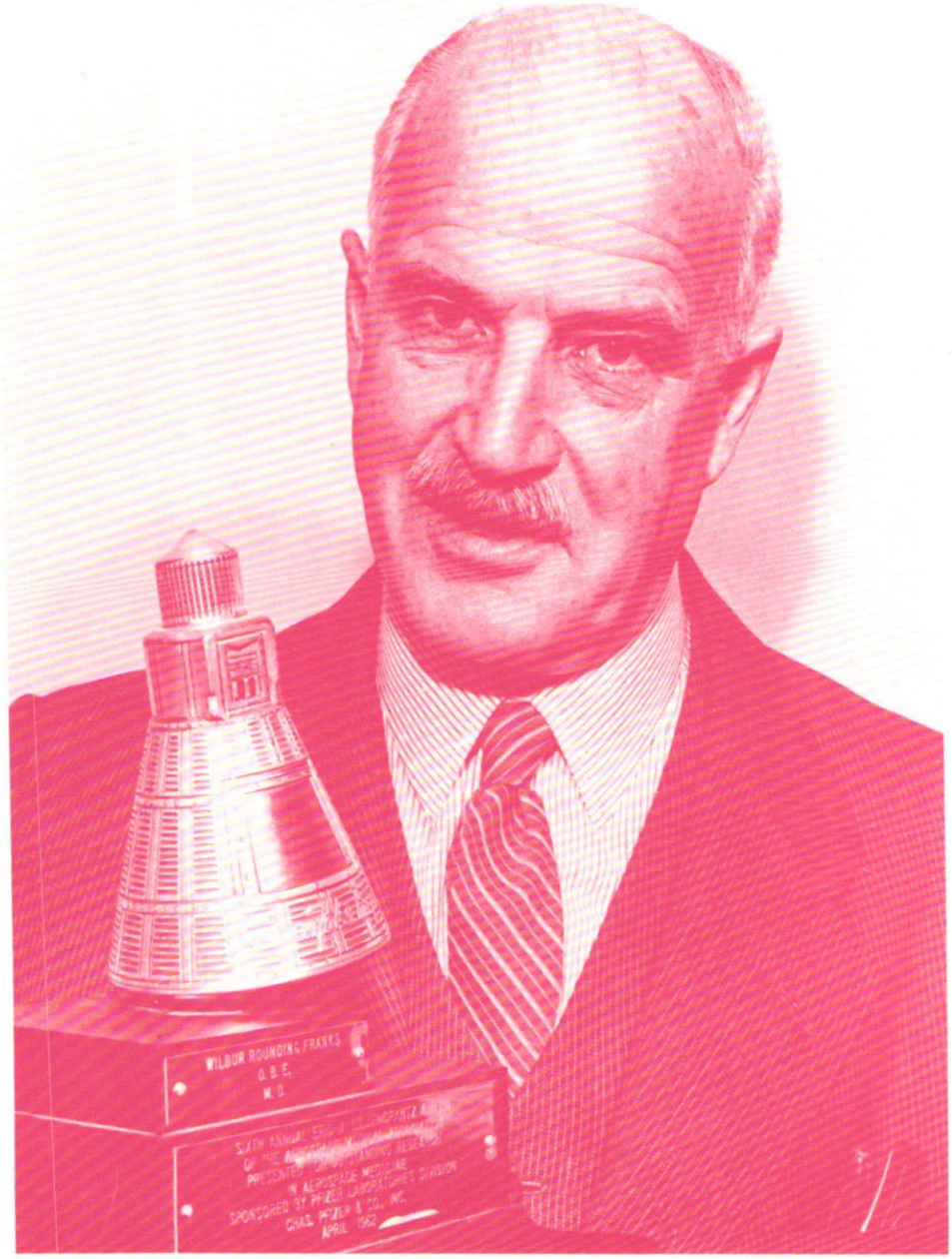
Proceedings of the Tenth Annual Meeting of the IUPS Commission of Gravitational Physiology

A Supplement to
The Physiologist
Volume 32, Number 1
February 1989

Published jointly by the American Physiological Society, the National Aeronautics and Space Administration, and The Galileo Foundation

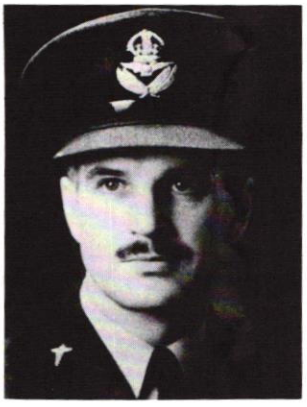


Wilbur Rounding Franks
1900-1986

Proceedings of the Tenth Annual Meeting of the IUPS Commission on Gravitational Physiology

October 9–14, 1988
Montreal, Canada

Orr E. Reynolds, Guest Editor



The Proceedings of the 10th Annual Meeting of the IUPS Commission on Gravitational Physiology is dedicated to Dr. Wilbur Rounding Franks, whose research program in Acceleration (G) Physiology and Aircrew G-Protection Systems at the Royal Canadian Air Force (RCAF), Institute of Aviation Medicine, Toronto during World War II (WWII), is well known throughout the world by all scientists knowledgeable in high G research. This dedication, however, must begin with historical recognition not to Dr. Franks but to Sir Frederick Banting, winner of the Nobel Prize in medicine for the discovery of insulin. Professor Banting, head of the Banting Institute for Medical Research at the University of Toronto, was concerned about the dim prospects of "peace in our time" following the Munich Conference in the fall of 1938. Consequently, at this time he "mobilized" his staff of brilliant research scientists for war by directing them to become aware of problems in war aviation medicine. Subsequently, Dr. Franks, Professor in the Banting Institute, directed his research efforts to problems with acceleration in military aviation (4).

The most significant problem in military aviation at that time was blackout, which occurred in pilots flying high-performance aircraft. This condition of blackout, which no doubt included G-induced loss of consciousness (G-LOC), clearly was limiting the performance capabilities of the most advanced fighter aircraft of the allies, as well as the Germans, in WWII.

It was well known at that time that blackout was caused by reduced arterial blood pressures (P_a) at head level during aerial combat maneuvers due to increased G exposures that resulted in increased intravascular hydrostatic pressures. Since blackout has a physiologic basis, it was expected that scientists with physiologic knowledge should become interested in this military problem.

Consequently, in the fall of 1939 at the outbreak of WWII, Dr. Franks changed his cancer research interests to solving the problem of blackout. He had noticed earlier during his laboratory research that while centrifuging glass test tubes at high speed and floating them in water, they would not break. He hypothesized that animals and perhaps humans could be protected in a similar manner. He therefore placed mice in water, centrifuged them at high speed, and found that their G tolerances increased dramatically; i.e., 240G was required to kill the mice submerged in water. But the problem was clearly one of applying this basic physiologic knowledge to pilots.

A simple crude water-filled suit was designed by Dr. Franks and tested on himself in an aircraft. The concept of water pressure counteracting the internal hydrostatic pressure during increased G exposures proved to be valid as he was able to tolerate much high G levels without blacking out. However, not unexpectedly, this first suit design required major modifications and, of course, needed a method of testing it. The use of an aircraft as a test bed proved to be troublesome, slow, quite dangerous, and unpredictable because of weather constraints. He soon decided upon building a human-use centrifuge as a method to test his anti-G suit, as well as conduct related physiologic experiments.

Working with Mr. G. A. Meek, a research fellow with Dr. Franks at the university, a centrifuge was designed. In 1940, funds were obtained from the National Research Council and private sources; construction began under the direction of Professor H. W. Price, Head of the Department of Electrical Engineering at the University. The centrifuge was completed in the summer of 1941 but was not used regularly until modifications were made to improve its operational capabilities early in 1942.

This centrifuge designed for physiologic experimentation was the first of six human-use centrifuges built by the allies in WWII. It proved to be exceptionally reliable, inexpensive (said to have cost the same as one fighter aircraft), and safe, providing 17,000 test runs on human subjects without a mishap.

Sir Frederick Banting realized the importance of Dr. Franks' anti-G suit early in its development to military aviation. He was killed in a plane crash in 1941 while on a mission to England to further its development involving the Royal Air Force (RAF).

The centrifuge that Dr. Franks designed and built was extremely advanced for that time. Color movies of subjects during G exposures were possible and by replacing the motion picture camera with a portable X-ray apparatus, radiographs of the chest were made of the subject during acceleration.

Well-engineered slip rings provided electrical connections between the gondola and the recording station. Physiologic monitoring of the subjects included but was not limited to electrocardiograms, electroencephalograms, light and buzzer signals that indicated the onset of blackout and unconsciousness, and an ear opacity test using a small photoelectric cell that measured blood flow reduction during increased G. The recording system also had a timer and an event marker so that the G level was continuously recorded using the numbers of revolutions per minute. Dr. Franks was also able to detect when a subject was having a convulsion in association with the G-LOC. The entire centrifuge room was air conditioned since he correctly perceived that G tolerance was reduced by as much as 1G with an increasing ambient temperature.

Although Dr. Franks' major focus was on developing a useable anti-G suit, he conducted numerous physiologic studies that developed this science to a high level of understanding and that must be considered his most important legacy. Interestingly he published very little in the open literature; i.e., only three abstracts in the *Journal of Phys-*

iology Supplement in 1945 concerning his work are known (1–3). However, he did write numerous reports to the Canadian Aeromedical Panel, National Research Council, and Flying Personnel Research Committee. These reports, however, are not readily available to Universities or Government laboratories. The most comprehensive review of his work appears in the *Journal of the Canadian Medical Services* (4). Any interested readers should read this report.

In this dedication because of the need for brevity we can only summarize his extensive physiologic experimental results:

1) accurately measured human-related tolerances to G that included greyout, blackout, and unconsciousness;

2) determined the importance of duration of G on relaxed tolerances and identified the existence of a cardiovascular accommodative response that increased G tolerance but did not identify this baroceptor reflex;

3) identified the convulsive nature and the ECG wave pattern of G-LOC;

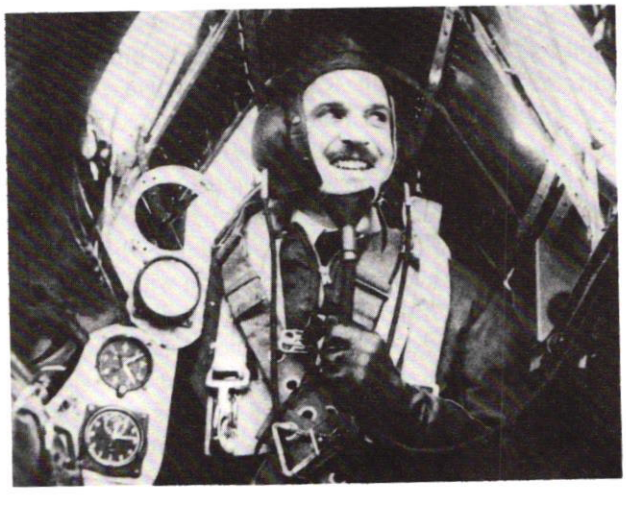
4) found that repeated daily G exposures did not change relaxed G tolerances and that these tolerances are not correlated with tilt-table tolerances;

5) determined that blood going to the brain was reduced in volume and with radiography revealed that a marked decrease in venous return occurred during positive G;

6) identified the beneficial effects of his anti-G suit in increasing G tolerance, improving venous return, reducing fatigue, and supporting people in shock. Interestingly, this application for treating people in shock by increasing vascular resistance with body compression predates the development of the Military Anti-Shock Trouser (MAST) by nearly thirty years!

7) noted that heart rate was slowed during exposure to 3 negative G, but once again did not identify the role of the baroceptor.

His research, however, focused on the development of an anti-G suit that eventually involved seven models. These anti-G suits were called the "Franks' Flying Suits" (FFS) with each succeeding model designated by increasing



Roman numerals as Mark I, Mark II, and finally Mark VII FFS. The earlier suits were water filled and did not require modifications to the aircraft nor any complicated anti-G valves for delivering air, which was required for air-pressurized suits that he had considered. In addition, air pressurization extracted a power requirement from reciprocating engines that was significant. The simplicity of the automatic application of hydrostatic pressure externally with the water suit, to counteract the same increased pressure inside the body during increased G, totally captured Dr. Franks' imagination, which resulted in his continued development of a series of water-filled FFS.

The first operational anti-G suit in WWII was the FFS Mark III that went into service in the Battle of Oran by the Royal Navy Fleet Air Arm, 1942. Not only were the pilots very pleased with the extra G protection, but in the Middle East, bailing out into the Mediterranean or the surrounding desert caused them survival problems due to lack of water. The water in the G suit gave them an extra water ration. Although this FFS was the first anti-G suit flown in combat, the air pressurization cut-away anti-G suit became the suit of choice for the allies in WWII and remains so today. Later versions of the FFS used pressurized air, and Dr. Franks developed a unique automatic bellows system for inflating anti-G suits.

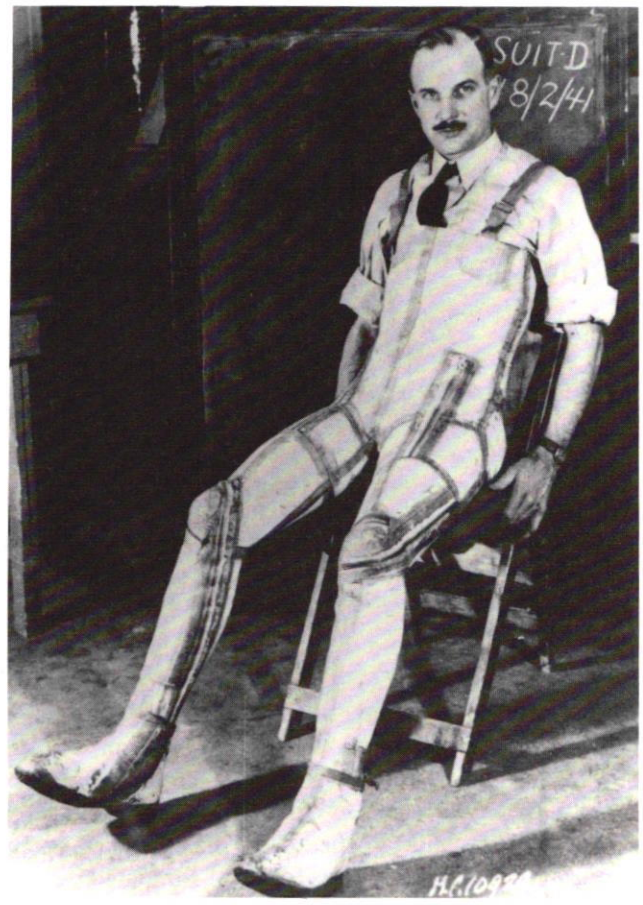
Dr. Franks was keenly aware of the danger of G to pilots and produced several instructional films about G for aircrew. These films had such titles as "Fit for G," "Gen on G," and "G Hygiene." The US Air Force became similarly aware of its danger some 30 years later developing similar types of G information; "G Awareness Kits," for aircrew of high-performance aircraft.

Wilbur Rounding Franks, OBE, CD, BA, MA, MD, at the age of 85, died on January 4th, 1986 at Sunnybrook Medical Centre, Toronto.

For his contributions to the advancement of aerospace medicine, he was made an Officer of the Order of the British Empire in 1944 and received the Legion of Merit from the President of the United States in 1946. The Aerospace Medical Association awarded him the Theodore C. Lyster Award in 1948 "for outstanding research in aerospace medicine" and the Eric Liliencrantz Award in 1962 "for outstanding research in the problems of acceleration and altitude." He was made a fellow of the Aerospace Medical Association (1950), the Canadian Aeronautics and Space Institute (1960), and an honorary fellow of the Ontario Medical Association (1985). He was appointed an Honorary Physician to the Queen in 1966 and Honorary President of the Canadian Society of Aviation Medicine in 1974.

In 1974 he was awarded the highest Canadian award for technical achievement in aerospace, namely the JAD McCurdy Award presented by the Canadian Aeronautics and Space Institute. He was elected a member of Canada's Aviation Hall of Fame in 1983 with the following citation:

> His invention of the Franks Flying Suit and the Human Centrifuge have been accepted throughout the aerospace industry. His significant contributions to research in aerospace medicine have been of outstanding benefit to Canadian aviation.



Dr. Franks, first and foremost, was a patriot who with enthusiasm interrupted his research career at the beginning of WWII to support the war effort for several years. He had a theory and developed it into a workable anti-G suit, which made an operational difference for the allies. In this process, he significantly advanced the physiologic knowledge of acceleration, the environment of the fighter pilot. His research contributions were so numerous and advanced that this knowledge is still used frequently by contemporary physiologists. A scientist can leave no greater legacy.

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IMPORTANT
PRELIMINARY ANNOUNCEMENT

Eleventh Annual Meeting
IUPS Commission on Gravitational Physiology
24–27 September 1989
Lyon, France

The Eleventh Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences will be held in Lyon, France, 24–27 September 1989.

Symposia by invited speakers, slide presentations of voluntary papers, and poster sessions dealing with the effects of physiological systems of humans, animals, and plants of changes in magnitude or direction of the force environment will be scheduled. The effects of weightlessness during space flight, acute and chronic acceleration, vibration, and the various forms of simulated weightlessness are included as well as consideration of the evolutionary consequences of gravity and the role of gravity in the manifestations of scale effects in animals and plants.

Your participation in the Commission Meeting is welcomed. Information and Call for Papers may be obtained from Orr E. Reynolds, Ph.D., Commission Business Officer, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814, USA.

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Opening Remarks

It is a great pleasure for me, as Chairman of the International Commission of IUPS on Gravitational Physiology, to welcome all of you to another of the Commission's Annual Meetings, the tenth of its kind. We are honored to be here in Montreal as a guest organization of the American Physiological Society (APS), and pleased to hold our Meeting jointly also with the American Society for Pharmacology and Experimental Therapeutics. From 1979 onwards, on the kind invitation of APS, every third of our annual meetings has been held in conjunction with an APS Fall Meeting. This is the fourth time that APS is our host, and for all the good things this privilege has brought us in terms of organization and meeting facilities it is my great pleasure to express our gratitude to the Society's Officers and Council, and especially to its Executive Director, Dr. Martin Frank, for his excellent guidance and generous help in the planning and realization of this joint venture.

The purpose of our Commission is, among other things, to promote scientific exchange within its area of interest. The Annual Meetings of the Commission are organized to meet the demand for such exchange. Research objectives include the effects of changes in the gravitational force environment on the physiological systems of humans, animals and plants. Further areas of interest are the physiological effects of the weightless condition during space flight, acute and chronic acceleration, and the various forms of simulated weightlessness. Also included are consideration of the evolutionary consequences of the earth's gravity, and the role of gravity in the manifestation of scale effects in animals and plants.

Our Commission takes great pride in having provided the forum for so many of our colleagues to come together here and present their investigations and discuss scientific matters of mutual interest. This year our Commission sponsors three Symposia with a total of 22 invited papers under the titles "Current Concepts in Gravitational Physiology" (organized by N. Pace and H. Bjurstedt), "Recent Space Flight Results in Gravitational Physiology" (organized by I.B. Kozlovskaya), and "Microgravity and the Lung" (organized by J.B. West). I would like to express our Commission's great appreciation for all the work expended in the organization of these Symposia. Besides the invited presentations at the three Symposia, two open sessions will be held with a total of 20 slide and poster presentations.

I would like to conclude these remarks by expressing our Commission's appreciation to the speakers and their coworkers for the hard work expended in the preparation of their manuscripts, and to all participants in this Commission Meeting who have come here from far-away corners of the world to make this Meeting a memorable experience for all of us. On behalf of the Commission I wish to extend my sincere thanks to the supporting organizations which have made this Meeting possible. Besides the American Physiological Society, our special thanks are due to the USSR Academy of Sciences, the National Aeronautics and Space Administration, and the European Space Agency.

Hilding Bjurstedt

Papers published in the Proceedings of the Tenth Annual Meeting of the IUPS Commission on Gravitational Physiology have been reviewed and approved by the Commission.

The Life Sciences Program at the NASA Ames Research Center: An Overview

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Shortly after the Aeronautical and Space Act of 1958, a presidential decision transferred the man-in-space program from the Department of Defense to the newly formed National Aeronautics and Space Administration (NASA). Project Mercury was the largest NASA effort at that time and relied heavily on Department of Defense Life Sciences facilities, personnel, and technology. For the first time, in 1959, Life Sciences appeared as a $1 million line item in the proposed NASA budget for FY60. A Bioscience Advisory Committee was convened under the chairmanship of Dr. Seymour Kety. The committee recommended that in addition to the operational biomedical support to project Mercury (provided by the Department of Defense) a "NASA intramural program in biology, medicine and psychology was needed, a broad and thoughtfully planned biomedical program of research extending from the most fundamental aspects to their most practical applications" (1). Both Goddard Space Flight Center and the National Institutes of Health were named as possible sites. Political wrangling ensued.

The eventual decision to place this program at the Ames Research Center, a long established NACA Aeronautical Center, was apparently influenced to a great extent by the wealth of excellent universities in the area and the ongoing simulation and centrifuge studies supporting aviation. Through the close research ties with this surrounding academic community, the extensive exchange of students and scientists, and the flexibility to administer some extramural funds, the program has been able to mobilize the relevant research talent and strive for excellence and innovation.

ARC VISIONS

The Center has focused on seven key visions for the future, of which three heavily involve life sciences: Space Sciences, Earth and Space Projects, and Humans in Space (Figure 1). In Space Sciences, fundamental questions relative to "origin, evolution and distribution of life in the universe" are addressed. Under Humans in Space, the goal is to obtain the knowledge and the supporting technology to make it possible for man to be in and explore the universe. Access to Earth orbit or outer space through Space Projects is essential to this program.

ARC LIFE SCIENCES

Activities in the Life Sciences cover a program to answer basic questions about the nature of life itself, and its evolution in the Universe from basic elements to the search for extraterrestrial intelligence. This also includes the study of the evolution and development of life on planet Earth and the global changes occurring today that affect life on Earth. Central to the program are the effects on living systems of the removal from Earth's gravity and magnetic fields. Thus fundamental research forms the basis of understanding and developing preventive and therapeutic support to enable man to live and work in space and to explore the universe. An extensive program in biomedical research, human performance and the development of advanced life support systems (from the physicochemical and bioregenerative needs of the spacecraft to space suits and portable systems for use on planet surfaces) form the core of the program (Figure 2). This program relies heavily on the development of advanced technology.

SIMULATION MODELS

Since access to space is limited, ground simulation models have been developed to enable the more extensive study of the effects of space. The clinostat has been a useful tool to null the effects of gravity in the study of plants. In rats, the tail-suspension model, developed by Morey-Holton et al. (2), has proven an invaluable experimental tool, with proven fidelity to the effects of actual space flight, at least for durations of 7-14 days (3). In humans, water immersion or head-down bedrest that mimics the headward shift in circulating blood volume, and the unloading effects of weightlessness on the musculoskeletal system, are used extensively to study the more prolonged effects of weightlessness. Actual weightlessness can be achieved for only a few seconds in parabolic flight. This has been useful in the study of disorientation, which is believed to trigger space motion sickness. Also in the human performance area, changes in sensory motor coordination can be detected even during the few seconds of weightlessness provided by parabolic flight or in increased gravitational loads on a centrifuge. ARC has a variety of rotating devices for studying in animals and humans the effects of increased gravity loads. These devices are also used in a research program designed to evaluate the requirement for artificial gravity as a countermeasure for the biomedical consequences of exposure to microgravity.

FLIGHT PROJECTS

Access to space for the study of biological systems is less than we would like it to be but nevertheless it has been quite extensive and diverse. Table 1 provides a historical summary of past Ames Life Sciences projects. They have included primates, rats, plants, insects, cells, tissues and microorganisms, as well as the crew whenever possible. Flight durations have ranged from a few days to 21 days on the soviet COSMOS biosatellite. On some COSMOS flights, 1 x G centrifugation of the animals was also provided with the results strongly suggesting that it reversed nearly all spaceflight effects (4). Highlights of the results are shown in Table 2.

Future flight activities include primarily those on Spacelab-1 and -2 (due to fly in 1990 and 1992 respectively), and continuation of our very fruitful collaboration with the U.S.S.R. COSMOS program. Also we are involved with other international partners on SPACELAB-J and the International Microgravity Laboratory. In addition, we fly experiments as secondary payloads and middeck shuttle payloads. Investigators on these experiments come mostly from the academic community but include a significant number of scientists at the Center.

BIOMEDICAL PROGRAM

Primary areas of research focus on the well-documented changes associated with spaceflight and the interaction among these. It is, for instance, now clear that muscle atrophy in the legs reduces the support provided to both bone and vasculature. Countermeasures designed to maintain muscle mass in the lower extremities may be partially, if not fully, effective in preventing bone and venous compliance. Data from head-down bedrest studies suggest that increases in circulating cortisol vary according to the "stressfulness" of the conditions of a study or a mission, rather than reflecting a specific effect of weightlessness on the pituitary-adrenal system. Thus the extent of cortisol-dependent effects on bone and the immune system would be expected to be reduced by training and by psychophysiological

intervention. Hence a change in one system which affects another must be taken into account in the search for the mechanisms involved in the development of biomedical and performance programs associated with spaceflight and in the development of appropriate countermeasures.

The effects of the headward shift in blood volume on the mechanisms regulating fluids and electrolytes and their cardiovascular consequences are one of our main areas of research (Figure 3). Using animals and man, we are investigating changes in cerebral spinal fluid pressure; central and peripheral regulation of the renin/angiotensin/aldosterone system and of natriuretic factors; the role of vasopressin (AVP) and its effects on thirst and kidney function; sodium (Na+)/potassium (K+) reabsorption; adrenal/renal and vascular sensitivity to neural and hormone stimulation; the roles of angiotensin, AVP, natriuretic factors, the autonomic nervous system, and K+ on orthostatic hypotension and on baroreflex sensitivity; the effect of changes in fluid and electrolyte balance on work performance; and thermoregulation. We are using this information to develop appropriate countermeasures.

We are trying to characterize the response of muscle to spaceflight and the rate and extent of muscle recovery post-flight, using rats in both flight and ground-based studies, as well as humans in bedrest studies. The effects of various exercise regimes are also being studied. We want to identify the endocrine and biochemical processes underlying the response, and to develop ways of preventing or counteracting the flight-associated muscle atrophy. Both humoral and biomechanical procedures are being investigated. Vandenburgh's elegant studies on the regulation of muscle fiber size in vitro support the notion that both tension and growth factors are required (4).

A small but elite group is addressing the problem of calcium and skeletal metabolism in spaceflight. Views have evolved over the years. It is no longer believed absolutely that bone density is equivalent to bone strength. In fact, results from rats flown on COSMOS where bone was labeled pre- and post-flight with tetracycline (5), as well as more recent human studies, have contested the long-held view that the primary effect of spaceflight was increased bone resorption (6). Calcium absorption and excretion by the gut, and kidney function and endocrine regulation of calcium, are increasingly gaining importance in the way we believe that bone changes in spaceflight (Figure 4). Our current research is focused on the defect in mineralization of bone (which we know occurs) and on its consequences in terms of the physical properties of bone and the calcium endocrine system.

Finally, we feel that with the exception of its importance to space motion sickness, the role of the central nervous system in the development of all spaceflight-associated changes in health and performance has been generally neglected by the agency. It should be considered, for instance, that the overt symptoms of sickness may be accompanied by more subtle but more pervasive readjustments of central nervous system (CNS) adaptive changes in networking and transmission. A strong neurosciences group and a growing psychophysiology nucleus is addressing both the obvious and the more subtle role of the CNS in the responses to spaceflight and the ability of man to effectively work in that environment, especially for long durations. Figure 5 shows that this research ranges from the study of the vestibular system and the neuromuscular junction to the neuroendocrine system and the psychophysiological factors underlying human performance and the response to spaceflight associated stresses. This work is complemented by the research of the Aerospace Human Factors Research Division, which is recognized internationally for its expertise.

CONCLUSION

This is by no means a comprehensive review of the Life Sciences program at Ames. Research stretches from the fundamental to the applied, from ground to spaceflight, and from single cell and isolated tissue to rat and man. Because of this active intramural program, Ames scientists collaborate actively with numerous university investigators and act as brokers enabling the academic community access to space. Our philosophy is that the best applied research is found where it is coupled with solid and innovative basic research.

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TABLE 1
REPRESENTATIVE AMES LIFE SCIENCES PAST PROJECTS

- **PAST**
  - **BIOSATELLITE 1, 2, 3 (1966, 1967, 1969)**
  - **JOINT US/USSR COSMOS BIOSATELLITES (1975-PRESENT)**
  - **PLANT GROWTH UNIT IN SHUTTLE MID-DECK (1983, 1985)**
  - **ANIMAL ENCLOSURE MODULE IN SHUTTLE MID-DECK (1983, 1984)**
  - **SPACELAB 3**
    - **HUMAN AUTOGENIC FEEDBACK EXPERIMENT FOR SPACE MOTION SICKNESS**
    - **RESEARCH ANIMAL HOLDING FACILITY TEST FLIGHT**
    - **ANIMAL PHYSIOLOGY EXPERIMENTS — "PARTS PROGRAM"**
- **OVER 100 LIFE SCIENCE FLIGHT EXPERIMENTS DEVELOPED AND FLOWN BY ARC**
- **OVER 50 CURRENTLY UNDER DEVELOPMENT AND AWAITING FLIGHT**

TABLE 2
HIGHLIGHTS OF RESULTS FROM
U.S. SPACEFLIGHT EXPERIMENTS

- PLANTS
  - PROBLEMS IN ORIENTATION
  - NORMAL GERMINATION AND EARLY DEVELOPMENT
  - LIGNIFICATION RATE DIMINISHED
  - ROOT CELL CHROMOSOME ANOMALIES
- INSECTS
  - ALTERED BEHAVIOR AND AGING
  - NORMAL DEVELOPMENT
  - NO SIGNIFICANT MUTATIONS
- ANIMALS*
  - DECREASED BONE GROWTH
  - DECREASED BONE STRENGTH
  - INCREASED RBC HEMOLYSIS
  - MUSCLE ATROPHY
  - ALTERED BEHAVIOR, BALANCE, AND COORDINATION
  - ALTERED LIVER ENZYMOLOGY
  - ALTERED BODY COMPOSITION
  - STRESS ASSOCIATED CHANGES
  - INTERFERON-GAMMA PRODUCTION DEPRESSED POSTFLIGHT
- MICROORGANISMS, CELLS AND TISSUES
  - NO SUBSTANTIAL CHANGES IN GROWTH AND REPRODUCTION

*1 X G CENTRIFUGATION OF ANIMALS DURING SPACEFLIGHT REVERSED NEARLY ALL SPACEFLIGHT EFFECTS

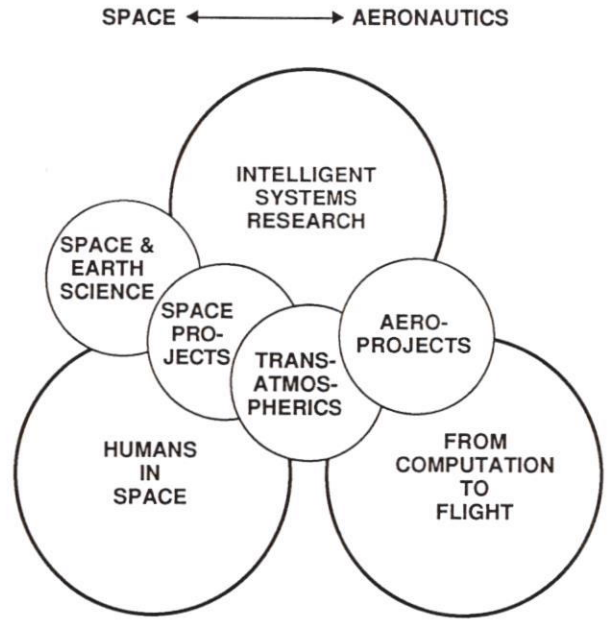

Fig. 1 The vision of Ames (1996).

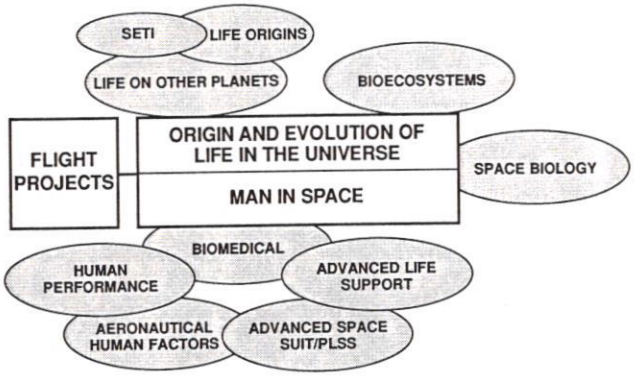

Fig. 2. Ames Research Center Life Science Program.

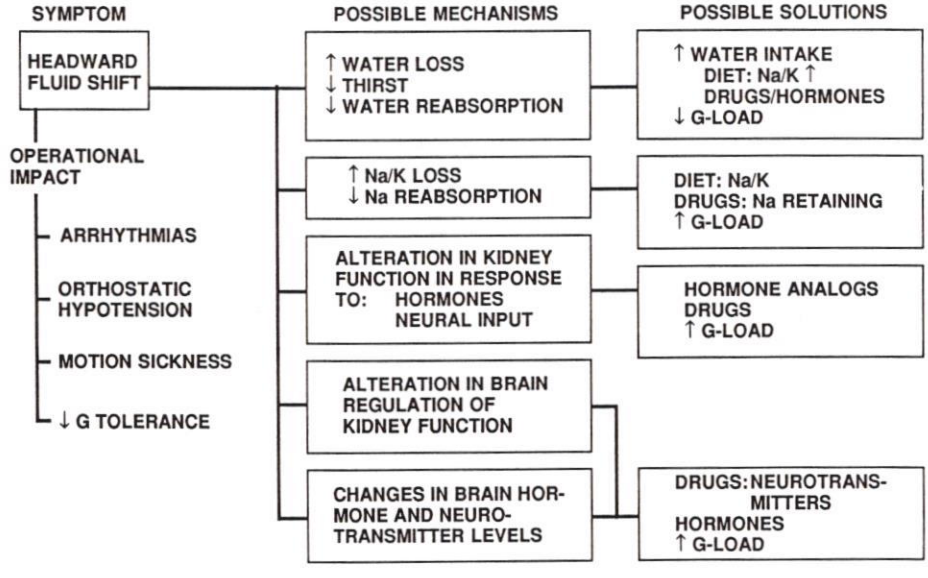

Fig. 3. Fluid and electrolytes.

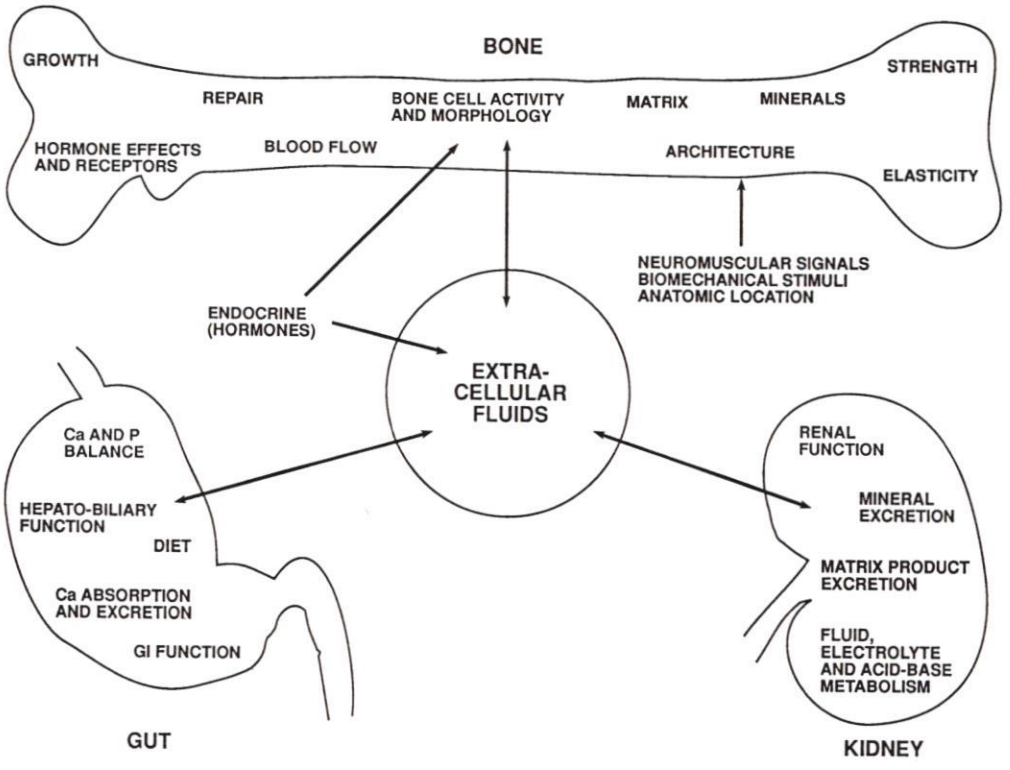

Fig. 4. Calcium and skeletal metabolism.

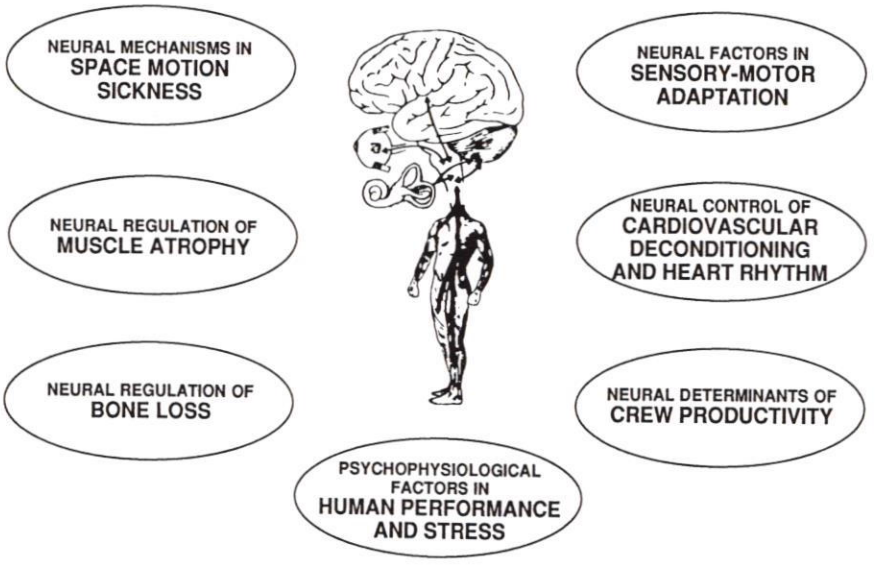

Fig. 5. Neuroscience/human performance.

Periodic Acceleration Stimulation in a Weightlessness Environment (PAS-WE): A New Science?

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Introduction

Although knowledge concerning the effects of weightlessness on all of the physiologic parameters of the human is limited, we do know enough to realize that null gravity is not a friendly environment. Research has clearly demonstrated that significant duration of weightlessness will cause muscle weakness, bone demineralization developing into osteoporosis, cardiovascular decompensation resulting in orthostatic intolerance, and suppression of the immune system that could result in disease such as cancer. Yet both Soviet and American space programs suggest that adaptation to this environment can be tolerated if for no other reason than that it is inevitable--at least until a rotating space station can be developed that will produce artificial gravity. However, such a massive undertaking as a rotating space station is several decades away while space crew in the meantime are developing what must be termed the Weightless Adaptation Syndrome - (WAS).

Humans have developed in a gravitational environment that has challenged and molded their major physiologic processes to an extent that these processes require gravitational stimulation. Humans probably will not be able to live indefinitely in a weightlessness environment without seriously jeopardizing their very existence as human beings.

Therefore considerable thought should be given to stimulating these physiologic processes with periodic artificial gravity in space using a centrifuge (2). Unfortunately, so little is known about periodic acceleration stimulation in a weightless environment (PAS-WE) that it is now only a hypothesis or possibly at best a theory. Certainly as knowledge is acquired in PAS-WE using acceptable scientific methods, a new science or at least subscience will emerge that will have evolutionary roots that began when animals first left the water to live on land and face the challenge of gravity.

We all know that gravity and acceleration are equivalent so that the ability to produce artificial gravity with centrifugation is a scientific fact. However, does the body require continuous exposure to gravity to produce the required gravitational stimulation for bodily processes? I suggest not, and in fact scientific evidence suggests that the only requirement for sufficient gravitational stimulation is short durations of PAS-WE on a regular daily, or perhaps less frequent, basis.

Evidence for Periodic Stimulation

Interestingly, there is little direct evidence that regular periodic exposure to gravity (using bed rest studies as the weightless environment) will prevent WAS and, in fact, no research directed at specifically preventing WAS has been conducted. Nonetheless, there are known physiologic responses that occur naturally or during research studies that strongly suggest that PAS-WE will be useful in space.

Daily Bed Rest:

Gravity does not stimulate us 24 hours a day in our posture (seated or standing) to maintain normal adaptation to earth's gravity. Bed rest has been documented during several research studies as an excellent simulation of weightlessness. Since we sleep in the prone or supine position several hours each day, we naturally maintain adaptation to gravity with daily periodic exposure to gravity--we usually spend about two-thirds of our time standing or seated. But is that much time required on a regular basis to maintain this adaptation? Direct research evidence suggests not.

Daily Three and Four-Hour Exposures to 1G:

A recent study has shown that the calcium metabolism bone deconditioning aspect of WAS produced by continuous bed rest can be prevented with daily four-hour exposures to 1G (11). Other physiologic conditions of WAS were not studied.

Increases in urinary calcium output from continuous bed rest were reduced to nearly pre-bed rest levels with three-hours of daily standing at 1G. Neither daily supine bicycle ergometry up to four-hours/day, nor sitting at 1G for eight hours/day had any effect on the increased urinary calcium from bed rest (9).

Periodic Acceleration Stimulation for Adaptation to an Increased Acceleration Environment:

Several research studies have shown that animals can physiologically adapt to an increased acceleration (above 1G) environment that is maintained continuously for extended durations of time--months that on occasion have been extended to years (12). Continuous exposure to increased G environment may be stressful at the beginning of the exposure that results in a disease of stress known as Chronic Acceleration Sickness (5). Chronic Acceleration Sickness can result in death or, with an adequate physiologic adaptation response, the animal can recover and thrive in this chronic increased G environment (6, 8).

The pathologic response to this exposure can be significantly reduced with fewer animals dying and more of them successfully adapting by gradually introducing them to this new dynamic environment. Two types of adaptation regimens have been studied: (a) slowly increase the level of

G over a series of several week-long exposures eventually reaching the desired level of increased G or, (b) short duration daily exposures at the desired level of G.

The results from this latter type of adaptation regimen of repeated exposure appears to be relevant to PAS-WE. It was found that four hours of repeated daily exposure to increased G was optimum for adaptation to the continuous environmental exposure. Shorter durations of daily exposure did not produce adaptation and longer durations were too stressful, eventually leading to death (7).

This study clearly shows that the basic physiologic mechanism instrumental in developing adaptation to G can be stimulated with periodic G exposures. It is reasonable therefore to extend this logic and theorize that adaptation to a "normal" G environment can be maintained in a reduced dynamic environment with similar periodic G exposures. Also, since we are only maintaining adaptation and not inducing it, less lengthy and perhaps less frequent exposure would be necessary.

Incidentally, periodic adaptation to acceleration or gravity is probably the "natural" approach that animals used during the evolutionary process of adapting to land from the physiologic weightless environment of water. Animals in this process of gravitational adaptation probably moved onto the land (with gravitational exposure) for short-periods each day, retreating back into the water for the remainder of the day to recuperate from the stress of gravity. Certainly, the gravitational adaptation regimen of slowly increasing gravity was not possible.

Periods of Exercise That Prevent Loss of Physical Conditioning:

Although exercise is not synonymous to gravitation as many treadmill studies have clearly shown, some basic physiologic pseudo-adaptive mechanisms to exercise may be similar to those mechanisms which produced adaptation to gravity. Specifically, the ability of the body to maintain a level of physical condition that must be obtained with training, requires considerably less effort than needed to develop that level of physical condition (1, 10).

The First Required Study

To determine if PAS-WE is an option to prevent WAS, a bed rest study that will measure all known physiologic parameters affected by weightlessness and simulated with bed rest must be conducted. This first study will have subjects in continuous bed rest until all physiologic parameters known to be affected by bed rest have significantly deteriorated; then the study can be terminated.

At the outset of this study, groups of subjects will each be exposed daily to a different period of time, standing beside the bed in order to determine the minimum amount of time necessary each day to prevent the threshold of deterioration for each physiologic parameter. Groups of these 1G daily exposures should include as a minimum: 30 min and one, two, four, and eight hour groups.

Each physiologic parameter may have different 1G daily exposure requirements to prevent deterioration that will begin to relate gravitational requirements to the different physiologic processes of the body--the big first step in understanding the physiologic basis of gravitational adaptation. However, for space application in preventing WAS, the 1G stimulation requirements of the most resistant physiologic prevention will have to be determined and used as a basis for developing acceleration schedules for the Space Station and for the trip to Mars to prevent WAS.

Follow-on studies will of course include: (a) different postures useful in preventing WAS (seated and squatting can be compared with standing); (b) different levels of G as related to duration requirements since periodic exposures to (for instance) 2G may very well reduce the required time of these acceleration exposures; i.e., 1G for one hour whereas 2G for only 30 min may be sufficient to prevent WAS; (c) the minimum radius centrifuge requirements that develop the threshold level of G throughout the body to stimulate the physiology to prevent WAS; (d) effect of various exercise activities combined with the periodic G exposure that may be useful to reduce the centrifuge exposure time; and (e) the minimum frequency of these periodic requirements (once a week may be adequate for the periodic acceleration exposure).

These proposed studies have been discussed previously in considerable detail (3).

We recently completed a G-tolerance study using a 5 ft (1.5m) radius centrifuge. Subjects were positioned so that they received acceleration stimulation that resulted in a head to foot inertial force. Their heads were approximately 2 ft (0.61m) from the center of rotation of the level of their buttocks in a squatting position. G exposures up to 7G at 76 rpm were well tolerated by all seven subjects. G-tolerance differences clearly showed that the baroceptors were stimulated suggesting that this radius centrifuge would be useful in Space Station for stimulating the cardiovascular system thereby preventing the reduction of orthostatic and G tolerances that occurs in space (4).

All of these studies will profoundly expand this fascinating science of acceleration physiology--specifically, the interaction of physiologic adaptation with G level, physical activity, G-level duration, and the G force vector.

Application of PAS-WE

Of course PAS-WE will have its first application in the Space Station to prevent WAS. Also, development of PAS-WE will expand our understanding of the basic phenomenon of gravitational interaction with basic physiologic functions. This knowledge gained with these bed rest studies can be validated in space with the Space Station centrifuge (2). Additional, more sophisticated, gravitational-physiologic adaptation studies can be conducted in space that will considerably expand our basic understanding of this phenomenon of the evolution of gravitational adaptation.

However, in addition to these obvious applications, the medical community also may benefit from this knowledge. A basic understanding of

the minimum G exposure and G vector requirements to prevent bone decalcification will be useful in orthopedic medicine on earth and eventually even in space. Perhaps daily exposure to 2G on earth may increase the rate of bone repair or prevent some types of osteoporosis.

Clearly PAS-WE is a science whose time has come; it can begin developing with some clearly-defined objectives such as those proposed in this article. I predict scientific rewards will be well worth the cost and effort.

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INTERSEROSAL PRESSURES AND CIRCULATORY HOMEOSTASIS DURING CHANGES IN THE GRAVITATIONAL INERTIAL FORCE ENVIRONMENT*

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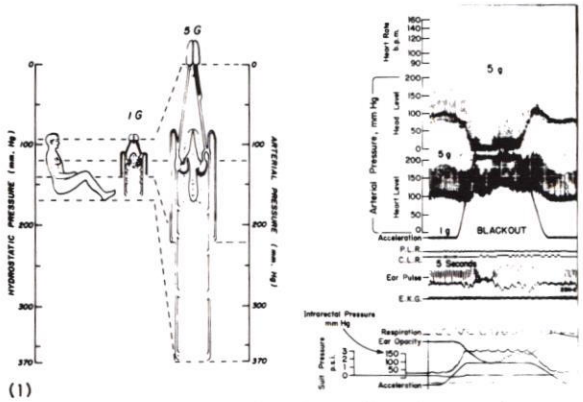
Introduction: The hydrostatic basis for the retinal and cerebral ischemic anoxia and resulting losses of vision and cognitive function during sustained exposures to G_z acceleration postulated by von Diringshofen in the early 1930s (1) is illustrated in the left panel of Figure 1.

COMPARISON OF MODEL WITH RECORDED CIRCULATORY EFFECTS OF 5 G_z ACCELERATION
(Male, 34 Years)



(1)

The 5 fold multiplication of hydrostatic pressure gradients in the systemic and pulmonary circulatory systems and within the chambers of the heart which, during such exposures, are an unavoidable consequence of the 5 fold increase in weight of blood and tissue, would be expected to cause severe circulatory effects, including cessation of cerebral blood flow and subsequent loss of consciousness, if the exposure were sustained for more than a few seconds.

However, simultaneous recordings in healthy humans of arterial pressures at heart and head level and other variables during exposures to these levels of acceleration (Figure 1, right panel [2-4]) indicate that this simple hydrostatic model of the circulation does not tell the whole story.

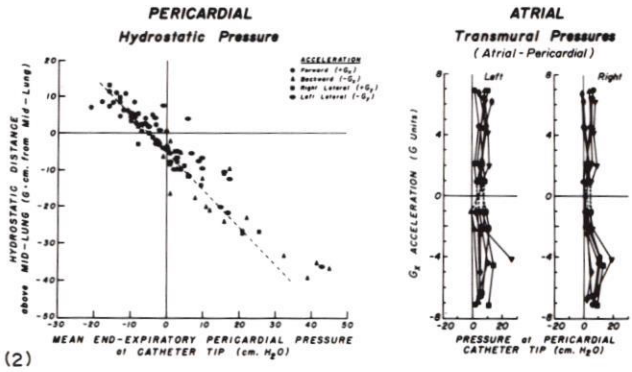
Note that although blood pressure fell to zero at head level and a brief loss of vision occurred, normal arterial pressure was maintained at heart level during the first 5-10 seconds of the exposure followed by a compensatory increase which restored circulation to the head so that vision returned during the last 10 seconds of the exposure.

Data accumulated progressively during the ensuing four decades indicate that magnification of interserosal pressure gradients in proportion to the gravitational-inertial force environment determined changes in weight of blood and tissue provide homeostatic mechanisms for effective protection of cardiovascular, respiratory, and cognitive functions under these conditions (5,6).

Results: Homeostasis of the abdominal circulation by proportional hydrostatic increases in interperitoneal pressure gradients during G_z acceleration as first proposed by Rushmer (7,8) have been documented both in man and animals (9,10).

That a similar hydrostatic behavior of intrapericardial pressure provides nearly perfect homeostasis of left and right atrial transmural pressures during exposures to increased force environments is documented in Figure 2 (10,11). The panels on the right indicate that, over an acceleration range of from +7 to -7G_x, right and left transmural atrial pressures are maintained essentially constant by simultaneous proportionate changes in pericardial pressure of from -20 to more than 40 cm H_2O as plotted in the left panel. These and similar data indicate that, in the intact thorax, the heart is encompassed by a hydrostatic pressure environment, which in effect functions as an essentially perfect anti-G suit for this anatomically flaccid organ (5,10,11).

HYDROSTATIC PERICARDIAL HOMEOSTASIS OF ATRIAL TRANSMURAL PRESSURES DURING 7 FOLD CHANGES IN THE FORCE ENVIRONMENT ABOVE AND BELOW ZERO G
(Simultaneous Values, 7 Dogs, Morphine - Pentobarbital Anesthesia)



(2)

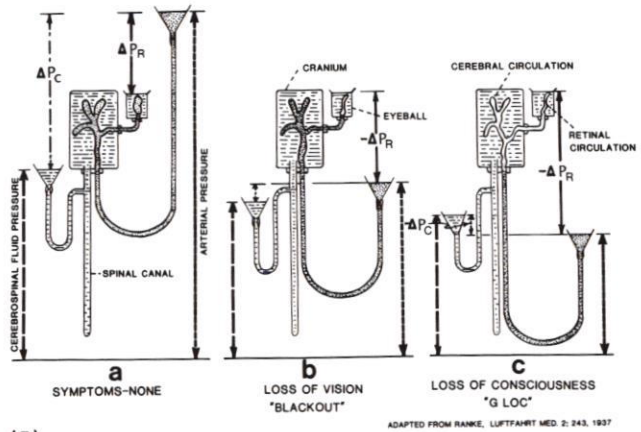
On the other hand, as shown in Figure 1, the disadvantageous superior position of the brain, and the consequent reduction of arterial pressure to zero at head level, precludes adequate protection of the cerebral circulation by physiologic mechanisms when in the erect position.

Never-the-less, the fact that there is an important degree of protection of cerebral perfusion during high G_z exposures is illustrated in the right panel. Note that arterial pressure at base of brain level was practically zero for the initial 8 seconds of this exposure and, although a transient loss of vision occurred, no detectable loss of cognitive function occured.

Since in a 1G environment this degree of cerebral hypotension for a period of 8 seconds would result in loss of consciousness, how is it possible to maintain consciousness during an exposure to 5G_z when arterial pressure at base of brain level falls to values near or at zero for periods of 5 to 10 seconds?

The explanation was formulated more than a half century ago in a paper by O.F. Ranke (12) as illustrated in Figure 3.

HYDROSTATIC MODEL: EFFECTS OF G_z ACCELERATION ON CEREBRAL AND RETINAL BLOOD PRESSURES



(3)

The left panel is at 1G. Arterial pressure represented by the fluid level on the right is greater than both the intraocular pressure (small inset) and the cerebrospinal fluid pressure, indicated by the fluid level on the left. At levels of acceleration

*Supported by an Alexander von Humboldt Award and DARPA/STO Contract No. N66001-87-C-0079.

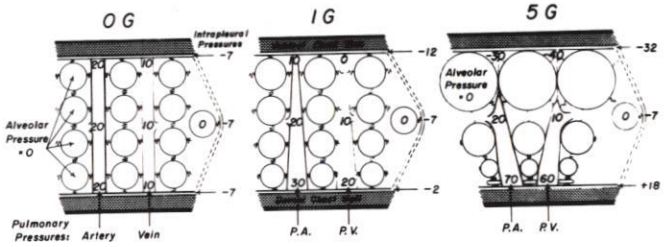
producing loss of vision (central panel), the hydrostatic level of the arterial column is decreased to less than intraocular pressure so retinal flow ceases and loss of vision results. However, in spite of the fact that the arterial hydrostatic level is decreased to below cranial level (represented by the inverted bottle), the more negative cerebrospinal fluid pressure maintains blood flow within the cranial vault so that consciousness is maintained.

At higher levels of acceleration (right panel), head level arterial pressure falls to levels considerably below cerebrospinal fluid pressure so cerebral blood flow ceases and consciousness is lost.

Unlike the cerebrospinal fluid system, the concept of hydrostatic gradients within the interpleural space is controversial (13,14). Never-the-less, hydrostatic considerations indicate that at 1G and, in proportion to the vertical dimensions of the lungs, arterial and venous pressures in the most dependent regions of the pulmonary parenchyma would, as indicated in the center panel of Figure 4, exceed the analogous pressures in non-dependent regions by about 20 cm of water. During an exposure to 5G these pressure differences would be increased 5 fold, to about 100 cm of water, as in the right panel of this figure (15).

DIAGRAM OF THE EFFECTS OF FORWARD (+G_x) ACCELERATION ON INTRATHORACIC PRESSURES

(Dorso–Ventral Dimension of Lung: 20 cm.)



Numerals = Pressures in cm. H_2O

0 Reference Level = Atmospheric Pressure at Mid Dorso–Ventral Chest Level

(4)

Because of the large differences between the essentially zero specific gravity of the intraalveolar respiratory gases and the surrounding blood and tissues, "nature's stratagem" for protecting the central nervous system, abdominal organs and the heart against changes in the force environment is not fully effective relative to the lungs (15,16).

The uniform occurrence of arterial desaturation with increases in the force environment (17,18) and the disruption of lung parenchyma in chimpanzees exposed to 40G in a water immersion capsule (19) highlight the susceptibility of the lungs to changes in the force environment and the possible validity of this model.

There are findings, however, which do not conform with the predictions therefrom that: 1) On the basis of the hydrostatic gradient in pulmonary artery pressure there should be a large increase in pulmonary blood flow to the dependent regions of the lungs concomitantly with a progressive decrease to the non-dependent regions; and 2) there should be a uniform gradient in pleural pressure encompassing the full vertical extent of the lungs of about 0.5 cm of water per cm of vertical height within the thorax.

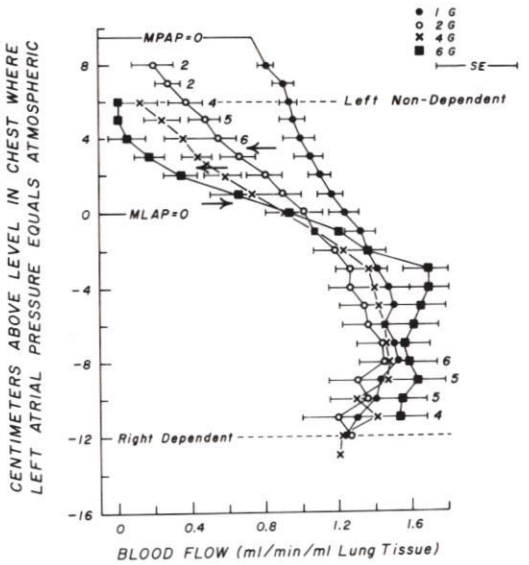
Measurements in dogs studied without thoracotomy indicate that neither of these predictions conform to the facts (Figure 5 [13,20]).

The progressive decrease in pulmonary perfusion with increasing vertical height in the upper region of the thorax conform to the model. However, there is a sharp inflection on each of the curves at about mid-lung level below which no further increase in lung tissue perfusion occurs.

This finding is compatible with the hypothesis that in the lower half of the thoracic cavity, there must be a homeostatic hydrostatic gradient which maintains essentially constant transmural vascular pressures within the dependent regions of the lungs.

EFFECT OF INCREASED FORCE ENVIRONMENT ON THE DISTRIBUTION OF PULMONARY BLOOD FLOW

(6 Dogs, Right Decubitus Position)

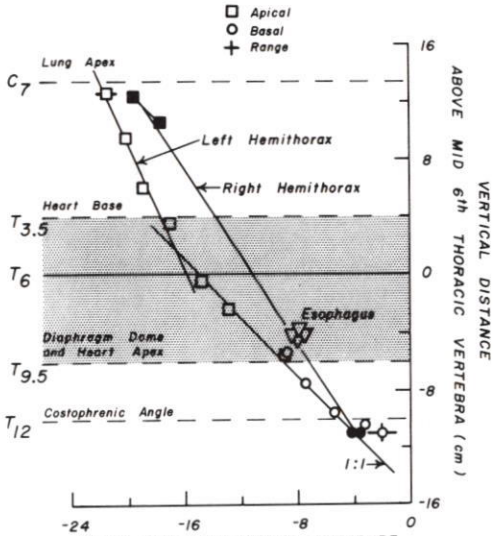


(5)

Measurements of liquid pleural pressures at different vertical heights in the thorax support this hypothesis (Figure 6 [13,21]).

COMPARISON OF PLEURAL PRESSURES IN RIGHT AND LEFT CHEST IN HEAD-UP POSITION

(Dog 25 kg, Morphine Pentobarbital Anesthesia)



(6)

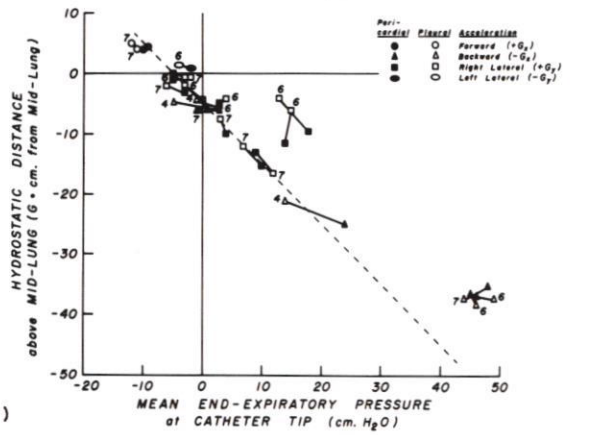
Note that the diagonal straight line extending from most dependent surface of the lung to the upper margin of the heart, which fits the experimental values from the left hemithorax, represents a hydrostatic gradient of 1 cm of water per cm of vertical height within the thorax.

Simultaneous measurements of pleural and pericardial pressures at contiguous sites in the thorax also document the presence of a hydrostatic pressure gradient in the lower segment of the thorax as indicated by the dashed line in Figure 7 (11).

If one grants there is a one-to-one hydrostatic pressure gradient within the lower half of the thorax,

what is the physiologic utility of the 0.2 cm of water per cm of vertical height relationship recorded over the upper one-half of the thorax as indicated in Figure 6. A vertical pressure gradient of 0.2 to 1 is too small to compensate for the one-to-one hydrostatic gradient in the pulmonary vasculature. On the other hand, from the viewpoint of the vertical distribution of pulmonary ventilation and, because of the near zero specific gravity of the intra-alveolar respiratory gases, homeostasis of regional pulmonary ventilation during a changing force environment would be best achieved if pleural pressures were independent of vertical height within the thorax, i.e. a vertical pleural and interstitial pressure gradient of zero.

COMPARISON OF PERICARDIAL AND PLEURAL PRESSURES AT CONTIGUOUS SITES IN RELATION TO HYDROSTATIC DISTANCES FROM MID-LUNG DURING TRANSVERSE ACCELERATION OF 1 TO 7G IN DIFFERENT BODY POSITIONS
(Values from 3 Dogs)



(7)

These considerations emphasize the unavoidable biophysical fact that because of the very large difference in the nearly zero specific gravity of the intra-alveolar respiratory gases and the much greater specific gravity of the surrounding blood and tissues, no matter what the actual magnitude of the vertical gradient in pleural and associated interstitial pressures may be over the range from 0, for ideal homeostasis of regional ventilation, to a value of 1.06, for ideal homeostasis of regional perfusion, there is no way, other than replacing the alveolar gas with a respirable liquid (22), to protect the anatomy and function of the lungs against changes in the force environment. Faced with this fact which is unalterable for air breathing animals, "Mother Nature" or, if you prefer evolution, has, by not fully understood mechanisms, devised a compromise.

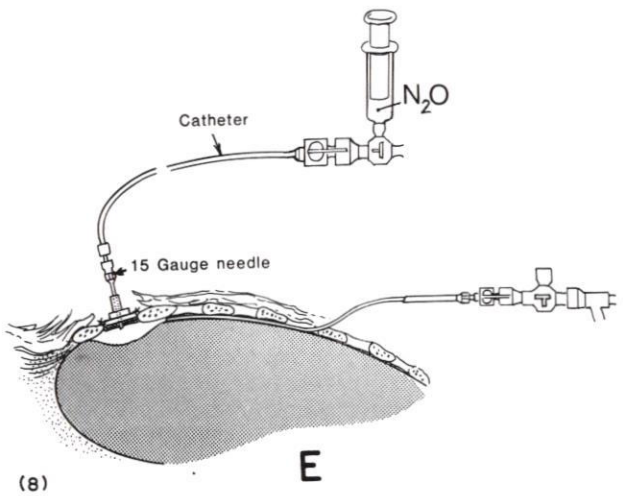
This compromise provides a one-to-one hydrostatic gradient in liquid pleural pressures in dependent regions of the thorax and thereby a nearly perfect homeostatic hydrostatic environment for the heart and pulmonary vasculature in the lower, approximately one-half segment, of the thoracic cavity.

The presence of much smaller vertical gradients in liquid pleural pressures in superior regions of the thorax, although it fails to maintain pulmonary blood flow does minimize the force environment dependent magnification of vertical transalveolar pressure gradients and the consequent vertical, nonuniformity of pulmonary ventilation and the potentially dangerous over-distention of non-dependent alveoli, associated with increases in the force environment as predicted by the model (Figure 4).

These pathophysiologic effects indicate that measurement of the time varying magnitude and spatial distribution of interpleural forces is of basic importance to respiratory and gravitational physiology.

There has been general agreement as to the magnitude of interpleural pressures at dependent sites in the thorax, and also a concensus, as indicated in this model, that the magnitude of the expansile forces acting on non-dependent regions of the lungs increases with vertical height in the thorax (15,23). However, because of the divergent values obtained by different measurement methods, there is little agreement concerning the magnitude of the vertical gradient in interpleural forces. These uncertainties stimulated development of a technique which would allow direct measurements of pleural liquid pressures with no or minimal distortion of the pleural space. A special wide-bore, barbed tip catheter and needle assembly has been used in anesthetized pigs and dogs to establish a liquid filled side portal to the pleural space without distortion of this space and without recourse to thoracotomy (24,25).

A 1.3 mm outside diameter catheter was inserted percutaneously into the pleural space as described previously (26) and its tip manipulated under fluoroscopic control to an apical and/or basal site in the right hemithorax. An incision was made along an intercostal space at the site of the desired pressure measurement so that a specially designed plastic needle guide could be sutured securely to the periosteal ligaments of adjacent ribs. Two hundred ml of nitrous oxide was then injected via the previously inserted interpleural catheter to create a gas pocket below the site of the needle guide. The 15 gauge needle sheathing the barbed tip of the special catheter was advanced, via the needle guide, through the intercostal musculature into the interpleural gaseous pocket. The needle was then drawn back along the catheter shaft, exposing the barbs at the tip of the catheter and the catheter withdrawn until the barbs were securely imbedded into the parietal pleura and underlying tissue so that the marginal wall of its pleural orifice was fixed in position and level with the surface of the parietal pleura (Figure 8).

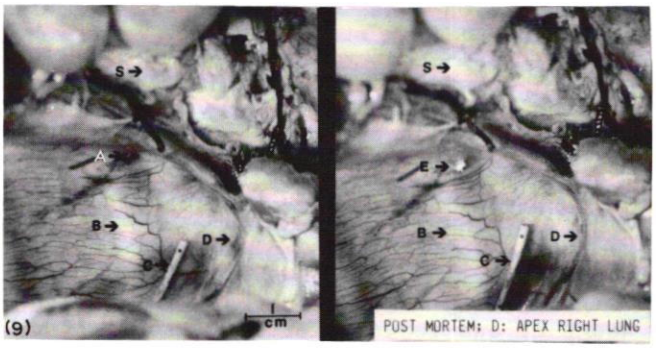


(8)

The nitrous oxide was withdrawn as completely as possible and, after a waiting period to allow complete absorption of any residual gas, pressures could be recorded simultaneously via both catheter systems with the animals in the horizontal, head-up and head-down positions. Studies using this technique have been carried out in anesthetized pigs at the Univ. of Kiel, West Germany (24), and in dogs at the Meakins-Christie Labs, McGill Univ., in Montreal (25).

Figure 9 is a post-mortem photograph showing, in the left panel, the barely discernible, in situ, parietal pleural orifice of the barbed tipped catheter (A) in the right apical second intercostal space of a pig along with the juxtaposed 1.3 mm diameter segment of the interpleural catheter (C). Inward pressure on its external shaft (right panel) provided increased visibility of the barbed tip (E) protruding from the parietal pleural surface. The lettered arrows S, B and D indicate the transected mid-line of the sternum, the second rib, and the

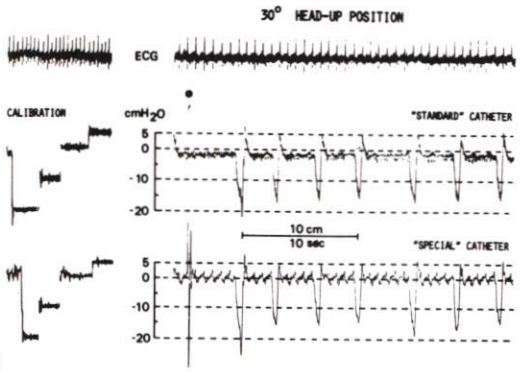
apex of the right hemithorax, respectively.



(9)

Simultaneous recordings (Figure 10) from this animal, when in the head-up position, document that simultaneously recorded pressures via the two systems are practically identical.

SIMULTANEOUS APICAL PLEURAL LIQUID PRESSURES



(10)

Average values recorded at the same vertical heights in the thorax were closely similar for the two systems as were the vertical gradients in pressure of about 0.6 cm of water per cm of vertical distance.

These data contradict the belief that distortion of the normally very thin, non-dependent pleural space produced by an interposed 1.3 mm diameter liquid-filled catheter invalidates the physiologic significance of pressures recorded from non-dependent regions in the thoracic cavity. Discussion: The data reported herein indicate that the magnification of interserosal hydrostatic pressure gradients in proportion to the gravitational-inertial environment determined weight of bodily tissues and fluids provide an important homeostatic mechanism for: 1) Maintenance of venous return from the abdominal contents; 2) maintenance of essentially normal cardiac function during changes in magnitude and/or direction of the force environment; 3) decreasing, within limits, the effects of the force environment on cerebral circulation; and 4) pulmonary function.

From the practical viewpoint, it is important that a fully horizontal position provides a high degree of protection to the cerebral circulation (12, 27). Consequently use of a fully horizontal cockpit configuration in current, very high performance fighter planes, would provide the physiologically most certain and safest means of preventing the currently serious acceleration induced loss of consciousness problem in the pilots of these planes (4,27,28).

However, due to the large difference in specific gravity of intra-alveolar respiratory gases and the surrounding blood and tissue fully effective protection of the lungs cannot be achieved by changes in body position (28,29).

Consequently, except for the impractical expedient of replacing the alveolar gas with a respirable liquid with a specific gravity closely similar to bodily tissues (22,30), the function and anatomical integrity of the lungs will remain highly vulnerable to changes in the magnitude and/or direction of the gravitational-inertial force environment. Furthermore, microgravitational physiologists may not fully realize that without knowledge of the unavoidable changes in interpleural and pericardial pressures at zero G, interpretation of the hemodynamic significance of atrial pressures is impossible (31). Discussion: Awareness and knowledge of gravitational-inertial force environment induced changes in interserosal pressures is an essential aspect of physiology, especially in air breathing animals, encompassing the micro-G environment of space, the 1G environment of planet earth, and the hyper-G environment associated with changes in direction and/or magnitude of velocity at any site in our Universe.

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RESPONSES TO CHANGED PERFUSION PRESSURE IN WORKING MUSCLES - FACTORS TO BE CONSIDERED IN EXERCISE TESTING IN SPACE FLIGHTS ?

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It is well documented that during dynamic exercise in the normal-gravity environment gravitational vectors acting along the blood vessels will influence muscle perfusion pressure by adding hydrostatic columns to the local arterial pressure (cf. Folkow et al 1971). Conceivably, such gravity-induced changes in muscle perfusion pressure will alter the physiological responses to exercise.

Accordingly, the present experiments were performed to study the influence of graded changes in muscle perfusion pressure on certain physiological responses to dynamic leg exercise. Muscle perfusion pressure was decreased by exposing the working legs to a supraatmospheric pressure of 50 mm Hg (Leg Positive Pressure, LPP) and increased by exposing the legs to a subatmospheric pressure of -50 mm Hg (Lower Body Negative Pressure, LBNP) (cf. Eiken 1987).

METHODS

All experiments were carried out in healthy subjects positioned supine in the opening of a pressure chamber with the legs inside the chamber and the feet strapped to the pedals of an electrically braked cycle ergometer, the axis of the pedals being at the level of the heart. Hermetic sealing of the chamber opening was provided by the use of a rubber diaphragm, which fitted around the lower portion of the abdomen or the upper portions of the thighs. A saddle-like support was used to avoid caudad displacement of the body as the pressure was reduced (LBNP), and craniad displacement was prevented by the use of shoulder supports as the chamber pressure was increased (LPP). Occlusion of leg blood flow was accomplished by inflating pneumatic thigh cuffs to suprasystolic pressures. Systolic arterial pressure (SAP) was measured by the auscultatory method from a brachial artery. Cardiac output (CO) was measured using an impedance cardiography assembly (cf. Eiken & Segerhammar 1988). Breath-by-breath inspiratory minute ventilation ($\dot{V}_I$) and end-tidal PCO_2 ($P_{ET}CO_2$) were recorded using standard techniques. Oxygen uptake ($\dot{V}O_2$) was determined using the Douglas bag technique, blood lactate concentration was determined in samples drawn from a fingertip.

The results given below refer to data collected in different experimental series during which the subject have conducted:

- *Exhaustive incremental-load exercise* in the control, LBNP, and LPP conditions (cf. Eiken & Bjurstedt 1987; Eiken 1988).
- *Constant-load exercise* with temporary applications of LPP and/or arterial occlusion (cf. Eiken 1987; Eiken et al 1988).
- *A training regime* consisting of four weeks of one-legged exercise. Each leg was trained one hour 3-4 times/week. Both legs were trained at identical external loads corresponding to 60-80% of LPP-peak load; one leg was trained in the LPP and the other in the control condition (Kaijser, Sundberg, Nygren & Eiken, unpublished observations).

RESULTS

Incremental-load exercise: Work performance was 9% higher during LBNP and 39% lower during LPP than in the control condition. Exposure to LPP augmented the exercise responses for CO and SAP, the differences from control values amounting to 15% for CO and 19% for SAP at LPP peak load. Exercise CO was lower in the LBNP than in the control condition, whereas SAP values were unaffected by exposure to LBNP (Fig. 1).

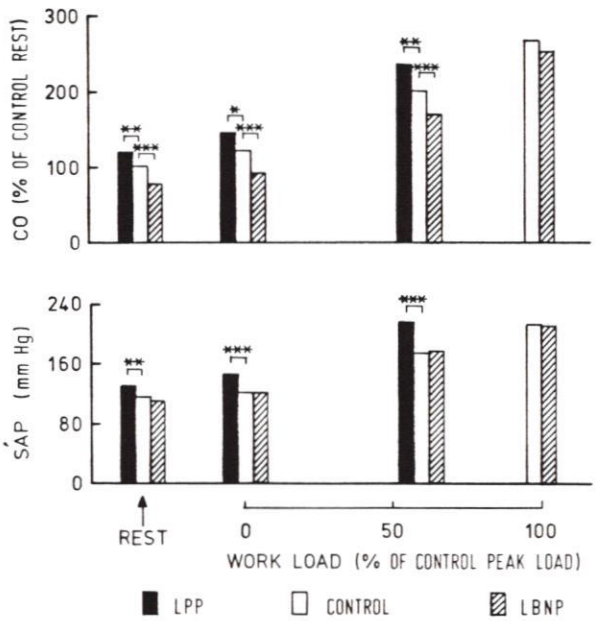

Fig.1. Comparisons of cardiac output (CO) and systolic arterial pressure (SAP) at rest and during exercise in the control, LBNP and LPP conditions. Exercise values are given at 0 W, and at peak loads attained in the LPP and control conditions. Work loads are given in per cent of control peak load.Values are means; n=7 for CO. n=8 for SAP. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The exercise responses for $\dot{V}_I$, $\dot{V}O_2$ and blood lactate concentration were

exaggerated by LPP and attenuated by LBNP. The differences between LPP and control values amounted to 36% for $\dot{V}_I$, 15% for $\dot{V}O_2$ and 56% for blood lactate at LPP peak load. The differences between LBNP and control values amounted to 15% for $\dot{V}_I$, 10% for $\dot{V}O_2$ and 20% for blood lactate at control peak load (Fig. 2).

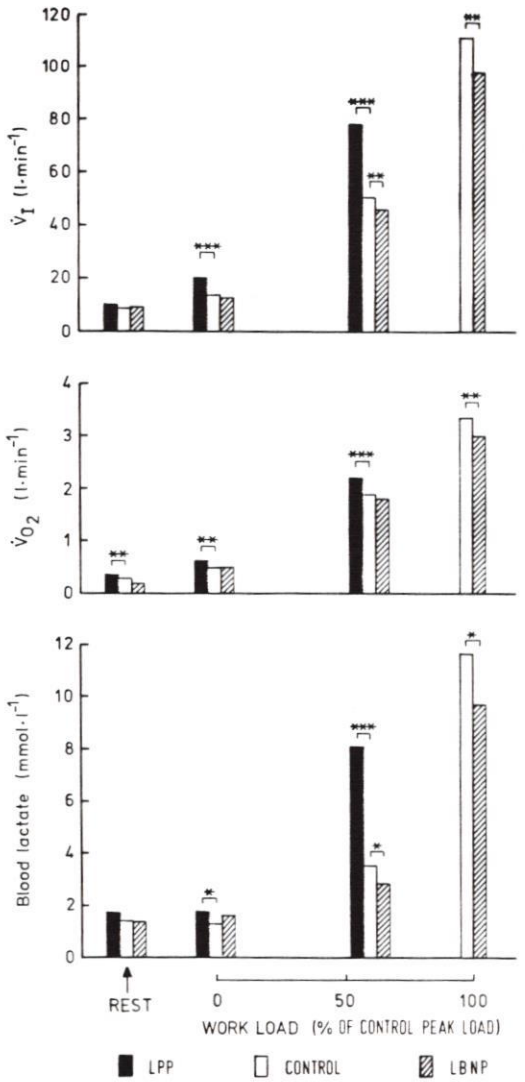

Fig.2. Inspired minute ventilation ($\dot{V}_I$), oxygen uptake ($\dot{V}O_2$) and blood lactate concentration at rest and during exercise in the control, the LBNP and the LPP conditions. Exercise values are given at 0 W and at the peak loads attained in the LPP and control conditions. Work loads are given in per cent of control peak load. Values are means,n=8. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. (From Acta Physiol. Scand. 131, (Suppl. 566).

Constant-load exercise. Temporary application (5min) of LPP during constant load pedaling at 120 W induced increases in $\dot{V}_I$ and blood lactate concentration and a drop in $P_{ET}CO_2$. A sudden release of the LPP led to a rapid fall in $\dot{V}_I$ and a sudden and sustained elevation of $P_{ET}CO_2$ in spite of a concomitant increase in blood lactate concentration (Fig. 3).

Fig. 4 shows $\dot{V}O_2$ during constant-load pedaling in the LPP and control conditions, where the measured values have been corrected for the estimated O_2 cost of breathing. Thus, the O_2 cost of breathing was determined from the standard curves of Barett et al (1958) and subtracted from the measured values. It can be seen that $\dot{V}O_2$ was substantially higher in the LPP than in the control condition, and that this discrepancy was abolished when the leg arteries were occluded by pneumatic thigh cuffs.

Long-term effects of LPP exercise. Fig. 5 shows the effect of a four week training regime on peak O_2 uptake both for the LPP-trained leg and the leg that was trained at normal atmospheric pressure. It can be seen that the training resulted in improvements in O_2 uptake which was more marked in the LPP-trained leg both when tested at normal ambient pressure and especially when tested during LPP exposure. Also, the training-induced improvements in exercise performance were larger in the LPP-trained leg than in the control leg especially when tested during exposure to LPP.

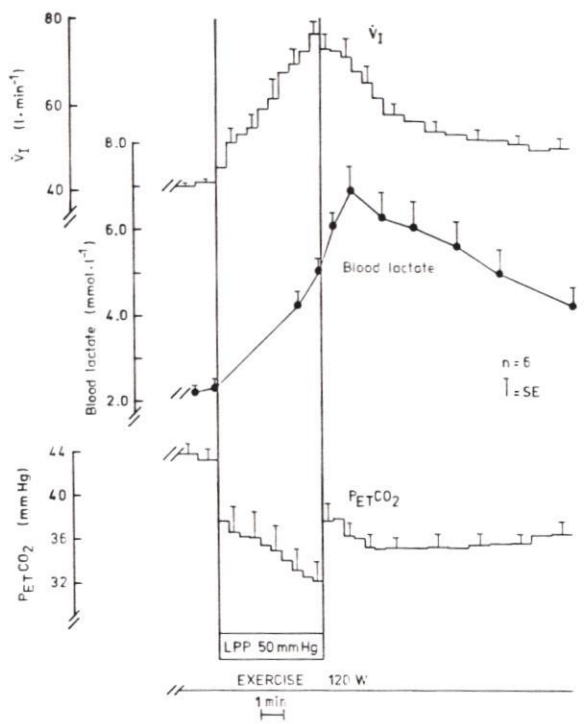

Fig.3. Effects of temporarily applied LPP on inspired minute ventilation ($\dot{V}_I$), blood lactate concentration, and end-tidal PCO_2 during constant-load pedaling at 120 W. Initial values represent data obtained during min 4 and 5 after onset of exercise (from Acta Physiol. Scand. 131,(Suppl 566).

DISCUSSION

The present results should be viewed in the context of the fact that perfusion pressure in the working muscles was reduced by LPP and increased by LBNP.

Acute responses to changes in muscle perfusion-pressure. It was found that exercise-induced increases in cardiac output were attenuated by LBNP, an effect which is attribuable to diminished circulating blood volume secondary to suction-induced sequestration of blood volume in capacitance vessels not affected by the action of the leg muscle pump. That work performance was improved by LBNP in the face of decreased cardiac output and increased muscle perfusion pressure, suggests that the limitation of work performance during supine cycling was set by the limitation of the circulatory system to distribute blood to or within the working muscles rather than by the cardiac pump to generate flow. In light of the finding that muscle perfusion pressure appears to be critical for work performance during supine cycling, a substantial reduction in work performance is conceivable in conditions where this pressure is decreased. This notion was supported by the observation that work performance was severely impaired by exposure to LPP.

That the exercise-induced increases in SAP were markedly exaggerated when muscle blood flow was restricted by LPP is attributable to increased exercise responses in both CO and total peripheral resistance, and may be explained by increased activation of muscle chemoreflexes by accumulation of metabolites. The exaggerated pressor response would then tend to reduce the existing flow-error (cf. Mitchell & Schmidt 1983).

The finding that exercise-induced responses of $\dot{V}_I$ were attenuated by LBNP and substantially exaggerated by LPP may in part be ascribed to humorally mediated changes in the respiratory drive resulting from perfusion-pressure dependent differences in the rate of blood lactate accumulation. However, the fact that a sudden release of LPP during constant-load pedaling led to a prompt fall in $\dot{V}_I$ and an increase in $P_{ET}CO_2$ in spite of a marked increase in the blood lactate level is strongly suggestive of other mechanisms capable of inducing exercise hyperpnea during LPP. Thus, it is likely that in conditions of flow-restricted exercise muscle chemosensors take part also in the development of exercise hyperpnea (cf. Asmussen & Nielsen 1964).

The present results show that exercise-induced increases in O_2 uptake and blood lactate concentration were both attenuated by LBNP and exaggerated by LPP. The changes in blood lactate levels are attributable to perfusion-pressure dependent variation in muscle blood flow, resulting in opposite changes in the share contributed by anaerobic metabolism to the energy release. That LPP exercise was associated with increased O_2 uptake at a given external work load may in part have been due to increased O_2 cost of breathing consequent to the exaggerated exercise hyperpnea. However, the findings that after accounting for the O_2 cost of breathing, exercise O_2-uptake remained higher during LPP than in the control condition, and that this discrepancy was abolished by arterial occlusion of the legs may suggest that part of the LPP-induced increase in O_2 uptake emanates from the working leg muscles. This, in turn, suggests reduced mechanical efficiency in the working muscles under conditions of muscle blood flow restriction.

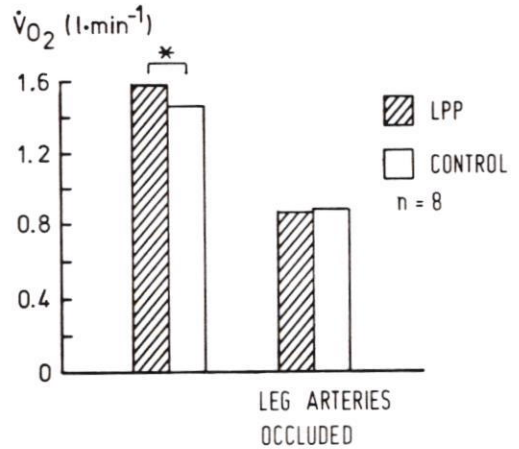

Fig. 4. Oxygen uptake ($\dot{V}O_2$) during constant-load pedaling (100 W) in the LPP and control conditions with and without occlusion of the leg arteries. All values have been corrected for the estimated oxygen cost of breathing (see text).

Responses to long-term changes in muscle perfusion pressure. The preliminary findings that the training effects on work performance and $\dot{V}O_2$ were more marked after training in the LPP condition suggest that for a given amount of external work, muscular training is more effective when performed in conditions of blood-flow restriction. This is in line with the concept that flow-restricted training exaggerates the formation of aerobic enzymes in the working muscles (Scherstén et al 1971; Jansson et al 1988).

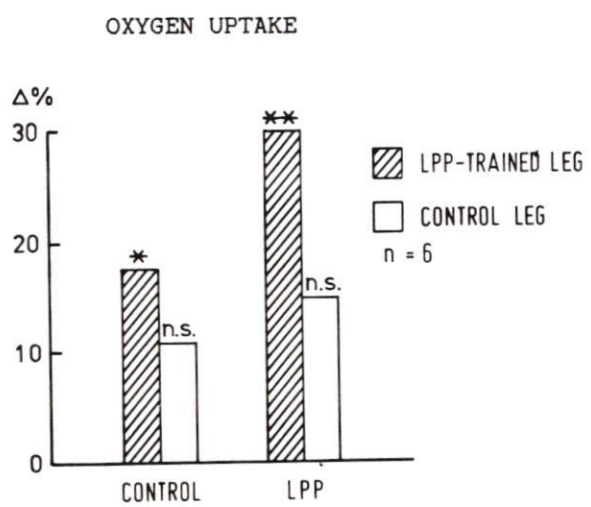

Fig. 5. Relative changes in peak oxygen uptake for each leg in response to a four week training regime (see text). Left two columns (control) represent improvments when tested at normal atmospheric pressure and right two columns *(LPP)* when tested during LPP exposure.

In summary, the present results show that changes in muscle perfusion-pressure alters both acute and long-term responses to exercise, a fact that might be considered when interpreting results from exercise studies performed in the space environment.

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CHANGES IN KIDNEY RESPONSE TO ADH UNDER HYPOGRAVITY: RAT MODELS AND POSSIBLE MECHANISMS

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Brattleboro homo- (DI) and heterozygous (HZ) rats with total or partial deficiency of the antidiuretic hormone (ADH) and also 10-14-day old Wistar rats irresponsive to ADH were used to examine the possible causes of inadequate responses to ADH in cosmonauts after space flights and in humans after prolonged bedrest in antiorthostasis (-6°). The experimental results suggested that changes in the intrarenal osmotic gradients, as well as in the aggregate state of the ADH receptors as a result of the decreased ADH blood level under hypogravitation, might be a cause of the inadequate response to ADH in cosmonauts during readaptation to the Earth's gravity.

Introduction

The system of regulating water salt metabolism is of great importance in maintenance of homeostasis during space flights and readaptation to the Earth's gravity (3,4,7). The antidiuretic hormone of the neurohypophysis (ADH) is a major regulator of body fluids osmolality. Impairment of the kidney concentrating ability has been observed in cosmonauts during the first days after space flights, when they readapt to the Earth's gravity (5). Decrease in the sensitivity to ADH has also been reported for conditions simulating weightlessness (immersion) in humans (1,5,6,8). The cause of this impairment in the cosmonauts and human subjects is unclear. It appeared worthwhile to compare the osmoregulatory function of cosmonauts after flights and volunteers after bedrest in antiorthostasis (-6°) and to examine the possible causes of inadequate response of the kidney to ADH using rat models with genetically and experimentally produced impairment of ADH secretion.

Methods

Our standard method was used to study the concentrating ability of the kidney in cosmonauts after space flights of different duration and in human subjects after antiorthostasis (-6°) (2,4). Brattleboro homo- (DI) and heterozygous (HZ) rats served as animal models of total or patial deficiency of ADH synthesis. Estimates of the ADH response were based on increase in urine osmolality (mosm/kg H_2O) produced by an s.c. injection of synthetic arginine-vasopressin (AVP, Sigma, 500 μU/100 g b.w.). The effect of ADH was prolonged by a s.c. injection of dDAVP (Spofa, 20 μg/100 g b.w. for 6 days). The secretion of ADH was suppressed by free access to a 4% sucrose solution in food-deprived HZ (11). The rats were killed by decapitation, the kidneys were removed. Renal tissue was dissected, the concentrations of urea and sodium were determined in zones of this tissue and the papilla to cortex concentrating gradients for sodium and urea were determined (9). Measurements of the specific binding of ADH in the membrane fraction from the papillary homogenates of young (10-14-day old) and adult rats and gel chromatography of solubilized ADH receptors were performed according to (10). Student's t-test was used for statistical treatment.

Results and discussion

After short (2-8 days) and long (more than 30 days) space flights, there was a marked retention of water and electrolytes in the cosmonauts (2,4,7). Decrease in diuresis was a response of osmoregulation to the sharp redistribution and deficiency in the volume of body fluids during landing. However, this decrease was not associated with a proportionate rise in urine osmolality. A similar distortion of this relationship, although less marked, was observed in human subjects after the antiorthostatic test (-6°) (2). What may be the cause of this distortion: an impairment of ADH secretion or an inadequate renal response to ADH? It is pertinent to recall that blood ADH and its urinary excretion were elevated after landing and remained so for 6 days in the Skylab astronauts (7). As to our present observations, the level of blood ADH was also considerably higher than before the flights and, what is noteworthy, the increase in ADH level was not associated with an increase in urine osmolality (Fig. 1). This excludes ADH deficiency as a putatitive cause of the distortion observed for urine osmolality and ADH blood level. It was much rather caused by changes in the renal mechanisms of the response to ADH.

The concentrating ability of the kidney and the response to ADH may fail under the effect of either a decrease in intrarenal concentrating gradients providing water reabsorption or changes in molecular events provoked by drastic hormonal fluctuations during flights-landings. Rat models were used in an attempt to explain why these mechanisms may fail. The aim was to elucidate the contribution of ADH to the efficiency of the renal concentrating function for the reason that the hormonal level is consi-

derably decreased during weightlessness.

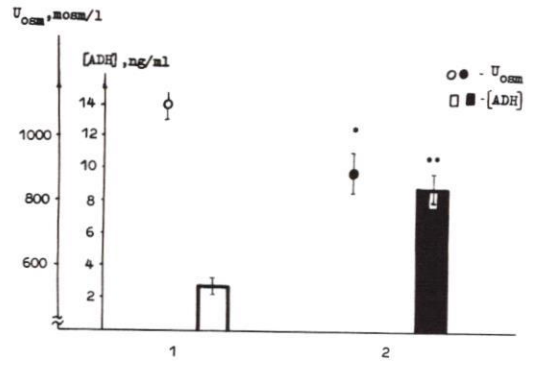

Figure 1. Urine osmolality (circles) and concentrations of ADH in the blood (bars) before (1) and after (2) space flights. Significant differences between 1 and 2, Student's t-test, *p<0.05; **p<0.01.

Good models were Brattleboro rats whose ADH synthesis is deficient (homozygotes, DI) or somewhat decreased (heterozygotes, HZ), compared to the normal Long-Evans rats. Polydipsia and polyuria are characteristic of DI, HZ do not differ in water consumption and osmolality from normal rats.

Kidney responsiveness to ADH is much lower in DI than in HZ (Fig. 2). In DI, a single injection of ADH produces an increase in urine osmolality not exceeding 500 mosm/l. Conversely, in HZ, ADH produces its increase up to 1500 mosm/l like in normal rats. In DI, only prolonged treatment with dDAVP leads to a normalization of the response to ADH and to a steep rise in urine osmolality.

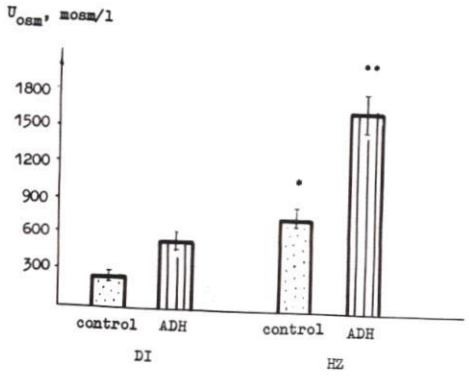

Figure 2. Urine osmolality in DI and HZ given a test injection of synthetic AVP (Sigma, 500 μU/100 g b.w., s.c.). Significant differences between DI and HZ, Student's t-test *p<0.05; **p<0.01.

Kidney irresponsiveness to ADH in DI appears to be the result of a sharp decrease in intrarenal concentrating gradients, mainly the urea gradient (Fig. 3). Administration of dDAVP restores the normal intrarenal gradients and, consequently, the concentrating ability. Thus the deficiency in the intrarenal osmotic gradient is due to the absence of ADH. We

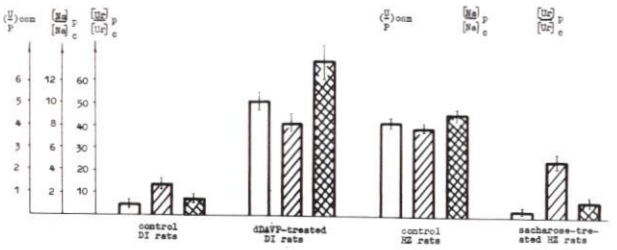

Figure 3. (U/P)osm, papilla to cortex gradients of sodium and urea concentrations in DI (control rats, ones treated with dDAVP for 6 days) and in HZ (control, before and after drinking of 4% Sucrose solution).

used the following device to suppress ADH secretion and to maintain ADH blood level stably low in HZ. These rats were deprived of food and given free access to a 4% Sucrose solution. They started to drink the solution in amounts making up 80-90% of their body weight; the resulting increase in the volume of circulating body fluids and decrease in sodium concentration in the blood led to a suppression of ADH secretion. Two-six days after maintenance on this regime, the HZ intrarenal urea gradient was sharply decreased, and the urine became hypotonic (Fig. 3). It should be noted that kidney sensitivity to ADH in these HZ decreased, like in DI lacking ADH. It took some time for the ADH response to normalize.

Decrease in the intrarenal osmotic gradient as a consequence of the low ADH under conditions of weightlessness and antiorthostasis appears to be the most likely cause of the decrease in kidney responsiveness to ADH during readaptation to the Earth's gravity. There exists the possibility of changes in the molecular mechanisms underlying the action of ADH being involved in this decrease.

We would suggest that ADH may induce the formation of a polysubunit structure of a functionally active receptor complex. Our suggestion is based on the results of studies of ADH reception in the developing kidney. It is known that rat kidney is not responsive to ADH to the age of 20 days. The lack of responsiveness is due to the incompetency of the intracellular mechanisms providing ADH effect, mainly to the immaturity of the ADH receptors compared to the adults. The specific binding of ADH is much lower in 10-day old rats irresponsive to ADH. The adults and 10-day old rats, however, did not differ significantly in receptor affinity (Fig. 4).

The increase in specific binding was associated with structural changes in the receptor complex, as evidenced by the results of gel filtration of the solubilized receptors. Fig. 5 shows the results of

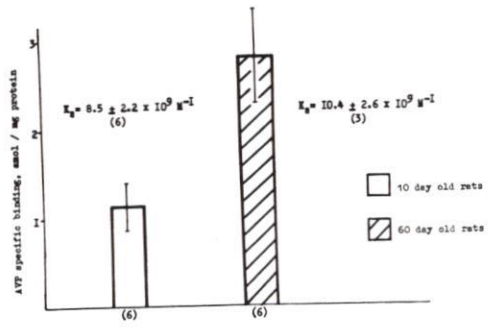

Figure 4. Specific binding of (^{3}H)AVP to the membrane fraction of renal papillary homogenate. The number of experimental determinations is in the brackets. Significant differences between 10- and 60-day old rats (*p<0.05).

the receptor complex formation in the presence of the ligand-ADH: the profiles of the specific binding were almost the same for the 14- and 60-day old rats, and there was a single peak corresponding to a protein of 500 kDa. After preliminary separation of the receptor complex from ADH, the profile of the "young" receptors was composite. It consisted of two major peaks, 45 and 13 kDa; and a minor one in the region of 500 kDa. However, in adults, in the absence of a ligand, there were also two peaks. The ADH receptor, which consists of several subunits, is disassembled during the period of irresponsiveness to ADH. In adults, the subunits are kept more together in the complex, and ADH as an aggregating factor is of less

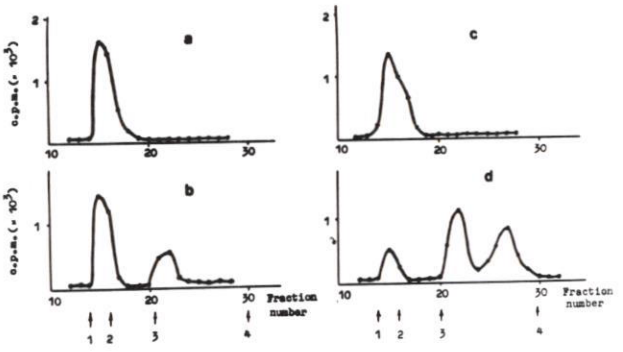

Figure 5. Separation of solubilized VP receptors of the rat renal papilla on a column of Sephacryl S-300. Adult rats, receptors in the presence (a) or in the absence (b) of (^{3}H)VP ligand; 14-day old rats, receptors in the presence (c) or in the absence (d) of the ligand. Arrows indicate the site of the elution of markers of different molecular weights: (1) blue dextran, (2) ferritin, (3) ovalbumin, (4) cytochrom C.

importance than in younger individuals. However, in adults, the concentration of ADH must be sufficiently high to maintain the stability of the molecular form of the receptor and, hence, its functional activity. With this in mind, ADH decrease in the blood for a long time, as might be expected in space flights and antiorthostatic tests, may lead to desagregation of the receptor complex and to a decrease in its functional activity.

The present experimental results suggest that changes in intrarenal osmotic gradients, as well as in the aggregated state in the ADH receptors, which can arise under hypogravitation, can result in an inadequate response to ADH in cosmonauts during readaptation to the Earth gravity.

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THE EFFECT OF SUSPENSION ON NICOTINIC ACETYLCHOLINE RECEPTOR NUMBER AND AFFINITY AT THE RAT NEUROMUSCULAR JUNCTION

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INTRODUCTION

It has been recognized for some time that exposure of man or animals to microgravity results in changes in certain physiological systems. One such change is the loss of mass and function in skeletal muscle, primarily of the weight-bearing muscles and in particular in the antigravity muscles such as the soleus. These changes can be largely attributed to the fluid shifts from the periphery to the head and torso and to a mechanical unloading of the load-bearing muscles. However, evidence of muscle catabolism, despite vigorous exercise regimens and dietary supplementation, suggests something more than disuse atrophy.

It has been well documented that associative and feedback mechanisms exist between nerve and muscle. And that interrupting these interactions can lead to changes in the muscle similar to those seen with space-induced muscle atrophy. It is also known that receptors at the neuromuscular junction are sensitive to changes in the system as evidenced by changes in receptor number in denervation studies. There exists flight data in support of a neuronal component. In particular the morphological study on biosputnik rat soleus muscle that showed synaptic vesicles to be decreased in both volume and number (1).

Our objective is to investigate a possible neuronal component, as measured by receptor affinity or number, to space-induced muscle atrophy.

METHODS

Weightlessness was mimicked using the Morey-Holton rat tail-suspension model (5). Five-week-old male Sprague-Dawley rats were suspended for 1, 3 or 8 weeks. Weight-matched and pair-fed controls were housed singly in identical cages. Receptors were examined in the unloaded phasic gastrocnemius and tibialis anterior and the tonic soleus, as well as the loaded phasic triceps brachii. Receptor affinity (Kd) and number (Bmax) were measured in competitive inhibition radioligand binding studies using the specific nicotinic acetylcholine receptor ligand, α-bungarotoxin. Data were analyzed using the paired t-test to compare experimental animals with their controls. ANOVA was used to test for differences between suspension groups and Fisher's least significant difference test was used to determine which group(s) differed significantly.

RESULTS AND DISCUSSION

Analysis of binding data from control animals (Table 1) indicates that a maturational change in receptor number and affinity occurs independently of experimental protocol. The Kd in the triceps brachii is increased between 6 and 13 weeks of age and in the soleus between 8 and 13 weeks of age (both $P < .01$). Bmax (in fmol/mg protein) is decreased between ages 6 and 8 weeks in the triceps brachii ($P < .05$) and the tibialis ($P < .01$). In general, as the animals age from 6 to 13 weeks, both Kd and Bmax decrease, indicating an increase in affinity and a decrease in receptor population or number. Similar trends are found in suspended animals.

Developmental elasticity in skeletal muscle innervation has been previously reported. That our data report a simultaneous increase in affinity as receptor populations decrease, suggests a refinement of the process begun with the loss of embryologic extrajunctional receptors (2) and polyneuronal innervation (3) and the formation of adult synapses (4). These data show that care should be taken when designing neuromuscular studies to match animal age as well as other experimental parameters. Care must also be taken when interpreting data to discriminate maturational from experimental changes.

In analysis of binding data between suspended animals and their controls, the Kd is significantly decreased only in the soleus after 3 weeks of suspension. The Bmax is increased in the triceps brachii after 3 weeks of suspension and in the gastrocnemius after 8 weeks.

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Table 1. Kd (nM ± SE) and Bmax (fmol/mg protein ± SE) versus age in control rats

		Age in Weeks		
		6	8	13
Gast.	Kd	.40 ± .02	.44 ± .06	.26 ± .09
	Bmax	12.8 ± 0.4	15.1 ± 2.3	2.7 ± 0.5 [b, d]
T. brac.	Kd	1.15 ± .39	.61 ± .18	.28 ± .04[c]
	Bmax	36.5 ± 5.8	16.0 ± 5.1[a]	7.8 ± 1.2[d]
Tib.	Kd	.46 ± .12	.39 ± .04	.26 ± .01
	Bmax	21.8 ± 2.3	8.1 ± 1.5[b]	5.9 ± 0.6[d]
Sol.	Kd	.31 ± .07	.83 ± .10[a]	.20 ± .0[b]
	Bmax	16.5 ± 2.1	20.6 ± 2.7	10.7 ± 0.1[a]

n = 2 for the soleus at 6 and 13 wks; n = 3 for all other values

[a] ($P < .05$) [b] ($P < .01$) values significantly different from preceding time
[c] ($P < .05$) [d] ($P < .01$) values at age 13 wks significantly different from values at age 6 wks.

BIOCHEMICAL AND HISTOCHEMICAL OBSERVATIONS OF VASTUS MEDIALIS FROM RATS FLOWN IN COSMOS 1887 (EXPERIMENT K608)

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INTRODUCTION

The vastus medialis (VM) in the rat is chiefly composed of fast twitch fibers, comparably divided between oxidative glycolytic and glycolytic types (1). In many respects it is comparable to the extensor digitorum longus (EDL), chiefly fast twitch fibers (97%), oxidative glycolytic and glycolytic (59 and 38%, respectively) and a few (3%) slow twitch fibers (1). The VM and EDL are not load bearing muscles and, in addition to morphological similarity, it is reasonable to assume that there is some functional and metabolic similarity. We obtained the VM because of its availability and it afforded us an opportunity to compare data with the EDL which has been used in both microgravity flights (2,4,8,11) and earthside experiments using simulation models (3,6).

The principal objectives were to ascertain VM responses to 12 days of microgravity. Three approaches were used: (a) histochemical evaluation of cellular morphology (fibers and capillaries), (b) assessment of biochemical composition (protein, RNA and DNA concentrations) and (c) estimation of metabolic activities and capacities (oxidative and glycolytic metabolism) and lipoprotein lipase activity.

MATERIALS AND METHODS

Muscles were obtained from five rats exposed to weightlessness, 12 days(F), and five rats in control groups basal controls (B), vivarium controls (V) and synchronous controls treated comparably to flight rats (S). Muscles were frozen in liquid nitrogen and shipped on dry ice to the University of Louisville. Samples from the belly of two muscles from rats in each group were examined histochemically for morphometric characteristics as in a previous report (7). Frozen sections were stained for ATPase activity, muscle fibers and capillaries were differentiated. Fiber area and density measurements were made and capillary distribution was assessed. Remaining muscle samples were lyophilized, weighed and powdered with a Wiley Mill; aliquots for protein (mg/mg dry wt,) RNA and DNA concentration determinations (ug/mg dry wt) as previously described (11), lactate dehydrogenase (LDH) (9), citrate synthase (CS) activities (u moles/min/gm) (10), and lipoprotein lipase (LPL) activities (nmoles FA liberated) (5).

RESULTS AND DISCUSSION

Vastus medialis showed the greatest loss when comparing F and B, and least when comparing F and V, only about 13% (Table 1). In terms of percent water, there is no difference between flight and control groups. Sample size is limited and conclusions about muscle mass changes must be considered cautiously.

There is some similarity in VM from 12 day rats and EDL from rats following seven days microgravity. The VM lost about 13% mass when compared with V controls and the EDL lost about 10% (11).

The VM is almost totally composed of type II fast twitch fibers. There was significant reduction in fiber area, about 30%, when compared to B controls (Table 2). The fiber area and fiber density in F and B controls showed an inverse relationship. Also, capillary density was increased in VM of F rats (Table 2). Such responses are evident in muscles that undergo disuse atrophy (7).

Protein concentrations were comparable in F, B and S subjects (0.7 to 0.8mg/mg dry wt); however, RNA concentrations were significantly reduced below B controls (Table 3). Biochemical profiles suggest that changes in the VM, after 12 days of weightlessness, were minimal. In this respect VM is comparable to EDL from Sprague Dawley rats exposed to seven days of weightless flight in the SL-3 mission (11).

Levels of LDH activity (>2100) are characteristic of fast twitch highly glycolytic (type II B) fibers (Fig. 1). Conversely, oxidative capacity measured by CS activity was low, also characteristic of fast twitch muscle (Fig. 2). LPL levels showed the lowest levels in the F rats (Fig. 3). This may suggest that the VM in F rats had a reduced capacity to utilize stored triglycerides for energy production.

Table 1 - RAT, VASTUS MEDIALIS AND BODY WEIGHTS

Groups	Body Wt(gm)	Muscle Wt (mg)	Muscle Wt/B Wt	%Water
Flight	303 ± 2.4	334 ± 41.1	1.1 ± 0.14	73.9 ± 2.16
Controls				
Basal	316 ± 8.3	586 ± 85.0	1.9 ± 0.28	74.2 ± 0.64
Vivarium	342 ± 7.7	386 ± 31.0	1.1 ± 0.08	75.5 ± 0.72
Synchronous	349 ± 5.8	427 ± 19.4	1.2 ± 0.04	75.8 ± 0.26

Mean ± S.E. N = 5

Table 2 - RAT, VASTUS MEDIALIS, Morphometric Measurements

Groups	Rat Number	Fast Twitch Fibers Cross Sec.* Area (μm^2)	Density** (Cells/mm^2)	Capillary*** Density (cap/mm^2)
Flight	8	3889	249	565
	10	3852	253	725
Controls				
Basal	7	5536	190	374
	8	5201	202	409
Vivarium	6	3469	239	554
	9	3940	247	634
Synchronous	7	5662	156	390
	9	3962	266	673

* number of cell areas measured; 40 or more
** number of cells counted; 70 to 100
*** number of capillaries counted; 300 to 700

Table 3 - RAT, VASTUS MEDIALIS, Biochemical Observation

Groups	Protein (mg/mg Dry Wt)	RNA (ug/mg Dry Wt)	DNA (ug/mg Dry Wt)
Flight	0.70 ± 0.07	5.5 ± 0.1	14.1 ± 0.6
Controls			
Basal	0.73 ± 0.06	6.0 ± 0.1	13.9 ± 0.4
Vivarium	0.88 ± 0.06	5.5 ± 0.1	12.9 ± 1.4
Synchronous	0.81 ± 0.06	5.1 ± 0.6	16.8 ± 0.9

Mean ± S.E. N = 5

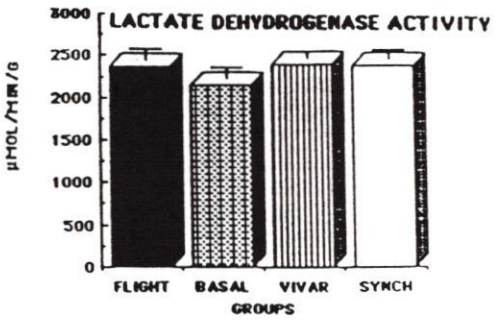

Figure 1 Vastus Medialis (5 each, M ± SEM)

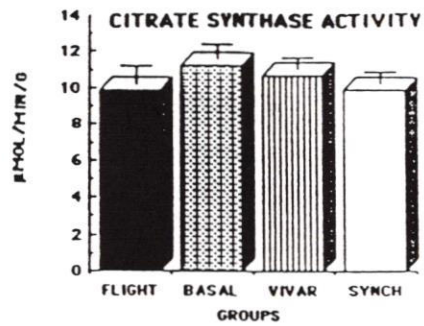

Figure 2 Vastus Medialis (5 each, M ± SEM)

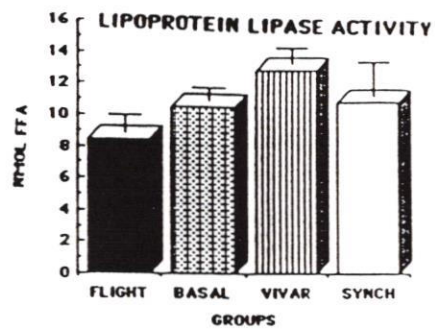

Figure 3 Vastus Medialis (5 each, M ± SEM)

CONCLUSIONS

The morphometric and histochemical results showed some decrease in fiber area in VM from flight rats. The biochemical data showed some decrease in RNA concentration in flight rats. The LPL measurements were lowered in F rats.

The VM and EDL have several features that are comparable in response to short term weightless flight. Thus, when considering opportunities to maximize use of muscle samples from the limited numbers of flight rats, the VM can be considered for studies of fast twitch muscle.

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Acknowledgment: Technical assistants Ken A. Mook, M.J. Dombrowski and N. Dugan, and Dr. Richard E. Grindeland, NASA-Ames Research Center, for the muscle samples. Also, Drs. E. Ilyina-Kakueva and V.S. Oganov, Institute of Biomedical Problems, Moscow, for their efforts in our behalf.

DECREASED SWELLING PRESSURE OF RAT NUCLEUS PULPOSUS ASSOCIATED WITH SIMULATED WEIGHTLESSNESS

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INTRODUCTION

The intervertebral disc is an important weight-bearing tissue of the spine. The disc consists of a negatively-charged nucleus pulposus (primarily proteoglycan) confined by a collagenous annulus (Fig. 1). The disc absorbs shocks and allows flexibility of the spine during various activities and postures. Increased loading on the spine during spinal muscle contraction or weightbearing causes fluid loss from the disc, whereas decreased loading (e.g. during sleep) allows fluid influx into the disc. Although the disc is implicated in the etiology of back pain experienced by astronauts during weightlessness, little is known about intradiscal fluid homeostasis during prolonged unloading. Therefore, this preliminary study examined swelling pressure within discs obtained from rats exposed to actual and simulated weightlessness and compared these pressures with those in several groups of control rats.

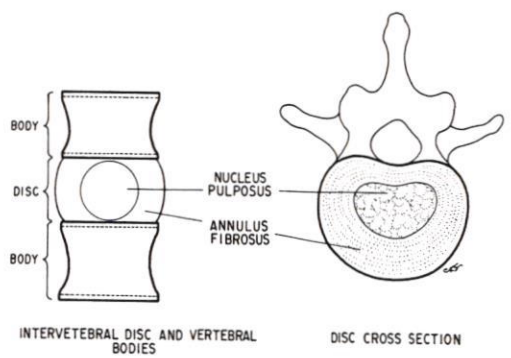

Figure 1. Each intervertebral disc consists of a central, gelatinous nucleus pulposus that is confined by an annulus fibrosis and two vertebral bodies.

METHODS

In studies of actual weightlessness, samples of nucleus pulposus were obtained from lumbar discs of:

1) five 300g male Wistar rats exposed to 12.5 days microgravity on Cosmos 1887
2) five 300g male Wistar rats (synchronous controls)
3) five 300g male Wistar rats (vivarium controls)

The Cosmos rats were re-exposed to over 50 hours of normal gravity prior to tissue harvesting. Spines were frozen in dry ice and shipped to the U.S. where nucleus pulposus samples were removed and swelling pressures were measured. Control samples were treated identically.

In studies of simulated weightlessness, samples of nucleus pulposus were obtained from lumbar discs of:

1) seven 200g male Sprague-Dawley rats exposed to 7 days of tail suspension using the model of Morey-Holton (3),
2) seven 200g male Sprague-Dawley rats (cage controls),
3) seven 200g male Sprague-Dawley rats (vivarium controls).

Samples of nucleus pulposus were frozen in dry ice and thawed immediately before each swelling pressure determination. Controls were handled identically.

Equilibrium swelling pressure was measured in a compression-type osmometer for 5-10 microliter samples (Fig. 2). Lumbar samples were pooled for each rat to obtain sufficient volume. Equilibrium was approached within 15-30 minutes, depending on the magnitude of swelling pressure.

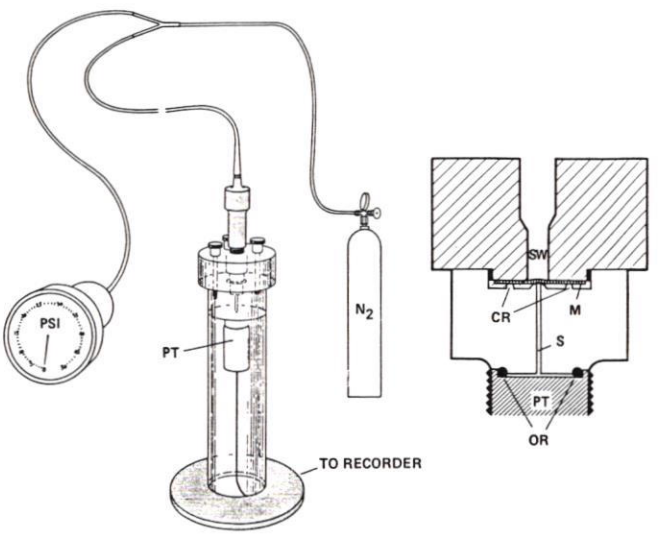

Figure 2. Compression-type osmometer for measuring swelling pressure of nucleus pulposus. Left: Plexiglas osmometer mounted on stand with nitrogen gas inlet at top. The osmometer is connected to a nitrogen gas source, precision pressure gauge, and pressure transducer (PT). Transmembrane pressure gradients are continuously measured by a strip-chart recorder. Right: Cross-section of osmometer with sealing of membrane by crimp rings (CR) on the screw-down Plexiglas plate. Nucleus pulposus is placed in the sample well (SW) on top of the membrane (M). Pressure gradients across the membrane are transmitted by the saline fluid column (S) and monitored by the pressure transducer (PT) fitted tightly to the bottom of the osmometer using an O-ring seal (OR).

RESULTS

For experiments involving actual weightlessness, swelling pressures were 690, 675 and 622 mmHg for Cosmos 1887 flight rats, synchronous controls, and vivarium controls, respectively (Table 1).

TABLE 1. COSMOS FLIGHT STUDIES

Swelling Pressures of Lumbar Discs
(mm Hg ± S.E.)

Cosmos Flight Rats (N = 5)	690 ± 45
Synchronous Controls (N = 5)	675 ± 32
Vivarium Controls (N = 5)	622 ± 25

There was no significant difference between any group using ANOVA statistics. For studies of simulated weightlessness, swelling pressures were 295, 610 and 527 mmHg for tail-suspended rats, cage controls and vivarium controls, respectively (Table 2). Intradiscal swelling pressure of tail-suspended rats was significantly lower than that of both groups of control rats.

TABLE 2. SIMULATED WEIGHTLESSNESS STUDIES

Swelling Pressures of Lumbar Discs

(mmHg ± S.E.)

Tail-Suspended Rats (N = 7)	295 ± 18 *
Cage Controls (N = 7)	610 ± 27
Vivarium Controls (N = 7)	527 ± 21

* Significantly less than both groups of controls.

DISCUSSION

Flight rats aboard Cosmos 1887 were re-exposed to normal gravity for over 50 hours prior to sacrifice and tissue harvesting. Based upon theoretical and experimental studies of fluid fluxes into and out of the intervertebral disc (7, 8), this time period is probably sufficiently long so that fluid contents of those discs exposed to actual weightlessness were probably fully re-adjusted to Earth's gravity. Additional studies of rats exposed to spaceflight and more rapid sacrifice after re-entry are needed to resolve this issue.

On the other hand, swelling pressure within discs of tail-suspended rats is about half that in control rats, suggesting that fluid moves into the disc during seven days of simulated weightlessness. It is known that the tail is exposed to tensile forces equivalent to about half of the rat's weight in the tail suspension model (2). In this regard it would be interesting to compare swelling pressures using this model developed by Morey-Holton (3) to that of Musacchia and co-workers (4). Moreover, longitudinal studies of swelling pressure after recovery from simulated and actual weightlessness are needed to document the time course of equilibration. Whereas short-term unloading of the spine may affect fluid movement into and out of the disc, long-term unloading may reduce cellular activity and proteoglycan production within the nucleus pulposus.

The rat is not an ideal model for understanding human adaptations to weightlessness in terms of its quadrupedal stature. However, rat discs may provide unique insights into the effects of unloading of spinal musculature on intradiscal pressure. In this regard, the relative effects of paralysis and/or electrical stimulation of spinal muscles on intradiscal pressures should be investigated.

During normal daily activity, the human spine loses about 1-2 cm in overall height (1, 6) and presumably this loss is regained during sleep and horizontal posture. It has been estimated that astronauts gain approximately 4-6 cm in height during weightlessness (5). Such spinal lengthening may help explain the etiology of back pain experienced by astronauts during weightlessness. However, detailed noninvasive-imaging studies of the disc and its ligamentous support structure are needed to address this issue.

ACKNOWLEDGEMENTS

We thank Dr. R. Grindeland, Dr. L. Keil, M. Vasques, M. Skidmore and J. Evans for transporting the samples safely to us and we thank the superb Cosmos Dissection Team for their assistance. We also thank C. Baldovino for assistance with manuscript preparation. This research was supported by NASA and by the Cosmos 1887 Rodent Parts Program.

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NORMALISATION OF BONE CELLULAR RESPONSES OCCURS BETWEEN 7 AND 14 DAYS OF SIMULATED WEIGHTLESSNESS IN RATS.

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INTRODUCTION

The tail suspension hypokinesia / hypodynamia model described by WRONSKI and MOREY-HOLTON (6) is a well documented maneuver to simulate weightlessness in terms of loss of bone and diminished bone formation (5). However, rigourous comparisons between experiments in real and simulated microgravity have never been performed.

In this study, two simulations were planned in order to be further compared with results obtained from the soviet Biocosmos 1667 (1 wk) and 1887 (2 wks) spaceflights. Rats used in real and simulated microgravity were of the same strain, age, sex and weight. Bone histomorphometric parameters were evaluated in the same weight-bearing bone, the tibia, at the proximal metaphysis level.

METHODS AND MATERIAL

Animals :

Two suspension experiments were performed : the one week suspension (experiment 1) was of the same duration than the Biocosmos 1667 spaceflight and the two week suspension (experiment 2) was similar in duration to the Biocosmos 1887 spaceflight. WISTAR rats of approximately 300g at the beginning of the experimental period were used in both experiments and spaceflights. Rats were acclimatized to our laboratory during one week with standard conditions of temperature (23°C ± 1°C) and light cycle (LD 12:12). Fourteen and 20 animals respectively in the experiment 1 and 2, were randomly divided into control and suspended groups. They were all housed individually. Suspended rats were lightly anesthetize with 30 mg/kg body weight of sodic thiopental (Nesdonal ® Specia Paris) at the time of tail preparation. They were provided standardized rat chow and tap water ad libitum.

Rats of the experiment 1 were intraperitoneally injected with 30 mg/kg body weight of demethylchlortetracycline (Ledermycine ® - Lederle laboratories - OULLINS - FRANCE) at day 0 and at day 6 (24 hours before sacrifice). Rats of the experiment 2 were similarly injected with Ledermycine ® at day 0 and at day 13. At day 7, they were injected with xylenol orange (50 mg/kg).

Finally, rats were sacrificed with a high dose of Nesdonal ®.

Histomorphometric analysis :

Tibial proximal methaphyses were embedded in methylmethacrylate. Longitudinal sections, 7 µm thick, were sawed for Goldner staining, used for bone mass and osteoid parameter measurements and for tartrate resistant acid phosphatase for the determination of the osteoclastic parameters (2). Sections 14 µm thick, observed under UV light, were used to determine the mineral apposition rate by dividing the number of day between labels.

Measurements of bone mass parameters were separatly done in the primary spongiosa and in the secondary spongiosa. The rate of longitudinal bone growth was determined by measuring the distance between labels which parallels the growth plate in the experiment 2. In both suspensions mean thickness of the primary spongiosa was determined in µm. The Bone Volume per Tissue Volume (BV/TV) was expressed in %. The Trabecular Thickness (Tb.Th) in µm and Number (Tb.N) in plate/mm reflected the spatial arrangement of trabecular structure.

In the secondary spongiosa, where remodeling occurs (1), the Number of Osteoclast per Bone Perimeter per Tissue Area (N.Oc+/(B.Pm/T.Ar)) expressed in cell/mm and the active resorption surfaces (Oc+.S/BS - Osteoclast Surfaces per Bone Surfaces) in % were measured.

Osteoblastic activity was determined by measuring :

- osteoid parameters : Osteoid Surfaces per Bone Surfaces in % at Cancellous (Cn OS/BS) and Endocortical (Ec OS/BS) levels and Osteoid Thickness in µm at the Endocortical level (Ec O.Th).
- Mineral Apposition Rate at Cancellous (Cn MAR) and Endocortical (Ec MAR) levels in µm/day.

RESULTS

In the experiment 1, rats were not weighted during the suspension period. At the beginning of the experimental period, suspended rats weighted 298.5 ± 24g, they weighted 284 ± 23g at the end. Control rats weighted 320 ± 5g at the beginning and 335.7 ± 18.5g at the end. Suspended animals all lost weight (0 to 30g). In the experiment 2, the first lost of weight during the first four days was followed by a gain which remains lower than in control group (fig. 1). No diet adjustment was made. Fed consumption was similar in control and suspended animals.

Tibial bone histomorphometric data are listed in Table 1. The comparison of histomorphometric data between 7d and 14d control groups reveals no difference. The thickness of the primary spongiosa is reduced by a factor of 1.5 after 7d suspension and of 3 after 14d suspension. In experiment 2, the bone formation rate is reduced by a factor of ~3.5. Trabecular bone volume is decreased in experiment 1and trabecular thickness is significantly diminished by 25% in experiment 1 and by 15% in experiment 2.

At the level of the secondary spongiosa, the bone volume is significantly reduced in both experiments (34% in experiment 1, 20% in experiment 2). Despite non significant difference between suspension groups and respective control groups, trabecular thickness is more affected in the experiment 1.

All bone resorption parameters are significantly increased in experiment 1. In experiment 2, values are greater than their respective controls but difference are not statistically different.

Trabecular formation parameters are decreased only in experiment 1. Mineral apposition rate between 7 and 14 days is not affected in experiment 2.

At the endosteal level, same results are observed for mineral apposition rates. However, the osteoid surfaces are decreased in both cases but with greater magnitude in experiment 1 (34% and 16% respectively). Thickness of the osteoid seams are similary affected in both experiments (27%).

DISCUSSION

The longitudinal bone growth, evaluated by measurement of the thickness of the primary spongiosa in both experiments and using tetracycline labeling in the 14d experiment, appeared to be twofold greater depressed in the 14d than in the 7d experiment. In a two weeks experiment, similar results were found in back-suspended rats whereas no change was observed in tail-suspended rats. Furthermore, the formers lost weight whereas the later did not (6). Authors concluded that these results may be indicative of a pronounced stress response in back-harness use.

At the level of the primary songiosa, the depressed longitudinal bone growth rate was associated with decreased trabecular bone volume in the 7d suspension and decreased trabecular thickness in both suspensions. At the level of the secondary spongiosa, the reduced mass of mineralized tissue observed in both experiments may be due to diminished rate of longitudinal bone growth. However, at this area, where remodeling occurs (1), the 7d suspended rats were more affected than the 14d. Consequently, a more severe desequilibrium in the bone cell population is suspected in the shorter experiment. This is confirmed by histomorphometric bone cell parameters : the osteoclastic population, determined with a specific histoenzymologic method (2) was increased by a factor of two whereas the trabecular osteoid surfaces and mineral apposition rate were decreased by 23% and 41% respectively. In the 14d experiment, these resorption and formation parameters tended to be

normal without statistical difference as compared with their controls. At the endosteal level, same trends were observed but osteoid parameters have not reached normal values, yet.

We suggest that two bone remodeling phases occurs in this model : the first is characterized by a severe imbalance in bone cell activities and results in bone loss, secondly bone cellular activities come back to equilibrium and bone tissue loss is no longer observed.

In WRONSKI and MOREY-HOLTON study (6), a loss of trabecular bone volume was noted in the proximal tibial metaphysis : in back suspended rats, the loss was evaluated to 52% as compared to control values. It was associated with stimulated bone resorption and inhibited bone formation. In tail-suspended animals, it was about 19% with no change in cellular activities. This last result was comparable to ours despite rats of different strain and age. Other studies confirmed that inhibition of bone formation is transient (3-4).

Additional works are needed to confirm the transient stimulation of bone resorption and to explore the importance and the impact of the stress response.

Acknowledgement : The authors are grateful to Yves BOYER for animal care. This work was partially supported by the Direction Recherche Etudes et Techniques (DRET).

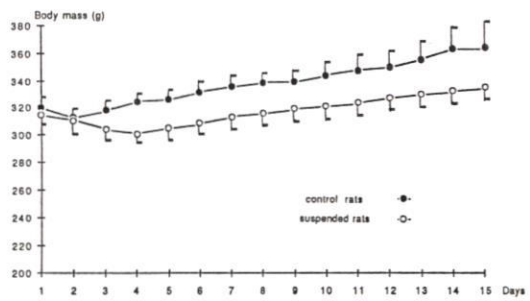

Fig. 1 : Body mass as a function of time in tail-suspended and control rats. The body masses of suspended and control rats were significantly different ($p < 0.001$) at the end of the experimental period.

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	7 DAYS		14 DAYS		CONTROL SUSP. 7 D. vs SUSPENSION 7 D.	SUSPENSION 7 D. vs SUSPENSION 14 D.	CONTROL SUSP. 14 D vs SUSPENSION 14 D.	CONTROL SUSP. 7 D. vs CONTROL SUSP. 14 D.
	CONTROL SUSPENSION n = 7	SUSPENSION n = 7	SUSPENSION n = 10	CONTROL SUSPENSION n = 10				
1° SPONGIOSA MEAN THICKNESS (µm)	366 ± 62	237 ± 37	113 ± 13	358 ± 49	p < 0,001	p < 0,0001	p < 0,001	ns
BONE FORMATION RATE (µm/d)	--	--	16,95 ± 4,82	63,99 ± 8,62	--	--	P < 0,001	--
BV/TV (%)	38,08 ± 4,9	30,1 ± 5,2	34,03 ± 4,49	35,87 ± 3,86	p < 0,02	p < 0,05	ns	ns
Tb. Th (µm)	30,7 ± 4,11	22,89 ± 2,55	29,7 ± 5,96	34,80 ± 2,35	p < 0,01	p < 0,002	p < 0,05	ns
Tb. N (/mm)	12,48 ± 1,5	13,2 ± 2,11	11,62 ± 1,20	10,40 ± 2,35	ns	ns	ns	ns
Tb. Sp (µm)	50,5 ± 9,33	54,6 ± 12,2	57,40 ± 8,57	62,20 ± 7,19	ns	ns	ns	ns
2° SPONGIOSA BV/TV (%)	15,9 ± 2,7	10,54 ± 3,48	11,62 ± 4,32	14,40 ± 3,40	ns	ns	ns	ns
Tb. Th (%)	34,8 ± 4,9	28,6 ± 4,48	40,20 ± 5,87	31,60 ± 2,99	ns	p < 0,001	ns	ns
Tb. N (/mm)	4,6 ± 0,66	3,6 ± 0,66	2,86 ± 0,80	4,45 ± 0,71	ns	ns	ns	ns
Tb. Sp (µm)	186 ± 32	255 ± 53	338 ± 117	161 ± 70	ns	ns	ns	ns
N.Oc+/(B.Pm/T.Ar) (o/mm)	3,35 ± 0,8	6,04 ± 1,79	5,27 ± 1,8	3,97 ± 1,14	p < 0,002	ns	ns	ns
Oc+.S/BS (%)	6,01 ± 1,36	12,45 ± 3,35	9,54 ± 2,72	6,88 ± 1,78	p < 0,001	p < 0,05	ns	ns
Cn OS/BS (%)	6,85 ± 1,79	5,28 ± 0,70	8,74 ± 2,36	6,62 ± 1,16	ns	p < 0,05	ns	ns
Cn MAR (µm/d) 1 to 7 d.	2,62 ± 0,35	1,55 ± 0,33	1,02 ± 0,42	2,35 ± 0,35	p < 0,001	ns	p < 0,001	ns
Cn MAR (µm/d) 7 to 14 d.	--	--	1,66 ± 0,13	1,88 ± 0,47	--	--	ns	--
Cn MAR (µm/d) 1 to 14 d.	--	--	1,12 ± 0,42	1,88 ± 0,35	--	--	P < 0,02	--
Ec OS/BS (%)	80 ± 10	53 ± 7	66,2 ± 9,72	78,9 ± 5,9	p < 0,001	p < 0,05	p < 0,01	ns
Ec O.Th (µm)	7,07 ± 0,52	5,18 ± 0,56	5,99 ± 1,25	8,12 ± 1,49	p < 0,001	ns	p < 0,01	ns
Ec MAR (µm/d) 1 to 7 d.	5,09 ± 0,80	4,8 ± 0,84	3,53 ± 1,47	4,83 ± 0,44	ns	ns	ns	ns
Ec MAR (µm/d) 7 to 14 d.	--	--	4,77 ± 1,18	4,73 ± 0,72	--	--	ns	--
Ec MAR (µm/d) 1 to 14 d.	--	--	3,91 ± 1,27	4,56 ± 0,8	--	--	ns	--

Table 1 : Quantitative bone histomorphometric data in the proximal tibial metaphysis of tail-suspended and control rats (see text for abbreviations).

MODIFICATIONS OF BONE ATROPHY SEEN WITH HINDLIMB SUSPENSION BY EXERCISE AND DOBUTAMINE

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Loss of bone mass during exposure to zero g has been well documented in both humans and experimental animals, and has been replicated with such ground-based models as hindlimb suspension (1). If this loss of bone is to be minimized during prolonged spaceflight, interventions must be tested for their effectiveness. The objective of this study was to investigate the interactive effects of endurance training and dobutamine injections on the cortical bone loss seen in rats during hindlimb suspension.

Exercise is generally assumed to increase the mechanical loading on bone and thereby increase bone mass. Shaw and co-workers recently investigated the effects of up to 1 1/2 hours of daily treadmill running on bone morphometry in rats subjected to hindlimb suspension (2). Their exercise protocol did not counteract and in some cases exacerbated the loss of bone and the decrease in its mechanical strength. However, their study design introduced a daily period of weight-bearing in addition to exercise stress during the suspension period. We wished to investigate the effects of endurance training previous to an uninterrupted suspension period.

Dobutamine is a synthetically produced catecholamine that has been shown to produce cardiovascular and metabolic changes similar to those produced by exercise training (3). Although there is no known direct effect of dobutamine on bone, we hypothesized that it may have a positive influence on bone mass as mediated by an improved maintenance of skeletal muscle mass.

METHODS

Forty-eight male Sprague-Dawley rats were assigned to either exercise training (EX) or sedentary (SED) conditions. The training protocol on a motorized treadmill progressively increased in speed and duration over 11 weeks: for the last 4 weeks, the animals were running at 30-31 m/min for 80 minutes at an 8% grade. This protocol has been previously demonstrated to result in dramatic improvement in cardiovascular function as well as skeletal muscle oxidative capacity (4).

Rats from both groups were then randomly assigned to either suspension (SUS) or control (NON-SUS) treatments for 14 days. The suspension apparatus used was that developed by Morey-Holton, with an x-y axis pulley system allowing free movement of the rat by use of its forelimbs. Each rat was suspended at a 30° head-down tilt via the tail traction method. Control rats were housed in identical cages for the 14-day period. To maintain their trained status, those trained rats not to be suspended continued their treadmill runs but at approximately 50% of the final treadmill duration.

Half of each group was given daily intraperitoneal injections of 2 mg/kg body weight dobutamine (DOB) during the suspension period; remaining rats received sham injections of saline (SAL). In addition, all rats were injected with bone labelling compounds on days 0, 6 & 12 of suspension.

Average age of the animal at sacrifice (by decapitation) was 155 days. Left hindlimbs were stored in 70% ethyl alcohol until the femurs could be processed for quantitative histomorphometry. Undecalcified 200 micrometer sections were analyzed with a Zeiss Videoplan digitizing system. Indices measured were: total cortical bone cross-sectional area; cortical thickness in anterior, posterior, lateral and medial quadrants; endosteal and periosteal diameters; and the average distance between fluorescent tags. The tag given at day 6 did not fluoresce adequately to be distinguishable, hence the reported periosteal formation distance is for a 12-day period. Results were analyzed by 3-way ANOVA with Tukey post-hoc testing for significant F values.

RESULTS AND DISCUSSION

As illustrated in Figure 1, suspension in sedentary rats produced the expected decrease in cortical bone area, as compared to the baseline control (SED/NON-SUS/SAL) rats (6.06 ± .21 vs. 7.33 ± .21 mm^2). Surprisingly, the training protocol appeared to produce similar decreases (average of -19.3%) in cortical bone area in three of the four trained groups. However, those trained rats that were suspended had bone areas equivalent to baseline control (6.99 ± .21 mm^2). Dobutamine appeared to maintain cortical bone area in SED/SUS rats as compared to baseline. The opposite effect occurred in the EX/SUS rats; that is, rats given dobutamine injections had smaller cortical bone areas relative to rats given saline.

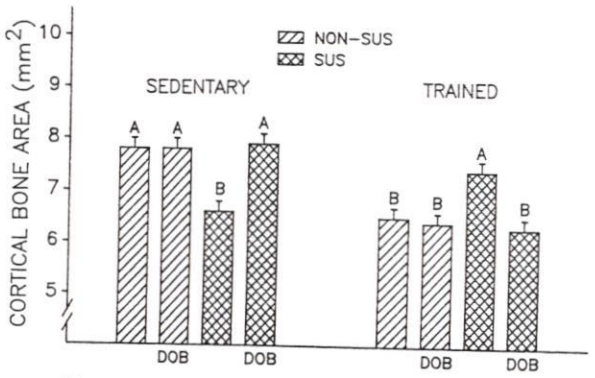

Figure 1 Cortical bone area after 14 days of hindlimb suspension (SUS) or control (NON-SUS) treatment; DOB = dobutamine-treated animals. Bars sharing a common letter represent group values that are not statistically different from each other ($p < .05$).

The most dramatic changes in regional cortical thickness occurred in the medial quadrant. As seen in Figure 2, training had a cortical thinning effect in all 4 EX groups, with an average decrease of 26% as compared to the baseline control animals. Neither suspension nor dobutamine affected this change. Suspension in sedentary rats produced a small but non-significant decrease in medial cortical thickness.

These changes in cortical thickness with training could be the result of a net bone loss at either periosteal or endosteal surfaces of the cortical bone. Only two groups exhibited significant changes in lateromedial endosteal diameter as compared to baseline: those trained rats on saline, both non-suspended and suspended (2.02 $\pm$ .06 and 1.99 $\pm$ .06 mm, respectively, vs. 1.78 $\pm$ .06 mm for baseline controls). Implied is an increase in net resorption for these two groups at the medial endosteum. Dobutamine produced no significant changes in this parameter except for the trained, non-suspended rats.

In examining the lateromedial periosteal diameter, an average decrease of 7.7% relative to baseline was observed in this dimension in all training groups except the EX/SUS/SAL rats. Also noted was a similar decrease in this periosteal diameter in SED/SUS/SAL animals; dobutamine appears to prevent the decrease in this diameter for this group.

Changes in the rate of bone formation at the periosteal surface, as reflected by the distance between fluorescent tags, are illustrated in Figure 3. Training alone did not produce a change in this parameter. However, in both groups of trained rats subjected to suspension, as well as SED/SUS/SAL rats, there was a significant decrease in periosteal formation as measured over 12 days (12.2 $\pm$ 3.2, 15.0 $\pm$ 2.4 and 17.7 $\pm$ 2.9 mm x 10^{-2}, respectively, vs. 26.0 $\pm$ 2.6 mm x 10^{-2} for baseline controls). Dobutamine produced no significant changes in periosteal formation at this site.

Changes in cortical bone parallel to those at the medial surface but of smaller magnitude were noted in the anterior quadrant. For example, cortical thickness here decreased an average of 19% in three of the four trained groups. However, no significant changes occurred in periosteal formation as measured by distance between fluorescent tags.

In conclusion, 14 days of hindlimb suspension produced significant decreases in cortical bone area, resulting at least in part from a decreased formation at the periosteal surface. Secondly, our vigorous training protocol produced a net loss of bone in non-suspended rats, but appeared to maintain bone area in suspended, trained rats. It should be noted that our trained rats had significantly lower body weights than the sedentary rats at the start of the suspension period, which may well explain the former effect.

Importantly, dobutamine did effectively prevent the loss of cortical bone with suspension in sedentary rats. Preliminary data indicates that loss of muscle mass with the suspension treatment was diminished in dobutamine-treated groups; this may provide one mechanism for the observed maintenance of bone mass. For trained rats, however, dobutamine appeared to counteract the maintenance of bone; the mechanism for this latter effect is unclear.

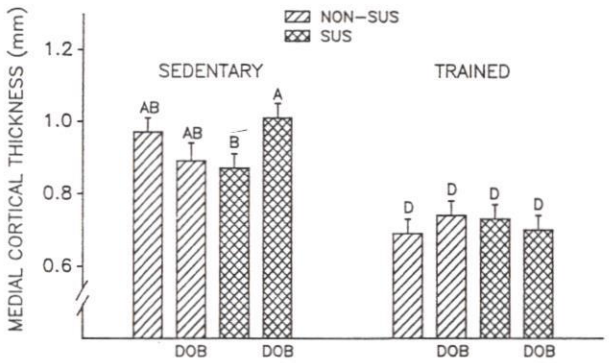

Figure 2 Medial cortical thickness after 14 days of hindlimb suspension; see Figure 1 legend for abbreviations.

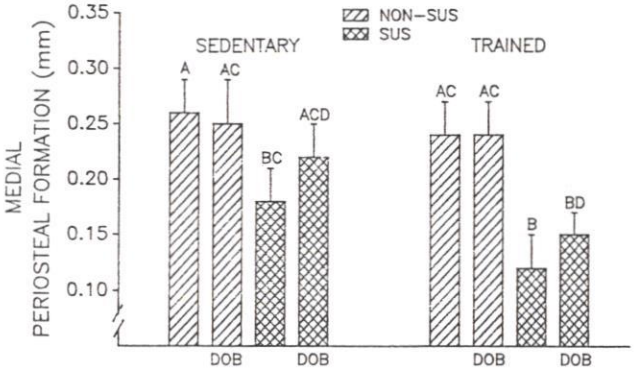

Figure 3 Medial periosteal formation over 12 days of experimental period; see Figure 1 for abbreviations.

ACKNOWLEDGEMENT

This work was supported by the Air Force Office of Scientific Research, Project #2312V6. We gratefully acknowledge Dr. Emily Morey-Holton for loaning the x-y axis pulley system for the hindlimb suspension cages.

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THE EFFECT OF MICROGRAVITY ON THE REPRODUCTIVE FUNCTION OF MALE-RATS

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Any new environment may be considered to be developed by a biological species if it retains normal reproduction and viability of progeny there.

Unfortunately effects of microgravity on reproductive function of mammals are conflicting and not numerous yet (3, 5-9, 11).

After 7-day space flight onboard biosatellite "Cosmos-1667" the male-rats* reproductive function has been appreciated in a wide complex program, including the citological and biochemical analysis of testis and epididymis, the assessment of sex behaviour, fertilization capacity, pre- and postnatal development and viability of the progeny of the male-rats mated with intact females. There were no differences between experimental and control groups in the mass and water content of the testis. Quantitative analysis of the smears, prepared of the testis homogenate, has showed no differences between flight and control groups both in whole testis cell content and in germinative, trophic and hormone-produced cells alignment. Sertoli cell cariometry has revealed no distinctions between the groups. No changes in epididymal weight, epididymal spermatozoa content (Fig.I) and the quota of spermatozoa with head abnormalities were found in the animals, which have been to the space as compared with control rats.

When flight males were mated with vivarium females during two weeks after the flight there were found no chages in sex behaviour and fertilizing capacity. The females, fertilized by flight males have shown no changes as compared with controls in the pre- and postimplantation mortality rate, litter size and sex ratio of the offspring. The number of abnormal and immature newborns was not increased either. The flight males' offspring was under observation during the first mouth after the birth. They have shown no deviations in postnatal growth and development, physical endurance, behaviour (10).

13-day space flight onboard biosatellite "Cosmos-1887" has also had no vital influence on the male reproductive system. The flight animals have shown a small, within 10% decreasing of testis and epididymis mass, caused by decreasing water content. At the same time total testis cell number and alignment of different cells of interstitium and spermatogonial epithelium were not changed (Fig.2). There were no differences between flight and control animals in total epididymal spermatozoa number (Fig.1) and the quantity of pathological and abnormal spermatozoa (2).

The changes of male reproductive organs in experiments with 7-day hympokinesia and immobilixation (the fixation in the back upward position by 2,5 hours during 7 consecutive days) were more expressed rather than after 7-day space flight. The immobilization has resulted in the decreasing of testis and seminal vesicles mass. The concentration of spermatozoa in hypokinetic and immobilized rat was lower, than in controls (Fig.1). In both model experiments the increasing of multinuclear spermatogonis, spermatocytes and spermatides was recorded (1).

In the years 60-70 the main method for simulating of microgravity effects in terrestrial conditions was hypokinesia (achieved by keeping the rats in the specific boxes of small size), which is superseded now by the "tail-suspension" model, permiting to simulate microgravity effects in the rat extremities (4). The last model is especially widely used in investigations of musculo-skeleton apparatus.

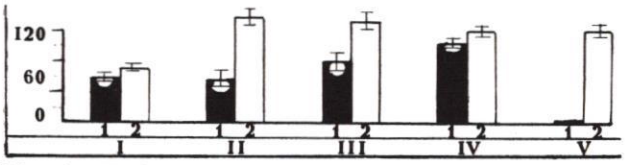

Fig.1. Epididymal spermatozoa content, mln: I-space flight, 7 days; II-immobilization, 7 days; III-hypokinesia, 7 days; IV-space flight, 13 days; V-tail suspension, 14 days. I-exptl group, 2-control group.

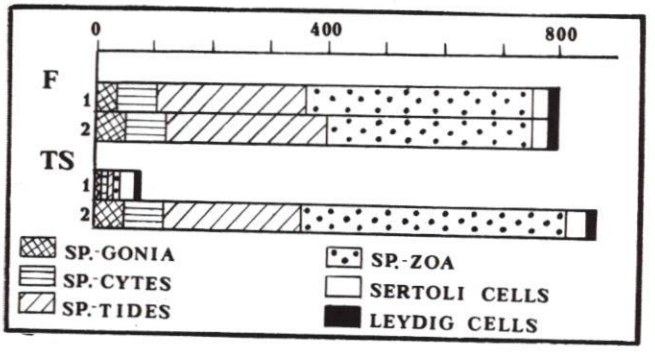

Fig.2. Total testis cell number (mln); space flight (F) and "Tail suspention (TS); I-exptl group, 2-control group.

We have studied the condition of the male-rats reproductive system after the 14-day tail-suspension. The testis of ex-

* Wistar strain rats, the breeding colony of the Institute of experimental endocrinology, CPS, SAS, Bratislava, Czechoslovakia.

perimental animals were cyanotic, flabby. The testis and epididymis mass of "suspended" rats were essentially (more than twice) lower, than in controls. The total epididymal spermatozoa number in control rats was about 123±II million while the tail-suspended rats have shown practically entire devastation of the epididymis: light microscopy preparation scanning has revealed only a few spermatozoa in the optic field (Fig.1). The testis of the control animals contained about 420 million of trophic, interstitial and spermatogenic cells and about 460 million of immature spermatozoa, and those of "suspended" rats - 69 and 9 million respectively. The whole number of the Sertoli cells and Leydig cells was slightly changed, while the spermatogonia, spermatocytes and spermatides number was sharply reduced (Fig.2). There were a lot of cells, particularly spermatides. on different stages of degeneration: with vacuolized cytoplasma, with picnotik and resorbing nuclei. The Leydig cells of "suspended" rats were hypertrophic. Their cytoplasma and round-shaped nuclei were significantly increased.

The cause for the changes in "tail-suspended" rat reproductive organs seems to be the anatomical dislocation of the organs (the shift into the abdominal cavity), resulting in the depression of the spermatogenesis. The disturbance of testicular blood supply has brought a definite contribution to those changes.

This phenomenon has no common features with the situation, observed after space flights, when the changes either were absent (Cosmos-1667), or turned out to be very small (Cosmos-1887). The results obtained make us decide negatively the question about the possibility to study microgravity effects on male reproductive system in model experiments with rat "tail-suspension".

It must be noted, that such evident changes in the reproductive organs, obviously, cannot possibly but affect the whole organism first of all, the hormonal status. These results are to be taken into consideration when using the "tail-suspension" model for the investigation of the different organs and functional system.

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PLASMA STRESS HORMONES IN RESTING RATS. EIGHTY FOUR DAY STUDY.

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Introduction

Concentration of plasma "stress hormones" (ACTH, corticosterone, prolactin and others) in resting, undisturbed rats is rather low and fluctuates in narrow limits (15, 16). Exposure to an unfamiliar cage (1, 5), handling of a rat (19), and many other environmental factors increase concentration of plasma ACTH, corticosterone, and prolactin. It seems, however, that plasma concentration of stress hormones can be elevated even in resting rats. Chronic exposure to a stress (for instance repeated or continuous cold exposure) increases plasma corticosterone concentration in resting rats (from a resting level of 8-12 g/dL to a "stressed" level of 23 g/dL)(2), but leaves plasma ACTH concentration unchanged, at a level of 50 to 150 pg/ml. Plasma prolactin concentration (resting value of 10 to 15 mg/ml) was not measured in this work. Similarly an increased plasma corticosterone concentration is found in resting rats after exposure to chronic stresses of other types (3, 4, 6, 7-10, 17 18). There are reports that plasma corticosterone increase in resting rats exposed to chronic stress last 40 days only and then corticosterone concentration returns to normal (11). Furthermore, it seems that various chronic stressors exert an inhibitory influence on prolactin.

In this work repeated stress (once every week during a period of three months) of handling and placing rats in unfamiliar cages ("sampling boxes") for 30 minutes was used to verify if such a chronic stress would lead to an increased concentration of plasma corticosterone in resting rats. The three month duration for the experiment was chosen because it simulates duration of our own cardiovascular rat experiment planned for NASA Spacelab flights SLS 1 and SLS 2.

Materials and Methods

Animals. Fifty-six male Sprague-Dawley VAF/Plus rats (virus antibody free, Charles River Laboratories, Inc.) were used in this study. The animals, weighing 60 g, were delivered to our animal quarters, singly caged and given water and food (Purina Rat Chow) ad libitum. Temperature in the animal rooms was maintained at 24 ± 1° C and the photo period was 12/12 hours, with the lighted phase starting at 8 am.

Cannulation. All the rats in this work were carriers of chronic aortic and of right ventricular heart cannulas needed for Spacelab experiments. At a body weight of 210 ± 10 g, the aorta of all rats was cannulated through the left common carotid artery with a PE-10 cannula (12-14). The opposite end of the cannula was exteriorized at the back of the animal's neck. Cannulation of the right ventricle of the heart was done simultaneously through the right jugular vein. The proper position of the cannulas was verified with a HealthMate FluoroScan. Before surgery the cannulas were sterilized for 72 hours in undiluted Sporocidin. One hour before surgery and immediately after surgery the animal was given 0.1 cc of Di-Trim subcutaneously. The surgery (under ketamine-xylazine anesthesia) lasted 8-10 minutes. The cannulas were filled with slightly heparinized saline. The distal part of the cannulas was exteriorized and heat sealed.

Blood sampling. Handling and restraint increase plasma stress hormone concentration. The increase persists for 20 minutes after an initial stimulus (15, 16). Our experimental animals had to be handled and placed in "sampling boxes" in order to withdraw the blood. After removal from its home cage, each rat was placed in a nontransparent sampling box, with a slot in the cover. The animal was free to move in the chamber while the aortic cannula was protruding through the slot. To avoid sampling from the stressed animal, blood from the aortic cannula was sampled 30 minutes later, after the altered plasma stress hormone concentration returned to the basal, resting value. A 0.3 ml blood sample was withdrawn. The blood sample was placed in an ethylene-diamine-tetracetate (EDTA) treated capillary blood collection container, shaken, and placed on ice. The whole blood sampling procedure lasted two minutes. All blood sampling was done between 8 am and 1 pm in order to avoid effect of circadian variations (15, 16).

Radioimmunoassays. Radioimmunoassays were used to determine the plasma concentration of ACTH, corticosterone, and prolactin (15, 16).

Experimental group. Thirty-six rats in this group were used to withdraw blood from the aorta once every week during a period of three months.

Control group. Arterial blood from twenty resting rats was sampled only three times starting in the middle of the third month period.

Results

Plasma ACTH. Figure 1 shows the plasma ACTH concentration of the rats in the experimental group (handled once a week). Plasma ACTH concentration decreased during the first two weeks (adaptation to a new environment). During weeks 3 to 24 the plasma corticosterone concentration of the resting rats stayed at the same low level. Plasma ACTH concentration of the control (non-stressed) rats had similar values as found in the experimental rats.

Plasma corticosterone. Figure 1 shows the plasma corticosterone concentration in the handled (experimental) rats. The plasma corticosterone concentration of these rats, after decreasing the first two weeks, stayed at the same level during the following weeks of handling and blood sampling. There was no difference between the "stressed" rats and the rats in the control (not handled) group.

Plasma prolactin. Figure 1 shows values of plasma prolactin concentrations in resting (chronically stressed) rats and plasma prolactin concentration in rats that were not handled (control group). There was no difference between these two groups.

Discussion

Few studies describe effects of chronic stress on pituitary-adrenocortical axis. Burchfield et al. (2) exposed rats for three months to chronic (continuous or repeated cold exposure) stress. Chronically stressed rats had an

elevated resting plasma corticosterone concentration. The increase was as much as it is seen in control but stressed animals as high as during an acute cold exposure. The observed increase was from 8-12 g/dL to 23 g/dL. The longer duration of the stress, the higher plasma corticosterone concentration was observed. However, in the same rats the plasma ACTH concentrations remained unchanged. Similar increase of plasma corticosterone in resting rats was found after chronic stress by other investigators (2, 4, 6, 9-11, 18).

In our study miniscule samples (0.3 ml) of arterial blood were withdrawn from fifty resting rats. The blood was withdrawn through a chronic aortic cannula once every week during a period of three months. Each animal was handled (placement in a sampling box for thirty minutes) before and after (return to home cage) the blood was withdrawn. The concentration of plasma ACTH, plasma corticosterone and plasma prolactin in the resting rats stayed the same during the three month duration of the experiment. The level of the plasma stress hormones in not handled, resting rats (control group) was the same as in lightly stressed (experimental) animals.

We conclude, therefore, that repeated mild stress of handling of rats once a week during a period of three months (as well as repeated blood sampling) does not effect the plasma stress hormone concentration in resting rats. Thus periodic handling of rats and periodic blood sampling - part of SLS 1 and 2 cardiovascular rat experiments - are unlikely to increase the level of plasma stress hormones and thus have an effect on the level of cardiac output and other circulatory parameters in the rats.

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(Supported by NASA contract NAS2-10527)

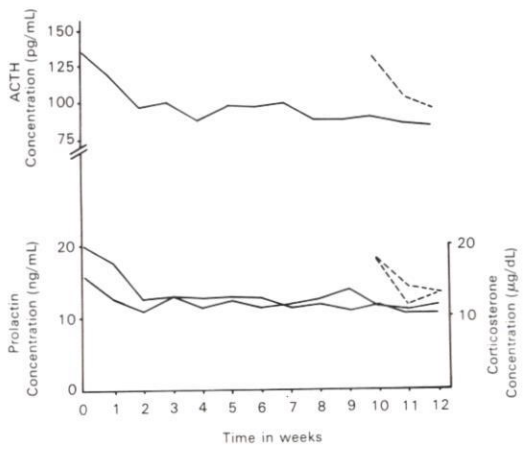

Fig. 1 Concentrations of ACTH, corticosterone and prolactin in plasma of resting rats.

EFFECTS OF TRANSITION FROM SUPINE TO UPRIGHT POSITIONS ON CENTRAL HEMODYNAMICS IN PATIENTS WITH CHEST PAIN SYNDROME.

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Although there have been numerous studies examining the effects of postural changes on the cardiovascular system of man (1,2), there is a paucity of information on central hemodynamics during transitions in posture (3,4). In part, the lack of information can be attributed to difficulties in maintaining hydrostatic reference levels during transition periods using the fluid-filled catheters commonly employed in clinical environments. In addition, fluid-filled catheters are subject to artifacts due to catheter motion, and they do not permit pressure recordings simultaneously from multiple sites with high-fidelity. The present study examined the effects of posture changes on right and left heart hemodynamic in man using multisensor micromanometry.

The study population consisted of nine male patients with chest pain syndrome (mean age = 44±11 years). Four patients in this group had nonsignificant coronary artery disease and normal hemodynamics at rest; five patients had angiographic evidence of significant coronary artery disease. Patients were brought to the catheterization laboratory in a fasting state either mildly or unsedated. Catheters were introduced by a right brachial approach. Either simultaneous right atrial (RA), right ventricular (RV) and pulmonary artery (PA) pressures and/or left ventricular (LV) and aortic pressures (Ao) were obtained using Millar #7F right heart and/or #8F left heart multisensor micromanometric catheters. All micromanometric pressures were initially referenced to pressures obtained with a fluid-filled catheter using an external strain gauge transducer positioned at the mid-chest level with patients supine.

Control supine hemodynamics were recorded from patients after a minimum of five minutes of stabilization in the resting state. Following the supine control period, patients assumed a sitting or standing position. After three to five minutes in the upright position patients resumed a supine position. Data were continuously recorded during postural changes.

Figure 1 demonstrates the effects of transition from supine to upright. High-fidelity right heart pressures, including right atrial, right ventricular and pulmonary artery pressures are shown in the upper section. High-fidelity left ventricular and aortic pressures and a fluid PA pressure are shown on the lower section. It is noted that during active transition significant alterations in left and right heart pressures occur. These alterations were attributed to the isometric and Valsalva strains made by patients during active transition. In addition to demonstrating the ability of catheter-mounted micromanometers to follow physiologic and hydrostatic pressure changes during transition from 0 to +1 Gz, Figure 1. illustrates the limitation of fluid-catheter systems when external strain gauge transducers are not moved to compensate for an altered hydrostatic reference.

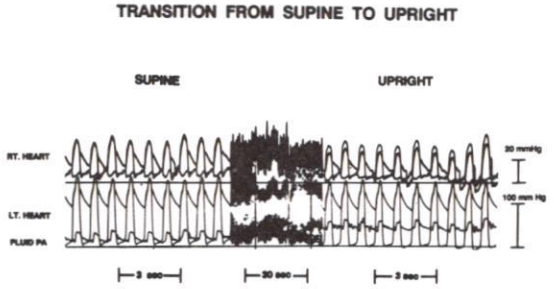

Figure 1

Since in the supine control period the micromanometric pressures were matched to pressures from a fluid catheter, small hydrostatic gradients between sensors were intentionally neglected during this period. Figure 2 shows that under this condition no gradient exists between Ao and LV pressures or PA and RV during late systole. Nor are gradients noted during late diastole between RA and RV sensors. It was assumed that during these periods the contribution of blood flow to pressure gradients between sensor sites is minimal. The appearance of gradients after changes in posture during these periods reflects hydrostatic gradients due to changes in the vertical distance between micromanometer sensors.

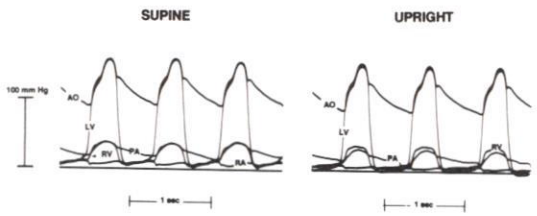

Figure 2.

The following Table presents data demonstrating the effects of posture change on heart rate (bpm), right and left heart pre-ejection and ejection times (msec), pressures (mm Hg), and dP/dt (mmHg/sec). The data were pooled from all patients and are presented as mean ± standard deviation (sd).

	CONTROL	UPRIGHT	SUPINE
HEART RATE	86(16)	95(13)	84(20)
RVPEP	69(12)	95(19)	66(9)
RVET	364(19)	251(84)	337(31)
PAs	33(8)	24(5)	35(3)
PAd	15(5)	10(4)	15(4)
RVs	33(7)	30(6)	36(5)
RVEDP	11(3)	6(5)	12(2)
RV dP/dt	292(83)	331(62)	367(98)
LVPEP	88(18)	106(20)	95(18)
LVET	310(21)	265(16)	317(21)
AOs	132(23)	125(22)	149(29)
AOd	81(12)	85(17)	72(12)
LVs	132(22)	132(22)	149(29)
LVEDP	16(7)	9(4)	20(4)
LV dP/dt	1273(259)	1518(362)	1265(213)

Where PEP = pre-ejection period, ET = ejection time, s = systolic, d = diastolic and dP/dt = first derivative of pressure.

From the above data, it is observed that transition to upright posture increases heart rate (10%), RVPEP (38%), LVPEP (20%) and RV dP/dt (13%) and LV dP/dt (19%); and decreases RVET (31%), LVET (15%), PA systolic and diastolic pressures (27% and 33%, respectively) and right and left ventricular end-diastolic pressures (45% and 43%, respectively). When supine posture was resumed, hemodynamic data returned to baseline. Immediately following transient hemodynamic changes, patients with heart disease had left ventricular end-diastolic pressures which were increased over supine control.

Also subtle changes were noted in the high-fidelity pressure wave forms which cannot be appreciated from the Table. For example, the left ventricular "a-wave" tended to disappear, while the right ventricular "a-wave", although diminished, was preserved in the patients studied. When pulmonary artery wave reflections were present in supine subjects, reflected waves were diminished while standing. Arterial wave reflections in the ascending aorta were less altered when systemic arterial pressure was maintained.

In summary, this study suggests that multisensor micromanometric catheters are useful in the assessing the central hemodynamic response during postural changes in man. The data presented demonstrate that "active" transition produces complex changes in left and right heart pressures which may be attributed not only to hydrostatic changes, but also to the transient effects of isometric and Valsalva strains. Finally, this study reports significant differences in the morphology of pressure wave forms in man upright compared to supine.

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EFFECTS OF SIMULATED WEIGHTLESSNESS AND SYMPATHECTOMY ON MAXIMUM $\dot{V}O_2$ OF MALE RATS

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INTRODUCTION

Maximum oxygen consumption ($\dot{V}O_2$ max) is considered to be one of the best indices of functional capacity; however, the effect of actual weightlessness on this parameter has not been reported at the present time. Studies utilizing models of simulated weightlessness have consistently reported reductions in $\dot{V}O_2$ max of 10-20% in humans and animals after exposures of 1-35 days (2,3,4).

The purpose of this investigation was to determine whether the sympathetic nervous system was associated with a reduction in $\dot{V}O_2$ max. Specifically, whether chemical sympathectomy and exposure to simulated weightlessness (Overton-Tipton hindlimb suspension model) would significantly alter $\dot{V}O_2$ max, treadmill run time, and mechanical efficiency during sub-maximal exercise. We hypothesized that chemically sympathectomized rats would have significantly lower values of $\dot{V}O_2$ max, run time, and mechanical efficiency than the saline injected controls exposed to the same experimental conditions.

METHODS

Mature female Sprague-Dawley rats were either chemically sympathectomized with injections of guanethidine sulfate (50 mg/kg) starting two days after birth (Symx), or, injected with equal volumes of saline (Sham). Prior to suspension, all animals were tested for $\dot{V}O_2$ max and randomly assigned to one of three groups for 14 days; namely, head down suspension at a 45 degree angle with the hindlimbs non-weight bearing (HDS), horizontal suspension with the hindlimbs weight bearing (HWB) or caged control (CC).

$\dot{V}O_2$ max was assessed by having rats run on a treadmill enclosed in an airtight plexiglass chamber as described by Bedford, et al., 1979 (1). Oxygen and CO_2 content was determined by using analyzers from Beckman and Applied Electrochemistry respectively. Data were collected prior to, and 7 and 14 days after the experiment began. At the time of sacrifice, arterial blood (descending aorta) was secured from anesthetized rats for the determination of plasma catecholamine concentrations using the radioenzymatic method of Passon and Peuler (5) as modified for commercial purposes (CAT-A-Kit, Amersham, Arlington Heights, Ill). In addition to O_2 max measurements, results were obtained for treadmill run time and mechanical efficiency at a given sub-maximal exercise condition.

RESULTS

The effectiveness of the chemical sympathectomy procedure is demonstrated in Table 1.

Table 1

The influences of chemical sympathectomy and suspension on plasma concentrations (pg/ml)

Group	N	Norepinephrine	Epinephrine
CC			
Symx	5	311± 38*	772±254
Sham	6-7	1098±128	566±191
HWB			
Symx	6-7	374± 99*	890±166
Sham	7	1011±180	602±181
HDS			
Symx	7	483± 73*	1198±367
Sham	7-8	821± 78	650±215

Values are means and SE, * denotes an inter-group difference that was statistically significant at 0.05 probability level.

Summarized in Table 2 are the findings pertaining to the physiologic responses from the various experimental groups.

TABLE 2

The influence of suspension on select variables

BODY MASS (grams)

Group	N	Before	Day 7	Day14
CC				
Symx	6	240± 8	254±10*	261±9*
Sham	8	275±16	285±15	296±16
HWB				
Symx	6	264± 4	247±10*	239±5*
Sham	8	255± 5	228± 8*	235±6*
HDS				
Symx	7	264± 8	245±11*	248±10*
Sham	7	249± 6	229± 6*	235± 7*

MAXIMUM OXYGEN CONSUMPTION (ml/min)

CC				
Symx	6	21.1±1.3	22.3±1.4	22.6±2.1
Sham	8	22.9±1.2	24.1±1.4	22.8±1.2
HWB				
Symx	7	23.0±1.1	22.0±0.8	22.5±1.2
Sham	8	21.9±0.9	20.0±0.7*	21.3±1.0
HDS				
Symx	7	21.3±0.7	21.7±0.7	21.8±1.3
Sham	7	23.4±0.5	20.7±1.0*	20.6±1.0*

MAXIMUM OXYGEN CONSUMPTION (ml/min/kg)

Group	N	Before	Day 7	Day 14
CC				
Symx	6	87.6±4.2	88.9±2.9	87.7±3.1
Sham	8	83.3±2.0	85.2±2.3	77.7±2.1*
HWB				
Symx	7	89.9±3.0	97.8±1.8*	96.1±3.9
Sham	8	82.6±3.1	81.9±2.5	89.8±3.3
HDS				
Symx	7	85.4±1.9	95.2±3.7*	93.7±4.5*
Sham	7	88.6±1.4	84.1±2.3	88.3±2.2

TREADMILL RUN TIME (min)

CC				
Symx	6	11.8±0.5	12.3±0.6	11.8±0.6
Sham	8	12.2±0.5	12.5±0.2	12.0±0.4
HWB				
Symx	7	12.6±0.5	12.3±0.5	12.4±0.5
Sham	8	13.0±0.5	12.7±0.5	11.8±0.3
HDS				
Symx	7	12.3±0.2	11.1±0.5*	10.6±0.4*
Sham	7	12.4±0.3	10.8±0.4*	10.8±0.3*

MECHANICAL EFFICIENCY (%)

CC				
Symx	6	26.0±1.9	24.3±5.2	23.0±4.5
Sham	8	22.8±2.8	22.8±2.6	25.7±2.0
HWB				
Symx	7	23.3±2.3	19.7±2.2	24.3±2.5
Sham	8	26.3±1.8	21.6±2.1	24.8±5.0
HDS				
Symx	7	22.4±1.5	16.8±0.7*	15.1±1.7*
Sham	7	27.7±4.5	25.6±5.6	26.0±4.5

Values are means and SE. * denotes an ANOVA determined difference from the pre-suspension value that was statistically significant at the 0.05 level.

It was evident that the chemical sympathectomy was effective in reducing the influence of the postganglonic fibers. Suspension was associated with significant decreases in body mass for the HWB and HDS groups while the CC animals exhibited an increase. Saline injected HWB and HDS demonstrated significant decreases in absolute $\dot{V}O_2$ max and treadmill run time, whereas the Symx had significant reductions in treadmill run times and mechanical efficiency. However, when $\dot{V}O_2$ was evaluated by body mass, Symx was associated with increases rather than decreases.

DISCUSSION

The major finding of this study was that suspended Symx rats did not demonstrate the decrease in $\dot{V}O_2$ max observed in the saline injected controls or in suspended rats from other investigations (2,3,4). The significance of this finding is unclear because HDS Symx rats did exhibit shorter run times and lower mechanical efficiencies than their pre-suspension values or their saline injected controls. Besides the functioning of an intact adrenal medulla, we are exploring aspects pertaining to lean body mass, cardiac output, blood flow (distribution) and muscle oxidative capacity to explain these observations.

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(Supported by NASA Grant, NAG-2-392.)

Calcium homeostasis in prolonged hypokinesia.

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Calcium metabolism, its hormonal regulation and renal calciuretic function were investigated in 20 healthy volunteers during 120- and 182-day head-down tilt studies. The content of total and ionized calcium and parathyroik hormone was found to increase during the study. The concentration of calcitonin increased at an early stage of exposure and decreased thereafter. This resulted in negative calcium balance which was on the average 5,6g per month. Measurements of iliac bone bioptates showed calcium decrease and phosphorus increase.

During long-term exposure to microgravity, changes in mineral metabolism, particularly calcium metabolism, may become one of the basic factors limiting space flight duration (1,2). It is important to investigate the mechanisms underlying these changes in order to predict possible undersirable effects and to develop pathogenetic countermeasures against calcium metabolism shifts.

Calcium metabolism was investigated during and after manned space flights (3, 4) as well as in simulation studies (5,6). One of the methods that can best simulate the physiological effects of microgravity on the ground is antiorthostatic hypokinesia or head-down tilt (7). This simulation causes metabolic shifts and bone changes that are similar in sign and magnitude to those observed in real space flights.

We examined 20 healthy volunteers, aged 25 to 44 years, who remained in the head-down position (-4°, -5°) for 120 to 182 days with no countermeasures used. During the 182-day bed rest study we measured total and ionized calcium, and parathyroid hormone in blood and calcium in response to lactate calcium loading (8). In the 120-day bed rest studies, which concentrated on metabolism, these measurements were supplemented with balance studies and, in the last experimental series, with iliac crest biopsy and bone examinations.

One of the basic parameters of homeostasis is invariability of the ionic composition of blood. Measurements of total calsium and its ionized fraction in serum during prologed bed rest give evidence that this exposure led to persistent, although moderate, hypercalciemia (Fig.1,2). In our studies, changes in the total calcium concentration occurred at late stages

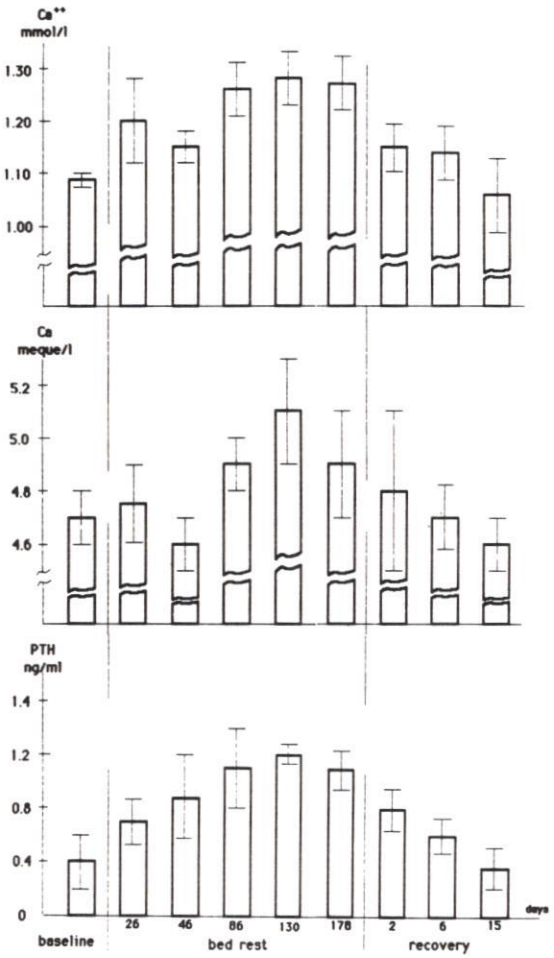


Figure 1 CONCENTRACION OF TOTAL CALCIUM, IONIZED CALCIUM, PTH AND CALCITONIN IN BLOOD DURING 182 BED REST STUDIES (N=6)

and remained within physiological limits; however ionized calcium was on the averrage 10% higher than the upper limit thorughout the entire bed rest. As a rule, higher calcium concentrations were seen after long-duration space flights and bed rest studies (3); during Skylab flights calcium content was also increased (9).

Figure 1 illustrates the content of PTH during the 182-day bed rest and figure 2 shows PTH and calcitonin concentrations during the 120-day bed rest study. These data demonstrate that during bed rest the PTH content was higher than before. The calcitonin concentration increased during the first month of study, then decreased gradually and by the end of study became lower than the baseline level.

Prolonged bed rest caused negative

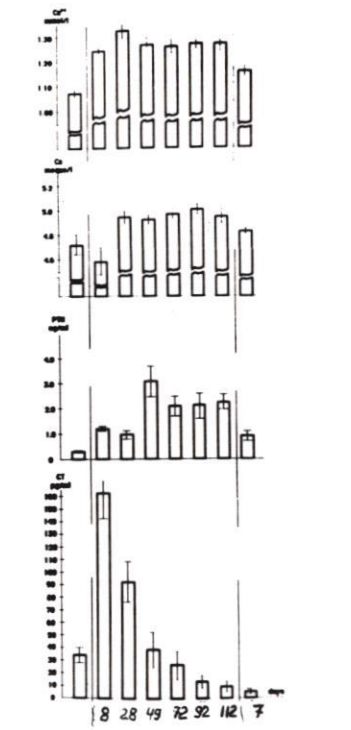


Figure 2 CONCENTRACION OF TOTAL CALCIUM, IONIZED CALCIUM, PTH AND CALCITONIN IN BLOOD DURING 120 BED REST STUDIES (N=9)

water and electrolyte balance. Body dehydration and enhanced excretion of sodium and osmotically active substances asscoaited with hemodynamic changes developed during an acute stage of adaptation to head-down tilt, whereas negative balance of mineral substances pooled in bones and muscles developed continuously and manifested throughout the entire bed rest study.

Figure 3 illustrates the development of negative calcium balance. The time course of this parameter may vary from one test subject to another but the values averaged per group increase steadily with exposure time. Our observations indicate that calcium losses during prolonged hypokinesia are persistent and progressive amounting to 5.6 g calcium per month on the average.

This finding is comparable with the results of balance measurements carried out by US investigators during long-term Skylab flights and simulation studies (6, 9). It is important to note here a large scatter of calcium loss data: as a result of 120-day bed rest, calcium losses varied from9.64 to 35.04 g in different test subjects. This observation is of practical significance - it should be taken into consideration when predicting the adverse effects of calcium losses and when developing individual protocols of countermeasures.

Our results indicate that negative calcium balance may be produced by both increased renal excretion of calcium ant its reduced intestinal absorption. During bed rest calcium excretion in urine increased by 5.35% and in feces - by 10.1% compared to the data obtained during the ambulatory period. Individual data suggest that negative calcium balance may evolve through one of the two mechanisms being predominant - this was the case with US Gemini-7 astronauts (10).

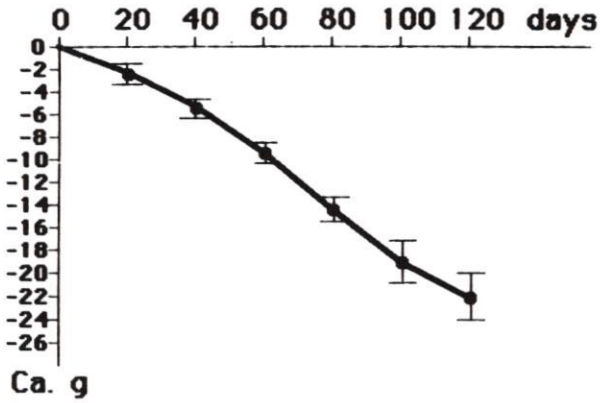


Figure 3 CALCIUM BALANCE IN 120-DAY BED REST STUDY (N=14)

Calcium loading tests conducted during 182-day bed rest showed that oral calcium lactate intake caused a greater than in the norm increase of calcium renal excretion, clearance and excreted calcium fraction (Fig.4).

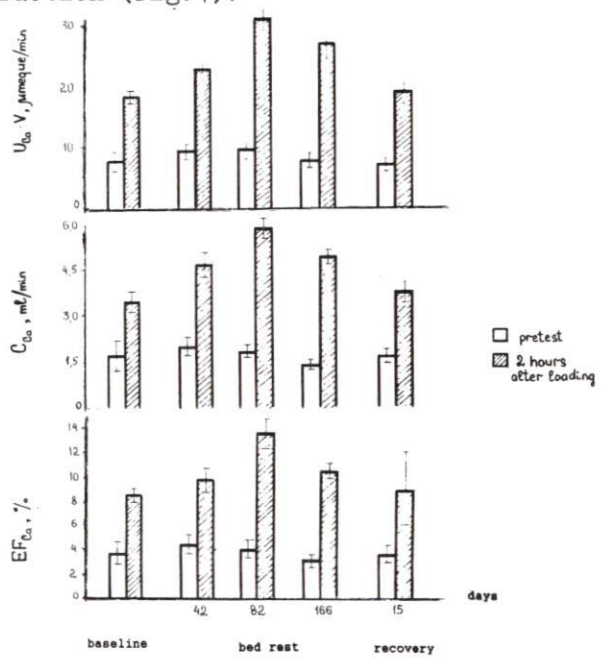
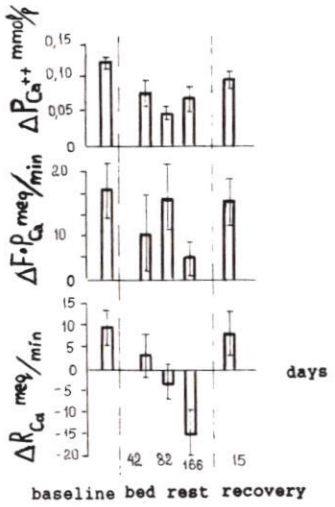


FIG.4 URINARY CALCIUM EXCRETION, CLEARENCE AND EXCRETED CALCIUM FRACTION DURING LACTATE CALCIUM LOADING TEST (N = 6)

During bed rest the rate of magnesium excretion in response to calcium loading was also higher than the baseline level; however the increase of the rate of calcium excretion was much greater. In response to calcium loading before bed rest, the ratio of urinary concentrations of Ca and Mg (U_{Ca}/U_{Mg}) grew by 43.2±4.8% as compared to the pretest level. On bed rest days 82 and 166 the ratio U_{Ca}/U_{Mg} after calcium loading increased by 95.0 ± 8.2 and 8 3.0 ±

4.9%, respectively. During hypokinesia the test specificity for calcium increased; this gives evidence that transport regulation in the nephron varies selectively during diminished motor activity.

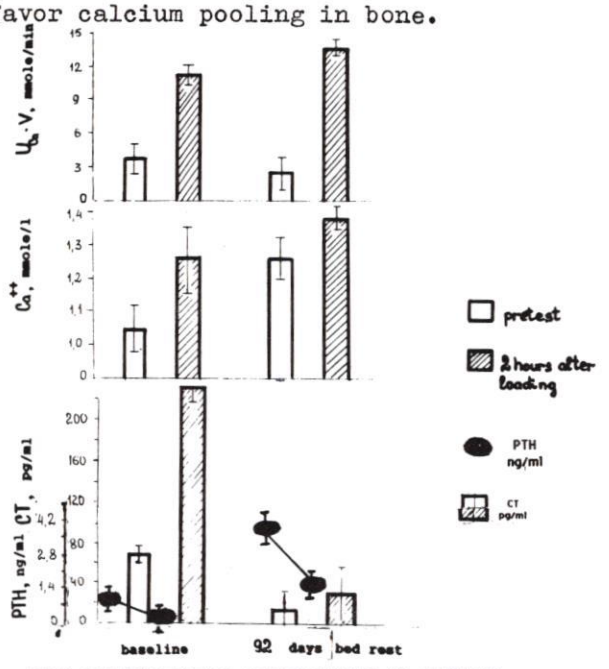
Ionized calcium and filtred calcium load during calcium loading tests increased in bed rest to a smaller extent than in normal activity; the reabsorption rate did not grow but decreased in contrast to the changes in the baseline period (Fig.5). It can therefore be concluded that enhancement of calciuresis in response to calcium lactate loading during bed rest was induced by a more significant decrease of its tubular reabsorption.



FIC.5 VARIATIONS IN FILTERED LOAD CALCIUM AND CALCIUM RENAL REABSORPTION DURING LACTATE CALCIUM LOADING TEST (N = 6)

Results of the investigation of the calciuretic function in response to the calcium loading test showed that the basic parameters characterizing calcium transport significantly correlated with an enhanced rate of renal excretion of calcium in response to the calcium loading test ($U_{Ca} \cdot V$) and total calcium excretion during the test. The glomerular filtration rate did not change substantially, water diuresis remaining normal. This allows measurement of the filtration charge with respect to an increase of ionized calcium. During the 120-day bed rest studies we investigated the renal calciuretic function and concentrated on the above intergrated parameters. As a result of the calcium loading test we obtained data characterizing hormonal regulation of calcium.

During normal motor activity increased levels of total and specifically ionized calcium in blood and in urine led to a decrease of PTH in blood and an increase of calcitonin by 2.8 and 3.8 times, respectively (Fig.6). Changes in the concentration of calcitrophic hormones in response to calcium lactate formed a consistent reaction to the hypercalciemic test. This reaction was used to normalize calcium in blood by reducing its intestinal absorption and inhibiting its reabsorption in renal tubules. Calcitonin may also favor calcium pooling in bone.



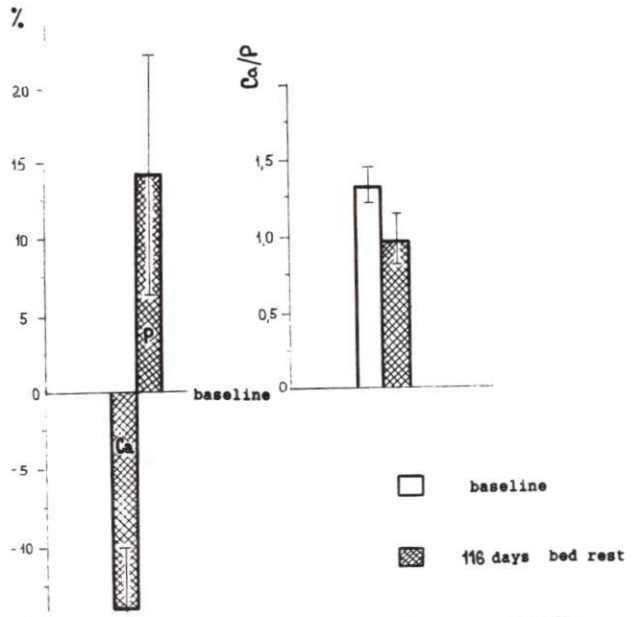
FIC.6 EFFECT OF LACTATE CALCIUM LOADING ON TOTAL AND IONIZED CALCIUM, PTH AND CALCITONIN IN BLOOD DURING 120- DAY BED REST STUDY (N = 6)

During hypokinesia the calcium loading test was conducted when the hormonal milieu was modified. The initial PTH concentration was significantly higher than before the test and the calcitonin concentration was markedly reduced. After calcium lactate uptake the trend of changes in calcitrophic hormones (Fig. 6) remained unchanges, PTH content decreased and calcitonin content increased, although to a lower degree than before the test (by 2 to 2.5 times). However, at the peak of the loading test the PTH level was significantly higher and the calcitonin level was significantly lower that before the test. In spite of this, the increment of the excretion rate and excretion of calcium during the test exceeded the baseline data. The adequate renal response to prevent an exceptionally high level of calcium in blood against a modified hormonal background is associated with a change in the cell sensitivity of renal tubules to PTH and calcitonin.

Of great interest are the data of bone studies performed on iliac crest bioptates. On bed rest day 116 spongy bone samples showed a 15% decrease of calcium and a similar increase of phosphorus (Fig. 7). The Ca/P ratio, which shows the relationship between the crystalline and amorphous moieties of the mineral component and influences bone strength, declined significantly (11). Morphological examinations on three bone samples suggest a slight enhancement of bone resorption (12).

Calcium metabolism and its regulation during prolonged head-down tilt change as a result of adaptive reactions aimed at maintaining blood calcium within physiological limits.

Functional unloading of the musculoskeletal system produces metabolic changes in bones, including changes of calcium metabolism regulators at the tissue and cellular levels which cause calcium transport via bone membrane to be suppressed. The next step may be stimulation of PTH secre-



FIC.7 VARIATIONS OF CALCIUM AND PHOSPHORUS CONCENTRATIONS AND Ca/P IN BONE BIOPTATES DURING 120-DAY BED REST STUDY (N = 5)

tion, leading to a higher content of ionized calcium and then calcitonin in blood.

In the situation when PTH and calcitonin increase and moreover when calcitonin decreases, PTH produces a predominant effect of bone: it stimulates calcium transport from the perilacunar space to extracellular fluid and bone resorption by osteoclasts.

The systemic and local regulatory shifts result in a reduction of the capacity of the labile fraction of calcium and an enhancement of its mobilization from the stable fraction of bone.

Diminished intestinal absorption of calcium and increased renal excretion, the purpose of which is to decrease calcium in blood, fail to make up for calcium loss from bone. Therefore ionized calcium in blood remains slightly increased, stimulating the systems responsible for calcium homeostasis, and causes progressive calcium loss.

This state may persist for several months under conditions of strong immobilization. Clinical observations suggest (13,14) that this may bring about manifest osteoporosis, impairment of metabolic processes in bone to such levels when calcium loss diminishes continuously and, in the long run, calcium reduction in blood.

Space medicine should focus on the mechanisms that determine the level of calcium metabolism changes and the rate of bone demineralization in the case of prolonged hypokinesia.

Space medicine should identify the effects of calcium metabolism changes, persisting for a long period of time, on the function of various organs and systems and specify the degree of the changes and the time when they may become critical. Space medicine should concentrate on the development of pathogenetic countermeasures and methods of modifying calcium metabolism during long-term exposure to hypokinesia and microgravity.

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MICROGRAVITY-INDUCED CHANGES IN HUMAN BONE STRENGTH

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A decrease of spongy tissue strength of iliac bone has been identified in healthy men under conditions of simulated weightlessness (bed rest). Countermeasures have significantly prevented this decrease. Additional investigations have indicated probability of degenerative-destructive bone change development under conditions of usual human activity as a consequence of bone strength decrease.

The effect of microgravity on bones has been thoroughly investigated from the theoretical point of view. Emphasis has been made on osteodystrophy, its parameters and mechanisms whereas applied aspects of the problem still remain inadequately understood. Among them attention has been focussed on bone strength variations in terms of crew safety in the landing vehicle. However, there are other issues that have great importance for practical purposes. These include, first of all, probability of destructive changes in bones during postflight normal activities and, second, the effectiveness of countermeasures applied to prevent bone strength variations.

In order to resolve the first problem, the following approaches were used:

- identification of the skeletal compartment where destructive processes are most probable;
- determination of the possible mechanism and characteristics of bone destruction in man in a normal environment;
- interpretation of the data obtained in relation to bone strength decline in microgravity.

The spinal column is most likely to show postflight bone changes. This assumption is supported, first, by a high incidence of its degenerative=destructive changes in a normal environment (5) and, second, by its weakness in biomechanical terms (3).

Our pertinent investigations included an experimental study of on-line degradation of spinal bones, examination of highly experienced parachutists, and an investigation of a loaded bone of rats flown on Cosmos=1129.

Bone destruction was investigated using the method of acoustic emission during mechanical loading of T_{II}-L_3 vertebrae segments. The method is based on the concept that materials disintegrate as a result of initiation and build-up of their structural lesions. The size and number of defects depend on structural heterogeneity. Formation of the defects causes emission of stress waves (acoustic effect) which can be recorded by a sensitive receiver of elastic vibrations. Spinal segments were loaded and biomechanical parameters were recorded using conventional equipment. The rate of segment deformation was consistent with the shock loading level. It was found that initial microfractures in the vertebral body developed in 10% cases at 2.4g and in 100% cases at 8g.

X-rays taken at an incremental step of 50 kgf showed that the central compartments of the vertebral body (in the nucleus pulposus projection) underwent the greatest longitudinal compression when compared to the anterior and posterior compartments. At a load of 8g the difference of deformation of the central and peripheral compartments was 2 or 3-fold. It can therefore be concluded that microfractures recorded by acoustic emission originate in the central compartments of vertebrae. It is not accidental that in response to significant loading macroscopic fractures evolve in the central portion of end plates of vertebrae.

Our observations raised the question whether degenerative=destructive changes in the spinal column may develop as a result of repeated exposure to medium-level mechanical loads. To answer this question, we performed X-ray examinations of 30 parachutists who made jumps for 3 to 34 years. In contrast to the matched controls, they showed a 6-fold incidence rate of Schmorl's nodule and a 4-fold incidence rate of spondylosis deformans. Schmorl's nodule develops as a result of destruction of the central portion of the end plate and prolapse of the nucleus pulposus into the spongiosa of a vertebrae. Our findings suggest that Schmorl's nodule is a condition resulting from a continuous build-up of microfractures of bone structures in the nucleus pulposus projection during impact loads occuring during parachute landing and from accumulated bone destruction. Spondylitis deformans can be viewed as a consequence of reparation responses to microfractures.

When applied to bone changes associated with space flight, the above data need to be taken into consideration with the following reservations. First of all, exposure to microgravity may reduce significantly the strength of vertebrae (4). Second, during readaptation bone properties vary in such a manner that microfractures originate at stresses far lower than in the norm. This is indicated by acoustic emission data obtained during mechanical loading of femoral heads of rats flown for 19 days on Cosmos-1129 (Table 1).

The tabulated data show that immediately after flight microfractures developed in flight and control rats at an identical rate. During the postflight reco-

Table 1.

Parameters of stress in rat femoral heads corresponding to the acoustic emission effect

Group	Stress, kgf/mm^2		
	Postflight	During recovery	
		6 days	29 days
Control	4.47±0.58	5.25±0.45	8.75±1.04
Flight	4.36±0.47	3.92±0.32	3.85±0.19*

*Differs significantly from the controls

very period the rat groups showed differences in the stress magnitudes inducing acoustic emission. As the recovery period continued, the differences grew. This observation has not yet found an adequate explanation and causes serious concern : if microfractures can build up in the spinal column exposed to low loads and if its strength declines then it may produce degenerative=destructive changes when postflight crewmembers lead a normal life.

Such changes can be prevented if the second problem-bone strength maintenance using adequate countermeasures - is resolved. As of today, this problem remains unresolved even from the theoretical point of view.

In order to clarify the above issues, we examined mechanical properties of iliac bone samples taken from 9 essentially healthy male test subjects, ages 27 to 42 years, who took part in a bed rest study for 370 days.

The test subjects were subdivided into two groups. Group 1 included 5 volunteers who remained in the head-down position (-5°) with no countermeasures used for as long as 120 days. During days 121 to 370 of bed rest they performed prophylactics. Group 2 consisted of 4 volunteers who used these preventive measures beginning with the first day of bed rest.

Bioptates were bone cylinders 6 mm in diameter taken across the iliac bone. In Group 1 test subjects, biopsy was made before bed rest (Subgroup 1), on day 120 (Subgroup 2) and on day 370 (Subgroup 3). In Group 2 test subjects, the procedure was performed before (Subgroup 1) and after (Subgroup 2) bed rest.

Bone samples were exposed to 23 tests in an Instron tensile testing machine equipped with a special designed device. The double section method was used and the stress profile and acoustic emission data were recorded. Mechanical properties are described in Table 2.

Table 2.

Mechanical properties of biopsy samples

Group	Subgroup	Deforming load kgf	Deformation of failure, mm	Rigidity, kgf/mm	Energy of failure, kgf/mm
	1 (pretest)	45.3±10.3	2.1±0.2	23.5±7.3	41.0±5.3
	2 (120 days)	18.4±1.84*	2.2±0.3	9.2±1.4*	24.2±3.4*
	3 (370 days)	29.3±3.9	1.6±0.2	19.2±3.7	29.8±6.6
	1 (pretest)	31.7±13.5	1.7±0.3	25.9±15.4	21.6±1.7
	2 (370 days)	29.9±13.9	1.3±0.2	29.0±16.8	19.1±6.3

As a result of 120-day bed rest when no countermeasures were used, the breaking load decreased by 59.4% when compared to the pretest level. In spite of this significant decrease of strength the bone samples fractured with similar deformation parameters which is in agreement with previous findings on human vertebrae (2). In other words, deformation is a critical parameter of failure for the cases under consideration. Rigidity and energy of deformation diminished by 61.0% and 41.0%, respectively.

The use of countermeasures after 120 days of bed rest led to a partial recovery of bone mechanical strength.

In this situation the level of deformation and energy was lower which may give evidence for a greater bone brittleness. It can therefore be concluded that exercise may in part make up for the effects of 120-day bed rest and simultaneously increase bone brittleness.

Group 2 test subjects who used preventive measures throughout the bed rest study showed the following changes: bone strength diminished only by 6.0%, rigidity increased by 12% and deformation and energy of failure decreased by 24.0% and 12.0%, respectively.

The fact that, as a result of countermeasures used in this group bone strength approximated the pretest level, rigidity increased and deformation and energy decreased gives evidence that the

samples under examination became more brittle. It is known from reported data that this phenomenon develops when the bone organic matrix exceeds a certain level of mineralisation (I), i.e. when bone is aging . It can therefore be concluded that countermeasures may inhibit both processes involved in bone metabolism : synthesis of new (poorly mineralized) bone matter and resorption of mature (highly mineralized) structures.

On the whole the above data indicate that adequate countermeasures may help maintain spongy bone strength in simulated microgravity.

This conclusion is very important for manned space programs. However, other changes of mechanical properties e.g. bone rigidity and energy of deformation, should be taken into consideration because they may influence bone resistance to impact stresses.

Resistance to external loads may also depend on the pattern of microfractures. This was investigated with the aid of acoustic emission. Unlike the responses of rat femoral heads, acoustic signals recorded during bioptate loading appeared from the onset of loading which seems to be typical of spongy bone fragment responses.

This effect may also be associated with the pattern of sample loading by the section method. Due to this, microfractures were characterized with respect to the acoustic signals from the areas of elastic and total deformation of samples (Fig. 1).

Figure I shows that accumulation of microfractures in biopsies taken before and 120 days after the bed rest study is very similar. After application of the countermeasures the rate of microfractures of samples under load increased in both test groups. The samples disintegrated completely when the number of microfractures was close in all groups. On the one hand these observations confirmed the concept that countermeasures may enhance spongy bone brittleness. On the other hand, they supported the hypothesis that a certain critical amount of microdefects in samples determined the time of their total failure regardless of structural changes in bone that are related to the effect of hypodynamics or countermeasures.

In summary, our results extend our knowledge about bone changes in real and simulated microgravity. This additional information lends support to the idea that 1) the strength of spongy bone of the axial skeleton of a normal man may

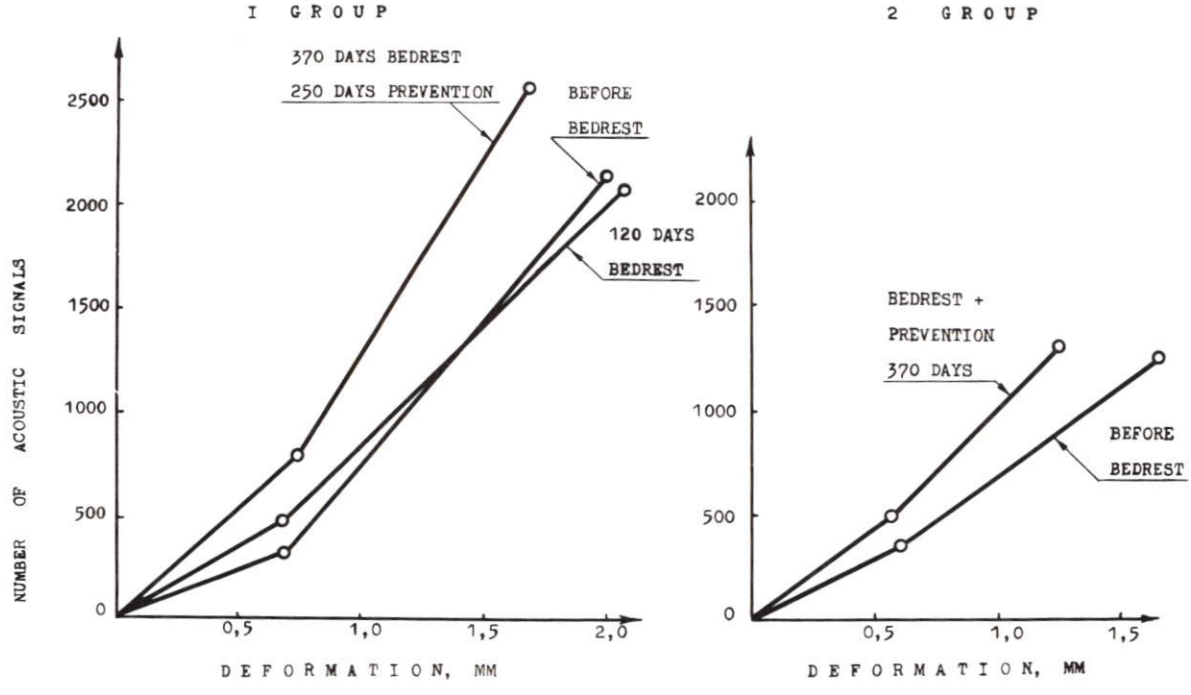

Figure I. Acoustic emission variations during bone sample testing

decline, 2) countermeasures may in principle prevent this decline, and 3) if no countermeasures are used the strength of spongy structures of the axial skeleton decreases, the risk of their postflight degenerative-destructive changes, particularly in the spinal column increases.

Although the countermeasures used in the study had a prophylactic effect on bone strength, they cannot be regarded as optimal because bone brittleness grew. Our study cannot clarify the differential effect of individual countermeasures on bone restructuring and structural changes of different type.

In order to answer the above questions, it is necessary to continue theoretical investigations and animal experimentation studies. This may help better understand bone dynamics and develop adequate countermeasures. The results obtained allow us to be optimistic when speaking about the possibility of preventing osteodystrophy in long-duration space missions, including those to Mars.

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STUDIES OF SPACE ADAPTATION SYNDROME IN EXPERIMENTS ON PRIMATES PERFORMED ON BOARD OF SOVIET BIOSATELLITE "COSMOS-1887"

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Studies of adaptation processes score an important place among those dedicated to the exploration of effects of microgravity on living organisms. It is obvious that alterations of activities of sensory systems adapted to gravitational field play a triggering role in the development of many if not all the disturbances characteristic for space adaptation syndrome (SAS). Vestibular disfunction is considered by many researchers as the most important factor. There are many hypothesis that link SAS to vestibular disfunctions, which can be provoked by microgravity, like changes of the paired function of two vestibular apparatus (1), the canal-otolith disbalance (2), alterations of the translatory functions of the otolithic system (3) etc. However, no one of these suggestions based on theoretical assumptions is proved because of lack of experimental data on vestibular state and function in microgravity. Therefore when the program of experiments on primates was planned a priority of about 60-70% of total experimental time was given to vestibular researches. Effects of microgravity on motor control, cardio-vascular system, higher nervous activity and some other functions were under study as well.

The same set of equipment has been used on board of "Cosmos-1887" as in the previous flights. It included the capsule for animal life maintenance that was supplied by special chair, animal fixation system, two feeders - for food and juice supply, a littering system and some devices, namely, the display for presenting of visual stimuli and two actographes - for upper and lower extremities. The scientific set included in its turn all the necessary sensors, transducers, amplifiers and other equipment which ensure recording and storage of all scientific information that was required.

Studies of vestibular functions in this flight were performed using the same tests as in previous flights, namely, eye-head coordination or gaze fixation reaction (GFR) - to reveal and evaluate alterations of canal system functions and lift reaction - to test the otolithic functions. As it was discussed earlier (4,5), GFR appears to be an adequate and informative method for studying the vestibular functions in microgravity since some of its characteristics, namely, amplitudes and velocities of the saccades, values of K_{VOR}, amplitudes and velocities of head movements are determined by and correspondingly reflect the excitability of the canal part of vestibular apparatus.

In the motor control studies the animals performed a complex test consisting of two parts. In the first one they performed phasic movements of pressing the lever of the actograph against a spring, in the second - they pressed the lever tonically sustaining it in a given position. Both - movements of the lever and EMG of the agonist-m. triceps surae and the antagonist-m. tibialis anterior were recorded.

The operational control for animal state was provided using the heart rate, the rate of breathing, and body temperature. Food and water consumption, speed and quality of experimental tests performance were also taken in consideration. Every once in a while the animals were shown on the TV screen.

The duration of flight this time came to 12 days and 15 hours.

During active phase of flight both animals were active; the signs of liquor redistribution as well as motion sickness were extremely mild, during test intervals both of them performed the task.

The heart rate changes in both animals were of the same direction with the increase during first two hours of flight up to 200 beats per minute in Yerosha data and to 136 - in Drema ones and subsequent development of bradycardia. The body temperature during flight was characterized by rhythmical changes with maximal values during the day time and minimal ones during nights.

During the flight Drema ate more than 3.3 kG of food and drank more than 850 ml of juice. As a whole his water and food consumption reached 3000 ml and 4600 kKal accordingly.

Yerosha data in this respect were quite different. On the second day of flight there was a breakage in the system of his food supply, so that from the third day this monkey did not get food any more. Being extremely active the animal set free one of his arm and starting from the 3-4th day of flight he couldn't perform the GFR task. However, his water consumption was relatively high reaching to the end of flight more than 2618 ml.

Eye-head coordination (GFR)

As we mentioned above the GFR is an appropriate test for evaluation of state and function of semicircular canal system. The number of characteristics of the reaction-like K_{VOR}, the maximal speed of saccadic movements, the velocity of head movements that causes saccade inhibition and others correlate directly with dynamic excitability of the vestibular input, the others - like amplitude and speed of head movements, accuracy of movements-reflect correctly the dynamic of adaptive processes in eye motor control systems under conditions of vestibulo-oculomotor disbalance. Studies of neuronal responses of different parts of the vestibular system, namely - fibers of the vestibular nerve, neurones of the vestibular nuclei, and the floccular zone of the cerebellar cortex widened significantly the basis of the experimental analysis.

As in the previous flights the transition from the gravitational field to microgravity didn't destroy eye-head coordination: on the very first day of flight both animals performed the task th ough a bit slower than on the Earth. The characteristics of the reactions - their precision, amplitudes and rate, however, changed drastically. The main feature of GFR performance under conditions of microgravity was a hypermetria: the movement amplitudes fluctuating around 40 degrees before the flight, in-flight increased steadily reaching the maximal value on 6th day of flight (Fig. 1).

As it is seen (Fig. 2) the hypermetria of gaze was linked closely to the hypermetria of the saccudic movements: the amplitude of saccades in both monkeys increased in microgravity reaching the maximum also on the 6th day of flight. Later the amplitude of saccades and gaze revealed the tendency to normalization but even on the 10th day both values were still well above preflight values.

Along with the increase of the amplitude there was also increase of saccade velocity. It is well known that amplitudes and durations of saccadic movements correlate. In preflight data the coefficient of correlation for these two values in Yerosha and Drema data was 0.9-0.98 and 0.76-0.95 correspondingly. In flight these values were still high, but in addition the approximation lines for inflight data situated above that obtained preflight, thus pointing out to the fact that the saccades of the same amplitude in microgravity lasted shorter and hence performed faster. These data are in good agreement with previous obtained in flight of biosatellites "Cosmos-1514" and partially (in 1 monkey) "Cosmos-1667".

As in previous flights in the microgravity the values of K_{VOR} increased.

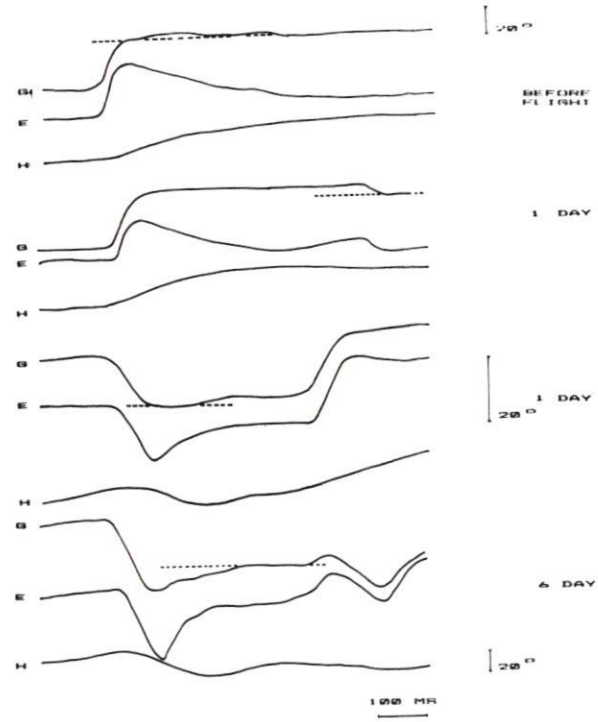

Figure 1. Eye-head coordination before and during space flight.
Positions of head (H), eyes (EOG) and gaze (G) are shown by corresponding letters.

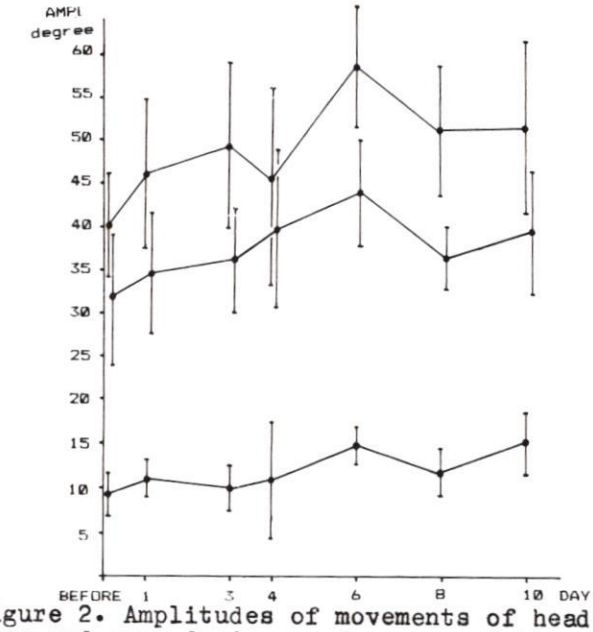

Figure 2. Amplitudes of movements of head, eyes and gaze during performance of the eye-head coordination.

However, the dynamic of changes was a little bit different with small but distinct decrease on the first day of flight, gradual increase to the maximal value to the 6th day and subsequent normalization on the 10th day.

Vestibular neuronal responses to canal stimulation

As it was already mentioned, in this flight recordings of vestibular neurones were supplemented by recordings

of vestibular nerve fibers and flocculus neurones. Comparative analysis of neuronal responses to the semicircular canal stimulations performed under different experimental conditions (in darkness, with head clamped etc., Fig. 3) allowed to prove the proper positioning of electrodes as well as their safety at the different stages of flight and after it (Fig. 3). The quality of neuronal registration was good; all the records analyzed contained not only mass-activity but activity of single units as well.

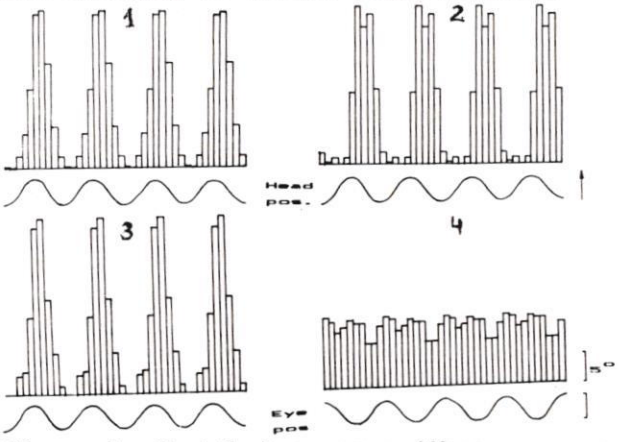

Figure 3. Vestibular nerve fibers response to canal (1-3) and visual (4) stimulation under 4 experimental conditions, namely: 1 - darkness; 2 - VOR suppression; 3 - light on; 4 - visual stimulation.

The data of analysis in which the relations between the head movements velocities and the amplitudes of integrated neuronal activities at any 50 ms were processed (Fig. 4) showed that the transition to microgravity was followed by distinct alterations of neuronal dynamic sensitivity. The most prominent these changes were in the vestibulocerebellum in which the value of this relation from the first till the 8th day of flight outnumbered the preflight value more than 3 times.

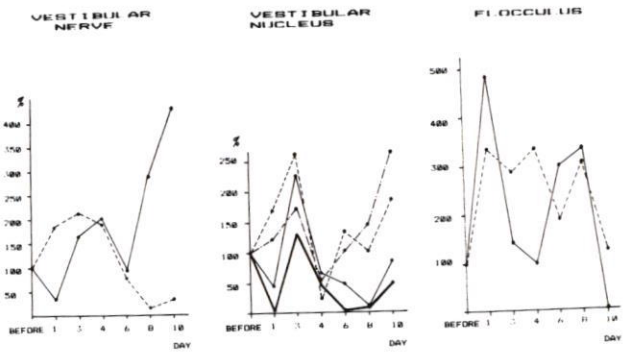

Figure 4. Alterations of the intensity of neuronal responses to canal stimulation during the space flight.

Vestibular neuronal responses to otolith stimulation

The neuronal responses of the same structures (vestibular nuclei, vestibular nerve, vestibulo-cerebellum) to the vertical displacement of the chair have been studied in order to reveal effects of microgravity on the otolith sensitivity. The amplitude of displacement was 45 mm; the duration of chair lifting on the ground was equal to 5 s, the duration of chair lowering - 0.6 s. In flight these values were equal correspondingly to and 0.9 s.

In order to analyze the neuronal activity, the whole cycle of chair movements was subdivided into 4 phases, namely: resting, lifting, going down and stop reaction and the mean amplitude of the activity integrated for 500 ms for each phase was calculated. The results of the comparative analysis of pre-and-inflight data are presented in Fig. 5. It is seen that the values of otolithic responses in microgravity changed considerably. The neuronal activity on the ground in monkey Yerosha did not change obviously at any phase but going down. In microgravity both dynamic phases - lifting and going down were followed by definite neuronal activation. The intensity of this activation outnumbered that "on the ground" by 1.5-3 times.

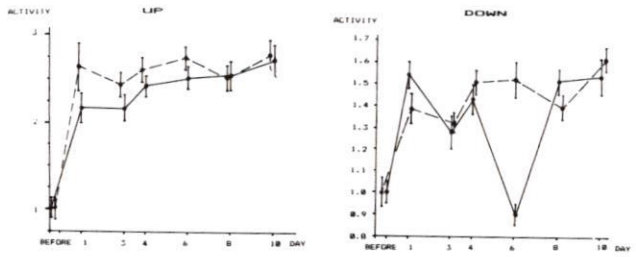

Figure 5. Alterations of the intensity of neuronal response of the n.vestibularis to otolith stimulation in Yerosha.

Thus the results of study gave another evidences to the fact of increased dynamic excitability of vestibular system to both canal and otolithic stimuli in microgravity. The most prominent changes were observed in vestibulo-cerebellum. The changes linked to canal functions showed a definite tendency to adaptation, being of maximal value at the 1st-3rd days of flight. The reactions of otolithic origin in this case were much more stable and didn't show definite signs of adaptation during 10 inflight days.

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Antioxidant enzyme levels (Figures 2 & 3) in the EXER groups were significantly greater when compared to their SED counterpart; the only exception was in CAT levels in the exercise trained H/H SAL group (p=0.06). All H/H SAL groups had lower CAT and SOD levels than the CON SAL groups and the H/H DOB groups were always significantly greater than the H/H SAL groups. Percent change and basic patterns of group differences were very similar for the two antioxidant enzymes.

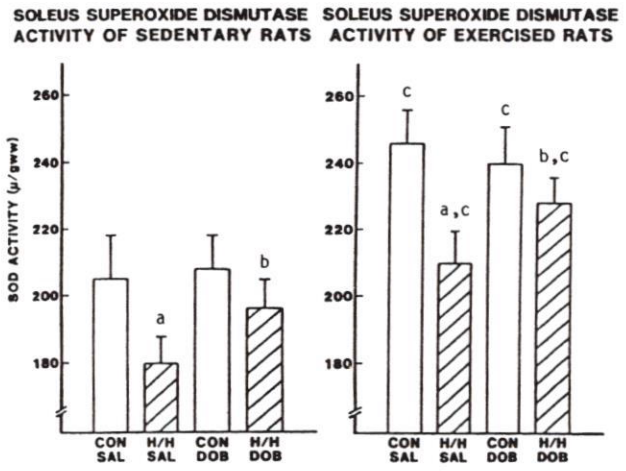

Figure 2. Superoxide dismutase activity.

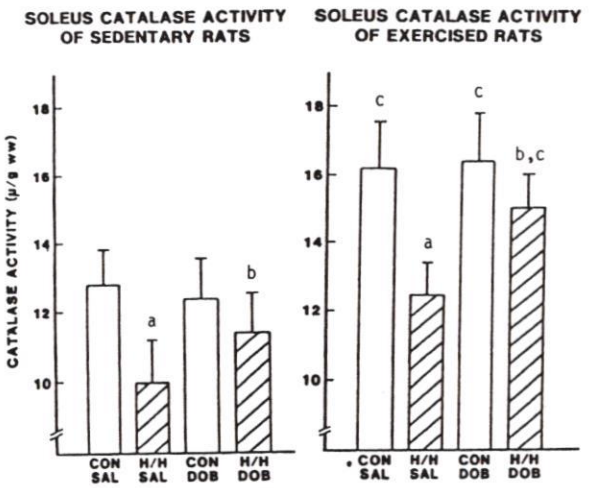

Figure 3. Catalase activity.

DISCUSSION

Recent results reported by Zezerov et al. (14) indicate that antiorthostatic (head down tilt) hypokinesia increases lipid peroxidation in muscle tissue of rats. Although antioxidant enzymes were not measured the authors postulated that reduced activity of antioxidant enzymes might have been a factor in the increased level of free radical lipid peroxidation. A short time later Kukhta et al.(7) provided evidence that supported this point of view when data indicating decreased activity of SOD and CAT as well as increased levels of lipid peroxidation with hypokinesia in rats were reported. Findings reported here that indicate significant decreases in antioxidant enzymes after fourteen days of H/H hindlimb suspension confirms results of these earlier investigations.

Aerobic exercise conditioning prior to suspension and IP injections of dobutamine during suspension were utilized in an effort to determine if these treatments could attenuate oxidative and antioxidant enzyme changes in the soleus muscle. Although there has been some controversy regarding antioxidant enzyme change with exercise, several investigators have shown increased levels of antioxidant enzymes with aerobic training (4,5). Information on dobutamine's ability to affect muscle change includes reported increases of CS (13), but there is no past information published concerning dobutamine's affect on antioxidant enzymes. In the present study eleven weeks of exercise training prior to suspension significantly increased levels of CS as well as SOD and CAT. Dobutamine during suspension helped maintain oxidative and antioxidant enzymes levels.

The data presented here demonstrates that H/H suspension not only results in decreased oxidative enzyme levels, but also results in decreased activity of two antioxidant enzymes that are necessary to prevent uncontrolled free radical reactions that can lead to oxidative tissue damage. Exercise training prior to simulated weightlessness, and dobutamine administration during the simulation, helped prevent large decreases in skeletal muscle antioxidant and oxidative enzyme levels that would otherwise occur.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. Emily Morey-Holton, Robert Hamlin and James Cooper for assistance and advice; Marvin Souder, Susan Young and Susan Bloomfield for technical support; and Doris Skinner for preparation of this manuscript. This work was supported by AFOSR/AFSC, USAF Project #2312V6.

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SKELETAL MUSCLE ANTIOXIDANT ENZYME LEVELS IN RATS AFTER SIMULATED WEIGHTLESSNESS, EXERCISE AND DOBUTAMINE

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INTRODUCTION

An organism's cells and tissues are protected against oxidizing free radicals by a number of complex mechanisms (2) and included among these are the actions of antioxidant enzymes. These naturally occurring compounds, or scavengers, react with free radicals without generating further radicals and act to quench chain reactions and decrease free radical oxidation of membrane phospholipids that can lead to tissue damage (11).

Recent evidence indicates that some forms of simulated weightlessness induce significant decreases in antioxidant enzymes (7) and significant increases in lipid peroxidation (14). A treatment that could have positive effects in maintaining or increasing antioxidant enzyme activity levels during the simulated weightless conditions of antiorthostatic hypokinesia or hindlimb suspension might be exercise training; another might be administration of dobutamine, a compound that has been shown to induce or maintain conditioning effects similar to those seen with exercise training (8,9,13). The purpose of this study was to determine if exercise training prior to hypokinesia/hypodynamia (H/H) hindlimb suspension and dobutamine (DOB) injections during suspension could have a positive effect on levels of oxidative and antioxidant enzymes in skeletal muscles of adult rats.

METHODS

Twenty-five male Sprague-Dawley rats were exercise trained (EXER) on a treadmill for eleven weeks and twenty-three were not (SED). The exercise program was a modification of the protocol developed by Holloszy (3). Animals from both the EXER and SED groups were randomly assigned to one of four additional treatment groups. Rats that received the H/H hindlimb suspension treatment were suspended ($\cong 30°\angle$) for fourteen days by the tail traction method originally described by Morey (10). Animals from the EXER group that were not suspended were placed on an exercise maintenance program of 40 minutes of exercise, three days per week, for the fourteen days. Half of the H/H group received intraperitoneal (IP) injections of 1 mg/kg dobutamine (H/H DOB) twice a day during suspension, while the other half received an equivalent amount of saline (H/H SAL). Control animals (CON SAL and CON DOB) were placed in a plexiglas cage identical to that of the H/H suspended rats and received the same type of injections of either SAL or DOB, but were not suspended. All animals were approximately 155 days old when killed. The soleus muscle, as well as a number of other muscles and organs, were quickly excised, wrapped in foil and quick frozen in liquid nitrogen. This tissue was stored at -70°C until the enzyme assays were performed.

Citrate synthase (CS), an oxidative training enzyme, was measured by the spectrophotometric method of Srere (11). The reaction involves measurement of activity by linking the release of CoASH to the colormetric agent 5,5-dithiobis-2-nitrobenzoate (DTNB). Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the destruction of the superoxide radical. This enzyme was measured according to procedures described by Keele et al. (6) and is based on the ability of this compound to inhibit the O_2 reduction of ferricytochrome C. Catalase (CAT) is an antioxidant enzyme that works in concert with glutathione peroxidase to keep hydrogen peroxide (H_2O_2) at a low steady state level. CAT activity was measured by the method of Cohen et al. (1) by titrating residual H_2O_2 remaining after a known time with permanganate.

Standard analysis of variance procedures were used to compare mean group differences based on the three treatments (exercise, suspension, dobutamine). When appropriate, Tukey's pairwise comparison was utilized as the post hoc test. All results are expressed as mean + standard deviation and $p<0.05$ was the established level of significance.

RESULTS

The soleus CS levels are summarized in Figure 1. Levels of CS for the H/H were all significantly less than their CON counterparts, except in the case of the H/H group that received both EXER and DOB treatments. The EXER groups had, in each case, significantly greater CS activity than their SED counterparts. Both H/H groups that received DOB had greater CS levels than the corresponding H/H SAL groups.

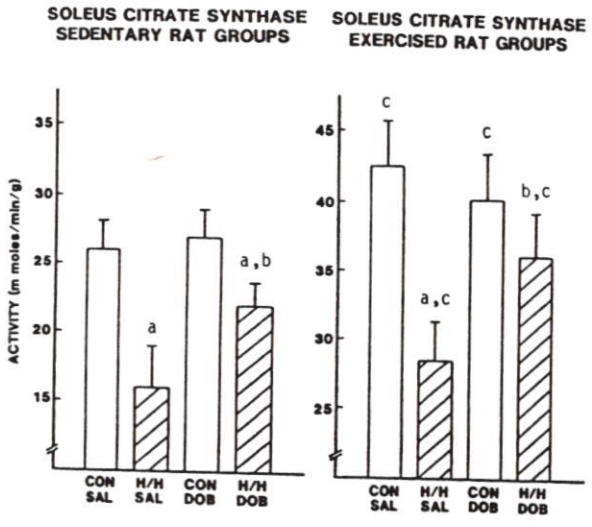

Figure 1. Citrate synthase activity.

Superscripts for all figures as follows:

[a]Significantly < CON group counterparts.
[b]Significantly > SAL group counterparts.
[c]Significantly > SED group counterparts.

3.5 µg/cm^2 Cell-Tak (CT, BioPolymers). Cultures were placed in a humidified 37°C incubator (5% CO_2) for attachment. After the attachment period, which was 2 hours in some experiments and 20 hours in others, 1.8 ml of medium was added to all cultures, air bubbles were removed from the hardware, 4 BEX units were placed in a Type I container (Fig. 1B), and both plates and hardware were returned to the incubator.

Cultures were observed daily for progression of chondrogenesis and scored according to these criteria:

6 = Cartilage nodules.
5 = Tightly packed aggregates of cells.
4 = Loosely packed clumps of cells.
3 = Homogeneous cell layers.
2 = Scattered cells.
1 = Complete detachment of cells.

After 5 days, cultures were fixed in 1% glutaradehyde in Sorenson's phosphate buffer, and stained with Alcian blue (pH 1.0) for detection of cartilage nodules (18).

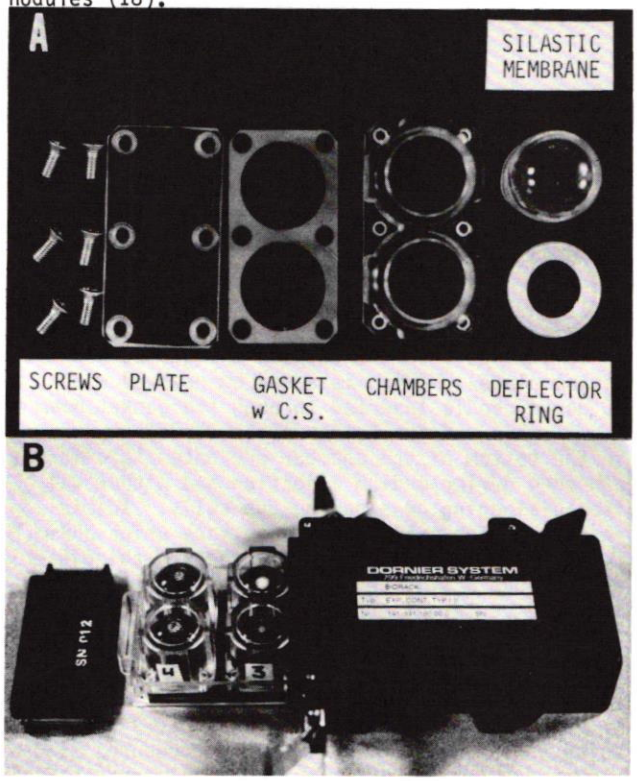

Fig. 1: A - Hardware components.
B - Assembled BEXs in Type 1.

RESULTS

Cartilage formation occurs in BEX hardware as shown by the presence of Alcian blue staining cartilage nodules (Fig. 2). Table 3 shows that cartilage formation is decreased when coverslips are coated with CT. Longer attachment time in CT-treated cultures also decreases cartilage formation. On uncoated coverslips, however, chondrogenesis proceeds during the 20 hr attachment period.

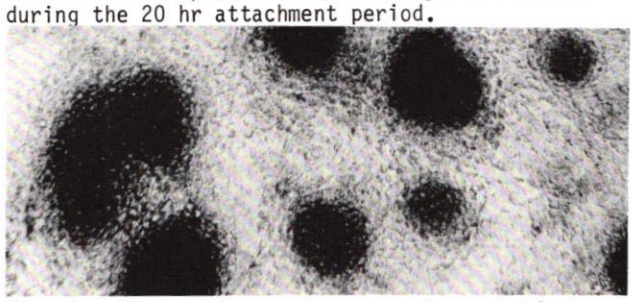

Fig. 2: Alcian-blue stained cartilage nodules.

TABLE 3: CARTILAGE FORMATION

2 hr attachment	Uncoated	CT-coated
CN	1* (8%)**	3 (33%)
BEX	2 (75%)	2 (25%)
20 hr attachment		
CN	1 (89%)	2 (83%)
BEX	1 (100%)	2 (38%)

*Day of first cartilage appearance.
**% cultures with cartilage.

DISCUSSION

BEX hardware supports chondrogenesis in micromass cultures. The progression of chondrogenesis in these cultures can be manipulated by use of CT as a coating and by the use of longer attachment times on CT-coated plates. Delay of chondrogenesis with the use of CT and with the longer attachment time can be attributed to the delay of cell rounding, a necessary precondition for cartilage formation (19). These results will be useful in trying to delay the onset of chondrogenesis until cultures are placed in the Biorack, approximately 20 hrs after handover to ESA. Cultures will also be kept in the cold (4°C) for that time period in an attempt to slow chondrogenesis. Cooling of cultures and further characterization of the matrix produced by micromass cultures in BEX hardware are currently being carried out in this laboratory. Although designed for the CELLS experiment, and for Biorack, the BEX hardware could be used for other cell culture experiments, for organ cultures, or for small plants or animals, and can also be used in ground-based clinostat experiments.

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ACKNOWLEDGEMENTS: M. Campbell, C. Williams J. Arizpe and IML team at ARC.

CONTINUING STUDIES OF "CELLS" FLIGHT HARDWARE

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INTRODUCTION

A basic question of space biology is whether changes in gravity (Δg) can be perceived at the cellular level. Previous studies with a variety of cells (Table 1) indicate that this is the case. Improvement of these studies, and confirmation of the results, was made possible by the development of European Space Agency's Biorack--a modular cell culture facility designed to fit into Spacelab (1). Biorack contains a freezer, a cooler, a glovebox for operations involving cultures, and two incubators, each with a 1 g centrifuge. The centrifuges make it possible for the first time to separate the results of spaceflight from the results of microgravity. Cultures to be flown on the Biorack are sealed in Type I or Type II containers, which fit into slots in the various Biorack components. Biorack was flown in October/November 1985 on the German Spacelab mission D1(STS 61-A). Results from this mission as well as further information on the Biorack have been published (1).

TABLE 1: EFFECTS OF Δg ON CELLS

WI-38 fibroblasts on Skylab 3: consumed 18% less glucose (2).
Paramecium aurelia: Proliferation increased by µg; decreased by 2 g (3).
Lymphocytes: 5 X increase in interferon production in µg (4).
Lymphocytes: Mitogen activation increased at 2,4,10 g (5,6); decreased by exposure to actual or simulated µg (7,8).
Lymphocytes, HeLa cells, chicken embryo fibroblasts, Sarcoma Galliera cells (rat), Friend leukemia virus-transformed cells: Increased proliferation at 10 g (6).
Rat pituitary cells: decreased growth hormone release in µg (9).

This paper describes preliminary studies for an experiment (CELLS) which was selected for flight as a Biorack experiment on International Microgravity Laboratory 1 (IML 1). The objective of the flight experiment is to examine the effect of microgravity *in vitro* on a cell known to be sensitive to gravitational changes *in vivo* (Table 2). Small, high density cultures of embryonic limb mesenchymal cells (micromass cultures) will be exposed to microgravity aboard the shuttle, fixed daily during the mission and examined on return for any effects of µg on cartilage differentiation. The well characterized micromass system is known to reflect changes in chondrogenesis occuring *in vivo* due to mutation or exposure to teratogens (10), so results should also indicate how *in vivo* limb development might be affected by µg.

TABLE 2: EFFECTS OF Δg ON CHONDROCYTES

Early ossification of femoral heads of centrifuged rats (11).
Delayed mineralization and abnormal collagen formation in growth plates of rats flown on Cosmos 1129 (12).
Suppression of morphogenesis in embryonic mouse limbs exposed *in vitro* to 2.6 g (13).
Decreased height and altered matrix in growth plates of rats flown on Spacelab 3 and Cosmos 1887 (14,15,16).

MATERIALS AND METHODS

Cell culture hardware: The main unit of the CELLS hardware is a polycarbonate culture chamber with two wells, and in each, a "bubble" or "blister" of a gas exchanging material (Dow Corning Silastic MDX-4-4210) which expands or collapses as medium is added or removed (Fig. 1A). A silicon rubber gasket and a polycarbonate bottom plate held in position by six small stainless steel screws complete the basic hardware setup. Cells are cultured on coverslips inserted between the gasket and the bottom plate. Medium is added or removed with a 26 gauge needle inserted through the gasket between the chamber and the bottom plate. A deflector ring in the bottom of the chamber prevents fluid forces produced during medium injection and withdrawal from dislodging or shearing the cells. Initial injections for cell culture are made using a Hamilton syringe, which is inserted through the gasket and through a silicon plug in the deflector ring into the center of the chamber. The "bubble" exchange hardware (BEX) was developed by NASA's Ames Research Center, with consultation and testing provided by our laboratory.

Cell cultures: Micromass cultures were prepared using a modification of standard procedures (17). Eleven day embryos (plug day = day 0) were obtained from timed matings of ICR mice (Harland-Sprague-Dawley). Pregnant females were sacrificed by cervical dislocation, embryos removed and placed in 37°C sterile Tyrodes solution (GIBCO). Fore and hind limbs were cut from embryos, rinsed with calcium/magnesium-free Tyrodes (CMF), minced, and treated for five minutes with 2.25% trypsin (GIBCO) in CMF. One ml of medium was added, minced tissue was pelleted, rinsed with CMF, and incubated in CMF for 30 min. Medium for these studies was CMRL 1066 (GIBCO) with 10% fetal bovine serum (GIBCO), 5 mM Hepes, 150 µg/ml ascorbate acid, and 50 µg/ml Gentamicin (Sigma). The CMF was replaced with 1 ml of medium, and limb buds were mechanically dissociated by flushing through a fine bore pipette. The cell suspension was filtered through a layer of 20 µ Nitex (Tetko), and cell number in the filtrate was determined using a hemacytometer. The final concentration of cells was adjusted to 4×10^5/20 µl drop.

For preliminary studies, 20 µl drops were placed on 15 mm diam Thermanox coverslips (Nunc) in prototype BEX hardware or in Corning 24 well plates. In some experiments coverslips were coated with

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Lymphocyte proliferative activity in the peripheral blood (Fig. 2) in group A subjects did not undergo noticeable changes except a decrease at the end of bed rest and on days 1 and 7 of recovery. In group B, the index showed similar dynamics, but the decrease started already during day 230 of HDT. It should be noted that the lowering of lymphocyte proliferative activity after the completion of 370-day HDT was more marked than after 120-day bed rest.

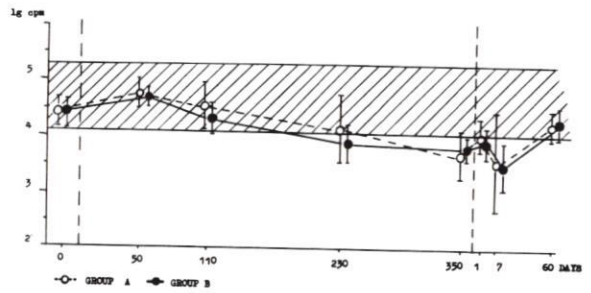

Fig. 4. Number of "active" T-lymphocytes in peripheral blood during 370-day head-down tilt.

Measurements of "active" T-lymphocyte count in peripheral blood (Fig. 4) showed that in group A the number of these cells increased on day 350 and on day 1 of recovery, but in group B the increase began on day 230.

The more marked changes in group B are in accord with the more complicated conditions of the study for this group. The subjects are exposed to "pure" hypokinesia during the initial 4 months of the study. It is noticeable that the dynamics of proliferative activity values and "active" T-lymphocyte count in the blood flow is practically identical, but the direction of changes was inverted. Apparently, the increase in relative number in the general T-lympocyte population of "active" T-lymphocytes which are known to respond less actively to mitogenic stimulation in vitro (3), is one of the reasons for a decrease in cell proliferative capacity.

On the other hand, it is obvious that the completion of prolonged hypokinesia and the beginning of recovery is a stressful situation for the subjects. And it is known that stress is accompanied by a suppression of T-subset of the immunity system. This may also be one of the causes of a more marked decrease in T-lymphocyte proliferation response during the first days after the completion of 370-day HDT. In turn, the lowering of supernatant activity in stimulated mononuclear cell cultures on days 1 and 7 of recovery practically to base-line values may have brought about by a suppression of T-cell activity. E.g., treatment of mitogen-stimulated lymphocytes with cyclosporin A (a T-cell suppressive agent) inhibits the synthesis of osteoclast-activating factor (4).

Now a discussion is going on as to the nature of local immunological factors activating resorbtion, and cells producing these factors. Among them are the osteoclast-activating factor, lymphotoxin, interleukin I, tumor necrosis factor. Dewhirst F.E.etal.(5) published convincing evidence on identical character of osteoclast-activating factor and interleukin-I-beta and suggested a hypothesis explaining the essence of lymphocyte and monocyte synergism in producing the resorbtion activating mediator by the by the fact that monocytes stimulate lymphocyte production of gamma-interferon which, in turn, causes synthesis of interleukin I by monocytes. Raisz LG (1) suggested that local factors similar in many respects may represent a single group of genes, which may originate not only from immunocompetent, but also from bone cells, thus mediating systemic hormone effect.

Immune cell functions may also be modulated by hormones regulating calcium metabolism in the body. It has been shown that on the membrane of lymphocytes and mononuclear phagocytes there are receptors to systemic hormones regulating calcium metabolism.

The probability of an increase in the synthesis of local mediator of immunological nature in systemic bone tissue damage is supported by the fact that in congenital osteopetrosis with a growing PTH and 1.25 $(OH)_2$ vitamin D_3 concentrations, there was an increased production of osteoclast-activating factor (6).

It is obvious that circulating immunocompetent cells producing humoral osteoclast function regulators, are capable under certain conditions to realize their effect in any part of the skeleton, accessible to them, given the presense of funcionally active receptors on bone cells. The clinical manifestation of the effect of local factors is most frequently associated with local inflammatory processes in particular with paradintitis. Systemic skeletal damage when this mechanism can be realized, has been established in rats with experimental aseptic inflammation (7).

Thus, the increase in the ability of immunocompetent cells to produce humoral factors activating resorbtion in bone tissue, that we saw in healthy persons exposed to prolonged bed rest, suggests a possible contribution of this mechanism to changes seen in the skeleton during hypokinesia.

The specific conditions necessary for in vivo realization if immunocompetent cell' potential to produce humoral regulators of regulators of bone cell functions, require further study.

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bone tissue was also taken into account (RJII), which made it possible to measure the response more accurately. The index was expressed as the test/control ratio of percent ^{45}Ca release from bone fragments.

Results and discussions

The study of supernatant resorbing activity in leucocytes cultures from 36 healthy males performing their routine daily activities, yielded similar results for RII and RI II, and allowed to determine approximate limits for physiological variation of the variable ($M \pm 2\sigma$).

In 14 of the 15 patients with periodontitis the resorbtion index exceeded the upper normal limit, which corresponds to data obtained by other workers (2) and confirms the informatory value of the method used during the study of persons exposed to 120-day HDT, RJI evaluation method was used. We failed to observe significant differences of mononuclear supernatant activity in peripheral blood of subjects from different groups. The dynamics of averaged individual values (Fig 1) shows that on days 28 and 112 of HDT there is a significant rise in HDT. Furthermore, in 7 of 12 subjects on day 28 and in 8 of 13 subjects on day 112 RJI exceeded the approximate limit of physiological variation. On day 7 of recovery the index shows a trend to decrease to baseline values, and remains elevated in four of 14 subjects.

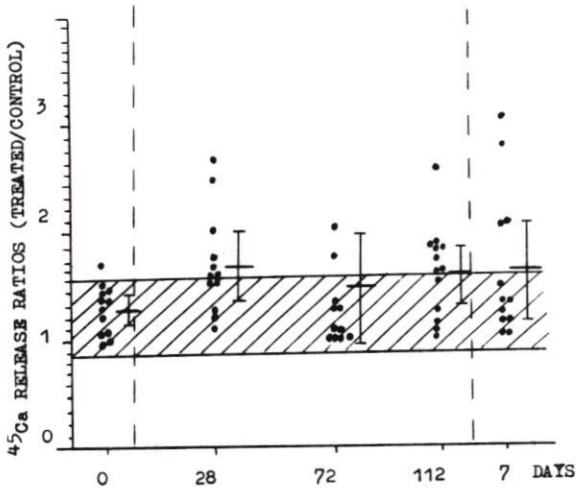

Fig. 1.Resorbing activity of phytohemagglutimin-stimulated leucocytes culture supernatants in healthy men during 120-day head-down tilt.

Lymphocyte proliferative activity showed little variability, with a tendency to decrease at the end of bed rest and on dat 7 of recovery. However, it remained within physiological normal limits.

Thus, subjects exposed to 120 HDT showed a significant rise in the ability of peripheral blood mononuclear cells to produce humoral mediators activating resorbtion in the bone tissue, with a tendency to restoration after completion of hypokinesia.

Further study was carried out during 370-day HDT using a more exact testing method (RJ II) aimed at obtaining data which would confirm the results at studying the dynamics of this index during more prolonged hypokinesia and determining one of the possible ways of in vivo realization of this mechanism through an evaluation of blood level of"active" T-lymphocytes which correlates, to a certain extent, with T-lymphocyte function (3).

Supernatant activity did not differ significantly between groups A and B. The averaged individual values (Fig. 2) were significantly increased, compared to base-line through the 370-day HDT study, returned to control values beginning with recovery day 1.

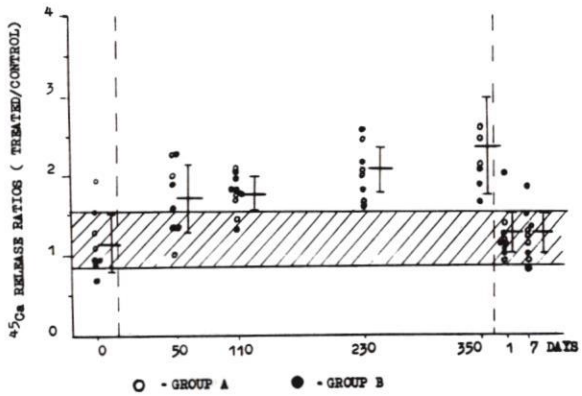

Fig. 2. Resorbing activity of phytohemagglutimin-stimulated leucocyte supernatants in healthy men during 370-day head-down tilt.

The value exceeded the upper normal physiological limit on day 50 in 4 of 8 subjects, and on days 230 and 350, in all subjects. The data obtained was in accord with the results of 120-day HDT. It was shown that the capability of mononuclears to produce mediators activating resorbtion, increases with the duration of bed-rest and is noted practically in all subjects. Lowering of the index during early recovery suggests a possible relation of mononuclear cell reaction to the subjects status, in particular to an increase of weight-loading of the skeleton.

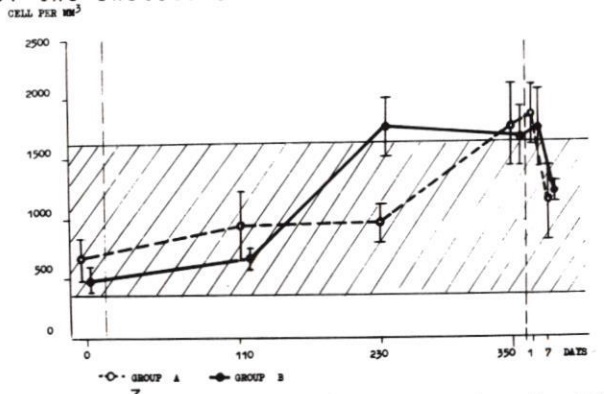

Fig. 3. ^{3}H-thymidine incorporation in 48hr. lymphocyte cultures of healthy men during 370-day head-down tilt.

IMMUNOCOMPETENT CELLS PRODUCING HUMORAL MEDIATORS OF BONE TISSUE MINERAL METABOLISM DURING SPACE FLIGHT SIMULATION

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Abstract

During 120-day and 370 day hypokinesia the possibility has been shown of manifestation of mechanism of immunological regulation of osteoclast functions. Supernatants of mononuclear peripheral blood cells which were in vitro nitogen-stimulated, had an increased potential for resorbtion in ^{45}Ca-labelled mice fetus long bone organ cultures. Resorbing activity was increased in mononuclear supernatants from some of the subjects exposed to 120-day hypokinesia and from all subjects exposed to 370-day hypokinesia. This variable returned to base-line values after completion of the bed-rest period. Lymphocyte in vitro proliferative activity decreased at the end of hypokinesia and during the initial days of recovery, and the number of active T-rosetting cells was, on the contrary increased. This suggested a possible activation of part of immunocompetent cell population potentially producing humoral regulators of bone cell functions in vivo. A study of a group of healthy males performing their routine daily activity and of patients with local osteoporisis (parodontitis) showed significant differences between normal subjects and patients, confirming the informative value of the method used and allowed to establish approximate limits of physiological variations of the values.

Introduction

In humans, prolonged space flight is associated with significant skeletal changes in particular, a decrease of bone mass. The loss of calcium which is the main component of bone mineral fraction, in astronauts, is as high as 0.3 - 0.4% of total body content per month. One of the factors underlying the described phenomena is an increase in resorbtion, but mechanisms leading to osteoclast activation during weightlessness, have not been adequately studied. Functional immunological status plays an important role in bone cell activity regulation, together with systemic hormones and local factors, produced both directly in bone tissue and in other tissues. Osteoclasts are cells of hematopoetic origin, in many respects similar to macrophages which mediate immune reactions; and their activity may be directly or indirectly regulated by lymphocines and monocines (1).

On the other hand, cosmonauts, after prolonged space flights frequently show changes in the immune system - both a decrease of integral functional and quantitative characteristics of T-lymphocytes and variation in regulatory cell activity, and in increase in some cases, of the number of T-cells actively forming rosettes with sheep red blood cells, and of cells mediating delayed hyper-sensitivity reactions (2). This, in turn, may affect interaction of immunocompetent and bone cells.

One of the most widely used models for studying some effects of wightlessness upon the human body is head down tilt (-4°)(HDT) which produces changes in the musculoskeletal and immune systems having much in common with those seen in cosmonauts. This allowed us to study some immunocompetent cell reactions with respect to cellular bone tissue elements.

The purpose of the present investigation was to study the dynamics of production of humoral mediators activating resorption, by in vitro stimulated immunocompetent peripheral blood cells during prolonged HDT.

Materials and methods

Sixty one healthy males participated in the study (15 were subjected to HDT for 120 days, and 10 - for 370 days; 36 subjects performed their routine daily activities. Fifteen patients with local osteoporisis (periodontitis) were also studied. Subjects exposed to HDT for 120 days were subdivided into 4 groups: 3 subjects used no countermeasures (I), 4 subjects performed physical exercise (II), 4 subjects received medication (III), and four subjects received medication and simultaneously performed physical exercise (IV). Subjects exposed to HDT for 370 days, were subdivided into two groups of 5 men each. One of them (group B) was exposed to bed rest for 120 days without any countermeasures. Countermeasures (physical exercise and medication) were begun in group A during the initial days of hypohinesia, and in group B,after after 120 days of HDT. The study was carried out during HDT and during recovery.

Peripheral blood cells were obtained by density gradient centrifugation on Ficoll-Hypaque. Cell syspensions were adjusted to a concentration of 10^6 cells per ml and cultured in medium containing phytohemagglutinin for 48 hours at 37°C. After completion of the culturing period, supernatants were freezed. The incorporation of ^3H-tymidine in DNA of lymphocytes was measured in cell pellets. The number of T-lymphocytes with high activity for sheep red blood cells ("active" T-lymphocytes) was determined and expressed as cells per mm^3.

The bone resorbing assay of supernatants was performed in ^{45}Ca- labeled mice fetus long bone organotypic culture. The supernatant activity was determined by the amount of ^{45}Ca released from bone tissue into the medium after 48-hour incubation at 37°C. The results were expressed as the CPM test/control ration, and called the resorbtion index(RJI). While measuring the RI value, ^{45}Ca release from embryonic

TEMPERATURE REGULATION IN RATS EXPOSED TO A 2 G FIELD

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ABSTRACT

The regulation of body temperature involves both homeostatic and circadian control systems. Both systems are influenced by exposure to hyperdynamic fields and demonstrate acute responses that eventually recover to an adapted level. This experiment examined both the homeostatic and circadian responses of body temperature to a separate environmental challenge (high frequency light/dark cycles) during exposure to a 2 G hyperdynamic field.

INTRODUCTION

Homeostatic and circadian systems are two important components in the regulation of body temperature (Tb) in mammals. Previous studies have shown Tb homeostasis to be affected by light/dark cycles and changes in the gravitational field. For example, monkeys exposed to a high frequency light/dark cycle for 24 hours (e.g., LD 2:2) exhibit a depression of Tb during the dark periods and an increase in Tb during the light periods when compared to the same periods under a normal control (LD 12:12) light/dark cycle (homeostatic effect) [3]. However, there is a diurnal fluctuation in the magnitude of the Tb changes caused by the high frequency light/dark cycles (circadian effect) [3]. Therefore, high frequency light/dark cycles can be very useful for testing the integrity of the homeostatic and circadian components regulating Tb homeostasis.

Rats exposed to a 2 G hyperdynamic field exhibit a decrease in mean daily Tb (homeostatic effect) [1,2,4] and total reduction in the circadian amplitude of Tb (circadian effect) [1,2,4]. Mean daily Tb returns near control levels approximately 5-6 days later. The circadian rhythm returns after approximately 7-10 days [1,2]. Although the homeostatic and circadian components appear to recover toward a new steady-state, it is not known whether these components have recovered their ability to respond to other environmental challenges. This experiment examined the integrity of the homeostatic and circadian neural components to regulate Tb in response to another environmental challenge under a hyperdynamic field.

MATERIALS AND METHODS

Rats were exposed to a continuous 2 G hyperdynamic field. The ability of the homeostatic and circadian components to respond to an environmental challenge was tested once a week with a high frequency light/dark cycle (LD 3:3). These high frequency LD cycles were presented during and after Tb's adaptation to the continuous 2 G field under a normal 12:12 cycle.

Eight male albino Wistar rats were surgically implanted with a biotelemetry unit (Mini-Mitter). Following recovery the rats were placed in a cage on an 18 ft diameter centrifuge. A receiver board under each rat cage was interfaced with a computer which recorded body temperature every 10 minutes. The animals were typically exposed to a 24 hour light/dark cycle composed of 12 hours of light and 12 hours of darkness (LD 12:12). At weekly intervals, the animals were exposed to a high frequency light/dark cycle (LD 3:3) for 24 hours. One week after the rats were exposed to a control exposure of LD 3:3 at 1 G, the animals were exposed to a continuous 2 G field for 6 weeks. Two days after the initiation of the 2 G field, the rats were again exposed to 24 hours of LD 3:3. Five additional 24-hr periods of LD 3:3 were presented at weekly intervals. The effect of LD 3:3 on Tb was quantified by calculating the difference in Tb during the light periods versus the dark periods.

RESULTS

Figure 1 illustrates the response of mean daily Tb and circadian amplitude of Tb following exposure to 2 G for all 8 rats. Mean daily Tb exhibits an immediate decrease following 2 G exposure. There is a subsequent recovery approximately 4 days later. Circadian amplitude of Tb does not exhibit an immediate decrease following 2 G exposure. Instead, circadian amplitude gradually becomes depressed by the sixth day. Circadian amplitude remains at this depressed level from days 6 to 8 at 2 G. There is a gradual recovery of circadian amplitude in Tb by the eleventh day of 2 G.

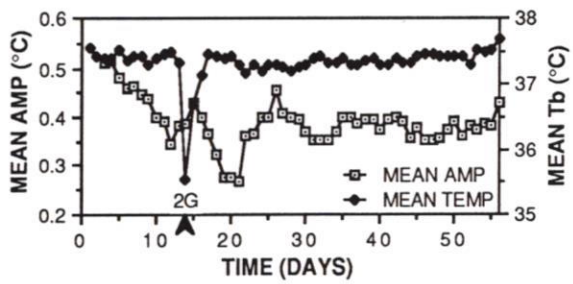

Figure 1. The effect of a 2 G field on average daily temperature (Tb), and circadian amplitude (Amp).

Figure 2 illustrates the effect of 24 hours of LD 3:3 on rat Tb at normal 1 G control conditions (Fig. 2A) and at three times during the 2 G conditions (Fig. 2B,C,D). At 1 G (Fig. 2A) the homeostatic Tb component responds to LD 3:3 with increases in Tb when the lights are turned off and decreases in Tb when the lights are turned on. In addition, the circadian Tb component is evident when the magnitude of the homeostatic Tb response is examined. The Tb change is larger during the night than during the day. Finally, note that there is a robust circadian rhythm in body temperature despite the LD 3:3 condition.

Figure 2B shows the effect of LD 3:3 on Tb after 9 days at 2 G (second LD 3:3 test at 2 G). The circadian rhythm of Tb is depressed and the effect of LD 3:3 on Tb is highly attenuated, if present at all. Figure 2C shows the effect of LD 3:3 on Tb following 16 consecutive days at 2 G. During the subjective night

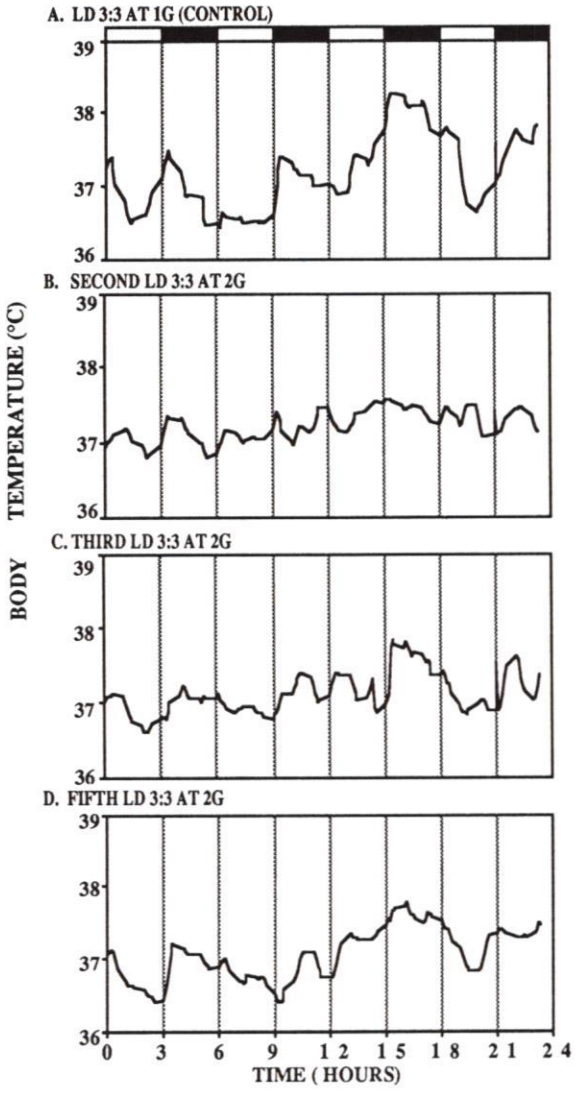

Figure 2. The effect of LD 3:3 on Tb.

there is a rise in Tb during the dark periods and a decrease in Tb during the second 3 hour light period. However, during the subjective day the Tb response is still highly attenuated, if present at all. Also, there is an increase in the amplitude of the circadian rhythm of Tb. Figure 2D shows the effect of LD 3:3 on Tb following 30 consecutive days of 2 G. Changes in Tb in response to LD 3:3 can be seen during both the subjective day and night. The changes are similar to that of the control, but smaller in magnitude. Similarly, the amplitude of the circadian rhythm has increased, but remains depressed below that of controls.

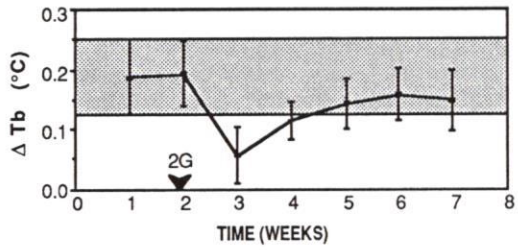

Figure 3. Recovery of Tb responses to LD 3:3.

Figure 3 summarizes the effect of LD 3:3 on Tb for all 8 rats, by comparing the differences in Tb during the light versus the dark periods ($\bar{X}$±S.E.). For the control 1 G (week 1) the mean Tb during the four 3 hour dark periods is 0.19 ± 0.05°C greater than the Tb during the four light periods. Following exposure to 2 G, the effect of LD 3:3 on Tb becomes severely depressed (0.06 ± 0.05°C) by week 3 (9th day at 2 G). By week 5, the Tb differences recover (0.16 ± 0.04°C) and are not significantly different from the controls.

DISCUSSION

The results of this experiment demonstrate that changes in light intensity produce changes in the level of rat Tb. Further, these changes are time of day specific in their magnitude and superimposed on the endogenous circadian variation in Tb. In the hyperdynamic environment, mean daily Tb decreases, the circadian rhythm of Tb is suppressed, and there is a deficit in the sensitivity of the thermoregulatory system to changes in light intensity.

Unlike previous studies that reported an immediate suppression of the circadian rhythm of Tb following exposure to hyperdynamic fields [1,2,4] this experiment demonstrated a gradual decrease in circadian amplitude. In addition, the effect of LD 3:3 on Tb was still apparent 2 days after exposure to 2 G. This also suggests that there is a gradual effect of 2 G on the sensitivity of Tb to high frequency light/dark cycles. It appears that both mean daily Tb and the circadian rhythm of Tb returns prior to the time when the thermoregulatory system can respond to changes in light intensity.

The data also suggests that for any regulatory system, the recovery of both the homeostatic and circadian components are necessary in order to functionally respond to an environmental challenge under a 2 G field. In addition, during the recovery of Tb, in response to LD 3:3, the changes in Tb recover during the subjective night before the subjective day. The causes behind these phenomenon will be important for understanding the effect of gravity on Tb regulation. Additional studies may be directly applicable for understanding the process of man's adaptation in space.

ACKNOWLEDGEMENTS

We would like to thank Wendy Lipman and Ina Green for technical support. This research has been supported by NASA Grant NAG 2-349.

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EFFECT OF INCREASED ACCELERATION ON LUNG EXPANSION IN DOGS: PRONE VS. SUPINE BODY POSITIONS.

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INTRODUCTION

Our previous studies using rib capsules in anesthetized dogs to measure pleural liquid pressure have indicated that the vertical gradient in transpulmonary pressure is greater in the supine position than in the prone position (5). Similar findings have been shown using the intraparenchymal lung marker technique (2) and lung density measured by computerized tomography (1). However, studies using implanted liquid filled catheters have shown the vertical gradient in pleural liquid pressure in the supine position was similar to that in the prone position, both under normal (earth's gravity) and increased acceleration loading (3,4). Accordingly, we investigated whether lung expansion would be greater in the supine than in the prone position under increased acceleration loading. We studied the vertical gradient in pleural liquid pressure under increased acceleration in prone and supine body positions.

METHODS

We used mongrel dogs (15-20 kg) that were anesthetized using sodium pentobarbital (30 mg/kg) and were allowed to breathe spontaneously. The rib capsule technique was used to measure pleural liquid pressure (5). Two rib capsules were installed, one located ventrally in the 4th rib, the other dorsally in the 6th rib. The ventral-to-dorsal distance between the capsules was ∿8 cm. Pleural liquid pressure was measured by transducer connected to the capsules in the supine and prone positions under earth's gravitational acceleration (G).

The dog was positioned in a 7.6 m radius centrifuge so that between 2G and 3G centrifugal acceleration, the resultant force due to acceleration was in a direction either ventral-to-dorsal (+Gx, supine position) or dorsal-to-ventral (-Gx, prone position), with a relatively small cranial-to-caudal vector that ranged in magnitude between 0.08G to 0.26G (Table 1). For acceleration in the prone position, the animal was positioned head-up at 20 degrees to the vertical direction and facing radially outward from the center of rotation. For acceleration in the supine position, the animal was positioned head down at 20 degrees to the vertical direction and facing radially inward to the center of rotation. Each animal was exposed to step increases in centrifugal (radial) acceleration of 1G, 2G, and 3G followed by return steps to 0G. Exposure at each level lasted for 1-2 minutes in order to reach a steady state response. The acceleration loading in the primary ventral-to-dorsal and dorsal-to-ventral directions and the excess loads in the cranial-to-caudal direction are shown in Table 1.

TABLE 1. Imposed G loadings in the centrifuge.

	PRONE	SUPINE
Primary dorsal-ventral or ventral-dorsal acceleration	Excess cranial-caudal acceleration	Excess cranial-caudal acceleration
1G	0G	0G
2.2G	+0.26G	-0.26G
3.2G	-0.08G	+0.08G

Pleural liquid pressures and systemic arterial pressure were measured by 3 saline filled transducers which were connected to the rib capsules and arterial catheter and mounted on the restraining cage. Pleural pressures and blood pressure were measured continuously as the centrifuge was rotated. To correct for increased centrifugal acceleration of the liquid column between each capsules and its transducer, a saline filled (reference) line was connected to the transducer and its opened end located at the capsule. The acceleration protocol was repeated with the transducers connected to the reference lines to provide the correct reference levels for the pleural pressure measurements.

RESULTS

Table 2 (mean ±SD, n = 5) summarizes measurements of pleural liquid pressure at functional residual capacity (FRC) at the different acceleration loadings in the dorsal-to-ventral direction (-Gx, prone position) and in the ventral-to-dorsal direction (+Gx, supine position). Note that pleural liquid pressure in both the ventral and dorsal lung regions became more negative with increased acceleration in the supine position. The overall effect was to expand the lung. By contrast, in the prone position, during increased acceleration loading, pleural liquid pressure became positive in the ventral lung regions with the pleural liquid pressure remaining constant in the dorsal lung regions. The overall effect was to cause the lung to be compressed in the prone position.

TABLE 2. Pleural pressure at different G loadings.

Accl.	Ppl (prone)*		Ppl (supine)	
	ventral	dorsal	ventral	dorsal
1G	-2.5±1.3	-5.5±1.3	-4.9±1.4	+0.7±2.4
2.2G	+4.2±3.2	-3.6±1.3	-8.7±3.9	-0.5±4.6
3.2G	+5.6±7.6	-2.3±3.3	-10.8±3.2	-2.0±4.3

*Ppl, pleural pressure, cmH_2O.

Table 3 (mean ±SD, n = 5) summarizes the gradient in pleural liquid pressure; that is, the difference between the two capsule pressures divided by the dorsal-to-ventral distance between the capsules per unit G loading, at the different acceleration loadings. Note that the gradient was about the same, between 0.47 and 0.77 $cmH_2O/cm/G$ in both body positions at all G loadings (4) except at 1G in the prone position where the vertical gradient was minimal (0.26).

TABLE 3. Gradients in Ppl at different G loadings.

Acceleration	$\Delta P/\Delta h$ ($cmH_2O/cm/G$)	
	prone	supine
1G	0.26 ± 0.25	0.73 ± 0.24
2.2G	0.47 ± 0.08	0.51 ± 0.14
3.2G	0.77 ± 0.06	0.51 ± 0.08

DISCUSSION

We have used the pleural liquid pressure at FRC as an index of lung expansion. This assumes that pleural liquid pressure is an accurate reflection of the pleural surface pressure or the pressure acting to expand the lung. Also, at FRC the alveolar pressure is equal to atmospheric pressure so that transpulmonary pressure is the difference between atmospheric pressure and pleural pressure. Thus the negative of the pleural pressure at FRC is the transpulmonary pressure. With these assumptions, we can draw certain conclusions from the pleural pressure measurments during increased G loadings.

First, the net effect of high acceleration is to expand the lung in the supine position and to contract the lung in the prone position. In the supine position at FRC, the nondependent lung regions at 3.2G expanded to twice its transpulmonary pressure at 1G. Higher accelerations may conceivably result in lung damage due to lung overexpansion. In the prone position at FRC, dependent lung regions were subjected to large compressive forces. Thus it is possible that breathing may become difficult at high acceleration in the prone position, because the respiratory muscles must provide a force to overcome the compressive forces on the lung in addition to the force required to expand the lung.

Second, the magnitude of the gradient in pleural pressure with respect to the ventral-dorsal dimension increased with acceleration, with a value of ~0.5 cmH_2O/cm per unit G acceleration, for both prone and supine positions. The normal prone position at 1G appears to be exceptional with the lowest gradient of 0.26 cmH_2O/cm.

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ACKNOWLEDGEMENTS

This research was supported by NIH grant HL 36597.

CHANGES OF MUSCLE FUNCTION AND SIZE WITH BEDREST

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Introduction

It is generally believed that atrophy and reductions of strength of lower limb muscle groups occur during space flight and that these responses may compromise ambulation upon return to 1-g, especially in an emergency egress situation (1). A few studies using lower mammals provide the only direct measures of the effects of orbital flight on muscle size and strength. Rats show structural and biochemical evidence of atrophy and reductions of force output ability of mainly antigravity muscles following space flight (1).

Indirect measures of size and of protein metabolism have been used to assess changes in skeletal muscle with orbital flight of humans. Reductions of thigh and calf circumferences and of lower limb volume and elevations in urinary nitrogen, phosphorous, amino acids and 3-methylhistidine all suggest muscle wasting with space flight (1). These responses appear to have functional consequences because reductions of strength have been reported for both Skylab and Salyut crews (1). The ability to develop force during isometric and "slow" speed isokinetic, concentric voluntary muscle actions decreases with greater changes in extensor than flexor and in lower than upper limb muscle groups (1).

To date, no studies of crew members have been done to directly assess alterations of muscle size as a result of space flight. Whether the type or speed of muscle action influences strength loss has also not been investigated. At present it is not possible to address these issues by studying only crew members. Logistics and time constraints greatly curtail their availability. We, therefore, conducted a study using headdown bedrest to examine the impact of a short term exposure to simulated microgravity on skeletal muscle.

Methods

Seven untrained healthy males 38±2 years of age served as subjects in this study after giving their informed written consent. They were housed in a bedrest facility (National Aeronautics and Space Administration, Ames Research Center, Moffett Field, CA) for a nine day pre bedrest control period (C), for 30 days of -6° headdown bedrest (BR) and for a five day post bedrest recovery period (R).

The *in vivo* torque-velocity relationship of the knee extensor muscle group of the dominant lower limb was determined the second day of the control period (C2) and the first day of the recovery period (R1). Dominance was determined by the handedness of each subject. Subjects were exposed to the muscle function tests twice during the week prior to C2 in an effort to train them to exercise. Torque-velocity relationships were established by measuring angle specific peak torque at four speeds (0.52, 1.74, 2.97 and 4.19 rad·s^{-1}) during unilateral concentric muscle actions and at three speeds (0.52, 1.74 and 2.97 rad·s^{-1}) during unilateral eccentric muscle actions. An isokinetic loading dynamometer (KIN/COM, Chattecx Corp., Chattanooga, TN) was used to measure and record velocity and torque for each muscle action (2).

Three voluntary maximal efforts were made at each test speed of concentric and of eccentric muscle actions and the highest torque developed at the angle of 0.78 rad below horizontal (the test angle) was considered angle specific peak torque. The position of the lever arm at the start of each muscle action was adjusted to ensure that minimal fatigue and a constant angular velocity was achieved before reaching the test angle (3,4). One min of rest was provided between different test speeds and 3 min between concentric and eccentric muscle actions. Tests with concentric muscle actions preceded those with eccentric actions.

Muscle biopsy samples were obtained from the vastus lateralis muscle as described by Bergstrom (5). They were taken the first day of the control period (C1) and the 30th day of bedrest (BR30) and processed for subsequent histochemical identification of fiber types and fiber cross-sectional area.

The histochemical processing of the muscle biopsies was essentially as described by Matoba and Gollnick (6). The distribution of fast- (FT) and slow-twitch (ST) fibers in each biopsy sample was determined from counts of all dark and light staining fibers in each section. The area of the fibers was determined with a computerized image analysis system. In most muscle sections there were fibers that appeared to have been cut tangentially to their long axis. Only fibers judged to have been cut perpendicular to the long axis were included in the analysis of fiber area. An average of 48 fibers (range 30 to 71) of each type per biopsy sample were considered in area analyses (7).

Results

Thirty days of -6° headdown bedrest decreased ($P<0.05$) force output of the knee extensor muscle group during maximal voluntary efforts (Fig. 1). The average decrease of angle specific peak torque across speeds of concentric and eccentric actions was 21 percent. The reduction of angle specific peak torque was not significantly ($P>0.05$) influenced by the type or speed of muscle action. For concentric muscle actions at the test speeds of 0.52, 1.74, 2.97 and 4.19 rad·s^{-1} angle specific peak torque decreased 24±7, 24±6, 22±5 and 18±6 percent, respectively. For eccentric muscle actions the decrease for each of the first three speeds was 22±6, 17±1 and 17±4 percent, respectively.

There was a decrease ($P<0.05$) in the cross-sectional area of both FT and ST fibers of the vastus lateralis following bedrest (Fig. 2). The area of FT fibers decreased 17 percent (4456±338 to 3672±177 μm^2). The area of ST fibers decreased 11 percent (4124±191 to 3656±207 μm^2). The magnitude of these responses was not different ($P>0.05$) between fiber types. The percentage of ST fibers in the biopsy sample of the vastus lateralis was not different ($P>0.05$) before (65 percent) and after (57 percent) bedrest.

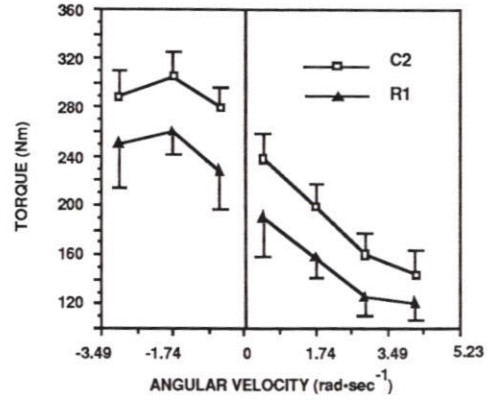

Fig. 1. The *in vivo* torque-velocity relationship of the knee extensor muscle group of seven subjects. Values are mean + or - SE. A negative test velocity indicates eccentric muscle actions. A positive velocity indicates concentric muscle actions. C2, the second day of the control period. R1, the first day of the recovery period. Across speeds of eccentric and concentric muscle actions the values for R1 are less ($P<0.05$) than those for C2.

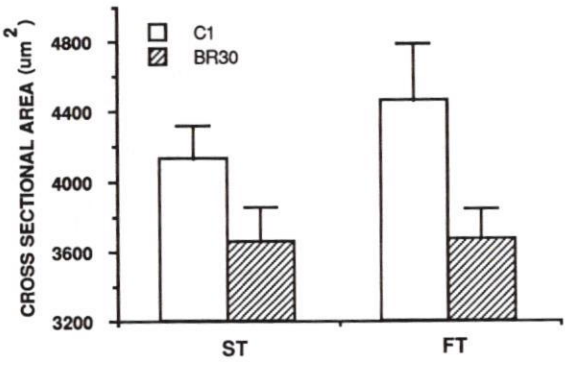

Fig. 2. Cross sectional area of ST and FT fibers. Values are mean ± SE in μm^2. VL, vastus lateralis muscle. C1, the first day of the control period. BR30, the 30th day of bedrest. BR30 values are significantly less than C1 values ($P<0.05$).

Discussion

A significant result of the present study was that 30 days of -6° headdown bedrest decreased force developed by the knee extensor muscle group during maximal voluntary efforts. The average reduction across speeds of concentric and eccentric muscle actions was 21 percent. Thornton and Rummel have reported that average torque developed during 10 concentric muscle actions (0.78 $rad \cdot s^{-1}$) of the hip and knee extensors decreases 22 percent pre- to five days post- four weeks of orbital flight (8). Although the testing methods used in the present study are different than those used by Thornton and Rummel, the variables measured should be related. It appears, therefore, that -6° headdown bedrest is a reasonable analogue to spaceflight since exposure to either intervention for about the same duration, i.e., 4 weeks, causes a similar relative reduction in muscle strength.

The mechanisms responsible for strength loss with bedrest are not well understood. A significant decreases in the cross-sectional area of ST and FT muscle fibers of the vastus lateralis was found in the present study. Computed tomographic analyses pre and post bedrest showed an 8 percent reduction ($P<0.05$) of the total cross-sectional area of thigh muscle (unpublished observations). Muscle atrophy, therefore, probably contributed to the loss of strength, but cannot solely account for it. The relative changes in muscle and mean fiber size (8 - 14 percent) were less than the relative change in strength (21 percent). Although electromyographic (EMG) analyses were not performed in the present study, alterations in motor unit recruitment could have contributed to the loss of strength. The crew of Skylab 2 showed changes in EMG activity of the gastrocnemius muscle postflight (9). The predominant bandwidth was shifted to a lower frequency when a unilateral "toe-raise" with 40 pounds was performed. After long-term Soviet missions, more than a two-fold increase of integrated EMG activity during a standard muscular effort has been reported (1).

Angle specific peak torque decreased as the velocity of muscle action increased during voluntary concentric efforts using the knee extensors. This has been reported by us and by others (3,4). In frank contrast, velocity had minimal influence on torque development during eccentric actions. Hanten and Ramberg have reported similar results in that torque does not increase as speed increases during eccentric actions of the knee extensors (10). This response is in marked contrast to that of isolated muscle stimulated electrically or of human forearm flexors activated voluntarily. For either, force is greater during eccentric than concentric actions and increases markedly as velocity increases during eccentric muscle action (11). Thus, neural inhibition that limits force development in the high- force, slow-speed region of the *in vivo* torque-velocity relation when concentric muscle actions are performed appears to play even a greater role in determining force during voluntary eccentric muscle actions (3,4). Whatever the factors that limit force development during voluntary muscular efforts, the culmination of their changes with 30 days of bedrest results in similar strength loss for eccentric and concentric muscle actions independent of velocity. The finding that the nature of the torque-velocity relation during concentric muscle actions was not altered by bedrest probably reflects in part that preferential fiber type atrophy was not evident (4).

Both FT and ST fibers showed a reduction in cross-sectional area after 30 days of bedrest. The fact that total cross-sectional area of the thigh also decreased with bedrest lends support to our finding of fiber atrophy, although we are aware of the limitations of using a single biopsy to determine fiber size (12). Moreover, atrophy is a general finding with a variety of conditions that result in unloading of skeletal muscle. It may appear surprising that there was little difference in the magnitude of atrophy between the two major fiber types in the present study. It is generally believed that ST fibers are more susceptible to unloading than FT fibers (13). However, this may depend on the muscle and the species studied and there may be an age dependent response concerning selective fiber type atrophy (14). Adult rats exposed to orbital flight or hindlimb suspension show much less of a predominance of ST fiber atrophy than young rats (14). Moreover, they do not exhibit a marked shift in the fiber type composition of skeletal muscle when exposed to these interventions (14,15). Thus, our finding of a similar percentage of ST fibers in the vastus lateralis pre and post bedrest seems reasonable.

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CAROTID BAROREFLEX RESPONSE FOLLOWING 30 DAYS EXPOSURE TO SIMULATED MICROGRAVITY

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INTRODUCTION

Astronauts who have been exposed to as few as 10 days of weightlessness have exhibited some degree of orthostatic intolerance as manifested by hypotension and tachycardia during standing postflight (2). With the development and occupancy of the Space Station, missions of greater than 120 days duration may become routine. The impact of such long exposures to microgravity may have profound adverse effects on blood pressure regulation following return to Earth and subsequently compromise the health, safety and productivity of astronauts after spaceflight.

The mechanisms of postflight orthostatic intolerance are unclear. Reduced blood volume contributes to, but is not a sufficient explanation for the inability of cardiovascular reflexes to adequately regulate blood pressure following exposure to actual or simulated microgravity (1). We considered the possibility that modification of baroreceptor input may alter carotid baroreflex response and compromise blood pressure maintenance during standing after re-entry.

We conducted the present study to determine 1) if there are alterations in the shape of the sigmoid baroreceptor-cardiac response curve and/or if resetting occurs following prolonged exposure to simulated microgravity (bedrest) and 2) if impaired baroreflex function, where evident, is associated with hypotension during post-exposure orthostatic stress.

METHODS

Eleven healthy, nonsmoking, normotensive men, with a mean (±S.E.) age of 38 ± 2 yrs gave written consent to participate as volunteer subjects after they had been informed of all procedures and possible risks. The regular daily activity levels of the subjects varied considerably from sedentary to running 5 miles/day.

The experimental protocol consisted of a 9 d ambulatory control period (C) followed by 30 d of bedrest (BR) in the 6° head-down position to simulate tmicrogravity and 5 d of post-bedrest recovery (R).

Each subject underwent a carotid-baroreflex test on the fourth day prior to bedrest (C4), on the third, twelfth and twenty-fifth days during bedrest (BR3, BR12, and BR25, respectively), and on the second and fifth days of recovery (R2 and R5). In addition, the subjects returned to the laboratory after 25 days of uncontrolled recovery (R30) for a final baroreflex test.

Baroreceptor-cardiac reflex responses were elicited by a complex sequence of pressure changes delivered to a neck chamber device. During held expiration, neck chamber pressure was raised to approximately 40 mmHg for about five heartbeats. Then, chamber pressure was lowered by successive 15 mmHg R-wave triggered decrements to approximately -65 mmHg. A test session comprised seven successful applications of pressure sequences. Neck chamber pressures and R-R intervals during the seven sequences were averaged for each test session. Systolic (SBP) and diastolic (DBP) blood pressures were measured before the first application of neck chamber pressure sequences. Baroreflex relations were reduced to the following parameters for statistical comparisons: baseline R-R (position of the operational point), range of R-R interval responses (buffer capacity), and maximum slope (gain).

Resting plasma volume (PV) was measured on C4, BR3, BR12, and BR25 using Evans blue dye and was estimated on R2 from changes in hematocrit and hemoglobin concentrations. Resting norepinephrine (NE) and epinephrine (E) were measured on C4, BR1, BR12, BR25, and R1 using a radioenzymatic assay.

Posture tests were conducted before bedrest and immediately upon the termination of the 30 d BR exposure (R1) to assess the responsiveness of the sympathetic nervous system. The posture test consisted of lying horizontal for 60 min followed by sitting for 60 min. Venous blood samples were drawn from the antecubital vein before sitting and at 5, 15, and 60 min after sitting up in both pre- and post-BR posture tests for the determination of NE and E. Immediately following the post-BR sit posture test, subjects underwent a 5-min stand test for determination of orthostatic responses. Blood pressure and heart rate were measured at minutes 3 and 5 of standing.

A repeated measures analysis of variance technique with contrasts was carried out to look for differences between measurements of the baroreflex relations taken during the control and experimental sessions. Syncopal and non-syncopal groups were compared with Wilcoxon Rank Sum test. A two-way ANOVA was run to determine differences in resting levels of NE and E across days. A three-way ANOVA using test days (pre-and post-BR), posture (supine vs. sitting), and subjects as factors was run to determine differences in the responses of NE and E during the posture tests.

RESULTS

The response relation shifted significantly on the R-R interval axis, but did not shift on the pressure axis (Fig. 1). There were parallel shifts in baseline R-R intervals (resting heart rate), which increased after the initial 2 hours of bedrest (BR1) but then progressively decreased through R2 and had not returned by R5. The position of the baseline HR (operational point) on the response curve was not altered by bedrest. The baroreflex response curves demonstrated that both the maximum slope and the range of the response decreased progressively with continuing bedrest (Fig. 2), remained attenuated through R5 and returned to baseline values by R30. Resting SBP, DBP, and mean arterial pressures did not change with bedrest.

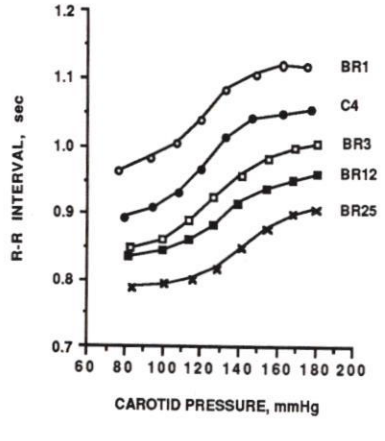

FIG. 1: Carotid-cardiac baroreflex response relations.

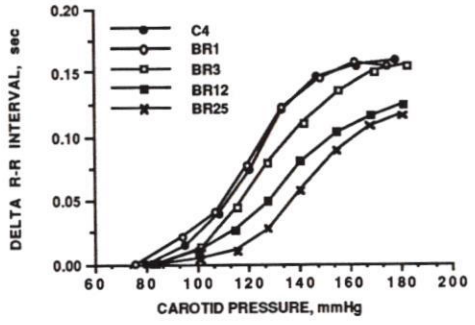

FIG. 2: Carotid-cardiac baroreflex responses as expressed relative to the change in R-R interval.

Compared to C4, resting NE and E were not significantly altered during bedrest. The elevation of NE induced by moving from the supine to upright sitting posture was significant ($p < 0.05$) but did not differ between pre- and post-BR posture sit tests. There was no alteration in E during the posture test and no differences between pre-and post-BR responses.

Complete data from the stand test were obtained on 10 of the subjects. Four subjects became syncopal during the 5 min stand test while the remaining six subjects tolerated the posture test with no noticeable difficulty. Although heart rate increased with standing in both syncopal and non-syncopal subjects, the increase was significantly less($p < 0.05$) in the syncopal group despite a greater unloading of the baroreceptors, i.e. significant reduction in SBP in syncopal subjects while non-syncopal subjects maintained SBP during standing. The reduction in maximal slope of the response curve from 4.0 msec/mmHg to 2.2 msec/mmHg for the syncopal group was significantly greater ($p = 0.042$) than the reduction from 3.1 msec/mmHg to 2.7 msec/mmHg in the non-syncopal group. The change in the maximal slope of the baroreflex response curve from C4 to BR25 and the change in SBP from supine to standing during the post-bedrest stand test were significantly correlated ($r = 0.75$, $p = 0.013$).

Mean resting PV for all subjects decreased by approximately 15% by BR3, but showed no further reduction through the end of bedrest; it was restored within the first day of post-bedrest ambulation. The time course of changes in PV and slope of the baroreflex curve were poorly correlated ($r = -0.17$, $p = 0.376$).

DISCUSSION

The shift of the carotid baroreflex relation along the R-R interval axis paralleled reductions and elevations in baseline HR such that the baseline R-R (operational point) remained in the same position on the response curve. This parallel shift in location of the reflex relation indicates a significant resetting of the carotid baroreceptors. This resetting may represent an appropriate adaptation which contributes to the maintenance of a constant resting systolic, diastolic and mean arterial blood pressure before, during and after bedrest observed in our subjects throughout the study.

Bedrest significantly reduced the responsiveness (gain) of the carotid sinus-cardiac baroreflex response. This effect was measured at day 12 of bedrest and persisted through at least 5 days of ambulatory recovery. Further, the buffer capacity of the reflex was reduced as indicated by the decrease in R-R range. Consequently, the capacity to respond and compensate for moment-to-moment changes in blood pressure was reduced by bedrest.

Several mechanisms may be responsible for the resetting and reductions in baroreflex responsiveness. It has been suggested that central blood volume changes may induce alterations in the response of the carotid baroreflex (4). In the present study, the time course of changes in plasma volume and slope of the baroreflex curve were not synchronous, suggesting that blood volume changes are an unlikely cause of the change in the responsiveness of the carotid baroreflex during bedrest.

The reduction in responsiveness of the carotid baroreflex could be associated with alterations in sympathetic and/or parasympathetic activity. Resting levels of plasma catecholamines in our subjects were not altered during bedrest. Further, catecholamines increased in a similar magnitude to a standardized orthostatic stress following compared to before bedrest. These results suggest that the baseline activity and responsiveness of the sympathetic system were not associated with the reduced responsiveness of the baroreflex. Since the cardiac response to carotid stimulation reflects cardiac-vagal responsiveness, our data suggest that a likely mechanism responsible for the attenuated carotid baroreflex response during bedrest was a reduced vagal activity. This is further supported by the significant reduction in baseline R-R (increased heart rate) which occurred during bedrest and was associated with the reduced maximum slope of the response curve.

During a 5-min stand procedure as an orthostatic test following bedrest, we observed that a subgroup of four subjects became syncopal. Compared to the subjects who were tolerant of the upright posture following bedrest, the syncopal subjects demonstrated an inability to increase heart rate despite a greater unloading of the baroreceptors. It is well documented that the inabilty to increase heart rate is a primary contributing mechanism to orthostatic intolerance. Although other factors such as reduced circulating blood volume, leg compliance, and attenuated responses of vasoactive hormones may contribute to orthostatic intolerance (1,3), there were no significant differences between our syncopal and non-syncopal subjects in these factors. The syncopal subjects demonstrated greater reduction in maximal slope and buffer capacity of their baroreflex response curve compared to the non-syncopal subjects. The degree of attenuation of the baroreflex response following bedrest was significantly correlated with a greater reduction in systolic blood pressure during standing. Although we cannot rule out the contribution of other baroreflexes, we have demonstrated that the reduction in the responsiveness of the carotid-cardiac baroreflex is associated with the inability to raise heart rate during an orthostatic stress following simulated microgravity, with a subsequent reduction in tolerance to standing.

To the extent that data measured in the head-down bedrest position can be used as an analog of physiological responses in microgravity, we hypothesize that as the duration of spaceflight increases, astronauts will increase their risk of postflight orthostatic intolerance due to the progressive attenuation of carotid-cardiac baroreflex sensitivity. Our results suggest that the development of an effective countermeasure for postflight orthostatic intolerance may require some technique of increasing vagal-cardiac activity and/or reversing the shift of the baroreflex curve upward (steeper slope), and to the left (response in the hypotension range) prior to re-entry.

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PLASMA ANF CONCENTRATIONS DURING HEAD-DOWN BED REST OF VARIOUS DURATION (from several hours to one month). Role of LBNP countermeasure.

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INTRODUCTION -

Soon after the discovery of the Atrial Natriuretic Factor (ANF) and its physiological properties (vasorelaxant, diuretic and natriuretic) it appeared that this new hormone could play an important role in volume regulation (1, 2), specially during the volume redistribution in conditions of weightlessness.

This role was brought out by the interrelationships observed between ANF and other hormonal systems involved in volume regulation : arginine vasopressin (AVP), the renin-angiotensin-aldosterone system and the sympathoadrenal system.

Considering the difficulties of sampling blood during spaceflight, the main source of our knowledge on ANF is weightlessness simulations : immersion, antigravity suit or bed rest and head-down tilt (8). The more convenient method, specially for long term simulations, is head-down bed rest (HDBR). Moreover, with this method, countermeasures such as Lower Body Negative Pressure (LBNP) or exercise can be easily studied.

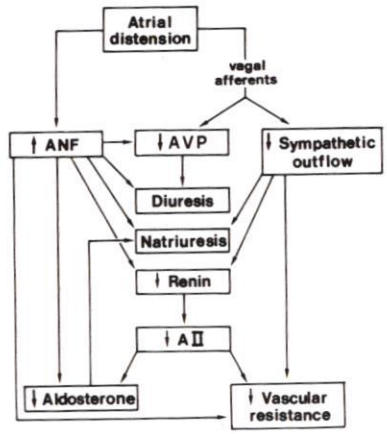
The aim of these studies was :
- To analyse ANF variations during simulated weightlessness of short and long duration,
- To demonstrate whether there was a variation in the response when head-down bed rest was realized during day or night,
- To study the relationship between ANF and sleep stages,
- To determine the role of LBNP on ANF secretion, LBNP being used as a countermeasure to prevent orthostatic intolerance in weightlessness or simulated weightlessness situations.

ANF was measured in blood collected in EDTA. Plasma was extracted with Sep-Pak C18 cartridge and determined by radioimmunoassay (9).

I - SHORT TERM EFFECTS

Short term effects of head-down bed rest -

The initial change induced by head-down tilt as well as spaceflight is a headward shift of fluid leading to an increased pressure in atria. Now in the regulatory mechanisms of ANF release, atrial distension is considered as one of the most consequent (1, 2). This distension also induces AVP and aldosterone modifications (Fig. 1).



SHORT TERM EFFECTS

Fig. 1

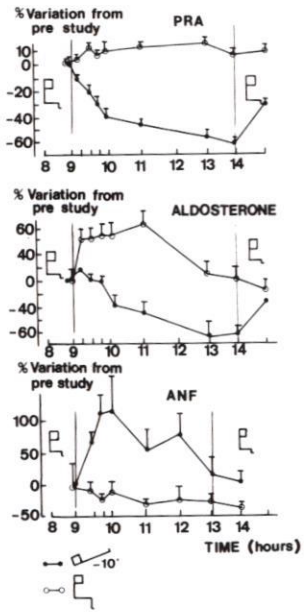


Fig. 2 - Comparison of the effects of head-down tilt (HDT) (-6°) on plasma renin activity (PRA), aldosterone and ANF. Data (±SE) are expressed as percent changes from pretilt period in seated position. At the end of the tilt period ANF returned to control value, but PRA and aldosterone were still decreased. Composite data from 2 protocols.

Indeed head-down bed rest or leg raising (17) induces an increase in plasma ANF but this increase is of short duration (concentration peak reaches a maximum in one hour and then decreases) in contrast (Fig. 2) to the time course of the renin-angiotensinaldosterone system, arginine-vasopressin and sympatho-adrenal system which remained inhibited for several hours (5).

This response could be modified by several factors :

- The elderly secrete more ANF than the young for the same cephalad fluid shift (more precisely for the same angle of head-down tilt) (11),
- a low sodium diet inhibits totally the ANF increase on adoption of the supine posture (19). Decreased plasma volume has the same effect (Fig. 3),
- when ANF concentration is maintained constant by infusion, the standing position induces an elevation of ANF with a decrease in MCR (Gillies). This is probably related to a postural change in renal blood flow.

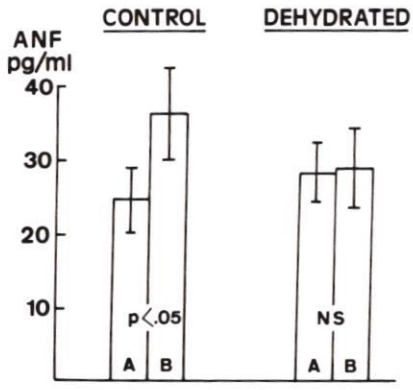


Fig. 3 - Effect of dehydration on plasma ANF concentration in 6 subjects.
A : 15 min. supine
B : 130 min supine
In the dehydrated condition ANF did not increase (unpublished data from B. Melin).

II - LONG TERM EFFECTS

The long term effects of head-down bed rest are less documented, but they are by far the most interesting for comprehension of the volume and electrolyte adaptations induced by weightlessness.

During a one month head-down bed rest (-6°) 6 young subjects were submitted to LBNP (see experimental schedule below). One year later they were submitted to the same protocol without LBNP.

EXPERIMENTAL SCHEDULE FOR LBNP

3 times a day (LBNP-30 mm Hg/20 min) for 3 wks
4 times a day for 4 days
6 times a day for 3 days } the last week

The preliminary findings in 3 subjects (Fig. 4) indicate that :

1°/ Plasma ANF decreases slightly during head-down bed rest without countermeasure. These results are comparable to those of C. Leach obtained during weightlessness (12, 13): after 175 h or 180 h ANF had decreased by 59%.

2°/ There is a difference between head-down bed rest alone and head-down associated with LBNP : ANF had a trend to decrease in the former case LBNP and to increase in the latter.

The effect of LBNP is less evident on PRA, but PRA seemed to be increased during both protocols.

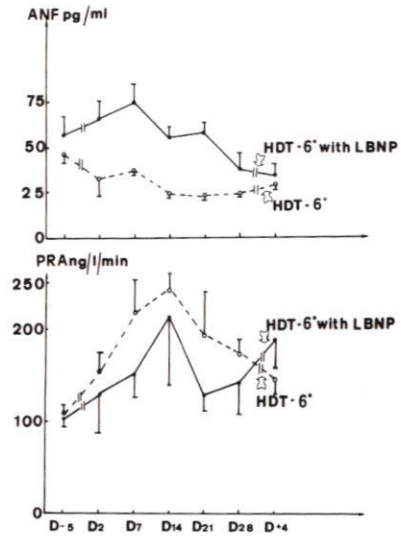
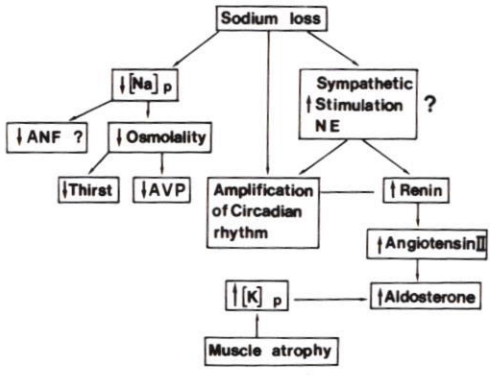


Fig. 4 - Time course of plasma ANF (top) and PRA (bottom) during a one month bed rest with and without LBNP.

Figure 5 summarizes the hypothesis which could be raised by the interpretation of these data and those of the literature : as other hormones implied in volume regulation, ANF respond acutely to volume disturbances and chronically to electrolyte disturbances (14).



LONG TERM EFFECTS

Figure 5

III - RELATION OF ANF OR SECRETION WITH TIME

Three connected problems can be raised :

- There is no circadian variation in ANF secretion,
- There is no relation between sleep stages and ANF secretion (4),
- ANF is not directly implied in the attenuated renal response observed when cephalad fluid shift is realized at night. Indeed, when in man or monkeys a central volume expansion is induced at night the diuresis and natriuresis are strongly reduced compared to the diuresis and natriuresis observed during the daytime (16). It was tempting to attribute this blunted response to a modification of hormone secretion during night. This is not the case because renin, aldosterone, AVP and ANF have the same time course during day or night after a central volume expansion (4, 16, 18). We thought the explanation for this phenomenon was a modification of the renal receptors and we therefore studied the renal glomerular receptors to ANF in the tail suspended rat (a model which produces a cephalad fluid shift analogous to head-down bed rest). After 2 hrs of suspension (3) the rats present the same increase in ANF, the experience being realised during day or night, but during night there is a decrease in the the number of receptors to ANF (Fig. 6). This could explain the differences observed in the renal response, but does not exclude other mechanisms implying hormones (renin, angiotensin, aldosterone or AVP) or hemodynamic modifications.

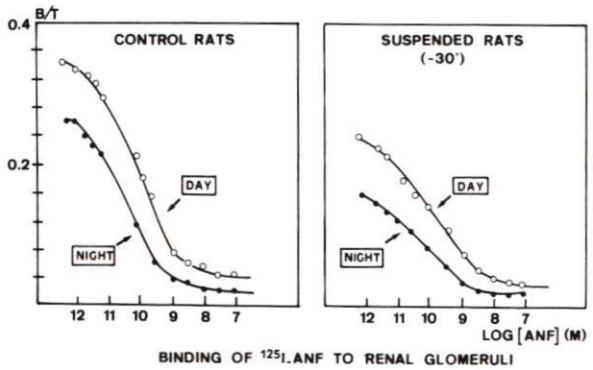


Fig. 6 - Representative curves of the binding of ^{125}I ANF to renal glomerule in the presence of increasing concentrations of unlabeled ANF. During night suspension there is a lower number of ANF receptors in both groups (results from ref. 3).

All these modifications are important not only because they account for the disturbances of the cardiovascular system (specially orthostatic intolerance) but also because they help to understand other pathological states such as space motion sickness (9, 12) ANF being implied in the water balance in the central nervous system (20).

CONCLUSIONS

ANF varies very rapidly during postural changes and returns to control value in 3 hours. This contrasts with PRA and aldosterone whose variations take place over several hours. The preliminary results of a one-month HDBR seem to indicate that ANF concentration has a trend to decrease, probably in relation to sodium loss. It would be very interesting to compare these data with those which will be obtained from the soviet station MIR in December 1988 during a one-month flight (ARAGATZ-Minilab).

Acknowledgments : CNES (Grant 1986-1987), DRET (87.056) Université CL. Bernard (UFR Grange-Blanche, Biologie Humaine), Centre Jacques Cartier and Fondation pour la Recherche Médicale. We thank M.J. CAREW for correction of the English.

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CENTRAL VENOUS PRESSURE IN HUMANS DURING SHORT PERIODS OF WEIGHTLESSNESS

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IN THE DISCUSSION of cardiovascular and pulmonary adjustments to weightlessness, the level of central venous pressure (CVP) is an important issue (17, 19). Weightlessness is hypothesized to induce an increase in CVP, which finds support in results from ground-based simulations where increases in CVP are well documented during water immersion (1, 4-6, 12, 15) or head-down tilted bed rest (2, 6, 12, 14). It is of special interest to know whether an increase in CVP induced by weightlessness is of such a magnitude that it can cause some degree of pulmonary interstitiel edema (West, this symposium).

On a few occasions, CVP or pulmonary arterial pressures have been measured directly in monkeys during weightlessness. During a 3-h orbital flight, systemic and pulmonary arterial pressures were measured invasively in an instrumented chimpanzee (7). The pulmonary arterial diastolic pressure was somewhat elevated with a subsequent reduction in pulmonary pulse pressure. On Biosatellite III, CVP was elevated during several days in a monkey (macaca nemestrina) to a level comparable to the supine position at 1 G (10). This indicates that at least a minor fluid shift occurs inflight compared with the upright sitting position on ground (+1 Gz).

Evidence of a fluid shift also exists from manned spaceflights. Thornton et al. (18) measured a decrease in leg volume and obtained photographic evidence of puffy faces on the astronauts, indicating a headward fluid shift. Kirsch et al. (8) estimated CVP from measurements with a peripheral venous catheter after 22 h of weightlessness during the Spacelab 1 mission. They concluded, however, that a decrease compared with the supine position on ground had occurred. With the use of the same method during the D-1 mission in Spacelab, a decrease in estimated CVP was observed as early as 20 - 45 min. inflight (9).

CVP has, however, never been measured directly in humans with a long catheter in an intrathoracic vein during weightlessness. Even though parabolic flight maneuvers have been extensively used in human physiology to create short periods of weightlessness (3, 12) most of these investigations have focused on vestibular research. Because CVP in humans has never been determined in weightlessness, the purpose of this study was to measure CVP in males during short periods of weightlessness (free fall) during parabolic flight in a jet aircraft. Thus we intended to examine the impact of the acute disappearance of hydrostatic gradients on CVP in humans.

METHODS

Fourteen healthy males participated in the experiment. Details of the methodology and the protocol have been outlined previously (16). Each subject had a 60 cm catheter (Intracath) inserted through a cubital vein. The catheter was connected to a disposable pressure transducer plastered to the skin at the level of the right atrium. The level of the right atrium was determined by x-ray. The flights were performed in a Gulfstream SMA-3 jet aircraft. The parabolic maneuvers were carried out as described by Michels and West (11) and was followed by a straight and level flight for 2 min. Thereafter a +2-Gz turn lasting for either 60 or 120 s was performed.

In Fig. 1, the G profile over time is demonstrated simultaneously with a CVP tracing during a typical parabolic maneuver. The aircraft ascended with either a 45 or 60 degree inclination. Thereafter the aircraft went abruptly into 0 G which on an average lasted for 23.3 seconds.

CVP was measured in one subject at a time. CVP was recorded a few minutes before each parabola at 1 G while flying straight and level both with the subject in the supine position (+1 Gx) on the floor and sitting upright (+1 Gz) in the flight seat. During the parabolic flight the subject sat upright in the flight seat without moving and a continuous CVP recording was performed over the entire parabolic maneuver. A similar tracing was carried out during the last minute of the +2-Gz period also with the subject sitting upright and relaxed. Due to a low-pass filter (in order to avoid noise) only the last 5 s of the CVP tracing during the 0-G period and the last 10 s during the +2-Gz period were used to measure CVP.

Heart rate (HR) was measured in seven of the subjects from ECG recordings monitored simultaneously with CVP. The G load was measured using a vertical accelerometer and recorded on the two strip-chart recorders parallel with the CVP and the ECG.

Data are presented as means +/- SE and evaluated statistically by two-tailed paired t-tests. A significance level of 0.05 was accepted.

RESULTS

Central Venous Pressure. A typical CVP tracing is demonstrated in Fig. 1 simultaneously with the G profile during a parabolic maneuver. CVP during the last 5 s of weightlessness (Figs. 2 and 3) was elevated significantly to 6.8 +/- 0.8 mmHg compared with 2.6 +/- 1.5 mmHg during upright sitting (+1 Gz, $P<0.005$) and 5.0

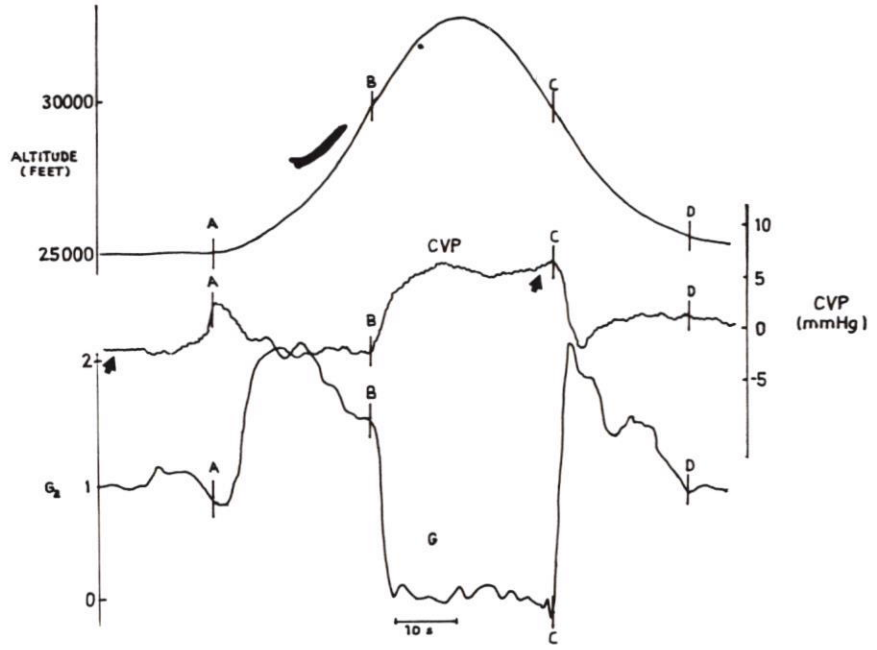

FIG. 1. A parabolic trajectory (top) is demonstrated together with a central venous pressure (CVP) tracing (middle), and a G profile (bottom) over time in one subject. Subject sat upright in flight seat during whole maneuver with G forces acting along z-axis of his body. A: aircraft starts parabolic maneuver with an inclination of either 45 or 60 degrees. B: aircraft abruptly goes into 0 G, which in this case lasted 26 s. C: 0-G period ends. D: aircraft is flying straight and level. CVP curve was dampened by a low-pass filter to avoid noise on curve so only last 5 s of tracing during 0 G was used to estimate CVP. Compared with CVP at +1 Gz (upright sitting) just before maneuver, it is evident that CVP value has increased during last seconds of weightlessness (arrows).

+/- 0.7 mmHg in the supine position (+1 Gx, $P<0.005$) at 1 G inflight. In Fig. 3 individual results are presented demonstrating that CVP in weightlessness increased significantly when compared to the supine position at 1 G. During the last 10 s of the +2-Gz period, CVP attained a value of 2.8 +/- 1.4 mmHg, which did not differ from CVP during upright sitting at 1 G.

Heart Rate. 1-G values for HR inflight (n = 7) were 65 +/- 7 beats/min in the supine position (+1 Gx) and 70 +/- 5 beats/min in the upright sitting position (+1 Gz). During weightlessness, HR increased to 79 +/- 7 beats/min, which was only significantly different from HR in the supine position at +1 Gx ($P< 0.01$). During the +2-Gz period, HR increased significantly to 80 +/- 6 beats/min.

DISCUSSION

Results from this study demonstrate that the disappearance of hydrostatic gradients during acute weightlessness induces a significant increase in CVP in humans compared with both the supine and upright sitting position at 1 G (+1 Gx and +1 Gz, respectively). Since the period of weightlessness was very short (23.3 s), the measured increase of CVP in this study was probably smaller than the expected increase during the first minutes of weightlessness in orbital flights.

Since the venous hydrostatic indifference point in humans is below the heart (2) it was of no surprise that CVP during weightlessness increased above the value measured in the subjects during upright sitting at +1 Gz. That CVP during weightlessness also increased above the level observed in supine subjects at +1 Gx could not have been predicted with certainty and is a major finding of this study (Fig. 3).

Theoretical considerations suggest that the +1.5 to +2 Gz load for 15-20 s during the pull-up phase of the parabolic maneuver might have attenuated the increase of CVP during the subsequent weightless period by a caudal displacement of blood. Since the upright sitting relaxed subjects went abruptly from +1.5 - 2 Gz into 0 G, steady state could not have been obtained during the 23.3 s of weightlessness. During the +2-Gz turns, however, CVP did not change significantly compared with the values obtained during upright sitting at +1 Gz. Counter-regulatory mechanisms - e.g. peripheral vaso-constriction and increased abdominal pressure (20) - may have accounted for the maintenance of CVP during this period. If this was the case, the compensatory responses were probably also elicited during the pull-up phase of the parabolic maneuver and may have counteracted the caudal displacement of blood. Therefore the influence of pull-up on our results is difficult to evaluate.

The measured increase in CVP could not have been caused by pressure changes in the cabin because the transducer measured the

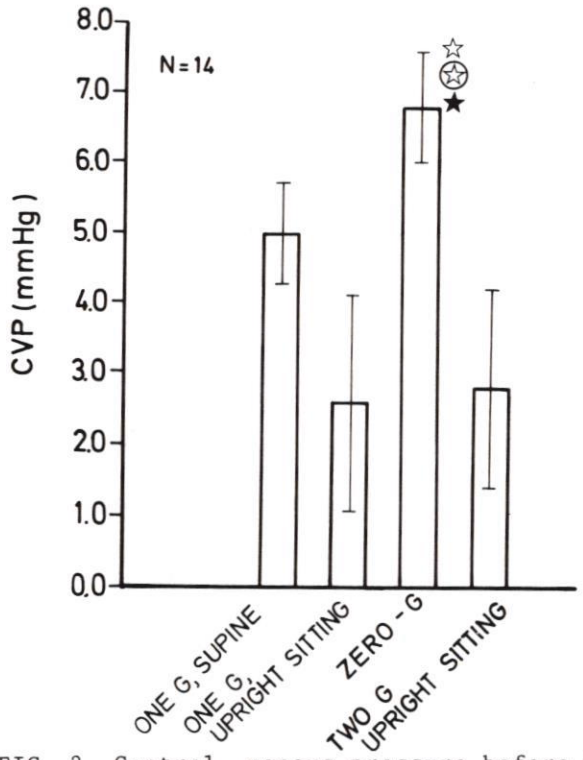

FIG. 2. Central venous pressure before the parabolic maneuver inflight (one G, supine and one G upright sitting), during weightlessness (zero-G) and during +2 Gz (two G upright sitting). Stars indicate significant difference from three other values

difference between intravascular and cabin pressure while the subjects breathed at cabin pressure. Furthermore, the aircraft was able to keep a constant pressure.

Compared with the supine position preflight, Kirsch et al. (8, 9) found a decrease in CVP estimated from peripheral measurements 22 h into the Spacelab 1 mission and as early as 20 - 45 min inflight on Spacelab D-1. Several explanations may be given to the discrepancy between the results of Kirsch et al. and ours. 1) The methodology was different in the two studies because Kirsch et al. used peripheral venous catheters. They were therefore only able to compare with the supine position on ground. Since the change in CVP from this position at 1 G to weightlessness is probably small it may be difficult to detect such a change with short peripheral catheters in a limited number of subjects. 2) Cardiovascular adaptation might have taken place as early as 20 - 45 min inflight accelerated by the supine leg-up position of the astronauts before and during launch (13). 3) Different states of hydration pre and inflight during the Spacelab missions may have confounded interpretation of the results. We found that the ground-based CVP values at the hospital 3-5 h before the flight were significantly higher in the supine position than in the same position inflight at 1 G (1 Gx). The reason for this may have been a slight dehydration because 11 of the subjects did not drink or eat from the morning of the experiment until the end of the flight.

The increase of CVP during weightlessness in our experiment by 1.8 +/- 0.5 mmHg compared with the supine position at +1 Gx (Fig. 3) is comparable to the increase of 2.0 mmHg measured during simulated zero gravity utilizing the -5 degree head-down tilted bed-rest model (14). The increase by 4.3 +/- 1.2 mmHg from upright sitting at +1 Gz to weightlessness (Fig. 2) is from one-fourth (1) to one-half (15) the increase observed during thermoneutral water immersion with the subjects in the upright sitting position (another model to simulate weightlessness).

Even though it has been clearly demonstrated in this study that CVP in humans increases during weightlessness, the physiological significance of this increase remains to be determined. This would require transmural CVP changes to be estimated e.g. by simultaneously measuring esophageal pressure as an estimate of intrapleural pressure. Thus by subtracting a change in esophageal pressure from a change in CVP during weightlessness would more accurately reflect changes in filling pressures to the right heart. A future obligation should be to do this.

The significant increase in HR during the +2-Gz period was expected (2, 20). The increase during weightlessness compared to the supine position at +1 Gx was in agreement with the reported increase of 9% during space flights (12). The very short period of exposure to weightlessness in our study, the pull-up phase of up to +2 Gz before weightlessness, and the possible psychological arousal may also have affected our HR responses.

Parabolic flights have an experimental advantage over space flights because they provide the only currently available means of direct transition from +1 or +2 Gz to 0 G without an intervening adaptation in the

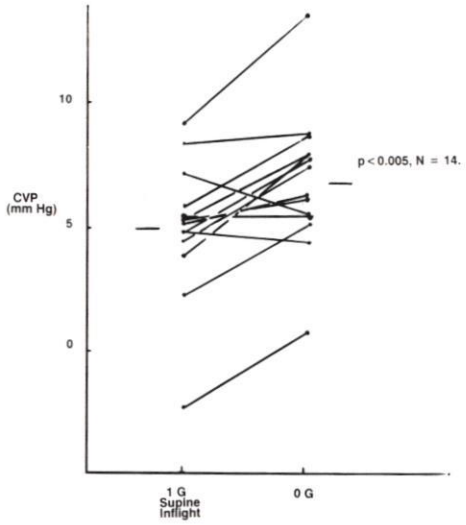

FIG. 3. Central venous pressure (CVP in mm Hg) in each subject (N=14) during +1 Gx (supine position inflight when aircraft was flying straight and level) and weightlessness (0 G) for 23.3 s. The individual values indicate that CVP increased during weightlessness when compared to supine at 1 G.

supine seated launch position. However, parabolic flights do not permit periods of weightlessness extended beyond 45 s (2).

In conclusion, the results from this study demonstrate that the acute onset of weightlessness in upright seated humans during parabolic flights increased CVP significantly compared with both the supine (+1 Gx) and upright sitting (+1 Gx) position, respectively, at 1 G before the maneuver. This suggests that CVP may also be increased during the initial period of weightlessness in orbital flights.

Acknowledgements

This work was supported by the Danish Space Board Grant 1112-33/85. We acknowledge the pilot, Major B. V. Jensen for his flying performance, the Royal Danish Air Force for the use of the aircraft, and the x-ray department at Hvidovre Hospital for assistance during catheter insertions. We are indebted to Mette Boesgaard for the expert preparation of this manuscript.

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CARDIORESPIRATORY RESPONSES TO SIMULATED WEIGHTLESSNESS IN MAN

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Experiments on 9 healthy male volunteers were done to study cardiorespiratory responses to the so-called dry immersion of 7 days in duration. During the first 7 hours of immersion the main parameters of central circulation were consistently high. Lung ventilation increased by 11.9-16.5%. By the 15th min of immersion, P_{aCO_2} grew by 1.4 mm Hg. P_{aO_2} and oxygen saturation of arterial blood changed insignificantly. On day 2 of immersion the central venous pressure, mean pulmonary pressure, stroke and cardiac indices decreased by 64.1%, 37.3%, 13.9% and 15.9%, respectively. Lung hyperventilation disappeared. The above cardiorespiratory responses seem to be typical of early stages of adaptation to fluid shifts in the cranial direction which occur in real space flight.

It is known that cardiovascular responses to space flight effects have been studied in great detail whereas the respiratory function in weightlessness remains inadequately understood. However, there is strong evidence that the respiratory function changes, probably, due to central circulation shifts. The data available are insufficient to determine the magnitude and mechanisms of cardiorespiratory responses to weightlessness. Hence, the importance of investigations of the cardiorespiratory function, especially during the first hours and days of exposure to real and simulated weightlessness. These investigations are needed to prognosticate reliably the development of human adaptation to weightlessness in long-term space flights.

The purpose of this study was to evaluate cardiorespiratory changes, gases and oxygen saturation of arterial blood in man during 7-day simulated weightlessness.

Methods

The experiments were performed on 9 healthy male volunteers (mean age 33 years, mean height 1.79 m, mean body weight 86 kg). The physiological effects of weightlessness were simulated by so-called dry immersion according to the method described elsewhere (16). Two days before immersion Swan-Ganz catheters with a thermistor were implanted into the pulmonary artery and Teflon catheters into the brachial artery, where they remained during 10-11 days (implantation was performed by V.V.Chestukhin). Cardiorespiratory measurements and blood withdrawal were made at rest every hour during the first 7 hours of immersion day 1 and then once a day during subsequent 6 days of immersion. Central venous pressure (CVP), pulmonary artery pressure (PAP) and systemic arterial pressure (AP) were measured by Siemens-746 electromanometers and recorded by Mingograf-82 ink recorder. Cardiac output was determined by the thermodilution technique using an Edwards Lab. computer (model 93A-131-7F) and thermodilution curves were recorded in parallel. Heart rate (HR) was calculated from ECG. Total lung resistance (TLR), right ventricle work (RVW), cardiac index (CI) and stroke index (SI) were calculated using standard formulas. Respiratory parameters were measured by a Beckman MMC model. Arterial blood gases were determined by an automatic AVL-940 gas analyzer. Hemoglobin was measured by the cyanmethemoglobin method. Statistical analysis was performed by the Student's t-test.

Results

During 7 days of immersion the health condition of the test subjects was adequate. Cardiorespiratory changes did not develop into pathological states.

During the first hours of immersion central circulation parameters were increased (Fig. 1). Absolute values of CVP, CI, SI, RVW and TLR were very close to the baseline levels recorded in the supine test subjects. During the 1st hour of exposure, some subjects showed a short-term but a marked increase of CVP, CI, SI and RVW. At the 15th min after immersion these changes averaged 12.8%, 8.2%, 6.2% and 6.4%, respectively, but were statistically insignificant as compared to the control. During this period, HR ranged from 72 ± 3 to 74 ± 3 beats.min^{-1} (2.8%), right ventricle end-diastolic volume (EDV) increased by 4% and end-systolic volume (ESV) decreased by 4%. Pulmonary artery systolic pressure grew by 4-4.5 mm Hg ($p > 0.05$). By the 7th hour of immersion initial EDV changes levelled off and ESV, RVW and TLR tended to decrease by 10%, 15.4% and 10%, respectively.

By immersion day 2, systolic, diastolic and mean PAP pressure decreased by 16.2%, 37.3% and 26.4%, respectively; by 3 day - CVP - by 87.2%, by 7 day - systolic AP by 8.5% (Fig. 2). After its decrease on day 3 CVP remained lower than the baseline value. HR varied insignificantly. The parameters calculated from the ther-

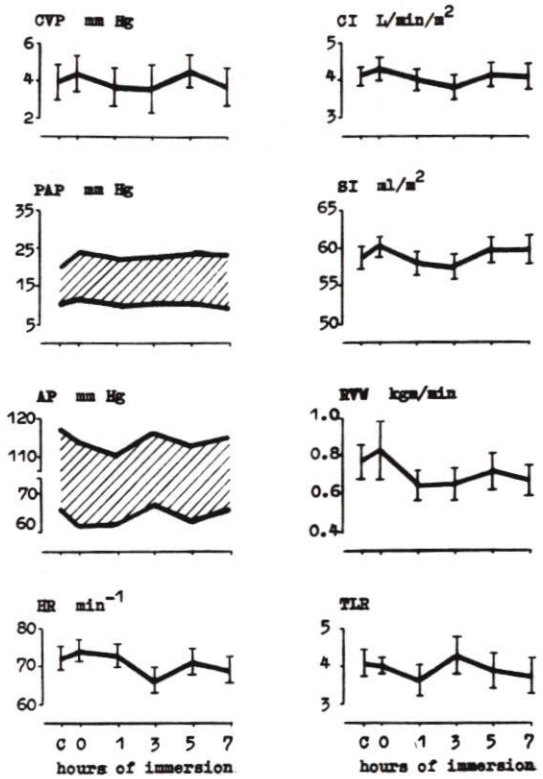

Figure 1. Variations in cardiovascular parameters during the first 7 hours of immersion.
C - control, * $p < 0.05$ as compared to control.

modilution curves also diminished significantly. As early as by 2nd day, CI and SI decreased by 15.9% and 13.9%, respectively. The blood volume in the right heart decreased by 57 ml (15%) and remained unchanged thereafter.

By immersion day 2 right ventricle EDV and ESV decreased significantly: EDV by 15% and ESV by 20%. RVW and TLR decreased by 38.5% and 37.1%. Subsequently, the parameters stabilized at these levels and remained practically unchanged till the end of the study.

The time course of the respiratory parameters, arterial blood gases and oxygen saturation are given in Figs. 3 and 4. Fifteen minutes after the beginning of immersion, lung ventilation ($\dot{V}_E$) increased by 16.2%. During 5th and 7th hours V_E was by 16.5% and 11.9%, respectively, higher than in the control ($p < 0.05$). Lung ventilation grew mainly due to the breathing frequency increase (f), which averaged 20.9-25.5% and due to a slight decrease (8.2%) of the tidal volume (V_T) ($p < 0.05$). The f values reached a maximum during the 1st hour of immersion.

Analysis of individual responses showed that during the first hours of immersion oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were higher than in the control. The maximum increase in $\dot{V}_{O_2}$ was by 15th min of immersion. It was 11.1% but was not significant ($p > 0.05$). On immersion days 2-3 $\dot{V}_E$, f as well as $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ diminished reaching the values recorded pretest in the supine position and remained essentially unchanged thereafter. On immersion days 4-7 these parameters were lower than before the exposure.

During immersion PO_2 and oxygen saturation of arterial blood did not change significantly. During the 1st hour of immersion PaO_2 tended to decrease /on the average by 2.6 mm Hg ($p > 0.05$)/ and then to increase by the 7th hour of exposure /by 3.4-6.1 mm Hg ($p > 0.05$)/. These changes in PaO_2 occurred together with a small but significant (by 1.4 mm Hg) rise of $PaCO_2$ at the onset of immersion.

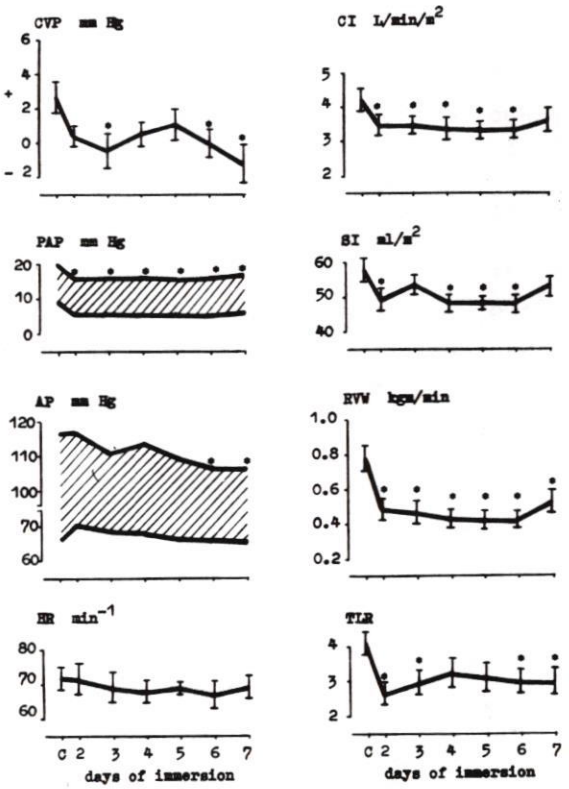

Figure 2. Variations in cardiovascular parameters during 7-day immersion.
C - control, * $p < 0.05$ as compared to control.

Discussion

Our investigations showed that immersion resulted in significant changes of circulatory and respiratory parameters that varied in a phase-like manner and depended on the test duration. One of the first responses was a rapid increase of CVP and PAP. Our observations agree with the literature data suggesting that during the first hours of immersion central circulation parameters are consistently high (2,13). A small additional rise in CVP, PAP and right heart volume at the initial phase of immersion when compared to the effect of the upright-supine

transfer can be associated with an additional gravity-induced blood shift in the cranial direction, with a rapid displacement of extracellular fluid to circulation and relative hyperthermia (13, 17).

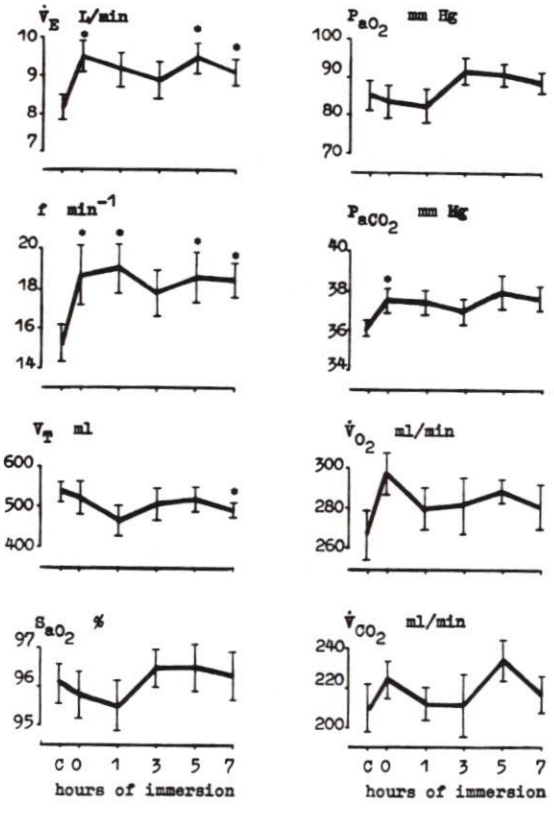

Figure 3. Variations in respiratory parameters during the first 7 hours of immersion.
C - control, * $p < 0.05$ as compared to control.

Venous return to the heart that grows at the onset of immersion increases the thoracic blood volume and stimulates compensatory reactions. At the early stage of immersion $\dot{V}_E$ and f increased as well. This result conforms to the findings of Golovkina (9), who reported a 7.7% increase of $\dot{V}_E$ and a 30% increase of f with no changes in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ and also to the measurements performed in "Voskhod-2", "Soyuz-4,5,6,7" (10), "Salyut-6" (8) and "Skylab" flights (14). In some space flights $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were also measured (10,14) but the data obtained are limited and contradictory.

A more marked increase in $\dot{V}_E$ as compared with $\dot{V}_{O_2}$ is suggestive of lung hyperventilation in our subjects during the first hours of immersion. The nature of this phenomen still remains obscure. On the one hand, enhancement of lung ventilation at the very onset of immersion may partly be produced by emotional reactions of the test subjects to an unusual environment. On the other hand, enhanced lung ventilation may serve as a compensatory response to reduced lung volumes, modified pulmonary circulation and impaired gas propagation in small airways. According to Tikhonov (18), during the first hour of immersion the functional residual capacity (FRC) decreased by 7%. This was followed by a 20% decrease in lung compliance and a 65-80% increase in airway resistance. It was shown (3,19) that on immersion days 1-2 the closing volume (CV) and its fraction in the vital lung capacity (VLC) increased. By increasing the fractional lung volume with altered ventilation, the above changes enhance the ventilation-perfusion maldistribution and may result in a larger venous admixture and drop in PaO_2 (1,4).

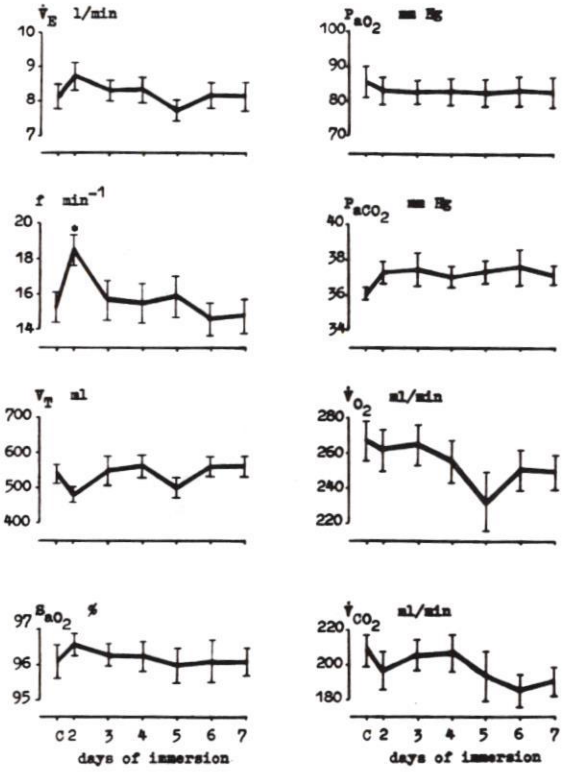

Figure 4. Variations in respiratory parameters during 7-day immersion.
C - control, * $p < 0.05$ as compared to control.

In this study, we did not reveal a statistically significant drop in PaO_2 or in oxygen saturation of arterial blood. On the contrary, after lung ventilation increased PaO_2 tended to grow. In this case, the above regional changes in alveolar ventilation seemed to be less pronounced and were completely made up for by a slight increase in the minute breathing volume. Hence, hyperventilation during the first day of immersion was an important compensatory reaction required to provide an adequate cardiorespiratory function and to maintain normal oxygenation of arterial blood during this transition period.

Our investigations demonstrated that the phase at which intrathoracic blood pressure grew occurred only at the very onset of immersion and was rapidly re-

placed with a longer and more pronounced phase at which this integrated parameter lowered. The data obtained are in good agreement with the time course of respiratory parameters described by Tikhonov (18). He found maximum changes in airway resistance and dynamic compliance during the first 30-60 min of immersion. Afterwards, these parameters tended to recover.

The above cardiorespiratory variations seen during the first 2-3 days of immersion may be produced by specific neurohumoral reactions (Gauer-Henry reflex) that diminished blood volumes in the thorax and lungs.

As follows from published data, at this stage of immersion the major portion of the fluid displaced towards the head is excreted from the body, the circulating blood volume decreases and pulmonary circulation seems to be improved (5,6,12).

According to our data, by this time period (i.e. by immersion day 2-3) lung hyperventilation was over. Thus, it can be supposed that at that stage cardiorespiratory parameters were more or less adapted to the effects of simulated weightlessness. Distinct parallelism between variations in lung ventilation and fluid-electrolyte balance at an early period of immersion suggests that hydrostatic changes and fluid redistribution play the key role as the stimulus triggering complicated mechanisms of adaptation of the human body on the whole and the cardiorespiratory system in particular.

In US Space Shuttle astronauts, CVP also increased at first and decreased by flight day 3 (15). It is not easy to interpret the nature of these CVP variations. According to Gauer et al. (7), CVP fall can indicate a circulating blood volume decrease. In our study, CVP fall can in part be a result of a greater contribution of chest suction to the adequate venous return under the conditions of negative pressure breathing and reduced circulating blood volume.

Lower right ventricle end-diastolic volume detected in our subjects on immersion days 3-7 may be considered as an early manifestation of cardiovascular deconditioning. At that period of time our subjects also showed lower values of V_{O_2}, V_{CO_2} and systolic AP that were adequated to the new requirements associated with reduced physical activity.

Another method used to simulate physiological effects of weightlessness is head-down tilt. Comparison of cardiorespiratory effects of 7-day head-down tilt and immersion shows that they have both similarities and differences. The differences can be seen mostly during the first hours of exposure and are probably associated with primary effects of immersion. For example, during head-down tilt PAP, RVW, TLR increase by the 7th hour of exposure reaching the values seen immediately after immersion (11). It is very likely that during the first hours of head-down tilt the above changes develop at a slower rate than during immersion; however at later stages they develop in a qualitatively similar manner.

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GRAVITATIONAL INFLUENCE ON SYSTEMIC ARTERIAL DYNAMICS USING A 3-ELEMENT WINDKESSEL MODEL

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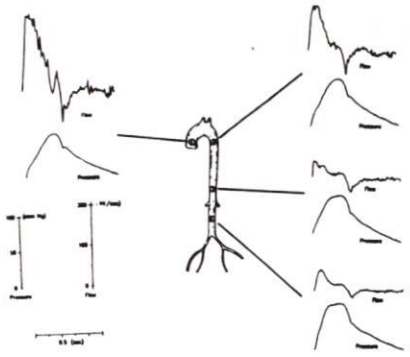
Most of the hemodynamic data known for man has been acquired from supine hemodynamic studies in a clinical cardiac catheterization laboratory (1-2). Some noninvasive work has been done which has looked at the postural effects of hemodynamics, but sophisticated studies of central circulatory dynamics are few in number (3). It is technically difficult to perform supine and upright studies in human subjects as most catheterization laboratories are not equipped with upright tilt tables. An animal model which is phylogenetically close to man would be an acceptable alternative in which to study some of these basic effects. We have evaluated the hemodynamic response to passive upright 70° tilt in six baboons to assess the effects of gravity on systemic compliance (C), aortic characteristic input impedance (Zc) and peripheral resistance (R) as determined by the three-element Windkessel model.

Methods

Baboons were studied in the cardiac catheterization laboratory at the Southwest Foundation for Biomedical Research in San Antonio, TX. Study animals were initially sedated with ketamine 30 mg/kg., and intubated. Sedation was maintained by intermittent boluses of Nembutal (3 mg/cc for 20-30 ml bolus). Local anesthesia for left carotid artery cutdown was achieved with 2% xylocaine by local infiltration. Left heart catheterization was performed using multisensor high-fidelity catheter that has a micromanometer located at the tip and a second micromanometer 3 cm proximal. An electromagnetic flow velocity probe was mounted with the proximal pressure transducer housing. This catheter was advanced through an arteriotomy in the left carotid artery and fluoroscopically positioned in retrograde fashion across the aortic valve. This allowed simultaneous measurement of left ventricular pressure, aortic root pressure and aortic root flow.

The catheter was manipulated into the descending aorta to record simultaneous pressures and flows at various sites (see Fig. 1).

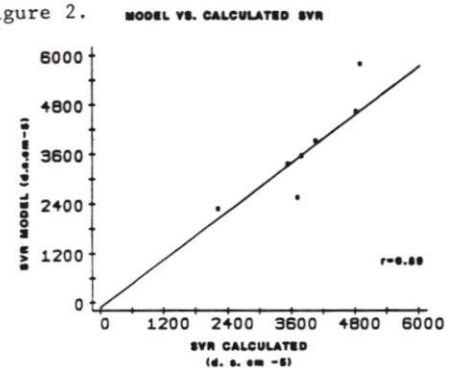


Full length angiography was then performed. Physiologic data were archived on 1" FM analog tape after passing signals through a low pass band filter with a corner frequency of 100 Hz and roll-off 3 dB/octave to avoid aliasing. The signals were simultaneously digitized at a 200 Hz sample rate with 12 bit resolution. Digital data were processed to average four representative beats of pressure and flow. Pressure and flow data were then submitted to three-element Windkessel model used to determine the parameters of C, Zc and R (4). The computer model accepts the input pressure and adjusts the parameters to determine a calculated flow and compares the calculated flow with the actual measure flow for goodness-of-fit. Using a modified Newton iteration technique, the model adjusts the parameters until the calculated flow returned by the model gives the best fit of the actual measure flow by χ^2 and returns the final parameters of C, Zc and R. The flow signal was calibrated to volumetric flow using thermodilution-derived stroke volume. The aortic dimensions from angiography were used to calibrate the descending aortic flow signals to volumetric flow.

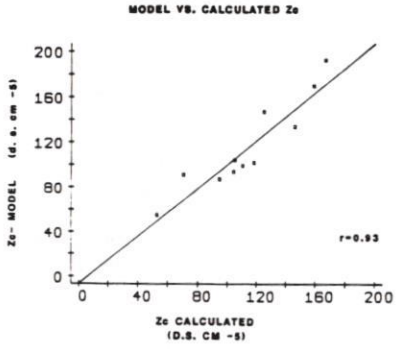
Independent calculation of Zc was performed using a Fourier analysis on simultaneous pressure and flow data. Input impedance was calculated by dividing harmonics of pressure by corresponding harmonic moduli of flow, and their phase angles were subtracted. The higher harmonic moduli were averaged to determine the characteristic input impedance, Zc. This Zc was compared to the Zc returned by the model. Systemic vascular resistance was determined by dividing mean pressure by mean flow in the usual fashion and compared to the value returned by the model. Compliance was estimated from the time constant, τ, of diastolic pressure decay of the aortic pressure waveform. Compliance was estimated from the relationship tau=RC. The compliance calculated from this two-element model was then compared to the compliance calculated by the computer model.

Results

The comparison of the characteristic impedance calculated versus model-determined is shown in Figure 2.



Comparison of the systemic vascular resistance by independent calculation with that of the model is shown in Figure 3.



The compliance determined by the two-element and the three-element model when the same order of magnitude but correlation with the line of identity was only 0.4. Supine to upright data is shown in the following table.

	Zc ($d \cdot s \cdot cm^{-5}$)	R ($d \cdot s \cdot cm^{-5}$)	C (cc/mmHg)
CM (supine)	140±16	3689±296	.37±.07
CC (supine)	151±38	3648±241	.36±.02
CM (tilt)	88±18	3931±396	.57±.10 *
CC (tilt)	113±21	3567±450	.42±.03

CM = computer model, CC = conventional calculations. Mean ± SE; * $p < .05$.

Conclusions

These data show that the three-element Windkessel model fit of pressure and flow to determine characteristic impedance, systemic arterial compliance and peripheral resistance produces similar results to independent calculations of these parameters. Additionally, the influence of posture in this primate model reveals that gravitational stress to passive upright tilt has its most prominent effect on characteristic impedance and peripheral resistance.

These results are limited by several factors: (1) The study is performed in sedated primates, and this may have significant adverse effect on baroreflexes. (2) Additionally, the Nembutal sedation resulted in steady state hypertensive and tachycardic study conditions. (3) Results may be different in the setting of chronically instrumented conscious primates, a study which is ongoing, or with active changes in posture.

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REGIONAL AORTIC PRESSURE APPARENT PHASE VELOCITY IN THE BABOON DURING PASSIVE 70 DEGREE TILT

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This study examined the effects of a passive change in position from supine to upright, that is from 0 to 1 Gz, on the regional vascular properties of the aorta. Regional vascular properties may be characterized by a number of parameters to include: pressure and flow measurements that yield impedance, geometric changes in the aorta and regional foot-to-foot pulse wave velocity or apparent phase velocity. We evaluated apparent phase velocity as a measure of vascular function because it offers much information such as: influence of wave reflections; it is proportional to local impedance; it is an indicator of elastance; and the mean of the higher frequency components yields wave velocity. We hypothesized that if the aorta is considered to be bathed in a fluid-like container, then there should be no hydrostatic gradient between the aorta and surrounding tissues in the upright posture, and therefore, no change in the apparent phase velocity with changes in position.

METHODS

Apparent phase velocity measurements use the results of the Discrete Fourier Transform (DFT) of two pressure pulses measured simultaneously from separate micromanometers. The DFT yields a series of sinusoids the sum of which equals the original signal. Each sinusoid in the series is a harmonic having a specific phase and amplitude. Apparent phase velocity (C_{app}) calculations use the difference in phase angle for each harmonic (3,4):

$$C_{app_n} = \frac{2\pi f n \Delta z}{(\phi_2 - \phi_1)_n}$$

where f is the fundamental frequency, n is the harmonic number, Δz is the distance between the sensor pairs measured in centimeters and $\phi_2 - \phi_1$ is the difference in phase angle between the sensor pairs at the n^{th} harmonic. Apparent phase velocity is also related to elastance by the equation

$$C_{app} = \sqrt{\frac{Eh}{2\rho R}}$$

where E is the elastic modulus, h is the wall thickness, ρ is the blood density and R is the lumen radius. Finally, mean C_{app} is related to impedance as

$$\bar{C}_{app} = \frac{Z_c A}{\rho}$$

where Z_c is the characteristic impedance and A is the cross sectional area of the vessel. This relationship assumes the phase of the global reflection coefficient is small and Z_c is mostly a real number.

Each of eight mature baboons (Papio anubis) were lightly anesthetized with nembutal prior to insertion of a Millar high-fidelity multi-micromanometer catheter using a left femoral approach. This catheter is 8F at the base tapering to 6F at the tip with a total of 8 sensors spaced 5 cm apart. The catheter was positioned under fluoroscopic guidance so that the tip was placed in the aortic root. Pressure signals were low pass filtered at 100 Hz and A/D converted at 200 Hz. Simulataneous pressure measurements were made from all 8 sensors first in the supine and then in the upright position.

Pressure data were analyzed by first selecting a single pressure pulse (three pressure pulses were used for each position), followed by subtraction of the mean (1), windowing with a Hamming window (2), readjusting for alterations in the mean, performing the DFT, calculating the phase angle and finally calculating the apparent phase velocity using the 4^{th} through 15^{th} harmonics. Stastistical analysis was performed using a two-way analysis of variance (ANOVA) comparing effects of sensor location and tilt. One-way ANOVA was performed within position and across sensors, and finally, a multiple comparison means range test was employed where appropriate to determine whether regional differences in $\bar{C}_{app}$ could be identified.

RESULTS

The baboons had elevated heart rates, pressures and cardiac outputs compared to unsedated animals ($p<0.05$) (Table 1).

BABOON HEMODYNAMICS

	Position	
	Supine	Upright
Heart Rate	112±25	119±25
Systolic Pressure	177±10	168±5
Diastolic Pressure	118±13	120±10
Cardiac Output	3.3±0.7	3.5±0.9

Table 1.

Aortic pressure waves were digitized as shown in Figure 1 and a single pressure pulse selected and processed in the time domain as previously described (Figure 2).

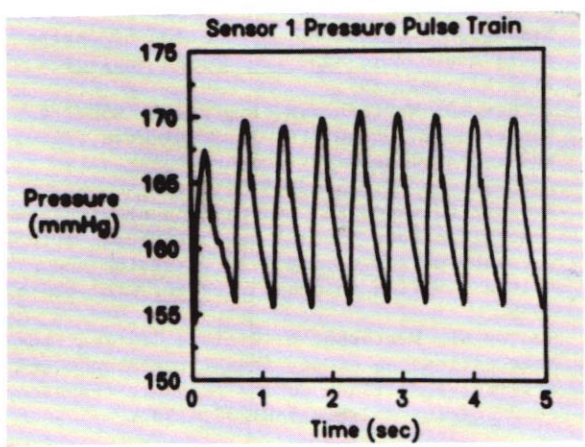

Figure 1.

The DFT was applied to the wave form of

Figure 2 yielding real and imaginary components for each harmonic. Phase angles were then calculated using the arctangent of the imaginary component divided by the real component. An example of the phase angle results for a single beat for all sensors is shown in Figure 3 where proximal sensors lead (are above) more distal sensors.

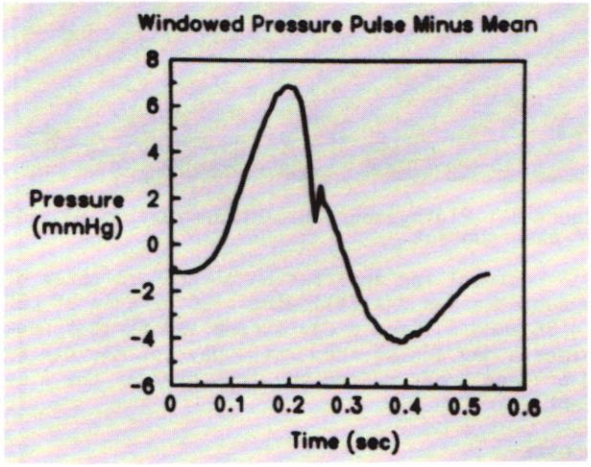

Figure 2.

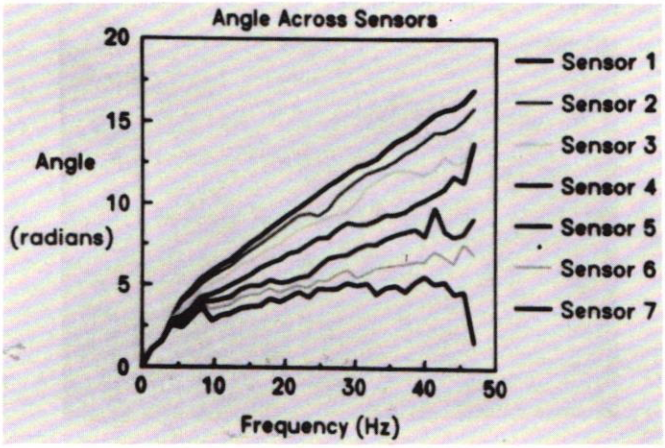

Figure 3.

Apparent phase velocity measurements were averaged across sensor pair and position with results shown in Table 2 and plotted in Figure 4.

APPARENT PHASE VELOCITY

Sensor Pair	Position	
	Supine	Upright
S1-S2	762±144	679±136
S2-S3	757±166	749±131
S3-S4	598±75	596±73
S4-S5	593±95	633±87
S5-S6	763±84	716±85
S6-S7	740±110	900±87

Table 2

Statistical analysis showed no difference between supine and upright regional $\bar{C}_{app}$. However, for the upright position, the most distal $\bar{C}_{app}$ was greater than the proximal $\bar{C}_{app}$ ($p<0.05$).

DISCUSSION

The processing techniques described here-in, specifically, removing large low frequency data

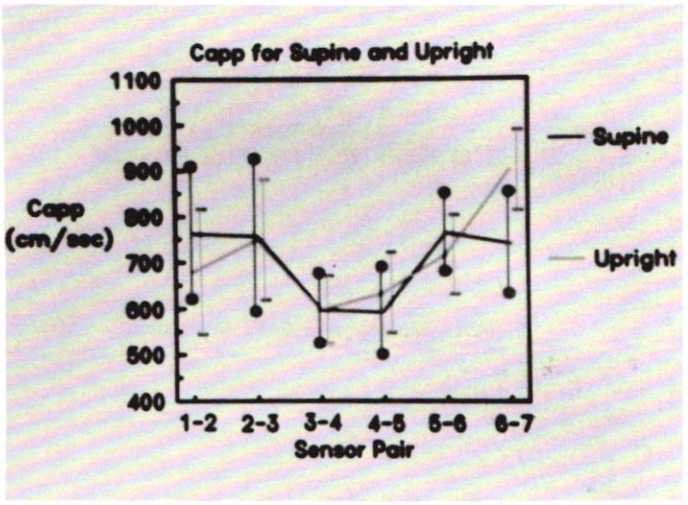

Figure 4.

below the frequency range of interest and windowing, reduces the standard deviations of apparent phase velocity measurements. When applying the Fourier transform, the mean and large low frequency components should be removed to reduce leakage (1) that spills into the frequency range of interest. The discontinuity between the ends of the extracted signal must also be removed by using a window other than the rectangular window (2). These two techniques reduce the standard deviation in $\bar{C}_{app}$.

High $\bar{C}_{app}$ values in the aortic arch may be a result of using 5 cm as the spacing between sensor pairs instead of linear distances. This distance was remeasured using cinifluoro and found to be 2.9 cm and 3.3 cm for sensor pairs S1-S2 and S2-S3, respectivly. As a result, $\bar{C}_{app}$ values for these pairs fell more in line with those values found in sensor pairs S3-S4 and S4-S5.

Although there was no difference in the mean apparent phase velocity measurements between the supine and upright position, it is worth noting that the pressures were quite high in the sedated baboon. Apparent phase velocity measurements are dependent on the mean pressures. It may be that in our preparation any hydrostatic effects of a change in position were masked by the high pressures seen in the sedated animal.

Since a significant increase in $\bar{C}_{app}$ was found between distal sensors in the upright position and no difference was found in the supine position, data suggest that there may be an increase in characteristic impedance in the upright posture which occures below the hydrostatic indifferent point. The reverse is suggested in the aortic root but is not significant. Future work with chronically instrumented baboons will test the trends of lower apparent phase velocities in the proximal sensors and higher measures in the distal sensors for the upright verses supine position.

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HEMODYNAMICS OF LEG VEINS DURING A 30 DAYS BED REST - EFFECT OF LOWER BODY NEGATIVE PRESSURE (LBNP)-

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Introduction

The response of lower limb capacitance vessels to microgravity is one of the determining factors of the orthostatic intolerance syndrome observed in astronauts when they return to earth. In order to test this response under simulated weightlessness (which has not been much studied in litterature) and to test the efficiency on this response of LBNP used as a preventive measure against cardiovascular deconditionning, we studied the leg venous compliance of six subjects exposed to head-down bedrest for thirty days (minus six degree head-down bedrest). Of the six subjects, three were regularly submitted to LBNP (LBNP subjects) ; the other three were not exposed to any countermeasure (control subjects).

Material and Methods

Leg venous compliance was measured by strain gauge plethysmography with venous occlusion.

The apparatus is the simplified Perivein system (ETNA, France). The cuffs for venous occlusion were applied around the whole thigh. The mercury strain gauge was placed around the calfs. Its high internal electrical resistance varies linearly with lengthening. Comparative lengthening of the strain gauge is proportionnal to the comparative variations of leg volume. Subjects were examined under standard conditions in the supine position . Their heels were elevated twenty five centimeters above the bed level.

Compliance is the change in volume for a given change in pressure and, in this experiment, it was assumed to be a change in leg venous volume (ml/100 ml of tissue) induced by cuff pressures of 25, 30, 35, 40, 45 and 50 mmHg.

For each subject, plethysmographic determinations were performed 4 times : in the pre-bedrest period, on day 6 of bedrest (D6), on day 20 of bedrest (D 20) and on day 5 of the recovery period (D + 5). (Figure 1).

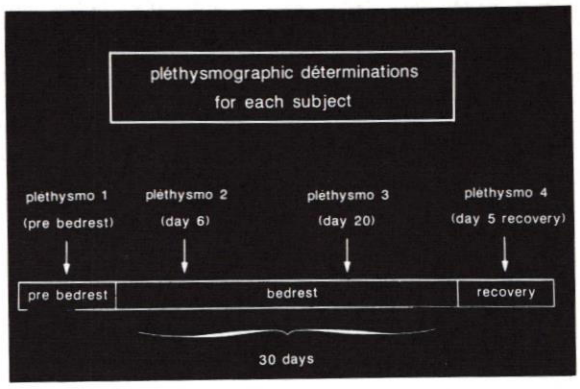

Figure 1 . Plethysmographic determination protocol for each subject.

Protocol for application of LBNP in three of the subjects was the following : 20 minutes of LBNP at - 35 mmHg 3 times a day during the first 3 weeks, 4 times a day during the first 4 days of the 4th week and 6 times a day during the last days of the experiment.

Results

Mean leg venous compliance for the 2 groups of subjects is plotted in Figure 2 in terms of percent change compared with pre-bedrest compliance at 25 mmHg of counterpressure.

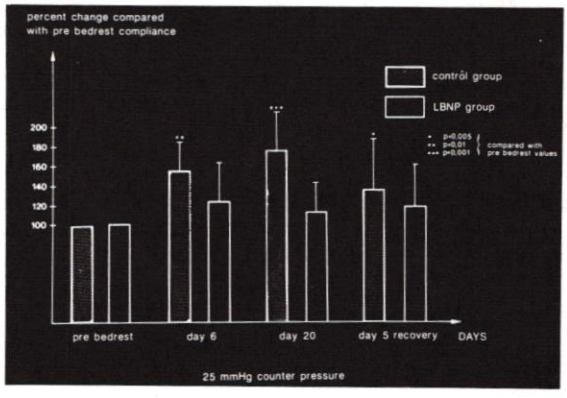

Figure 2 . Mean leg venous compliance for the two groups of subjects at 25 mmHg. Percent change compared with pre-bedrest compliance.

Percent change are presented in hatched bars for control subjects and in open bars for LBNP subjects. So there is a progressive increase in leg venous compliance at 25 mmHg for control subjects during exposure to head down bedrest. (D6 and D 20 of bedrest). This parameter tends to return but incomplety to initial values on D + 5. Comparatively, leg venous compliance in LBNP subjects does not differ significantly throughout the entire experimental period ie during pre-bedrest, bedrest and post-bedrest.

Figures 3 and 4 show the mean change in leg venous volume plotted against the different corresponding cuff pressures. Figure 3 represents 4 different curves of the Δvolume-cuff pressure relationship corresponding to each period of plethysmographic determination (pre-bedrest, D6, D 20 and D+5) in control subjects.

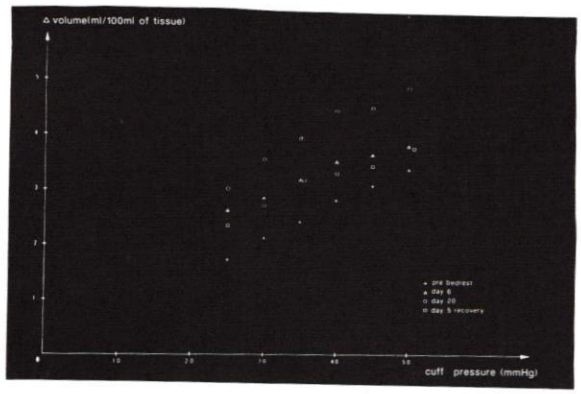

Figure 3 . Δ volume-cuff pressure relationship in control subjects.

It appears that changes in venous volume differ significantly according to the different bedrest periods at any counterpressure, Figure 4 represents the same curves determined for LBNP subjects. Δ volume-cuff pressure relationship does not differ significantly depending on the given bedrest period.

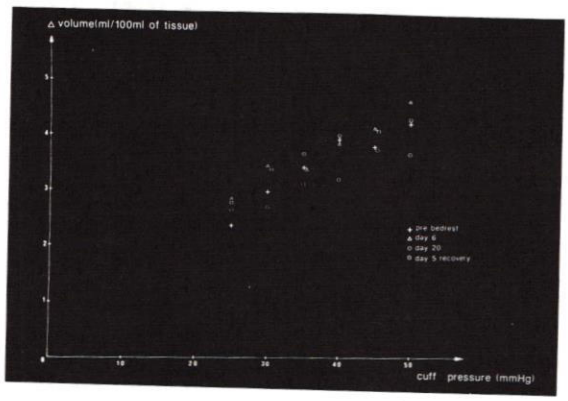

Figure 4 . Δ volume-cuff pressure relationship in LBNP subjects.

Discussion

This experiment shows that :

- - 6° head down bed rest is a good model to simulate the effects of microgravity on the capacitance vessels of the lower limbs. In the absence of manned spaceflights, we have the assurance to use a good experimental model to study more comprehensively the venous system with regard to the effects of microgravity.

Exposure of subjects to the LBNP protocol used in this experiment is an efficient way to counteract the opposite effects of microgravity on capacitance vessels. Since a partially efficient effect of LBNP on orthostatic tolerance has already been demonstrated, it is obvious that this latter finding is associated with LBNP induced improvement of the capacitance vessel reactivity. This evidences the fact that sensitivity to orthostatic stress is partly related to changes in leg venous compliance.

Considering these results, we can wonder what are the mechanisme involved in venous dilatation under weightlessness and in the improvement by LBNP exposure . We can hardly settle this question because of the lack of litterature data. However, several points have to be emphasized :

- primarily, the increase in leg venous compliance is evident at low levels of counterpressure (25 mmHg or minus). It is possible that this fact is in relation with the increased volume of the zone of free distensibility as it was assumed by **Jonhson** and his colleagues. In fact, if pressures lower than normal prevail in the veins, due to a diminished plasma volume, they tend to become elliptical or flat and could accomodate relatively large volumes of blood before any change in pressure occurs. This is the so called zone of free distensibility. LBNP could act in reducing the volume of this zone in maintaining a quasi normal plasma volume.

- the increase in leg venous volume is also obvious at high levels of counterpressure (35, 40, 45 and 50 mmHg). At those levels, transmural pressure across vein wall must result in stretching of venous musculature. Increased compliance of the veins, reduced tone of supporting muscles in proximity to the veins or even diminished tissue pressure could account for the greatest changes in venous volume in weightlessness : to what extent LBNP act on those mechanisms is unknown. Further studies concerning this problem would contribute to a better understanding of physiological mechanisms involved in the regulation of the venous system during adaptation to microgravity.

In summary, these results demonstrate a progressive increase in the distensibility of the veins of the lower limbs during an experiment of microgravity simulated by means of bedrest with a LBNP "protecting effect". Tney arise the question of mechanisms involved in venous dilation during exposure to weightlessness.

DIFFERENCE IN CARDIOVASCULAR RESPONSES TO BLOOD POOLING PATTERNS BETWEEN LBNP AND HEAD UP TINTING STIMULATED AFTER SUPINE CYCLING IN WOMAN

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To investigate a relation of the change in the leg volume to the cardiovascular adjustments to gravitational stimulus given just after a mild supine exercise by 40° head up tilting (HUT) and LBNP, 5 female students participated as subjects. LBNP was estimated to almost correspond to 40° HUT in each subject Calf volume identified as leg volume increased during the post exercise in order of the control, LBNP, and HUT. Averaged changing rate of leg volume (Δ LV) of 5 subjects during post exercise was significantly greater in HUT ($p<0.05$) and in LBNP ($p<0.05$) than in the control. Although the gravitational stimuli were loaded by different methods , the Δ LV was significantly correlated to stroke volume (SV) ($p<0.01$), cardiac output ($p<0.01$) and total peripheral resistance ($p<0.01$). The significant relations suggest that the decrease in venous return and thus central blood volume, assumed by SV, with increasing leg volume become a triger to facilitate the sympathetic nervous functions to maintain arterial blood pressure during post mild exercise.

Leg volume increasing with pooling blood in the lower body by gravitational stimulus should become one of important variables of cardiovascular adjustments to post-exercise.

In the present study, cardiovascular responses to the change in leg volume has been investigated as suddenly exposing female subjects under 40° head up tilting (HUT) and some LBNP just after mild supine exercise. The LBNP was estimated to be as almost the same degree of gravitational stimulus as the LBNP produced in 40° HUT each subject.

Methods

Five healthy female students participated to the study as subjects. Mean age, height, weight, VO_2max and LBNP load were 22.4years old, 158.0cm, 51.2kg, 40.68ml/kg/min, and 35.4mmHg, respectively. The experimental protocols and a formula of determination of LBNP were shown in Fig. 1. Through the experiments, VO_2 was determined by a metabolic analyzing system which was consisted of mass-spectrometer, gas flowmeter, and computer. EKG and heart rate (HR) were continuously observed on the visual face of an EKG monitor.

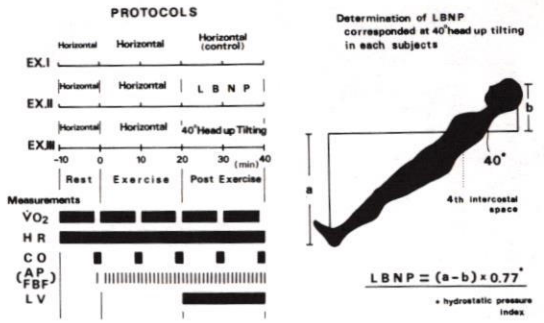

Fig. 1 The protocol of experiments and a formula which was used to determine the LBNP load in each subject.

Arterial blood pressure in the left upper arm was measured by an auscultation method. Cardiac output (CO) was measured by means of acetylene gas rebreathing method with a mass-spectrometer. Forearm blood flow (FBF) in the right arm was measured by means of a rubber straingauge plethysmograph. The changing rate of calf volume inentified as the change in leg volume (Δ LV) was measured by means of a rubber straingauge plethysmograph.

Result

In Fig. 2, The averaged (± SD) HR and mean arterial pressure (MAP) of the subjects was shown at 10 min. and 20 min. during post exercise in each experiment. The HR at 10 minutes was about 13% higher in LBNP ($p<0.01$) and 27% higher in HUT ($p<0.01$) than in the control. At 20 minutes, the HR was about 24% higher in HUT ($p<0.01$), and about 17% higher in LBNP ($p<0.01$) than in the control. But the difference between the two HR at 10 min. and at 20 min. in LBNP was not significant. The averaged MAP given at measuring points were not significantly different between each other in the three experimental conditions.

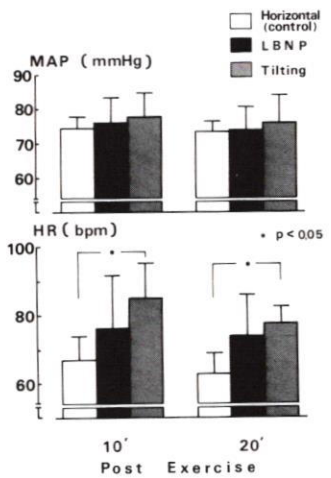

Fig. 2
Averaged (± SD) heart rate (HR) and mean arterial pressure (MAP) of the 5 subjects at 10 min. and 20 min. during post exercise.

In Fig. 3, the averaged (± SD) Δ LV and stroke volumes (SV) of the subjects were shown at 10 min. and 20 min. during post exercise in each experiment. The averaged Δ LV at 10 min. were about 320% higher in HUT and 260% higher in LBNP than in the control, while the Δ LV given was not significantly different between HUT and LBNP. At 20 min., Δ LV was about 430% higher in HUT and 280% higher in LBNP than in the control. Further Δ LV at 20 min. in HUT was significantly greater than in LBNP ($P<0.01$) The averaged SV at 10 min. during post exercise was about 50% lower in HUT, and about 34% lower in LBNP than in the control, but not significantly different. At 20 min., during post-exercise, the SV was about 40% lower in HUT, and about 33% lower in LBNP than in the control but not significantly different.

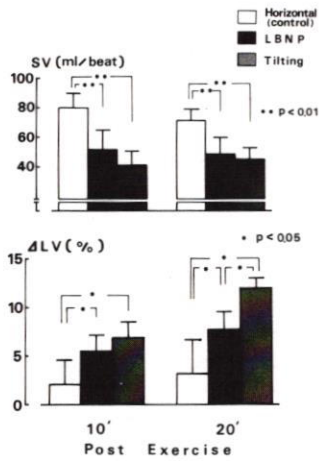

Fig. 3
Averaged (± SD) changing rate of leg vilume (Δ LV) and stroke volume (SV) of the 5 subjects at 10 min. and 20min. during post exercise.

In Fig. 4, the relationships of Δ LV to CO, SV and total peripheral resistance (TPR) were shown. All of the relationships were significantly correlated. That is, the Δ LV increased was directly related with TPR ($p<0.01$), but inversely related to SV ($p<0.01$) and CO ($p<0.01$

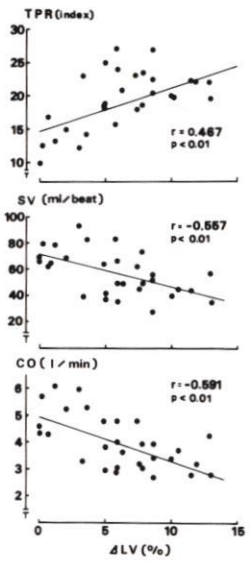

Fig. 4
The relationship of changing rate of leg volume (Δ LV) to cardiac output (CO), stroke volume (SV) and total peripheral resistance (TPR).

Consideration

In the present study, when the gravitational stimulus was loaded just after mild supine exercise, calf volume identified as a changing rate of the leg volume greatly increased 3 or 4 times the control values given in the horizontal rest during a 20 min. period in the post exercise. Although LBNP was estimated to almost correspond to 40° HUT in each subject, the increment in the Leg volume was greater in HUT than in LBNP. This is probably that the pooling pattern of blood in the lower body part was different due to the different stimulation methods with HUT and LBNP. However, the difference in Δ LV indicated in the two gravitational stimulation methods could not be solved by the present results. On the otherhand, even if there were significant increases in Δ LV with gravitational stlmulations more than the control, MAP was slightly, but not significantly increased in order of the control, LBNP, and HUT. Also VO_2, ventilation and FBF were not significantly affected by the increase in Δ LV. However, CO and SV were significantly increased with increaseing leg volume during post exercise. In addition, despite of non-significant decrease FBF had a decreasing tendency with increasing Δ LV, and TPR was significantly correlated with the Δ LV. These results suggest that sympathetic nervous activity facilitate to maintain or increase arterial blood pressure, even if CO is decreased with decreasing SV by a gravitational stimulation. The fact that SV was significantly correlated to Δ LV suggests the the decrease in SV with increasing Δ LV becomes a triger to facilitate the sympatetic nervous function during post mild exercise.

In conclusion, when gravitational loads are stimulated just mild supine exercise by means of HUT or LBNP the cardiovascular responses to increased leg volume are adjusted by sympathetic nervous functions which is facilitated by the activities of the baro-receptors seated on the carotid sinus, aorta, and heart. These baro-receptors are stimulated by lowering the perfusing pressure with decreasing venous return and the central blood volume as suggested by the decrease in SV.

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INCREASING CENTRAL BLOOD VOLUME WITH HEAD-OWN TILTING WOULD INHIBITE WATER INTAKE DURING MILD PEDALING AT 25℃ AND 35℃ ROOM TEMPERATURES IN WOMAN

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Water intakes during prolonged mild exercise in the two body positions of head-up tilting (HUT) and head-down tilting (HUT) were investigated with measurements of body temperatures and cardio-vascular responses under the two enviromental temperature (ET) of about 25°C and 35°C. Through the experiments, VO2, HR, arterial blood pressure, cardiac output, fore-arm-blood flow, mean skin and external audiomutus temperatures were measured. Water volume intaken at 50 min. of exercise in HUT was significantly greater in 35°C than in 25°C ($P<0.01$). In the HDT, the water intaken was almost the same as each other given in the ETs. The water intake was significantly correlated to the values of stroke volume (SV) ($p<0.01$). The presented results suggest that the increase in central blood volume identified as SV inhibits water intake during mild exercise in spite of the difference not only in body positions but also in ETs.

In the many previous studies, it has been provided that water intake is increased by the hyper-osmolerity or the hypo-volemia of body fluid. In our previous study presented in ICPS meeting of 1986, we have reported that water intake during prolong mild exercise at 26°C room temperature was increased by the posture of head-up tilting (HUT) as compared with the head-down tilting (HDT). And the water intake was significantly corelated to stroke volume, but not to body temperature. Therefore, it was concluded that the central blood volume and also brain blood flow maintained in HDT inhibited the center of water intake, because of lowering activity of the water intake receptors. In the present study, we have tried to confirm the previous conclusion, so that water intake during prolonged mild exercise in the two body positions of HUT and HDT was investigated with measurements of body temperatures and cardio-vascular responses under the two enviromental temperature (ET) conditions of about 25°C (averaged 26°C) and 35°C (averaged 37°C).

(Methods)

Five female students participated as the subjects in the present study. Their averaged values of body weight 54.8kg and height 159.2cm were almost the same as standard Japanese young women's levels. Their average VO2max was 40.86 ml/kg/min., and also included in Japanese standard values of young woman. Before the experiment, the subjects were informed details of the study and could not intake any water and food over the two hours before the start of experiments. After the rest, a 300 kpm/min ioad exercise was performed for one hour in 10°HDT or HUT. Water of about 7.5°C was at will drunk at 50 min. during exercise by the subjects. The water volume drunk was determined as water intake during exercise in each subjects. Through all experiments, oxgen uptake (VO2), heart rate (HR), arterial blood pressure, cardiac output (CO), fore-arm blood flow (FBF), mean skin temperature (TSK) and external audiomutus temperature (TEAM) were measured. VO2 was determined by a metabolic analysing system which was constructed with mass-spectrometer, gas flow meter and computer. HR was counted on the record of EKG by computer system. Arterial blood pressure was measured by mean of auscultation method in the right upper arm. CO was measured by acetylen gas rebreathing method, which the gases were analysed by a mass spectrometer. FBF was measured in the left fore-arm by means of rubber straingauge plethysmography. TSK and TEAM were measured by a thermister system.

(Results and Consideration)

Comparing TSK between the two body positions, it was not significant difference not only in 26°C but also in 37°C. However TSK in 37 C was significantly higher than it in 26°C in each of HDT and HUT ($P<0.01$). On the otherhand, TEAM during exercise was not significantly affected by the difference not only in the body position but also in ETs. HR during exercise was significantly increased by HUT comparing with HDT in each ET ($P<0.01$). Also, it was significantly increased by 37°C condition as compared with 26°C ($P<0.01$). VO2 during exercise was not significantly different not only in the two body positions but also in the two ETs. FBF was significantly increased by HDT as compared with HUT in each ET ($P<0.01$). The significant difference in FBF between the two positions was greater in 37°C than in 26°C. MAP was not so different not only in the two body positions but also in the two ETs, even if, it is slightly higher in HDT than in HUT under each ET condition. SV during exercise was significantly higher in HDT than in HUT under each ET condition ($P<0.01$). However, SV given in each body position was not significantly affected by

the difference in the ETs.CO during exerci -se was not significantly different in the two body positions and in the two ETs,alth -ough it is slightly higher in HDT than in HUT in each of the two ETs.The upper part of Figure 1 shows the averaged volume wat- er intake of the five subjects given at 50 min. during exercise in each experiment. Also , lower part of the figure shows the averaged decreases in body weights (Wt) in he five subjects between before and after the experiment.

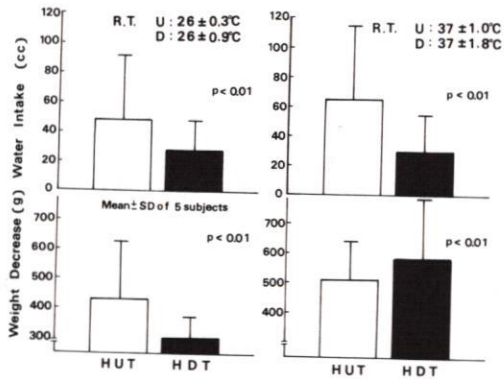

Figure 1.Water volume intaken during exercise and the differ -ence body weight between befo -re and after exercise

The decrement of the Wt. in 26˚C was signi- ficantly greater in HUT than in HDT(P<0.01). However,the decrement under 37˚C was ra- ther significantly greater in HDT than in HUT(P<0.01).The water intake in HUT was sig -nificantly greater in 37˚C than in 26˚C(P<0.01). However, in this case of HDT, the water intake was almost the same as each other given under the two ETs.

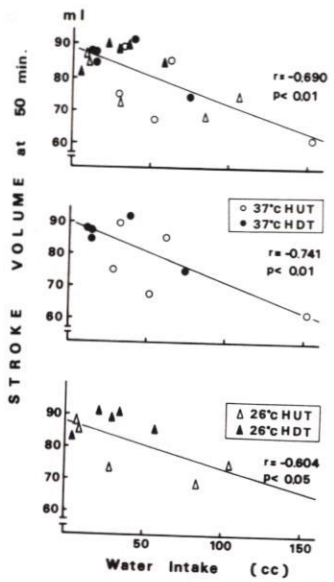

Figure 2. The relationship bet- ween water intake and stroke volume

Figure 2 shows the relationship between between water intake and SV, which were given just before drinking water in each ET of 26˚C and 37˚C,as collecting the all of the data.The water intake was signi- ficantly correlated to SV(P<0.01).The high significant correlation coefficients show that water intake was depend on the decrea- sing degree in the central blood volume identified as SV.On the otherhand, the re- lationship between water intake and TEAM did not show any significant correlation. That is, it is indicated that water intake during mild exercise was independ on chan- ing in the body temperature.

(Conclusion)

In the presented results, water intake du- ring prolonged mild exercise was smaller in HDT than in HUT not only under moderate ET condition but also under high ET condi- tion.The water intake was also independent on the sweat rate indicated by the Wt dec- rement given after the exercise and also on chan ging in the body temperature .On the otherhand,,the significant relationship between the water intake and SV suggests that the change in the central blood volume is one of the great influence factor on the water intake, so that water loss related to gravitation stimulus is strongly affected by the blood volume pooling in the upper body.In conclusion, we could confirm that the maintained central blood volume in the HDT inhibited the activity of the water intake center,because of lowering activity of the thoracic water intake receptors due to central blood pooling and of maintaining or little bit increasing in brain blood flow.

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PILOT PERFORMANCE IS INCREASED AFTER ALTERNATING HYPO- AND HYPERGRAVITY STATES

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Artificial gravity represents the only real solution of imminent deterioration of the astronauts working abilities during long lasting space flights. This is induced by circulatory disorders and general asthenization due to weightlessness.

The idea of artificial gravity was considered theoretically in the very first times of astronautics by Tsiolkovskiy (1895). More recently Kotovskaya (1981) explored the problem of the magnitude of the accelerative force, necessary to the substitution of the influence of gravity. Important problems of practical realization of this complex and complicated task have been elsewhere stressed by Berry and Gazenko.

During space flight artificial gravity can be induced e.g. in a system of twin rotating spacecrafts. Then the activities of astronauts will necessarily be performed under artificial gravity at one time, but in full weightlessness at another one. This is generally believed to induce the symptoms of kinetosis with decrease of the astronauts performance.

In our experiments the problem of alternating zero- and hypergravity states on pilot performance was considered.

First, our experimental subjects, six experienced pilots, learned to fly the Keplerian trajectory. Thus they experienced upon themselves the influence of weightlessness.

During following experiments, the influence of weightlessness was simulated as ten expositions to 40-50 seconds of zero-gravity in a L-410 light transport plane, flying repeatedly appropriate Keplerian trajectories. During transitions from one zero-gravity state to the other one the force of + Z acceleration reached about 2-3 g. Experimental subjects floated freely in the cabin space during the zero-gravity state. The whole flight lasted about 30 min.

An objective in-flight performance test was elaborated, consisting from a fixed set of 8 flight aerobatics elements, flown by the tested subject with a twin-seat L-39 training light jet-fighter plane. Pilot performance was judged directly during the flight by an experienced pilot-instructor, using a uniform scaling procedure. The whole test flight was judged as arithmetic mean from the scale values for each element from 1 (best) to 5 (worse). At the same time the course of the +Z acceleration force was registered continuously during the whole flight as an objective expression of the pilots manoeuvring performance. The results of both methods were compared and evaluated for each flight separately.

Heart beat to beat frequency was stored during the whole experiment in a ZAK-BIOPORT monitoring system.

The in-flight test was repeated after exposition to the weightlessness flight and the changes in manoeuvring performances were compared for both tests, together with the changes of the heart beat frequencies.

Control experiments were performed with the in-flight test repeated after a 30 minutes period of inactivity (sitting in a pilot rest-room) by 2 pilots (8flights).

Results

Pilot performance

Flight I.	Flight II.	
2.50 ± 0.80	1.45 ± 0.71	after weightlessness
2.0 ± 0.01	1.54 ± 0.31	control--inactivity

The second in-flight test was performed better. No difference was found after the period of alternating gravity conditions if compared with controls.

Heart rate changes

Flight I.	Flight II.	
96.62 ± 22.67	91.05 ± 25.39	after weightlessness
90.95 ± 21.57	91.72 ± 23.29	control--inactivity

The differences in the heart beat frequency are unsignificant.

Discussion

Negative changes in pilot performance after alternating gravity conditions were feared. Evidently in experienced subjects fine motor skills were not influenced negatively by exposition to alternating zero- and hypergravity conditions. Moreover, the process of adaptation during repeated complex flights remained unaffected by expositions to weightlessness in our experiments.

We suppose that experienced pilots are able to adapt better to a changing zero-hypergravity milieu than to a full long lasting weightlessness. This necessarily remains to be proved during real space flights.

Summary

An increase of performance of six experienced pilots in a complex in-flight aerobatics test repeated after a period of alternating zero-hypergravity conditions, lasting for 30 minutes, was observed, if compared with the same test before such exposition.

There was no difference in the increase of the pilot performance, if the in-flight test was repeated after a period of rest of the same duration.

We conclude that in experienced pilots adaptation to a complex in-flight test remains uninfluenced by alternating zero-hypergravity conditions, realized in a light transport plane.

Perhaps adaptation to alternating gravity conditions is implemented better than to full weightlessness in experienced subjects. This is to be proved in real space flights.

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SIGNIFICANCE OF LIGHT AND SOCIAL CUES IN THE MAINTENANCE OF TEMPORAL ORGANIZATION IN MAN

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Future space flight will expose humans to exotic environmental conditions (e.g., microgravity, altered light/dark (LD) cycles) and a social environment characterized by some degree of social isolation and enforced small group confinement. Such confinement and isolation has commonly resulted in boredom, irritability and social withdrawl (1). In addition, alteration of social or LD cycles in such environments has been associated with fatigue, insomnia, performance degradation and gastrointestinal dysfunction (2).

This study was designed to simulate several environmental conditions which will be encountered in future space missions, such as long-term confinement and isolation, exclusion of periodic environmental synchronizers, altered work/rest schedules, and the change in group composition following exchange of crew members. We focused on the relative importance of two environmental synchronizers (e.g., light and social interaction) in the regulation of biological rhythms, including heart rate (HR), rectal temperature (RT), rest/activity (RA), feed/fast, and selected urinary and plasma parameters.

METHODS

Two 20-24 year old male subject triads (North Room, NR and South Room, SR) were confined to separate 3.4 x 5.2 meter rooms for 105 days (d). These two groups were deprived of external time cues and outside communication was limited to voice contact via the intercom. A third triad (ER) was confined to the test center during the study and served as non-isolated controls. The subjects (Ss) were supplied ad libitum meals and recreational materials during the study. The experimental design is summarized in Figure 1.

Figure 1. Experimental design.

		ROOM (SUBJECTS)		
		SOUTH	NORTH	EAST
REGIMEN	DAYS	(1-3)	(4-6)	(7-9)
1	(1-27)	16L:8D	16L:8D	16L:8D
2	(23-43)	24L:0D	24L:0D	16L:8D
3	(44-63)	16L:8D	16L:8D	16L:8D
4	(64-83)	24L:0D	16L:8D	16L:8D
	SUBJECTS 3 AND 5 EXCHANGED ON DAY 84			
5	(84-105)	16L:8D	24L:0D	16L:8D

LIGHT INTENSITY WAS 1076 LUX IN 16L:8D, 162 LUX IN 24L:0D, SUNLIGHT-STIMULATING FLUORESCENT LIGHTS, VITALITE, DUROTEST, CORP.

At the start of regimen 5 (d84), subject S3 (SR) was exchanged with subject N5 (NR). Rectal temperature (RT) and heart rate (HR) circadian rhythm (CR) data were obtained continuously every 30 min via biotelemetry. A rest-activity (RA) data set was constructed from times of subject activities, and from HR data using the Kronauer (3) algorithm. Selected urinary components (e.g., cortisol) were obtained from ad libitum urine voids.

RESULTS AND DISCUSSION

All of the subjects completed the study and their anecdotal experience was one of progressive boredom rather than increasing stress. Evidence for the role of social interation in the regulation of CR within isolated triads was shown by a) the occurrence of coincident changes in CR period and phase (Figure 3) during LL; b) significant differences ($F=41$, $P<0.001$) between NR (24.8hr) and SR (24.3 hr), CR period lengths, determined by linear regression of daily acrophase estimates during LL, and c) convergence of CR period lengths in NR Ss after the subject transfer (increase of 0.2-0.3 hrs in S3 ($F=5.6$, $P<0.05$), decrease of 0.3 hrs in N4 ($F=15$, $P<0.0001$), and decrease of 0.1 hrs in N6 ($F=5.6$, $P<0.05$). The occurrence of coincident CR phase changes (4) and CR period convergence (5) within isolated subject groups, and CR period length differences between isolated groups (5) has been previously reported.

The expected difference between CR parameters in LL and LD reported in previous studies, using singly isolated Ss, were not observed in this study, using isolated triads. For example, the RT, CR phase shifted only 0.3^{o} with respect to sleep onset, between LD and LL, compared to a reported shift of $47\text{-}84^{o}$ (4,5), the RT, CR amplitude decreased only 9.3% compared to the reported 45% decrease (5,6), and the mean minimal RT increased only $0.03^{o}C$, compared to the reported $0.4^{o}C$ increase (6). These results indicate that mutual social CR synchronization maintains the properties of entrained CR in free-run LL conditions.

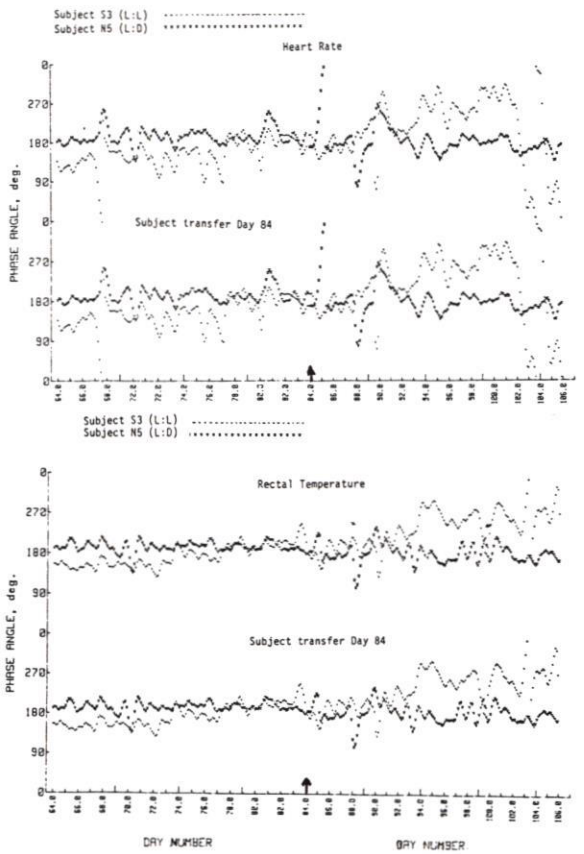

Figure 2. Pergressive (moving) RT and HR acrophases computed at 3-hr intervals (complex demodulates) for subjects S3 and N5 during regimens 4 and 5. Subjects S3 and N5 were exchanged between SR and NR groups on day 84 (arrow). This is a double plot, X axis begins on d64, ends on d106.

After the subject transfer, subject N5 was exposed to Ss S1 and S2, whose RT acrophases were delayed 3.7 hr relative to N5, and whose Cr's underwent a 6-7 hr phase advance to achieve entrainment to the new LD regime. Although N5 was maintained in regulated LD photoperiods throughout regimens 4 and 5, the CR of N5, post-transfer, showed unstable phase shifts, a 6 hr dissociation between RA and RT, CR, and desynchronization (180° phase jump) in the HR rhythm (Figure 2). This shows that social factors can modulate CR and, even temporarily override LD cycle synchronization of a previously entrained subject.

After the subject transfer, the CR of subject S3 were initially 1.5 hr phase advanced relative to the other NR Ss, but mutual group CR synchronization rapidly occurred, However, on d96, the RA, CR of S3 began to dissociate from the other Ss (Figure 3), followed by dissociation the the RT, CR on d99. Internal rhythmic dissocitaion between the RA and RT, CR of S3 later occurred on d102 (Figure 3). This rhythmic dissociation in S3 was associated with a substantial increase in performance error, cortisol and mean HR levels following subject transfer and numerous instances of hostility directed toward the technical support staff during the study. These changes were not observed in S3 during other photoperiod transitions, nor in N5, after the subject transfer. Subject S3 also had the earliest RA acrophase, highest HR, CR amplitiude, second highest RT, CR amplitude in LD and the shortest CR period lenghts in LL of the experimental Ss.

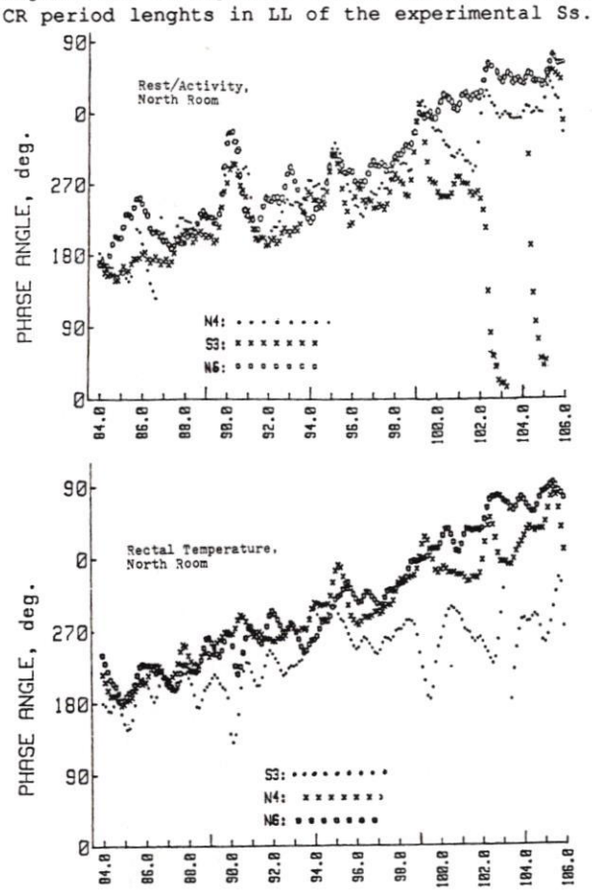

Figure 3. Pergressive (moving) RT and RA, CR acrophases computed at 3 hr intervals (complex demodulates) for NR subjects S3, N4, and N6 during regimen 5 (LL). Dissociation of the CR of subject S3 from the group is indicated on days 96 (RT) and 99 (RA).

Therefore, either psychophysiological stress induced by the study conditions or unfavorable CR chronotype characteristics may be responsible for the breakdown in social and internal CR entrainment in subject S3 after the subject transfer.

These results show that social contact can affect physiological processes in a positive (e.g., promote CR synchronization) or negative (induce CR dysrhythmia assicaiated with psychophysiological evidence of stress) manner. Further work is needed to clarify individual behavior and CR characteristics which promote adaptability to long duration space mission analog environments. Countermeasures could include behavioral measures such as selection for optimal within crew and crew/ground staff compatability, attenuation of contact between crew members on different RA schedules, and promotion of periodic group interaction and performance tasks. Environmental measures could include preadaptation to mission RA schedules, maintenance of regular RA schedules, and manipulation of ambient temperature and light intensity and spectral characteristics to promote CR entrainment.

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and an individual who appeared to function quite normally both during the test sessions and at all other times. The only hint of a problem was poorer than average pre-flight performance when asked to hop rhythmically up and down on both feet as part of another experiment. This was similar to the behavior of other subjects asked to perform the same task immediately post-flight, when vestibular function is thought to be temporarily suppressed. It is known that normal people can adopt a wide variety of motor strategies, with some more dependent on vision, some using vestibular inputs to a greater extent, and so on. One could speculate that this subject's normal mode was to be strongly "visual" and much less "vestibular". If that were true, it is interesting that he was the only subject out of the four tested in-flight who seemed to be totally immune to space motion sickness.

In summary, this experiment has demonstrated that otolith-spinal reflex function changes during prolonged exposure to weightlessness. The extent and nature of the change may depend on the subject's pre-flight motor strategy. Post-flight, the response to sudden falls returns to normal very quickly, possibly assisted by a temporary substitution of visual for vestibular inputs. The latter suggestion will be the subject of future flight experiments.

Acknowledgements

The authors wish to thank the Payload Crewmembers of Spacelab-1 and D-1 for their dedication and hard work before, during and after these flights: Guy Bluford, Bonnie Dunbar, Reinhard Furrer, Owen Garriott, Byron Lichtenberg, Ernst Messerschmid, Ulf Merbold, Wubbo Ockels and Robert Parker. Also deserving special acknowledgement are: Howard Jones, Ed Boughan, Bill Mayer, Walter Kucharski and Suzanne Watt for the design, building and certification of equipment; Gloria Salinas for crew training and coordination; Mike McEwen for support during the flight; Bob Clark for support during pre and post-flight testing; Alanna Smith for data analysis.

This work has been supported by the Medical Research Council of Canada, the Department of National Defence, Canada (including the Defence and Civil Institute of Environmental Medicine) and the National Aeronautics and Space Administration. The support of these agencies is gratefully acknowledged.

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The apparent decline of the in-flight response suggests that postural reflexes mediated by otolith-spinal pathways gradually disappear during prolonged space flight. Unfortunately, the inherent variability of biological data combined with a limited number of samples makes it difficult to draw any firm conclusions. Therefore, the in-flight data have been analysed further.

Each time a subject was tested, a graph of EMG response as a function of stimulus strength was plotted, and a linear regression line was fitted to those data. The slope of this line is a better estimate of the sensitivity of the otolith-spinal system than measurements made at single stimulus amplitudes. These slopes, normalized with respect to the average of all of that subject's pre-flight results, have been plotted as a function of time in-flight in Figure 5. Two subjects demonstrated a marked decrease in otolith-spinal sensitivity (slope) during their week in space. Another showed only a slight decline, however, and the fourth subject (data indicated by unfilled circles as in the previous figure) actually became more responsive with time. If the early in-flight results of the first three subjects (days 0 and 1) are compared to their late in-flight results (days 6 and 7), there is a statistically significant difference ($P<.05$). If the subject indicated by unfilled circles is included, the statistical significance disappears.

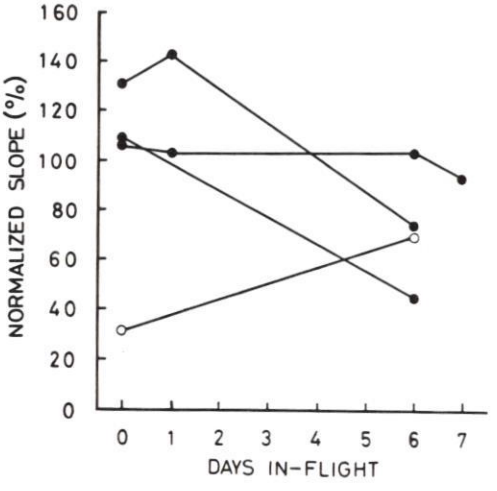

Figure 5. Otolith-spinal sensitivity as a function of days in weightlessness. The subject indicated by unfilled circles is the same one as in Figure 4.

Discussion

The present experiments are subject to certain limitations, and these must be considered if the data are to be interpreted correctly. In particular, it is difficult to compare the pre and post-flight results with those obtained in-flight. The acceleration stimulus produced by the elastic cords, while closely similar to that resulting from gravity, decreased with time during each fall. Furthermore, the accelerating force was applied to the torso by the harness but to every part of the body by gravity. The instrumentation used to amplify, filter and record the EMG responses pre and post-flight was different from that used in-flight. While every attempt was made to treat the data equally in these two situations, there were inevitable differences in the level and quality of noise contaminating the EMG signal, and this could be significant in those cases where the latter was of small amplitude. These limitations do not apply, however, if the pre and post-flight data are considered independently of the in-flight results. In this case, the biomechanics and data handling were identical for all subjects and all tests.

It was noted in Figure 4 that if the results of the very first session were excluded for each subject, the response to sudden falls did not change despite repeated exposure to testing. The average response recorded during the first session tended to be somewhat larger than normal, however, and a careful fall-by-fall examination showed that the first two or three falls were mostly responsible. Based on the studies of Wicke and Oman (9), this was probably the result of a rapidly adapting startle response, which never reappeared once the subjects were familiar with the testing method. While present, it had a proportionately greater effect when added to the relatively smaller otolith-spinal reflexes resulting from the lesser acceleration stimuli.

The results shown in Figure 4 also provide little if any evidence that the response to sudden falls was altered post-flight, despite the fact that crewmembers showed obvious defects in postural control while being tested, especially on the day of landing. This may be the result of a rapidly acquired compensation in which visual inputs substituted for disrupted vestibular sensation, a phenomenon that has been demonstrated in animals and which could have occurred in the 1.5 to 8 hours which passed between landing and initial testing (8). Objective tests of visual dependence of returning crewmembers have shown increased reliance on vision in about the same time period (1,10). It is also known that visual inputs can have an effect on the response to sudden falls (9).

Perhaps the most interesting observation of this experiment was the apparent gradual decline of otolith-spinal reflexes during prolonged exposure to weightlessness. Three out of four subjects showed this effect at every size of acceleration input. However, the subject indicated by unfilled circles in Figures 4 and 5 actually increased his response.

Why did the response to sudden falls decrease with time in-flight in three subjects? Habituation is unlikely, since there is no evidence of it occurring during the intensive post-flight testing period. More likely, the gradual loss of otolith-spinal reflex function was the result of an attempt by the nervous system to adapt to disrupted vestibular function in the absence of a defined goal (8). It appears that postural control is a meaningless concept in weightlessness.

Why did this phenomenon not occur in the fourth subject? This individual was identified as anomalous during the pre-flight test sessions, when he produced a very unusual EMG pattern during sudden falls. The variability of his responses was by far the greatest of any of those tested, both within and between sessions, and the responses themselves were often very small. Later, his first in-flight test demonstrated far more suppression of the early burst of EMG activity than was seen in any other subject (Figure 5). This was followed by an increased response toward the end of the flight. Did this crewmember lack the discipline and rudimentary physical skills necessary to perform the experiment correctly? In fact, he was a skilled jet pilot, a cooperative and thoughtful subject,

For each strength and type of stimulus, the EMG responses to about 15 consecutive drops were rectified and averaged. As noted by Lacour et al. (4), the early burst actually consists of two subcomponents. The two could usually be distinguished in our averaged responses by a sharp increase in slope of the curve (the second sub-component was often much larger than the first), or by the presence of 2 distinct humps with a "valley" in between (Figure 3). The latency of the dividing line between the two sub-components varied somewhat between subjects, but was quite constant from test session to test session for any given subject. While two sub-components can be defined in this way, it must be assumed that some overlapping occurs.

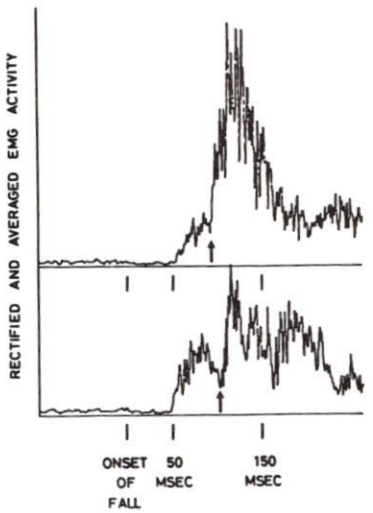

Figure 3. Rectified and averaged EMG responses to a step change of 1.0g recorded on the ground from 2 different subjects. The onset of fall and the limits of the early burst are shown. The arrows indicate the division of each early burst into first and second sub-components.

It has been shown that the two sub-components can change independently during prolonged exposure of subjects to the supine position, and that whereas the first is not susceptible to voluntary control, the second can be influenced by mental set (7). For this reason, the present analysis was limited to measuring the mean amplitude of the first sub-component of the EMG response to sudden falls.

Four Spacelab-1 crewmembers were tested repeatedly before and after flight, with two of these able to participate both while in space and on landing day (7). Five Spacelab D-1 astronauts have been tested in a similar fashion, with 2 carrying out the experiment in-flight and 3 being tested within a few hours of landing. The data obtained during these two flights have been combined in this article.

Results

Following each test session with a given subject, the mean amplitude of the first sub-component of the EMG response was determined for each of the three stimulus magnitudes. When pre, in and post-flight testing was completed, these data were normalized with respect to the average of all of that subject's pre-flight responses to a step change of 1.0g.

The results obtained from all subjects have been collected and presented in Figure 4. Normalized mean EMG amplitude has been plotted as a function of time before, during and after flight. The results obtained from one subject who will be discussed later have been indicated by unfilled circles. As mentioned previously, data were obtained using three different stimulus amplitudes at each session, but it should be noted that two of the three in-flight stimuli were greater than those used on the ground, since it was anticipated that the subjects would be less responsive in weightlessness.

Linear regression lines have been fitted to these data using the method of least squares. This has been done to assist visual interpretation of the results, and does not imply that a linear function produces the closest fit. In each case, two lines have been calculated for the pre-flight data, one which is based on all results and another which ignores data obtained during the initial testing session, when the subjects were becoming accustomed to the experimental procedures. The latter line has a slope which is close to zero in all cases, whereas the former has a slight downwards trend when the two smaller stimuli were used. Post-flight, the response to sudden falls was very similar to that seen pre-flight, although the reaction to the strongest stimulus may have been somewhat depressed. By way of contrast, all lines fitted to the in-flight data indicate a gradual decline of the response. Regardless of whether the data were obtained before, during or after flight, however, the size of the EMG response was always proportional to the size of the acceleration stimulus.

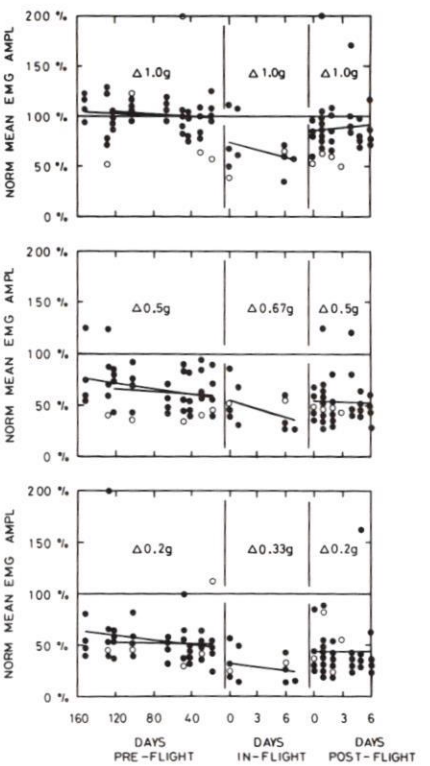

Figure 4. Normalized mean amplitude of the EMG response to sudden falls as a function of time before, during and after flight. The size of the acceleration stimulus is indicated on each section of each graph. Linear regression lines have been added to assist in the visual interpretation of the data. One subject who tended to produce unusual results is indicated by unfilled circles.

OTOLITH-SPINAL REFLEX TESTING ON SPACELAB-1 AND D-1

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Introduction

The vestibular labyrinth consists of two parts which are anatomically and functionally distinct. These are the semicircular canals, which transduce angular head movement, and the otolith organs, which sense linear acceleration and gravity. When man goes into space, cancellation of the gravity vector must lead to immediate and significant changes in the function of the latter organs. This in turn should lead to disordered reflexes and possibly motion sickness. Further changes in otolith function would then be anticipated as the nervous system tries to adapt to the new acceleration environment.

At this time, the only generally accepted method for the objective and selective testing of the human otolith organs is measuring ocular counter-rolling during static lateral tilt. While this technique has been adopted by clinical investigators on the ground (2), it cannot be used in the absence of a gravity vector. Methods which apply transient linear accelerations are necessary if otolith function is to be measured in space.

Methods

The experiments reported here took advantage of short-latency postural responses to sudden loss of support (5). The controlled, natural stimulus to the otolith organs was sudden, unexpected falls, resulting in step inputs of linear acceleration (Figure 1A). On orbit, a harness and suitably adjusted elastic cords provided the accelerating force (Figure 1B). The magnitude of the stimulus was adjusted by counter-weighting the subject on the ground, or by varying the number of elastic cords while in space, allowing three different step amplitudes to be employed each time a subject was tested.

The muscular response to this stimulus was recorded as electromyographic (EMG) activity in gastrocnemius and soleus (Figure 2). EMG activity occurring between 50 and 150 msec after the onset of fall has been called the "early burst" (7). It is considered to be the result of an otolith-spinal reflex, being time-locked to the moment of release, too rapid for a voluntary response (5), and absent in labyrinthectomized cats (6) and monkeys (4) as well as labyrinth-defective humans (3).

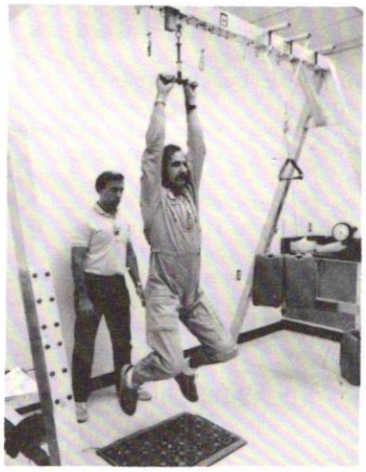

Figure 1A. Drop-testing on the ground. The subject is hanging by his arms from a handle which will be released unexpectedly. The resulting transition into free-fall will provide a 1.0g step change of acceleration.

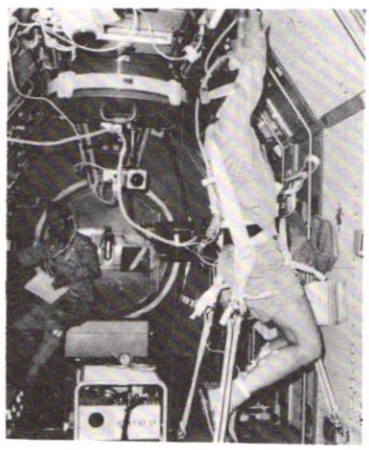

Figure 1B. Drop-testing in Spacelab. The subject is also hanging from a handle which will be released suddenly and unexpectedly. The accelerating force is being provided by a harness and elastic cords, however.

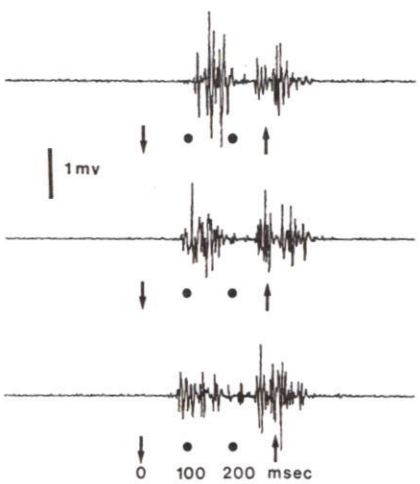

Figure 2. EMG responses to 3 consecutive falls with accelerations of 0.5g. The downwards arrow indicates the moment of release and the upwards arrow contact with the ground. The EMG occurs in 2 bursts, an early one related to the onset of fall and a later one which controls landing on the ground.