carboxylic acids, H ₂ CO, HCN	aldehydes, alcohols, ketones, hydrocarbons, HCN, amino acids
CO, NH ₃ , H ₂	HCN, amino acids	
CO ₂ , CO, N ₂ , H ₂	HCN, amino acids	
CO, N ₂ , H ₂ O	"	hydrocarbons, alcohols, ketones
CO ₂ , N ₂ , H ₂ O	HNO ₃	formic acid, H ₂ CO

acids.²² Since this historical experiment, nearly all the biogenic building blocks have been successfully synthesized under simulated prebiotic conditions. Seventeen of the 20 amino acids that occur in proteins have been found. In addition, purines and pyrimidine bases, the nitrogenous compounds found in nucleotides, and lipid-like materials able to form membranes, have thus been detected.

Miller's laboratory synthesis of amino acids occurs efficiently when a reducing gas mixture containing significant amounts of hydrogen is used. The true composition of primitive Earth atmosphere remains unknown, although geochemists now favor a non-reducing atmosphere dominated by CO₂, N₂ and H₂O. A variety of possible atmospheres ranging from highly-reducing (H₂, NH₃, CH₄, H₂O) to non-reducing compositions have been exposed to different energy sources to determine their potential for permitting abiotic organic synthesis (Table 4). In non-reducing atmospheres, the production of nitrogen-containing compounds appears to be inhibited.²³ If the early Earth atmosphere was dominated by CO₂ and N₂, then the methane-rich atmosphere postulated by Miller would not be a possible route to amino acids on Earth.

Import of Extraterrestrial Organic Compounds

Extraterrestrial material may have been an important source of organic compounds that triggered life on Earth by evolving in liquid water. Contemporary experimental support for such an alternative scenario can be found at three distinct levels:

1. At ground level (meteorites).
2. At deep-ice level (micrometeorites).
3. At stratosphere and Earth-orbit level (cosmic dust).

Table 5. Distribution of Carbon in Murchison Meteorite

Compounds	Abundance	
	%	ppm
Acid-insoluble carbonaceous phase	1.3 – 1.8	
CO ₃	0.2 – 0.5	
Hydrocarbons and lipids	0.07 – 0.11	
Carboxylic acids		~350
Amino acids		10 – 30
Ketones and aldehydes		17
Urea and amides		<2 – 15
Alcohols		~6
Amines		~2 – 3
N-heterocycles		<2 – 40
Total carbon	2.0 – 2.58	

Meteorites. The study of meteorites, particularly the carbonaceous chondrites that contain up to 5% of organic matter, has allowed a close examination of extraterrestrial organic material (Table 5).

The 17 amino acids found in the Murchison meteorite²⁴ are present as a mixture of L and D enantiomers in equal proportions, unlike the amino acids found in the proteins of the biomass which all have the L configuration. Amino acid precursors or their condensed forms are also present.²⁵ However, no clear-cut evidence has been found for the presence of peptides; compounds of a limited number of amino acids.^{26,27} In addition to these fundamental building blocks of proteins, some of the purine and pyrimidine bases of RNA and DNA,²⁸ some hydrocarbons and fatty acids²⁹ have been identified (reviewed in ref. 30, 31). Since there is evidence for intense meteorite bombardment more than 3.9 billion years ago (10^4 times the present flux), meteorites may have been an important source of organics on the primitive Earth.

Micrometeorites. These particles, also referred to as cosmic dust or interplanetary dust particles, have been extracted from deep-sea sediments,³² from cryoconite (black sediment) collected from the melt zone of the Greenland icecap,³³ and directly from Antarctic old blue ice.³⁴ In the latter study, a constant high percentage of 30% of unmelted chondritic micrometeorites from 100 μm to 1000 μm in size has been observed, indicating that many particles cross the terrestrial atmosphere without drastic modification due to thermal treatment.

Most of the unmelted micrometeorites are composed of porous aggregates of tiny

grains embedded in an amorphous component which appears to consist of a carbon/oxygen-rich pyrolyzable material. This combination may have served the same purpose as the charring composite material used as thermal protection for re-entry vehicles.

Stratospheric Dust. Brownlee demonstrated for the first time in 1978 that extraterrestrial particles may be recovered in the stratosphere by high-altitude aircraft.³⁵ Particles of 5 to 50 μm have been successfully collected by U-2 aircraft. Mineralogic and chemical analysis allowed distinction of extraterrestrial materials from terrestrial contaminants and showed a wide diversity of extraterrestrial particles. On the basis of major-element composition related to the mineral phase, two classes are distinguished: "chondritic particles", which are analogous to carbonaceous chondrites, and "non-chondritic particles".

Two sub-classes of chondritic particles can be distinguished; anhydrous and hydrous. The anhydrous chondritic particles are highly porous aggregates of grains. The pore spaces in the particles may have originally been filled with ice. The hydrated particles are more compact and the bulk of their mass is contained in silicates with a hydrated layer-lattice (clays) in addition to olivine, carbonates, and carbonaceous materials.

Most chondritic particles contain carbonaceous material. Unfortunately, it is still poorly characterized. Raman spectra and associated luminescence observations indicate the presence of highly disordered carbons.

Chondritic interplanetary dust particles bear many similarities to the fine-grained matrices of the carbonaceous chondrites. They share the common property of having a bulk composition similar to that of the solar photosphere. It must be noted that carbon concentrations of chondritic dust particles are generally higher than for any chondrites and are closer to solar abundance than those of chondrites.

Earth Orbit Dust. About 10,000 tons of cosmic dust that reach Earth's atmosphere each year³⁶ are derived from a number of parent bodies. The flux of 100 μm particles is $1 \text{ m}^{-2} \text{ yr}^{-1}$ and that of 10 μm particles is $1 \text{ m}^{-2} \text{ day}^{-1}$. Comets and asteroids are believed to be the major sources of cosmic dust, although their relative contributions are difficult to quantify.³⁷ Interstellar dust grains should also enter the solar system as the sun sweeps through the interstellar medium. However, when entering the solar system, their orbit and speed will be drastically modified as a consequence of collisions with interplanetary particles, especially in the asteroid belt and in dust tails of comets, or as the result of orbital perturbances by gravitational effects of other celestial bodies.³⁸ Therefore, it is difficult, if not impossible, to determine whether their origin is outside of our solar system.

Surfaces of spacecraft returning from space, such as the windows of *Apollo* and *Skylab* and the thermal blankets and aluminum louvres of *Solar Max*,³⁹ provide proof that analyzable particle residues can be returned from low Earth orbits.

To date, *COMET* is the only experiment specially designed to collect and return

dust particles to Earth for analysis.⁴⁰ Cosmic dust impacting with the *Salyut 7 Space Station* in October 1985 at an altitude of 350 km when the Earth crossing the swarm associated with the Giacobini-Zinner comet was analyzed. X-ray and ion mass spectrometry analysis showed different classes of grains, identified by their chemical composition. Although space debris of terrestrial origin accounted for the most numerous impacts, many impacting grains had a composition compatible with an extraterrestrial origin. Among these, some have a carbonaceous chondrite-type composition. Several ionic spectra indicate the presence of carbon associated with other light elements. They resemble the spectra of "CHON-rich" grains detected in the coma of comet Halley.¹³ The presence of CHON-rich grains in a cometary swarm indicates that these compounds can survive in the solar UV flux for thousands of years.

Role of Solar UV-Radiation in the Evolution of Life

It is generally accepted that the atmosphere of the early Earth did not contain any ozone. Therefore, during the first billion years, solar UV-radiation below 290 nm did penetrate the atmosphere and reached the surface of the Earth. This is the period when the decisive processes of abiotic organic chemical evolution, appearance of life, and diversification of anaerobic bacteria took place on Earth.⁶ Presently, insight in these processes has mainly been obtained from geological and paleontological observations, and from laboratory studies simulating the conditions of the early Earth. Space experiments using solar UV as an authentic energy source represent a new approach towards understanding the history of life on Earth. They can especially contribute to the understanding of the formation and stability of organic molecules in a prebiotic environment.

C. Organic Chemistry on Other Planets of our Solar System

Mars

The early histories of Mars and Earth clearly show some similarities. This is inferred from the existence of large valley networks and different channels, which attest that liquid water was once stable on the surface of Mars.⁴¹ Therefore, one of the major goals of future exploration aims at shedding more light into our understanding of the processes of organic chemical evolution on early Mars, the fate of organic material brought to Mars by comet or meteorite impact, and the chances for life to have appeared and evolved on early Mars.⁴² In all these processes, solar UV-radiation may have played a decisive role.

Since planetary missions are expensive and rare, the experiments have to be optimized to reach unequivocal results. Preparatory studies under conditions simulating early Mars are mandatory; in the laboratory as well as in space which provides solar UV-radiation as a unique energy source.

Present-day Mars has a surface temperature of 140–295 K, and water is present as ice, perhaps even in liquid form in some niches (hydrothermal vents) or underground. This means that the prerequisites for supporting life may be present on Mars. Therefore, the *Viking* missions carried out a number of experiments to search for indications of life forms on Mars^{43,44} with emphasis on microbial activity in Martian soil. The results are ambiguous; they are consistent with a biological as well as a non-biological explanation. The non-biological explanation assumes an active photochemical process in Martian soil, driven by the high influx of solar UV. Space experiments, using the extraterrestrial UV-flux modified with respect to Martian conditions acting on simulated Martian soil, will help to understand the *Viking* experiments and prepare for designing more appropriate exobiological experiments on future Mars missions.

Jupiter and Saturn

On Jupiter, hydrocarbons including acetylene, methane, propyne, ethane, propane, and benzene, as well as hydrogen cyanide have been observed. However, any organic compounds formed in the higher atmosphere by electric discharges, thunder shock waves, or ultraviolet irradiation are thought to be rapidly destroyed by hydrogenation and to be reconverted to the primary ingredients (hydrogen, ammonia, methane, water, and hydrogen sulfide) due to vertical atmospheric cycling. Thus, conversion of simple gaseous compounds to complex organic molecules seems unlikely on Jupiter. A similar organic chemistry can be assumed to occur on Saturn.

Titan

Titan, the largest satellite of Saturn, offers a natural “control” reactor to study chemical evolution of organic matter in the absence of liquid water. On the one hand, Titan resembles the Earth. It is large enough to have retained a dense atmosphere mainly composed of nitrogen and methane (1.5×10^5 Pa surface pressure). It is small enough to allow atmospheric hydrogen to escape. It is likely that Titan’s surface is covered by an ocean of liquid methane and ethane. On the other hand, the temperature is very low, around 175 K at the tropopause (the top of the troposphere), and around 95 K at the surface. Under these conditions, liquid water will be totally absent from this celestial body.

Infrared experiment, IRIS, on board *Voyager I* detected several hydrocarbons and nitriles, including compounds with saturated and unsaturated carbon chains⁴⁵ (Table 6). The formation of nitrogen-containing organic molecules requires a high energy source, probably electrons from Saturn’s magnetosphere or from galactic cosmic rays. It should be noted that UV-radiation does not reach the surface of Titan. Most of the compounds present in Titan’s gas phase must condense as aerosol clouds in the low stratosphere because of the low temperature. Submicron particles

Table 6. Organic Molecules Detected on Titan

<i>Compound</i>		<i>Physical State</i>	<i>Prebiotic Interest</i>
ethane	CH ₃ — CH ₃	gas	
ethylene	CH ₂ = CH ₂	gas	
acetylene	CH ≡ CH	gas	
propane	CH ₃ —CH ₂ —CH ₃	gas	
methylacetylene	CH ₃ —C ≡ CH	gas	
diacetylene	HC ≡ C—C ≡ CH	gas	
hydrogen cyanide	H—C ≡ N	gas	source of purines and amino acids
cyanoacetylene	HC ≡ C—C ≡ N	gas	source of pyrimidine
cyanogene	N ≡ C—C ≡ N	gas	condensing agent
dicyanoacetylene	N ≡ C—C ≡ C—C ≡ N	solid	

formed by photopolymerization of acetylene, ethylene, and hydrogen cyanide in the higher zones of the atmosphere will serve as nucleation centers for the condensation processes leading to aerosol formation.

In ground-based simulation experiments, N₂—CH₄ mixtures have been submitted to electric discharges,⁴⁵ or to high energy protons (1.5 MeV) and electrons (10 MeV).⁴⁶ Hydrocarbons and nitriles identical to those detected in Titan's atmosphere have been obtained in these experiments.

Titan has been considered as a natural laboratory in our solar system where organic chemical evolution still occurs. Solar UV is one of the energy sources, driving the production and condensation of organic gases in the upper regions of the atmosphere.⁴⁷ The space environment can be used to perform experiments simulating the chemistry of the organic gas clouds in Titan's atmosphere. This information should be valuable for the preparation of the exobiological experiments of the *Cassini* mission to Titan.

III. SURVIVAL OF RESISTANT LIFE FORMS IN SPACE

A. Panspermia Revisited

The Earth is the only planet in our solar system known to support abundant life today. During more than 3.5 billion years, life has successfully expanded over the entire surface of the Earth after it adapted to a variety of environmental extremes. The question arises whether our biosphere is an isolated system in the universe, or whether mechanisms exist by which living material can be exchanged between celestial bodies.

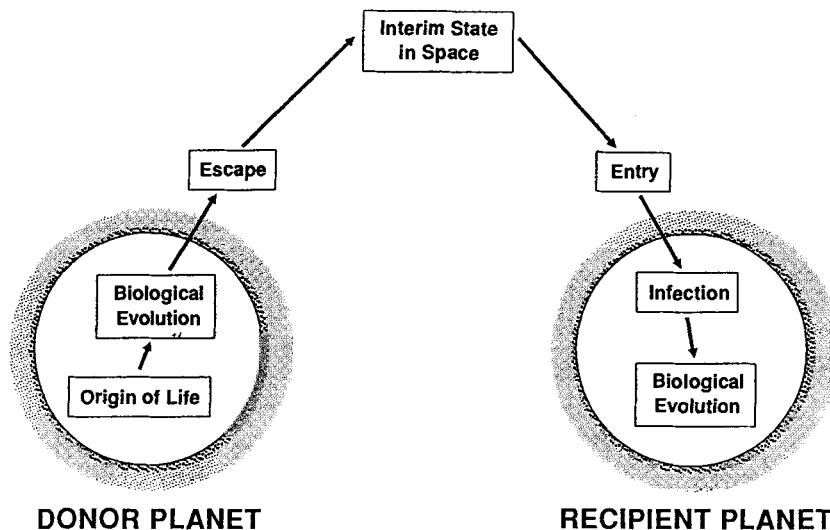


Figure 2. Hypothetical scenario of an interplanetary transfer of life (from ref. 57)

The idea of interplanetary transfer of life was originally put forward by Richter and Arrhenius (Panspermia).⁴⁸ Recent discoveries have given support to this idea.⁴⁹ These are: (1) the detection of meteorites, some of them being of lunar and some probably of Martian origin;⁵⁰ (2) the probability of rocks to reach escape velocities by the impact of a big meteorite on a planet;^{51,52} (3) the capability of bacterial spores to survive a simulated meteorite impact;⁵³ (4) the high UV-resistance of bacterial spores at deep space temperature;⁵⁴ (5) the very early appearance of autotrophic carbon fixation on Earth, as concluded from isotopic analysis of 3.8 billion years old sedimentary rocks;⁵⁵ and (6) the likelihood of artificial or directed panspermia by space missions to other planets.⁵⁶

So far, the scenario of an interplanetary transfer of life (Fig. 2) involves many hypothetical steps. We do not know whether "panspermia" is likely to have occurred in the history of our solar system, or whether it is feasible at all. With space technology, a new tool is available for testing experimentally whether microorganisms are capable of surviving some of the steps required. The following steps can be studied experimentally: (1) escape process, (2) interim state in space, and (3) entry process.

Escape Process

Dynamic forces like gravitation, meteorological drifts, thermal movements, magnetic or electric fields, and solar radiation pressure are considered insufficient to accelerate small particles of the size of a microorganism or small rock fragments

to reach escape velocities.⁵⁸ More promising processes to accelerate to such velocities may be volcanic eruptions, fly-by meteorites,⁵⁹ or meteorite impacts.^{51,52} One out of 10,000 spores of *Bacillus subtilis* have been found capable of surviving the low frequency shockwaves of a simulated meteorite impact of 42.5 GPa.⁵³

Interim State in Space

In space, microorganisms must cope with an interplay of various adverse environmental factors, such as high vacuum, intense radiation, and extreme temperatures. Together, these factors will set a definite barrier for active biological processes. The chances for living forms in an anabiotic state to survive such unfavorable conditions are being determined in space experiments, as well as in laboratory facilities simulating these space parameters. The results will be discussed below.

To travel from one planet of our solar system to another (e.g. from Mars to Earth) by random motion, a travel time of several hundred thousands to millions of years has been estimated.^{52,60} As far as interstellar transport is concerned, the clouds of gaseous and particulate matter between the stars move in a random fashion at speeds of about 10 km/s. If a bacterial spore is captured in such a cloud, it will be swept along with the gas. Given the distance between neighboring stars of 0.3 to 3 light years, this corresponds to a passage time of 10^5 – 10^6 years. Thus, for interstellar infection, microorganisms would have to survive at least for 10^5 – 10^6 years in such a cloud.⁵⁴ Results of the exposure of microorganisms to space conditions for up to several years are available.⁶¹ More data on long-term effects are required; they may allow extrapolation to astronomical time spans, as required for interplanetary or interstellar transport of life.

Entry Process

Space-travelling microorganisms, which have been captured by the gravitational force of a planet or moon, will have to survive entry and landing. To study processes that may bring microorganisms from space to a planet, a probable approach might be to drop a “contaminated artificial meteorite” by space mail to Earth.

B. Air Spores in High Altitudes

The troposphere is teeming with tiny organisms, the air spores. They comprise minute life forms, such as viruses, bacteria, algae, microfungi, spores of fungi, moss or fern, pollen, minute seeds, and protozoan cysts. Outdoor air contains hundreds to several thousands of airborne organisms per cubic meter.⁶² Hence, the atmosphere can be described as a spore suspension that generally decreases in concentration from ground level up to the tropopause.⁶³ It is constantly replenished by various microbiota from soil, water, and other sources.

The air spores have to cope with severe imponderables connected with take-off, aerial transport, and subsequent landing. Mechanisms of take-off are either active liberation processes, such as explosive ascii of some *Ascomycetes*, or squirting mechanisms of some *Phycomycetes* to propel spores into the air. Or else, they are passive in nature, using external energy, such as convection currents, wind, mist pick-up, or rain-splash, respectively.⁶²

Once in the air, air spores will be dispersed mainly by atmospheric turbulences, which are strongly influenced by weather conditions. Close to the Earth surface, the laminar boundary layer, still and windless, acts as a dust trap. Aerosols which have sunk through it come to rest at the Earth surface. However, local eddies may penetrate this layer and sweep away dust particles. When entering the turbulent boundary layer, they are rapidly carried laterally or upwards by eddies.

Vertical transport reaches up to an altitude of 100 to 1000 m, where the outer frictional turbulence layer marks the upper limit to which spores or other aerosols can be raised by frictional turbulence. From about 1 km above ground up to the tropopause, the convection layer dominates in which particles can enter by large scale convection currents when the Earth surface is heated up by sunshine. They may be carried by "bubbles" of heated air that arise under conditions of thermal instability. Thermals, or warm ascending currents of air, can raise air spores up to an altitude of 15 km.⁶²

Distribution of air spores including their identity, behavior, movements, and survival in the troposphere, are well understood. Spores are especially adapted to survive this inhospitable environment, characterized by low temperature, drought, low pressure, and solar radiation.

With increasing height, the concentration of air spores falls off. By means of balloons and rockets, viable microorganisms have been collected up to 77 km in a few experiments (Table 7). They were predominantly black conidia and spores of fungi. It is assumed that pigmentation offers a selective advantage for the spores because it protects them against the intense solar UV-radiation.

It still is an open question as to what natural dynamic forces can transport air spores to the stratosphere or beyond, and, if they happen to arrive there, what protection mechanisms are required for them to survive this journey. Space technology offers an opportunity to sample particles of biological interest in the stratosphere and in Earth orbit. This will provide substantial information towards an understanding of the changes of concentration of air spores with height, and of their circulation over the surface of our planet. This information is also important for assessing the chances and limits of the interplanetary transfer of life.

C. Environmental Parameters of Interplanetary Space

The interplanetary space (Table 8) is characterized by the following four parameters: (1) high vacuum, (2) intense particle radiation, (3) electromagnetic radiation, and (4) extreme temperatures.

Table 7. Air Spora Sampled at High Altitudes

Year	Mission	Altitude (km)	Device	Organisms detected		
				Species	Total No.	Ref.
1936	Balloon: Explorer	11–21	Sterile cylindrical sampling device descending by parachute	• bacteria Bacillus sp. • fungi Rhizopus sp. Aspergillus sp. Penicillium sp. Macrosporium Micrococcus Alternaria Cladosporium	-0.14 m ⁻³ 64	
1967	Balloon	20–30	Drawing large volumes of air by a fan through polyurethan foam filters		-0.003 m ⁻³ 65	
1966	• Gemini 8 Agena 126.34 d • Gemini 9A 16 h 47 min • Gemini 12 6 h 24 min	~400 288–320 288–310	Sterile surface of freeze-dried methylcellulose	no viable microorganisms	0	66
1975	Meteorological rockets	48–85	Nutrient medium in polyethylene bags, γ -ray sterilized, exposed on the forepart of the rocket during ascent	Micrococcus Mycobacterium Circinella Penicillium Aspergillus	31(48–77 km) 67 0(>77 km)	
1979	Meteorological rocket	51–81	MIR 1: Sterile cylindrical collector, coated with silicone oil, divided in 3 sections, moving along three nozzles during rocket ascent	bacterial cells fungal spores	no numbers given	68

Table 8. Comparison of Environmental Conditions of Interplanetary Space, High and Low Earth Orbit and Simulation Facilities on Ground

Parameter of Interest	Interplanetary Space	High Earth Orbit (≈ 1000 km)	Low Earth Orbit (≤ 500 km) :			
			Free Flying	Satellite	Space Station	Groundbased Simulation Facilities
Space vacuum						
Final pressure/Pa	10^{-14}	10^{-7}				
Residual gas/cm $^{-3}$	1 H	5×10^4 H				
		5×10^4 He				
		1×10^3 O				
Solar UV radiation						
Intensity/Wm $^{-2}$	variable	1360				
spectrum	unique	unique ¹				
Ionizing radiation						
galactic radiation		galactic radiation				
solar radiation		solar radiation				
		radiation belts				
Temperature/K	4	wide range				
Microgravity/g	$< 10^{-6}$	$\sim 10^{-6}$				
Space environment (all factors)	unique	unique				

¹see Fig. 3

²sources of contamination: waste dumping (H₂O, organics), thruster firing (H₂O, N₂O, NO) (ref. 69)

³South Atlantic Anomaly

High Vacuum

In free space, pressures down to 10^{-14} Pa prevail. Within the vicinity of a planetary body, the pressure may significantly increase due to outgassing. In low Earth orbit, pressure reaches 10^{-4} to 10^{-6} Pa. The major constituents of this environment are molecular nitrogen and oxygen, as well as highly reactive free oxygen and nitrogen atoms. In the vicinity of a spacecraft, the pressure is further increased, depending on the degree of outgassing. In the *Shuttle* bay, a pressure of 3×10^{-5} Pa was measured, after bay doors had been open for more than four days.⁷⁰

Intense Particle Radiation

The particle radiation of our solar system is composed of galactic and solar radiation. The galactic cosmic radiation, when entering our solar system, is composed of protons (85%), electrons and alpha-particles (14%), and heavy ions (1%) of charge $Z > 2$, the so called HZE particles.⁷¹ The solar particle radiation, emitted in solar wind and during solar flares, is composed of 90 to 95% protons, 5 to 10% alpha-particles, and a relatively small number of heavier ions. In the radiation belts in the vicinity of the Earth, protons and electrons are trapped by the geomagnetic field.

Electromagnetic Radiation

The spectrum of solar electromagnetic radiation spans over several orders of magnitude, from short wavelength X-rays to long wavelength radio frequencies. At the distance of the Earth (1 AU), the fluence rate of solar radiation amounts to 1360 W/m^2 (1 solar constant), 45% of which are attributed to the infrared range, 48% to the visible range, and 7% to the UV range.⁷²

Extreme Temperatures

In Earth orbit, the following energy sources exist: solar radiation (1360 W/m^2); Earth albedo (480 W/m^2); and terrestrial radiation (230 W/m^2). Energy is emitted to the frigidity of free space (4 K). The resulting temperature of a body, determined by absorption and emission of energy, as well as by surface, size, and mass of the body, can reach extreme values.

D. Responses of Microorganisms to Space

The vast and empty reaches of space have been considered to be extremely hostile to all forms of life. Above all, the high vacuum represents a definite barrier for active biological processes, such as growth, metabolism, and reproduction. However, some living organisms have developed capacities to survive unfavorable

conditions in a dormant state, and to resume metabolic activity when conditions change to a more favorable state. Examples are bacterial spores, certain fungal conidia, macrocysts of the slime mold *Dictyostelium*, brine shrimp cysts, and dry larvae or adults of certain species of nematodes.

The survival of resistant microbial forms in the upper atmosphere or in free space has been investigated *in situ* by exposing resistant microorganisms to the upper atmosphere or to space by means of balloons, rockets, and spacecraft. Their response to the space environment or to certain related factors has been analyzed after recovery (Table 9). These investigations have been complemented by studies in the laboratory in which certain parameters of space (high vacuum, extreme temperatures, UV-radiation of different wavelengths, and ionizing radiation) were simulated. The microbial response (physiological, genetic, and biochemical changes) to selected factors applied separately or in combination have been determined.⁷⁹

Microorganisms were exposed to selected spaceflight factors, such as different spectral ranges of solar UV-radiation, space vacuum, and cosmic radiation, applied separately or in combination. Physiological, genetic, and biochemical studies were then performed. Special attempts were made to detect the vacuum-sensitive sites of a bacterial cell and to determine the biochemical and biological processes induced by solar UV-radiation as well as by the combined action of solar UV and space vacuum. The radiobiological consequences of a hit by a single HZE particle of cosmic radiation were investigated. To date, the maximum exposure time of microorganisms to space that were investigated after recovery was 5.8 years during the LDEF mission.⁶¹

Response to Vacuum

Spores of *Bacillus subtilis* wildtype survive extended periods of vacuum exposure by almost 100%.^{61,78-79} Nevertheless, the genetic material is affected, indicated by an increased mutation rate, delayed germination, cross-linking of DNA and protein, and the requirement of cellular repair processes to restore viability after vacuum exposure.⁷⁸

When exposed to vacuum, the spores become dehydrated. When the exposure time is extended, free water is removed first; then the hydrate water, and finally chemically bound water and other volatile molecules are removed. There are many effects on membranes and macromolecules, such as DNA and proteins.^{80,81}

Solar UV-Radiation

Solar UV-radiation has been found to be the most deleterious factor of space as tested with dried preparations of viruses and bacterial or fungal spores (Table 9). The incidence of the full spectrum of solar UV-light (> 170 nm) kills 90% of *B. subtilis* spores within seconds. To reach the same effect on Earth, an approximate

Table 9. Summary Results of Space Experiments to Test Responses of Microorganisms to Selected Parameters of Space

Year	Mission	Assay System	Test Parameter	Phenomenon Studied	Major Findings	Ref.
1965	Balloon flights 35 km alt. 6 h Rockets 150 km alt. 3 min	Poliovirus Bacteriophage T1	Stratosphere Free space with/without protection from solar UV	Inactivation Inactivation	Little loss in viability High inactivation by solar UV radiation	73 73
		B. subtilis spores Penicillium spores	Free space	Inactivation	Little or no loss in viability	
1966	"Luster" rocket 77–149 km alt. 204 s	Bacteriophage T1	Solar UV	Action spectrum of inactivation	High inactivation by direct exposure to solar UV; max. effect by 250-λ<280 nm	74
	Gemini 9 300 km alt. 16 h 47 min	Bacteriophage T1	Free space with/without protection from solar UV	Inactivation	Solar UV and/or soft X rays responsible for killing in space	75
	Gemini 12 300 km alt., 6 h, 47 min	TMV B. subtilis spores P. roquefortii spores				
1972	Apollo 16 transearth coast 10 min exposure	B. subtilis spores	Solar UV (254 nm, 280 nm) and space vacuum	Inactivation, mutation induction	Space vacuum enhances lethal effect of solar UV in wild type strain, not in repair deficient strain.	76 77
1983	Spacelab 1 240 km alt 10 d, 8 h, 47 min	Bacteriophage T1 B. subtilis spores	Solar UV (254nm) Solar UV (>170 nm, 200, 240, 260, 280 nm) and/or space vacuum	Inactivation, repair mutation induction, photoproducts in DNA and protein (Action spectrum of inactivation)	Full spectrum of solar UV (>170 nm) kills spores within seconds. Synergistic action solar UV and vacuum for all wavelengths tested. Formation of "vacuum-specific" photoproducts.	78
1984– 1990	LD50F ≤ 500 km alt. 2107 d	B. subtilis spores	Free space or selected parameters	Inactivation, chemical protection	Survival in space vacuum ≤10%. Survival in free space ≤ 10 ⁻⁴ . Protection by glucose, salts or by thick layers of spores.	61

1000-times longer exposure to sunlight is required (Fig. 3). This difference is attributed to the ozone layer that protects the biosphere from the most harmful fraction of solar UV-radiation (< 295 nm). Action spectra of the solar photons (160 to 320 nm) for killing of bacteriophage *T1* or *B. subtilis* spores closely correlate with the absorption spectrum of DNA, indicating that DNA is the critical chromophore for lethality (Fig. 4). A second proof that UV-radiation damage is the main cause of inactivation in free space was obtained with *Tobacco Mosaic Virus*. After exposure to space, this virus showed structural changes which are typical for UV-induced damage.⁸²

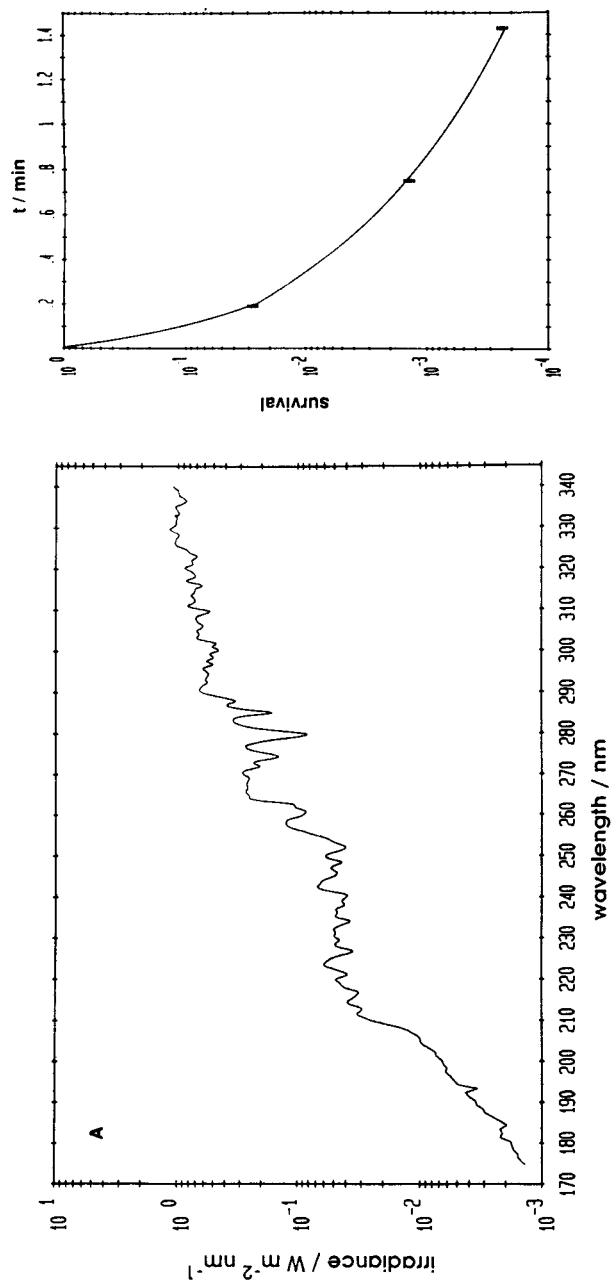
Considerable information on the far-UV photochemistry of nucleic acids and related compounds under Earth conditions has accumulated during the past three decades (for reviews see refs. 83–85). Various chemical and biochemical assays offering high sensitivity and accuracy are now available for monitoring the formation of the main photoproducts of far-UV-irradiated cellular DNA. However the exact biological role, and in particular the mutagenic effects, of the DNA photoproducts remains to be assessed. The four main classes of DNA photoproducts are the cyclobutadipyrimidines, (6-4)pyrimidine-pyrimidone adducts and their Dewar valence isomer,⁸⁶ as well as 5,6-dihydro-5-(α -thyminyl)thymine (TDHT). There is also a relative paucity of information on the photochemical reactions induced by vacuum-UV-light, the other important component of solar radiation, in DNA and model compounds.

Combined Action of Solar UV-Radiation and Space Vacuum

When spores are simultaneously exposed to solar UV-radiation and space vacuum, they respond with an increased sensitivity to UV,⁷⁸ to a broad spectrum of solar UV-light (> 170 nm), as well as to selected wavelengths.⁷⁸ Several attempts have been made to isolate and identify thymine photoproducts generated in the DNA of resting bacterial spores when exposed to UV-light in a vacuum. In various Earth and space experiments, two thymine decomposition products, namely the cis-syn and trans-syn isomers of cyclobutadithymine (Thy< \rightarrow >Thy) as well as DNA-protein cross-linkings were tentatively characterized in addition to TDHT, the so-called “spore photoproduct”^{78,87,88} (Table 10). From the efficiency of repair processes (photoenzymatic repair and spore photoproduct specific repair), it is concluded that photoproducts other than cis-syn Thy< \rightarrow >Thy and TDHT seem to be responsible for the UV supersensitivity of spores irradiated under vacuum conditions.⁷⁸

Combined Action of UV-Radiation, Vacuum and Low Temperature

During the major part of a hypothetic journey through space, if shielded from solar thermal radiation, microorganisms will be exposed to the cold emptiness of space with temperatures down to 4 K. At these low temperatures, thermodynamic



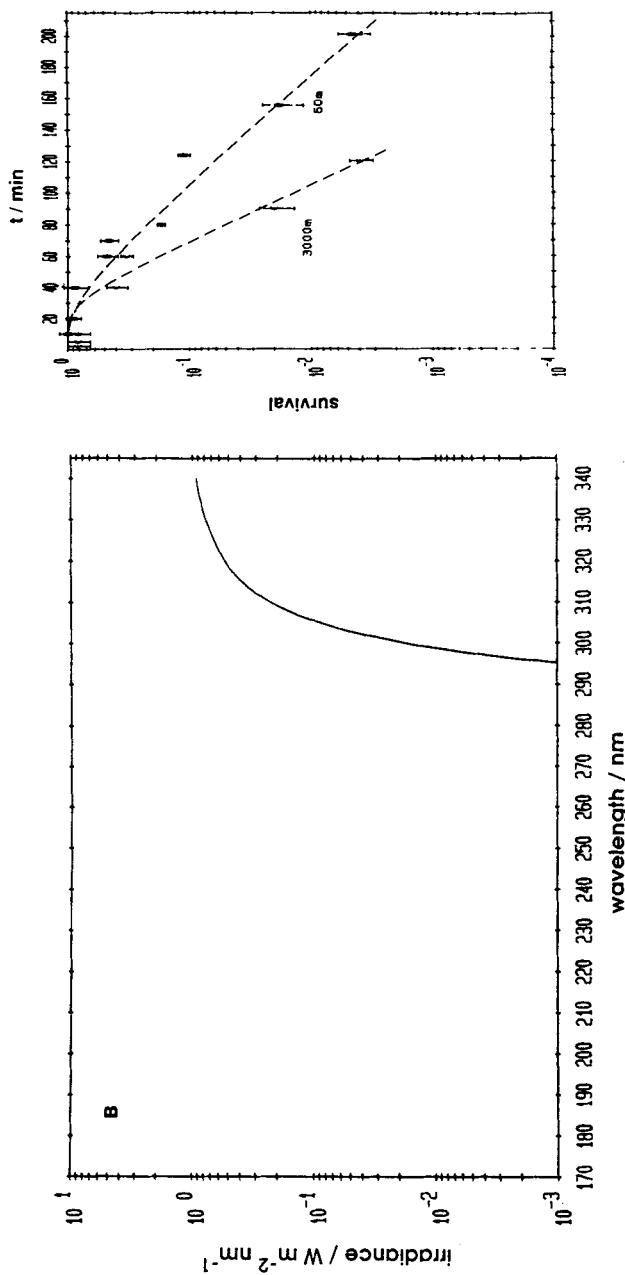


Figure 3. A. Solar extraterrestrial spectral irradiance ($170\text{--}340\text{ nm}$)⁷² and survival of spores of *B. subtilis* after irradiation by solar UV-light of spectral range above 190 nm; results of an experiment on SpaceLab 1. B. Solar spectral irradiance at the surface of the Earth and survival of spores of *B. subtilis* after exposure at different elevations on Earth. Note that the time scale has been changed by a factor of 1/140 compared to A.

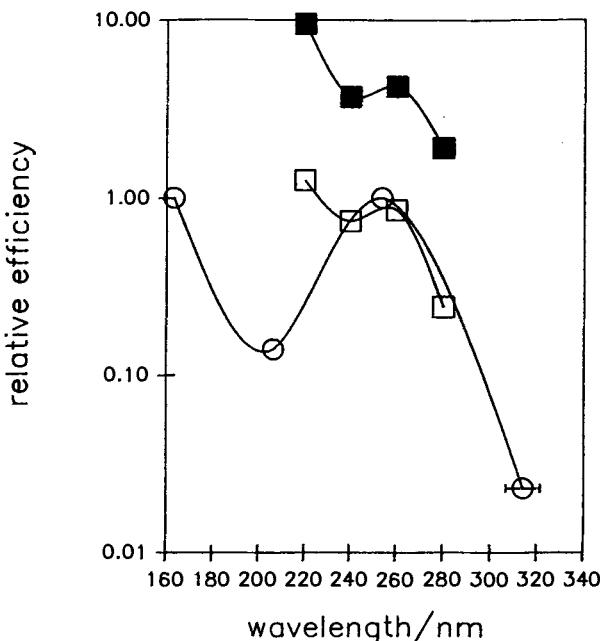


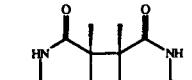
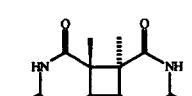
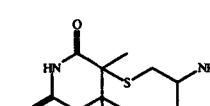
Figure 4. Action spectra for lethality induced by extraterrestrial solar UV-radiation in bacteriophage T1 (open circles) (from ref. 74) and in spores of *B. subtilis* after irradiation at atmospheric pressure (open squares) or in space vacuum (closed squares) (from ref. 78). Results have been normalized to the response at 254 nm.

and chemical reactions are nearly frozen. Thus the photobiological response to solar UV-radiation may be completely different from room temperature conditions. Laboratory experiments under simulated interstellar space conditions point to a remarkably smaller damaging effect of UV-radiation at these low temperatures than at room temperature. *B. subtilis* spores, exposed simultaneously to UV-radiation (>110 nm), vacuum, and low temperature (10 K) in space simulation experiments, showed an unexpectedly high survival, even at very high UV fluences.⁵⁴ The temperature profile of bacterial spore UV-sensitivity shows a maximum at 190 K. Therefore, it appears that at increasing distance from the sun, the lower temperature will first lead to an increase in sensitivity and, further out in the outer solar system, to a drastic decrease in sensitivity.⁵⁴

HZE Particles of Cosmic Radiation

It has now been well documented that exposure of living systems to space ionizing radiation has deleterious biological effects (recent reviews in refs. 89 and

Table 10. Photoproducts, Identified in Bacterial Spores that were Irradiated with Extraterrestrial Solar UV-Light (>170 nm); Results of SL1-Experiment ES 029 and Laboratory Experiments^{78, 86-88}

Photoproduct Identified in Spore DNA	UV- Irradiated in <i>vacuo</i>	UV-Irradiated at 1 atm
cis-syn Thymine dimer cytosone-thymine dimer		+
trans-syn-Cylobutadithymine		+
5,6-Dihydro(α -thimyinyl)thymine		+
DNA-protein crosslinking		++

90). Among this large variety of radiation, which covers a wide spectrum of energy, it appears that HZE particles are the most effective species. To understand the ways by which cosmic radiation particles interact with biological systems, methods have been developed to precisely localize the trajectory of an HZE particle relative to the biological object, and to correlate the physical data of the particle relative to the observed biological effects along its path. Visual track detectors were used that were sandwiched between layers of biological objects in the resting state (Fig. 5). A variety of test systems were used, such as viruses, bacterial spores, plant seeds, or shrimp cysts. Injuries to these objects were traced back to the traversal of a single HZE particle, such as somatic mutations in plant seeds, developmental disturbances and malformations in insect and salt shrimp embryos, or inactivation in bacterial

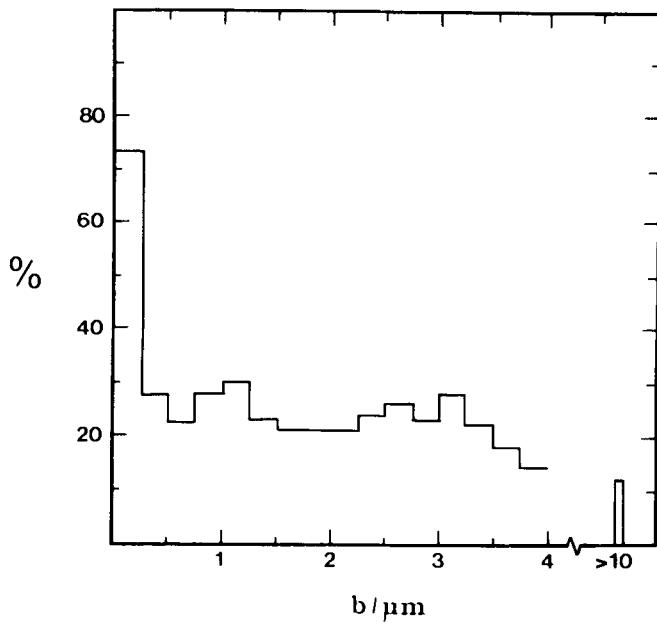
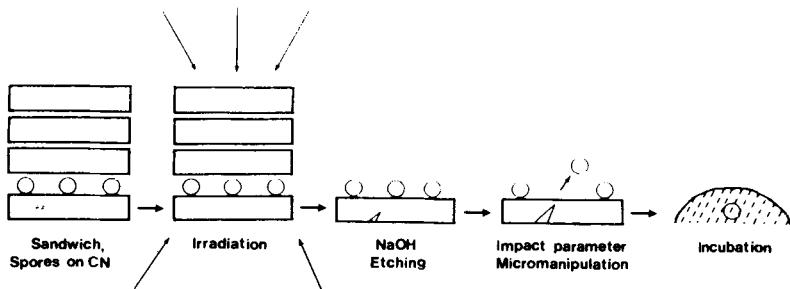


Figure 5. Inactivation (%) of *B. subtilis* spores by single HZE particles ($Z \geq 12$, $\text{LET} \geq 200$ keV/ μm) of cosmic radiation as a function of the radial distance, b , of the center of the spore from the trajectory of the particle. The spores are mounted on a nuclear track detector; after irradiation, the spores and the detector are analyzed separately and the rate of inactivation is correlated to the track parameters (see upper part). Results of the Biostack experiment (from ref. 95)

spores (reviewed in refs. 90–94). Particularly interesting is the observation of a radial long range lethal effect of single HZE particles on individual spores of *B. subtilis*. However, not every spore, even if centrally hit, was inactivated⁹⁵ (Fig. 5).

HZE particles of cosmic radiation are thought to set the ultimate limit on the survival of spores in space, since they penetrate even high shieldings. The maximum time of a spore to escape a hit by a HZE particle (e.g. iron with a LET value >100 keV/ μ m), has been estimated to amount to 10^5 to 10^6 years.⁷⁹

E. Perspective for Future Research

To disentangle the network of potential interactions of all space parameters on a biological organism, the following research strategy should be pursued. First, each space parameter should be separately studied for its impact on biological integrity. Second, space parameters should then be combined one by one and the effectiveness of their combined action should be quantified. This approach will allow to identify types of interaction, whether additive, synergistic, or antagonistic.

To date, exobiological experiments in space have mainly focused on the microbial response to single parameters of space, such as solar UV-radiation, space vacuum, or cosmic radiation, as well as to the combined action of two parameters such as solar UV-radiation and space vacuum. These data need to be confirmed and expanded. There is a real need to gain further insight into the molecular aspects of the biological action of solar radiation, and to find a mechanism for the synergistic action of vacuum and UV-radiation. Identification and quantification of the main DNA photoproducts are necessary for a better understanding of their biological role. Furthermore, space experiments should include those at low temperatures as an additional decisive parameter of space.

Emphasis should be laid on studies of photochemical and photobiological consequences of irradiation with polychromatic solar UV-light including the vacuum-UV range. Lethal effects of polychromatic UV-radiation have already been described for the range of solar UV-light reaching the surface of the Earth (reviewed in ref. 96). Even more important will be the evaluation of the biological effects of the extraterrestrial spectrum of sunlight. This may produce effects that are one or more orders of magnitude greater than the action of terrestrial solar UV-radiation. Various mechanisms have been suggested to explain the increase in photosensitivity, including a possible increase in the quantum yield of specific DNA photoproducts, an inhibition of DNA repair ability for some damage, or a deficiency in this ability.

Appropriate test systems might include the following:

- airborne microorganisms which are commonly in resting or temporarily inactive state, either as spores with inherent resistance mechanisms against environmental extremes, or as spores modified by desiccation and starvation;

- microbial communities from soil, rocks, or desert, which live either inside rocks and sandstone of hot and cold deserts,⁹⁷ or in association with desert varnish;⁹⁸
- anhydrobiotic organisms that have developed molecular strategies to survive dehydration, e.g. by accumulation of polyols like trehalose;⁹⁹ and
- archaebacteria, a diverse collection of phenotypes adapted to extreme environmental conditions,¹⁰⁰ which probably have significantly contributed to the organic matter content of sediments since the Precambrian period.

Protection mechanisms, internal and external, may enable organisms to cope with the hostile environment of space for extended periods of time. Such mechanisms may prevent or repair damage produced by UV- or ionizing-radiation, vacuum, or freezing; or by a combination of these space parameters.

Protective compounds known to be internally accumulated include polyols, such as trehalose, sucrose, polysucrose, and inositol. They protect both liposomes and membrane vesicles, prepared from the dry-sensitive bacterium *Micrococcus luteus*, from disintegration and loss of membrane-bound functions by vacuum exposure. The polyols can be present on either side of the membrane.¹⁰¹ Polyols, like trehalose, present in high concentrations (20% of dry weight), appear to interact by hydrogen bonding with the phosphate groups of phospholipids,¹⁰² and thus stabilize the membrane structure in the absence of water.

An example of external protection is the coating of bacteria by a mantle of some material which attenuates the solar UV-radiation by several orders of magnitude. Such a mantle could consist of any of the following materials: dust, rock, clay, abiotically produced organic matter, meteorite dust, proteins, sugars, or a thick layer of microorganisms, ice, or soil (artificial comets or meteorites).

IV. CONCLUSION

The primary goal of exobiological research is to understand the processes leading to the origin, evolution, and distribution of life on Earth or elsewhere in the universe. In this endeavor, space technology now plays an important role by offering opportunities for exploring our solar system, for collecting extraterrestrial samples, and for utilizing the peculiar environment of space as a tool.

In the chain of evolutionary steps towards the emergence of life, prebiotic chemical evolution is crucial. This process first occurred in the interstellar medium, where more than 50 different organic molecules have been detected. Organic compounds are found in cometary coma and tail, indicating a dynamic organic chemistry already operating at the beginning of our solar system.

For the supply of organic molecules on the primitive Earth, two plausible pathways are considered: terrestrial production of organics and/or import of extra-terrestrial organic matter. In experiments simulating the putative conditions exist-

ing on the early Earth, nearly all building blocks of life have been synthesized, such as amino acids, purines, pyrimidines, and lipid-like compounds. However, in a non-reducing atmosphere, such as now favored by geochemists, nitrogen-containing compounds are unstable. Therefore, incoming extraterrestrial material may have to be considered as an essential source of organics on the primitive Earth. Contemporary witnesses are meteorites, collected on the Earth surface, micrometeorites found embedded in deep-ice level, and cosmic dust, sampled at stratospheric and Earth-orbit levels. All three have yielded specimens which contain organic compounds up to 5% by weight.

Of the other planets of our solar system, Mars and Saturn's satellite Titan are of special interest to exobiologists. Since the early history of Mars seems to bear certain similarities to that of the Earth, future Mars exploration will concentrate on elucidating the chances for life to have appeared and evolved there. Titan has been considered as a natural "control" reactor where organic chemical evolution still occurs in the absence of liquid water.

The discovery on the Earth surface of rocks of lunar origin, and probably also Martian origin, indicates that matter can be exchanged naturally between the planets in our solar system. Therefore the idea of "Panspermia" has been revisited, particularly in terms of the escape process, the fate of resistant microorganisms in space, and the entry process. Planetary missions provide an artificial means for such a transport.

The environment of space is extremely hostile to all forms of life when encountered without any protection. This is attributed to the complex interplay of various adverse factors, such as high vacuum, solar electromagnetic radiation, corpuscular radiation of solar and galactic origin, and extreme temperatures. Yet, viable microorganisms have been collected at altitudes of up to 77 km.

During the *Gemini*, *Apollo*, *Spacelab* and *LDEF* missions, exposure of various microorganisms to space conditions has demonstrated the enormous lethal potency of solar UV-radiation. The UV-sensitivity of microorganisms is enhanced in the vacuum of space. This synergistic effect has been ascribed to the production of specific photoproducts in the DNA which are less susceptible to cellular repair mechanisms during subsequent growth. However, when efficient shielding is provided against the influx of solar UV-radiation, bacterial spores cope with space for more than 5 years without any loss of viability.

The highly penetrating heavy ions of cosmic radiation might set the ultimate limit for survival, since effective shielding is nearly impossible. It has been estimated that a spore might travel through space for more than 10^5 years without being hit by a heavy ion. Similar time spans of 10^5 to 10^6 years have been estimated as the mean travelling time for a spore from one planet of our solar system to another by random motion.

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CHEMICAL SENSORS FOR SPACE APPLICATIONS

Sjoerd L. Bonting

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I. INTRODUCTION

There will be a great need for a wide variety of chemical analyses, both for biomedical experimentation and for the monitoring of water and air recycling processes on *Space Station Freedom*. The infrequent logistics flights of the *Space Shuttle* will necessitate onboard analysis. Carrying out traditional bioanalytical procedures during spaceflight is complicated for several reasons. Sample treatment is more difficult in a weightless environment than on Earth. Use of toxic reagents can only be permitted under conditions of double containment. Analytical instruments take up space and use power. In addition, the usual bioanalytical procedures are rather time-consuming. Crew time, pressurized volume, and power are always scarce resources in space missions.

During the relatively short missions of the *Space Shuttle*, onboard analysis is mostly avoided by performing tests immediately before and after flight, in humans as well as in animals; in the latter case there are also ground control animals. Any samples obtained during flight are preserved and returned to the ground for analysis.

However, with the advent of the *Space Station Freedom*, onboard analysis of a large number of analytes can hardly be avoided. Experiments of months duration will be performed to study the long-term effects of space conditions on living organisms. In such experiments, it will be very important to be able to measure various biochemical parameters at frequent intervals during the experiment in order to monitor the course of adaptation of the organism to space conditions. However, logistics flights of the *Space Shuttle* will be at intervals of at least three months. This means that the analytical results on samples returned to Earth would become available with several months delay, which is obviously undesirable. Thus the ability to perform onboard analysis of a variety of biochemical parameters becomes very crucial for space biology research on *Space Station Freedom*. This will also

be true for other long-term missions in the future, like a manned mission to Mars or a permanently manned Lunar base.

During such long-term missions there will also be a need for a closed or nearly closed environmental life support system (CELSS), which provides regeneration of the gas atmosphere and the water used by crew members and other living organisms. This will require the monitoring of a number of chemical parameters. If the monitoring can be carried out continuously, then this provides the possibility of optimizing and controlling the regeneration processes.

This chapter explores the possibility of using chemical sensors, rather than more conventional techniques for chemical analysis onboard *Space Station Freedom*. The term, "chemical sensors", rather than "biosensor", is used because neither the analyte nor the selector in the applications considered here need be of a biochemical nature. While (bio)physical sensors have already been used rather extensively in spaceflight, the development and utilization of chemical sensors is still in its infancy. In particular, we shall discuss the use of chemical sensors for space biomedical research and for the monitoring of water recycling.

II. ADVANTAGES OF CHEMICAL SENSORS FOR ONBOARD ANALYSIS

A chemical sensor is a device that consists of a selector which selectively interacts with a chemical substance (analyte) present in a mixture of substances, and a transducer which produces an electric signal in response to the interaction of analyte and selector. The transducer signal, thus, provides a quantitative and selective measurement of the analyte. Chemical sensors can be distinguished by the type of selector or the type of transducer used in its construction. The main types of selectors used so far are: polymeric membranes, glass membranes, other membranes, enzymes, antibodies, and cells. The main types of transducers used at present are: potentiometric, amperometric, ISFETs, optical systems, piezoelectric crystals, and thermal devices. The various types of transducers and selectors will be described in more detail below.

The use of chemical sensors offers considerable advantages over conventional analysis onboard a spacecraft. Sensor measurements do not require extensive sample treatment before analysis. For example, a sensor can be placed in a blood or urine sample and, in many other cases, it can simply be inserted in or be placed on the organism. In the case of CELSS processes, sensors can be placed in the flow of air or water. This means that far less crew time is needed than for conventional methods of analysis.

In addition, sensors, and even ancillary instruments, are small compared to conventional analytical instruments, and their power consumption is low.

In principle, chemical sensors can provide (near) real-time monitoring of many important analytes. In some cases they can even provide continuous monitoring of

such analytes. Space experiments are often monitored, and even controlled, by scientists on Earth. This requires that data be transmitted to the ground, preferably near real time. Here sensors have the advantage that their output is usually in digitized form, which lends itself to rapid transmission to the ground. If the sensors are made to transmit their output by telemetry, then this has the additional advantage of allowing free movement of the experimental subject.

The use of sensors thus provides an efficient use of the scarce resources of crew time, pressurized volume, and power, both in space biology experimentation and in monitoring the operation of life support systems. For these reasons, the American and European space agencies (NASA and ESA) have both initiated programs devoted to the study and development of chemical sensors for spaceflight.¹

III. SENSOR TECHNOLOGIES TO BE CONSIDERED

A. Sensors Already Used in Spaceflight

To date, only physical sensors have been used in space biology research, because these sensors have been available for some time, while chemical sensors are still under development. Table 1 shows sensors used during five missions. The first four missions listed were unmanned satellites, *Biosatellite III* of NASA, and *Cosmos* of the Soviet Union. The animals carrying sensors on these missions were small primates (*Macaca* and *Rhesus*). The fifth one was a *Shuttle/Spacelab* mission carrying an improved version of the Research Animal Holding Facility (RAHF) with rats. The duration of these missions ranged from 5 to 13 days. Under development by NASA are sensor systems for the *Cosmos 1992* primate flight

Table 1. Sensor Technologies in Current Use

<i>BIOSATELLITE III</i>	1969	Temperature, pressure, pO ₂ , pCO ₂ in capsule Food and water consumption EEG, EOG, EMG, brain temperature ECG, respiration Venous and arterial pressure catheters
<i>COSMOS 1514</i>	1983	Temperature and activity sensors Carotid pressure and flow cuff
<i>COSMOS 1667</i>	1985	Carotid pressure and flow cuff
<i>COSMOS 2044</i>	1992	EEG, EOG, EMG Activity and skin temperature sensors
<i>SPACELAB SLS-1</i>	1991	Implanted sensors for body temperature, venous pressure, and aortic blood flow

Abbreviations: EEG - electroencephalogram, EOG - electro-oculogram, EMG - electromyogram,
ECG - electrocardiogram

experiment, the *SLS-2* cardiovascular rat experiment, and the *SLS-3* rhesus research facility. For the *Cosmos 1992* mission vestibular neural response (VNR), EEG, EMG, and EOG measurement systems are being developed, which make use of modular hybrid electronics. The rhesus project will use implanted ECG and deep body temperature transmitters, and hardwired, skin-mounted EMG and EEG measurement systems. The *SLS-2* mission will monitor cardiac output by means of pulsed Doppler ultrasonic flow measurement and arterial pressure, both using implanted biotelemetry systems.

B. Sensors Needed in the Future

Table 2 lists a number of analytes for which chemical sensors would be useful in space biology experiments. The list is not exhaustive; eventually nearly every biochemical parameter would be of interest. A prototype ionic calcium sensor using a coated wire electrode with reference electrode has been developed, which is to be incorporated in a totally implantable biotelemetry system for chronic studies of untethered animals.² Not all chemical sensors need to be implantable. For example, for the analysis of urine samples, the sensor could be dipped into the fluid. For blood analysis an implanted sensor or a skin sensor is preferable. Where this is technically not feasible, the alternative would be to draw a blood sample and to place a drop of blood on the sensor. The latter procedure is being followed with currently available blood glucose sensors for diabetics.

The purity of recycled water is of vital importance for the crew during long-term space missions, as on *Space Station Freedom*, a Lunar base, or a Mars mission. For this purpose, various parameters will need to be monitored, such as pH, conductivity, turbidity, total organic carbon (TOC), microbial count, presence of toxics like ammonia, heavy metals, and hydrocarbons. On-line monitoring would be the preferred method, although for less critical parameters periodic monitoring, once every 1 to 4 weeks would be acceptable.

Table 2. Analytes For Which Chemical Sensors Are Needed

Ions	Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , Cl ⁻ , PO ₄ ³⁻ , HCO ₃ ⁻ , F ⁻ , pH
Gases	O ₂ , CO ₂ , H ₂ , CO, NH ₃
Metabolites	Glucose, lactic acid, creatinine, cholesterol, etc.
Enzymes	Alkaline phosphatase, SGOT, CPK, LDH, etc.
Hormones	ACTH, ADH, adrenaline, aldosterone, cortisol, growth hormone, etc.

Table 3. Sensor Requirements for Spaceflight Use

Operating in microgravity	Minimal size and weight
Accurate	Low power consumption
Stable	Minimal complexity
Easy or automatic calibration	Easy to service
Long lifetime	Materials space-qualified
Biocompatibility (for invasive sensors)	

C. Requirements for Sensors Used in Spaceflight

Obviously, sensors to be used for operation during spaceflight have to satisfy a number of requirements, some dictated by the conditions peculiar to the space environment; others to ensure the safety of the mission and the crew onboard. The requirements are listed in Table 3.

“Operating in microgravity” means that the functioning of the sensor does not depend on gravity. “Accurate” means not only that the analyte concentration is provided within a certain percentage error, but also that the specificity of the sensor is such that no serious interference from other substances present in the sample occurs. “Stable” means that the sensitivity of the sensor does not change appreciably during the period of measuring.

In most cases the sensor produces a signal in the form of an electric current or voltage that requires calibration in terms of analyte concentration. The calibration process should be “easy or automatic,” requiring little crew time. Automatic calibration can in some cases be achieved by the use of a reference sensor inserted in a standard solution.

“Long lifetime” refers both to shelf-lifetime of the sensor, and to the continued functioning of the sensor during an experiment of long duration. Ideally, a sensor should not have to be replaced during the course of an experiment.

“Biocompatibility” is, of course, a strict requirement for all invasive sensors, those that are to be used inside the human or animal body. In the first place this means that the sensor must be constructed of non-toxic, non-corroding materials. As yet, an unsolved problem with invasive sensors is that eventually a series of tissue reactions occurs which increasingly impairs the function of the sensor.

The requirements for minimal size and weight and low power consumption will be clear from what has been said in the previous sections. Minimal complexity and easy servicing are requirements typical for spaceflight conditions where crew time is a precious commodity.

All materials onboard a NASA spacecraft must conform to the requirements set by NASA in terms of structural stability, flammability, explosiveness, and toxicity.³ Extensive testing is required before any equipment is space-qualified.

IV. TYPES OF TRANSDUCERS

A. Potentiometric Transducers

In potentiometric transducers, an electrode, covered by an ion-specific membrane, registers a potential difference relative to a reference electrode—a logarithmic function of the concentration of the measured ion. A concentration range of 10^{-5} – 10^{-1} M can thus be measured (ref. 4, p. 135–152). The drawback of this type of transducer is that the selectivity of the membrane is limited, so there will be interference from other ions, particularly those present in high concentration. For a dominant ion, like sodium in blood and urine, the method can be suitable. Ion-selective electrodes are commercially available.

An interesting development is the light-addressable potentiometric sensor (LAPS), which is essentially a silicon-based pH meter.⁵ A silicon chip is coated with a 0.1-μm insulator layer of silicon oxynitride, which is pH-sensitive over a

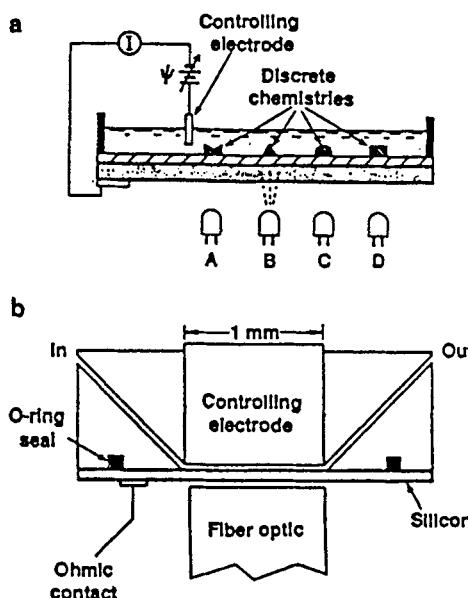


Figure 1. Principle and electric circuit of the LAPS device. a. A silicon plate with a surface insulator of silicon oxynitride (diagonal lines) in contact with an electrolyte solution is photoresponsive to the light-emitting diodes A, B, C, and D. The resulting ac photocurrent I in the external circuit depends on the applied bias potential ψ . b. Arrangement of the microliter flow cell. The reference Ag/AgCl electrode and the flow channels are incorporated in a plunger, which is moved down to 0.1 mm above the coated silicon chip.

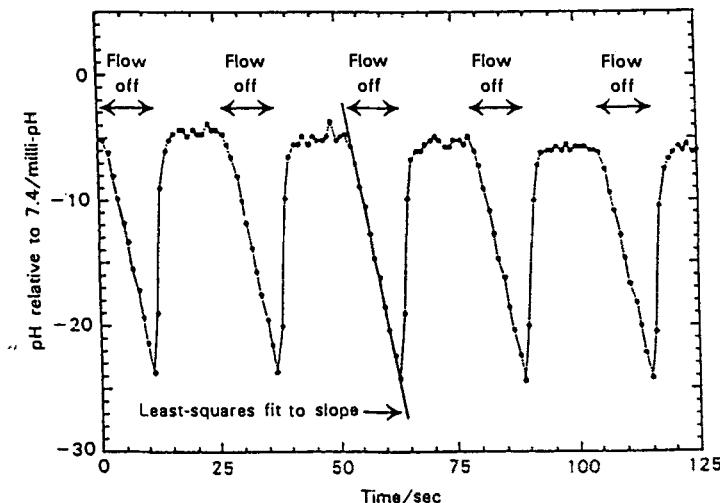


Figure 2. Plot of pH vs. time obtained with the LAPS device. Measurements are made at one second intervals during perfusion with weakly buffered nutrient medium. Several cycles of 10 seconds stopped flow and 15 seconds resumed flow are plotted. The slope of the plot during stopped flow gives the rate of acid production. Values obtained during sample flow can serve as reference pH.

pH range of 2 to 12 due to the proton binding capacity of the Si-O and Si-NH₂ groups on its surface. The chip forms the bottom of a microliter flow cell, in which a Ag/AgCl reference electrode is placed. Between chip and Ag/AgCl electrode, a bias potential ψ is applied, which sweeps from 0- to 1-V once per second. This depletes the silicon of charge carriers at the insulator interface. In the charge-depleted state, an intensity-modulated (10 kHz) light-emitting diode (LED) produces an alternating photocurrent I , which is measured with a low-impedance a.c. ammeter (Fig. 1). Plots of I vs. ψ obey the Nernst equation, and shift to the right with increasing pH. When acid-producing cells are present in the flow cell, changes in pH value can be traced by rapid sampling of I at a median value (Fig. 2). The sensitivity of the device is such that the acid production of as few as 1000 mammalian cells can be measured. The LAPS device can also measure changes in redox and membrane potentials, and it has been adapted for immunoassays by means of the sandwich technique.

B. Amperometric Transducers

In amperometric transducers a constant potential is imposed on a noble metal electrode (platinum, gold, silver, mercury) at which the ion to be measured is reduced (but not other ions requiring a higher potential). A current flows that can

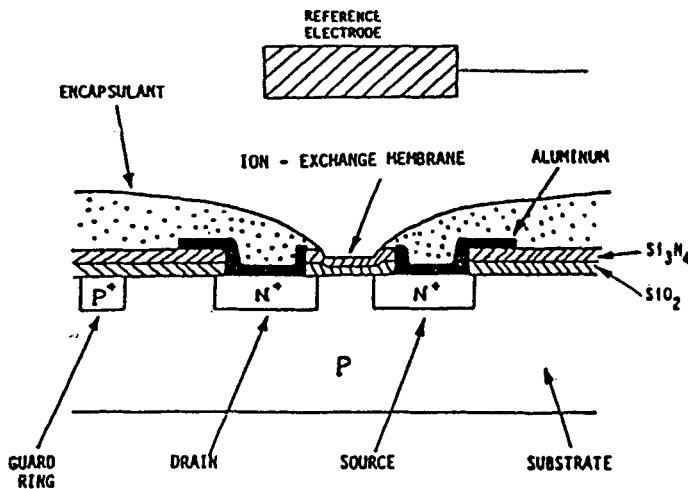


Figure 3. Schematics of an ion-sensitive ISFET connected to a constant current controller and reference electrode.

be measured and is proportional to the concentration of the ion being reduced (ref. 4, p. 165–179). This principle also works with non-ionic substances that can be reduced at the electrode. A well-known example is the Clark oxygen electrode, where oxygen is reduced to hydroxyl ions. Many organic compounds can also be determined in this way. The selectivity by means of the imposed potential is limited, but can be improved by covering the electrode with a selective membrane. Amperometric transducers are commercially available.

C. Ion-Sensitive Field Effect Transistor (ISFET)

This device consists of a transistor in which the silicon oxide insulating layer is covered by an ion-sensitive layer instead of a metal layer (Fig. 3). When an ISFET is in contact with a solution containing the target ion, a potential difference relative to a reference electrode exists which is logarithmically related to the ion concentration (ref. 6, p. 325–358; ref. 7, p. 171–190). Advantages of the ISFET are its small size, which permits its integration with other sensors on a single chip (Fig. 4), and its very short response time (< 0.1 sec). Drawbacks are the long-term drift in the baseline, problems with adhesion of the ion-sensitive membrane, and leakage through the encapsulation (often epoxy resin). An ISFET pH sensor, incorporated in a catheter for medical use, is commercially available. ISFETs for other ions suffer from limited selectivity, short lifetime, drift, the difficulty of providing a reliable reference electrode, and limited biocompatibility for invasive use. A combined pH

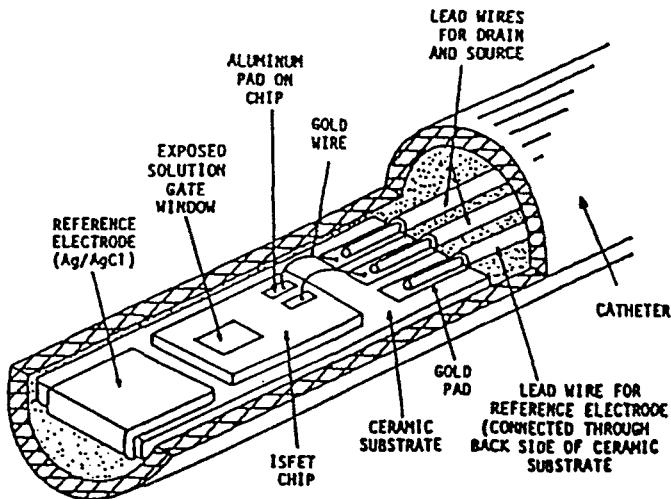


Figure 4. Catheter tip packaging of an ISFET sensor.

(Al_2O_3 membrane) and pCO_2 (Si_2N_4 membrane) ISFET is commercially available. An ammonia sensitive ISFET has also been described (ref. 7, p. 186–188).

D. Optical Transducers

Three types of optical transducers have been developed (ref. 4, p. 599–701).

*Evanescent Wave Optic Fiber System.*⁸ The optic fiber acts as a waveguide by total reflection of the light beam at its surface. Some of the light leaves the fiber and penetrates the surrounding medium for about half a wavelength, called the evanescent wave (Figs. 5 and 6). The fiber is coated with a thin layer of a transparent sensor substance. Binding of the analyte to the sensor substance may cause a change in light absorption or fluorescence, which can be measured by a photometric device. This principle is used in immunosensors, where the sensor substance is an antibody against the analyte and the analyte displaces a fluorescent derivative.

Surface Plasmon Resonance (SPR) Technique (ref. 4, p. 661–663). Here a totally reflecting prism surface is coated with a thin film (60 nm) of gold or silver (Fig. 7). A scan of the intensity of the reflected light beam as a function of the incident angle of the light shows a sharp dip at a particular angle. When the analyte adsorbs at the metal surface, the reflection minimum shifts to a lower angle; this

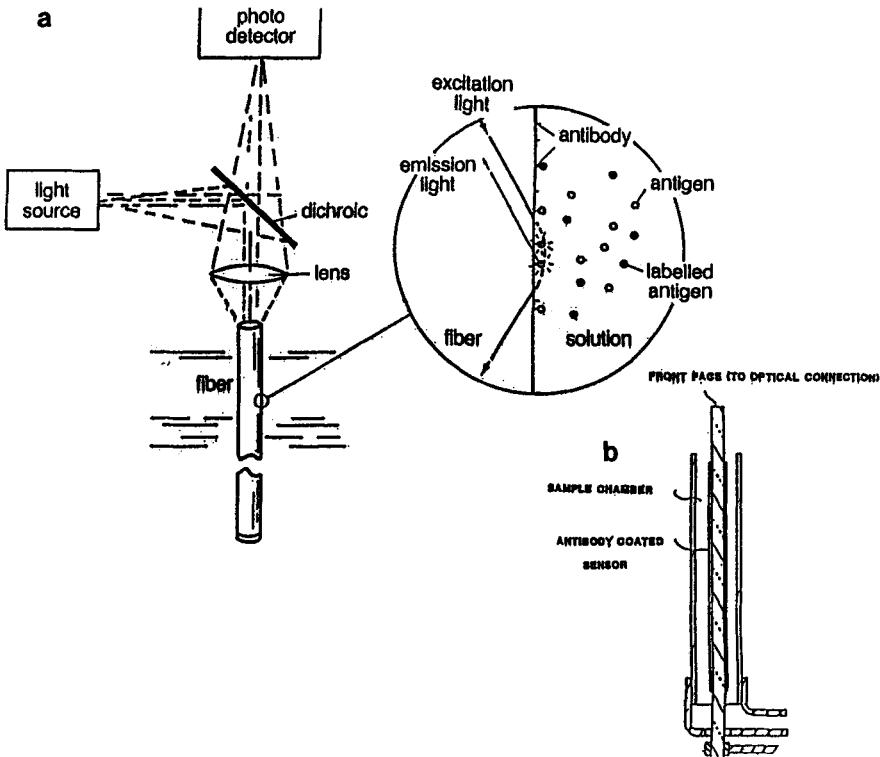


Figure 5. Evanescence wave optic fiber system. **a.** Optical arrangement; **b.** Ligand coated optic fiber.

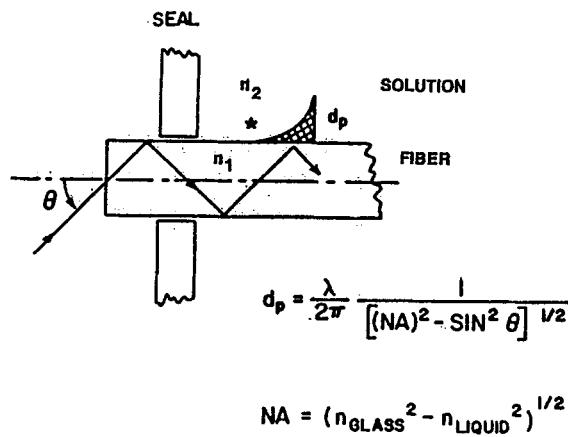


Figure 6. Optic fiber with internal reflection and depth of penetration in medium.

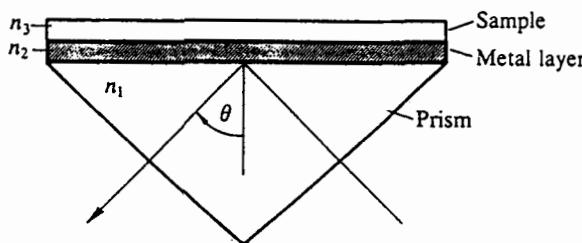


Figure 7. Principle of the surface plasmon resonance (SPR) sensor.

shift is proportional to the amount of analyte bound (Fig. 8). This system is also used as an immunosensor. The advantage of this technique is that it is direct and no fluorescent derivative is needed. The disadvantage of the SPR device is that it is not suitable for miniaturization, and that its use is limited to batch-wise analysis in fluid droplets placed on the prism surface.

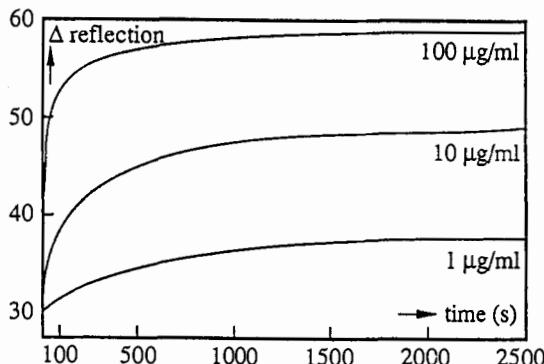
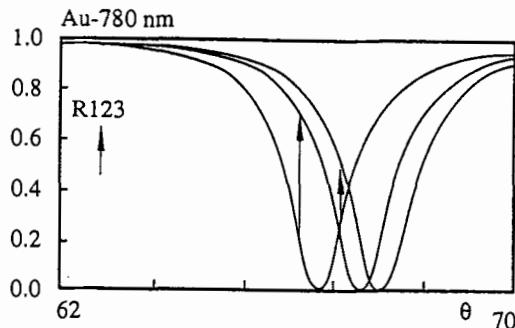


Figure 8. Measurements with an SPR sensor. Top: Dip shift observed after antibody-antigen reaction; Bottom: Signal as a function of time.

Near-Infrared Spectrophotometric Device. In this instrument, the intensity of diffusely reflected light at one or two characteristic wavelengths is measured. Optic fiber wave-guides are usually employed. An example is the skin oximeter, which measures oxygen saturation of hemoglobin through the skin by determining the hemoglobin/oxyhemoglobin ratio with a two-wavelengths technique. Near-infrared light has the advantage of penetrating the skin and subcutaneous tissue rather well. This offers the possibility of a non-invasive sensor.

E. Piezoelectric Transducers

Applying an alternating potential to a piezoelectric crystal like quartz causes the crystal to vibrate at a resonance frequency determined by its dimensions and mass. A change in mass causes a proportional change in resonance frequency which can be measured very accurately. This means that the adsorption of very small amounts of analyte can be observed. By applying suitable coatings on the crystal, a wide variety of analytes can be measured (ref. 4, p. 551-571). The system has been successfully used in volatile and gas-phase analysis, but application in the fluid phase has proved difficult because of high dissipation of energy in a fluid. This problem has been overcome by means of the surface acoustic wave (SAW) technique. A gold crystal with a resonance frequency of 9-14 MHz and coated with an antibody displays a drop in frequency of 100-300 Hz upon the binding of as little as 10^{-12} g of antigen.

F. Thermal Transducers

In this system, the thermal effect of an enzymatic reaction is measured (ref. 4, p. 572-595). Two parallel columns are used, one of which contains an immobilized enzyme. An analyte reacting with the enzyme will cause a temperature change in the enzyme containing column. The temperature difference between the two columns is measured with a transistor.

V. TYPES OF SELECTORS

A. Polymeric Membranes

Various polymeric membranes, such as polyvinylchloride, polystyrene, and cellulose acetate, act as molecular sieves in that they will pass gas molecules but not water molecules or other larger molecules. They have been used in gas sensors for the potentiometric and polarographic determinations of oxygen and carbon dioxide in blood and other fluids, and as a protecting and preselecting film in enzyme sensors (ref. 6, p. 197-276).

B. Glass Membranes

The most important use of glass membranes is in the pH electrode. The type of glass used swells and partially "dissolves" in contact with the solution. The resulting basic silicate groups strongly interact with hydrogen ions. By changing the oxide composition of the glass, the membrane can be made sensitive for other monovalent ions, like Na^+ , K^+ and Ag^+ , though with less specificity than in the pH electrode. Glass membranes are used with potentiometric transducers and ISFET transducers (ref. 4, p. 135–152).

A variation of the use of an ion-sensitive glass membrane is the application of an ion-sensitive coating on a silicon chip as in the LAPS device (see Section IV-A).

C. Ionophoric Membranes

Ionophores are neutral hydrophobic organic compounds that form a complex with a specific cation such as valinomycin with K^+ and nonactin with NH_4^+ (ref. 6, p. 226–243). When one of these compounds is incorporated in a polymeric membrane, the latter will then become permeable for the cation with which the ionophore complexes. Such membranes are used with potentiometric and ISFET transducers.

D. Enzymes

The great specificity of enzymes for their substrate is utilized in enzyme sensors (ref. 4, p. 180–425). The analyte serves as the substrate, while a product or reactant of the enzyme reaction is determined by the transducer. For this purpose, the enzyme must be immobilized on or near the transducer. Various methods of immobilization have been applied: (1) adsorption with or without cross-linking; (2) enclosure between two membranes or in liposomes; (3) entrapment in a gel matrix; (4) incorporation in a polymer film during its polymerization; (5) covalent binding to a metal or glass surface (ref. 4, p. 85–99). The response time is in the order of 1 to 2 minutes.

An example is the determination of glucose by means of the enzyme glucose oxidase, which catalyzes the following reaction:



The glucose concentration can be measured in four ways. First, the disappearance of oxygen is measured by means of an oxygen electrode. Second, the formation of H_2O_2 is measured by converting it to oxygen and water with the enzyme catalase and determining oxygen with an oxygen electrode. Third, an electron transfer mediator like ferrocene can be used to replace oxygen as a reactant, and the amount of reduced ferrocene is measured amperometrically (ref. 7, p. 23–30). The advan-

tage of using ferrocene as a mediator is that it makes the glucose sensor insensitive of the oxygen concentration. Finally, the heat of reaction can be determined by means of a thermal transducer.

E. Antibodies

The great specificity of the reaction between an antigen and its antibody is utilized in immunosensors (ref. 6, p. 297–304; ref. 7, p. 97–124). The antibody is immobilized at the transducer surface (ref. 4, p. 85–99). The specificity is high, but the response time is long (in the order of 60 min). Repeated use of the sensor is possible if the binding strength between antibody and antigen is not too high. For this reason, a part of the antibody molecule (e.g., the heavy chain), is sometimes used instead of the complete molecule. Antibodies have been used in combination with potentiometric, amperometric, ISFET, and piezoelectric transducers, but mostly they are used with optical transducers (evanescent wave optic fiber and SPR technique). Immunosensors can be used for the determination of any analyte for which an antibody can be produced. They are particularly advantageous for the assay of hormones, bacteria, and viruses.

In the case of an optic fiber, this is coated with the antibody. Two methods for measurement of the antigen can be used. In the *competitive method*, a fluorescent-labeled derivative of the antigen is bound to the antibody and fluorescent excitation light is guided into the fiber. The resulting fluorescence from the labeled antigen enters the fiber and is measured by a photometric device. When the fiber is then exposed to the native antigen analyte, part of the labeled antigen will be displaced and the fluorescence will be decreased proportionally. In the *sandwich method*, the antigen analyte is allowed to bind directly to the antibody on the fiber in the presence of a fluorescent-labeled derivative of the antibody, which then binds to the antigen. The fluorescence of this “sandwich” enters the fiber and is measured by the photometric device. In neither case does fluorescent light from unbound fluorescent molecules enter the fiber. The two methods are schematically presented in Figure 9.

In the SPR technique, when antigen binds to the deposited antibody, the reflection minimum shifts to a lower angle, proportional to the amount of antigen bound. The advantage of this technique is that it is direct and no fluorescent derivative is needed.

In the piezoelectric technique, binding of antigen to the antibody-coated crystal is measured as a downward shift in the resonance frequency of the piezoelectric crystal.⁹ Electrochemical measurements (potentiometric, amperometric, ISFET) of an antigen analyte are possible by using antigen labeled with an enzyme such as alkaline phosphatase, or glucose oxidase and an electrochemically active substrate in a competitive assay (ref. 7, p. 100–124).

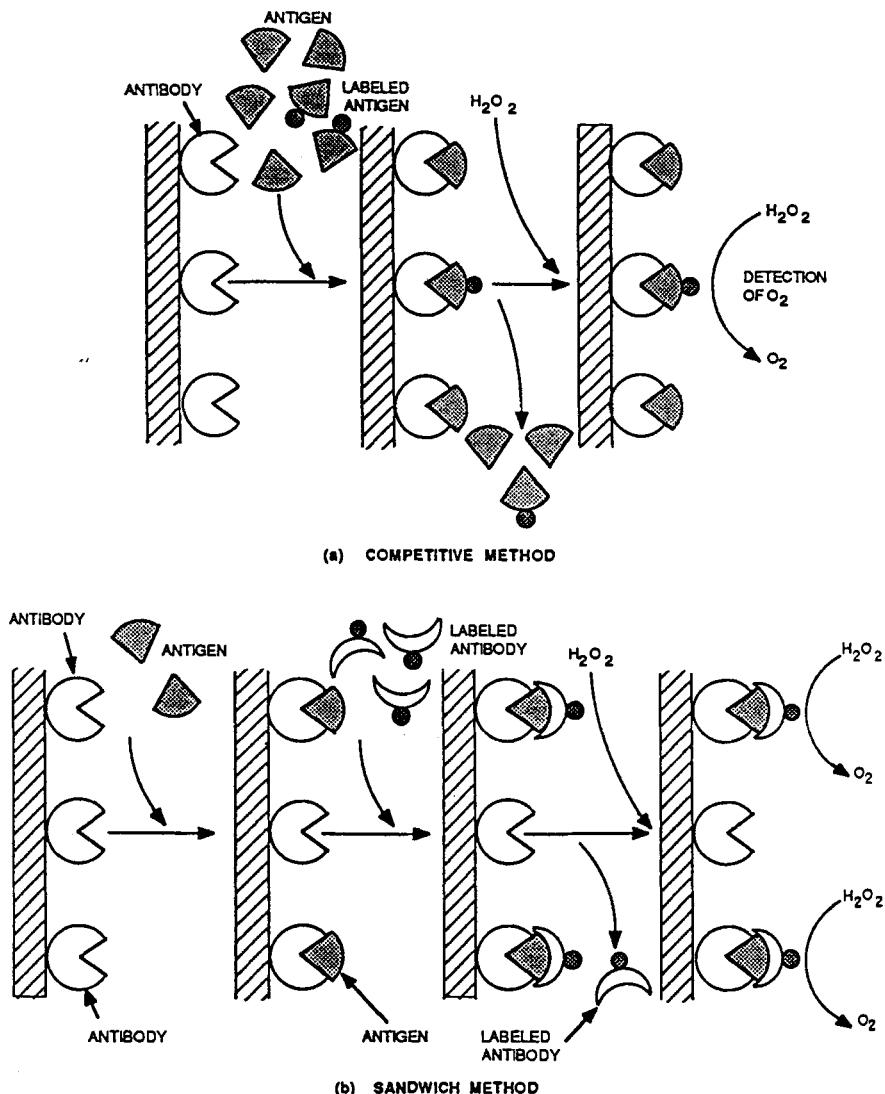


Figure 9. Principle of the competitive and sandwich methods in immunoassays with the evanescent wave optic fiber technique.

F. Cells

Using a cell instead of an enzyme as selector has the advantage that (1) enzyme activities in an immobilized cell are often more stable than those of isolated enzymes, and (2) multi-enzyme selectors can be utilized (ref. 6, p. 294-297; ref. 7,

p. 155–170). The usual combination is with an oxygen electrode, since mostly oxygen consumption is measured. The response is relatively slow, in the order of 10 to 20 minutes. A shelf life of 20 to 30 days at 4°C can be achieved. The dynamic range is often larger than that in enzyme sensors. Examples of analytes which have been determined with a microbial cell sensor are: glucose, ethanol, glutamine, aspartate, methane, biochemical oxygen demand (BOD), creatinine, and urea. In the last two cases, a microorganism is combined with an enzyme (creatinase and urease, respectively).

Plant and animal cells have also been used (ref. 4, p. 30–59). Examples are: glutamine (pig kidney cells, NH₃-sensor), adenosine (mouse intestinal cells, NH₃-sensor), adenosine mono-phosphate (rabbit muscle, NH₃-sensor), dopamine (banana pulp, O₂-sensor), tyrosine (sugar beet, O₂-sensor), and cysteine (cucumber leaf, NH₃-sensor). The NH₃-sensor is a potentiometric transducer consisting of a modified glass electrode. The dynamic range is large; the response time is in the order of 5 to 10 minutes; and the lifetime of the sensor is 30 days or more at 4°C, and 2 to 7 months at –25°C. It should be kept in mind that cells are sensitive to elevated temperatures and pH changes.

VI. SENSORS FOR SPACE BIOMEDICAL RESEARCH

A. Facilities and Activities on *Space Station Freedom*

Space biomedical research is concerned with the study of the effects of space conditions, particularly microgravity, on the living organism. Such studies can have a fundamental aim (how does gravity affect living organisms?), as well as an applied aim (how does long-term spaceflight affect humans?), and (how can countermeasures be developed against these effects?). For this purpose, *Space Station Freedom* will have an integrated biological research facility, the key element of which is a standard-type habitat that with certain adaptations can accommodate and maintain various animal and plant species. This facility has been described in detail in Volume 1 of this series.¹⁰

A stationary holding unit accommodates the habitats with subjects to be maintained at zero gravity. A 2.5 m diameter centrifuge holds the habitats to be maintained at artificial gravity for control or experimental purposes. A glovebox permits handling the subjects for experimental purposes and for transferring them to a clean habitat under conditions of bioisolation.

The habitats are 44 cm wide and 51 cm deep; the heights are 30 cm (rodents), 49 cm (plants or restrained small primate) or 60 cm (unrestrained small primate). They have a standard interface plate at the rear with connections for power, data, water, and air. Each habitat contains an exchangeable specimen chamber suitable for the subjects to be accommodated. The functions integrated in the habitats are shown in Figure 10. There are provisions for 20 data channels, to be divided between

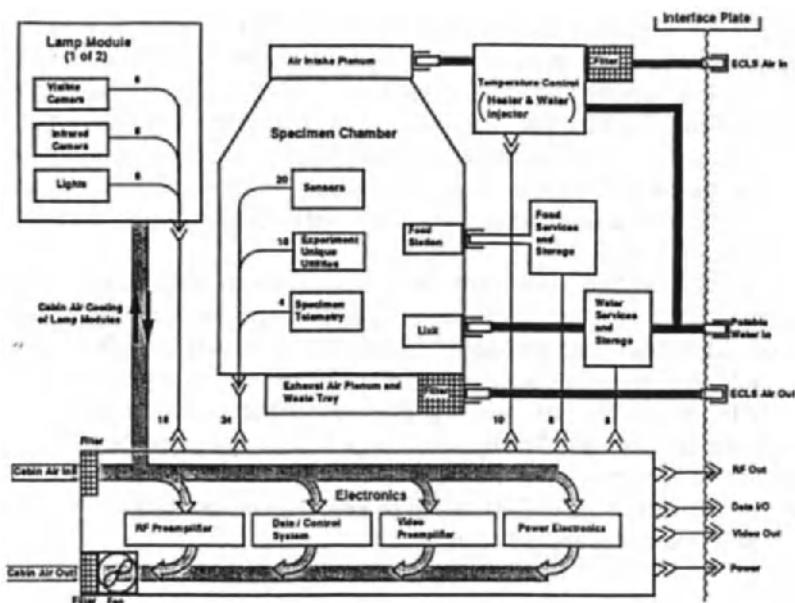


Figure 10. Functions integrated in the habitats for Space Station Freedom. (Top center) location of the exchangeable specimen chamber. The other components are fixed parts of the habitat: lamp module (with lights and cameras; top left), temperature control system (top right), food and water services and storage (right), and electronics system (bottom). (Reproduced with permission from ref. 10)

radiofrequency and hard wire transmission depending on the experimental protocol. In this way up to 20 sensors can be deployed in each habitat.

The large, variable speed centrifuge has a threefold function: (1) to provide 1-G controls for animals and plants; (2) to provide storage of animals at 1 G before the start of an experiment; and (3) to conduct experiments at other G-levels (0.001–2 G by adjusting the rate of rotation), such as gravity-threshold studies. The 2.5 m diameter rotor can accommodate up to 12 habitats, including 6 of 60 cm height (Fig. 11). Centrifuge and holding unit will provide the same services to the habitats: power, water, air, data exchange, habitat monitoring and control, and waste water collection.

Crew members, when serving as subjects for studies of human physiology in space, will have to be monitored. In addition, there will be a need for monitoring the health of crew members. For both purposes, chemical sensors can be very useful. Data transmission by telemetry has the advantage of not hindering normal activity of the subject.

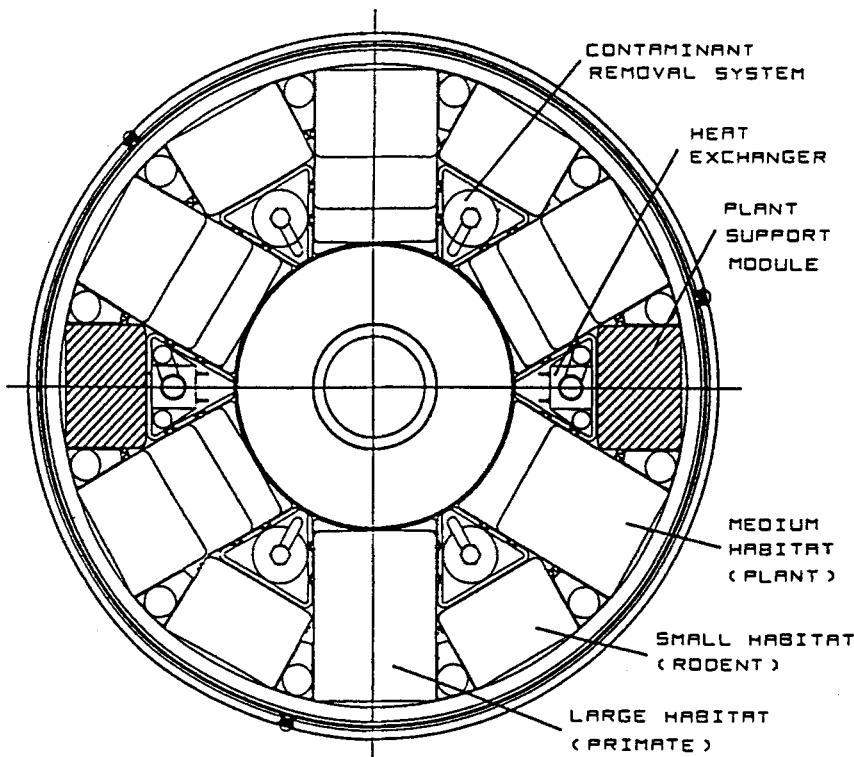


Figure 11. Centrifuge rotor with layout of habitats. Note that six high and six low habitats can be accommodated simultaneously. When plant experiments are conducted, the place of two low habitats is taken by two plant support modules containing bottled gases and plant nutrient media. (Reproduced with permission from ref. 10)

B. Analytes to be Determined

Examples of analytes to be determined are listed in Table 2. Table 4 shows the combinations of selectors and transducers that have been used on Earth and the categories of analytes for which each of these sensors has been found suitable. Here we shall consider the use of these sensors in spaceflight for biomedical experimentation as well as for monitoring of water recycling. For use with small experimental animals (rodents, squirrel monkeys), both transcutaneous and invasive sensors will need to be small and rugged. It would simplify crew training in utilization and servicing of the sensor systems and logistics, if the same sensors would also be used for human subjects. The lifetime of all sensors should ideally be 1 to 3 months in use, and no less than 3 months in low temperature storage. It should not be necessary to manufacture a sensor (applying fresh selector) onboard.

Table 4. Possible Combinations of Selectors and Transducers

	<i>Selector</i>			
	<i>Membrane</i>	<i>Enzyme</i>	<i>Cell</i>	<i>Antibody</i>
Transducer:				
Potentiometric	×			×
	ions			
Amperometric	×	xx	×	×
	ions, O ₂		any substrate (metabolites)	
ISFET	×			×
	ions			
Piezoelectric	×			×
	gases			
Optic Fiber				x any antigen (esp. hormones)
SPR				x any antigen (esp. hormones)
Diff. Reflect. NIR	transcutaneous oximetry, glucose determination			
Spectrophotometry				

x: possible combination. xx: most suitable combination.

For cations, the potentiometric sensor (ion electrode) seems to be the most suitable device, both for invasive and non-invasive applications. For some cations and anions, as well as for dissolved oxygen, the amperometric method would be suitable. The ISFET would be ideal from the point of view of miniaturization and integration of several sensors into a single chip, were it not for the many technical problems that still surround its manufacture and use. The LAPS device does not have these technical problems, but in its present form is not suitable for invasive use.

For the wide variety of metabolites encountered in biomedical research, amperometric sensors with immobilized enzymes appear to offer the best possibilities, both for invasive and non-invasive use. For any substances that cannot be determined in this way, particularly hormones, an optical immunoassay would be indicated. The evanescent-wave optic fiber device, which can be incorporated in a hypodermic needle would offer the best possibilities for invasive use. The SPR device, which cannot easily be miniaturized, appears to be unsuitable for invasive use, and is probably less rugged and easy to use than the optic fiber device. For non-invasive use, the LAPS device in the immunoassay adaptation could be used.

The application of diffuse reflectance, near-infrared spectroscopy with fiber

optics should be considered for transcutaneous oximetry and possibly glucose determinations.¹¹⁻¹³

C. Some Sensor Problems and Their Solutions

Chemical sensors, ideal as they may seem for spaceflight application, still pose several problems and limitations:

- leakage of the bound selector reagent, causing a gradual decline in sensitivity;
- slow reaction between reagent and analyte, causing low sensitivity;
- strong binding of analyte to reagent, as in antibody/antigen reactions, precluding continuous monitoring;
- lack of selectivity of the selector (as in the case of ion electrodes and ISFETs with an ion-specific membrane), leading to interference from unwanted molecules and thus to faulty measurement of the desired analyte;
- invasive sensors eventually cause tissue reactions which increasingly impair the function of the sensor (a period of approximately 9 days is maximal at present for an invasive glucose sensor); and
- long-term drift in the baseline, such as occurs in ISFET sensors.

Advanced polymer technology is being used to remedy some of these problems.¹⁴ Trapping the selector molecule (e.g., an enzyme), and any auxiliary compound (e.g., an electron transfer agent like ferrocene) in a polymer matrix of polysiloxanes and polyethylene oxide sharply reduces reagent leakage (lifetimes of a year), while still providing fast migration of the analyte. To provide fast analyte migration, a sulfonated polydimethyl siloxane membrane of only 40–100 nm on a porous aluminum oxide base (for stability) has been used.

Another technique is to cross-link a polymer (e.g., poly-N-vinyl pyrrolidone) by gamma-irradiation, making it into a molecular sieve that will keep out molecules larger than the analyte, and thus improve selectivity.

Polymers can also be used as “wires” for the transfer of free electrons from the enzyme to the electrode. Polyvinyl pyridine is linked with organic osmium compounds. The polymer chains link up with the enzyme as well as with the electrode; the osmium atoms transfer the electrons along the polymer chain with a much higher current density than in a conventional enzyme electrode.

To increase the lifetime of an immunosensor, a supply of the fluorescent antibodies binding to the analyte in the sandwich technique can be enclosed in a polymer reservoir. Polyethylene vinyl acetate has been used for this purpose.

VII. SENSORS FOR MONITORING OF WATER RECYCLING

A. Types of Water Use

Three types of water can be distinguished in terms of usage: potable water, personal hygiene water, and laundry water. Standards have been set for each type by NASA¹⁵ and are shown in Table 5.

The highest quality requirements must, of course, be maintained for potable water, needed for drinking, food preparation (hydration and washing), and internal use (tooth brushing, eye wash, mouthwash, and douches).

Personal hygiene water, used for washing persons and dishes, constitutes the second largest use of water. This must be substantially free of pathogens and toxic compounds. Excess iodine or other bactericides can be tolerated, since the amount ingested will be small. Total dissolved solids (TDS) requirements are mild, as long as adequate washing action is achieved.

Laundry water, the largest single use of water, has the lowest quality requirements. A moderate accumulation of organics [140 ppm total organic carbon, (TOC)] and salts (500–1000 ppm TDS) can be tolerated. Bactericides, chlorine, or iodine may be used to kill microorganisms. Control of pH, oxidation potential, conductivity, turbidity, and TOC are sufficient for satisfactory performance.

B. Sources and their Hazards

In recycling, water is derived from different sources, each with its own hazards: humidity control condensate, wash water, urine, reduction of respiratory CO₂, and solid organic waste.

Humidity control condensate, deriving from human breath, perspiration, shower, and clothes-washing, will be contaminated with human microorganisms which could multiply in the system. There may also be toxic corrosion products from the plumbing. Organic contaminants will be insignificant if atmospheric particulate and absorbent filters are used.

Wash water from shower, hand, and dish washing will obviously contain many contaminants of all three categories. Urine will have as possible contaminants: ammonia, salts, various bioorganic compounds, and microorganisms. Pretreatment with hypochlorite or potassium mono-persulfate can fix ammonia and inhibit microbial growth.¹⁶

Respiratory carbon dioxide may be reduced with hydrogen to carbon and water by either the Bosch or Sabatier process. The resulting water may be added to the potable water supply. Any microorganisms will be heat-sterilized. Organic contaminants will be negligible, but inorganic contaminants may result from corrosion.

Solid organic waste can be oxidized to carbon dioxide and water. The former can be reduced, leading to more water. Organic contaminants will be negligible as long as complete oxidation is achieved. Inorganic contaminants may result from corro-

Table 5. Maximum Contaminant Levels (MCL) for Potable Water and Hygiene Water

Parameter	Potable		Parameter	Hygiene	
Physical		Bactericides, mg/l			
Total solids, susp./diss., mg/l	100	500	Residual iodine, min.	0.5	0.5
Color True, Pt/Co	15	15	Ibid., max.	4.0	6.0
Taste and odor, TTN/TON	3	<3			
pH	6–8	5–8	Aesthetics, mg/l		
Particulates, max. size, µm	40	40	Cations	30	
Turbidity, NTU	1	1	Anions	3	
Dissolved gas (free at 35°F)	none	none	CO ₂	15	
Free gas (at STP)	none	none			
		Microbial			
Inorganic constituents, mg/l		Bacteria, total count, CFU/l		10	10
Ammonia	0.5	0.5	Anaerobes	10	10
Arsenic	0.01	0.01	Aerobes	10	10
Barium	1.0	1.0	Gram positive	10	10
Cadmium	0.01	0.01	Gram negative	10	10
Calcium	30	100	E. coli	10	10
Chloride	250	250	Enteric	10	10
Chromium	0.05	0.05	Viruses, PFU/l	10	10
Copper	1.0	1.0	Yeasts and molds, CFU/l	10	10
Iodine, total	15	15			
Iron	0.3 0.3		Organics, µg/l		
Lead	0.05	0.05	Total acids	500	TBD
Manganese	0.05	0.05	Cyanide	200	TBD
Magnesium	50	50	Halogenated hydrocarbons	10	TBD
Mercury	0.002	0.002	Phenols	1	1
Nickel	0.05	0.05	Total organic carbon (TOC)	500	10,000
Nitrate, NO ₃ -N	10	10	TOC, less non-toxics	100	1,000
Potassium	340	340	Specific toxics	TBD	TBD
Selenium	0.01	0.01	Total alcohols	500	TBD
Silver	0.05	0.05			
Sulfate	250	250	Radioactive constituents		
Sulfide	0.05	0.05	to conform to Fed. Register, vol. 51, no. 6,		
Zinc	5.0	5.0	1986, App. B, Table 2, col. 2		
Fluoride	–	1.0			

Source: ref. 3. Additional maximal levels in mg/l in potable water: Sodium 150, Carbonate 35

sion of system materials. Biological contamination will also be negligible because of heat sterilization.

C. Sensor Systems for Monitoring

In general, it is desirable to have on-line monitoring of the water coming from the recycling system before it enters the storage tank, because this will give immediate warning before an entire tank of water has become contaminated. However, this will be possible only for a limited number of parameters, such as pH, conductivity, oxidation potential, color, and turbidity. It may also be desirable to monitor the water leaving the storage tank, particularly for microbial growth which might take place during storage.

On-line monitoring is also needed if corrective action is to be applied when a given parameter becomes abnormal. For example, an iodide electrode sensor could be made to control a reoxidation electrode in a recycling path, which would convert iodide back to iodine in order to retain bactericidal action. A calcium electrode could control the addition of calcium to improve the taste of the water.

Periodic monitoring, once per 1 to 4 weeks, will generally be carried out in a batchwise operation. This could be applied to the detection of microbial growth and to the measurement of organic and inorganic contaminants in storage water.

D. Microbial Monitoring

Many pathogenic organisms (bacteria, e.g., *E. coli*, *Streptococcus*, *Pseudomonas*; fungi, e.g., *Penicillium* and *Aspergillus*; yeasts, e.g., *Candida*) could be present in the waste water, and the development of disinfectant-resistant strains during recycling must also be considered.¹⁷ Long-term use of the water distribution system will favor the development of a biofilm within the system, which may harbor organisms and protect them from the residual bactericide. In the absence of sunlight, algae should not be a concern.

Quality maintenance of the water system during a long-term mission will require maintaining a continuous residual bactericide downstream of the recycling process, and a method of restoring system integrity in the event of system contamination. Microorganisms and their toxins will be removed by iodinated resin beds in the water recycling system.¹⁸ Iodine is considered to be the primary bactericidal agent. A suitable iodine sensor will be provided for maintaining the required iodine concentration (0.5–6.0 mg/l). An alternative bactericide, such as chlorine or ozone, will be available in case of the presence of organisms that are resistant to iodine. The possibility of flowing the water past an ultraviolet source should also be considered.

Toxins present a danger only when the recycled water is heavily contaminated with one or more microorganisms. If contamination is properly controlled, there

will be no health risk from toxins. This means that it is sufficient to monitor microbial growth.

In the storage water, microorganisms can also be expected to develop. There is no sense in looking for any specific organism until contamination has been discovered. Thus, in first instance, monitoring of microbial growth in general is indicated. Since the total microbial count in potable water is less than 10 colony forming units per liter,¹⁹ it will probably be necessary to have a bypass at the entrance, as well as at the exit of the storage tank through which some of the recycled water passes. The monitor would trap the organisms and at periodic intervals would signal their presence.

General Detection of Microbial Growth

This can be accomplished in several ways:

1. detection of ATP consumption by means of the luciferin/luciferase technique;
2. detection of carbon dioxide production by means of radioisotope assay or infrared absorption;
3. increase in conductivity in the growth medium;²⁰ and
4. detection of microbial acid production by means of the LAPS device (see Section IV-A).

The LAPS device could probably be adapted to detect the presence of 10 CFU/l (the maximally permissible level; Table 5) by fitting the sensor cell with a microbial filter to retain any microbes present in the water. The water to be tested would be perfused through the cell for a period of 24 hours. Then a growth medium would be perfused for 2 hours to permit multiplication of the microbes to the number minimally required for the detection of acid production.

Specific Detection of Microbial Growth

This may be necessary once microbial growth has been detected. There are currently three techniques available for this purpose:

1. The use of test kits which contain differential media to which fluorogenic or colorigenic compounds have been added.²¹ The filter on which the microorganisms have been collected is cultured, and the results can be available in 18 to 24 hr.
2. The phage method, in which for the detection of an organism like *E. coli*, a culture of the *E. coli* phage is placed with the sample on an agar medium. After 4 to 6 hr of incubation virus plaques can be observed.
3. The use of an immunoassay with an antibody against the organism to be

detected. This can be done with any immunosensor system described in section V-E. It could also be done with an adapted form of the LAPS device having a biotin-coated membrane and a urease-labeled antibody. Urease-catalyzed hydrolysis of urea to ammonia and CO₂ induces a pH change which is recorded by the LAPS device, with a total assay time of only 20 min.²²

E. Toxic Metals

Distilled water is hard on metals, and may for instance, leach out chromium from stainless steel. Microbial growth may lead to a biofilm on the walls of pipes and vessels, which can cause corrosion of a material like stainless steel.²³ When an anti-fouling agent like butyl-tin is used, there is the possibility that tin would be present in the recycled water.

There are now commercially available ion-selective electrodes for ammonia, cadmium, calcium, copper, lead, potassium, silver, and sodium,²⁴ and for a number of anions (bromide, carbonate, chloride, cyanide, fluoride, fluoroborate, iodide, nitrate, nitrite, perchlorate, and thiocyanate). The sensitivity is 10⁻⁶ M or better, which is of the order of the maximal contaminant levels (MCL) for these metal ions in potable water in *NASA-STD-3000*. The response time is approximately 60 sec and continuous measurement is possible. A multiple probe for the simultaneous determination of a number of metal cations has been developed.²⁵ A compact unified ion-selective electrode with simple laser spectrophotometry has been described, which can provide reproducible and sensitive measurements of potassium, calcium, ammonium, chloride, nitrate, and phosphate.^{26,27}

Another method for the determination of heavy metal ions is potentiometric stripping analysis.²⁸ The method is based on potentiostatic reduction and amalgamation (solution in mercury) of metal ions followed by potentiometric measurement. The glassy carbon working electrode has a film of mercury on its surface. The metals are accumulated in the mercury film to a much higher concentration than their level in the sample solution, thus providing a very low detection level (approximately 10⁻⁵ mg/l). The dynamic range is large (10⁻⁵ to 1 mg/l), so dilution is usually not necessary. Another advantage is that several metal ions can be determined simultaneously in each other's presence. However, the method is limited to metals which form an amalgam: Zn, Cu, Pb, Cd, Sn, Tl, In, Bi, and Ga. The metals Fe, Co, and Ni, which do not form amalgams, can be determined by allowing them to form metal-ligand complexes which are adsorbed on the mercury film. Hg and As can be determined with a gold-plated electrode, since they dissolve in gold. The analysis can be automated by means of a microcomputer, and takes only a few seconds. There is no interference from high concentrations of organic compounds and from dissolved oxygen. Although the electrode regenerates automatically after each run, the analysis has to be carried out in a batchwise operation.

F. Toxic Organics

Prior assessment of the contaminants to be expected in the recycled water must provide the information necessary to define the toxicological monitoring requirements. MCL values must be set for the toxic contaminants that can be expected. Monitoring techniques must be developed, which can provide direct measurements (on-line or periodic) of these substances.

Organic contaminants that might be present in the *Space Station* atmosphere, and thus could be found in water are: dichloromethane, butanol-1, ethanol, m-xylene, methyl ethyl ketone, acetone, propyl acetate, halon-1301, methyl isobutyl ketone, propanol-2, butanal, cyclohexane, toluene, cyclohexanol, methane, methyl acetylene, 111-trichloroethane, and methanol.¹⁵ In waste water plasticizers (released by plastics), soap and detergents might be present.

For the determination of such a diverse group of compounds, a non-discriminating sensor will be needed. This might be accomplished with an evanescent wave fiber optic probe where the adsorption of organic molecules causes a change in refractive index at the fiber surface, and thus a change in the intensity of light passing through the fiber. The total organic compound (TOC) sensor being developed by Teknekron,²⁹ which depends on the adsorption of a variety of organic compounds to the silanized surface of an optic fiber, is considered to be a promising approach. The only limitation is that it may not be reversible. Reversibility of optic fiber sensors is a general problem. Although regeneration in strong acid is possible, this requires recoating of the fiber with the selector element, usually an antibody. Replacement of the fiber is therefore preferable. At the Naval Research Laboratory, Washington, D.C., a connector system is being developed which will allow replacing only the active end piece of the fiber, thus reducing the costs.

A prototype fiber optic instrument has recently been constructed, which contains the laser light source, the optical elements, the photodetector, and a microcomputer to provide the analytical data.³⁰ Tapering of the active fiber end by dipping in hydrofluoric acid can increase the sensitivity of an immunosensor 20-fold. Thus, bacterial toxin can be determined in a concentration of 10 µg/l. Fibers have to be selected for low intrinsic fluorescence.

Immunosensing has also been adapted to a continuous flow system for the detection and monitoring of organic toxics and drugs.³¹ Here the antibody is covalently immobilized on Sepharose 4-B beads, and the antibody binding sites are saturated with antigen carrying a fluorescent label. The column with the beads is subjected to a continuous flow of buffer. When the analyte is introduced into the buffer stream, labeled antigen is displaced and is detected downstream. The antibody is retained on the beads for reuse. Concentrations of 50 µg/l and quantities of 5 nanograms can be detected within 45 seconds with a bed volume of 200 µl and a flow rate of 0.3 ml per minute. The column can be used for at least 60 assays before it has to be replaced. The instrument is small and portable, and it can be operated by non-technical personnel.

Table 6. Water Quality Monitoring Schedule for Potable and Hygiene Water

<i>Parameter</i>	<i>On-Line</i>	<i>Batch</i>	<i>Periodic</i>
Conductivity	x	x	-
pH	x	x	-
Turbidity	x	x	-
Color	-	x	-
Temperature	x	-	-
Ammonia	x	x	-
Iodine	x	x	-
Selected specific ions	-	x	-
Other inorganic constituents	-	o	o
Total organic carbon (TOC)	x	x	o
Selected organic constituents	-	x	o
Total bacteria	-	x (bypass)	-
Yeasts and fungi	-	-	x
Bacterial identity	-	-	x
Viruses	-	-	-
Radionuclides	-	x	o
Dissolved gas	-	x	-
Free gas	x	x	-

x: monitoring required

o: monitoring requirements and schedule dependent on water use and recycling process

-: monitoring not required

Source: ref. 3.

G. Suggested Monitoring Strategy

Monitoring of the water entering the storage tank provides a check on the proper functioning of the recycling system. Monitoring of the water leaving the storage tank is necessary because microbial growth may take place there, and toxic materials might enter the tank.

A limited number of mostly physical parameters, like pH, conductivity, temperature, and turbidity, should be monitored on-line. All other parameters can probably be monitored periodically in batchwise operation (Table 6). This is particularly suitable where no continuously operating sensor is available. In order to limit the number of sensors, those with a multiple function should be given preference.

Microbial testing should in first line be on a general basis. Only when microbial growth is detected, will it be necessary to determine the specific microbe(s) present. The method of choice for detecting general microbial growth might be the light-addressable potentiometric device (LAPS). This method will probably be sensitive

enough for the detection of microbial growth in water, particularly when a microbial filter in a bypass is used to collect the organisms. For detecting specific microorganisms, the simplest system might be a commercially available test kit.

Inorganic cations and anions can be determined with sufficient sensitivity by means of an ion-selective electrode (cations: Ag, Ca, Cd, Cu, Na, NH₄, K, Pb; anions: Br⁻, CO₃⁼, Cl⁻, CN⁻, F⁻, I⁻, NO₃⁻, NO₂⁻, ClO₄⁻, CNS⁻) and by means of potentiometric stripping analysis (Zn, Cu, Pb, Cd, Sn, Tl, In, Bi, Ga; Fe, Co, Ni; Hg, As). These two techniques can probably satisfy all needs for analysis of inorganic cations and anions.

Total organic carbon can be determined with an optic fiber technique which is currently being developed. Once there is insight in which toxic organics may be expected, optic fiber or flow immunosensors can be developed for each of these compounds.

It appears that a relatively small number of sensors may be sufficient for adequate monitoring of the water recycling process and of the quality of the recycled water.

VIII. SUMMARY AND CONCLUSION

There will be a great need for a wide variety of chemical analyses, both for biomedical experimentation and for the monitoring of water and air recycling processes on *Space Station Freedom* and later long-term space missions. The infrequent logistics flights of the *Space Shuttle* will necessitate onboard analysis. Chemical sensors offer several advantages over conventional analysis onboard a spacecraft. They require less crew time, space, and power. A chemical sensor consists of a selector which selectively interacts with the analyte present in a mixture of substances, and a transducer which produces an electric signal in response to the interaction of analyte and selector. The transducer signal thus provides a quantitative and selective measurement of the analyte. Types and requirements for chemical sensors to be used in biomedical experimentation and monitoring of water recycling during long-term space missions are discussed.

With chemical sensors, a wide variety of analytes can be determined selectively without separation steps. In principle, chemical sensors can provide (near) real-time monitoring of many important analytes. In some cases they can even provide continuous monitoring of such analytes. The sensors, and even the ancillary instruments, are small compared to conventional analytical instruments. Their power consumption is low. Sensor measurements do not require extensive sample treatment before analysis. In most cases a sensor can simply be inserted in, or be attached to, the organism; or be placed in the water flowing through the water recycling system. Since the sensor signal can usually be provided in digitized form, rapid transmission to the ground is possible. The use of sensors thus provides an efficient use of the scarce resources of crew time, pressurized volume, and power.

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