Proceedings of the 13th Annual Meeting of the IUPS Commission on Gravitational Physiology

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Proceedings
of the
13th Annual Meeting
of the
IUPS Commission on
Gravitational Physiology

September 29–October 3, 1991
San Antonio, Texas, USA

Hilding Bjurstedt, Guest Editor

International Union of Physiological Sciences
Commission on Gravitational Physiology

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Dedication

Orr Esrey Reynolds
1920–1991

The Proceedings of the 13th Annual Meeting of the IUPS Commission on Gravitational Physiology are dedicated to the memory of Orr Esrey Reynolds, a devoted supporter of space biology in general and of gravitational physiology in particular. His friendship, wise counsel, and enthusiastic participation in the affairs of the Commission will be keenly missed. He died on March 30, 1991 in Fort Lauderdale, Florida, USA.

Orr Reynolds was born in Baltimore, Maryland on March 3, 1920 and received the BS and MS degrees in zoology from the University of Maryland during the years of World War II. On completion of his MS degree in 1943, he obtained a position as an assistant physiologist in the National Institutes of Health (NIH) Laboratory of Industrial Hygiene to work on problems relating to high-altitude physiology. Under the guidance of A. C. Ivy, then the civilian scientific director of the Naval Medical Research Institute in Bethesda, he carried out thesis research entitled “The effects of discontinuous exposure to 18,000 feet simulated altitude,” which led to the award of the PhD degree by the University of Maryland in 1946. In 1944 he applied for and received a commission in the US Navy Hospital Specialist Corps as an aviation physiology training officer. His uniformed Naval career culminated in assignment to the Aviation Physiology Research Division of the Bureau of Medicine and Surgery in Washington, DC, where he was given the task of reviewing research proposals.

On separation from the Naval Reserve in 1946, Reynolds accepted a civilian position with the Navy's Office of Research and Inventions, again reviewing research proposals in physiology. Within a year the Office of Research and Inventions became the Office of Naval Research and he was appointed its first head of the Physiology Branch.

It was at this time that Reynolds began to display his remarkable talents as a science administrator. In 1948, he was appointed Director of the Biological Sciences Division of the Office of Naval Research, a position he held until 1957. During these years he oversaw the development of a ground-breaking research grant program of support in the basic biomedical sciences that served as a model for the evolution of the massive support in this field now being provided by additional federal government agencies, including other branches of the Department of Defense (DOD), National Institutes of Health (NIH), National Science Foundation (NSF), and National Aeronautics and Space Administration (NASA). With great insight Reynolds championed the importance of basic research across the broad reach of experimental biology. He actively encouraged proposals in the then-arcane areas of biochemistry that have now become the explosive field of molecular biology. He fostered investigations in neurophysiology, cardiorespiratory physiology, and endocrinology. He evinced keen interest in supporting environmental physiology, in particular the physiology of deep-sea diving, high altitude, cold and heat exposure, and chronic centrifugation. In addition he funded research in areas of direct practical concern at the time, such as the complex problems of burn shock and combat stress. It can be said without exaggeration that Orr Reynolds was a force majeure in the burgeoning of basic biomedical research that occurred in the first decade after World War II.

In the spring of 1957 Reynolds took up a new challenge at the very beginnings of space science research carried out in orbiting artificial satellites. He accepted appointments as Director of the Office of Science of the DOD, with the task of expanding overall support of fundamental research by the military. One of his responsibilities was the administration of investigations to be carried out in Earth orbit for the International Geophysical Year (IGY) by payloads to be flown initially by the US Navy Vanguard rocket system, at that time under development. The IGY began officially on July 1, 1957, but Vanguard experienced launch vehicle technical problems. On October 4, 1957, the USSR successfully launched Sputnik 1 as the first man-made satellite. Sputnik 1 was quickly followed into orbit on November 1, 1957 by the much larger Sputnik 2 with the dog Laika as a passenger, thus opening the age of space physiology.

It was not until January 31, 1958 that the US Army Ballistic Missile Agency successfully launched Explorer 1 as the first US satellite into orbit, and on March 17, 1958 Vanguard 1 was finally placed into orbit. Both of these small spacecraft carried Geiger counters that produced the data revealing the presence of the Van Allen radiation belts about the Earth for the first time. Subsequently, on November 12, 1960, the US Air Force launched Discoverer 17 with a payload of cell cultures and microorganisms. After two days in orbit the reentry capsule was successfully retrieved in midair during parachute descent for the first US biological payload recovery. All of these came under Reynolds’ administrative purview.

During his years as Director of the DOD Office of Science, Reynolds was involved with the administration of other major projects such as the development of weather satellite systems, the magnetic field facility at the Massachusetts Institute of Technology, and the two-mile-long linear accelerator at Stanford University. He also traveled abroad on a great deal, championing the cause of basic research in other countries. However, in 1961 it became increasingly evident that the principal support for basic research was steadily shifting from...

the military to the civilian agencies of the federal government. In mid-1961 he was invited by Homer Newell, then head of the Office of Space Science of the three-year-old NASA, to assume leadership of the Bioscience Program. Reynolds accepted the position in early 1962 with a mandate “to apply the capabilities of space flight, which NASA is developing, to advance our fundamental knowledge in biology.”

With his characteristic elan, Reynolds rapidly began the building of a solid base of research grants to fund investigators who would carry out spaceflight experiments in three principal areas: gravitational biology, radiobiology, and exobiology. He was also instrumental in establishing two major NASA flight programs to be dedicated for research in these areas: the Biosatellite Program and the Viking Program.

The Biosatellite Program was to comprise a series of six unmanned spacecraft based on the well-tested Discoverer parachute recovery system to carry experiments designed to examine the effects of weightlessness and space radiation on biological test objects. The program was approved by Administrator James E. Webb of NASA in December 1962, and Biosatellite 1 was launched with a payload of microorganisms, insects, plants, and frog eggs on December 14, 1966. After functioning successfully for the scheduled three days in orbit, the retrorocket misfired and the reentry vehicle could not be recovered. Biosatellite 2 was then launched on September 7, 1967 with an identical payload, and this time successful recovery ensued with a consequent harvest of scientific results.

The next step, Biosatellite 3, was far more ambitious. The payload was a heavily instrumented 5.5-kg macaque monkey scheduled to be recovered after 30 days in orbit. The 700-kg spacecraft was launched on June 28, 1969, but although the animal tolerated the first few days of weightlessness quite well, its physical condition began to deteriorate. By the eighth day in orbit a decision was reached to initiate recovery of the spacecraft, which occurred 8.8 days after launch. The animal was found to be in shock, and attempts to revive it failed. In retrospect it became evident that the instrumentation of the animal represented a physiological overload which even the ground control animals could not tolerate. However, despite the shortened flight of Biosatellite 3, a number of valid observations of the effects of weightlessness on a primate were made during the first few days. Among these, contrary to expectation, were the findings that central venous pressure changed very little and that a diuresis did not occur. Although three more Biosatellites had originally been scheduled, a decision was made by NASA to cancel these flights.

The Viking Program was designed to land two spacecraft on the surface of Mars to seek evidence for the possible presence of living organisms. While the experiments and the hardware were being developed, Reynolds successfully led a campaign in the Committee on Space Research (COSPAR) of the International Council of Scientific Unions (ICSU) to reach the important international agreement that spacecraft to be landed on Mars should be sterilized so that biological contamination from Earth would not occur and confound the search for Martian organisms. To accomplish this he became a member of COSPAR Working Group 5 for Space Biology in 1965 and continued as such until the reorganization of COSPAR in 1979.

One of the experiments to be flown in the parallel Biosatellite Program was to measure the urine calcium, creatine, and creatinine excretion rates of the Biosatellite 3 primate. Reynolds perceived this as an opportunity to provide a flight test of the miniaturized, automated chemical analyzer being developed by the Jet Propulsion Laboratory for the Viking Program. As a result of his decision the analyzer was adapted for the urine measurements and successfully performed continuous analyses throughout the Biosatellite flight. Similar analyzers were used for the historic Viking 1 and 2 Mars landers in the search for life on Mars in 1976. This episode serves well as an illustration of the remarkable talent of Orr Reynolds.

From 1966 to 1975 Reynolds played a major role as a member of the Editorial Board in the development of the landmark three-volume work entitled “Foundations of Space Biology and Medicine,” published jointly by NASA and the USSR Academy of Sciences under the general editorship of
Melvin Calvin and Oleg G. Gazenko. This comprehensive scientific and technical treatise summarized biomedical results of the first 15 years of space flight and has served as an invaluable handbook for the field as well as an example of outstanding international cooperation.

In 1970 Reynolds resigned from NASA to become the Education Officer for the American Physiological Society, and in 1973 he assumed the position of Executive Secretary-Treasurer. In this capacity he became active in the affairs of the International Union of Physiological Sciences and provided invaluable advice to the newly formed IUPS Commission on Gravitational Physiology. With his encouragement the Commission decided in 1978 to establish annual open, international meetings for the exchange of research results by investigators in gravitational physiology. The first meeting was held in October 1979 in conjunction with the fall meeting of the American Physiological Society in New Orleans. A grant from NASA and the cooperation of Reynolds as volunteer editor and business officer made possible the publication of the Proceedings of the meeting as a supplement to *The Physiologist*. Aside from the replacement of NASA by the Galileo Foundation in 1988 as a funding source, the arrangement has continued through this, the 13th Annual Meeting.

In 1983 Reynolds was appointed a member of the Commission on Gravitational Physiology by the IUPS Council and served in this capacity until his death. He also continued to serve as editor of the Proceedings. When the Galileo Foundation was organized in 1987 he agreed to become a member of its International Advisory Panel. Thus Reynolds played a key role in the evolution of the annual meetings of the IUPS Commission on Gravitational Physiology, and he leaves a lasting legacy for us all.

The stature of Orr Reynolds is reflected in the formal honors that he received. He was awarded the US Navy Meritorious Service Medal in 1957 and the NASA Exceptional Service Medal in 1970. International honors include receipt of the Semmelweis Medal of the Semmelweis Medical University, Budapest in 1978 and the Yuri Gagarin Medal of the Soviet Academy of Sciences, Moscow in 1985. He received the Ray G. Daggs Award of the American Physiological Society in 1987, and in that same year the Society established the Orr E. Reynolds Award presented annually to the author of the best historical article published in one of its journals.

The protean nature of his scientific interests was matched in his personal interests. Although Reynolds was born in Baltimore, his family moved to Washington, DC when he was a few months old. He spent much of his boyhood there and then spent two years in San Antonio as a high school teenager in the early 1930s. At this time he became an expert horseman and acquired the skill of guitar playing and some Texas mannerisms he never quite lost. He joined a jazz quartet that performed on a local radio station, and after he and his family returned to Washington, DC from San Antonio he formed a dance band during his college years at the University of Maryland. In this period he also took up the sport of falconry and developed a keen interest in marine biology, taking summer courses in 1939 and 1940 at the

Chesapeake Biological Laboratory on Solomon Islands, Maryland.

In 1955 Reynolds purchased an 80-acre farm in Frederick, Maryland where he lived with his wife Maxine and daughter Caroline. In his spare time from his prodigious ONR and NASA duties he managed a herd of black angus cattle and grew the hay to feed them. He ran his cattle ranch for more than 10 years but gave it up when Maxine fell into ill health. Following her death Reynolds married Marjorie Potts in 1971, and Orr and Margie ornamented many of the Commission Meetings until recently when his health failed. It was his great desire to attend the San Antonio Meeting, not only for the science but also to revisit his teen-age haunts. It is fitting therefore to dedicate these Proceedings to Orr Reynolds, who is most certainly a part of them in many ways.

References


The Physiologist, Vol. 35, No. 1, Suppl., 1992
Important Announcement

14th Annual Meeting
IUPS Commission on Gravitational Physiology
September 29–October 3, 1992
Berlin, Germany

The 14th Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences, cosponsored by the International Society for Gravitational Physiology, will be held in Berlin, Germany, September 29–October 3, 1992.

Symposia by invited speakers, slide presentations of voluntary papers, and poster sessions dealing with the effects on the 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.

The following are the scheduled four half-day symposia.

Gravitational Effects on Embryogenesis and Development in Animals
K. Boda, Organizer
Wednesday, September 30

Gravitational Physiology in the Mir Program
I. B. Kozlovskaya and K. Kirsch, Organizers
Thursday, October 1

Current Concepts in Gravitational Physiology
A. Berthoz and J. Seylaz, Organizers
Friday, October 2

Gravity and the Immune System
I. V. Konstantinova and L. Macho, Organizers
Saturday, October 3

Your participation in the Commission meeting is welcomed. Information and Call for Papers may be obtained from Lorraine Tucker, American Physiological Society, 9650 Rockville Pike, Room 4402, Bethesda, MD 20814-3991, USA.

The deadline for the receipt of abstracts is June 15, 1992.

Financial Support

Appreciation is expressed for the following contributions during 1991–1992 to the Galileo Foundation for support of the activities of the IUPS Commission on Gravitational Physiology.

Institutional Grants

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<td>Laboratory of Environmental Physiology, Claude Bernard University, Lyon, France</td>
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Individual Donors

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International Society for Gravitational Physiology

At its business meeting on September 29, 1991 in San Antonio, Texas, the members of the IUPS Commission on Gravitational Physiology unanimously decided to request that the Galileo Foundation establish the International Society for Gravitational Physiology as the body responsible for continuation of the Annual Open Meetings commencing in 1992. The 14th Annual Meeting, to be held in Berlin, Germany during September 29-October 3, 1992, would be under the joint sponsorship of the IUPS Commission and the new Society.

The Board of Directors of the Galileo Foundation agreed to carry out this task and proceeded forthwith to appoint a Council of Trustees to administer the activities of the International Society for Gravitational Physiology. The Council comprises the current membership of the IUPS Commission on Gravitational Physiology as follows: Chair, Hilding Bjurstedt, Stockholm, Sweden; Augusto Cogoli, Zürich, Switzerland; Oleg G. Gazenko, Moscow, USSR; Karl Kirsch, Berlin, Germany; Inesa Kozlovskaya, Moscow, USSR; Ladislav Macho, Bratislava, Czechoslovakia; Nello Pace, Berkeley, California; Paul E. Pilet, Lausanne, Switzerland; Hisashi Saiki, Tokyo, Japan; Jacques Seylaz, Paris, France; Arthur H. Smith, Davis, California; Zhuang Xiangchang, Beijing, China. It is intended that the Council will act in parallel with the IUPS Commission.

On October 1, 1991 the Organizational Meeting of the Council of Trustees was held with Hilding Bjurstedt presiding as chair. Rules and Regulations of the International Society for Gravitational Physiology were adopted, and future activities of the Society were considered. Karl Kirsch, as organizer of the 1992 Berlin Meeting, was appointed the first President of the Society in accordance with the Rules and Regulations. David Cardus, as organizer of the 1993 Barcelona Meeting, was also appointed as the first President-Designate of the Society.

It was unanimously agreed that the present format of the Annual Meetings and publication of the Proceedings be continued unaltered. However, after the 1992 Berlin Meeting the Proceedings will be published independently and no longer as a supplement to The Physiologist. A Publications Committee of the Society was appointed to consider other publications as well. The Committee is chaired by Jacques Seylaz with Karl Kirsch and Ladislav Macho as members.

It was further agreed that in the near future an invitation will be sent to all authors of papers published in the Proceedings of the 13 Annual Meetings to date to become charter members of the new Society. Dues will not be required for membership, and members will receive the publications of the Society and announcements of future meetings. The Galileo Foundation will continue to provide subvention for the costs involved.

Queries concerning the International Society for Gravitational Physiology may be addressed to the Chair of the Council of Trustees, Hilding Bjurstedt, Environmental Physiology Laboratory, Karolinska Institutet, S-10401 Stockholm, Sweden.
Introduction

The International Commission of IUPS on Gravitational Physiology held its 13th Annual Meeting September 29–October 3, 1991, in San Antonio, Texas, USA. The Commission joined the American Physiological Society in its specialty meeting during this period, which was devoted to “Interaction of the Endocrine and Cardiovascular Systems in Health and Disease.”

The scientific program of the Commission’s Annual Meeting included four symposia with invited papers dealing with 1) “Gravitational Cell Physiology” (organizer A. Cogoli), 2) “Current Concept in Gravitational Physiology” (organizer N. Pace), 3) “Physiology of High-G Loadings” (organizer R. R. Burton), and 4) “Mathematical Modelling in Gravitational Physiology” (organizer R. Latham). A symposium with invited papers sponsored by the Commission and devoted to “NASA Spacelab Life Sciences-1 Mission: Preliminary Findings” was also held. The number of invited papers belonging to these symposia totaled 43. In addition, the program contained 5 open sessions on a variety of topics in the gravitational area, with the number of voluntary papers submitted totaling 57.

This Supplement to The Physiologist contains the Proceedings of the meeting. By previous agreement between the Galileo Foundation and the Institute of Biomedical Problems, Moscow, certain papers are also included that were presented at an International Symposium on “Cosmos” Biosatellite Experiments, held in Leningrad, August 12–15, 1991. The Symposium was organized and sponsored by the Institute.

The Commission expresses its gratitude to the Galileo Foundation for spiritual and material support of Commission activities in connection with its meeting in San Antonio and especially for making possible the publication of this Supplement.

To Dr. Martin Frank, Executive Director of the American Physiological Society, the Commission extends its sincere appreciation for all the help received from the Society in organizing the Commission’s 1991 Annual Meeting.

Hilding Bjurstedt
Chairman

Papers published in the Proceedings of the 13th Annual Meeting of the IUPS Commission on Gravitational Physiology have been reviewed and approved by the Commission.
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Gravitational Physiology in the 1990s

Hilding Bjurstedt
Department of Physiology
Karolinska Institutet, Stockholm, Sweden

I have been asked to talk about gravitational physiology in the 1990s. To do this, I should make clear at the outset that I do not intend to give a “shotgun” review of everything that can be included in this subject. Rather, I shall select a few areas that have caught my imagination and that hopefully may also engage your interest. The limited time available for my survey precludes an account of the spectrum of gravitational forces, to which current aerospace technology exposes human subjects. The very strong gravitational forces, up to nine times normal or above, which are encountered during operation of high performance aircraft, tax the tolerance of the human body, especially its circulatory system, to its limit. Although the physiological responses to this type of stress are of great interest, I shall here have to leave this area aside in favor of the gravitational stresses of space travel.

It should also be made clear that my talk will not so much be about space travel as it will attempt to show that gravitational physiology features serious questions of a fundamental nature that go far beyond the problems encountered in spaceflight or any envisioned voyage to Mars. They all center around the largely unknown role of gravity in the physiological equation.

Before I go any further, a few background notes may be appropriate. Gravitational physiology may in generic terms be defined as the study of the loading effects of earth’s gravity on the structure and function of living organisms. One approach to such study is to observe the effects of removing “effective” gravity made possible by orbital spaceflight, a condition that will here be referred to as “microgravity” to comply with current usage. To simulate the effects of microgravity on humans, often necessary for both ethical and practical reasons, ground-based forms of gravitational unloading have been widely employed. Useful human models include bed rest and water immersion. Examples of animal models are various kinds of restraint and hindlimb suspension. Another approach is to investigate effects of increased gravitational forces produced in ground-based centrifuges.

Gravitational physiology is intimately associated with the advent of the space age. In recognition of the rapid development of gravitational physiology, the International Union of Physiological Sciences in 1974 decided to form a special Commission for the promotion of scientific activities in this field. The rapid development that has subsequently marked research in gravitational physiology is underscored by the fact that since the Commission in 1979 began sponsoring annual meetings, the names of over 1,000 scientists have appeared as authors of about 600 papers published in the Proceedings of the first 10 Annual Meetings. The truly international nature of these annual meetings is evident from the fact that no less than 23 countries have been represented at these meetings.

Having been asked to look into the crystal ball for directions in which gravitational physiology may be headed in the next 5 to 10 years, it occurs to me that it may be appropriate to first state some basic facts that may serve to put my subject in perspective, namely that 1) many links are missing in our understanding of the chain of events that lead to adverse effects in the microgravity environment; 2) in prolonged human spaceflight, we have so far attempted to counteract these effects by interventions that have necessarily been of a makeshift character because control experiments have been lacking; 3) by regularly employing such countermeasures, we have compromised the body’s spontaneous adaptation to microgravity, and we therefore do not know if, with time, the body can truly adapt to this environment; and 4) assuming that such adaptation is possible, which by no means is certain, we do not know whether on return to earth’s gravity after long-term residence in space certain adaptive changes will become apparent as irreversible or otherwise pathological.

To make my presentation digestible, I shall have to leave out many important areas in gravitational physiology. In the following, I shall therefore give the present status of some selected problems of immediate concern that fall in the categories of microgravity effects on cardiopulmonary, musculoskeletal, and certain aspects of neurovestibular function. A brief account of current knowledge in each area will be given, and significant gaps in our knowledge will be emphasized. While most of these gaps will not be filled during the next 10 years, some ideas will be presented on what can be done to narrow some of them. Emphasis will be on mammalian, predominantly human, physiology.

Cardiopulmonary Function

The microgravity effects of spaceflight on human cardiovascular functions have many features in common with those caused by water immersion and by prolonged bed rest. When the hydrostatic pressure components normally present in the upright body position are abolished or neutralized, blood volume in the highly distensible low-pressure system is shifted in the headward direction. This initiates a series of adaptive responses in the cardiovascular system as well as in several other organ systems. Prominent among these are a progressive decrease in total blood volume, development of ortho-
static intolerance, reduced heart size, and impaired cardiac performance.

**Regulation of Blood Volume**

Observations during the Skylab missions provided the first evidence that exposure to microgravity may cause headward displacement of up to two liters of blood from the legs. In-flight measurements as well as bed rest studies have demonstrated that the initial increase in central blood volume induces compensatory losses of water, sodium, potassium, and calcium through the kidneys. As a result, total blood volume is gradually diminished. Losses of up to one liter during several months of bed rest have been reported. The time course of microgravity-induced changes in central blood volume is not known. In early adaptation to bed rest, measurements of central venous pressure by indwelling catheter have indicated a prompt rise in pressure, with a duration of about one hour and a subsequent fall below control level. Corresponding measurements during the early phase of spaceflight have so far failed to show the expected increase in central venous pressure.

Ground-based experiments and measurements during spaceflight have helped to elucidate some of the neuroendocrine mechanisms through which fluid and electrolyte regulation by the kidneys is effected. Central hypervolemia induces increased urinary output by stimulating cardiopulmonary stretch receptors that cause reflex suppression of antidiuretic hormone. The central overload also decreases aldosterone in the renin-aldosterone-angiotension system (RAAS), so that sodium reabsorption in the kidney is suppressed. In addition, release of atrial natriuretic peptide from the heart is increased. The end result of these and other changes is diuresis and a progressive decrease in circulating blood volume.

The influence of each of these and other neurohumoral mechanisms responsible for blood volume regulation is well recognized, but the nature of the integrated mechanism that governs the blood-volume response to microgravity is not known. Further investigations, supported by bed rest studies, are needed to characterize all of the cardiovascular, renal, endocrine, and neuroregulatory forces at play during spaceflight as well as their time courses over varying durations of exposure. The concomitant changes in central venous pressure should be monitored by indwelling catheter during selected periods. Results from short- and long-duration studies of this kind will not only increase our understanding of blood volume regulation and its dynamics but will also indicate if microgravity offers any unique advantages for the study of human cardiovascular physiology.

**Orthostatic Intolerance**

Upright posture normally places a heavy strain on the cardiovascular system's protective reflexes; eventually syncope will ensue unless the leg muscle pump is activated or cardiac filling is otherwise ensured. Already in the early 1960s, it became evident that even a few days of spaceflight may markedly reduce orthostatic tolerance. Postflight tests on the tilt table or with lower body negative pressure (LBNP) show a more marked narrowing of pulse pressure and increase in heart rate than before the flight, and the time until signs of presyncope become apparent is shortened.

The bed rest model has been used extensively to study the mechanisms responsible for orthostatic intolerance. This dysfunction is also a salient feature in certain clinical states of pathological blood volume depletion or other conditions of severe fluid loss. But the orthostatic intolerance induced by microgravity or bed rest seems to be far more complex than simply a response to general hypovolemia. The possibility of a blunting of cardiopulmonary or arterial baroreflex influence has been explored. In certain clinical conditions, such as diabetics, alcoholics, postinfectious, and other types of neuropathy, orthostatic hypotension is caused by defective links in various chains of autonomically generated responses. However, the responses to head-up tilt and LBNP following exposure to microgravity and bed rest are generally brisk and include a greatly increased heart-rate response and an exaggerated peripheral vasoconstriction.

Even after several decades of extensive research, the basic causes of microgravity-induced orthostatic intolerance are still unknown. It can be assumed that the understanding of the nature of the decreased cardiovascular competence will depend on increased knowledge regarding the neurohumoral alterations that are involved in the regulation of blood volume. It is worthy of note that orthostatic tolerance is often increased in certain clinical conditions, e.g., hypertension and congestive heart disease. For this and other reasons, continued studies of the effects of vasoactive drugs will probably be rewarding. The condition is of great interest from the point of view of basic physiology and is also of considerable operational importance.

**Cardiac Dimensions**

Combined data on cardiac dimensions after bed rest and after spaceflight have shown a decrease in total heart size in the supine position, with a decreased preload and a depressed stroke volume. Reductions in filling pressures have been confirmed by direct measurements of central venous pressure. Whether changes in the contractile state occur with time in human subjects is an unsettled question. Ultrastructural myocardial abnormalities have been observed in rats exposed to microgravity for 20 days. So far there appears to be no serious effect of microgravity directly on the human heart. However, the question whether long-term spaceflight may lead to involutional myocardial degeneration needs attention.

**Cardiac Performance**

Marked increases in resting cardiac stroke volumes have been a consistent finding in orbit, at least during the early phases. Since heart rates are decreased, changes in cardiac output are not striking. Cardiac pump function is normally the limiting determinant of dynamic exercise capacity as measured by maximal oxygen uptake. Data on oxygen uptake during maximal exercise in the microgravity environment are
missing, but bed rest studies have shown a significant decrease already after 24 hours. After 3 weeks maximal heart rate did not change, but there was a 25% decrease in stroke volume, with consequent proportional decreases in cardiac output and maximal oxygen uptake compared to the responses before bed rest. Measurements during submaximal exercise after up to 84 days in space have also shown substantial decreases in stroke volume and a relative tachycardia compared to preflight data. After bed rest, relative tachycardia is present during both rest and in submaximal exercise in the supine position.

The mechanisms responsible for the changes in cardiac performance in microgravity and during bed rest are unknown. Information on the effect of microgravity on maximal oxygen uptake is especially desirable, since this variable reflects the overall functional capacity of the cardiovascular system. Because of the loss of muscle mass and strength, and accompanying reduction of muscle blood flow that occur in space, the possible impact on cardiac performance of a generalized increase in venous compliance or subtle changes in the distribution of regional blood flow needs to be investigated.

**Pulmonary Function**

Of all organs in the body, the lung is particularly sensitive to changes in the gravitational load. It is well recognized that gravity causes vertical gradients in the distribution of both ventilation and perfusion that create regional variations in ventilation-perfusion relationships and in gas exchange. Many aspects of pulmonary function can therefore be expected to be altered in microgravity.

However, little is known as to how various lung functions adapt to the microgravity environment. Observations during parabolic flights (<30 sec) indicate that gradients in exhaled gas concentrations may in part be explained as gravity induced. It is known that certain features of single-breath nitrogen washouts have a nongravitational origin, which is also apparent from the results of parabolic flights. The same may be true for the so-called cardiogenic oscillations in exhaled gas concentration, although such oscillations have been reported not to be present during parabolic flight. The use of the microgravity environment will help to unmask the roles of gravity in creating the gradients and oscillations in expired gas concentrations and may throw light on the nature of the nongravitational mechanisms involved. To gain detailed information about alterations in ventilation, blood flow, gas exchange, alveolar size, pulmonary capillary blood volume and diffusing capacity, and the influence on the pulmonary interstitium, observations made in parabolic flights need to be confirmed and extended during longer exposures to microgravity both at rest and during exercise, using a battery of modern single-breath, multibreath, and rebreathing methods.

A decrease in vital capacity in microgravity has been a consistent finding. A causal relationship with the headward displacement of blood volume may exist. The possibility that pulmonary interstitial edema may be present during certain phases of prolonged exposures merits attention. Although microgravity may improve pulmonary gas exchange at rest, nothing is known about its effects on lung functions during exercise. It is of particular interest to investigate whether the degree of microgravity-induced pulmonary congestion and interstitial edema and accompanying effects on pulmonary diffusion capacity may be great enough to affect maximal exercise tolerance. The consequences of such changes on respiratory control, on arterial blood gases, and on the acid-base status need to be established in space both for normal workloads and for maximal exertion.

**Countermeasures**

Various interventions have been employed during spaceflight to counteract hypovolemia, orthostatic intolerance, and impaired cardiac performance. Principal approaches have included in-flight dynamic and resistance exercise, repeated application of LBNP to redistribute blood volume, and preentry oral administration of fluids and electrolytes. Post-flight palliative measures include the use of antigravity garments to support orthostatic tolerance.

Because of the lack of in-flight control experiments, it is difficult to evaluate the relative efficacy of the countermeasures employed so far. Current consensus is that in-flight dynamic exercise regimens are valuable in counteracting not only cardiovascular deconditioning but also the deleterious effects of microgravity on the musculoskeletal system. However, we have very little understanding of the exact physiological effects of the various types of physical exercise on the phenomenon of cardiovascular deconditioning. It may be noted that in bed rest studies even vigorous dynamic exercise has failed to prevent development of orthostatic intolerance and decrease in exercise capacity in the upright position is not prevented.

There is an urgent need for systematic investigations of the efficacy of more structured in-flight exercise regimens, including heavy-resistance training. This matter will be further discussed in connection with microgravity-induced atrophy of skeletal muscle and loss of calcium mineral from bone.

In bed rest studies, prolonged and repeated application of LBNP and other means of simulating normal hydrostatic gradients have been shown to present or minimize hypovolemia and orthostatic intolerance. The mechanisms underlying the beneficial effects of these procedures are not well understood and warrant further investigation. Reexpansion of the blood volume to pre-bed rest levels and also fluid and electrolyte replacement have yielded variable results with regard to orthostatic tolerance. The role of salt and water loading immediately before reentry and the use of sodium-retentive drugs and other agents as countermeasures need clarification.

Systematic studies of different measures to minimize cardiovascular deconditioning should be designed to gain a better understanding of their relative efficacy. In-flight and post-flight control experiments are necessary. At least in short- and medium-duration flights, some crew members should not receive any in-flight countermeasures. This is necessary if we are ever to understand the full impact of microgravity.
on cardiovascular and pulmonary function. Once it has been established which countermeasures offer clear advantages, questions may be addressed as to their optimal use during long-term spaceflight, when they should be started, and whether they have to be used throughout the flight.

**Musculoskeletal Function**

Prolonged exposure of humans to microgravity leads to progressive muscle atrophy and loss of bone mass, or osteopenia. Serious muscle weakness and the prospect of bone fractures are major dangers posed by spaceflights lasting months or years. If permitted to run their courses, they may severely compromise the ability of crew members to do physical work in extravehicular activities and on return to earth. Although clearly intertwined, their underlying basic mechanisms are poorly understood. This area of research is virgin soil where modern methods in muscle and bone physiology offer abundant possibilities.

**Muscle Atrophy**

The gradual loss of muscle mass during spaceflight predominantly affects the lower extremities, which are no longer weight bearing. There are significant decreases in muscle tone, muscle strength, and working capacity, with reductions in muscle strength being greater in extensor than in flexor muscles. There is also indirect evidence of loss of muscle protein, with increases in urinary nitrogen, phosphorus, and amino acids. In the Skylab studies, nitrogen loss continued unabated for up to 84 days, accompanied by losses up to 30% of muscle mass and strength in the legs. Ground-based clinical experience indicates that normal muscle mass and function can be regained even after years of disuse, and there is as yet no conclusive evidence that microgravity has any unique or specific effects on muscles that cannot be adequately reproduced by enforced inactivity on the ground.

Bed rest and plaster casting are useful models of gravitational unloading for experiments in humans. Accumulated data from bed rest studies show atrophy and strength losses preferentially affecting antigravity muscle groups in the trunk and legs, which contain a high proportion of slow-twitch fibers, and that these changes are associated with increased excretion of nitrogen and phosphorus. Significant loss of strength does not become evident until after about two to three weeks. Studies with plaster casting have indicated decreased whole muscle and fiber size, reduced aerobic oxidative potential and protein turnover, and reduced electromyographic activity. Several of these changes are similar to those obtained from spaceflight.

As mentioned previously, physical exercise regimens of various kinds have regularly been employed during spaceflight to counteract cardiovascular deconditioning. They have also served as countermeasures against muscle atrophy and osteopenia. Unfortunately the influence of the various regimens employed, which have included dynamic, endurance-type training as well as resistance training, has not been systematically studied. The lack of controls have precluded any evaluation either of the progress of atrophy of specific muscle groups as uncompromised by exercise or of the specific efficacy of the different regimens employed. It should be recognized that most crew members have been in a state of high athletic conditioning before flight, so that the relative inactivity during flight may have led to deconditioning independently of any specific effect of microgravity. The data on space disuse muscle atrophy so far obtained from human spaceflight cannot therefore be regarded as optimal.

Valuable information on structural and cellular changes in muscle has been obtained from observations in rats exposed to microgravity for up to a few weeks and also in ground-based experiments with gravitational unloading by hindlimb suspension and other forms of restraint. Postflight observations in rats in the Cosmos series have shown loss of muscle force and elasticity and both structural and biochemical evidence of loss of muscle mass, especially in the “antigravity” soleus muscle, with reduction of the cross-sectional area of slow-twitch fibers and specific changes in enzyme activity. A partial transformation of slow-twitch fibers into fast-twitch ones has been reported. In control experiments with centrifugal force substituted for earth gravity, these muscle changes were largely absent. Rat experiments in Spacelab 3 have shown corresponding decreases in muscle mass and protein content, especially in extensor muscles, with shift of muscle metabolism in the glycolytic direction.

There is insufficient information on the central question of how structural characteristics and the various functional capacities of skeletal muscle are affected by unloading, and during remodeling brought about by different kinds of conditioning. The basic mechanisms in these processes as they occur on earth and in space are poorly understood. We do not know the end point of space disuse muscle atrophy. Progress in these areas is necessary in order to design rational countermeasures.

A primary problem that should be addressed is what alterations occur with time in structural and functional characteristics of human skeletal muscle in space and in ground-based models of gravitational unloading, such as bed rest and casting. Reductions of force output, endurance, fatigue, and recovery abilities need to be investigated and compared in flexor and extensor muscle groups, particularly in the legs and under both concentric and eccentric contractions. To understand the observed changes, we must search for explanations on the cellular and ultrastructural levels. This can be done in bed rest and casting studies using histochemical, biochemical, and ultrastructural analyses of biopsy samples from the muscle groups studied. In this way, with the support of animal experiments, our knowledge of muscle physiology and the cellular and molecular mechanisms underlying muscle atrophy and remodeling on earth and in space will be increased. The new information gained from such studies is likely to have an impact on muscle research far beyond spaceflight.

The design of rational countermeasures to muscle atrophy and dysfunction depends on knowledge of the type of muscle groups and fiber types that are primarily affected. Of immediate concern is to determine the underlying causes and
to search for specific exercise regimens that are efficacious in promoting muscle remodeling during gravitational unloading. The importance of isometric and isotonic exercise, as well as of concentric and eccentric dynamic exercise, in the process of remodeling of various types of muscle groups should be determined. It is worthy of note that there are few data on the effects of heavy-resistance training of specific muscle groups engaged in normal everyday standing and walking.

Osteopenia

Loss of calcium and phosphate with associated osteopenia, or loss of bone mass, is at present probably the most significant hazard for long residence in microgravity. It can be regarded as a functional adaptation of the musculoskeletal system to the absence of gravitational loading. The mineral loss in the heel bone, as estimated by radiographic densitometry preflight and postflight in Skylab and Salyut missions, amounts to about 1% per month, and there is no indication that this rate abates with longer periods. If gravity is the basic factor maintaining normal bone metabolism, then bone loss during spaceflight could continue indefinitely. Photon absorption measurements show about 20% loss of bone in the tibia for the long-term Soviet missions.

There are indications that bone mineral loss occurs at a much faster rate in leg bones than in the rest of the body. It has been estimated that after one year, bone could fracture under the stress of heavy work, such as may be required in extravehicular activities, or upon return to the one-g environment of the earth. It is not known whether microgravity osteopenia is completely reversible if permitted to progress uncompromised by any countermeasures.

Accumulated data indicate that there are similarities between the osteopenia of microgravity and that occurring during bed rest. Continuous loss of bone mass occurs in both instances, although at different rates. In general, exposure to microgravity is characterized by a slower loss of bone mass, by persistent and stable hypercalcemia, and by earlier and more sustained calcitriuria. With long-term exposure to microgravity, the increased urinary calcium excretion shows no sign of abating.

The cellular mechanisms responsible for microgravity osteopenia are largely unknown. Histomorphometric analyses in rats in the Cosmos biosatellite series and in Spacelab 3 have provided evidence for suppressed formation as well as increased resorption of bone tissue. There is also indication that significant changes in bone cell function and metabolism occur already within 8 days. A 17% decrease in total calcium in these animals over a few weeks has been reported. Findings in the rat are difficult to apply to adult humans, because bone tissue in rats does not mature. Nevertheless, current concepts of the cellular mechanisms underlying microgravity osteopenia are largely based on comparisons between results from rat experiments in space with those obtained in ground-based immobilization or suspension studies. Results from suspension studies in rats suggest that bone loss resulting from unloading of the skeleton is local rather than systemic. Observations in restrained monkeys indicate that loss of cancellous bone from the axial skeleton was significantly greater than the loss of cortical bone from extremities.

As a countermeasure against microgravity osteopenia, vigorous physical exercise of various kinds has regularly been pursued by crew members. So far those types of physical exercise that have been employed during spaceflight and that have largely been based on activity rather than force have yielded variable results and have not appreciably decreased calcium loss or been demonstrably effective in preventing loss of bone mineral. The lack of experimental controls defies any attempt to explain these failures. In bed rest studies, similar failures of physical exercise in the supine position have been documented, whereas a few hours of standing or walking each day have been effective.

Dietary and biochemical measures have largely been ineffective in preventing calcium loss. Among biochemical approaches in humans, the use of diphosphonates that prevent the rise in urinary calcium and tend to inhibit bone resorption has shown some promise in bed rest osteopenia, whereas administration of calcitonin, one of the calcitropic hormones, has not been effective.

Information is missing on many critical processes in the chain of events that characterize the osteopenia of spaceflight. Because of the slow development of some of the processes involved, many questions that have to be asked are not easily resolved. A major investment in research is therefore needed to make it possible to arrive at preventive procedures that may permit safe exposures to microgravity of really long-term duration. It seems appropriate to proceed along two main lines of research, namely 1) to further investigate the presumably central role of muscular inactivity in the development of bone mineral loss and 2) to continue current basic studies of the cellular mechanisms involved in bone development, formation, and resorption and of the interrelationships of the bone remodeling system and the calcium homeostatic system. Many of these studies are best carried out in adequate animal models.

The osteopenia of microgravity can, as already mentioned, in certain respects be regarded as secondary to muscle atrophy, since calcium mineral loss is almost certainly triggered by the absence of forces that normally support the body weight. It may be assumed that the search for optimal exercise regimens to prevent the muscle atrophy of microgravity will also benefit the prevention of osteopenia. The biomechanics of microgravity is unknown territory. A primary objective is to investigate how microgravity affects the normal compression forces acting on the bone and the shearing and tensile forces acting on the periosteal surfaces. With such knowledge it would be possible to mimic the gravity-dependent skeletal stresses and strains and thus replace the missing mechanical input to the bones. Other objectives of immediate concern are to determine the time course of calcium loss in crew members with progressive extension of flight duration, particularly whether calcium loss will continue beyond some critical level of bone density, and to continue metabolic studies with controlled diets. Progress in these areas is necessary before spaceflight of several years.
can be considered, as would be required for Mars exploration.

**Neurovestibular Function**

I finally wish to draw your attention to some problems belonging to the area of neurosensory physiology. In spaceflight the gravity signals from the otolith organs are physiologically "dissected" from the vestibular/neurosensory system. As a result, the otolith discharge behaves as it would do under only one special terrestrial condition, namely that of free fall. The free-fall signaling from the otoliths in the space environment contradicts the visual and proprioceptive inputs, which tell the subject that he is in a stable environment. The resulting conflict between inputs to the vestibular system disrupts the sense of gravity and initially causes disorientation and disturbances in postural control. The development of space motion sickness during this phase may be regarded as a generalized averting reaction. These vestibular phenomena are unique to microgravity and cannot be adequately simulated on earth.

Space-related neurosensory physiological research has long centered on the neurovestibular area because of its importance for the problem of space motion sickness. I shall therefore limit my account to this special case of intravestibular sensory conflict, whose manifestations can have severe disruptive effects on crew efficiency and be a source of potential problems in early extravehicular activity and during arrival and transfer at space stations. Symptoms usually start within an hour of exposure and increase in intensity over a period of hours to reach a plateau lasting for two to three days, after which they rapidly subside and resolve. Vomiting, loss of appetite, headache, and malaise are prominent symptoms. Space motion sickness afflicts roughly half of all space travelers and has therefore become a significant operational concern particularly for short-lasting space missions.

Investigations into the area of space motion sickness can be roughly classified in the following categories: 1) identification of basic mechanisms responsible for space motion sickness, 2) provocative testing and prediction of individual susceptibility, 3) prevention and management of symptoms.

**Identification of Basic Mechanisms**

The intravestibular mismatch of sensory signals previously referred to, reinforced by head movements, is commonly thought to be the primary cause of space motion sickness. However, the basic central circuitry responsible for motion sickness is at present unknown. It is believed that the mismatch of sensory inputs is relayed to the area postrema adjacent to a vomiting mechanism located in the medulla. It is noteworthy that individuals without a functioning labyrinth are not susceptible to motion sickness. Continued testing of classical theories of terrestrial motion sickness is required, including the possible presence of a chemoreceptor trigger zone and a vomiting center. Since many symptoms are subjective, the generation of motion sickness is difficult to study in animals, and efforts to identify an adequate animal model of motion sickness are therefore of prime importance.

The ultimate goal of identifying the basic mechanism of space motion sickness is closely related to the understanding of the mechanisms responsible for orientation and postural control on earth and the manner in which these mechanisms adapt to microgravity. This is a task of vast proportions, requiring intensive ground-based and in-flight investigations in both humans and animals and the use of special vestibular research facilities.

**Provocative Testing and Prediction of Individual Susceptibility**

The motion sickness experienced on the ground and in conventional flight can readily be provoked by the use of devices that produce different combinations of labyrinth and visual stimulation. One of the problems with the use of such tests is that none of the stimuli produced mimics the free-fall information from the otolith organs typical for the microgravity environment. This may account for the fact that none of the many ground-based tests designed have helped to predict individual susceptibility in space.

Valuable information has been obtained from provocative tests during exposure to microgravity. Initially, head movements tend to exacerbate symptom development, which supports the notion that space motion sickness is not primarily a result of the cephalad redistribution of fluid that occurs during the initial phase of exposure to microgravity. Head movement tests, which stimulate the otolith organs and are performed after adaptation over several days, have shown decreased susceptibility to in-flight motion sickness, which suggests that the central processing mechanism has learned to disregard certain types of input from the otolith organs. The kernel of the matter seems to be the role of adaptation of otolith function to microgravity and its influence on orientation and sensory motor control and how this adaptive process is related to the development of space motion sickness. Devising rational predictive tests for use on earth rests on an understanding of the nature of this process. To attain such understanding requires extensive in-flight experiments on humans in both short- and long-duration missions with the use of facilities of delivery of controlled provocative motion, such as vestibular research facilities, moving platforms, and variable-G centrifuges.

**Prevention and Treatment of Space Motion Sickness**

Among the various methods explored in the past to suppress the symptoms of space motion sickness are biofeedback training, preadaptation procedures, and drug therapy. Biofeedback, involving the individual's monitoring and conscious control of his autonomic responses such as heart rate and blood pressure, has in individual cases been effective in suppressing symptoms of motion sickness in the one-g environment. The efficacy of this method for preventing symptoms of space motion sickness should be further explored.
To attain preflight adaptation to microgravity by the use of graded exposure to provocative vestibular stimulation is a possibility of potential value, which needs further investigation. The successful use of preadaptation measures would seem to depend on whether provocative tests can be devised that bring out visual and semicircular canal signals at the expense of otolith and other gravity-sensing cues. Drugs used to treat space motion sickness are largely the same that are used against motion sickness in the normal-gravity environment. To develop a rational basis for further development in effective drug therapy is of major importance. Increased understanding of the basic mechanisms responsible for space motion sickness, employing adequate animal models, is a prerequisite for progress in this area. In-flight drug testing on crew members using provocative stimulation will be needed, even if operational demands will put a limit to such testing.

Having received briefly some unresolved questions in selected areas of gravitational physiology, and having in very general terms indicated what is needed to narrow the gaps in our knowledge in these areas, I wish to add some concluding remarks. Gravitational physiology is in its infancy. If we are to have a future of humans in space, we have a long way to go to ensure protection against the adverse physiological effects of microgravity. Relatively few physiological experiments have been flown, and the availability of experiment opportunities in human physiology will continue to be limited, which in turn will impede the carrying out of adequately controlled and replicated experiments. One unavoidable approach to the study of human adaptive responses to microgravity, and their interrelationships, is to permit the adaptive process to run its course in a sufficient number of crew members without countermeasures of any kind. However, both ethical and operational considerations will limit the duration of such control exposures.

In the next 10 years we will learn a little more how human physiology is affected by the microgravity environment. Valuable information on the cardiovascular, pulmonary, renal, and neuro-vestibular responses will be gained from missions with durations as short as one week. Long-term exposures in space stations will permit acquisition of new data on the progress of these responses with time and on the relative efficacy of various countermeasures. Exposures of months to years may be required for corresponding investigations regarding the more slowly developing alterations that occur in musculoskeletal and other functions. However, most of the needed information in all these areas will no doubt be gained from relevant ground-based research on both human and animal subjects, with unlimited access to new and sophisticated methods and the possibility of manipulating the experimental material at will. Substantive and continuous spinoffs from such research will, as in the past, contribute importantly to terrestrial medical research and practice.

And yet, it is likely that by the turn of the century the understanding of the effects of long-term microgravity on human physiology will at best be fragmentary, particularly the potential for irreversible adaptive changes. It is axiomatic that all problems connected with the physiological decay caused by microgravity can be solved by substituting centrifugal force for gravity during spaceflight. Man-rated centrifuges on board space stations will materialize for the study of threshold effects, i.e., to determine how much of earth’s gravity we need to bring with us into space for really long-term residence. The engineers will eventually step in and provide centrifugal force as the ultimate protective device. Nevertheless, even if such a development is bound to happen sooner or later, we cannot forget about the fascinating effects on the absence of effective gravity, perhaps the most familiar and, at the same time, the least understood of all environmental forces.

Suggested Readings


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INTRODUCTION

In this communication some rather unexpected experimental data important for the basic concept of food assimilation will be considered.

In higher organisms the small intestine is a principal organ of nutrition which performs vital functions of digestion and absorption of nutrients and a function of the biological barrier separating external and internal media of the organism [Revs: 3, 4, 7, 10, 14, 15]. These functions are attributed mainly to the mucosal layer consisting of functionally polarized cells which perform membrane hydrolysis and transport [Revs: 2, 3, 13, 14]. The functions of the enterocyte have been studied intensively during the last decades and some important discoveries appeared to be significant not only for the understanding of main regularities of food assimilation but for fundamental rules of living systems as well.

According to the current views nutrients hydrolyzed and resorbed by the epithelial layer easily permeate through highly permeable structures of the basement membrane. Then they enter blood and lymphatic vessels of the stroma.

However, it is necessary to emphasize that a fundamental idea according to which only a mucosal layer is the only digestive and barrier sheet is not experimentally proved.

To study this problem our Lab developed a new technique which allows us to separate three layers of the small intestine (mucosal, submucosal and muscular-serosal ones). This permits to analyze their physiological functions.

METHODS

The three small intestinal layers: mucosal (epithelial), submucosal and muscular-serosal ones were preparatively isolated in male Wistar rats (180-200 g, fed or fasted for 3 days), with a technique developed by our Lab (Fig. 1).

To separate the layers the small intestine (without duodenum) was excised and washed with the calcium-free Ringer solution (about 0°C, pH 7.0). Then the small intestine was everted, filled with the same solution and placed into 2 mM EDTA for 10 min. After the treatment three layers (mucosal, submucosal and muscular-serosal ones) of the small intestinal tissue were isolated. Each of them was washed (three times) with the Ringer solution to remove EDTA. The quality of the separation of mucosal and submucosal structures was checked morphologically and by measuring the activity of sucrase as a marker brush border enzyme [Rev 16]. The layers were isolated either from the entire intestine or its proximal and distal portions.

The activities of sucrase (EC 3.2.1.48), maltase (EC 3.2.1.20), alkaline phosphatase (EC 3.1.3.1), alanine aminopeptidase (EC 3.4.11.2), peptidases (EC 3.4.13--) hydrolyzing diglycylglycine and dipeptides: glycyglycine, glyclyglucine, L-leucylglycine and glycol-L-α-alanine (not identified enzyme) were tested as described previously [16, 17].

The enzyme activity was expressed in μmoles of the hydrolysis products per 1 min per 1 g of the wet weight of the tissue and per 1 mg of protein.

The distribution of enzyme activities in the supernatant and sediment fractions was examined after the ultracentrifugation (100 000 g, 30 min) of homogenates of the mucosal, submucosal and muscular-serosal layers.

To study the absorption of tri- and dipeptides the small intestinal intact and deepithelized everted sacs filled with the Ringer solution (pH 7.4) were incubated in 5.0 mM diglycylglycine or some dipeptide solutions for 20 min at 37°C. The concentration of the substrate and its hydrolysis products was de-
terminated by high performance liquid chromatography (HPLC) of their dansyl derivatives [13] in both fluid filling the sac and the incubation media.

The data obtained were statistically evaluated using the Student t-test and non-parametric test. The main results are presented below.

RESULTS

1. The distribution of enzymes in isolated intestinal layers

We have found sucrase (a marker enzyme of the enterocyte brush border membrane) only in the mucosal layer.

Maltase activity was concentrated in the mucosal layer but it was found in the submucosal and muscular-serosal layers as well (9% and 4%, respectively, as compared to enzyme activity in the mucosal layer taken as 100%).

Alkaline phosphatase (AP) and alanine aminopeptidase (AAP) activities were shown to be present in all three layers of the small intestine, their levels being significant in the submucosal and muscular-serosal layers. In particular, the activities of AP and AAP were 19% and 16% in the submucosal layer and 17% and 14% in the muscular-serosal one.

Maximal activities of peptidases splitting diglycylglycine, glycyl-\(\alpha\)-L-alanine and glycyl-L-leucine have been found in the mucosal layer. The activities of these enzymes were characterized by relatively high levels in the submucosal layer (29%, tripeptidase; 48%, glycyl-\(\alpha\)-L-alanine dipeptidase; and 33%, glycyl-L-leucine dipeptidase) and in the muscular-serosal layer (27%, 46% and 36%, respectively).

The distribution of glycyglycine dipeptidase in isolated intestinal layers was quite unexpected. Maximal enzyme activity has been found in the muscular-serosal layer (254% as compared to that in the mucosal layer). This activity was similar in the mucosal and submucosal layers.

In summary, the data obtained allow us to postulate that in addition to an enterocyte enzymic layer there exists a post-epithelial enzymic system, in particular a significant peptidase system which possibly is very important in the digestive and barrier functions. Different experimental data obtained by us support this point of view.

2. Nutritive adaptations

It has been widely accepted that brush border enzymes are involved in nutritive adaptations [5, 6, 15]. In particular, the system of maltases, aminopeptidases etc. On the other hand, cytosolic enzymes of the enterocyte do not participate in food-dependent adaptive reactions.

Using the new technique of isolated layers of the small intestine this problem was re-investigated in our Lab. (Enzyme activities of fed and fasted for 3 days animals were compared). We have demonstrated a complex structure of the enzyme adaptation. In particular, adaptive changes of maltase activity in mucosal, submucosal and muscular-serosal layers were found. In fasted rats maltase activity in corresponding layers of the small intestine was 53%, 20% and 24%, respectively, as compared to that in fed animals.

However, some other brush border enzymes, for example, AAP do not react significantly to food load and fasting for 3 days.

On the contrary, typical cytosolic enzymes such as glycyl-\(\alpha\)-L-alanine and glycyl-L-leucine dipeptidases are involved in the adaptation in the mucosal, submucosal and muscular-serosal layers. In fasted for 3 days rats the activities of these dipeptidases were 78% and 74% in the mucosal layer, 43% and 70% in the submucosal layer, 36% and 48% in the muscular-serosal one, respectively.

In other words, an intestinal enzyme adaptation is not only the enzyme adaptations of the enterocyte layer. This is rather an integrative adaptive reaction of the small intestine as a whole organ, including mucosal, submucosal and muscular-serosal enzyme systems.

It is of interest that sometimes we could observe the adaptation of only submucosal layer enzymes without the adaptation of the mucosal component.

3. Membrane-bound and cytosolic enzymes in isolated small intestinal layers

The enzymes under study may be divided into two groups: membrane-bound enzymes and cytosolic ones [Revs: 8, 11]. However, this widely spread classification is based on studies using the enterocyte fraction or more often nonpurified intestinal tissue preparations. We investigated the subcellular distribution of the same membrane-bound and cytosolic enzymes of isolated intestinal layers using ultracentrifugation technique. In this case the results obtained were sometimes unexpected and different from widely accepted views.

In these conditions alkaline phosphatase demonstrated cellular localization typical for membrane-bound enzymes in all three layers (about 85-95% of the AP total activity).

Maltase behaved as a brush border enzyme in the mucosal and submucosal layers (98% and 88% of maltase activity, respectively, was found in the sediment). But in the muscular-serosal layer about half of the activity was observed in the supernatant.

An increase of enzyme activity of the cytosol fraction was found in the submucosal and muscular-serosal layers for several enzymes. For example, AAP activity
of mucosal homogenates was sedimented after the ultracentrifugation (95%). In the submucosal and muscular-serosal layers sedimented AAP activities were 53% and 35%, respectively.

At the same time, some peptidases demonstrated the distribution typical for cytosolic enzymes in all three layers. The activity of Glycyl-L-leucine dipeptidase was predominant (some 90%) in the supernatant. After the ultracentrifugation of mucosal, submucosal and muscular-serosal homogenates peptidase activity against glycyl-g-L-alanine in the supernatant was some 75, 90 and 85%, respectively.

These data were not only unexpected. They demonstrated that the same enzymes in the mucosal, submucosal and muscular-serosal layers seemed to perform different physiological functions the analysis of which is an important and interesting task for future studies.

4. Peptide hydrolysis and transport through an intact and deepithelized intestinal wall

In conclusion we shall consider some direct evidence concerning that enzymes of subepithelial structures are involved in the hydrolysis of food substrates during their absorption.

To solve this problem a special approach was developed (Fig. 2). In the experiments two types of everted sacs have been used. One of them is a classic sac which consists of the mucosal, submucosal and muscular-serosal layers (intact sac) [2, 12]. The second type of the sac (prepared from an intestinal segment of the same animal) consists only of the submucosal and muscular-serosal layers (deepithelized sac).

![Fig. 2. Schematic representation of two types everted intestinal sacs for the studying membrane hydrolysis and transport.](image)

The transport of different di- and tripeptides was investigated. The qualitative determination of peptides and their hydrolysis products in the incubation medium and an internal fluid of sacs was carried out using the high performance liquid chromatography.

In the preliminary experiments we have established experimental conditions convenient for the experimental analysis of the role of mucosal and submucosal peptidases during the transmural peptide transport.

Fig. 3 demonstrates the absorption and intensive hydrolysis of glycylglycine by intact everted sac wall during its transmural transport. It is interesting that a diffusional resistance and significant hydrolysis of this dipeptide takes place when deepithelized everted sacs are used.

![Fig. 3. Transmural transport of glycylglycine and its hydrolysis product by everted intact and deepithelized intestinal sacs.](image)

Vertical line, glycine concentration in mucosal (M) or serosal (S) medium, mM. Mean (n=4). Incubation time, 20 min. Details see in "Methods".

The comparison of properties of intact and bilayer sacs allows us to conclude that subepithelial structures are an important diffusional and enzyme-active barrier for peptides under study.

CONCLUSION

Thus, the use of new experimental approaches in combination with the available ones allowed us to discover unknown earlier fundamental functional characteristics of the small intestine as an organ performing vital digestive-transport and barrier functions. However, one should take into consideration that we have made only first steps in this field.

The results obtained require to make several conclusion. The perfect and reliable functioning of the small intestine as a biological barrier and a digestive
layer is achieved by the additive interaction of enzyme systems of the enterocyte layer and the submucosal one. Such a doubling of the functions is an expression of an important biological principle described first by Barcroft [1].

An important role of the submucosal layer is demonstrated not only by a high level of some enzyme activities comparable to that of the mucosal layer but by a direct comparison of the peptide hydrolysis during their absorption by intact and denuded segments of the small intestine. At the same time a number of problems is to be further analyzed.

Thus, using a new technique of isolated intestinal layers we have found that, in addition to enterocyte hydrolytic enzymic system [Revs: 2-4, 7, 10, 13, 14, 15], there exists an important and specific hydrolytic system in submucosal structures. They are involved in nutritional adaptations and in the splitting of food substances during their absorption. The existence of mucosal and post-mucosal hydrolytic systems enriches significantly the current concept of food assimilation and the idea of biological intestinal barrier. Finally, the above data are useful for the interpretation of the normal function of the small intestine and simultaneously for the better understanding of different forms of the intestinal pathology.

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CELL IN GRAVITATION FIELD

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Cell organization should be, to our mind, considered from two points of view: on the one hand, cell represents chemical reactor which functions in accordance with regularities of biological thermodynamics, not depending on gravitation forces; on the other hand, it is a mechanically reconstructed existing in gravitation field in strained condition, due to resistance to gravity [10].

The influence of gravitation forces is also dual. Direct mechanical action of gravity force on individual cell and intracellular structures as mass carriers should be distinguished from nondirect influence of gravity on biological cell status via alteration of physico-chemical parameters of environment.

In accordance with this, in analysis of the extent of influence of gravitation field tension on cell level and of causes for any changes in cell structure and functions, it is important to use as an object for the study of the adequate models corresponding to cell types in their morpho-functional properties and environment. Namely, monolayer or multilayer cell culture developing on solid substratum (in vitro); association of individual cells or free living unicellular liquid-inhabiting organisms (in vivo); cells functioning within single multicellular plant or animal organism (in situ).

Let us consider in more detail two former models (cell types). We will consider cells developing on solid substratum as major model for studying direct action of gravity force, free living unicellular organisms inhabiting liquid being taken as a model for the study of indirect effect of this factor. As to the cells functioning within single multicellular organism, their interaction in gravitation field is completely determined by regulatory processes of the highest level, nervous and hormonal, which may be reported elsewhere.

Direct influence of gravity on cell (in vitro)

It is well known that mechanical cell forces occurring in gravitation field are the result of two oppositely directed processes: space randomization of intracellular elements of different density and their tendency to sedimentation with various rates under the influence of gravity. The extent of the latter tension depends on the cell size and initial distribution of intracellular structures of varying mass. The longer the cell and higher the quantity and density of cell organelles, the stronger is gravitation dependence, and contrary, diminishment of cell and cell organelles sizes is followed by decrease in the index of cell dependence on gravity.

A number of hypotheses explaining mechanical cell rigidity and stability of its functioning in gravitation field have been put forward which claim the principle of retaining positional homeostasis. The term "positional homeostasis" introduced into gravitation biology by G. Nace [7] is by now interpreted in more broad sense and points to active maintenance of stable state and optimal cell orientation in gravity force field as well as to retaining its morpho-functional status under conditions of alteration of gravity field tension within definite limits of values. No doubt that some energy expenditure is required for maintenance of positional homeostasis, this constituting a noticeable value of cell energy expenditure. Most important is retaining positional homeostasis in early morphogenesis during the period of highest dynamic development of the system.

Since physico-chemical processes taking place in developing systems occur in heterogeneous medium with complex rheological properties and are, in majority of cases, accompanied by various mechanical motions specified by both internal and external forces, including gravity, deformations may well occur. When active deformation takes place, cell shape, volume and sizes are being changed. This process is usually characterized by latent period necessary for reception and realization of mechanical impulse, gravitation stimulus, in particular, and proceeds with active participation of membranes and elements of cytoskeleton and intracellular organelles.

It is well known that cell membranes are in quasi-elastic liquid state and as viscous-elastic bodies, which accounts for their elastic properties and high mechanical rigidity. It is ascertained that biomembrane elasticity defined by the Young modulus varies within the cell limits 10^8 - 10^9 dyn/cm [11]. To compare the values of mechanical deformations which may be a result of cell weight effect on basal membrane in gravity, some calculations were made. Cell models were regarded which are most often encountered in nature: cylindrical, cubic and globe-shaped of equal sizes. The results demonstrated that maximal pressure of cell mass on basal membrane constitutes 10^8 dyn/cm. Hence, we may conclude that membrane possesses significant rigidity capacity and remains intact under such deformations. But then, deformations mediated by gravity may lead to structural cell rearrangements caused by interaction of cytoplasmic membranes with contractible cytoskeleton elements.

Cytoskeleton, an indispensable portion of all eucaryotic cells, is a mobile component constructed of microtubes and microfibrils [5] and has functional and structural function. Cytoskeleton determines cell shape, the extent of its mobility, the nature of intercell contacts and, at the
same time, participates in regulation of metabolic processes. As some specialists believe [1,3,6], cytoskeleton may act as non-specialized universal sensor of gravitation within a cell. Permeability of cell membranes and contractile elements of cytoskeleton clearly point to the possibility of ensuring autoregulatory cell processes on the principle of reversibility of mechanical deformations occurring in gravity field. This means: mechanical deformations mediated by gravitation may directly influence individual cell. Most pronounced this influence is seen in cell cultures developing on solid substratum.

In addition to membranes and cytoskeleton contractile elements, nucleus directly acts in realization of the mechanism of gravitation impact on cell. Nucleus is the largest organelle of eucaryotic cell. Its density is 1.5 times higher than that of cytoplasm. Spatial disposition of nucleus within cell is important for realization of cell division algorithm and is one of significant members of positional homeostasis. Since nucleus is associated with membrane surface and cytoskeleton elements, force action such as mechanical deformation leading to change in nucleus disposition in cell may affect metabolism and determine largely the rhythm of cell division. Moreover, as a result of increase in nucleus area and its spatial location in gravitation field, cell sprawling takes place. Such effects may be as well stimulated by rearrangement of other cell organelles: plastids, mitochondria, etc.

Thus it may be stated that mechanical deformations caused by changes in size and direction of gravitation vector lead to direct influence of gravitational forces on cell. As noted above, most pronounced these effects are in cell cultures developing on solid substratum, where the nature of intercellular contacts and the adhesion extent are well expressed. In conditions of gravity absence (weightlessness) or minimal values of this factor (microgravitation), deformations in mechanical connections may cause changes in cell physiological characteristics.

Indirect influence of gravity at cell level (in vivo)

Let us regard the influence of gravity on liquid inhabiting cells. In majority of cases, these are unicellular organisms actively moving in liquid or floating cell suspensions passively distributed in medium. For three-dimension-distributed in liquid culture, physico-chemical parameters of medium, such as hydrostatic pressure, gradients of substrates concentrations, conditions of gas-liquid exchange, sedimentation rate, convective flows are very important. However, gravitational impacts on unicellular organisms depend both on individual properties and regularities of behaviour of the whole cell population as a single biosystem.

Almost all unicellular freely-floating organisms possessing their own movement apparatus demonstrate negative geotaxis and positive oixyaxis. Due to this, in liquid media they form species-specific gradients of concentrations which are actively maintained by gravitation-dependent bioconvection. Physico-chemical and hydrodynamic basis of bioconvection, the phenomenon of its formation and maintenance are described in literature in detail [9,12].

The presence of bioconvective flows in liquid medium is mediated by difference in weight values of cell suspensions and layers of cell culture located within a father narrow surface range (1.5-2 cm) on the gas-liquid separation border. When critical value is reached in densities of higher and lower layers of cell culture \((P - P) = \), the former falls down avalanche-like and the latter takes its place. In this way, periodical exchange of culture portions ensures uniform oxygen supply. Since the effects of gravity, \( P = \), gravity force should directly participate in this process. In other words, bioconvection is gravitation-dependent process and consequently, alteration in intensity of gravitation field must lead to redistribution of cell culture or unicellular organisms and thus affect vital activity of single cell and the culture as a whole.

Experimental studies performed in laboratory conditions using centrifuge at 2, 3 and 5g confirmed theoretical calculations [11]. Double-phase nature of hypergravitation effect on growth dynamics of cell culture and unicellular organisms was revealed. Acceleration of growing biomass in experimental culture was observed at initial stage. However, stimulation process was slowed down rather quickly. The period of stimulation depended on two factors: cell size and cell mass volume. The higher these values, the sooner the curve broke down and shorter stimulation period. Then the stage of growth brake came. Thus, the results of a series of experiments carried out on various representatives of unicellular organisms demonstrated that stimulating effect of hypergravitation was connected with the ability of gravity to switch on the mechanisms of bioconvection initiation. Importance of the effect obtained for applied interests is, to our mind, of great value in Earth and space biotechnology, because it becomes possible to optimize cultivation conditions for various cell types operating with gravity as an alternative factor.

No spontaneous bioconvection is initiated under conditions of space flight, since no mechanisms underlying this process operate. Stimulation of cell division rate and increase in culture biomass observed by specialists in numerous space experiments is of the other nature. It is connected, in the first place, with more uniform distribution of both cells and high-molecular substances (nutrient substrata and products of vital activity) under microgravitation as well as with changes in physico-chemical parameters of cultivation medium. Therefore, it is evident that to elucidate regularities of cell culture behaviour in liquid and in gravitation field, information on environment is needed, in addition to the data on cell morphological status.

Such approach to the problem enables us, first, to furnish an explanation for mechanisms responsible for stimulating effect of microgravitation observed in a number of space flight experiments performed on cell culture, and secondly, to reveal some novel regularities of cell culture behaviour in gravity field. In particular, it was shown, in which way cell shape and sizes are interrelated with gravity.

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It is established that gravity for large multicellular organisms is a factor limiting their sizes. And body mass growth leads to non-uniform growth of body shape. Width increases quicker than length. It is not so at the cell level. As cell sizes increase, the ratio of their length to width increases. For example, if one disposes unicellular organisms under study in the order of increasing size, one can see following picture: the ratio of length to width is equal 10x10 mkm for Chlamydomonas reinhardtii, this being 50x30 mkm for Tetrahymena pyriformis, 100x30 mkm for Euglena gracilis and 200x70 mkm for Paramecium aurelia. We believe that the main reason for this is that medium resistance to floating cell is determined by its frontal portion. Therefore, less energy is spent to resist medium by a cell growing in length quicker than in width. Moreover, it should be kept in mind that each cell or unicellular organism rises separately (spending energy to resist gravity), while all cells fell down assembled, without energy expense. This, to our mind, may explain some peculiarities of different type cells' behaviour in gravitation field.

Analysis of the results of studies at cell level obtained aboard spacecrafts indicates that microgravitation conditions cause various responses in various cells: in some cells these conditions stimulate growth and metabolic activity, whereas in others they lead to retardation of the processes of vital activity [8, 2].

So, what are the reasons for so different, even contradictory results? We will try to elucidate them, using the data obtained in later research carried on aboard biosatellites "Smos 1887" (1974) and "Cosmos 2044" (1989), in experiments "Cytos" and "Protoplast", respectively. In the "Cytos" experiment free floating unicellular organisms Tetrahymena pyriformis served as objects of study. Significant increase in culture biomass took place during 14 days of flight, due to augmentation of cell proliferative activity. In the "Protoplast" experiment with isolated protoplasts of raps and carrot (Brassica napaus, Daucus carota) exposed to microgravitation during the same period of time significant decrease in biomass growth and general metabolic cell activity for many other indexes took place.

It should be brought to mind, first of all, that the area of free floating unicellular organisms is a thin layer on the liquid-gas border. In the conditions of normal gravity on Earth certain quantity of energy should be spent to survive as long as possible in this area. In conditions of microgravitation, in addition to alteration of behavioral properties of the culture itself, spatial redistribution of two-phase system's components (gas-liquid) occurs. As a result, numerous gas bubbles are formed in liquid, which enlarges markedly the area of phases separation and consequently favours culture growth. Also, as it is not necessary for cells to spend energy for resisting gravity, the energy released may be used for other purposes. To our mind, stimulating effects of space flight on cell growth retardation and metabolic activity decrease in the "Protoplast" experiment and the like, where cells were lacking motion apparatus and passively distributing in microgravity inhabited. In particular, loss of weight in the absence of gravity may lead to negative results, owing to disturbance of intercellular contacts, blocking signals needed to ensure connections between cell and environment, the absence of available nutrients and accumulation of metabolic products. Thus, it may be asserted that dynamics of growth and distribution of unicellular organisms and separate cells in gravitation field has multifactor foundation. The effects of altered gravity, including microgravitation, at cell level are the summed up result of shift in physiological characters of cells per se, and physico-chemical parameters of environment as single biosystem.

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EPIDERMAL GROWTH FACTOR INDUCED SIGNAL TRANSDUCTION IN A431 CELLS IS INFLUENCED BY ALTERED GRAVITY CONDITIONS

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INTRODUCTION

In recent years it has become clear that microgravity may affect cell growth and differentiation both in vivo and in vitro. The evidence for this notion is manifold and includes observations on a variety of human mammalian and non-mammalian animal cell types (1) and plant cells (2). Although this might be taken as indicative for an effect of gravity on well conserved regulatory mechanisms of growth and differentiation, no clues as to what molecular mechanisms are involved have been obtained so far. Based on the original observations that prolonged space flights affect the responsiveness of the immune system, a number of in vitro experiments were performed, in which it was shown that hypergravity results in enhanced cell proliferation, while microgravity had the opposite effect. Most noticeable however, was the severe depression of the activation of T lymphocytes by the artificial mitogen concanavalin A (ConA) under microgravity conditions (3-5). The activation of lymphocytes by ConA is representative for the common mechanism by which plasma membrane receptors exert control over cell proliferation and differentiation. In view of far reaching consequences of gravity on cell proliferation during prolonged space flights and for reasons of space technology, we feel it important to identify the target mechanisms involved. Therefore we have studied the effect of gravity on polypeptide growth factor induced signal transduction.

Polypeptide growth factors have been demonstrated to play an important role in the regulation of cellular proliferation. Epidermal growth factor (EGF) is one of the best known growth factors to date. EGF exerts its effects by binding to a specific, plasma membrane located receptor, the EGF-receptor (EGF-R), which exhibits intrinsic protein tyrosine kinase activity. Binding of EGF to the EGF-R causes activation of the tyrosine kinase and subsequent induction of signal transduction, involving a cascade of cellular responses such as the breakdown of a particular class of plasma membrane phospholipids, the formation of the second messengers diacylglycerol and inositol triphosphate, increase in protein kinase C activity, and intracellular Ca²⁺ concentrations. Depending upon the cell type, the activation of protein kinase C and the Ca²⁺ pathways are independently or in combination sufficient to elicit within minutes transcription of various genes, i.e. c-fos and c-jun (6) (Figure 1).

![Figure 1. Schematic representation of early cellular responses to EGF.](image)

We have studied the effects of microgravity on EGF-induced signal transduction by determination of a plasma-membrane related response, i.e. EGF-induced receptor redistribution; a cytoplasmic response, i.e. EGF induced changes of actin microfilament organization; and a nuclear related response, i.e. EGF-induced expression of c-jun and c-fos proto oncogenes. Experiments to test whether microgravity affects EGF-R mediated signal transduction in human A431 epidermoid carcinoma cells and to specify microgravity-sensitive molecular events have been carried out during two ESA-sounding rocket missions (Maser 3 and 4), which allowed exposure to microgravity for 7 minutes. The study was supported by ground-based clinostat experiments to simulate low gravity conditions and by centrifugation experiments to induce hypergravity.

RESULTS AND DISCUSSION

To investigate the effect of gravity on EGF-induced expression of the protooncogenes c-fos and c-jun, A431 cells were cultured in the CIS plunger box units and stimulated with EGF or medium alone for 6 minutes after microgravity was reached. Subsequently, RNA was isolated from the cells and analysed for c-fos and c-jun transcripts by RNase protection. As shown in Figure 2, no c-fos transcript was detected in un-stimulated cells, neither in the 1-G control experiment, nor in the microgravity samples. In contrast, a strong expression was detected in the 1G control samples treated with EGF for 6 minutes. However, the expression of the c-fos gene was decreased by about 50% under microgravity conditions (Figure 2).
Figure 2. Microgravity decreases EGF-induced c-fos expression:
A431 cells were cultured on thermanova coverslips and mounted in the CIS plunger box unit and assembled in the CIS module on the MASER sounding rocket. As soon as microgravity was reached the cells were treated with EGF (+ EGF, 6 min) or with medium alone (-EGF, 6 min). As a control one sample was lyzed after launch immediately when microgravity was reached (-EGF, 0 minutes) to study the effect of the high g-values reached during the launch of the rocket. Simultaneously a 1-g reference experiment (ground) was performed identically to the microgravity experiment (flight). After all samples were washed and lyzed, RNA was isolated and analyzed for c-fos transcripts by RNase protection.

Similar results were obtained for expression of the c-jun gene. These results were supported by ground-based clinostat experiments, and clearly demonstrate that EGF-induced signal transduction is sensitive to gravity alterations.

In order to determine the molecular target(s) for the gravity effects of signal transduction, the effect of microgravity was studied on EGF-induced receptor clustering. Binding of EGF to its receptor causes a redistribution of the receptor in the plane of the membrane (7), and as such this receptor clustering constitutes one of the first responses in the EGF-induced signal transduction pathway. As shown in Fig. 3, exposure of the cells to microgravity for 6 minutes did not affect the receptor distribution significantly as compared to normal gravity conditions, indicating that the gravity sensitive targets in the signal transduction pathway are located down-stream of the receptor.

A variety of different signal transduction pathways lead to a rapid increase of c-fos expression (8). To examine if the observed modulations of c-fos expression by gravity changes are specific for EGF-induced signal transduction, we studied the effects of microgravity on c-fos expression induced by the phorbol ester TPA, the Ca2+ ionophore A23187 and the activator of protein kinase A, forskolin. As shown in Fig. 4, both EGF and TPA-induced c-fos expression was decreased under microgravity conditions, whereas forskolin and A23187-induced c-fos expression remained constant.

Figure 3. Effect of microgravity on EGF-induced receptor distribution.
A431 cells were cultured on thermanova coverslips and mounted in the CIS plunger box unit and assembled in the CIS module on the MASER sounding rocket. As soon as microgravity was reached the cells were treated with EGF (+ EGF) or with medium alone (-EGF). Simultaneously a normal gravity reference experiment was performed. Cells were fixed, immunogold labeled, frozen and freeze fractured as described (12).

Figure 4. Gravity alterations differentially modulate distinctive signal transduction pathways:
A431 cells cultured and mounted in the sounding rocket (0G) as described in the Legend of Fig. 2, and the 1G reference setup (1G), were treated for 6 min. with EGF (100 ng/ml), A23187 (2.5 μM), TPA (100 ng/ml), forskolin (10 μM) or in medium alone (6CON), after which the cells were lyzed. As a control to determine the effect of high G-levels reached during the launch of the rocket, cells were lyzed directly after reaching microgravity in the rocket (CON 0). RNA was isolated and analyzed for c-fos and B-2 microglobulin (B2M) expression by RNase protection.
The data obtained so far demonstrate that c-fos and c-jun gene expression, as induced by EGF or by the phorbol ester PMA, is severely inhibited under microgravity conditions, but no effects of gravity changes were observed on the expression of these genes induced by the Ca$^{2+}$ ionophore A23187 or by the cAMP elevating stimulant forskolin (9, 10). These results indicate that microgravity affects specifically the signalling pathway mediated by protein kinase C. In addition we demonstrated that microgravity has a significant effect on EGF-induced cell rounding (Figure 5) and actin microfilament organization (11, 12).

![Graph showing the effect of gravity on EGF-induced cell rounding.](image)

**Figure 5.** The effect of gravity on EGF-induced cell rounding:
The effect of simulated gravity (clinostat) and hypergravity (centrifuge) on EGF-induced cell rounding was determined by the percentage of rounded cells of the total. Data were obtained from three separate experiments. From each culture, 8 to 10 samples were assayed, each comprising approximately 60 cells (Bars represent S.E.M.).

0G: EGF treated cultures at simulated microgravity (N=35)
1G: EGF treated cultures at normal gravity reference (N=53)
10G: EGF treated cultures at high gravity (N=24)

Whether this is a direct effect of microgravity on actin polymerization/dem.polymerization or an indirect effect, possibly through protein kinase C, remains to be established. Altogether these data provide strong support for the notion that microgravity affects growth factor receptor mediated signal transduction, possibly because of a gravity-sensitive component in the cellular cytoskeleton. This suggestion may however have far reaching consequences, since the cytoskeleton has been demonstrated to play a crucial role in cellular physiology, because the cytoskeleton is directly involved in the maintenance of cell structure, in intracellular targeting of proteins, in protein synthesis and most likely also in targeting of mRNA’s. Modulation of the function of the cytoskeleton by gravity may therefore influence fundamental cellular processes and consequently may have a high impact on several biotechnological aspects. Our future research is aimed to study the gravity sensitive component(s) in more detail.

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GRAVITATIONAL EFFECTS ON PARAMECIIUM AND LOXODES

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INTRODUCTION
Many motile cells show a clear orientation in the gravity field (geotaxis/gravitaxis), however, the gravistimulus-response chain is not yet identified. Recent investigations support the hypothesis that gravitaxis is the result of an active physiological mechanism and cannot be explained by a pure passive mechanism (2,11,14,18,24,25). In case of Paramecium no graviresponse has been demonstrated so far (16). For Loxodes the so-called Mueller Organelles are proposed to be graviorganelles (Fig. 1) (8,9,13). In order to characterize and compare the gravireactions of Loxodes and Paramecium we investigate the swimming behavior of these two organisms under different conditions of gravity. In the present paper we describe the methods we use to modify the influence of gravity and the results of the observed behavior are discussed. This will include the fact that mechanoresponses in ciliated protozoa - especially in Paramecium - involve numerous steps which are obviously comparable to stimulus response chains in tissue cells (21,22,23).

METHODS
Cell cultures: Paramecium aurelia (wild type) was cultivated with Enterobacter in phosphate-buffered straw medium (pH 7.2). Loxodes striatus was cultivated in deoxygenated soil extract medium with Euglena gracilis as feeding organism. Cells were placed in a round observation chamber (MBE-ERNO, Bremen, FRG) (Ø30 mm; depth 0.5 mm), consisting of titan, viton-O-rings (Busak & Luyken, Stuttgart, FRG) and red filter glass (RG 645, Schott & Gen., Mainz, FRG).

Different g-conditions:

180°-turn: By turning a vertically positioned cuvette upside down the influence of gravity was inverted (5).

Hyper-gravity: Experiments under the conditions of up to 5 x g were performed in a low-speed centrifuge microscope NIZEMI (part of the IML-2 equipment) proposed by Briegleb and coworkers (6,15) and constructed by Dornier (Friedrichshafen, FRG). During rotation the direction of the acceleration vector is changed relative to the observation chamber.

Simulated weightlessness: By means of a fast-rotating clinostat, developed by Briegleb and coworkers (3,4,5), the influence of gravity was canceled. A clinostat microscope was used to observe the cells and a test-tube clinostat for cultivation of cells under simulated 0 g.

Real near weightlessness: Free-fall conditions for 6 min of a quality of 4·10^{-2} x g were received during the parabolic flight of a sounding rocket TEXUS 27, launched from Kiruna (Sweden) (17).

Microscopy: The swimming cells were observed in dark field mode with a 1.6 x objective. Documentation of movements was achieved by a CCD camera (Aqua TV, Aqua, Kempten, FRG or AVT BC-2, AVT-Video-Technik, Aalen, FRG).

Computer analysis: Real time image analysis was performed by means of the method of Haeder (10,12). Deviation angles of the cells from the predetermined stimulus direction (acceleration vector), speed of movement and pathways of individual swimming tracks were analyzed using custom made computer programs (12). The degree of orientation was quantified by the Rayleigh Test (1). An r-value near 1 (or 0) indicated a high (or low) degree of orientation, 0.2 was set as the limit for random distribution.

RESULTS

180°-turn: After 10 minutes of adaptation
Loxodes showed a bilateral orientation along the gravity vector with a slight preference downwards (60% of the cells). By a 180°-turn the distribution of the cells became unipolar by enhancing their positive gravitaxis (r-value = 0.68). Randomly swimming (r-value = 0.18) Paramecium cells showed a similar behavior, however, with an opposite sign. The number of negative gravitactic paramecia increased significantly by inverting the influence of gravity. In addition, the orientation towards the direction of the g-vector became very precisely (r-value = 0.69), while the mean swimming velocity was not influenced.

Hyper-gravity: Experiments under the conditions of hyper-gravity (up to 5 x g tested) revealed the following results: At 5 x g paramecia did not sediment and the precision of their negative gravitaxis was even increased. An improved precision of positive gravitaxis along the increasing acceleration vector was also observed in case of Loxodes. Due to the fact that we used a static cuvette and not a swing-out chamber in the centrifuge microscope it was possible to determine the reaction time that Paramecium and Loxodes need to adjust to the turning acceleration vector. Both organisms reacted within 30 s to a turn of less than 10 degrees.

Low gravity: Starting conditions for all experiments were cell cultures of Paramecium with a precise negative gravitaxis. The high degree of orientation was induced by either leaving the cells within a closed observation chamber (diminishing oxygen concentration) and/or turning the observation chamber by 180° (see above). After onset of "functional weightlessness" (5) in a sounding rocket or in a fast-rotating clinostat three reactions were observed (for more details see 17). During the first seconds (30 s in case of the rocket, 40 s in the clinostat experiment) paramecia maintained the swimming direction which had been induced by the last effective acceleration. Within the next 20 s a near reversal of the swimming direction occurred followed by the expected random swimming.

Independently from the swimming direction of the cells the individual swimming paths remained more or less straight during the 6 min lasting rocket experiment as well as during 3 h of clinorotation. Immediately after stopping the clinostat and after retrieval of the rocket-payload, paramecia showed again a precise negative gravitaxis. The effects of vibration and hyper-gravity on orientation and swimming velocity were tested separately. From these results we can conclude that the observed behavior during the rocket flight and during clinorotation were induced by the compensation of gravity. Identical experiments with Loxodes on the fast-rotating clinostat are under investigation.

![Figure 2](image)

Figure 2. Circular histograms showing the movement vectors of Loxodes after an adaptation time of 10 min (a) and after turning the cuvette upside down (b). 350 tracks were registered for each histogram. Notice that the bilateral movement is changed into an unilateral one.

**DISCUSSION**

The fact that Loxodes shows a positive gravitaxis under oxic and Paramecium a negative one under anoxic conditions demonstrates the influence of oxygen on sign and precision of gravitaxis (8,14). The opposite signs of gravitaxis are determined by the different oxygen demands of our test organisms. While Paramecium is normally found in oxic areas of a water column, the microaerophilic Loxodes stays at the oxic/anoxic border. By using gravity for orientation these protozoa will reach a water layer with suitable living conditions. This behavior enables us to optimize their gravitaxis before exposing them to the condition of functional weightlessness. Furthermore, we are planning experiments with Loxodes under low oxygen pressure to induce negative gravitaxis (8). Under these conditions we will test whether the swimming velocity of Loxodes depends on the swimming direction (gravikinesis) as it was shown for Paramecium (14,24,25).

We expect that a 180°-turn must have a strong effect on a primary g-receptor, similar as a permanent disturbance in a slow-rotating clinostat; the latter may even lead to destruction of the pressure sensitive structures within the cell (20). The fact that Loxodes and Paramecium can precise their orientation after the turn proves the physiological nature of their gravi-orientation.

We predicted that low gravity would induce
phobic reactions which are known for photosynthetic flagellates after taking away the directing light source (7). The straight-on movement during the first seconds of reduced gravity shows that the coordination of the ciliary beat works independently from the g-receptor. This might suggest a second order regulation mechanism of the beat. In this particular case, deviation of the swimming path caused by an irregular beat of the body cilia should be registered (perhaps by the elongated immobile cilia at the posterior end) and used to correct the swimming direction (idiopathic?) (17).

First analysis of the swimming velocities showed that cells swam faster under the conditions of weightlessness.

Electrophysiological experiments demonstrated that frequency and direction of the ciliary beat are regulated by the polarization status of the cell membrane (for review see 22,23). This leads to the conclusion that after the transition to low gravity paramecia were not depolarized because no backwards swimming occurred. However, the increase in swimming velocity gives a hint that the membrane might be hyperpolarized.

The reversal of the swimming direction 40 - 60 s after onset of functional weightlessness might be the result of spontaneous turns or of 180°-turns after hitting the wall of the observation chamber. Due to the fact that no spontaneous turns were observed the second possibility is more reasonable. However, the observed "reflecting" angles under 1 x g conditions do not explain the behavior under near and simulated weightlessness. By using a modified observation chamber we are going to observe the behavior of paramecia during hitting the wall.

The random distribution of the cells 80 s after onset of low gravity during the flight and 120 s after starting the clinostat proves gravity to be the stimulus for orientation.

Similar reactions of paramecia during real low gravity and simulated weightlessness demonstrate that the fast-rotating clinostat is a necessary supplement to space experiments.

Due to the distinct reactions of Paramecium and Loxodes to changes of the g-influence they are suitable for determination of the threshold for gravity-perception in single cells (planned on the IML-2 mission). The use of genetic mutants and specific metabolic inhibitors to modify the graviresponse are our further approaches to restrict the list of postulated g-receptors (2,16).

A first comparison of the reaction times of Loxodes and Paramecium to turning acceleration vectors showed no significant difference. Multigeneration experiments under the condition of simulated weightlessness on a test-tube clinostat with special regard to the morphology of the Mueller Organelle will clarify its function as g-receptor.

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motor response in Paramecium. II. Methods and
HYBRID FORMATION AND METABOLISM OF PLANT CELL PROTOPLASTS UNDER MICROGRAVITY

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INTRODUCTION

In plants somatic hybridization and the exchange or recombination of organelles between incompatible species can be achieved by fusion of plant cell protoplasts. Early attempts to induce protoplast fusion successfully employed high pH and calcium, polyethylene glycol or dextran. Another approach is fusion of protoplasts by the reversible electric breakdown of their plasma membranes (1). With this method, particles are first brought into close membrane contact by a weak alternating electrical field ("positive electrophoresis"), followed by a field pulse of high intensity and short duration which induces local membrane reorganization at the contact areas of the two spheres. This approach has been shown to be an efficient alternative to conventional methods as it avoids chemical interference of the fusogens with cell properties. Furthermore, if selected hybrid cells are to be formed, electrowelding is the method of choice.

Inspection of the considerable potential offered by this field pulse technique, optimum yields of hybrids are only obtained, if the electrically aligned cells do not change their position for a limited time after pulse application. Only then, persistent membrane continuities can develop. Under terrestrial conditions, however, gravitational and convective forces severely interact. For example, cell nuclei or organelles, such as chloroplasts or amyloplasts, are not centered and thus cell rotation occurs. It is thus imperative to apply and to perpetuate field strengths higher and longer than necessary in order to keep the fusion partners aligned. In addition to suboptimal fusion yields, this can cause a decrease in hybrid viability.

These limitations become even more stringent when protoplasts of different specific density are to be fused, such as cells with or without vacuoles. Such types of protoplasts are present in differentiated leaf (vacuolated) and in meristematic tissues (largely free of vacuoles), respectively. They can also be formed experimentally by evacuolization of, e.g., ordinary leaf cell protoplasts. Such a kind of fusion is of interest with respect to the hybridization of cereal grains: Up to now the best approach in order to regenerate grass species is via protoplasts from embryogenic cell suspension cultures (e.g. 2). These protoplasts, similar to those from meristems, are not vacuolated and possess a very dense cytoskeletal matrix. Their specific density is thus different from vacuolated protoplasts such as those from leaf mesophyll.

A successful heterospecific fusion of both types of protoplasts (embryogenic x differentiated) would be very important in order to get more information about (1) the regulation of cell division and regeneration in grass species, which appears to be different from those of dicotyledonous plants (e.g. Solanaceae; fusion between vacuolated and vacuole-free protoplasts offers a lot of advantages over fusions between protoplasts of comparable properties (less vacular interference; fusion products with intermediate specific density, etc.).

Due to detrimental effects of differential sedimentation velocity of protoplasts of different specific density it was our hypothesis that electrowelding under microgravity should considerably increase yields of viable fusion products.

There is also evidence from experiments with cell cultures and animals that changes in gravitation should interfere with cell metabolism. For example, it has been shown that under μg unicellular organisms (Paramesium; (3)) exhibit reduced mobility but increased rates of cell division. It was suggested therefore that a surplus of energy resulting from decreased motional activities is available for other cellular processes. It appeared thus interesting to screen for possible metabolic responses to changes in gravitational forces.

Here we report on two aspects of effects of μg on plant cell protoplasts, yield and viability of fusion products after electrowelding, and changes in pool sizes of adenylates (ATP, ADP) and of fructose 2,6-bisphosphate, a metabolite regulator of glycolysis. The experiments were performed as part of the sounding rocket program TEXUS (TEXUS 17, 21, 25). Possible changes in adenylate ratios were also tested on a fast rotating klinostat (4).

METHODS

Plant material. Preparation of vacuolated (P+) and evacuolated (P-), mesophyll protoplasts of Nicotiana tabacum (cv. Samum) or Avena sativa, determination of protoplast and fusion product viability, and enrichment of heterospecific 1:1 fusion products was as detailed elsewhere (5,6,7). Hardware design for radio-controlled electrowelding under microgravitation and data transmission. The module used for the experiment with TEXUS 17 and 21 consisted of the fusion chamber, two storage units for vacuolated and evacuolated protoplasts respectively, a reservoir for fusion medium, a peristaltic pump, and facilities for optical control of the interelectrode space of the fusion chamber (7). Connections between the different containers were made from silicon tubings (inner diameter: 1 mm). In order to compensate for changes in volume during resuspension of the protoplasts and filling of the fusion chamber, all storage units (containers for protoplasts and fusion medium) were closed at one end by flexible membranes (Freudenberg, Weinheim, F.R.G.). This design was modified for TEXUS 25 according to the sketch given in Fig. 1. Facilitated resuspension of protoplasts by a stirring bar resulted in very homogeneous protoplast mixing and chamber filling. In order to introduce significant numbers of protoplasts into the fusion process a meander-shaped fusion chamber was constructed (Fig. 1). The electrodes (stainless steel) were fabricated by spark-erosion (Witte, Barskamp, F.R.G.). Owing to an electrode distance of 1.2 mm, a gap height of 8 mm, and a total length of the interelectrode space of 520 mm, about 5 ml of protoplast suspension could be exposed to the necessary electric field. For optical control of the interelectrode space, the electrode housing was made from plexiglass. The fusion chamber was mounted on a moveable platform. By means of a step motor it was

Figure 1. Sketch of the electrowelding module TEM 06-5.

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thus possible to adjust the position of the chamber in the direction of the light path of the microscope optics (perpendicular to the chamber surface; total magnification 100 to 200×).

The complete experimental unit (except optics) was mounted on a separate platform which could be introduced into the module through a lateral window. This allowed for late access of our samples to the assembled payload at about 2 h before take off. Thermal control of the cell containers was achieved by Pelletier elements. This was necessary in order to maintain the protoplast suspensions (a) at 6-8°C until about 10 min before take off, (b) at 20°C during the electroporation experiment.

**Conditions for electroporation of vacuolated and evacuated tobacco mesophyll protoplasts (TEXUS 18)**. About 80 s after launch the peristaltic pump was activated and both types of protoplasts (0.4 ml each) were suspended by dilution in their storage units to 8 ml with fusion medium (0.5 M sorbitol). By changing the pumping direction the protoplasts were withdrawn from their respective chambers under continuous mixing in a ratio 1:1. Times for mixing and filling were 15 to 20 s each (20°C). After ending the filling procedure, protoplasts were collected by a weak alternating field (2 MHz, 180 V/cm, peak to peak) under optical control and as soon as short protoplast chains were visible the fusion pulse was applied (0.9 kV/cm; 50 μs). Part of the processing of the sample was in an automatic mode but critical steps (pump activation, flow direction and pulse application) could be directed by telecommand.

**Evaluation of fusion yield and physiological integrity of fusion products (TEXUS 17)**. Within one hour after pulse application (reference test 1 g as well as experiment under microgravitation) the protoplast suspension was washed out of the fusion chamber by about 50 ml of 0.5 M sorbitol, containing 1 mM CaCl₂ and 5 mM Mes-KOH (pH 6). Preseparation of particle populations of different densities was by sedimentation for 1 h under 1g (6°C). This procedure was repeated with the remaining supernatant. For enrichment of heterospecific 1:1 fusion products the pellets obtained by the centrifugation were combined with the pellet resulting from centrifugation of the second supernatant (10 min, about 50g) and layered on top of a preformed Percoll gradient (6). The yield of heterospecific fusion products (vacuolated x evacuated protoplast) was determined microscopically by counting aliquots of the initial washout on a hemocytometer (Fuchs-Rosenthal).

**Physiological integrity of fused protoplasts was tested by their individual ability to evolve oxygen in the light.** The assay which employed aerotactic bacteria (*Pseudomonas aeruginosa*) is described in detail elsewhere (8).

**Culture of protoplasts/fusion products and regeneration of plants (TEXUS 21, 25)**. For sterile culture, pulse-treated protoplasts were washed once with 0.5 M mannitol buffer, containing 1 mM CaCl₂, 5 mM 4-morpholinol-[3]ane-sulfonic acid (MES, pH 6.0), and resuspended in modified 12a medium (9) which contains macro- and micro-elements, vitamins, amino acids, 4.7 mg ml⁻¹ p-chlorophenoxacetic acid, and 1 mg ml⁻¹ kinetin, all supplemented with 3% (w/v) sucrose and 9% (w/v) mannitol (see (10)). Aliquots of the pulse-treated suspension were embedded in 1% (w/v) low gelling temperature agarose (Sea Plaque, FMC Bio-Products, Rockland, USA). Droplets (100 μl each) of the agarose protoplast suspension were transferred into petri dishes (6 cm diameter) and incubated in the dark at 22°C and in the presence of feeder cells (tobacco mesophyll protoplasts or carrot suspension culture cells; 50,000 and 100,000 *μl⁻¹; (10)). Tobacco mesophyll protoplast feeder cells were diluted every 10 d with 1 ml osmoticum-free culture medium. Carrot suspension culture cells were diluted in intervals of 4 d by replacing 1 ml of suspension by 1 ml of culture medium with decreasing osmolarity. After 6 weeks the agarose lenses free of feeder cells were transferred onto agar medium. Several days later microcalli were dispersed on the agar. After another 2 weeks the calli were transferred on shoot regeneration medium. Developing shoots were cut off and rooted (10).

**Assay of pool sizes of metabolites (TEXUS 25)**. For the analysis of changes in pool sizes of metabolites a module was constructed (MBB/ERNO) which contained 31 sample containers with adjustable volume (0.2 to 1 ml; pressure-driven piston) and a pressure-driven injection device for a quenching agent (Fig. 2). Oat mesophyll protoplasts (0.2 ml, 0.2x10⁶, Hampp and Ziegler 1980) were filled into the sample cavities (now set to 0.2 ml) about 2 h before lift off and kept at 6-8°C. The sample temperature was increased to 20°C and the whole device activated 30 s before launch (injection of 0.8 ml ethanol into the first sample); the remaining samples were then mixed with the quenching agent in 14-s-intervals. Ethanol was taken because it allowed for the assay of both acid-stable (ATP, ADP) and acid-labile (fructose 2,6-bisphosphate) compounds from the same extract. Assay of adenylates was by luminesmetry (11), fructose 2,6-bisphosphate was determined as described in (12).

**RESULTS AND DISCUSSION**

**Electroporation of a Mixture of Vacuolated and Evacuated Tobacco Mesophyll Protoplasts under Terrestrial Gravitation (1g)**.

The experimental setup as described above was used for control experiments under terrestrial gravitation. In order to obtain optimum fusion rates the fusion chamber was filled under different spacial orientations. Best results were obtained with the long side of the meander perpendicular to the gravity field (see Fig. 1). Independent of the chamber orientation, the mixing and filling procedure resulted in a very homogeneous distribution of both vacuolated and evacuated protoplasts (Fig. 3) over the total length of the interelectrode space. Immediately after filling, however, a rapid separation of both particle populations started, i.e., while the vacuolated protoplasts remained suspended the evacuated ones sedimented. This led to a very inhomogeneous distribution of both types of protoplasts within seconds (see (7)) and could only be slightly retarded by the immediate application of the alternating electric field (positive dielectrophoresis). Thus, primarily homospecific pair and chain formation occurred (P⁺ x P⁺; P⁻ x P⁻). As a consequence, electric pulse induced fusion created only small numbers of P⁺ x P⁻ fusion products (between 0.7 and 1.0% of the total protoplast population).

**Electrofusion under Microgravitation**

Radiocontrolled mixing and filling of the fusion chamber under microgravitation caused a homogeneous distribution of both protoplast preparations which was completely stable when the...
filling step was ended. The only visible movement of the protoplasts relative to each other occurred when the collecting ac field was applied. After a 20-s-ac field protoplast pairs started to grow into chains of several protoplasts and thus the alternating field was switched off. By setting a square pulse (0.9 KV/cm, 50 μs) fusion was initiated, and again no particle movement was visible. This is in significant contrast to fusion under 1g: here, sedimentation and convectional forces induce movement of the fusion partners relative to each other which eventually leads to a breakdown of newly formed membrane continuities. Thus, typically a post-fusion weak alternating field has to be applied in order to prevent a separation of the fusion partners. This, however, affects the viability of fusion products (unpublished observation). Our experimental data show that this is not necessary under weightlessness.

Microscope analysis of the exposed protoplast suspension after retrieval (less than 1 h after fusion) showed a significantly increased portion of fusion products, of both homo- and heterospecific nature. Evaluation of about 1,000 protoplasts (vacuolated and evacuated) yielded about 120 clearly distinguishable 1:1 fusion products, i.e. about 12% of all cells submitted to the fusion procedure. This is about 10 to 15 times more compared to fusion under terrestrial conditions with the same hardware and, in real numbers, about 0.5 * 10^6 heterospecific 1:1 fusion products out of 4 * 10^6 protoplasts introduced into the fusion chamber.

As far as fusion products from protoplasts with comparable specific density (P(+1) x P(+2) x P(-1) = P(-2)) could still be identified without doubt (about 2 to 4 h after ug fusion), the yield with respect to the total number of vacuolated protoplasts was about twice compared to terrestrial conditions (10.5% instead of 4.5%). This is, however, an underestimate. Owing to the more or less rapid reorganisation of a vacuolated fusion product from tobacco mesophyll protoplasts we were possibly only able to identify a fraction of the totally formed fusion products. Under terrestrial conditions only up to 50% of the fusion products recognizable within minutes after electrosfusion can be identified by recounting 2 h later (storage at 4°C). As the microgravity-exposed samples were kept under comparable conditions, the real increase in yield could be considerably higher. Such an evaluation is not a problem with fusion products formed from vacuolated x evacuated protoplasts. Here the viscosity of the cytoplasm of the evacuated partner is that high that complete mixing takes up to more than two days.

Centrifugation of the pulsed protoplast suspension on a preformed sigmoidal Percoll gradient yielded a fraction enriched with up to 27% heterospecific 1:1 fusion products. Fusion products from this fraction were assayed for physiological integrity by qualitying their individual ability for photosynthetic oxygen evolution (8). This test system which employs aeroresistant bacteria (Pseudomonas aeruginosa) indicated that nearly all fusion products (more than 90%) obtained by electrosfusion under microgravitation were viable according to this standard. This is significantly more compared to terrestrial conditions (about 50 to 60%).

Regeneration of Tobacco Plants from Pulsed Suspensions of Vacuolated and Evacuated Mesophyl Protoplasts

Finally, with the fusion experiment during the TEXUS 21 and 25 missions we showed that the whole experimental procedure can also be performed under sterile conditions. As a result we obtained hybrid plants resulting from the fusion of vacuolated tobacco mesophyl protoplasts. These regenerates expressed morphological characteristics which were intermediate to those of the parental plants, Nicotiana tabacum (cv. Samsun; evacuated protoplasts) and Nicotiana rustica (vacuolated protoplasts; Fig. 4).

Pool Sizes of Metabolites During Transients in Gravitational Forces (TEXUS 25).

Figs. 5 and 6 show changes in the ratio of ATP/ADP of of the amount of fructose 2,6-bisphosphate in extracts of oat mesophyll protoplasts. In both examples there is obviously some impact of the decrease in gravitation down to less than 10^-5 g. The ratios of ATP/ADP started to fluctuate and the pool size of fructose 2,6-bisphosphate increased upon transition to μg. In control experiments we could show that under the conditions described respiration of protoplast suspensions was rather steady (oxygen electrode). Thus, the alterations shown should not be dependent

upon changes in oxygen availability. This is supported by the reference experiment (1g) which did not result in comparable variations of pool sizes or adenylate ratios.

From literature and own experiments (13) we know that an increase in fructose 2,6-bisphosphate indicates an increase in glycolytic activity, and thus possibly a higher demand for metabolic energy (increased rates of active transport due to decreased rates of
Figure 6. Levels of fructose 2,6-bisphosphate in oat mesophyll protoplasts. Samples as in Fig. 5.

diffusion under μg because of larger unstirred layers (no convection)?). At the moment we do not want to comment these findings to much, because they only result from one single experiment.

Pool Sizes of Adenylates during Transients in Gravitational Forces (klinostatt experiment).

In order to obtain additional data on possible metabolic responses to changes in gravitation we performed experiments on a klinostatt (Dr. Brégleb, DLR, Köln, FRG; see also (4)). Oat mesophyll protoplasts (100 μl; 10^5) were pipetted into glass capillaries (inner diameter, 1.35 mm; length 4.6 cm) and rotated at 60 rpm (highest g-force: 1.6*10^5). The samples were quenched by rapid mixing with 400 μl of 12.5% (w/v) HClO4 at the times given (Fig. 7). After an initial decrease (30s) there was a steady increase of the ATP/ADP ratio. Again this result is rather preliminary as it results from only two independent trials. On the other hand, however, it is to some degree in accordance with the μg-experiment and merits further investigation.

CONCLUSION

We have shown that electrofusion under conditions of weightlessness can be used to significantly increase not only the yield of viable fusion products from parental protoplasts with considerable differences in specific density, but also in general, as detrimental effects caused by particle sedimentation and convectional forces are excluded. Recovery of fusion products under terrestrial conditions allows for the regeneration and propagation of hybrid plants.

Our preliminary experiments on metabolic responses during g-transients suggest adaptations of the energy metabolism.

Figure 7. Adenylate levels and ratios of ATP/ADP in oat mesophyll protoplasts after different times of rotation in a klinostatt.

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EXPERIMENTS WITH SUSPENDED CELLS ON THE SPACE SHUTTLE

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Abstract

Spaceflight experiments since 1981 have demonstrated that certain cell functions are altered by micro-g. Biophysical models suggest that cell membranes and organelles should not be affected directly by gravity, however, the chemical microenvironment surrounding the cell and molecular transport could be altered by reduced gravity. Most experiments have used suspended live cells in small chambers without stirring or medium exchange. Flight results include increased attachment of anchorage-dependent human cells to collagen coated microcarriers, reduced secretion of growth hormone from pituitary cells, decreased mitogenic response of lymphocytes, increased Interferon-alpha by lymphocytes, increased Interleukin-1 and Tumor Necrosis Factor secretion by macrophages. Related experiments on cells immediately postflight and on procaryotic cells have shown significant changes in secretory capacity, cell proliferation, differentiation and development. Postulated mechanism include altered cell-cell interactions, altered calcium ion transport, effects on cell cytoskeleton, transport of transmitters and interactions with receptors. The discussion includes use of new molecular methods, considerations for cell environmental control and a preview of several experiments planned for the Shuttle and Spacelab flights to study the basic effects of microgravity on cellular physiology and potential interactions of spaceflight with radiation damage and cellular repair mechanisms.

INTRODUCTION

Early predictions about the lack of effects of reduced gravity on cells were based on calculations that no significant gravity-dependent movement occurs within the cell(1). Although movement of some intracellular organelles within plant cells has been clearly demonstrated, analogous motions within mammalian cells are not likely due to the presence of the tightly packed cytoskeleton, intracellular membranes and organelles.

Prior to 1981 the few experiments that were conducted in space showed some changes in bacterial growth, but no major changes in mammalian cell growth and proliferation (2). Since 1981 experiments flown on the Space Shuttle and Spacelab have shown that indeed there are significant changes in cell functions of both eu- and prokaryotic cells during spaceflight. These include 2-4x increase in the proliferation rate of Paramecium sp., while intracellular calcium was decreased (3), increased growth rates of Bacilli (4), and increased resistance of E. coli to antibiotics (5). In vitro human kidney cells attached more readily in micro-g to collagen coated microcarrier beads and to each other (6). The potential consequences of compromised immune cell functions have prompted several studies of lymphocyte proliferative responses and cytokine secretions during spaceflight. Lymphocytes were induced to secrete 4 to 8x more alpha-Interferon (IFN-α) than controls (7), while postflight response of lymphocytes to an gamma-Interferon (IFN-γ) inducer showed that the cells could not respond as well as ground controls (8). The blastogenic response of cultured lymphocytes to mitogen stimulation was decreased more than 90% (9), whereas in flight 1-g control experiments showed only a 50-60% loss in mitogenic response (10). Micro-g experiments during parabolic flight showed that T cells and monocytes were activated by cellular interactions but not by phorbol myristate acetate activation of protein kinase C (11). Experiments on STS-8 and SL-3 showed that pituitary cells witheld their growth hormone (GH) during spaceflight (12). Furthermore, pituitary cells (somatotrophs) transplanted from flight animals into hypophysectomized animals postflight did not release normal amounts of bioactive GH thus bone growth was depressed in the host animals. However, the prolactin secretions from those same pituitaries were normal. More recent experiments conducted on STS-37 and STS-43 studied early secretion of Interferons by lymphocytes and cytokine production by macrophages (13). It was confirmed that IFN-α secretion in micro-g was increased 2x in the first 14 hours after stimulation by Poly I:C, while IFN-γ was increased 3x by 24 hours after stimulation with Concanavalin A. Also it was shown on STS-37 that a macrophage cell line secreted 3x more Interleukin-1 (IL-1) and 63% more tumor necrosis factor-α (TNF) in 12 hours following lipopolysaccharide (LPS) stimulation. On STS-43 the experiment was repeated with samples taken 24 hours after LPS stimulation and the TNF production was approximately 3x that of the ground, while the IL-1 production was 2.1x the controls.
BIOPHYSICAL PROCESSES

A number of gravity-dependent biological and physical processes could be responsible for these cellular effects esp. in micro-g cultures of suspended cells. These are reviewed by Todd (14) and relate to those cells which respond to gravity as well as those cells that are affected by gravity. As buoyancy-driven convection is eliminated in micro-g solute-driven flow (Marangoli flow) and diffusion become important processes that establish chemical homeostasis in the immediate vicinity of the suspended cell. Diffusion and Brownian motion become effectively slower in the absence of buoyancy-driven convection thus metabolite transport to and from the cell (and inside the cell?) is reduced in micro-g. Stokes sedimentation, droplet sedimentation and isothermal settling of the cell and other suspended particles is virtually eliminated in micro-g. Surface and interfacial tension become relatively more important, esp. at hydrophilic and hydrophobic surfaces. The lipid bilayer of the cell's plasma membrane, transmembrane proteins that make up the surface charge character of the cell glycocalyx and local ion concentrations in the fluid microenvironment immediately surrounding the cell all determine the effective surface tension of the cell membrane and biophysical interactions of dissolved proteins with membrane receptors. In the absence of gravity-driven convection cell-cell collisions will be reduced in suspension cultures and the normal transport of chemical signals between cells is reduced to an effective diffusion rate of about 1 mm. per 40 minutes, thus affecting cell-cell communications. The cell is a complicated structure containing multiphasic aqueous and lipid chemical phases and physical compartments among the solid cytoskeleton and internal membranes. Cellular solutes must be partitioned among these phases and thus dramatic reduction of gravity could affect physiological signaling and perhaps intracellular cytoplasmic streaming.

CULTURE ENVIRONMENT

All cells exhibit homeostatic responses to adverse changes in their local environment. The microenvironment surrounding the suspended cell must be carefully controlled to avoid misinterpretations about whether or not the changes in cell function during spaceflight are due to the absence of gravity or to other environmental conditions. Launch vibrations, accumulated radiation doses, fluid shear and temperature changes are the most likely to cause significant changes in cell function. Cell stress can result in dramatic temporary changes in cellular physiology as illustrated by the rapid synthesis of "stress proteins" and temporary cessation of normal protein synthesis during responses to slightly elevated temperatures, abnormally low glucose levels and calcium or oxygen deprivation(15). Collectively, the stress proteins appear to serve as protective agents to enhance the survival of the cell in a hostile environment. This stress response involves a rapid collapse of vimentin-containing intermediate filaments inside and surrounding the nucleus. Mitochondria and ribosomes relocalize, ribosomal RNA synthesis and ribosomal assembly is repressed. Some of the stress proteins exhibit a role in steroid hormone receptor functions and cell proliferation. Low level fluid shear can induce hormone secretions (16) and stress response proteins in mammalian cells (17). If rapid changes in the culture environment cannot be avoided spaceflight experiments must be planned so that the cells have recovered from the stress response prior to execution of the inflight protocol. The design and operation of the cell culture system is crucial to the avoidance of unnecessary cell stress and confusing responses. Local environmental requirements are often different for cell proliferation and growth phases as compared to maintaining the cells at confluence. During proliferation quantitative measurements are largely confined to attachment, viability, DNA precursor uptake rates and proliferation rate. In stable maintenance culture it is easier to quantitate cell survival, cell turnover, respiration, nutrient depletion rates, product secretion rates and changes from aerobic to anaerobic metabolism. New spaceflight culture systems must be designed to accommodate all of these factors.

FUTURE EXPERIMENTS

Many of the approved cell biology flight experiments for 1992-94 will use in vitro suspended cells in experiment specific hardware flown on the Space Shuttle. There is an obvious emphasis on immune cell function since previous studies showed postflight changes in crewmembers that included monocytes, reduced levels of T lymphocytes, reduced blastogenesis capability of their lymphocytes and reduced delayed skin hypersensitivity responses shortly after return to Earth. Studies prior to 1985 often have been inconclusive and contradictory, esp. when investigators try to relate invivo dysfunctions to invivo cell distributions using pre- and postflight samples. Also many of the early findings were typical of a stress induced immune suppression which confused the evidence for possible direct effects of microgravity on cellular functions.

The immune cell studies to date show that it is quite likely that the observed changes during spaceflight are a result of direct effects of microgravity at the cellular level. Meanwhile, other flight investigations have raised issues of potential synergism between microgravity and
radiation effects on biological organisms. Since immune cells are known to be the most radiosensitive cells of any in the human body it is most likely that any synergism between microgravity and radiation will be characterized during flight experiments on immune cell functions. Selected experiments should be developed to study both effects on the same flight. Other specific studies on the immune cells are needed to: 1) determine the functional responses of human T-lymphocytes activated during space flight by specific and non-specific antigens in terms of blastogenic transformation and ability to produce monokines and lymphokines; 2) examine the morphology, cell aggregation, adherence, and oxidative metabolism of neutrophils and monocytes. Also measure the markers for the C3b receptors such as the OKM-1 and LFA-1 antigens in neutrophils from crew members who demonstrate postflight neutrophilia; 3) examine the generation and function of cytotoxic T-cells against appropriate allogeneic stimulator cells; 4) determine the changes in morphogenesis of monocytes over a period of 7+ days, changes in subsets of lymphocytes, and the effects of various stress hormones on subpopulations of human lymphocytes in space.

Other important space cell biology experiments include: 1) studies of cultured endothelial cells to determine contact inhibition and the production of basement membrane; 2) measuring the response of endocrine cells to hormone releasing and inhibitor factors, such as somatomedin and somatostatin for secretion of pituitary growth hormone; and 3) examining the formation and growth of hybridomas in space.

CELL CULTURE FLIGHT HARDWARE

Except for the Biological Specimen Test Apparatus (SO-15 Experiment) flown on ASTP in 1975, most cell biology experiments have been conducted in small holding chambers or syringes stored at ambient cabin temperature or placed in a constant temperature incubator. There was no provision for active stirring and mixing, no oxygen or carbon dioxide control and pH was largely determined by the buffering capacity of the culture medium which had to last from pre-flight loading of the cells until the experiment was concluded. Examples of these hardware include the Carry-on incubator with moveable piston culture chambers; the Biorack Type I culture chambers; the Bioprocessing Modules flown on STS-7, 37 and 43; the Cell Syringes flown on STS-37 & 43.

There is a need for new designs of cell culture maintenance hardware that is dedicated to space cell biology flight experiments. It is also important to establish the critical ranges of environmental control that are required to maintain the cells in a homeostatic condition and to carefully record culture environment data during the flight to characterize those conditions which could cause cell stress or other responses. Examples are temperature profiles, vibrations levels, acoustic, EMI and radiation doses.

Figure 1. Schematic of Cell Syringes

The newest generation of cell cultures systems are represented by the Dynamic Cell Culture System (DCCS) developed by the ETH in Zurich, Switzerland, the Application Specific Experiment Cell Culture (ASECC) system and the Space Bioreactor developed by NASA's Johnson Space Center. The DCCS uses an internal osmotic pump to circulate medium. It has two chambers (200 µl) one that is operated in a batch mode and the other which is operated in a perfusion mode. It is designed to fit into a Biorack Type I container (18). The ASECC has the capability for two or more culture chambers machined into a plastic housing that provides nutrient medium circulation, pH control, oxygen and chemical fixation to each chamber. A built-in microprocessor controller maintains the culture environment and archives critical data in a non-volatile memory for postflight analysis. This system is quite suitable for small replicate cultures maintained for 3 to 7 days in an Middeck incubator.

Figure 2. Schematic of the Applications Specific Experimental Cell Culture System (JSC).
The Space Bioreactor is designed to grow or maintain 5 x 10^8 cells in a 500 ml vessel for up to several weeks. It provides low shear mixing and continual perfusion of culture medium through an oxygenator and then through the culture vessel. This is adaptable for suspensions of anchorage-independent cells or for anchorage-dependent cells attached to microcarrier beads (19). The Space Bioreactor would be suitable for studies of space radiation on cellular DNA damage and repair phenomena wherein 10^9 cells must be maintained for 60 days in order to accumulate enough radiation to elicit measurable genetic damage (~10^2 double stranded DNA breaks) for statistical analysis of dose responses important to radiation health and crew protection on long missions. Other cell culture systems are also being developed for studies of bioprocesses that can be enhanced in microgravity. These systems will also be available for basic cell biology flight experiments.

REFERENCES
LIGHT-POTENTIATED MAIZE ROOT GRAVITROPISM: IS PERCEPTION LOCALIZED IN THE ROOT CAP?

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INTRODUCTION
Light has been shown to promote georeactivity among roots of various maize cultivars (5,3). Most investigators believe that light signals downward curvature during the formation of a growth inhibitor in the root cap (1,4). This putative inhibitor ultimately becomes asymmetrically redistributed in the growth zone of the horizontally positioned root where the elongation rate at the bottom of the growth zone is reduced leading to downward bending.

Wilkins and Wain (6) demonstrated indirectly that, in maize, the root cap serves as the initiation point for light-induced primary root gravitropic curvature. These investigators exchanged the caps of light- and dark-treated roots and then subjected the re-headed roots to gravitropic stimulation in the dark. The result revealed a significant increase in positive gravitropic curvature among dark-exposed roots now containing light-exposed caps as compared with light-treated roots with dark-exposed caps.

There are no reports to date in which slit lamps, lasers or optical fibers have been used to selectively irradiate regions on intact, gravitostimulated roots. Such a demonstration would establish more firmly that the cap is indeed the initiation point for light-stimulated gravitropic bending. I sought to test this assumption directly using light from a helium neon laser with intact maize primary roots.

MATERIALS AND METHODS

Cv. Merit seeds were imbibed for 2 hours and germinated for 44 hours in the dark. Following germination, straight primary roots 1 to 1.5 cm in length were positioned horizontally in front of a metric gridded-background in a growth chamber (27°C; relative humidity, +95%). The laser irradiations were carried out at the beginning of the 3-hour georeaction period. A 0.5 mw helium neon laser (λ 6328 angstroms, Spectraphysics Model 155) was used in conjunction with an 77'' focal length positive lens, a λ 653 nm interference filter and a .01 cm pin hole (used to reduce stray reflections). The light intensity was adjusted by interchanging a series of Kodak neutral density filters. The laser light source and the optical components were mounted on an x-y-z table also containing a telescope which was triangulated with the light beam prior to the start of each trial (Figure 1). The width of the focused laser beam was measured by translating a 7 μm pin hole across the beam and measuring light intensity as a function of distance from the central laser spot. The radius of the focused beam measured at the 1/e² point was .02 cm. Dim green background light, λ 517 nm, intensity 1.71x10⁻⁷ W/m², was used for sighting purposes during the experimental period. Precise placement of the beam was accomplished by exposing each root to a brief low-intensity laser pulse, intensity 5.5x10⁻⁷ W/m² or about .2% of the base-line experimental exposure and of less than 10-second duration.

Infrared photographs were taken at 10-minute intervals during gravistimulation. The photographic slides were viewed under a microscope and data recorded with the aid of a protractor imprinted on the reticle.

RESULTS AND DISCUSSION

Horizontally-positioned roots whose caps were exposed to He Ne laser light developed significant positive gravitropic curvature. Laser light of equal intensity and duration shown on more proximal regions of primary roots had a reduced effect on the gravitropic response.

A detectable response above the dark control level was demonstrated with light directed on the root cap (exposure time: 60 seconds; intensity: 2.75x10⁻⁷ W/m²). Exposures of equal intensity and duration made 0.3 and 0.5 cm from the root cap, as well as on the base of the root, produced positive gravitropic curvatures which, like the dark controls, were considerably below the response level of cap-irradiated roots. Significant differences were assessed using Student’s t-test. Differences between cap-irradiated and non-cap-irradiated roots including dark controls, were most apparent 2.5 hours following laser irradiation. No significant differences were encountered.
when making pair-wise comparisons of mean curvatures for non-cap-irradiated roots, including dark controls at the 2.5-hour mark.

Cap-irradiated roots were also shown to develop greater positive curvatures than comparison roots when the mean of all angles recorded between 2 and 3 hours were computed. Here too, there were no significant differences between dark controls and non-cap-irradiated roots when pair-wise comparisons were made of values over the same time period (Table 1). These results support the contention that the root cap is the principal, if not the only, locus of interaction with light which triggers rapid positive gravitropic movement in maize primary roots.

<table>
<thead>
<tr>
<th>SITE OF IRRADIATION</th>
<th>CURVATURE AT +2 hr, 30 min.</th>
<th>AVERAGE CURVATURE AT +2 hr</th>
<th>NUMBER OF ROOTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROOT CAP</td>
<td>30.4 (18.8)</td>
<td>28.5 (17.0)</td>
<td>28</td>
</tr>
<tr>
<td>3 mm. FROM CAP</td>
<td>29.4 (17.1)</td>
<td>28.3 (17.2)</td>
<td>25</td>
</tr>
<tr>
<td>5 mm. FROM CAP</td>
<td>19.9 (14.0)</td>
<td>18.6 (12.2)</td>
<td>25</td>
</tr>
<tr>
<td>BASE OF ROOT</td>
<td>13.4 (11.8)</td>
<td>14.3 (13.4)</td>
<td>20</td>
</tr>
<tr>
<td>DARK CONTROLS</td>
<td>16.2 (13.5)</td>
<td>16.4 (13.4)</td>
<td>50</td>
</tr>
</tbody>
</table>

A slightly puzzling matter emerging from the data was that, though the absolute trough of gravitropic curvature was reached at 2 hours or 2 hours 10 minutes in all groups tested, it was not possible to demonstrate significant differences of the type outlined above at these more natural time points. Still, at both of these intervals (2 hours and 2 hours 10 minutes) the mean angle of descent generally increased with greater distance from the root cap (Table 1).

A point of potential confusion in the interpretation of the gravitropic response data presented here arises from the finding by Mandoli and Briggs (2) that light piping (internal reflection) occurs with He Ne irradiation of root tissue. In fact, I did not discover a reliable method for directly measuring the beam width on root tissue. However, simple visual inspection made clear that referred laser light caused a slight reddish glow on adjacent root tissue up to 0.5 cm from the central and intensely bright laser spot.

As reported above the profile of the He Ne laser beam used in my investigations showed a decline of 1/e² at a distance of 0.02 cm from the center of the focused beam. From Gaussian decay alone, we note that the beam intensity declines from 2.75x10⁶ W/m² to 5.5x10⁵ W/m² in moving from the center of the beam to a point 0.1 cm away. Therefore, it is clear that any light effect induced as far as 0.5 cm from the target would not be produced by Gaussian decay; rather it must arise from the processes leading to internal reflection. A gravitrophic effect could be induced in a region of the root removed from the point of incidence if, via light piping, a threshold level for gravistimulation had been exceeded at the removed site.

In order to nullify any confusion arising from this effect, I attempted to demonstrate light-potentiating gravitropic curvature at the point where the effect was just detectable above the dark control level. If such a point could be established for cap-irradiated roots, and no such response found upon exposure of more proximal regions, then the primacy of the cap as the perceptive organ in maize root gravitropism would be established unequivocally. Such a point of barely detectable curvature proved difficult to demonstrate in part because of the large variability in gravitropic response levels shown in cv. Merit primary roots. To begin with, in order to find the point of barely detectable curvature, sample sizes would have to be large enough to produce a response just above the dark control level. This task proved too taxing because of the difficulty of the experimental protocol and because of the limited number of roots which could be accommodated in each trial.

In spite of having abandoned, for the present, the task of establishing an absolute threshold level for light-potentiating root gravitropism, I believe that it is safe to conclude from the data presented here that the cap is the most sensitive, if not the only, region on the Merit maize primary root important as a receiver of light in gravitropism. Any apparent rise in the amplitude of positive gravitropic curvature shown in the data describing roots irradiated 0.3 or 0.5 cm from the root cap likely arises from the effect of referred light which reaches the cap tissue via internal reflection.

REFERENCES
RECEPTOR-MEDIATED ENDOCYTOSIS IN OSTEOSTBLASTIC CELLS UNDER "GRAVITATIONAL STRESS"

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INTRODUCTION

Experiments aboard the Space Shuttle and Cosmos Biosatellites have shown that cellular activation can be altered when cells are exposed to a spaceflight (1). It has been confirmed in vitro, in parabolic flight on lymphocytes (8), and in sounding rocket on cultured epidermal cells (2). The decrease of cell activation due to microgravity was also demonstrated in vivo. For example, the analysis of bone metabolism of rats and monkeys flown aboard the soviet 2044 Bioscosmos, revealed that the activity of osteoblasts, the bone-forming cells, is strongly decreased during spaceflight (15). On the other hand, it has been shown that cell proliferation is activated by hypergravity (15). Moreover, it was recently demonstrated that a 5G level could stimulate DNA synthesis, in a time dependent manner, in bone osteoblast-like cells (10). Thus, it appears that gravity variations can induce cellular activity modifications.

Cell activation often requires an interaction between a ligand and its plasma membrane receptor. Ligand binding on the cell surface is followed by a signal transduction and in some cases, the ligand-receptor complex is down regulated by internalization. This process, called Receptor-Mediated Endocytosis (RME), is an ubiquitous mechanism (3). It has been demonstrated on osteoblasts for many ligands, like insulin (7) or transferrin (TF) (5). RME is an active mechanism, closely related to the cytoskeleton and to the microtubular system. It is a reflect of the state of activation of the cell, and gravity variations may cause modifications of RME regulatory process. The different steps of RME can be divided in 3 main phases. First takes place the binding phase corresponding to the interaction between the ligand and its specific membrane receptor. The second phase corresponds to the clathrin-mediated internalization of the ligand-receptor complex, by the mean of coated pits and coated vesicles. In the third phase are found the intracytoplasmatic events, recycling or degradation, through endosomes and lysosomes.

Insulin and TF have been shown to bind to osteoblastic cells on specific receptors (5,11) and to stimulate bone formation (7). The receptor-ligand complex is then internalized by RME (14). Visualization of this complex in transmission electron microscopy can be made effective by coupling the ligand to colloidal gold particles (4).

The aim of the present work was to determine, by the mean of the quantitative analysis of the three phases previously described, whether RME is sensitive to alternative and cumulative gravity variations.

METHODS

Rat osteogenic sarcoma cells (ROS 17/2.8), which have been shown to exhibit multiple characteristics of osteoblast phenotype, were plated in 30 mm diameter Petri dishes and cultured to confluence in Dulbecco’s Modified Eagle Medium (DMEM) with 5% fetal calf serum. Twelve hours before the flight, the cells were incubated with fresh DMEM without fetal calf serum to avoid the presence of endogenous mediators. Twelve nm diameter gold particles were produced and labeled with insulin and TF according to a previously described procedure (4,12).

The internalization experiments were performed on June 6th 1990, aboard the French "Zero G" Caravelle based at the Air Force Test Flight Center in Bretigny (France). The gravity level obtained reached about 10+ G during the 20 s of the paraboles. The average hypergravity level was 1.8G, in two periods of about 20 seconds each.

Cells were maintained in presence of the ligands during 2 hours, at +4°C, in order to saturate the receptors. RME was initiated in flight, by transferring the cells from +4 to +37°C in an incubator provided by CNES (the French National Space Agency). The Petri dishes were maintained watertight by means of a rubber joint. Two groups of cells were studied. The first one was an onboard control group, in which cells were incubated for 18 minutes inflight, before the beginning of the parabols sequence. Thereafter, "G-stimulated" cells were incubated for 18 minutes, i.e. 5 parabols. At the end of the 18 minutes of internalization, cells were fixed with glutaraldehyde, and kept at +4°C (figure 1).

![Figure 1: Experimental protocol. G level and incubation temperature (Temp.), in function of time. (Fix. = fixation).](image)

All the manipulations were performed in a watertight glove box. After landing, cells were classically processed for transmission electron microscope observation. They were post fixed with osmium tetroxide, dehydrated and embedded in Epon resin.

Cell cross ultra-thin sections were obtained from the center of the Petri dishes with a Leica Ultracut ultramicrotome, and were observed with a Philips CM10 transmission electron microscope, at a magnification of 11500.

The different gold-labelled structures were counted and distributed among the three different groups previously described. Results are expressed as percentage of variation of the distribution. A Student test was performed for statistical analysis (P<0.05).

RESULTS

Microscopic observations showed that it was possible to observe the different steps of RME (i.e. binding, coated pits, coated vesicles, endosomes) in the "G-stimulated" cells as well as in the control cells.
The differences in the distribution of the labelled structures through the different steps of RME for "G-stimulated" and control cells are exposed in figure 2. After 18 minutes of incubation the membrane binding was lower in the G-stimulated cells than in the control group (-18.7%, P < 0.05). No difference was seen between the two types of cells for the second group of events (i.e. the coated structures). There was also a significant difference for the intra cytoplasmic labelling in the "G-stimulated" cells compared to the control cells (+21.0%, P < 0.05%). Similar trends in the results were found with TF.

Figure 2 : Differences in the distribution of the labelled structures through the different steps of RME for "G-stimulated" and control cells (expressed in percentage of variation, * = P<0.05). 1 : binding, 2 : coated structures, 3 : endosomes and lysosomes.

DISCUSSION

In our study, ROS osteoblast-like cells were preferred to bone derived primary cells. Indeed, they are a classical model in bone cell physiology, and have a higher proliferation rate (9). They have been extensively characterized (9), and are known to express receptors for insulin and TF (5,7,11). Those ligands play an important role in bone cells by regulating their physiology (5,7,11). Receptors for insulin and TF are down regulated by RME (5,7). The widely used gold labelled-ligand technique is adapted to the quantification of the different steps of RME (4) and is easy to perform. A time of incubation of 18 minutes, used in this study, was sufficient to observe the main steps of RME, from the binding to the endosomes (7). It also corresponded to the time allowed by the flight schedule. Since we stopped the experiment after 18 minutes, we could not find labelled lysosomes.

RME is an active mechanism, closely related to the cytoskeleton. It plays an important role in the regulation of cell activity. The down regulation of the number of receptors on the cell membrane is one of the mechanisms used by the cells to regulate their metabolism (3). Our results suggest that parabolic flight-induced G-stimulation could accelerate internalization processes. This acceleration could be related to an increase of cellular internal movements, coupled to an acceleration of cytoskeletal movements. This could be the reflect of a higher state of cell activity.

Usually, the gravitational biology experiments are conducted under continuous G conditions, i.e., microgravity or hypergravity. In this experiment, we investigated the effect of a periodical variation of the gravity vector. The effect of such a G-level switch can be compared to the effects of other intermittent stimulations, for example, the pulsed electromagnetic fields (13) or the intermittent compressive forces (6), which are known to influence bone-cell metabolism.

In conclusion, our results demonstrate that, during parabolic flight induced "G-stimulation", RME is not only preserved, but is also accelerated.

ACKNOWLEDGMENTS

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ULTRASTRUCTURAL ASPECTS OF CHONDROGENESIS IN "CELLS" FLIGHT HARDWARE

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INTRODUCTION

The embryonic mouse limb first appears at about ten days of gestation, at which time it consists of undifferentiated mesenchymal cells covered by an ectodermal layer (1). The mesenchymal cells have numerous processes, with which they communicate across the relatively large intercellular spaces (2). Chondrogenesis within the developing limb begins with the formation within the limb of aggregates of cells known as the prechondrogenic blastemata. Cells within the blastemata become closely associated in a step known to be necessary for chondrogenesis, but which can be replaced by a change in shape (3). This close association and concomitant shape change result in the turning on of the genes for production of cartilage macromolecules—collagen Type II and cartilage proteoglycan, and the cells rapidly become separated again as matrix is secreted into the intercellular space (1,2).

The events in cartilage development within the limb can be duplicated in vitro, using the high density system known as micromass culture. In micromass cultures, the high density of the cell inoculum directs the cells to develop in the cartilaginous pathway (3). The micromass system has been used in many studies in efforts to dissect out the various steps in chondrogenesis, and duplicates effects of mutation or exposure to teratogens.

Since previous studies in this lab and others had shown chondrogenesis to be altered by exposure to altered g in vivo or in organ culture, we proposed that the micromass system be used to examine the question of whether differentiating chondrocytes placed in culture retained their ability to sense gravitational changes (4,5). This experiment, now known as CELLS, was selected for flight on International Microgravity Laboratory I.

Previously we reported various hardware configurations that were shown to support chondrogenesis, as assessed by Alcian Blue staining of cartilage specific macromolecules. The present study reports ultrastructual aspects of chondrogenesis in micromass cultures grown in BEX (bubble exchange) units identical to those that will be used for flight.

MATERIALS AND METHODS

Cell culture hardware: The main unit of the CELLS hardware is a two-welled polycarbonate chamber into which is inserted what is known as the "double bubble"—a gas exchanging membrane of a Dow-Corning Silastic elastomer, molded in the shape of two domes or "bubbles" surrounded by a flange. The "bubbles" inflate or collapse as medium is added or removed. Within each bubble is a deflector ring, which controls fluid forces due to medium injection or withdrawal. A silicon rubber gasket, a polycarbonate bottom plate, and a stainless steel support plate, held in place by six screws, complete the assembly. Medium is added or removed through the gasket. Initial injections for micromass culture are made through a silicon-plugged port in the deflector ring.

Cell preparation: For this experiment, single cell suspensions were prepared from the fore and hind limbs of 12.5 day embryonic ICR mice as previously described. Briefly, minced limb buds were trypsinized (2.25% trypsin in calcium-magnesium-free Tyrodes), rinsed, incubated in CMRL, then dissociated in medium (CMRL with 10% fetal calf serum with 50 

\[ \text{mM} \] Gentamycin sulfate, 95 mg/ml ascorbic acid and 5mM HEPES). After filtration through 20 \( \mu \)N filter, the cell concentration was adjusted to 4 X 10^6/200ul and a 20 ul drop was placed into the center of the deflector ring onto a Thermax cover slip coated with 5 \( \mu \)g Coll-Tak. After a two hour attachment period in an incubator (37°C, 5% CO₂), the unit was filled with medium, all air was removed, and four units were placed in air-tight Type I containers, which were refrigerated at 4°C for 24 hrs, to mimic an inflight holding step. Cultures were then placed in a 37°C incubator and medium was changed every 24 hours. Control cultures were set up on coverslips in the 8 center wells of a Corning 24-well plate.

After 7 days of culture, cells were fixed in 1% glutaraldehyde in 0.1M Sorensen's phosphate buffer (pH 7.4) for 8 days—a period of time corresponding to length of fixation for the mission. After rinsing with Sorensen's buffer, cultures were postfixed in osmium, dehydrated through an ethanol series and infiltrated with Spurr. Portions of coverslips bearing cartilage nodules were cut out and embedded in Spurr. After taking thin sections for orientation purposes, thin sections of nodule regions were cut, stained with uranyl acetate and lead citrate, and examined in a Jeol 100 CXII transmission electron microscope.

RESULTS

Light Microscopy

Thick sections of a nodular region show rounded chondrocyte-like cells in the center region of the nodule, and flatter, more fibroblastic-like cells covering the nodule surface, and along the bottom of the nodule adjacent to the plate.

Electron Microscopy

Chondrocytes from control and BEX cultures are shown in Figure 1. The high nucleocytoplasmic ratio typical of chondrocytes was seen in the rounded cells in the center of the nodule. The N/C ratio was less in the fibroblastic-like cells. All cells had numerous polysomal complexes and a greatly expanded rough endoplasmic reticulum, with numerous associated mitochondria. The cytoplasm also had many vesicles—some, containing flocculent material, are presumed to be secretory. Others appear to be endocytotic, possibly resulting from uptake of
debris from necrotic cells.

Rounded cells communicate via cell processes across the intercellular spaces separating them, and where there were broad areas of cell contact--mostly in the regions with flatter cells--junctions were seen. A few necrotic cells were seen and occasionally a cilium. Although there was no difference between the controls and the cells grown in BEX hardware, it should be noted that in neither case was there the scalloped cell membrane typical of actively secreting chondrocytes fixed in this conventional manner. Matrix was present in the intercellular spaces (collagen fibrils and proteoglycan granules.)

![Figure 1: Electron micrographs of cells in control (A) and BEX (B) cultures. R, rough endoplasmic reticulum; N, nucleus; arrow indicates matrix.](image)

**DISCUSSION**

The structure of the cartilage module in the control plates and in hardware was identical to that described by others, and the "perichondrial layer" is similar to the layer seen in cartilaginous limb elements developing in vivo, and in aggregates of cartilage formed in rotation culture (6).

The classic description of a chondrocyte as having a scalloped cell membrane has been shown to be due to conventional fixation techniques, and is not found in tissue fixed with cationic dyes such as ruthenium hexamine trichloride in the fixative (7). Since no cationic dyes were included in the fixative used here, it is not clear why the chondrocytes exhibit a rounded cell profile, with no scalloping.

The expanded RER, the filled secretory vesicles, and the presence of matrix in the intercellular spaces indicate that the cells are active metabolically. Immunological studies in this lab show that cartilage proteoglycans and collagen Type II are present in the matrix of cultures grown in BEX hardware (8). It may be that the chondrocytes are in late hypertrophy, in which case rounded membranes can be seen even with conventional techniques (9). We have not observed any high molecular weight molecules in the smears from the cells; the presence of high molecular weight molecules in the fix-medium mix might serve as osmotic protection. The length of the fixation period is the most atypical portion of the EM procedure, and may be somehow contributing to the rounding of the cells. Additional studies are underway on samples fixed with a more conventional time (2 hrs).

Since the lack of scalloping is seen in both controls and cells grown in hardware, it is not attributable to any changes in the cells occurring because of the hardware itself.

No matter how elegant and potentially enlightening an experiment proposed for space may be, it cannot be carried out in the microgravity environment without appropriate hardware. Not only must the hardware be workable in a space environment (9g), it must be made of material that meets NASA safety requirements, provide the proper level of containment, and fit the envelope in which it is to be flown. For cell culture experiments, the hardware must be biocompatible with the cells and support the cellular activities in a manner not significantly different from the normal 1g operation. Cell culture hardware must also be easily sterilizable. The hardware developed for the CELLS experiment fits safety and operational criteria, and has no effect on differentiation of the cell cultures that will be flown within it. The unit is easily adaptable to other systems, including not only cells, but small aquatic organisms as well.

Culture hardware for the CELLS experiment was developed under the direction of the Life Sciences Payload Office at NASA's Ames Research Center, with testing carried out in the PI's lab at the University of Texas Dental Branch. Significant suggestions for development were provided by Paul Todd of the National Institute of Standards and Technology and Bill Scheld of Phytoresource, Inc. Phytoresource, Inc. was also responsible for development of the "double bubble" design.

**REFERENCES:**


PROTEOGLYCANs IN MICROMASS CULTURES OF EMBRYONIC MOUSE LIMB MESENCHYMAL CELLS: PRELIMINARY STUDIES FOR THE "CELLS" SPACEFLIGHT EXPERIMENT

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INTRODUCTION
The well documented response of the skeletal system to microgravity includes significantly altered differentiation and matrix organization in space flown animals (1). Among the ultrastructural alterations reported in these in vivo studies are the changes in proteoglycan granule (PGG) sizes and density (#granules/mm²) found in tibial epiphyseal growth plates of spaceflown rats (2). It is not known whether this is due to changes in PGG production and/or aggregation in microgravity. Proteoglycans have important roles in cartilage development and abnormalities in proteoglycans can have serious consequences, affecting collagen fibril formation, matrix hydration capacity and/or mineralization (3).

Complexities inherent in whole animal systems and the lack of in-flight animal sacrifice has made it necessary to develop cell culture systems to address mechanisms of gravitational responses in the skeletal system. One such system will be flown on the Spacelab mission MML-1 (International Microgravity Laboratory-1). This experiment, US6-CELLS, consists of a set of 40 micromass cultures of embryonic mouse limb mesenchymal cells that will be exposed to microgravity and compared to 3 sets of controls (centrifuged IgG controls aboard the space shuttle, ground controls, and centrifuged IgG ground controls). Inflight fixation of these cultures and determined intervals is expected to provide a detailed profile of chondrocyte differentiation under microgravity, including immunolocalization of proteoglycans.

There is no information on proteoglycan localization in micromass cultures from embryonic mouse limb mesenchymal cells. Prior to beginning immunological studies on cultures grown in flight-like hardware, it was necessary to locate proteoglycans in control micromass cultures set up in Corning 24-well plates. Since Cell-Tak® coated coverslips will be used for flight, the effect of the Cell-Tak® coat on proteoglycan production and localization was determined.

This portion of the study describes preliminary Ig experiments conducted to establish the localization of chondroitin sulfate (the predominant glycosaminoglycan in cartilage proteoglycan), 4-sulfated and 6-sulfated chondroitin sulfates, and keratan sulfate in micromass cultures of embryonic mouse limb mesenchymal cells grown on coated coverslips in standard 24-well Corning plates.

METHODS
CULTURE PREPARATION: Micromass cultures were prepared using a well characterized culture system previously described (4). Fore and hind limbs of 11.5d embryonic ICR mice (Harlan-Sprague-Dawley) were dissected and 20µl drops of cell solution (4x10⁵ cells/drop) were deposited on uncoated or Cell-Tak® coated (5µg/µl) Thermolon® coverslips in the inner 8 wells of 24-well Corning plates. After a 2h attachment period at 37°C, 5% CO₂, 3mL of medium ( Gibco's CMRL 1066, plus 10% FBS; 100µg ascorbic acid/ml, and 50µg/ml Gentamicin, 5% Hepes) were added. Because these cultures serve as controls to the spaceflight experiment, they were slowly cooled to 5°C to arrest cellular activities for approximately 24h, when post launch activation was simulated. Cells were incubated at 37°C, 5% CO₂, allowed to grow and differentiate, and finally fixed in 1% formalin in 95% ethanol for analyses.

IMMUNOSTAINING: Monoclonal antibodies from ICM Biochemicals (Lisle, IL 60532) were used to localize chondroitin sulfate, keratan sulfate, and 4-sulfated and 6-sulfated chondroitin sulfate. After fixation, samples were rinsed with PBS. For localization of 4- and 6-sulfated chondroitin sulfates, samples were first digested with 0.02µ/ml chondroitinase ABC Protease free (ICN, Biomedical) in 0.1M Tris-HCl pH8 containing 0.03M sodium acetate at 37°C for 40 min. (The 4- and 6-sulfated monoclonal antibodies used react with the chondroitin sulfate "stubs"—4S, 6S— which remain after extensive Chondroitinase ABC digestion. The antibody specificity is according to the sulfated position (4S, 6S) of these "stubs". The monoclonal antibodies should be reactive with PG from any species which contain chondroitin sulfate chains susceptible to ChABC digestion and which are sulfated in the proper position.) Samples were rinsed with PBS and evaluated using immunoperoxidase or immunofluorescence.

IMMUNOPERIODXIDASE: After quenching of endogenous peroxidase activity (0.6% H₂O₂ in methanol) for 1h and rinsing in PBS, non-specific reaction was blocked with 1% normal serum (VECTORSKTSTM E, Elite ABC kit, Vector Laboratories, Burlingame CA). Samples were incubated with the primary antibody overnight at 4°C, 1:1000 in PBS. Controls received only PBS. Samples were rinsed and incubated with secondary antibody biotinylated for 1h RT. After rinsing, samples were incubated with ABC reagent, rinsed, and reacted with DAB substrate for 10 min. Samples were mounted and observed under a light microscope.

IMMUNOFLOUORESCENCE: Some samples were post-fixed in cold methanol:acetone (1:1) for 10 min, (then air dried and stored at -20°C if not processed immediately). All samples were rehydrated or rinsed in PBS. Samples were incubated with primary antibodies 1:50 dilution in PBS, overnight at 4°C. Controls received only PBS. After rinsing with PBS, samples were incubated with goat anti-mouse IgG fluorescein conjugated affinity purified antibodies, diluted 1:20 in PBS, for 1h at room temperature in the dark. After rinsing, samples were mounted in anti-fade mounting medium and observed under an Olympus BX2 microscope.
RESULTS

Positive immunofluorescence for proteoglycan DI 4S, proteoglycan DI 6S, and chondroitin sulfate, appears to be concentrated in differentiated cartilage nodules. The cells between nodules also showed some positive staining, but to a lesser degree. In these regions, staining was stronger for chondroitin sulfate, less for 6-sulfated chondroitin sulfate, and very weak for 4-sulfated chondroitin sulfate. Keratan sulfate did not show a positive reaction in the cartilage nodules (Figure 1).

A similar pattern of reaction was observed with immunoperoxidase staining (not shown) except that the antibody concentration required was much lower.

There was no interference with immunolocalization of the antigens by using Cell-Tak® coated coverslips, however, the coverslip itself is source of some background.

Figure 1. Immunofluorescent staining of embryonic mouse limb mesenchymal cultures allowed to grow for six days. Antibodies were directed against: a. native chondroitin sulfate, c. 4-sulfated chondroitin sulfate, d. 6-sulfated chondroitin sulfate, e. keratan sulfate, f. PBS control, and b. shows phase contrast of a. Notice the reaction of cells being recruited in g. and h.; both are pictures showing another area of the same culture a. (bar=0.1mm)

DISCUSSION

Embryonic mouse limb mesenchymal cells grown on Cell-Tak® coated Thermowax® coverslips in standard 24-well Corning plates differentiated into cartilage nodules which enlarged by recruitment of surrounding cells (4), during the 6 day culture period. Native chondroitin sulfate, and 4- and 6-sulfated chondroitin sulfates gave a positive reaction concentrated in the differentiated cartilage nodules. Cells between nodules also showed some positive staining thought to be a first stage in their recruitment into the nodules. This is supported by the observed relationship between the amount of positive staining cell layer and the number of nodules in the area (Figure 1-a,g,h). The observation that in these regions, staining was stronger for 6-sulfated chondroitin sulfate and weaker for 4-sulfated chondroitin sulfate, may be related to the highly 6-sulfated chondroitin sulfate chains typical of hypertrophied cells (3). The presence of hypertrophied cells within nodules was confirmed by ultrastructural analyses (5). Keratan sulfate did not show a positive reaction in the cartilage nodules, but there was a weak positive reaction in the cell layer region. The lack of staining within the nodules seen in all antigens tested may be due to lack of penetration of the antibody into the nodule--either due to the nodule dimensions, or the presence of a perichondrium.

Because steric hindrance may affect the results and because of the small number of in-flight samples, additional studies are underway to develop an immunostaining procedure using sections of micromass cultures grown on coverslips in flight hardware.

Based on the information from previous flights, cartilage differentiation is expected to be altered in space. Some effects may be on the timing of differentiation; production, secretion, and aggregation of matrix may also be affected. Monoclonal antibodies to the various components of cartilage matrix can be used to address these questions.

REFERENCES:

ACKNOWLEDGEMENTS

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GRAVITATIONAL EFFECTS ON MAMMALIAN CELLS

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INTRODUCTION

It is known that mammalian cells in culture respond to hypergravity or microgravity. For example, HeLa cell's proliferation was reinforced by hypergravity (1). In experiments carried out in the Skylab 3, the proliferation of WI38 cells, derived from human fetal lung, under microgravity did not differ from those of the ground controls, while glucose consumption by the cells decreased in the microgravity (2). In addition, [3H]-thymidine incorporation into DNA of C6A-activated human lymphocytes decreased during space flight (3). On the other hand, analysis of medical data from astronauts after space flight showed that the bone was one of the organs affected by microgravity. However, the exact mechanism for responses of various mammalian cells to gravitation has not been clarified. The present study examined differences in the gravity perception of the cells by comparing the influence of hypergravity on proliferation between osteoblasts and other cells and by examining the production of prostaglandin E2 (PGE2), a factor that may mediate cell responses.

MATERIALS AND METHODS

Cell culture: Three cell lines MC3T3-E1 derived from neonatal mouse calvaria (4), HeLa derived from human uterine carcinoma, and JTC-12 derived from monkey renal tubules (5) were used. MC3T3-E1 cells were cultured in the Alpha Modification of Eagle's Medium (Flow), HeLa cells were cultured in the Eagle's minimum essential medium, and JTC-12 cells in the DM-160 medium (Kyorin). Each medium was supplemented with 5% fetal calf serum (Flow). The pH of each medium was adjusted to 7.3, using 20 mM HEPES and 0.1% NaHCO3.

Assay for cell growth: Cells were inoculated in six-well multi-plates (Falcon 3046), at 1 x 10^4 cells/well. They were incubated in a humidified CO2 incubator at 37°C for 24 hours.

To study the effect of hypergravity on cell growth, each plate was sealed with a 0.5 mm thick silicon sheet, and placed in a cell culture centrifuge at 5, 10, 20 or 40G for 72-hour. The plates for control were placed on the 1ID of the cell culture centrifuge and incubated for 72 hours. At the end of centrifugal incubation, cells in each well were counted with a Coulter counter (model ZM, Coulter Electronics Inc.). The cell number to relative the counts for the 1G control group was calculated.

To study the effect of the conditioned culture media on the cell growth, MC3T3-E1 cells, which had been precultured for 24 hours in un-conditioned media, were cultured in the 4G conditioned media or 1G conditioned media. At the end of 48 hours stationary culture, cells in each medium were counted. The 40G of 1G conditioned media were prepared as follow: MC3T3-E1 cells were first subjected to 24-hour stationary culture. Then, renewing the medium, they were subjected to 24-hour centrifugal culture at 40G. The medium was collected to serve as a 40G conditioned medium. Conditioned media, obtained in a similar way at of 1G, served as controls.

To study the effect of indomethacin (IND) on the cell growth, MC3T3-E1, HeLa and JTC-12 cells were inoculated in six-well multi-plates. After 24 hours of stationary culture, each medium was renewed with the one containing 10^{-6}M of IND followed by 48-hour centrifugal culture at 40G.

Assay for prostaglandin E2 (PGE2): MC3T3-E1 cells were seeded in the six-well multi-plates at 3 x 10^4 cells/well. After 48 hours of stationary culture, the cells were subjected to centrifugal culture at 5, 10, 20 or 40G for 8 hours. At the end of culture, the medium was collected and centrifuged at 5000g for 5 minutes. The supernatant was collected for PGE2 radioimmunoassay using the prostaglandin E2 [125I]-RIA kit (NEG).

Northern analysis: HeLa cells were inoculated into 25cm² culture flasks. After 24 hours of stationary culture, the cells were incubated at 18, 35 or 70°C for 120, 30, 80, 120, or 360 minutes. Total RNA was extracted in 4M guanidine isothiocyanate + 0.5% sodium lauryl sarcosinate solution. Then, the specimen was re-extracted with phenol/chloroform (1:1 V/V) at 60°C, followed by ethanol precipitation at -30°C. A 10 or 15 μg of the RNA sample was loaded on the 1.5% agarose gel for electrophoresis. RNA was then transferred onto nylon membranes. After cross-linking by the routine method, RNA was hybridized with a32P-labeled probes. By nick translation, the Clal-EcoR1 fragments of exon 3 of human c-myc gene were labeled with a[alpha-32P] d CTP. Hybrids were detected by autoradiography using X-ray films[6].

RESULTS

When MC3T3-E1 cells were cultured for 72 hours under high levels of gravitation, the cell proliferation was suppressed (8% reduction in cell counts) under 10G gravity as compared to the 1G controls (Fig. 1). Under 20G and 40G gravity, on the other hand, cell
proliferation was significantly promoted by 1G and 27%, respectively as compared to the 1G controls.

** Statistically significant difference from the 1G at p<0.01

Figure 1. Effect of hypergravity on proliferation of MC3T3-E1 cells (7).

We then examined the involvement of humoral factors in the promotion of cell proliferation under high gravity levels by examining MC3T3-E1 cells which were incubated in conditioned media after 24-hour culture at 1G or 40G. Cell numbers in the 40G conditioned media were 106% of the numbers in 1G conditioned media. Thus, a small but significant promotion of cell proliferation was noted in the 40G conditioned media, suggesting the involvement of humoral factors in the promotion of cell proliferation in 40G centrifugal cultures.

We then attempted to identify the humoral factors involved in promotion of cell proliferation under high gravity levels. For this purpose, we cultured MC3T3-E1 cells centrifugally in media containing 10⁻⁶M of IND which is a prostaglandin-synthesis inhibitor. As shown in Fig. 2, cell proliferation was markedly suppressed in the 40G IND-treated media to a level comparable to the 1G controls without IND treatment. Cell proliferation was also significantly suppressed in the 1G culture when treated with 10⁻⁶M of IND.

** Statistically significant difference from the 1G at p<0.01

Figure 2. Effect of indomethacin on the enhanced growth by 40G in MC3T3-E1 cells (7).

We next studied the effect of 40G centrifugation on the proliferation of other cell lines. For JTC-12 cells, 72hr incubation at 40G caused a 108% increase in the proliferation of the cell, compared with 1G control. Treatment with IND suppressed this enhancement at 40G. When the same experiment was repeated with HeLa cells, the 40G culturing enhanced proliferation by 134% over the 1G control. Here, however, in contrast to the case with MC3T3 and JTC-12 cells, IND did not statistically affect the enhancement of HeLa cells at 40G (Fig. 3).

** Statistically significant difference from the 1G at p<0.01

Figure 3. Effect of indomethacin on the enhanced growth by 40G in HeLa cells (7).

These results indicate that the action of IND varies among different cell lines.

The humoral factors involved in the promotion of MC3T3-E1 and JTC-12 proliferation under high gravity levels seemed to be prostaglandins.

Fig. 4 shows PGE₂ production following 8-hour incubation of MC3T3-E1 cells at 1, 5, 10 or 40G. The amounts of PGE₂ produced at 5G or 10G did not differ from those at 1G. At higher gravity levels, PGE₂ production increased in a gravitation-dependent manner, showing a 20% increase at 20G and a 55% increase at 40G as compared to the 1G controls.

** Statistically significant difference from the 1G at p<0.01

Figure 4. Effect of hypergravity on PGE₂ production of MC3T3-E1 cells.

Table 2 shows PGE₂ levels in the media after 48-hour incubation of HeLa and JTC-12 cells at 40G. PGE₂ level in 1G cultures of HeLa cells was 0.70 ± 0.16 pg per 10⁶ cells and that in 1G culture of HeLa cells was 0.76 ± 0.33 pg per 10⁶ cells.
cells. These levels were lower than those for MC3T3-12 cells. PGE₂ production by HeLa and JTC-12 cells during 48-hour 1G incubation did not differ from that during 48-hour 1G incubation.

Table 1. PGE₂ PRODUCTION OF MC3T3-E1, HeLa AND JTC-12 CELLS.

<table>
<thead>
<tr>
<th>CELL LINES</th>
<th>PGE₂ (pg/10⁵ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1G</td>
</tr>
<tr>
<td>MC3T3-E1</td>
<td>474 ± 12.3</td>
</tr>
<tr>
<td>HeLa</td>
<td>0.70 ± 0.16</td>
</tr>
<tr>
<td>JTC-12</td>
<td>0.76 ± 0.33</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D.

Thus, proliferation of MC3T3-E1, HeLa and JTC-12 cells was promoted under high gravity levels. Hypergravity-induced promotion of PGE₂ was only marked for MC3T3-E1 and hardly seen for HeLa and JTC-12 cells.

Analysis of the time course of c-myc gene expression during high gravity (70G) incubation of HeLa cells disclosed an approximately two-fold increase, as compared to the controls, in the gene expression 30 minutes after the start of high gravity exposure and this elevation was maintained for 6 hours (6).

DISCUSSION

The proliferation of MC3T3-E1 cells following 72-hour incubation at 20 or 40G was promoted by 10-27% as compared to the 1G controls. Promotion of proliferation was also observed for HeLa and JTC-12 cells at 40G. In this connection, Tschopp et al. reported that the proliferation of HeLa cells, chicken embryo fibroblasts and human lymphocytes was promoted by 20-30% when incubated at 10G. Kumel et al. (6) reported promoted proliferation of HeLa cells when incubated for 72 hours at 18, 35 or 70G. The results from the present study are consistent with those reported by Tschopp et al. and Kumel et al., except that our study disclosed a biphasic influence of high gravity on MC3T3-E1 cells, i.e., suppressed proliferation at 10G and promoted proliferation at other gravity levels.

We attempted to clarify the mechanism for promotion and suppression of MC3T3-E1 cell proliferation under high gravity from the aspect of humoral factors. When MC3T3-E1 cells were incubated in conditioned media at 40G, cell proliferation was promoted. This suggests that some growth factor is produced by cells under high gravity and released into the media. In the presence of IND, the effect of high gravity in promoting the proliferation of cells was suppressed, suggesting the involvement of PGs in the hypergravity-promoted proliferation of cells.

Based on the view that PGE₂ is a humoral factor involved in this effect of hypergravity, we determined PGE₂ levels in cell cultures after 8-hour exposure to 5-40G gravitation. This analysis disclosed that PGE₂ production by MC3T3-E1 cells was stimulated by high gravity, and that the amounts of PGE₂ production by these cells showed a gravity-dependent increase for a range of 10-40G.

The effect of PGE₂ on the proliferation of cells has been reported by many investigators. Hakeda et al. (8) reported that treatment of culture media with a low level (10⁻⁷M) of exogenous PGE₂ suppressed the DNA synthesis of MC3T3-E1 cells, and that treatment with 10⁻⁶M or higher levels of PGE₂ promoted their DNA synthesis, thus suggesting a biphasic effect of PGE₂ on the proliferation of MC3T3-E1 cells. This finding of Hakeda et al. is noteworthy when it is compared with the biphasic effect of high gravity observed in the present study. However, because the PGE₂ level in our 10G cultures (0.71 ng/ml) was lower than the levels of PGE₂ added to the medium in the above-mentioned studies, we cannot conclude that the suppressed proliferation at 10G is attributable to PGE₂. Furthermore, we previously reported that PGE₂, added to 20G and 40G cultures, which showed promoted cell proliferation, were 0.97 and 1.36 ng/ml, respectively, which were not consistent with the values reported by Hakeda et al. Moreover, the effect of high gravity was not completely suppressed in the present study. These results suggest that some unknown factors are involved in the suppression of MC3T3-E1 proliferation at 10G and the promotion of their proliferation at 40G.

In the present study, all three cell lines MC3T3-E1, HeLa and JTC-12 cells showed promoted proliferation under high gravity. PGE₂ levels in the culture of MC3T3-E1 cells increased under high gravity, while no such increase was observed for HeLa cells. Thus, the mechanism for hypergravity-induced promotion of cell proliferation seems to vary between different cell lines. In this connection, we previously reported that the enhanced proliferation of HeLa cells at 18, 35 and 70G was due to shortened cell cycle, in particular the G₁ phase, and that this effect of high gravity is mediated by the expression of mRNA of c-myc gene (6). A similar genetic study is needed for the other cells.

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NEUROGRAM ANALYSIS
TESTED ON SYNTHETIZED
MULTIUNIT FIRING PATTERNS

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INTRODUCTION:
The term 'neurogram' is used here for multiunit recordings, typically obtained by chronically implanted metal semi-microelectrodes of 3-50 μm tip diameter. Some kind of its integral (2) can serve as a general indicator of the activity of a linearly coded, homogenous neuron population.

However, this paradigm cannot be used for the cerebellar cortical activity, as the dominant Purkinje-cells are nonlinear units with complex spike-generating mechanism.

Therefore, Pc firing patterns, separated from cerebellar cortical neurograms, will be subjected to a structural analysis, formerly developed for the monitoring of elementary adaptive events in Pc activity (3,4).

PROGRAM FOR UNIT PATTERN EXTRACTION
The main analytic program begins with an early identification of peaks, based on a two-parameter schedule. The trap parameters could be used for an off-line adaptive optimization in a 3D-net, where the narrow plateau of the perfect parameters ranging between the downhill saturation of the hits and the uphill false positives could be readily detected.

After all the peaks have been identified, using the optimal trap parameters, the serial frequency-diagrams of interspike intervals for prefixed amplitude classes were constructed, where the different profiles of signal and noise appear clearly distinct enabling us to introduce an objective noise elimination (Fig. 1).

On final identification, five parameters for each spike were taken (1), 3 for peak amplitudes and 2 for the intervals between them. These were then used to separate spikes of common cellular origin, therefore of similar shape, even if they are distorted by overlapping with other unit amplitudes. Scattering diagrams of paired parameters (5) were prepared for future cluster analysis.

It is obvious that, given the increasing noise level — the number of recorded units, the under- and overshoot of spike discrimination and of clustering may reach a critical level, where the analysis becomes unreliable, and the factual error remains obscure.

\[ \text{Fig. 2. Frequency distributions of preliminarily detected peaks of a 1-Second-long record. (X: intervals length, log. scale, Y: frequency of appearance, Z: prefixed amplitude classes). The profiles are distinct below versus above a 1.5 mV threshold level. This can serve an objective type signal/noise discrimination.} \]
SYNTHETIZED TEST PATTERNS

Because of the problems outlined above, we decided to synthesize test patterns of different complexity, on which an analytic program could be evaluated precisely.

A set of standardized spike shapes of the cerebellar Purkinje cell, obtained by averaging 'simple spikes' of stable homogenous PC extracellular records were built in stochastic sequences, statistically following up the feature of the frequency diagram of interspike intervals of a spontaneously firing realistic Purkinje-cell (Fig.2). 2-4 of such unit sequences of different amplitude and/or frequency were then superimposed for a (cerebellar cortical) neurogram-like test-file, completed with different levels of 'white noise'.

All the ingredients of the test patterns were recorded into separate index-files.

When elaborating spike-pattern identifying programs or looking for optimal trap parameters, a continuous feedback (including a known ratio of the under- and overshoot) ought to be present. Also the hits and false positives should be evaluated on the basis of a well-defined number and of the feature of the constituents, provided by test files.

CONCLUSIONS:

The test files offer the possibility of enhancing the efficacy of an analytic program or its selected parameters (including an off-line adaptivity), and also of detecting and realizing the limitations of extraction of unit patterns from neurograms end of the multiunit analysis in general.

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RESPONSES OF GRAVITY LEVEL VARIATIONS ON THE NASA/JSC BIOREACTOR SYSTEM

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INTRODUCTION

A Couette flow bioreactor was developed for culturing mammalian cells. The system was designed to emulate aspects of a microgravity environment by providing a uniform low shear three-dimensional environment of controllable shear levels. The purpose was to allow positive control of performance of suspension systems and anchorage-dependent tissue culture in a low gravity (low shear) three-dimensional culture environment. A major design requirement was based on the need to suspend cells and aggregates of cells on microcarrier beads (emulated as a particle) with continuous perfusion while maintaining the minimum shear environment possible in unit gravity. The design provided sufficient circulation for adequate oxygenation and mass transfer of nutrients to the cells through mechanical methods. Conventional bioreactors were difficult to characterize the flow field and resulting hydrodynamic stresses. Special attention was required to develop a rotational suspension system without the inherent mechanical agitation mechanisms with corresponding shear effects which damage delicate cells and is hypothesized to degrade the formation of three-dimensional tissue-like structures. Microgravity is hypothesized to provide the best environment where the hydrodynamic variables within a bioreactor can be controlled over a wide operating range in order to optimize the assembly of tissues from elementary cells and substrates. NASA rotating-well perfusion vessels, partially simulating microgravity hydrodynamics, have shown excellent performance on Earth. We need to project if a further improvement could be realized in actual space flight where the residual gravity-induced stresses are removed. This Couette flow bioreactor upon hydrodynamic characterization will be utilized to introduce very low level homogeneous shear levels, of graded intensity, to understand the role of low shear levels on the in situ formation of living tissue.

MATHEMATICAL MODEL

The bioreactor to be modeled here is designed to provide a Couette flow field with nearly uniform shear stress throughout the vessel. The vessel consists of two concentric cylinders both 11 cm long with the outer cylinder having a radius of \( r_o = 4.0 \) cm and rotating at \( \omega_o \) rpm, while the inner cylinder has a radius of \( r_i = 2.86 \) cm and rotates in the same direction at \( \omega_i \) rpm. The end walls of the vessel are fixed with the outer cylinder and rotate with it. The narrow gap of 1.14 cm between the cylinders was completely filled with culturing medium into which particles (models of aggregates of cells) and microcarrier beads were introduced. Figure 1 provides a schematic representation of the vessel. Cylindrical coordinates \((r, \theta, z)\) will be used to indicate positions within the vessel where \( r \) is the radial component outward relative to the cylinders with \( r_i \leq r \leq r_o \), \( \theta \) is the angular component measured positively in the direction of rotation of the two cylinders, and \( z \) is the axial direction oriented horizontally in a gravitation field with \( 0 \leq z \leq 11 \) cm.

Assumptions for this mathematical simulation are:

1. The culturing medium, or substrate, was a Newtonian fluid with constant density \( \rho_f = 1.02 \) g/cm\(^3\) and constant viscosity \( \mu = 0.0015 \) g/cm sec;
2. Particles were spherical in shape with diameter \( d = 175 \) μm and density \( \rho_p = 1.04 \) g/cm\(^3\), did not interact with one another, and did not affect the flow of the culturing medium;
3. The flow of the culturing medium caused by the rotation of the concentric cylinders was a laminar, axially symmetric Couette flow and was modeled by the Navier-Stokes equation; and
4. The forces acting on a particle were drag from the fluid's circulation, buoyancy from the gravitational force relative to the difference between the densities of a particle and the fluid, and centrifugal force from the rotation of the vessel.

If \((u, v, w)\) represent the (radial, circumferential, axial) components of the velocity of the flow field as functions of time \( t \), then the Navier-Stokes equations for the flow field are:

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( ru \right) + \frac{\partial w}{\partial z} = 0
\]  

(1)

\[
\frac{D u}{D t} = \frac{v^2}{r} - \frac{1}{\rho_f} \frac{\partial p}{\partial r} + \frac{\mu}{\rho_f} \left( \frac{\partial^2 u}{\partial z^2} \right)
\]

(2)

\[
\frac{D v}{D t} = \frac{1}{\rho_f} \frac{\partial p}{\partial \theta} + \frac{\mu}{\rho_f} \left( \frac{\partial^2 v}{\partial r^2} \right)
\]

(3)

\[
\frac{D w}{D t} = \frac{1}{\rho_f} \frac{\partial p}{\partial z} + \frac{\mu}{\rho_f} \left( \frac{\partial^2 w}{\partial r^2} \right)
\]

(4)

and \( P \) is the fluid pressure measured in g/cm\(^2\).

If \((r, \theta, z)\) represent the position of a particle at time \( t \), then the equations of motion of a particle are:

\[
m \frac{d^2 r}{d t^2} = - \alpha \left( \frac{d r}{d t} \right) \cdot \beta g \cos \theta + \beta r \left( \frac{d^2 r}{d t^2} \right)^2
\]

(5)

\[
m \frac{d^2 \theta}{d t^2} = - \alpha \left( \frac{d \theta}{d t} \right) \cdot \beta g \sin \theta
\]

(6)

and

\[
m \frac{d^2 z}{d t^2} = - \alpha \left( \frac{d z}{d t} \right) \cdot \beta g
\]

(7)

where \( m = \rho_f \pi d^2 / 6 \), \( \alpha = 3 \mu \), \( \beta = (\rho_p - \rho_f) / 2 \mu \). The initial value problem was a two time-scale, three-dimensional, second order system of ordinary differential equations. Both time scales came from the large difference between the rate of rotation and the rate of circulation in the secondary flow. As a two time-scale approach, Equations (5) - (7) gave a stiff system of ordinary differential equations with a 6 x 6 Jacobian matrix having at least two large eigenvalues and at least two small eigenvalues requiring Gear's stiff method for trajectory calculations.
RESULTS AND CONCLUSION

Numerical models of the rotating bioreactors developed by the NASA Johnson Space Center Biotechnology Group indicate a partial microgravity simulation with respect to the major trajectories and circulation time frame of a slowly sedimenting particle's motion. Figures 3 and 4 illustrate the particle trajectories under one g and 10⁻⁴ environment. Comparisons of the trajectories in Figure 3 (unit gravity) with the corresponding trajectories in Figure 4 (microgravity) indicate

that, except for a gravity-induced spatial oscillation on each revolution, a slowly sedimenting particle in unit gravity will follow the same major trajectory as a particle in microgravity even though the strengths of the dominant forces controlling the dynamics are quite different. Off-bottom suspension of microcarriers in unit gravity was attained without turbulence in a rotating bioreactor. However, there is still a distinct advantage to operating the bioreactor in microgravity since the total force on a particle would be significantly reduced by eliminating the gravity-induced oscillatory particle motions. This motion is the primary cause of residual shear stress in the rotating culture vessels operated in unit gravity. Figure 5 demonstrates the result of incremental increases in shear stress forces in the rotating-wall bioreactor and its effect on three-dimensional tissue assembly. Condition #1 represents shear stress force of approximately 0.51 dyne/cm² increasing to condition #4 which represents 0.92 dyne/cm². The results show that small tissue masses (i.e., those of 2-5 complex microcarriers in size) experience little or no effect from increased shear forces. However, as the size of these complex microcarrier aggregates increase over time (condition #1 vs. condition #4) a severe effect is experienced on the three-dimensional tissue's ability to complex and grow. As particles (representing larger tissue segments) of increased sedimentation rates are cultured, the reduction in hydrodynamic stresses attainable by operation in microgravity is increased. The total force on a particle in a rotating bioreactor was less than the values reported by other researchers using different vessel geometries to model hydrodynamic effects on cell growth. The hydrodynamic conditions in the Couette bioreactor are well defined and nontrubulent making it a good choice for studies relating hydrodynamics to cell or tissue culture performance. Furthermore, the tumbling effect may facilitate energy dissipation by the particle leading to a force-per-unit cross-sectional area on a particle that is less than the analysis of hydrodynamic forces would indicate. In either unit gravity or microgravity it appears that the greatest potential for cellular damage occurs when the particle is near the vessel wall or collides with the wall. Increasing the differential rate of rotation strengthens the secondary flow but does not appear to entrap a particle within the flow. Faster rates of rotation decrease the time between impacts with the vessel walls, thereby increasing the number of collisions. A larger gap between the inner and outer cylinders would decrease the number of collisions but would weaken the circulation of the fluid from the secondary flow. Further study needs to be conducted to determine optimal design and operating conditions to optimize mass transfer while minimizing hydrodynamic forces.

REFERENCES
DELIVERY OF RECOMBINANT HUMAN GROWTH HORMONE TO RATS DURING EXPOSURE TO MICROGRAVITY ON NASA SPACE SHUTTLE DISCOVERY

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Genentech, Incorporated
South San Francisco, California 94080

INTRODUCTION

Growth hormone (GH) can normally promote statural growth and anabolism by direct actions on bone, muscle and liver. During exposure to the microgravity of spaceflight, bone mineral and muscle mass is lost, and GH secretion from the pituitary is impaired (1). Indeed, the GH secreted appears to have less biological activity (2). From these data, we hypothesized that GH treatment could attenuate the tissue loss induced by microgravity. Accordingly, we attempted to treat male rats with recombinant human GH (rhGH) or excipient via osmotic minipumps. However, there was no experience on earth orbit either with delivering a protein to research animals or with osmotic pump performance. There was also concern that the increased radiation in space could damage the protein.

METHODS

Weanling male rats (Sprague-Dawley, Charles Rivers) were implanted subcutaneously with osmotic minipumps containing rhGH or vehicle 1 d prior to launch. The samples were formulated in a diluent [5 mM phosphate, pH 7.8, 0.1% F68 (detergent)] at approximately 20 mg/mL. The "control" sample was recovered from an rhGH osmotic pump that had not been inserted into a rat, and that was not on Discovery. The 16 animals selected to fly or to provide ground controls were randomised by weight, loaded into the Animal Enclosure Modules (AEM) and then inserted into middeck lockers of Space Shuttle Discovery (STS-41). Food and water was provided ad libitum, and the light-dark cycle was 12:12 h. The AEM's and animals were monitored daily by Mission Specialists Bruce Melnick and William Shepard. The flight lasted for 4 d, and immediately after landing, the rats were judged healthy by 2 veterinarians. The animals were quickly transported to Genentech, and were sacrificed 4-6 h after the landing of Discovery. The pumps were removed, and the remaining pump solutions were both measured for rhGH content by immunoassay and studied analytically to prove that the pumps performed as specified and that the rhGH was intact.

The rhGH samples (125 mcL, about 625 mcg) were diluted with 400 mcL of milli Q water. Each sample was exchanged into 1 mL of digest buffer, pH 8.3 using NAP5 columns from Pharmacia. Trypsin (Worthington) was added (6.25 mcL/6.25 mcg) and the sample was incubated at 37°C. After 2 h a second aliquot of enzyme (6.25 mcL/6.25 mcg) was added to make the final concentration of enzyme to substrate 1:50 (w/w). The samples were incubated for a further 2 h at 37°C and then the digestion was stopped by the addition of 100 mcL 1N HCl. Each sample was analyzed on a Hewlett-Packard 1090 M high pressure liquid chromatography (HPLC) at 214 nm using a Nucleosil C18 column (4.6 x 150 mm, 5 micron, 100 A) with a temperature of 40°C, and a flow rate of 1 mL/min. The mobile phases consisted of solvent A: 50 mM sodium phosphate, pH 2.85, and solvent B: acetonitrile. After an initial 5 min hold at 0% solvent B, a 0.33% per min linear gradient to 40% solvent B was performed. This was followed by a 2.0% gradient to 60% solvent B before re-equilibration in the initial solvent.

Reversed-phase HPLC analyses were performed on a Hewlett Packard 1090M. rhGH samples (5 mcL) were diluted to 100 mcL with milli Q water and injected onto a Vydac C4 column, (4.6 mm x 25 cm, 5 micron, 300 A). Flow rate was 1 mL/min and the eluate was monitored at 214 nm. Solvent A was 0.1% trifluoroacetic acid (TFA) in water. Solvent B was 0.1% TFA in 95% acetonitrile, 5% water. After an initial hold for 10 min at 50% solvent B, a 1% per minute linear gradient was performed to 70% solvent B. The analysis was held at 70% solvent B for 10 min before returning to the initial conditions for reequilibration.

RESULTS AND DISCUSSION

Both the flown and ground control rats appeared normal and continued to gain weight during the flight days. The only anomaly was that the AEM temperatures increased each day such that peak temperatures on day 3 and 4 were 35°C and 36°C, respectively. All other parameters were normal. The humidity was 18-30% and the pressure was 14.66 - 14.74 psi during the flight. Serum levels of rhGH at landing were 36.1 ± 5.6 ng/ml (n = 8) in the flown and 39.3 ± 5.0 ng/ml (n = 11) in the ground control animals (mean ± SEM), whereas there was no rhGH in excipient rat blood. These values indicate that physiologic levels of GH were achieved throughout the flight.

No major differences were found between the tryptic maps or HPLC profiles of rhGH recovered from the osmotic pumps. The tryptic profiles are essentially identical except for a slight decrease in the amount of tryptic fragment T9 (Fig. 1). This is not unexpected since this hydrophobic peptide has previously been shown to have variable and poor recoveries. Some minor differences are also evident between control and flown samples. The small peak at approximately 21 minutes (fragment T14c) is noticeably smaller in the control sample. This peak is a chymotryptic-like cleavage and is not inhibited by lowering the pH to quench; variable
amounts are usually observed. The flown and ground samples show an increase in a small peak at 52.5 min when compared with the control. This is a typical pattern observed when deamidation occurs.

Reversed-phase HPLC analyses (data not shown) indicated that all samples recovered from osmotic pumps inserted into rats had a slight increase in the amount of non-dissociable dimer (1.12%) when compared with the sample that was not implanted into the animals (0.47%). A similar comparison indicated that all samples recovered from pumps in rats had some broadening and a slight tailing of the main peak as well as a small new peak (7.5-8.5 min) when compared to the non-implanted control. This new peak is probably oxidized rhGH. The minor broadening of the main peak is typical of heated 37°C rhGH samples. The exact cause is unknown but is likely due to many conformational forms of rhGH being partially resolved. When pump contents were compared between flown and ground control rats, there were no detectable differences.

The volume and immunoassay analysis of the osmotic pump contents are listed in Table 1, confirming that the

<table>
<thead>
<tr>
<th>TABLE 1. VOLUME AND IMMUNOASSAY ANALYSIS OF PUMP CONTENTS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microgravity</td>
</tr>
<tr>
<td>Expected</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Volume (mL)</td>
</tr>
<tr>
<td>Fill vol.</td>
</tr>
<tr>
<td>End vol.</td>
</tr>
<tr>
<td>rhGH</td>
</tr>
<tr>
<td>Initial (mg/mL)</td>
</tr>
<tr>
<td>Final (mg/mL)</td>
</tr>
<tr>
<td>Delivery (µg/h)</td>
</tr>
</tbody>
</table>

*Mean ± SD (n = 8-11). End volume and final rhGH concentrations were measured after pump removal from the rats.

ACKNOWLEDGEMENTS

We thank Astronauts W. Shepherd and B. Melnick, as well as Dr. T. Bowman, D. Mortensen and the Assay Services Group for their technical support. We also thank Dr. Wes Hymer and the Penn State Center for the Commercial Development of Space. The help and patience of our dedicated colleagues at NASA Ames Research Center and Hanger L at Kennedy Space Center was invaluable. We dedicate this work to the memory of Matthew Matlock.

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2. GRINDELAND, R., W.C. HYMER, W. VALE AND P. SANCHENKO. Growth hormone regulation, synthesis and secretion in microgravity. NASA report on Cosmos 2044 (Oct '89) findings.
AORTIC WAVE REFLECTION AND INPUT IMPEDANCE AS A FUNCTION OF POSTURE IN A CHRONIC PRIMATE MODEL

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¹USAARL, Ft Rucker, AL, ²C.E.R.M.A., Brétigny-sur-Ogrie, France, and ³Armstrong Laboratory, Brooks AFB, San Antonio, Texas

INTRODUCTION

Most data on central circulatory hemodynamics in man, formulated in hydraulic loading terms, is known only for the supine position, largely due to practical and ethical constraints. The upright position, however, is the most relevant to daily patient activities. The aortic input impedance and pulsatile power provide further understanding into central systemic pressure-flow relationships. Furthermore, pressure and flow wave contours are significantly affected by wave reflections. Therefore, we have evaluated some parameters of central circulatory hemodynamics in a conscious, chronically instrumented primate as part of a study to describe effects of changing gravitational stress on these variables.

METHODS

We have selected the mature baboon as our human surrogate and have completed analysis in 9 subjects prior to and 5 baboons after surgical implantation of transducers. Presurgery catheterizations were performed under ketamine and pentobarbital sedation. The femoral approach was used to introduce a balloon-tipped thermodilution catheter into the pulmonary artery and a multisensor Millar catheter retrograde across the aortic valve. The left heart catheter permitted simultaneous recording of LV, Ao pressures and aortic root flow (Fig 1.) Cardiac outputs were obtained by thermodilution. Data were recorded supine, then with 70° tilt.

Surgical implantation is performed under general anesthesia via left thoracotomy. Transducers implanted include: aortic and left ventricular pressure cells (Konigsberg Instr.), EMF probe around the proximal aorta, fluid lines in RA and LA, LV endomyo-

Cardiac crystals, and and IVC occluder cuff (Fig. 2).

Figure 1. Presurgery catheter position and sample data.

Figure 2. Implantation of Transducers.

Ao=Aortic pressure; EMF=Electromagnetic flow velocity; LV=Left ventricular pressure; PCWP=Pulmonary capillary wedge pressure

Before and after surgery study animals were trained to accept a specially constructed chair. Tilt studies were performed supine and with 90° upright tilt. Peripheral resistance (Rp) was calculated as mean AoP by mean Ao flow. Simultaneous pressure and flow data were submitted to Fourier analysis and impedance determined by dividing harmonics of pressure by corresponding harmonics of flow. Moduli from the 3rd to 15th harmonic were averaged to determine characteristic impedance (Ze). The backward reflected pressure wave (Pb) was determined by: Pb = (Pm - Ze*Fm)/2 where Pm and Fm are measured pressure and flow, respectively. Compliance (C) was calculated using a three element Windkessel model.
RESULTS

The mean aortic pressure (Ao$_m$) was not significantly different between supine and upright for presurgery studies. Cardiac output (CO), heart rate (HR), and Rp were significantly different (See Table 1). The aortic input impedance was not remarkably different between the two conditions. In support of this similarity, Zc supine and upright were not significantly different.

Table 1. Hemodynamics of presurgery tilt study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Supine</th>
<th>Upright</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao$_m$ (mmHg)</td>
<td>104 ± 4</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>3.0 ± .2</td>
<td>2.5 ± .2*</td>
</tr>
<tr>
<td>HR (b/min)</td>
<td>119 ± 5</td>
<td>133 ± 6*</td>
</tr>
<tr>
<td>Rp (d<em>sec</em>cm$^2$)</td>
<td>2953 ± 342</td>
<td>3432 ± 412*</td>
</tr>
<tr>
<td>Zc (d<em>sec</em>cm$^5$)</td>
<td>70 ± 11</td>
<td>72 ± 17</td>
</tr>
<tr>
<td>C (cc/mmHg)</td>
<td>1.31 ± .3</td>
<td>1.27 ± .13</td>
</tr>
<tr>
<td>Wp (mWatts)</td>
<td>85 ± 7</td>
<td>71 ± 9</td>
</tr>
<tr>
<td>Wm (mWatts)</td>
<td>688 ± 52</td>
<td>588 ± 51</td>
</tr>
<tr>
<td>Wp/Wt</td>
<td>11.4 ± 1.3</td>
<td>10.7 ± 1.3</td>
</tr>
<tr>
<td>Pb (mmHg)</td>
<td>19.5 ± 2</td>
<td>16.6 ± 1.9*</td>
</tr>
</tbody>
</table>

Mean ± SEM; *p < .05 n=5
Wp,m,t=pulsatile, mean, and total power.

Data after surgical implantation revealed similar results with changes from supine to upright with exception of amplitude of calculated backward pressure. See Table 2.

Table 2. Postsurgery hemodynamics supine and upright in conscious state.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Supine</th>
<th>Upright</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ao$_m$ (mmHg)</td>
<td>120 ± 5</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>3.2 ± .2</td>
<td>2.3 ± .1</td>
</tr>
<tr>
<td>Rp (d<em>sec</em>cm$^2$)</td>
<td>3022 ± 189</td>
<td>3998 ± 211*</td>
</tr>
<tr>
<td>Zc (d<em>sec</em>cm$^5$)</td>
<td>126 ± 16</td>
<td>135 ± 10</td>
</tr>
<tr>
<td>C (cc/mmHg)</td>
<td>2.2 ± .6</td>
<td>1.6 ± .3</td>
</tr>
<tr>
<td>Wp (mWatts)</td>
<td>62 ± 8</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>Wt (mWatts)</td>
<td>956 ± 87</td>
<td>734 ± 57</td>
</tr>
<tr>
<td>Wp/Wt</td>
<td>6.5 ± .8</td>
<td>5.5 ± 1.1</td>
</tr>
<tr>
<td>Pb (mmHg)</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
</tr>
</tbody>
</table>

Mean ± SEM; *p < .05 n=5

DISCUSSION

We have evaluated central hemodynamics with an emphasis on aortic input impedance and arterial wave reflection in a primate model in both the sedated and conscious conditions. We found that mean pressure was maintained in both conditions when changing from supine to the upright posture by a significant increase in Rp and HR although CO was modestly reduced. In addition, mean and pulsatile resistive loads were greater for the conscious condition (sup and up) compared to the sedated acute catheterization study.

Although there was a significant increase in the steady (Rp) load upright compared to supine there was no significant change in the pulsatile load given by Zc. This is not unexpected since Ao$_m$ was not significantly changed. Similarly, and probably for the same reason, C determined by the 3-element Windkessel model was also unchanged. Thus, pulsatile loading characteristics are apparently unchanged in this model with postural stress from the baseline steady state. Of note is that the percentage of pulsatile to total power (Wp/Wt) is less for the conscious condition compared to that with sedation.

The presurgery input impedance looks very similar for supine and upright states, however, Pb is significantly reduced for the upright posture. This in part may be attributed to a diminished stroke volume (SV), however an even greater reduction in SV in the conscious state was not associated with a change in Pb. A change in regional compliance of the central aorta, i.e. a change in transmural gradients, with the upright posture may alter attenuation coefficients and hence reduce Pb. The greater autonomic response in the conscious animals may compensate resulting in Pb which is unchanged from the supine value. The present data do not permit analyses separating possible mechanisms. In summary, reflex mechanisms maintain the ventricular/vascular coupling relationship for changes with upright posture. Barbiturate sedation may alter these effects such that wave reflection returning to the aortic root is diminished when upright.

REFERENCES


VENTRICULAR/VASCULAR COUPLING UNDER HYPERGRAVITY IN A CHRONICALLY INSTRUMENTED CONSCIOUS PRIMATE MODEL

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1C.E.R.M.A., Bretigny-sur-Orge, France; 2USAARL, Ft Rucker, AL; and 3Armstrong Laboratory, Brooks AFB, San Antonio, TX

INTRODUCTION

One parameter of cardiovascular function frequently assessed is the loading condition upon the heart. Usually, this load is formulated in terms of peripheral vascular resistance (mean pressure divided by mean flow). The pulsatile load upon the ventricle is formulated in terms of the aortic input impedance (pulsatile components of pressure divided by pulsatile components of flow). Pulsatile load is only a small percentage of mean load but is essential to the understanding of pressure-flow relationships (3,4) and of the left ventricle/vascular coupling which can also be assessed by the ratio pulsatile power/total power of the left ventricle (1,2). Previous data obtained in man and nonhuman primates during centrifugation have been expressed only in terms of mean load.

Therefore, we have evaluated the change of aortic characteristic impedance and left ventricular pulsatile power in a conscious, chronically instrumented primate under conditions of increased head-to-foot gravitational stress.

METHODS

Four baboons underwent surgical implantation under general anesthesia via left thoracotomy. Aortic bioinstrumentation included an electromagnetic flow probe (EMF) on the proximal ascending aorta, and a Konigsberg pressure transducer positioned downstream close to the EMF. Left ventricular instrumentation included a Konigsberg pressure transducer positioned through the apex and three pairs of piezoelectric crystals (DIM) using transit time sonomicrometry to measure left ventricular endocardial axis (base-apex, anteroposterior and septolateral).

Centrifugations started after a recovery period of at least 4 weeks. Centrifugation profile was as follows: 3 G/s onset run, 15 s Gz plateau, deceleration. Successive runs were separated by a rest plateau at 1.4 Gz. Gz levels performed were 2, 3, 4, 5, 6, 7, 8 and 9 G. The EMF signal (cm/s) was calibrated in volumetric terms (m/s) by using the stroke volume values measured from the DIM.

Fourier analysis was applied to simultaneous aortic input pressure and flow, and their respective harmonic components were used to determine aortic input impedance (4). Moduli of the impedance from 5 to 15 Hz were averaged to determine the characteristic impedance (Zc). Peripheral resistance (Rp) was the impedance modulus obtained at zero frequency. Fourier analysis of pressure and flow were also used to calculate pulsatile power (Wp), total power (Wt) and the Wp/Wt ratio (2).

RESULTS

Hemodynamic parameters (for baseline, 3, 5, 7 and 9 Gz plateaus) obtained during steady state hemodynamics are given in Table 1. The mean aortic pressure (AOPm) was not significantly different between Gz plateaus and baseline (1.4 Gz). Cardiac output (CO) decreased, heart rate (HR) and left ventricle dP/dtmax increased, but the change was not statistically significant at each Gz level. Peripheral resistance (Rp) increased with Gz level but was only significant at 3 Gz. Note that Rp decreased at 9 Gz, because of the very low value of AOPm. Characteristic impedance (Zc) and the Wp/Wt ratio were unchanged.

DISCUSSION

In spite of the limited number of subjects (n=4), this preliminary study tended to show that the cardiovascular system tries to maintain the ventricular/vascular coupling described in terms of impedance and power. Indeed, Zc was unchanged and Wp always accounted for a little fraction of Wt at each Gz level. We noted a phasic response of central hemodynamics to Gz stress. Aortic (AOP), left ventricular (LVP) pressures and aortic flow (Flow) were changing during Gz plateaus, defining four phases during the centrifuge run. Phase A is the phase before the onset run (baseline at 1.4 Gz). Phase B is the first part of the Gz plateau: AOP, LVP and Flow decrease. Phase C is the last part of the Gz plateau: AOP, LVP and Flow increase and tend to be steady (data in table 1 were obtained during phase C). Phase D is the phase just after the deceleration. Finally, phase C corresponds to the effects of Gz stress plus the effects of the baroreflex response (compensated phase). Phase B corresponds to the effects of Gz stress only, without baroreflex response (because of the delay in physiological response time). Phase D corresponds to the continued baroreflex response in the absence of Gz stress.
Table 1. Hemodynamics during baseline and compensated phase.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>3 G</th>
<th>5 G</th>
<th>7 G</th>
<th>9 G</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOP (mmHg)</td>
<td>111 ± 2</td>
<td>127 ± 6</td>
<td>118 ± 7</td>
<td>104 ± 14</td>
<td>65.85 ± 20</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>3.6 ± .34</td>
<td>2 ± .27</td>
<td>1.6 ± .35</td>
<td>1.5 ± .50</td>
<td>1.5 ± .28</td>
</tr>
<tr>
<td>HR (b/min)</td>
<td>135 ± 7</td>
<td>191 ± 19</td>
<td>196 ± 26</td>
<td>219 ± 13</td>
<td>229 ± 5</td>
</tr>
<tr>
<td>dP/dt (mmHg.s-1)</td>
<td>2655 ± 316</td>
<td>5661 ± 952</td>
<td>5245 ± 648</td>
<td>4007 ± 1273</td>
<td>4043 ± 1605</td>
</tr>
<tr>
<td>Rp (dyn.s.cm-5)</td>
<td>2199 ± 169</td>
<td>3522 ± 351'</td>
<td>6477 ± 1951</td>
<td>5507 ± 1953</td>
<td>2585 ± 503</td>
</tr>
<tr>
<td>Zc (dyn.s.cm-5)</td>
<td>96 ± 10</td>
<td>82 ± 8</td>
<td>94 ± 13</td>
<td>117 ± 50</td>
<td>66 ± 15</td>
</tr>
<tr>
<td>Wp (mW)</td>
<td>108 ± 28</td>
<td>74 ± 28</td>
<td>43 ± 20</td>
<td>81 ± 75</td>
<td>40 ± 35</td>
</tr>
<tr>
<td>Wp/WT (mW)</td>
<td>9.15 ± 1.67</td>
<td>7.28 ± 1.93</td>
<td>8.37 ± 2.58</td>
<td>9.51 ± 3.39</td>
<td>6.74 ± 2.71</td>
</tr>
</tbody>
</table>

Mean ± SEM (n=4; mean weight = 26 ± 1.3 kg); * p<0.05 when compared to baseline (paired t-test)
See text for abbreviations

Figure 1. Aortic root wave contour during A, B, C, D phases

X axis: time. One scale = 50 ms (A,B) or = 20 ms (C,D).
Y axis: mmHg

To explain pulsatile load data during the compensated phase C, we have selected one beat of AOP for each phase at 7 Gz (see Figure 1) from one baboon. Ascending aortic wave contour presented a mid-to-late systolic peak, as the result of pressure wave reflected from peripheral site(s) returning to the heart. This systolic peak disappeared in phase B and the reflected wave appeared later in diastole, suggesting the pulse wave velocity was decreased, maybe due to an increase of aortic compliance, with a low mean pressure. With compensation, wave contours tended to revert towards baseline with a higher mean AOP. The reflected wave appeared earlier than in phase B, suggesting a stiffer aorta in phase C. Rp and stroke volume increase (for a higher mean AOP) may also contribute to increase reflection wave. This preliminary study showed that the cardiovascular system attempts to revert towards baseline and thus to maintain the efficiency of the ventricular/vascular coupling in the baboon submitted to hypergravity.

REFERENCES
THEORY OF MECHANICAL INTRACRANIAL HYPERTENSION UNDER RAPID ONSET RATE (ROR) ACCELERATION
MODELING OF INTRACEREBRAL BLOOD FLOW

*Gaffié D., **Guillaume A., **Quandieu P., ***Lébaert Ph.

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INTRODUCTION

Changes in the symptomatology of inflight loss of consciousness affecting fighter aircraft pilots exposed to rapid onset rate accelerations motivated the search for an etiology (Quandieu and al., 1990, 1991) different from that usually known for gradual onset rates (GOR): Hypoxia. A physical model representing blood flow in cerebral vessels is proposed in order to analyze pathophysiological mechanisms inducing this type of inflight loss of consciousness. An essential aspect of our model is that it allows superimposition of the heart pulsed action with an outside field of disturbances caused by aircraft motion, and therefore to study its effect on blood flow.

MATHEMATICAL FORMULATION

We consider a simple vascular network from the arteriole to the venule. This network is modeled by a tube which initial cross-section and stiffness depend on its longitudinal direction.

This tube represents a system of main blood vessels considered to be on line.

Problem equations are written in a vector form:

\[
\text{DIV} \vec{V} = \Phi \quad \rho \frac{\text{DIV} \vec{V}}{\text{Dt}} = \text{DIV} \sigma + F
\]  

\( V \) is blood flow velocity, \( \Phi \) is the source term due to the contribution of peripheral vessel and trans-capillary diffusion, \( F \) is the load, \( \sigma \) is stress tensor, expressed as a function of the hydrodynamic blood pressure and of viscous stress tensor, as follows.

According to behavior low (we are considering blood as a newtonian fluid) stresses can be connected with deformations. Local equations are described in a system of cylindrical co-ordinates. We suppose that flow and tube deformation, with respect to the tube axis, are symmetrical. In order to evaluate the order of magnitude of the various terms of the equations, a assumptotic study was made considering parameter \( \varepsilon \), the ratio of the resting radius to the length of the tube, to be much smaller than the unit. The number of Reynolds is considered to be approximately one in order to maintain the influence of viscosity effects. A system of simplified equations is obtained (order 0 in \( \varepsilon \)). A onedimensional formulation is deduced from integration of this system on any cross-section of the tube (Gaffié and Guillaume 1991). We, finally, obtain:

\[
\frac{\partial S}{\partial t} + \frac{\partial (S.U)}{\partial z} = \frac{\partial}{\partial z} \left( \frac{\partial S}{\partial z} + \frac{\partial (S.U)}{\partial z} \right) - \Phi + \frac{\partial}{\partial z} \left( \frac{\partial S}{\partial z} + \frac{\partial (S.U)}{\partial z} \right) \times (U)
\]

U and S are the main variables of the problem respectively the mean blood velocity and tube cross-section. \( \tau_p \) is the mean parietal stress resulting from viscosity. Parameter \( \alpha \) corresponds to the integration of non-linear terms.

Determination of \( \tau_p \) and \( \alpha \) as a function of U and S is obtained using a law of homothetic profil for the longitudinal component of local blood velocity.

DESCRIPTION OF OUTSIDE FIELD OF DISTURBANCES

Fluid and wall motions are not independant; it is therefore necessary to define a additional relationship to express the coupling between the mechanical properties of the fluid and those of the wall (Kamm and Shapiro, 1979). This is all the more important as brain tissue deformations under load induce stress distribution (external pressure) along blood vessels.

\[
P - Pe = K(z).f\left(\frac{S(z,t)}{S_0(z)}\right)
\]  

P - Pe is the transmural pressure and \( K(z) \) is the stiffness coefficient of blood vessel.

When pressure is distributed in an elastic medium, it creates surface waves which propagate at a certain celerity \( C \). By analogy with problems of gas dynamics, we show that this celerity is dependent on transmural pressure (ie determined by transmural pressure):

\[
C^2 = \frac{S}{\rho} \frac{\partial (P - Pe)}{\partial S}
\]
We can observe (figure 1) that parietal wave celerity significantly decreases when the vessel is partially crushed. It is not excluded that, in this case, blood flow velocity can become higher than the wave celerity: transition from a subcritical regimen \((U < C)\) to a supercritical regimen \((U > C)\).

RESULTS AND CONCLUSION:

The first numerical results were obtained for a equivalent tube of constant initial cross-section and stiffness. Blood mass losses or supplies due to secondary vessels have not been taken into consideration.

A external pressure distribution depending an time and simulating changes in intracranial stresses applies to the side walls of the tube. The Fz component of the volume force is given as a linear ramp of 18 G/s.

In figure (2) the instantaneous field of velocities is represented by isovelocity curves. We observe backflow of blood when the field of disturbances is applied. This backflow could be the cause of increased intracerebral stresses. The mechanisms of blood flow limitation is associated with flow characteristics. In figure (3) the changes in the non dimensional \(M\) number, defined as the ratio of flow velocity over parietal waves celerity, are represented. This results have been obtained for conditions of disturbances corresponding to a load of 6 G reached at a rate of 6 G/s. The supercritical regimen which is reached after 2.45 s. is unstable. Return to the subcritical regimen takes place with a transition shock defined as a discontinuity of certain physical magnitudes, in this case the blood velocity regimen.

The last calculation presented (figure 4), is a preliminary result obtained from the generalized model.

A quasi-steady regimen has been observed in the capillary network which qualitatively corroborates physiological observations. The overall modeling of the system in parallelle (source term) will be more specific after a thorough analysis of the case where blood flow only results from the pumping action of the heart.

A sensitivity study of the various parameters in this problem and in particular the varying stiffness of equivalent tube and initial cross-section as a function of the longitudinal direction, will optimize the investigated system.

A model describing cerebral blood flow has been develop in order to validate the hypothesis of mechanical intracranial hypertension as an etiology of inflight LOCs observed in fighter pilots exposed to rapid onset rate accelerations. Numerical results show that a supercritical regimen can be reached under certain conditions of acceleration. Return to the subcritical regimen is associated with upstream blood sequestration which increases brain stresses.

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MUSCULAR ADAPTATIONS INDUCED BY
DOBUTAMINE AND THEIR INFLUENCE
ON G-TOLERANCE IN MINIATURE SWINE

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INTRODUCTION
The occurrence of acceleration (+G_y) induced loss of consciousness (G-LOC) in pilots flying high performance aircraft imposes serious limitations during training and operational activities. Although utilization of the anti-G suit and the anti-G straining maneuver are the more common means of operationally increasing +G_y tolerance, the straining maneuver have examined the usefulness of physical conditioning. While several studies have indicated that aerobic training did not result in improved tolerance (4,5), Epperson et al. (4) has demonstrated that an exercise conditioning program of weight training resulted in improvement in +G_y tolerance during simulated aerial combat maneuvers.

Dobutamine is an inotropic synthetic catecholamine which is used clinically in the treatment of congestive heart failure. This agonist improves cardiac output by augmenting stroke volume through enhanced left ventricular contractility. Chronic administration of dobutamine produces adaptive changes similar to those observed with exercise training (3.6,11,13). Most of the work relating to adaptive changes has emphasized hemodynamic improvements (3,11), but some investigators have reported changes in skeletal and cardiac muscle tissue (1,6,13) after treatment with dobutamine.

The purpose of this experiment was to determine if chronic administration of dobutamine could induce muscular adaptations in miniature swine, and since, whether these changes could influence the animals' +G_y tolerance. The effect of acute beta blockade with Brevicloc, a beta-1 antagonist, on the +G_y tolerance of control and treatment animals was also examined. Results pertaining to the acute and adaptive cardiovascular responses which occurred during this study are reported elsewhere in these proceedings.

METHODS
Sixteen male miniature swine (Hanford strain, Charles River Inc.), 6-7 months of age, were utilized in this study. During a 3 week quarantine and stabilization period, intravenous (IV) catheters were surgically placed in the animals' right jugular vein to allow for chronic administration of dobutamine. After sufficient recovery, animals were randomly assigned to either a saline control (SAL) group or a dobutamine treatment (DOB) group. All animals received IV infusions of SAL or DOB for 2 hours a day, 5 days per week for approximately 7 weeks. During the first week of the infusion period, treatment animals received 20 μg/kg/min of DOB to allow for a gradual acclimation to the tachycardia produced by DOB. A treatment dose of 40 μg/kg/min of DOB was administered during the 6 week adaptation period (weeks 2-7). An equivalent volume of SAL was infused into control animals over the same time periods. All infusions were stopped 48 hours prior to centrifuge testing.

After 6 weeks of treatment, animals were tested on the Dynamic Environmental Simulator (DESC) to determine +G_y tolerance. During the first series of centrifuge exposures (RUN 1), animals were subjected to 3, 5, 7 and 9 +G_y forces. The 3, 5, and 7 +G_y plateaus were 2 minutes in length, with a 10 minute rest period between each plateau. The +G_y run was continued until the animals lost consciousness. Investigators determining the point of loss of consciousness by closed circuit television were not aware of the animals' treatment group. Thirty minutes following RUN 1 the same acceleration profile was repeated with beta-blockade (RUN 2). Ten minutes prior to the second centrifuge exposure each animal received an IV priming dose of Brevicloc (esmolol HCl), delivered at a rate of 500 μg/kg/min for 1 minute. A maintenance dose (100 μg/kg/min) was continued throughout RUN 2.

Muscle biopsies from the gastrocnemius (GASTROC) were taken on the day of the centrifuge testing prior to the surgical instrumentation of the animals. Tissue samples from the sartorius (SARTOR) muscles were taken during surgery. Similar samples were removed from the soleus (SOL) and left ventricle (L VENT) at necropsy, which occurred approximately 48 hours after completion of the centrifuge tests.

Citrate synthase (CS) activity was determined by the spectrophotometric method of Srere (12). A slight modification of the method described by Cohen et al. (2) was used to measure catalase (CAT) and procedures outlined by Keele et al. (10) were used to determine superoxide dismutase (SOD) activity. Fiber typing was based on the histochemical determination of actomyosin ATPase (7).

RESULTS
Findings from this study demonstrate that chronic administration of dobutamine does induce adaptive changes in the cardiac and skeletal muscle of miniature swine. Results from the muscle enzyme analyses are summarized in Table 1.

<table>
<thead>
<tr>
<th>Table 1. MUSCLE ENZYME ACTIVITY (MEAN ± SE).</th>
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<tr>
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<td>---------------------------------------------</td>
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<tr>
<td>CITRATE SYNTHASE (umol/min/gram)</td>
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<td>SOLUS</td>
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<td>SUPEROXIDE DISMUTASE (μ/gram)</td>
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<td>SOLUS</td>
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<td>L VENT</td>
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*Significantly greater than corresponding SAL value (p<0.05).
With the exception of SOD in the GASTROC, DOB animals displayed significantly greater levels of enzyme activity (CS, CAT, SOD) for each of the tissues examined (GASTROC, SOL and L VENT). The % slow twitch muscle fibers in both the SARTOR and GASTROC were also significantly greater in the DOB group compared to the SAL group (Figure 1). Adaptive changes attributable to dobutamine infusion were also evidenced in treatment animals by a statistically significant increase in heart weight expressed both in absolute terms and as a ratio of heart weight to body weight (Figure 2).

When +Gz tolerance values (expressed as minutes before G-LOC during the +Gz exposure) were compared, there were no significant differences between DOB and SAL groups, or between RUN 1 and RUN 2. There was, however, a significant interaction between treatment groups and centrifuge runs. When time to G-LOC was expressed as difference between RUN 1 and RUN 2 (Figure 3), DOB was able to effectively attenuate the significant decrease in time to G-LOC seen in SAL animals during the second exposure which occurred with beta-1 adrenergic blockade (RUN 2). Due to the design of this experiment it was not possible to determine if the DOB was acting to attenuate the effect of repeated exposure to +Gz or to beta-blockade, but only that it helped with regard to a comparison of both.

**DISCUSSION**

Chronic dobutamine treatment resulted in significant increases in cardiac and skeletal muscle oxidative and antioxidant enzyme levels. These results support findings from previous reports that have examined adaptive and maintenance effects of chronic administration of DOB in rats (3,6), dogs (11), and humans (13). Significant cardiac hypertrophy and increases in % slow twitch fibers in skeletal muscle were also apparent in the DOB group. Although nonpathological hypertrophy has been reported in DOB treated animals (1), changes in fiber type has not. Findings reported here are all similar to results previously reported regarding muscle tissue adaptations induced by exercise (3,6,9).

Although the muscular adaptations induced by DOB did not result in an improved +Gz tolerance during the initial centrifuge run, it is possible that these adaptations did help to prevent the significant decrease in +Gz tolerance experienced by the SAL animals during the second +Gz exposure which included beta-1 adrenergic blockade. Further work is required to effectively differentiate DOB's ability to impact on the effects produced by repeated +Gz exposure versus those produced by beta-blockade.

**ACKNOWLEDGEMENTS:** We thank Drs. R.R. Tuttle and R.L. Hamlin for expert assistance in this study.

**REFERENCES**

RAPID ONSET RATE, G LOSS OF CONSCIOUSNESS (ROR G-LOC): MECHANICAL INTRACRANIAL HYPERTENSION. A NEW CONCEPT?

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INTRODUCTION
Fighter pilots exposed to +Gz accelerations during steep turns sometimes experience inflight loss of consciousness (G-LOC). During gradual load application, the centrifuge force draws the blood toward the lower limbs.

Blood depletion in the brain causes a grey-out followed by a black-out and eventually G-LOC if acceleration continues. This hypoxic etiology is commonly accepted. However, changes in the symptomaticology of G-LOC have been observed in more sophisticated new aircraft with high acceleration rates (high G.s\(^{-1}\)) : prodromic gray-and black-outs no longer occur and loss of consciousness is sudden, followed by lacunar amnesia. The theory of hypoxia no longer seems sufficient to account for the involved pathophysiological mechanisms. A biomechanical approach of this problem was therefore proposed : G-LOC might be caused by an excessively high increase in intracerebral stresses (4,5). The hypothetical mechanisms involved during high G.s\(^{-1}\) are described as mechanical intracranial hypertension (MIHT).

THE CONCEPT OF MIHT
The manner in which the load is applied seems to be determining in the occurrence of G-LOC. When acceleration rapidly occurs, the analysis of times characteristic of the problem shows that the time characteristic of the external disturbance can be considered as being of the same order of magnitude as the time associated with the mechanical response of the system, but is shorter than the time characteristic of physiological responses (baroreflexes). Blood mass inertia prevents immediate migration of brain blood toward the lower part of the body. In addition, blood migration caused by centrifuge forces could be impaired, as shown in Gaffié’s model (2).

The comparison with gradual onset rates for the same level of acceleration shows a relative increase in the total volume comprised within the skull. This volume increase enhances intracerebral stresses which are already high under load. LOC could then occur past a certain stress threshold. This concept of MIHT is a general concept which encompasses several hypothesis on cell perturbations :
- hypothesis of electrical effects : deformations induced by intracerebral stresses could be associated with a synchronous discharge of neurons similar to the epileptic process, or on the contrary, with soderation of all neuronal activity.
- hypothesis of mechanical effects : the increased pressure inside the skull could cause a herniation, but this totally reversible phenomenon would be highly transient.
- hypothesis of vascular effects : aside from the direct effect of load on blood flow, an increase in intracerebral stresses is suspected, resulting in vessel deformations.

New perturbations in blood flow and changes in flow rate distribution could result from these deformations, certain areas being over-abundantly irrigated while others are insufficiently perfused. In each one of these alternatives, impaired diffusion could reduce cell oxygen supply.

ANALYTIC TESTING OF HYPOTHESES
These hypotheses have been subjected to analytic testing, based on a decoupled behavioral study of viscoelastic matter (the brain), peripheral fluids (CSF), and blood flow through the system.

2.1 - CSF flow
Since the CSF is considered as a viscous and incompressible fluid a numerical model is used to determine the fluid velocity profile when it is redistributed around the brain (represented as a simple sphere), and to evaluate stresses applied to the brain surface by the field of velocities (3).

2.2 - Brain behavior
A model established according to a code with finite elements (6) is used to determine intracerebral stresses under load. When different onset rates are applied, significant stresses develop at the base of the brain, and in structures surrounding the cerebral trunk (3). These stresses increase with higher onset rates.

2.3 - Blood flow model
The basic model (2) is to represent blood flow through an arteriola modelled by a simple elastic tube with constant cross-section and stiffness called elastic equivalent. The resulting fluid/wall system is subject to the simultaneous action of :
- pulsed flow generated by the heart pump.
- the outer field of volume forces.
- surface forces resulting from increased stresses within brain tissue under the effect of acceleration (see 2.1).

This model shows that blood flow rate can be restricted, even with back flow, when a load is rapidly applied. This phenomenon could increase intracerebral stresses.

A more complex model is currently developed (Gaffié, cf infra), taking into account the concept of vascular network.

3- ARTERIAL PRESSURE AND CRITICAL MIHT THRESHOLD
Considering the non-reproducibility of G-LOC under indentical loads (or presumed to be so), we tend to believe that, if it exists, the critical threshold is reached all the more rapidly as a large blood volume is trapped inside the skull. This is so when arterial pressure reaches its peak.

3.1 - Time course of cardiac revolution and moment when load is applied
Under conditions of gradual onset rate (0.5 G.s\(^{-1}\)) the heart of a fighter pilot has time for 12 cardiac cycles at 120 bpm before is reached an acceleration of +6 Gz, for example, but it has only time for a single cycle at the same heart rate if the onset rate is 12 G.s\(^{-1}\).

The effects of these two loading regimes on the cardio-vascular system could be very different.
first case (GOR) blood migration toward the lower part of the body reduces ventricular preload, associated with a decrease in arterial pressure and the action of regulatory mechanisms. In the second case (ROR), acceleration applies over a single cardiac cycle; the ventricular preload is a direct function of the instantaneous level of acceleration. Concomitance of the first systolic wave with ROR acceleration could, perhaps, correspond to the critical MIHT threshold.

Two contradictory hypothesis may be considered:

a) The beginning of a systole coincides with the beginning of acceleration.

The effects of acceleration are first felt at the beginning of relaxation. Under 12 G.S⁻¹ the plateau (at 6G) is reached at the beginning of the following systole. Preload is maximum during the isovolumic systolic phase since it is applied to an end-diastolic volume whose mass, accelerated several times, acts on the ventricular motor pressure. Postload is, apparently, also very high during the isotonic systolic phase since the normally favorable effect created by blood pooling in the venous compliant system does not occur. Peripheral arterial resistances are increased sixfold, due to the rapid application of the field of forces.

b) The beginning of a diastole coincides with the beginning of acceleration.

Phenomena are not synchronized. The increasing load during the isotonic phase has an unfavorable but limited effect since the peak mechanical load is reached at the end of the relaxation period when the "solid displacement" effects of intravascular fluid appear.

3.2- To complete this chronological approach of the "cardiac cycle - accelerations" relationship, a model had to be developed to take into account mechanical stresses in the left ventricle (1). In the left heart this model shows a decrease in intraventricular pressure at the beginning of diastole and an increase in end-diastolic volume. In other words, ROR + Gz accelerations seem to improve the diastolic suction pump effect of the heart. Thus, when no physiological regulation applies, centrifuge forces resulting from ROR load seem to enhance blood inflow during diastole depending on the moment when they apply and could therefore largely increase the systolic stroke volume, and so arterial pressure during the following ventricular contraction.

The hypothesis of a chronological effect on the systolic pressure when both events are concomitant may be complemented. Four effects occur (figure 1), three are directly related to the acceleration onset rate. Two of these three effects are primary: chronological and volumetric effects, and the third, i.e. the effect of Starling (heterometric adjustment of the myocardium) is secondary and due to the increase in systolic stroke volume.

The fourth effect is autonomous, it affects the neuro-hormonal adjustment of the cardiac function (heterometric adjustment) and the adjustment of vascular motricity.

CONCLUSION

A mechanical approach brought forth a new concept of mechanical intracranial hypertension to try and explain changes the symptomatology of inflight ROR G-LOC. This concept is based on the principle of a sudden increase in intracerebral stresses. Mathematical models were developed to analyse the behaviour of the various structures:
- pressure distribution in the CSF,
- distribution of stresses and deformations in the brain,
- changes in pulsed blood flow inside the confining skull.

The first results show that ROR + Gz could increase mechanical stresses within nerve tissue. The intracranial pressure (threshold) which can coincide with loss of consciousness is discussed with respect to the instantaneous arterial pressure and cardio-vascular parameters.

Coupling of these models should provide optimal results to analyze interactions between hypothetical phenomena described here, and to improve experimental protocols, as experimental verification is an indispensable phase.

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REFERENCES:

ENTRAINMENT OF CIRCADIAN RHYTHMS IN THE RAT BY DAILY ONE HOUR G PULSES

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INTRODUCTION

Previous studies have demonstrated that the circadian system is sensitive to the effects of chronic exposure to hyperdynamic fields via centrifugation. For example, there are significant changes in the circadian amplitude of rodents and non-human primates following changes in the level of the gravitational field (1,2). In addition, the circadian rhythms of body temperature and activity may exhibit period changes following exposure to hyperdynamic fields (4).

The change in endogenous period of the circadian system as a function of gravity suggests that daily alterations in G fields may be capable of synchronizing or entraining the circadian system. Therefore, this study examined the effect of daily one hour G pulses via centrifugation on circadian rhythms of body temperature in rats.

METHODS

Three principal criteria must be met in order to demonstrate circadian entrainment (3). First, the period of the external synchronizing agent must equal the period of circadian rhythm of the organism in the absence of any other potential environmental time cues. Second, a stable and reproducible relationship must exist between the phase of the environmental synchronizer and the phase of the circadian rhythm of the organism. Third, the environmental time cues must exert phase control over the entrained rhythm. For example, when the time cue is removed the rhythm must begin to free-run from the point determined by the synchronizing agent and not the phase that would have been predicted by the prior free-run of the organism.

In this experiment, the following protocol was used to examine each of these principal criteria. Six male Wistar rats were anesthetized and implanted with a biotelemetry transmitter (Mini-Mitter) in the peritoneal cavity. The transmitter allowed the collection of body temperature every 10 minutes via a receiver. The data was collected and stored on a microcomputer data system (DataQuest). The animals were individually housed and visually isolated within modules on an 18 ft diameter centrifuge. The animals were allowed to free-run and express their endogenous circadian period in constant light (LL; 30 lux) and ambient temperature (Tañ; 26°C) at 1G for 19 days. Following a stable free-run baseline period, the animals were exposed to a daily 1 hour G pulse from 1 to 2G. The centrifuge was turned on from 2-3 PM local time each day. After 28 days of daily centrifugation, the animals were returned to the constant conditions of baseline.

After the experiments were completed, actograms of the data allowed visual inspection of the circadian patterns through each of the three phases of the experiment. In addition, cosinor analysis was used to quantify the daily phase of the body temperature rhythm.

RESULTS

All six animals exhibited the same basic pattern of results. Each animal exhibited an initial free-run with a circadian period greater than 24 hours. Following the initiation of daily one hour 2 G pulses, all animals exhibited a 24 hour rhythm within two to three days. At the end of the daily G pulse period, the animals were again allowed to free-run. In every case the animals re-established a free-running period in excess of 24 hours. Furthermore, the results of this preliminary experiment indicate that the phase control was established by the G pulse time cycle rather than being masked by it.

The data plotted in Figure 1 is an actogram of the daily body temperature rhythm phase from a single animal. The phases are the calculated times of the
maximum of the rhythm as determined by cosinor analysis. The time of centrifugation is indicated by the shaded area on days 11-39. A dashed line calculated from the 1G Pre phase data is projected through the experiment. The line reveals the predicted time of the onset phase of the body temperature rhythm had the G cycle been masking the body temperature rhythm than modifying the circadian clock. This would have been the time that the phase free-run would have ensued if the temperature rhythm had not been entrained by the daily G pulse.

DISCUSSION

The results of this study suggest that the temperature rhythm can entrain to the daily cycle of centrifugation. First the 24 hour period of 2 G pulse exposure resulted in a 24 hour period in the circadian rhythm of body temperature. Second, the phase of the 2 G pulse period produced a relatively stable phase relationship with the circadian rhythm of body temperature in all the animals. Third, when the animals were released from the 2 G cycle into the constant 1 G conditions, the temperature rhythms free-run from the point of entrainment, rather than the predicted phase had the rhythms been masked.

Synchronization of the circadian system of mammals to environmental time cues other than a light-dark cycle is not generally observed (3). Reports of other environmental time cues (such as food cycles, pressure cycles, or temperature cycles) indicate these are weaker than the light-dark cycle, as well as species specific. Thus, the apparent ability of alterations in gravity to produce circadian synchronization is both interesting and somewhat unexpected.

Although further studies will be required to validate and understand this phenomenon, several issues must be resolved. First, what is the sensitivity and the threshold of gravity's effects on circadian rhythms? Second, can this be a useful tool to help adapt individuals to shifts in time either on the ground, or, possibly more importantly, in space?

REFERENCES


ACKNOWLEDGEMENTS

This study has been supported by NASA Grant NAG2-2195.
HEART RATE ULTRADIAN RHYTHM IN PILOTS
SUBJECTED TO HYPERGRAVITATIONAL STRESS

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INTRODUCTION.
As far as +Gz acceleration tolerance in humans is concerned the cardiovascular systems plays a major role. The circulatory system control mechanisms are activated in response to gravitational stimulus. They act to provide the adequate blood supply to vital organs of the body and first off all to central nervous system. Acceleration applied in +Gz axis results in blood volume shift to lower body, the decrease in cardiac output and lowering of aortic blood pressure (4). The heart rate (HR) increase is a compensatory reaction for maintaining of the cardiac output at the proper level. In the first phase of hypergravitation stress the HR increase is due to the reflex induced by decompression of sinus and aortic baroreceptors (4).

The knowledge of cardiovascular system regulatory mechanism activated in hypergravitation stress is necessary for planning of prophylactic measures for improving flying personnel acceleration tolerance and prognostic purposes. To study the hemodynamics in normal and pathological conditions the heart rate spectrum analysis is commonly used (2,6).

In recent paper this method was used for evaluation of cardiovascular system response during centrifuge acceleration tolerance test.

METHODS.
The study was performed on 5 subjects, military jet plane pilots aged 23-30 with normal body weight. The were subjected to routine acceleration (+Gz) tolerance test on centrifuge in Polish Air Force Institute of Aviation Medicine in Warsaw. The acceleration onset rate was 0.1 G/s and the test was performed up to the acceleration threshold value when the subject reported the gray-out effect. The ECG was continuously monitored for 30 minutes prior and 20 after the centrifuge test, using MR-14 Medilog cassette recorder. The tapes were then replayed on Medilog Analyzer (Oxford Medical System) and digitized by microcomputer controlled data acquisition system. The beat-to-beat HR was calculated by detection of R-R intervals and then instantaneous HR was converted to equally spaced samples by linear interpolation. From this time series the trend was removed by degree polynomial approximation optimal in MSE sense. Then HR power spectrum was estimated with FFT algorithm.

Figure 1. (Top) The HR time trend recorded during the centrifuge test in 23 years old pilot covering rest and restitution period. (Bottom) The HR power density spectra corresponding to consecutive segments of HR time series as denoted above. The supine position HR baseline spectrum shown in inset A.

RESULTS:
In all subjects tested the onset of +Gz acceleration was accompanied by HR increase. The beat-to-beat HR values were in the range 157-197 bpm (beats per minute), with maximal +Gz from 6.9-7.7 G. The typical time trend of heart rate is shown on Fig.1. and Fig.2. upper. The signal was partitioned into segments with the length equal to duration 90 sec. The power spectrum was estimated for consecutive segment and shown on Fig.1.

The baseline spectrum was estimated for bed rest condition and is shown on the inset A in Fig.1. three basic components are observed for the rest condition namely: low frequency (LF) component, (0.01-0.05 Hz), mid-frequency (MF) (0.05-0.15 Hz) and high-frequency (HF) one in the band (0.25-0.35 Hz).

The power spectrum of HR fluctuations was not affected significantly by the application of acceleration stimulus below the frequency of 0.15 Hz. The return to the baseline pattern was observed in the 5 th minute after the acceleration stimulus termination (9th and 10th segment). The segments corresponding to periods immediately before the acceleration onset (subject expecting the test to start) and after the test are characterized by power increase in the MF band.

To study in more details the changes of HR spectrum in the phase of acceleration onset the corresponding segment was divided into two epochs (denoted B1 and B2) as shown in the upper part of Fig.2. In most of the cases studied in the early phase of acceleration the periodic components of 0.9 Hz frequency appeared which is not present in any other stage of the experiment as shown in the set of frequency spectra in the lower part of Fig.2. (B2). In the B2 no cyclic components were found segment.
DISCUSSION.

Analyzing the heart rate fluctuation in rest condition the authors had found three main frequency components similar to those obtained by other authors (1,2,3,6,7,8) as far as the frequency bands are concerned. It is generally accepted that LF frequency HR oscillations are related to thermoregulatory fluctuations of vaso-motor tone and regulatory action of renin - angiotensin control system. Oscillations in MF band result from blood pressure variability and HF component corresponds to respiratory frequency. It was also shown that HR spectral components are affected by sympathetic and parasympathetic nerves activity. The spectrum modulated in MF and HF band is mediated by parasympathetic system. Pharmacologic blockade of this system results in lowering of the corresponding spectral peaks (2,3). The simultaneous blockage of the whole autonomic nervous system is blocking the HR oscillations in all three frequency bands (2,8).

Parasympathetic and sympathetic nerves activity is associated with the various factors like mental and physical effort (task), emotional stress or acceleration. Sekiguchi et al. (7) had shown that in spite of HR increase, the power spectrum density for centrifuge test (2-4 G) revealed that the small frequency component at 0.1 Hz was considered to be the same as in the rest condition. It was suggested that HR increase is due to the changes in arterial blood and intra-thoracic pressures.

This fact was confirmed by the authors as shown in Fig.2-Bl whenever the flattening of the spectrum was observed in the centrifuge test phase with maximal +Gz values close to gray-out effect.

REFERENCE.


EFFECTS OF ACCELERATION STRESS ON THE SECRETION OF
ATRIAL NATRIURETIC PEPTIDE (ANP) IN RATS

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INTRODUCTION
Recently, the heart has been considered as an
endocrine gland involved in the regulation of body
fluid volume and blood pressure. Atrial myocytes
contain a family of biologically active peptides
stored in specific atrial granules. These
peptides, termed atrial natriuretic peptides
(ANPs), have potent natriuretic, diuretic, and
vascular smooth muscle relaxant activities(1). The
increase of atrial volume change and atrial
contraction frequency have been considered as
principal stimuli for ANP secretion(2-4). It is
well known that an exposure to acceleration
stress, in man, is accompanied by severe reduction
of venous return to heart, reflex increase of
heart rate and some endocrine responses, such as,
increased secretion of renin and corticosteroids(5,6). But, in small animals, these
responses to acceleration stress showed
 differences in part to those of man. And the
response of ANP secretion by acceleration stress
has not been reported yet. So we examined the
effects of acceleration stress on the plasma ANP,
atrial ANP, plasma renin concentration (PRC), and
heart rate in rats.

METHODS
Male Wistar rats weighing about 200g were
used. Four groups were exposed to +Gz
acceleration. Three groups were exposed to +Gz,
one time per day for 1 day (Day 1), 3 days (Day 3),
7 days (Day 7), respectively. Animals were
exposed to gravitational forces up to +Gz. An
onset rate was 0.35G/sec. Peak G was maintained
for 60s followed by deceleration to control. One
group of rats was used to obtain heart rate
responses via sternal and leads at Day 1 and Day
3. All animals were unanesthetized. Rats were
decapitated immediately after last exposure.
Plasma and atrial ANP and PRC were measured by the
method described previously(7).

RESULTS
When acceleration started, rats began to
perforee straining maneuver. Heart rate began to
decrease from onset of acceleration and
maintained significantly low level after reaching
to peak +Gz. Heart rate changes response to first
and third exposure to +Gz were not different. So
data of those were mixed (Fig. 1). Plasma ANP
levels significantly decreased on the 3rd day
after exposure to acceleration stress. ANP content
in both right and left atria significantly
increased on the 3rd day. But, the responses of
plasma and atrial ANP were not further accentuated
on Day 7(Fig. 2). Plasma renin concentration did
not change by acceleration stress(Fig 3).

Figure 1. Heart rate responses to +Gz acceleration
stress( n=6 ). HR, heart rate. Values are
means±SE. *P<0.05 vs. Control period before
acceleration stress.

Figure 2. Effects of acceleration stress on the
ANP levels in plasma and atrium. Open bars
indicate the values of right atria and hatched
bars indicate the values of left atria. Numbers in
parenthesis are the number of experimental
animals. *P<0.05 vs Day 0.

Figure 3. Effect of acceleration stress on the
plasma renin concentration( PRC ).
DISCUSSION

The present study demonstrates that the +Gz acceleration stress in rats causes a decrease in plasma ANP level and an increase in atrial ANP content.

Among the suggested factors regulating the secretion of ANP, atrial distension and its contraction frequency have been suggested as an important stimuli(2,4). We think that both a decrease in atrial volume due to peripheral pooling by +Gz force and a slight decrease in heart rate result in a decrease in plasma ANP level. Acceleration stress increases blood levels of glucocorticoids (6). Recently Peter et al. reported that glucocorticoid increases the secretion and synthesis of ANP, and content of ANP mRNA in atria(8). In this study, the increased atrial content of ANP may be caused by a reduced secretion from the atria and/or an increased synthesis of ANP by augmented corticosteroid level after acceleration.

Heart rate responses to acceleration stress were variable to animal species. In conscious man(9), heart rate usually increases and blood pressure falls after onset of acceleration stress. In dogs(10,11), some animals show an increase, while others show a decrease in heart rate. In rats(12), +Gz or +Gx acceleration resulted in a reduction in heart rate. The heart slowing observed during +Gz stress has been previously reported (10-12). This probably represented a reflex baroreceptor response to increasing systemic arterial pressure during performance of maximum straining maneuver. These suggest that reflex compensatory mechanisms during acceleration stress are more effective in smaller animals, such as rats, than larger animals. But the exact nature of these species differences are unclear.

In this study, plasma renin concentration was not altered by +Gz stress. Stone and Alexander(11) reported that tissue blood flow to the renal cortex was found to remain within normal limits up to +Gx in the supine and 10° head-up position. Although the extent of acceleration stress and position of animals are different from their study, the renal blood flow of rats may not be changed to the extent to increase renin secretion in this study.

In summary, +Gz acceleration stress in rats causes a decrease in plasma ANP level and an increase in atrial ANP content, and this may be due to decrease in both heart rate and cardiac volume. But mechanisms for decrease in heart rate and no alteration in PRC by acceleration stress in rats are not clear in this study.

ACKNOWLEDGEMENTS

This research was supported by a research grant from the medical department of Republic of Korea Army.

REFERENCES

LACTACIDEMIA IN NON HUMAN PRIMATES EXPOSED TO REPEATED HIGH SUSTAINED +Gz ACCELERATION

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INTRODUCTION

Baboon lactacidemia prior and after centrifugation was one of several parameters measured in a more general investigation of the effect of repeated high, i.e. 8-9 Gz sustained (more than 15 seconds), accelerations on the cardiovascular system, and of potential pathology. Lactacidemia was measured as control of muscular work and index of anaerobic metabolism.

METHODOLOGY

Protocols for acceleration exposure, training and blood sampling in kinetic analyses were described in detail in the article "Changes in renin-angiotensin response in non human primates exposed to repeated high sustained accelerations". More specifically, the determination of lactacidemia is done:

1. on venous blood sampled immediately prior to and after centrifugation, or at corresponding hours during post-training recovery.

2. In 5 baboons of the experimental batch, (where PRA and catecholamine concentration were observed) with the following schedule: lactacidemia was measured at the end of the adaptation period (+Gz), at the 2nd, 6th, 16th, 24th, 32nd and 40th exposures at 8Gz, and also 3 weeks after training during the recovery period.

In 2 baboons regularly exposed to + 8Gz accelerations, which training had to be interrupted after 29 exposures because of poor tolerance. The same sampling schedule was applied.

RESULTS

Results obtained on the 5 baboons were used for a statistical analysis with two factors ANOVA (exposure and repeat effects):

- the difference between prior to and after centrifugation means was significant with p < 2^-10 (p = 0.0014)
- the difference between means of various periods was significant with p < 5^-10 (p = 0.0021).

Table I shows mean results for each animal of both groups during acceleration exposure - mean of the values of 1st sample, 2nd sample and their difference. Figure 1 shows the graph of mean lactic acid concentrations prior to and after centrifugation, that is N^1 and N^2.

<p>| TABLE I |
| Lactacidemia prior to (N^1) and after (N^2) centrifugation in animals exposed to repeated HS+Gz |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Animal</th>
<th>N^1 mmol/l</th>
<th>SD</th>
<th>N^2 mmol/l</th>
<th>SD</th>
<th>Δ N^2-N^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.57</td>
<td>±0.24</td>
<td>4.73</td>
<td>±0.52</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.62</td>
<td>±0.31</td>
<td>5.7</td>
<td>±0.56</td>
<td>4.08</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.85</td>
<td>±0.07</td>
<td>4.76</td>
<td>±1</td>
<td>2.91</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2.05</td>
<td>±0.39</td>
<td>5.51</td>
<td>±0.64</td>
<td>3.46</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>±0.22</td>
<td>4.79</td>
<td>±0.74</td>
<td>2.79</td>
<td></td>
</tr>
<tr>
<td>m ± SD</td>
<td>1.82</td>
<td>±0.09</td>
<td>5.1</td>
<td>±0.21</td>
<td>3.28±0.23</td>
<td></td>
</tr>
</tbody>
</table>

During training the mean difference for the 5 animals A, B, C, D, E, between samples N^2 and N^1 is 3.28 ± 0.23 mmmol/l. It is physiologically significant (higher than 1.5 mmmol/l). For animals I and J this difference is only 0.89 ± 0.17 mmmol/l.

Table II shows mean results for each animal during post-training recovery period, and figure 2 shows the graph of mean lactic acid concentrations (N^1 and N^2) during recovery. During this period, the mean difference between sample N^2 and N^1 is 0.26 ± 0.09 mmmol/l for the group of 5 animals, and 0.13 ± 0.09 mmmol/l for the group of 2 animals.

<p>| TABLE II |
| Lactacidemia in resting subjects |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Animal</th>
<th>N^1 mmol/l</th>
<th>N^2 mmol/l</th>
<th>Δ N^2-N^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.93</td>
<td>1.41</td>
<td>-0.52</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.2</td>
<td>1.53</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.32</td>
<td>1.65</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.89</td>
<td>0.82</td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1.13</td>
<td>1.2</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>m ± SD</td>
<td>1.29±0.17</td>
<td>1.32±0.15</td>
<td>0.026±0.09</td>
<td></td>
</tr>
</tbody>
</table>

Sampling hours are the same as during +Gz exposure.
CONCLUSION

It is interesting to observe that the 5 animals with greatly enhanced lactacidemia under centrifugation had good tolerance to acceleration whereas the two baboons with no increase lactacidemia had poor tolerance to acceleration training. These results which will have to be confirmed on a larger number of animals corroborate results observed on humans (1, 2, 3).

REFERENCES


CHANGES IN THE RENIN-ANGIOTENSIN RESPONSE OF NON-HUMAN PRIMATES EXPOSED TO REPEATED HIGH SUSTAINED +GZ ACCELERATION.

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INTRODUCTION

The plasmatic-renin-activity (PRA) changes in response to repeated high-sustained + Gz accelerations, that is accelerations lasting more than 15 seconds, and of an intensity at least +8 or +9 Gz, reached with a jolt of 1 Gz per second, were studied in the baboon. The PRA is taken as the parameter of system renin-angiotensin activity (SRA).

This investigation was part of a more general study of the potential development of cardiovascular physio-pathology resulting from repeated HS + Gz. During the same experience, the plasmatic catecholamines changes were studied and biochemical parameters such as lactacidemia, blood glucose measured. The animal experimentation was conducted in accordance with recommendations from the declaration of Helsinki on animal use and care.

METHODOLOGY

The schema of the figure 1 shows the profile of one exposure to accelerations. This profile was developed by Borredon (1). One exposure included three plateaus of +9 Gz, lasting 30 or 40 seconds each. Each plateau was reached with a jolt of 1 Gz/s. A recovery period of two or three minutes took place between every two plateaus.

The experiment was made on five male baboons Papio Papio (weighing 10 kg at the beginning of the experiment). They were not wearing anti-G suits. During the experiment, animals were awake and placed in restraining chairs. Each animal was used as its own control.

The experimental protocol included three periods:

- adaptation period during which animals were exposed to an acceleration of +1 Gz on the centrifuge during 40 seconds, and this for three times
- training period during which animals were exposed to +8 Gz acceleration, twice a week for twenty two weeks, according to the previously described profile
- post-training recovery period when hemodynamic parameters were measured twice a week for 8 weeks, after the end of training.

The plasma renin activity (PRA) was evaluated in a 5 points kinetic analysis. Five samples were taken as follows at the same hour of the day, for the three different periods. 
- first sample (S1) : the animal was awake, sitting upright in its restraining chair for fifteen minutes. This sample was used for the determination of baboon resting PRA.
- second sample (S2) was carried out immediately prior to centrifugation when it took place, or at the corresponding time when the animal remained in the laboratory
- third sample (S3) was realised immediately after centrifugation (adaptation and training), in fact 2 minutes after the end of the third plateau.
- fourth sample (S4) was carried out thirty (30) minutes after the end of centrifuge exposure.
- fifth sample (S5) was realised two hours after centrifuge

Blood samples were collected from the caudal vein or from the tibial artery. Measurement were made using radioimmunology : kit SB-REN-2 (ORIS ).

Seven kinetics were made according to this sampling schedule, distributed as follow :
- the first was made at the end of the adaptation period.
- the five subsequent kinetics were regularly distributed over the training period. They were made at 8th, 16th, 24th, 32nd and 40th exposures.
- the seventh kinetic was made 3 weeks after training, during the recovery period.

RESULTS

Results of the kinetics are presented on table I. On the graph of the figure 2 only 3 kinetics are represented to make think clear.

The mean PRA value at rest, increased from 7.9 ±1.5 ng/ml/h during adaptation period to 14 ± 2.6 ng/ml/h at 32 exposures (+8 Gz) and to 16.9 ± 1 ng/ml/h at 40 exposures (+8 Gz). Half an hour after the centrifugation, (sample S4) the mean PRA value was 11.9 ± 1.8 ng/ml/h for +1Gz adaptation period, it increased to 18.2 ±1ng/ml/h at 32 exposures (+8 Gz) and to 18.5 ± 0.7 ng/ml/h at 40 exposures (+8 Gz).
TABLE I

<table>
<thead>
<tr>
<th>PRA ng/ml/h</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
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<tr>
<td>Periods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Adaptation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>K1</td>
<td>7.88 ±1.47</td>
<td>8.56 ±0.95</td>
<td>9.14 ±1.29</td>
<td>11.96 ±1.83</td>
<td>12.66 ±1.65</td>
</tr>
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</tr>
<tr>
<td>Training</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 exposures (K2)</td>
<td>6.54 ±1.65</td>
<td>9.34 ±2.11</td>
<td>10.88 ±1.66</td>
<td>12.39 ±2.17</td>
<td>12.48 ±2.65</td>
</tr>
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<td>n=5</td>
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<tr>
<td>16 exposures (K3)</td>
<td>12.68 ±1.68</td>
<td>14.32 ±2.23</td>
<td>12.46 ±2.37</td>
<td>15.12 ±1.67</td>
<td>15.95 ±1.70</td>
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<tr>
<td>24 exposures (K4)</td>
<td>11.36 ±3.18</td>
<td>15.72 ±3.31</td>
<td>15.84 ±3.17</td>
<td>17.78 ±2.55</td>
<td>17.10 ±2.69</td>
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<td>n=5</td>
</tr>
<tr>
<td>32 exposures (K5)</td>
<td>14.04 ±2.55</td>
<td>16.14 ±2.18</td>
<td>15.77 ±2.24</td>
<td>18.16 ±0.99</td>
<td>17.14 ±1.52</td>
</tr>
<tr>
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<td>n=5</td>
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<tr>
<td>40 exposures (K6)</td>
<td>16.93 ±0.97</td>
<td>17.80 ±0.86</td>
<td>17.77 ±0.76</td>
<td>18.52 ±0.65</td>
<td>18.73 ±0.32</td>
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<tr>
<td>Post training</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>K7</td>
<td>8.20 ±2.78</td>
<td>18.3 ±2.70</td>
<td>16.76 ±3.52</td>
<td>18.74 ±3.31</td>
<td>18.54 ±3.22</td>
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</tr>
</tbody>
</table>

S = sample
n = number of value; mean ± SE (standard error)

The centrifugation at 1 Gz (adaptation period) or 8 Gz (training period) takes place between S2 and S3.

The statistical analysis ran on 5 complete kinetics, i.e. all points were calculated with 5 values. The five kinetics are those made during adaptation period, post-recovery period and 8 th, 24 th and 32 nd exposures. We used ANOVA with nested factors.

Mean values of the five considered kinetics were significantly different with p < 2%. If the 3 kinetics of the training period are considered, their mean values are significantly different with p<1%, which means that repeated exposures to + Gz accelerations enhances significantly PRA activity and these increase is related to the number of exposures.

The mean value of samples, taken as a whole, is significantly different with p < 1%. If the samples of adaptation period are considered, they are different with p < 5% (p=0.037) : the two by two comparison shows that S4 and S5 are significantly different of S1. The mean values of samples of training period are not significantly different. The mean value of post training recovery period samples are significantly different with p <1% (p=0.005): the two by two comparison shows that S2, S3, S4 and S5 are significantly different of S1.

These results shows:
1) that +Gz repeated exposure induced a general increase of PRA
2) the first sample which is the rest value is the one which increase last ; it is also the first one to diminish during the recovery period.

DISCUSSION

The PRA secretion could be induced by the enhance sympathetic activity which stimulate 81 adrenergic receptors in the juxtaglomerular cells. It could be induced by the drop of the arterial pressure. It is admitted that, when the arterial pressure falls, stimulation of SRA interferes less rapidly than the bulbar baroreflex (nearly a two minutes latency and All effect on arterioles is maxima in 15 or 20 minutes). But the repetition of exposure could stimulate this system.

On the other hand, the interferences between the sympathetic system and All (particularly in noradrenergic transmission (3)) are probably very important and could act together to develop an +Gz adaptative response*.

Moreover, the renin is more and more widely considered as a stress hormone (2).

These causes could all act concomitantly.

CONCLUSION

Measurements of PRA were made on non human primates which were regularly exposed to +Gz acceleration. These repeated exposures enhanced all values of PRA. This increase induces certainly a rise of plasmatic All and consequently an arteriolar vasoconstriction as well as interference with the sympathetic system.

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HEMODYNAMIC EFFECTS OF DOBUTAMINE AND THEIR INFLUENCE ON G-TOLERANCE IN PIGS

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INTRODUCTION

Acceleration induced loss of consciousness (G-LOC) is a serious problem encountered by pilots flying high performance aircraft. The physiologic basis for G-LOC is the inability of the cardiovascular system to maintain blood supply to the brain in the presence of rapid sustained acceleration (Wood, 1987). Anti-G protective procedures currently in use are directed primarily toward increasing venous return to the heart. A physical conditioning program involving weight training has also been shown to enhance G-tolerance (Epperson, 1982).

Dobutamine is a synthetic catecholamine with beta-1 agonist activity. It is employed clinically in the treatment of patients with congestive heart failure. Dogs administered dobutamine for a period of 5 weeks displayed changes in cardiovascular function similar to those seen following a program of physical conditioning (Liang, 1979). Improvements in cardiac function, seen in human patients subsequent to dobutamine administration, have been shown to persist for several weeks following withdrawal of the drug (Liang, 1984) (Unverferth, 1980) (Leler, 1982).

The primary objective of this study was to determine if the chronic administration of dobutamine to the pig would increase the animal's G-tolerance. A secondary objective was to determine the effect of a beta-1 antagonist (Brevilbloc) on G-tolerance in animals which had previously received dobutamine.

MATERIALS AND METHODS

Twenty miniature swine (Hanford strain), 6-7 months of age, were purchased from Charles Rivers Inc., Wilmington, Massachusetts. Upon arrival, the animals underwent a 3 week quarantine and stabilization period during which time they were adapted to restraint in a Panepinto sling. Also during this period, intravenous catheters were surgically placed into each animal's right external jugular vein. The catheters were exteriorized in the midscapular region of the animal's back, thereby providing the central venous access needed for chronic dobutamine administration.

Following the stabilization period, pigs were randomly assigned to either treatment or control groups consisting of 10 animals each. Animals in the treatment group received an initial dose of dobutamine (20 mcg/kg/minute), 2 hours per day for 3-5 days. This dose was then increased to 40 mcg/kg/minute, 2 hours per day, 5 days per week for the remainder of the 7 week treatment period. Control animals received saline (1 ml/minute) in a similar treatment regimen. Blood pressure and heart rate data were recorded for each animal prior to and during the 2 hour drug infusion period using a Dinamap 8100 vital signs monitor. Dobutamine infusion was stopped 48 hours prior to centrifuge testing.

Immediately prior to centrifuge testing, the animals were anesthetized for placement of additional vascular catheters. The femoral vein was used to provide vascular access for a Swan-Ganz catheter which was then advanced through the pig's right heart and into the pulmonary artery. A Millar catheter was inserted into the animal's femoral artery and positioned in the ascending aorta. Following surgery, the pigs were allowed to recover from anesthesia while resting in a canvas sling mounted on the animal platform of the laboratory's human centrifuge.

The centrifuge paradigm consisted of subjecting the pigs to gravitational forces of 3, 5, 7, and 9 Gz (Run 1) followed by a 30 minute rest period. This acceleration paradigm was then repeated (Run 2). The animals remained at each of the G plateaus for a period of 2 minutes with a 10 minute rest period between plateaus. The 9 Gz run was continued until it was determined that the pig had lost consciousness, which was assessed by observing the animal via closed circuit television.

Resting blood pressure and cardiac output recordings were made immediately prior to each of the two centrifuge runs. Ten minutes preceding Run 2, each pig received a priming dose of Brevilbloc (esmolol HCL), delivered intravenously at the rate of 500 mcg/kg/min for 1 minute. A maintenance dose of Brevilbloc (100 mcg/kg/min) was then continued for the period prior to and during a second 3, 5, 7, and 9 Gz acceleration.

Following centrifuge testing, pigs were returned to the vivarium and anesthetized for removal of the Swan-Ganz and Millar catheters. The pigs were allowed to recover from anesthesia and recuperate for a period of 48 hours. At this time the animals were euthanized and a complete necropsy examination was performed. Tissue samples were collected for histology and oxidative enzyme determinations.

RESULTS

The results of this study indicate the presence of a sustained significant (P<.001) increase in heart rate during the period of dobutamine infusion (Graph 1). There was also a progressive and significant (P<.001) decrease in mean blood pressure in dobutamine treated animals over the 7 week period of drug infusion (Graph 2). Heart rate and blood pressure recordings taken while the animals were at rest were not statistically different. There was, however, a significant (P<.007) increase in resting cardiac output in those animals which had received the 7 weeks of dobutamine treatment.

None of the pigs experienced G-LOC during the 3, 5, or 7 Gz accelerations. Evaluation of the time to G-LOC data for the 9 Gz acceleration, performed using 2-way analysis of variance,
demonstrated a significant (P=.031) probability of treatment vs. run interaction. Further statistical analysis revealed no significant differences in time to G-LOC between the control and dobutamine treated animals either during Run 1 or Run 2. This data did, however, indicate a significant (P=.038) decrease in G-LOC times for saline treated control animals following Brevibloc administration. Times to G-LOC before and after Brevibloc administration for the dobutamine treated animals remained relatively constant (Graph 3).

In addition to the above objective recordings, the pigs were subjectively evaluated for appetite and general behavior during the 48 hour post centrifuge period. The control animals appeared noticeably fatigued following the second centrifuge run and required 24 to 36 hours to fully recover from the effects of acceleration testing. Those animals who had received dobutamine appeared more resistant to the effects of acceleration, and resumed normal feeding and activity patterns within 12 hours following removal from the centrifuge.

CONCLUSIONS
The results of this study indicate that the chronic administration of dobutamine does not increase the time to G-LOC in the pig. This finding may be related to the fact that no detectable decreases in resting heart rate or blood pressure were noted following chronic dobutamine administration. Lack of changes in these parameters would suggest that any cardiovascular changes which may have taken place were not analogous to those associated with true exercise training. The progressive decrease in mean blood pressure seen during dobutamine infusion has at least two possible explanations. First, dobutamine induced receptor down regulation may have resulted in an overall decrease in systematic vascular resistance. Second, dobutamine may have affected an increase in arterial compliance. The presence of either of these conditions would help to explain the reduced G-tolerance seen in dobutamine vs. saline treated animals during the initial centrifuge testing (Graph 3, Run 1).

Of interest in this study was the lack of significant change in time to G-LOC for dobutamine treated animals seen following the administration of Brevibloc. The apparent failure of beta-1 blockade to influence time to G-LOC in the treated animals may indicate that the drug is affecting hemodynamic mechanisms outside of the heart. Changes in muscle oxidative and antioxidant enzymes, fiber type and cardiac hypertrophy for dobutamine vs. saline treated animals are reported elsewhere in these proceedings.

ACKNOWLEDGEMENTS
The authors would like to thank Dr. R. Hamlin and Dr. R. Tuttle for their assistance in completing this study.

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VENOUS RETURN, GRAVITATIONAL STRESS AND PHYSICAL TRAINING.

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INTRODUCTION.
It has been demonstrated that endurance training increases lower limb venous return (3, 4, 5, 6). Tilling has been shown to predispose endurance trained subjects to orthostatic intolerance because of the greater pooling of blood in the lower extremities (6). But venous distensibility is not sufficient to define venous emptying characteristics and therefore venous return, which is determined to consider when studying cardiovascular adaptations to a given stress. Increased venous distensibility is not necessarily associated with decreased venous return (4) because emptying parameters may be modified to insure adequate venous return. The aim of this study was to test the hypothesis that increased lower limb venous distensibility is (or is not) associated with modified parameters of venous emptying and impaired anatomic-functional characteristics of lower limb veins.

MATERIAL AND METHODS.

Subjects.
21 subjects participated in this experiment. They were endurance trained (runners,7), strength trained (body-builders and weightlifters, 7) or sedentary subjects (7). Endurance and strength trained subjects had been trained for several years and regularly participated in national or international sports contests. All subjects were given a questionnaire and a physical examination in order to dismiss from the investigation any individual with a cardiovascular pathology, especially venous, and any subject with a family history of venous insufficiency. All gave their written consent after being informed of all protocol details.

Methods.
Venous distensibility and emptying have been investigated using the postural plethysmography. Postural plethysmography consists in measuring leg volume changes during a tilt-table test. Venous distensibility is measured when the table is positioned at an angle of 30 degrees relative to the horizontal plane and venous emptying is studied when the table is brought back to the horizontal position.

Leg volume changes were measured using a mercury strain-gauge plethysmograph (Perivém, Jansen Scientific Instruments, Etna, Paris, France). The principle and limitations of mercury strain-gauge plethysmography were described elsewhere (8).

Analysis of results.

Figure 1 shows the plethysmographic signal obtained on a leg during a tilt maneuver. Leg volume increases during tilt at a rate depending on the difference between arterial input filling the leg and venous output from the same limb. When volume equilibrium has been reached, the curve becomes a plateau. Leg volume tends to return to its initial value during horizontal tilt, first rapidly, then more slowly. The shape of the curve established during this protocol is identical to the plethysmographic curve obtained during venous occlusion. Several parameters were measured:

1) filling volume $FV$ (ml.100 ml$^{-1}$) which is the difference between the maximum plateau volume and point O. It expresses maximum blood volume pooled per 100 ml of tissue;
2) half-emptying time $T_{1/2}$ (seconds) which is the time necessary to empty half the venous volume, and
3) venous output at the 6th second of venous emptying $V_{O6}$ (ml.100 ml$^{-1}$) which reflects the active and/or passive behavior of venous walls, venous elasticity and resistance to venous flow.

Statistical study.
The individual measure of each parameter is the mean of measurements made during 3 successive tilt maneuvers. We carried out measurements on the right and left leg of each individual. The means were calculated for each group, and a two-factor analysis of variance was made (training factor for 3 schedules: endurance, strength and sedentary subjects; side factor for two schedules: right leg (RL) and left leg (LL)). The right-left comparison has been added to the test to distinguish general from local factors in differences usually observed between the right and left side. If a significant difference appeared in the global test for a given factor, inter-schedule comparisons were made using a student t test, the significance level was set at 0.05. All results are expressed as ‘mean ± SE’.

RESULTS.
The analysis of filling curves (figure 2) shows a significant increase in filling volume of endurance subjects compared to strength trained and sedentary subjects (p < 0.001) endurance trained vs strength trained subjects, and endurance trained vs sedentary subjects (RL and LL). We observe an assymmetry between the two legs in all groups but the difference is not significant.

The half-emptying time $T_{1/2}$ (Fig. 3) is not different between subjects and shows no asymmetry between right and left leg.
The venous distensibility measured by mercury strain gauge plethysmography and Trendelenburg maneuver. After 8 weeks of training, venous pooling in the foot had noticeably increased. Pawelczyk et al. (7) measured leg compliance by impedance plethysmography and venous occlusion in 10 subjects submitted to aerobic training for 7 weeks. After training calf volume changes were only observed at the highest occlusion pressures (80 and 100 mmHg).

These investigations show that there are various ways to measure limb venous distensibility, and that these various techniques all come to the same conclusions i.e., lower limb venous distensibility is increased by endurance training. The originality of the techniques used in this study was the employment of mercury strain gauge plethysmography to measure leg volume changes during vertical tilt (a technique differing from that of Convertino who used the impedance plethysmography), and also the additional study of venous emptying characteristics with parameters defining an emptying time constant (T_{1/2}), venous elasticity and venous flow resistance (VO_{2}). In fact, venous distensibility is not sufficient to define emptying characteristics and therefore venous return, which are important to consider when studying cardiovascular adaptation to a given stress. Increased venous distensibility is not necessarily associated with decreased venous return (4) because emptying parameters may be modified to ensure adequate venous return.

The venous distensibility of endurance trained subjects participating in our study is significantly higher than that of sedentary subjects. However, this greater distensibility observed in endurance trained subjects is associated with a similar half emptying time T_{1/2} and a significantly higher VO_{2} if compared with values obtained in sedentary subjects. In other words, regarding lower limb venous emptying, endurance trained subjects behave, compared to sedentary subjects, as if they had to assure blood return to the heart of a much greater quantity of blood in the same time as that required for sedentary subjects to eject a smaller blood volume. This proves that the active behavior and the elasticity of venous walls in endurance trained subjects are perfectly maintained (1), which is compatible with conservation of the integrity of anatomo-functional characteristics of the lower limb venous walls in endurance trained subjects.

Strength trained subjects have properties of venous distensibility and emptying not significantly different from those of sedentary subjects, which proves that muscle-building exercise does not modify venous vascular characteristics, at least for lower limbs.

In conclusion, these findings show that the lower limb venous overdistensibility seen in endurance trained subjects is not accompanied with impaired venous return from this area.

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GRAVITY AND THE ONTOGENY OF ANIMALS

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Earth's gravitational field imposes an omnipresent challenge to terrestrial animals. The argument that gravity may have influenced the shape and mechanical structure of animals through natural selection is logical and supported by experimental evidence (1-4,12). It seems logical to suspect that each species is prepared during the course of ontogeny and maturation with the tools to adapt structurally to and behaviorally to the 1.0G environment of the adult. But what if the embryo, so prepared, emerges from ontogenesis only to find it is weightless or under the excess load of a hypergravic field?

Mature terrestrial animals appear to have abundant regulatory mechanisms available for adaptation to moderate chronic changes in the gravitational field (e.g. 0 to 3G, (5-12). There is evidence in mature animals, for example, that gravitational loading and unloading produces an adaptive increase or decrease in skeletal mass respectively (12). But has the process of natural selection produced organisms manifesting analogous 'gravity-sensing' regulatory mechanisms that operate during ontogeny? Or is it the case that, since earth's gravitational field strength has remained constant over billions of years, organisms have evolved fixed developmental programs that proceed with little or no adjustment, and hence without regard to the gravitational environment?

Searching for gravity's roles:

One means of identifying roles for gravity in ontogeny is to examine the effects of altering the gravitational field on developing organisms. A number of potential actions of gravity at the level of the cell have been considered in some detail recently (13). And indeed there is evidence for gravitational effects at this level. Space flight experiments studying developing insects have provided some evidence that the weightless environment may compromise, directly or indirectly, cellular chromosome repair processes, especially repair of the damage produced by radiation (14). More recently investigators have offered evidence that randomizing the incidence of the gravitational vector (e.g. by using a clinostat) on cell cultures can lead to changes in morphology and cell-to-cell interaction in vitro (15-24).

The effects of changing the direction of the gravitational vector during early amphibian embryogenesis have long been cited as evidence for gravity's potential role in the process of polarizing morphological axes (23-35).

Although some evidence suggests that randomizing the G vector can result in normal morphological development, there remain issues about gravity's role in establishing the position of the neural plate in relation to the sperm entry point (25). Space flight experiments scheduled for 1992 are poised ready to decide whether or not gravity is an essential cue for polarization in these species.

G-Loading:

The effects of exposing embryos to increased gravitational loads have been evaluated in many species. Smith and Abbott (36) showed that avian embryos were most affected by increased G loads during very early development (especially E1-E4) and during hatching. The most notable abnormality was an inhibition of the axial skeleton. This appears to be a consistent finding in mammals as well, especially in reducing the length of bones (37-44). In mammals, ruling out maternal effects as potential confounding influences during these experiments is extremely important. The findings of Duke and colleagues (37,44) report that excess G (2.6G) can suppress morphogenesis of cultured embryonic mouse limbs. This observation shows that the effect can be produced in the absence of maternal changes and may suggest an independent role for gravity in the formation of skeletal elements during ontogeny.

Recent experiments in the weightlessness of space:

Experiments conducted in space are complicated by an array of technical and logistical problems that in many instances limit interpretation of results. A few examples of these difficulties are worth noting. Flight duration may be limited and exposure to weightlessness transient, occurring only during portions of the normal period of ontogeny. A flight control centrifuge may not be available. Moreover, animals may be exposed to the effects of launch and/or landing acceleration and vibration profiles thus making it difficult to isolate the effects of weightlessness (45). Moreover, it is conceivable that such complicated dynamics could even negate the effect of microgravity on some developing systems altogether. In spite of these difficulties, a great deal of information has been gleaned from such research.

Brief periods (less than 7 days) of weightlessness during the later stages of development seem to have only minor effects on subsequent postnatal survival and behavior on earth in birds and rats (46-55). Five gravid rats flown on the COSMOS 1514, upon return to earth gave birth to 48 pups (46), representing the first mammals to undergo a portion of gestation in the absence of gravity. In depth evaluation of postnatal sensory and behavioral development in these pups revealed few abnormal findings (47). Similarly, the growth and behavior of all 8 chicks exposed late in development to 5 days of space flight (E9-E14) aboard the Space shuttle Discovery (STS-29) were found to be normal (50-55). However, despite normal behaviors and normal vestibular gross morphology (53,55), vestibular thresholds were significantly higher in flight animals compared to synchronous but not vivarium controls (49). In the latter case, synchronous ground controls were exposed to launch vibration profiles and had the lowest thresholds of all groups. The effects of vibration alone on vestibular
development are otherwise unknown and may be significant.

The findings were quite different for younger chick embryos (E2-E7) housed simultaneously in the same incubators aboard the shuttle Discovery (STS-29). In this case all died after developing for various periods of time in space before returning to earth (52).

Tadpoles (56) and killifish (57), exposed during ontogeny to near weightlessness reportedly exhibited abnormal swimming patterns suggestive of vestibular abnormalities upon return to earth. These deficits also occurred in the absence of any gross morphological changes in the vestibular organs (56).

The results of these studies in general suggest that brief exposure to the weightlessness of space appears to be well tolerated at least for late periods of ontogenesis. Moreover, animals have few problems adapting to 1.0G once returned to earth. However, the death of the younger group of embryos and the findings of elevated vestibular thresholds leave open the question of the exact nature of changes occurring during and following space flight. In considering these issues it is important to be cautious in our interpretation of findings since only in one case (56) was a flight centrifuge control group studied and flight animals experienced both launch and landing of the shuttle and other space craft.

In my view, the most impressive experimental result to date is the successful incubation and hatching of 4 quails in March, 1990, aboard the soviet MIR space station by Boda and coworkers (58-61). Forty-eight fertilized eggs were cooled to arrest development (early gastrula stage) and then placed aboard the MIR space station. Development was initiated in space by raising the temperature of the incubators and thereafter development was allowed to proceed until hatch. Upon hatching, animals appeared morphologically normal. The animals, however, were disoriented and unable to execute common meaningful locomotor behaviors including feeding.

The results in quails, although lacking in detail, appear to suggest that in warm blooded vertebrates morphological development can proceed to completion in space once initiated in the early gastrula stage. Detailed morphological studies are underway. Unfortunately results may be few due to the limited amount of tissue and poor fixation. The behavioral findings of the hatchlings raise important questions about how well natural selection on earth has prepared terrestrial organisms for development in space? Without further study, it is not possible to know whether the altered behavior was due to an altered sensory-motor system or one that is normal but utterly useless when experiencing a weightless environment for the first time?

Summary comments:

The observation that increased G load can change the course of skeletal development in embryos raises the question of whether these changes reflect appropriate adaptations and hence the existence of some regulatory mechanism? Unsettled in this regard is the question of the appropriateness of the skeletal response. Do changes better prepare the embryo for postnatal life under increased G load?

With the successful MIR experiments, we now have an idea of what happens when the embryo emerges only to find itself weightless. It is difficult to understand the unusual behaviors without seeking an answer to the question of whether they resulted from an altered sensory-motor organization (which may have been produced by the absence of gravity during ontogeny), or whether a fixed program of ontogenesis, a program that otherwise has been successful for millions of years, was for the first time issuing inappropriate motor instructions for surviving and adapting to the postnatal environment?

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CONTROL OF CIRCULATORY FUNCTION IN ALTERED GRAVITATIONAL FIELDS

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INTRODUCTION

Terrestrial animals, especially taller upright species such as humans, are well adapted to a normal 1-g field. Physiologic mechanisms regulate blood pressure and flow to whole organs as well as to localized portions of the microcirculation. Such mechanisms are available to maintain blood pressure and flow to the brain upon assuming upright posture (13). In this review of circulatory function, peripheral control mechanisms will be emphasized along with their importance for responding to gravitational stress.

When the human body is exposed to hypergravity conditions such as on a centrifuge or jet aircraft where +Gz increases to several times that on Earth, blood pressure and flow decrease in the head and increase in dependent tissues (17). On the other hand, when exposed to hypogravity or microgravity conditions, blood pressure and flow may increase in the head and decrease in dependent tissues (Fig. 1).

Cardiovascular adaptations to microgravity include: 1) a shift of blood and tissue fluids from lower to upper body, causing headward edema (14), 2) reduced blood volume, and perhaps, 3) altered autonomic control of the circulation. Aside from edema and possibly lowered inflight exercise capacity, these adaptations are relatively benign and probably constitute appropriate responses to the low circulatory stress of the space environment. However, crew members returning from both short- and long-duration space flights experience orthostatic intolerance (hypotension) and decreased aerobic exercise capacity. Orthostatic intolerance has been identified as one of the most serious problems for Shuttle crew members if emergency egress is required immediately after landing.

Post-flight orthostatic intolerance may be caused by lower inflight blood volume (about one liter), altered baroreflex function (3), post-flight pooling of venous blood due to leg muscle atrophy (2), and pooling of tissue fluids (6). Orthostatic tolerance, as measured by LBNP tolerance, decreases within a week after exposure to microgravity (9). The post-flight recovery of orthostatic tolerance is not well documented for either Soviet or American space programs.

On Earth, microgravity conditions have been simulated by water immersion, supine bed rest, and 5-6° head-down tilt (HDT). The latter procedure is the most widely-employed model for studies of the cardiovascular system because many of the above-identified manifestations of space flight exist during and after HDT (Fig. 2).

The increase of urine output, a prominent feature of most models of simulated microgravity, has not been documented in actual microgravity as yet. This may be related to the dehydration and supine, knees-up posture of crew members preceding launch.

MECHANISM OF HEADWARD EDEMA FORMATION DURING HEAD-DOWN TILT

1. Transcapillary Fluid Pressures

The gravitational gradient of capillary blood pressure from head to foot, previously documented by Levick and Michel (10), is lost in microgravity (Fig. 3).

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Because a relatively high-resistance pathway exists for fluid to move through extravascular tissues (5), tissue fluid is redistributed primarily via the micro- and macrocirculation during changes in gravity or posture. Fluid transport between the capillary blood and interstitial fluid (fluid between cells) is governed by the Starling equation:

\[ J_c = L_p A [(P_c - P_l) - \sigma c (\pi_c - \pi_l)] \]

where:
- \( J_c \) = transcapillary fluid filtration
- \( L_p \) = fluid conductivity across capillary wall
- \( A \) = capillary surface area
- \( P_c \) = capillary blood pressure
- \( P_l \) = interstitial fluid pressure
- \( \sigma c \) = reflection coefficient for plasma protein
- \( \pi_c \) = blood colloid osmotic pressure
- \( \pi_l \) = interstitial fluid colloid osmotic pressure

To understand the mechanism, magnitude and time course of facial puffiness that occurs in microgravity, seven volunteers were tilted 6° head-down for 8 hours, and all four Starling transcapillary pressures were directly measured before, during, and after tilt (11). Head-down tilt (HDT) caused facial edema due to a significant elevation of microvascular pressures as measured in the lower lip: capillary pressure increased from 27.7 ± 1.5 mm Hg pre-HDT to 33.9 ± 1.7 mm Hg by the end of tilt (Fig. 4).

Subcutaneous and intramuscular interstitial fluid pressures in the neck (manifestations of edema) also increased as a result of HDT, while interstitial fluid colloid osmotic pressures in these tissues remained unchanged. Plasma colloid osmotic pressure dropped significantly after 4 hours of HDT (21.5 ± 1.5 mm Hg pre-HDT to 18.2 ± 1.9 mm Hg at 4 hours HDT), suggesting a transition from fluid filtration to absorption in capillary beds between the heart and feet during HDT (Fig. 5). After 4 hours of seated recovery from HDT, lip microvascular pressures (capillary and venule pressures) remained significantly elevated from baseline values, despite significant HDT diuresis and the orthostatic challenge of an upright, seated posture. Post-tilt

Subsequently, fluid pressures elevated significantly by the end of tilt (Fig. 6). Regional distributions of blood volume and capillary blood pressure (mm Hg) at head, heart, and feet before, during, and after 6° HDT.

2. Microvascular Flow Responses
To explore further the mechanism of facial puffiness, headache, and nasal congestion associated with microgravity, the postural responses of the cutaneous microcirculation in the forehead and dorsum of the foot of eight volunteers were studied by changing body position on a tilt table and measuring blood flows with a laser-Doppler flowmeter (11). Raising arterial pressure in the head by tilting from the 60° head-up to 6° head-down posture significantly increased forehead cutaneous flow by 25.5 ± 7.2%. Increasing arterial pressure in the feet by moving from a 6° head-down tilt to a 60° head-up posture significantly decreased foot cutaneous flow by 46.5 ± 12.0%.

![Figure 5: Plasma colloid osmotic pressure decreased significantly after 4 hours HDT. Lower bar indicates period of HDT (From reference 11).](image)

![Figure 6: Regional distributions of blood volume and capillary blood pressure (mm Hg) at head, heart and feet before, during and after 6° HDT.](image)
To investigate the possibility that these opposite responses are modified by simulated microgravity, identical tilt tests were repeated after 7 days of 6° head-down tilt bed rest (1). On the 1st and 2nd days after bed rest, microvascular flows in the head increased during the head-down tilt test (60° to -6°) by $39.3 \pm 8.6\%$ and $15.5 \pm 5.9\%$, respectively. Microvascular flows in the foot decreased during the head-up tilt test (-6° to 60°) by $60.4 \pm 8.8\%$ and $45.8 \pm 18.7\%$, respectively. These responses were not significantly different from those recorded before bed rest (1). However, longer-term, bed-rest studies may be necessary to produce "vascular deconditioning" sufficient to alter these responses significantly.

Cutaneous microcirculatory flow in the feet is well regulated to prevent edema when shifting to an upright position, whereas there is little regulation in the head microcirculation with head-down tilt. The development of arterial and microvascular adaptations to gravitational blood pressure gradients (e.g., smooth muscle hypertrophy in arteries of the feet) is well documented in tall species such as humans and giraffes (7). It is expected that some or all of this vascular adaptation may be lost during long-duration flight. Tissue hypertrophy following increased stress or tissue atrophy after decreased stress is documented in musculoskeletal tissues, and similar responses were recently proposed as a universal law for blood vessels (4).

The lack of regulation in the forehead cutaneous microcirculation increases capillary flow during a head-down tilt maneuver (60° to -6°), and consequently increases fluid filtration into the interstitium. This phenomenon further explains the facial edema associated with simulated or actual microgravity. These results also have important implications to long-duration missions because some cosmonauts have facial edema throughout their mission (up to one year). If intracranial edema also forms during microgravity, crew performance may be compromised by increased intracranial pressure (ICP). Therefore, future studies should: 1) investigate the post-tilt recovery period for longer times, 2) investigate ICP in rhesus monkeys during a future Cosmos mission, and 3) develop a noninvasive ICP technique to study crew members during actual microgravity.

**CIRCULATORY STRESSES APPLIED BY POSTURE, CENTRIFUGATION AND LBNP**

Development of countermeasures for long-duration spaceflight should consider regional circulatory stresses. To date, exercise hardware and protocols for use in microgravity exposure have not incorporated blood pressure gradients within the body similar to those existent on Earth. Theoretically, to maintain normal structure and function of vascular tissues, the distribution of transmural pressures should closely parallel that present in a 1-g field (Fig. 7).

![Figure 7](image_url)

*Figure 7:* Distributions of mean transmural pressures with stresses applied by upright posture, centrifugation, and LBNP: (A) upright posture on Earth where $G_2$ stress is uniformly one over entire body, (B) 25-foot centrifuge in space with $1-G_2$ at feet, (C) 6-foot centrifuge in space with $1-G_2$ at feet, and (D) lower body exposed to uniform stress of -100 mm Hg in space. Transmural pressures are gradients of pressure across the arterial wall. Extravascular tissue fluid pressures normally range between 0-10 mm Hg.
INTEGRATED "EXERCISE AGAINST LBNP" COUNTERMEASURE FOR LONG-DURATION SPACE FLIGHT

Theoretically, an integrated countermeasure for extended exposure to microgravity should combine high loads on the musculoskeletal system (16) with normal regional distributions of transmural pressure across blood vessels. Recently, we documented that about 100 mm Hg LBNP provides a footward force equivalent to 1-g body weight (8). Dynamic loads during running exercise within a chamber at -100 mm Hg may provide inertial forces on the musculoskeletal and cardiovascular systems similar to those present during identical exercise on Earth. At the foot, these accelerative loads may generate intermittent transmural pressure impulses of 200 mm Hg or above and the levels normally present with LBNP or standing upright posture on Earth (Fig. 9). An anti-LBNP suit with highest tissue compression below the waist and lowest compression at the feet may be necessary to provide an Earth-like gradient of transmural pressure. Recent experience in our laboratory has documented that the stress of LBNP is uniformly distributed to all extravascular tissue fluids in the LBNP chamber, but that intravascular pressures within the chamber are not significantly altered. Muscle contraction, however, increases intramuscular fluid pressure (12), so that the stress of LBNP is counteracted in activated muscle. In fact, dynamic leg exercise doubles LBNP tolerance, in part by skeletal muscle pumping of venous blood (15).

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GRAVITY AND THE REGULATION OF CARBOHYDRATE METABOLISM

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The knowledge of the effects of altered gravity on the function and metabolism of animal cells or whole organism is still rather limited. However, it is important to understand the influence of altered gravity conditions for determination of the capacity of cells and organism to cope with the load of gravitational stress.

The important role of carbohydrate metabolism to cover energy requirements of living cells and organism is evident. It might seem logical to assume that activity in microgravity conditions would require less energy than in one g gravity, since work associated with counteracting the force gravity is eliminated. However, the data acquired from cosmonauts during space flight missions revealed that the overall energy consumptions in flight were similar to those observed on the ground. With the prolongation of space flights the gradual elevations in energy input in humans flight is presumptual evidence of either increasing energy output or decreasing metabolic efficiency (1,2). This resulted in loss of body mass in astronauts.

However they have been difficulties in obtaining the data on the changes of carbohydrate metabolism during altered gravity conditions in human subjects and experimental animals because it was not possible to avoid the interventions into these studies and to overcome the additional factors: 1. wide variability in individual tolerance; 2. extensive use of contrameasures (e.g. use of therapeutics for neurovestibular symptom, cardiovascular deconditioning, muscle atrophy and bone degeneration); 3. limitation of sample size (small number of individuals in space); 4. restriction in biomedical observations due to operational program during space flight; 5. differences in the type of space missions (various duration of space flights, extravehicular activities); 6. variability in the postflight readaptation process.

There are two kinds of the effects of gravity changes on the organism:

1. Specific effects connected to gravity force:
   - circulatory changes and redistribution of body fluids
   - changes in interaction of organs and tissues
   - changes in structure of cells and tissues in distribution of subcellular component
   - changes of energy metabolism

2. Nonspecific effects connected to overall the conditions:
   - changes due to stressogenic stimuli.

Effect of gravity on cell metabolism

Gravitational effects on cellular levels are well documented in plant cells (3). In extensive studies of the gravity effects on mammalian cells showed that mitogenic stimulation of human lymphocyte in cultures by concanavalin A is almost completely depressed under microgravity and that hypergravity enhanced the stimulation of lymphocytes by concanavalin A as well as the proliferation of other mammalian cells (4,5). It was also observed that the cytoskeletal structure of cell may be affected by gravity (6). By using a culture of human A 431 epidermoid carcinoma cells (Groot et al., 7) induced cell rounding and protooncogen c-fos expression are decreased under the microgravity conditions in clinostat, whereas it is enhanced by hypergravity. It was discussed that even if the force of gravity at subcellular level is extremely weak, the fine structure of the cell and the non-linearity of the most of its molecular systems may provide an amplification required to allow the cells to sensing gravity (6). By comparison of different cell lines and hybridome cells Lorenzi et al. (4) demonstrated that each cell line developed its own reaction and adaptation to gravitational environment. They also found, that gravity could directly affect the glucose consumption in mammalian cells. An important decrease of glucose utilization was found in virus transformed leukemia cells (Friend cells) after exposure to hypergravity in centrifuge and hypogravity in clinostat. Also in K-526 cells the glucose consumption was depressed in microgravity.

In human fibroblast it was showed that the activity of glycolytic pathway seemed to be not disturbed by hypergravity conditions (no changes in glycerol-3-phosphate dehydrogenase and pyruvate kinase activities after 6 days in 15 g) whereas activity of the pentose phosphate shunt (glucose-6-phosphate dehydrogenase) was slightly increased. It was suggested that hypergravity exposed cells need more energy for spreading and migration and this energy could be provided by an increase of the glucose oxidation via the pentose pathway. Conversely, the glucose consumption in lung fibroblast culture exposed for 28 days to microgravity was noted (9). These experiments demonstrated a direct action of gravity changes on the cells. Molecular mechanism of cell adaptation to altered gravity, including microgravity, when acting directly, is a chain of successive alterations on the level of membranes and elements of cytoskeleton (10). The important changes of insulin binding to membrane fraction of adipocytes were found in rats exposed to microgravity for 14 day space flight (FIG. 1) suggesting the changes in cell membrane.
FIG. 1 Insulin binding capacity of liver cell membrane of rats after space flight. AC-intact control, C-controls (hypokinesia), S-synchron model group, A-antiorthostatic hypokinesia, F-space flight.

However no effect of space flight was noted on insulin binding in liver. These results are in agreement with the observation of Lorenzi et al. (4) that there are differences in the response of various cell type to alteration of gravity and that no general rules on g-sensing of mammalian cells can be applied.

Changes of carbohydrate metabolism in microgravity

Studies in human subjects. In multicellular organism the gravitation effects are largely mediated by changes in regulatory mechanisms on highest level, in nervous and humoral system. Since in experimental animals and also in human subjects the levels of hormones and metabolites in blood were examined mostly during the postflight period, besides the microgravity also the interference of hypergravity and other stress factor during the landing have to be taken into account in evaluation of space flight effects. There are only limited inflight observations of carbohydrate metabolite and hormone plasma levels performed in different time of space flight. Leach-Hanton and Rambut (11) presented that in first days of space flight there are no significant changes in plasma glucose and in insulin levels, the slight increase of cortisol was noted on the days 5 and 6. In cosmonaut on the station MIR an elevation of plasma norepinephrine (NE) levels was noted on inflight day 9, the epinephrine (E) levels were similar as in preflight period (12).

With prolongation of flight between 30-82 days the plasma glucose and insulin (INS) levels in Skylab crewmembers were decreased, cortisol levels were unchanged or slightly increased (11). Also in cosmonauts from Saljut-7 - Soyuz-T mission it was found that fasting plasma glucose levels are lowered on mission days 48 and 60, the glycemic curve after the glucose load was flattened (13). These data suggest that decrease of the glucose levels in basal conditions and after a glucose load recurred during the 2nd flight month is reflection of lower rate carbohydrate hydrolysis and absorption in gastrointestinal tract. However at lower flight stage, on mission day 88 and at the beginning of the 7th flight month an increase of the blood glucose levels was noted in fasting samples and after glucose load suggesting a delayed glucose utilization (14). During the 8th month of flight the plasma glucose values and glycemic curves of two cosmonauts were not different from the preflight tracing and in third crewmember the fasting blood glucose level was similar as preflight values and the glycemic curve was flattened. Therefore for exact evaluation of the rate of glucose utilization it seems useful to perform an intravenous glucose tolerance test and to calculate as coefficient of glucose utilization (15).

The plasma levels of hormones involved in the regulation of carbohydrate metabolism (NE, E, INS) were not significantly changed at later flight stage only slight increase of cortisol was noted on inflight day 82 (11).

The increase of blood glucose levels was noted in 1-4 postflight days, later in recovery period the values of glycemia were similar to preflight level. These elevated plasma glucose levels are in agreement with increased plasma concentrations of catecholamines, growth hormone and in several cosmonauts also plasma cortisol. The levels of insulin were increased in the first day of the recovery period. In spite of higher insulin levels the glycemic curves showed a decrease of the rate of glucose utilization in first day recovery, later during postflight period no changes of fasting plasma glucose values and glucose tolerance curve were noted (13,14).

After long-term space flight (150-326 days) a moderate increase of lactate and pyruvate values were observed in several cosmonauts. These indicate the enhancement of glucose metabolism through the glycolytic pathways and slower utilization of the active metabolism in agreement with this explanation the measurement of isocitrate dehydrogenase (ICDH) and malate dehydrogenase (MDH) and its mitochondrial iso-enzyme MDH-3 in blood demonstrated a decline of the activity (14) however after 7 days the enzyme activities were partially or completely recovered.

Studies in experimental animals. The advantage of the simultaneously running experiments with rats exposed to space flights was a possibility to study the influence of microgravity on metabolic processes and enzyme activities in various organs. However at present time there are no inflight data available from animals and the effects of space flight were evaluated only on the base from postflight samples.

The increase of plasma glucose levels was repeatedly observed in rats immediately after short or long term space flight (16). This is in agreement with slight increase of catecholamines and corticosterone in plasma (FIG.2) both hormones with hyperglycemic and gluconeogenic action. An important increase of the activity of enzymes involved in the processes of gluconeogenesis fructose-1,6-diphosphatase (FDP) and phosphoenolpyruvate carboxykinase (PECK)
was noted in liver. The activity of glucose-6-phosphatase in liver was decreased suggesting that the glucose produced by gluconeogenesis is used predominately for the synthesis of glycogen. Therefore the significant increase of glycogen content in liver was demonstrated (FIG. 2) in flight rats.

The increases of plasma glucose, corticosterone and insulin levels and the liver enzyme activities are probably due to the effect of prolonged state of weightlessness, because no differences in plasma hormone levels and in the activities of enzymes in liver were found in control and flight animals being during the space flight in artificial gravity on the board of biosatellite (FIG. 3). However the plasma content of epinephrine and norepinephrine was not corrected by artificial gravity during the flight suggesting that their increase after flight is predominantly due to other stress stimuli.

FIG.2 Changes of metabolite and hormone levels, in % of controls during short and long-term space flight. The high performance aircraft may expose the pilots to rapid onset of high sustained G-loads and the physiological limitations of the human tolerance to G-acceleration could be reached or even be exceeded at these flights. A great individual variability in exposure to high G-load in different subjects was noted (19). Many environmental and physiological factors may interfere with G-tolerance e.g. heat stress, dehydration, hypoglycemia, hypoxia, alcohol or minor illness. Several experimental observations were performed to describe the hypergravity effects on cardiovascular and nervous system, skeletal muscular system and endocrine response (19), however there are only limited metabolic changes. Pace and Smith (20) in their study on gravity and its metabolic effects pointed out on the influence of gravitational loading on metabolic energy requirement in relation to body mass of animals. Further it was showed that the hypergravity above some threshold becomes a stressful stimuli and the animals could adapt in certain extent to chronic exposure to hypergravity. Smith and Burton (21) were successful in selection of an acceleration tolerant strain of birds and it was suggested that the selection process has a metabolic basis. Important metabolic alterations like increase of blood glucose and fatty acids, decrease of free amino acids in plasma, progressive elevation in liver glycogen deposition were described in rats exposed to hypergravity (4.5 g) for varying periods ranging from 0.5 to 96 hours (22).

It was found that in hyper-G stressed animals the rate of gluconeogenesis is increased (23) and that the elevation of gluconeogenic activity is due to an increase in mobilization of gluconeogenic substrates.

FIG.3 Effect of artificial gravity during space flight in rats. CS-corticosterone, GLY-glycogen, GLU-glucose, NE-norepinephrine, INS-insulin, EP-epinephrine, A-controls, F-flight, FC-flight in centrifuge, S-synchon model control group, SC-synchron model in centrifuge. The activity of enzymes involved in oxidation of glucose metabolites was also determined (17). It was found, that the activity of malate dehydrogenase (MDH) and isocitrate dehydrogenase (ICDH) in liver mitochondria is similar as in control rats. In cytoplasmatic fraction from liver cells the activity of MDH and ICDH was decreased in flight rats, the MDH activity was lower also in animals in artificial gravity. The changes in the activities of these dehydrogenase in cell plasma could resulted in alteration of the ratio of oxidative and reductive form of NAD and NADP in mitochondria and cytoplasm of liver cells and these changes could affect the processes of glycolysis and gluconeogenesis (17). Also the activities of more than twenty enzymes involved in the metabolism of lipids and carbohydrate in liver were estimated in rats exposed to space flight (18). It was found that the activities of most of enzymes appeared to be unaffected by microgravity conditions. However, the activities of glycogen phosphorylase, α-glycerol phosphat acyl transferase and 6-phosphogluconate dehydrogenase were decreased in comparison to those in control and animals in artificial gravity (18). All these results showed that gravity changes affected the metabolism of glucose and the changes were normalized in postflight period between 7 to 25 days.
FIG. 4 Changes of hormones and metabolite in plasma (p) and liver (1) in rats exposed to hyper- and microgravity. (1) see reference 23, (2) reference 16, (3) reference 17.

from peripheral tissue to liver (e.g. lactate, aminoacids, glycerol, free fatty acids). This is a result of increases of plasma hormone levels - corticosterone, glucagon and catecholamines (22, 23, 24). The most important role of corticosterone was suggested because in adrenalectomized rats no hyperglycemia and no elevation of glyco-
gen deposition could be demonstrated. Furt-
er a sustained hyperglycemia in spite of incre,
crease of plasma insulin levels in hyper-
G exposed rats was noted. The reason of this 
discrepancies needs further studies. The  
possibility is that catecholamines might pro-
moine insulin counterregulation by inhibi-
ing the insulin stimulated glucose uti-
ization by peripheral tissue. The  
comparison of the changes in glucone 
metabolism and plasma hormone levels showed  
(Fig. 4) that gravity changes resulted in  
the development of similar reaction (hyper-
glycemia, liver glycogen deposition) in rat  
exposed to hyper-G or weightlessness prob-
ably due to stress effect of increased or  
decreased gravity and restraint conditions.  
Also in human subjects an important activa-
tion of pituitary adrenal system was found  
and exposure to hypergravity, however,  
significant effect of hypergravity on plas-
ma glucose, growth hormone and prolactin  
was found. Both hormones are "stress  
hormone" and probably relatively short  
exposure to stress stimuli may explain the  
lack of response in carbohydrate metabolism  
(25).

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ADAPTIVE CAPACITIES OF ANIMALS IN WEIGHTLESSNESS AND HYPERGRAVITY

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When animal studies on "Cosmos" biosatellites were started, it was already clear that humans can live and function effectively during weightlessness. A large body of information was accumulated concerning the effect of space flight factors upon various aspects of human vital activity. However, many questions remained unsolved. Among others, the following question remained open: what is the cost of adaptation to weightlessness, is it associated with occult visceral pathology and unfavourable remote consequences.

An answer to this question can only be based on regular systematic experiments with animals, primarily mammals, exposed on board space vehicles during flights of varying duration. Only such experiments could allow to perform a detailed study of structure and metabolism of various tissues of the body exposed to weightlessness, to use various provocative tests and rather complicated ones; to study the remote consequences of space flight effects, and finally, to ensure statistical significance of the material obtained.

Wistar rats from "Stolbovaya" nursery, as well as Wistar-SF rats (pathological factor-free) from the nursery of the Institute of Experimental Endocrinology, S.A. Sci (Bratislava, CSFR) were used.

Our research was started with evaluating the status of the animals-male Wistar rats - which were, during the space flight and readaptation, relatively resting, without additional loading (Cosmos-605, 722, 1129). This was associated with a moderately marked stress response; changes in the musculo-skeletal system, viscera, in the blood system which developed during the flight were reversible and completely normalized by day 29 of readaptation. The life span of the animals returning to the ground after spaceflight was unchanged. The level of dominant lethal mutations in mature spermatozoa and stem cells of spermato genesis in experimental animals was also unchanged (Gazenko et al., 1980; Serova, 1980). The main task of next stage of our research was to study postflight state of animals not only at rest but at different provocative situations.

On board "Cosmos-1129", a special experiment was performed to evaluate the animals' response (male Wistar-SF rats) to acute and chronic immobilization stress during the post-flight period.

Four animal groups were used. Group I rats were sacrificed on the final day of the 18.5-day mission 7 to 10 hours after return to the ground. Group II animals were not exposed to any additional effects during readaptation, and were sacrificed 6 days after flight. Group III animals were also sacrificed on day 6 of readaptation, but before that, unlike group II, they had been exposed to 2.5-hour immobilization stress tests (fixation in the "back up" position) on recovery day (day 0), and then on days 3, 4, 5 and 6 of readaptation.

Space flight was expected to substantially lower tolerance to additional functional loading of sufficient intensity affecting an organism on its return to the ground. However, despite the fact that the stress-tests we used were severe enough and the first test was performed only a few hours after recovery (when inflight stress-inducing factors were still present as those developed on return to terrestrial gravity), the first test and the subsequent ones were tolerated by the flight rats quite satisfactorily. There were no significant life-threatening changes of stress-test responsiveness in the experimental group as compared to control.

A number of response parameters in the flight and control groups during a series of tests were practically identical. This was true for changes of thymus mass and the number of thymocytes, of plasma epinephrine concentration. Other stress-response parameters, e. to changes of corticosterone adrenal and plasma levels, spleen mass, as compared to initial values on the first recovery day, were lower in the experimental than in the control group. Parameters of the third group were characterized by a more marked response to a series of stress tests in flight animals; these included epinephrine level and the adrenal tyrosine-hydroxylase activity.

As a result of an "overlapping" of changes developed during additional stress-tests and those occurring after flight, by the end of testing experimental animals had a lower thymus and spleen mass as compared to control, a higher plasma corticosterone concentration, a lower epinephrine adrenal content with an increased activity of the key enzyme of its synthesis-tyrosine hydroxylase, and a blood epinephrine level identical to control. This suggested a more intensive epinephrine utilisation by the tissues.

The essence of stress response is the mobilization of energy and structural resources of the body directed to the systems functioning at a higher level. Thus the fact that animals after space flight maintain many parameters of the stress-induced response at the initial control level, can be regarded as positive. At the same time, even when the response of experimental animals to immobilization...
stress was of the same magnitude as that of control animals, it develops when similar changes have already appeared by the return to Earth. These changes are practically added to the former changes. As a result of this, by the end of immobilization tests, the variation, or number of parameters from the physiological standard in flight group was greater than in the controls. This can hardly be viewed as a favourable prognosis, given a complication and prolongation of natural loading and special provocative tests during the pre-flight period.

Having obtained these results and designing the study as a gradual complication of loading situations the animals were exposed to, we searched for a still more universal test which would require, on the one hand, mobilization of resources and straining all regulatory systems (like a stress-test), and on the same time specifically load individual organs and systems which show, during weightlessness, sufficiently marked, if reversible, changes (bone tissue, erythrocyte system, etc.). Searching for such a universal loading test, we thought of mother-fetus system as a good experimental model for the study of mechanisms of physiological weightlessness-induced effects, and first of all for evaluating adaptive potentialities of the body under these circumstances. Our choice was based on the fact that pregnancy is necessarily associated with an extensive activation of anabolic processes, mobilization of calcium to build the fetal skeleton, activation of the erythrocyte system, and a number of other changes, directly opposite to changes observed in-flight. The effect of weightlessness upon the fetus and maternal organism, and to be determined by its metabolic and hormonal status.

These considerations formed the basis of the rat embryological experiment on board "Cosmos-1514" which was to provide the answer to a question of critical importance: is it possible, during spaceflight, not only to maintain physiological function of an adult organism, but to form functions of a developing fetus? The answer was "yes".

Exposure of pregnant animals to weightlessness (5 days) during the trimester of pregnancy was associated with serious alterations in the maternal organism. Their integral expression was a delay of body mass gain in flight female rats (average 60 g) as compared to ground-based synchronous control, i.e. actually 1/4 of their own mass. Nevertheless, parameters of reproductive function were virtually unchanged. Flight fetuses showed their own developmental stages, which was reflected in a delay of body mass gain and of the development of ossification segments. However, differences between the groups were small (not more than 10%), and were subsequently quickly smoothed over. The total mass of fetuses in a single mother during weightlessness on the final flight day was similar to that in vivarium control females. Thus, de-

spite a marked loss of their own mass due to increased catabolic processes during weightlessness, in flight female rats anabolic processes related to fetal growth were activated to the same extent as in control animals. During lactation flight rats maintained normal maternal behaviour and provided the necessary amount of milk for their pups.

Since both weightlessness and pregnancy are associated with serious changes of water and electrolyte metabolism (first of all calcium), when planning the experiment we expected the combined effect of these factors to be serious, perhaps even catastrophic changes of calcium metabolism. Indeed, there was a marked (over 50%) decrease in calcium content in the liver and kidneys of flight female rats. Less marked were changes in the skin; in bone tissue the changes were still more marked. The maternal body was capable of supporting homeostasis of the developing fetus: its tissue concentrations of sodium, potassium, calcium and magnesium under experimental and control conditions were similar. The high effectiveness of maintaining fetal homeostasis during space flight is also confirmed by the absence of serious differences between the progeny of the flight and control groups in the status of the central nervous system which was investigated for 3 months of postnatal life, using a battery of various tests corresponding to each age group; from simple tests for the newborn to complicated tasks in labyrinths of varying designs.

At the autopsy of animals part of whose prenatal development occurred under weightlessness, on days 15, 30, and 100 of their life, no differences were found between experimental and control groups in organ mass, water metabolism, as well as in that of electrolytes, fats, nucleic acids, biologically active substances. Thus, exposure to weightlessness during the fetal period, at the stage of formation of viscera and mechanisms controlling their function, had no effect on the rate of growth of the fetus and the level of metabolism in them at various stages of postnatal development, up to puberty. The only serious difference between experimental and control groups noted in this part of the study was altered collagen metabolism in bone tissue and skin of animals developed during weightlessness. This suggests some delay in the development of connective tissue. The differences between groups were an increased content of soluble collagen in the skin and bone tissue of experimental animals; an increased content of type III collagen characteristic of growing embryonic tissues (but not adults). Causes of these changes remain unclear; the presence of similar changes in the skin and bone tissue suggests their systemic nature.

Results of Cosmos-1514 embryological experiment and associated ground-based model experiment are in principle of development of mammalian fetus during exposure of the maternal organism to weightlessness, in-
dicate at the same time the possibility of quite serious changes in individual animals. If the majority of animals tolerated weightlessness without irreversible pathological changes and complications during readaptation, individual rats showed serious alterations (such as stillborn pups in one of flight females, or the birth of weak litters which died during the early days of their life). The existence of individual differences in the responses to environmental effects, including weightlessness and hypergravity, is not a new fact: it is the subject of discussion by a number of researchers. Performing experiments with male rats we also noted individual differences in weightlessness-induced changes of body and organ mass, blood pattern, and other parameters. It is experimenting with pregnant animals, however, that shows how great the individual variability can be: from absolute well-being (the birth of a healthy viable litter) to complete failure (stillbirth). Analyzing the reasons for individual differences one can suppose that they appear in intact animals due to gradual (in a number of generations) accumulating changes in the genetic apparatus which for a long time (for example when the animals were kept in a vivarium without any additional effects) remain neutral or almost neutral, i.e. having no effect upon the fate of the individual and the population as a whole. Under stressful circumstances these differences become the basis for the differentiation of animals in their resistivity. Under extremal circumstances they obviously can lead to the death of some of them. There are reasons to suppose that in prospective studies when an attempt is made to obtain several generations of mammals on board a space vehicle, selection (as is usually the case under natural conditions) will take place at the populational level by means of coupling of animals which are better adjusted to weightlessness. At the same time it is possible that individual animals will drop out at various stages of the study. In ground-based model centrifuge experiments with 2G we could demonstrate the possibility of realizing, under hypergravity, of all the main stages of prenatal and early post-natal development of rats: fertilization, embryo implantation, fetal development, birth, and lactation of progeny. Mechanisms of hypergravity-induced effects at various stages of development were different. As far as animal breeding under 2G is concerned, the main difference as compared to control was increased time from joining males and females to coupling. When rats were centrifuged during implantation of the embryo (day 7-th of gestation), the main difference from control was termination of pregnancy in some rats. When the females were centrifuged from days 13 or 14 of gestation (the fetal period), the fetuses of experimental rats slightly lagged behind control fetuses in body mass and length of ossified segments of the skeleton. Pups which were left on the centrifuged after

The results obtained allow to evaluate adaptive potentialities of mammals during exposure to weightlessness as high enough, and to give a favourable prognosis concerning the possibility in principle of a complete cycle of prenatal development, and long-term (throughout the life) existence in weightlessness of animals of this species and of species with similar levels of resistivity and responsiveness. However, serious changes in individual animals at various stages of adaptation cannot be excluded.

References
AN ASSESSMENT OF SUSPENSION SYSTEMS: MODELS THAT REPRODUCE RESPONSES TO WEIGHTLESSNESS

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INTRODUCTION

There are several ways to examine physiological responses to weightlessness. However, on a long term basis of days or weeks the only absolute approach is with exposure to hypogravity seen with exposure to space flight. As with many questions in physiology, investigators have developed animal models that reproduce many responses which occur during space flight.

Many models have been developed to assess muscle and bone disuse and cardiovascular responses, and each presents specific advantages and some limitations. Limb casting and joint pinning positions muscles in stretched or contracted positions and results in specific angles that determine the degree of passive stretch or shortening of a given muscle. Tenotomy presents muscle in which the length may be shortened below resting length and contractile activity becomes fully isotonic. Denervation eliminates nerve to muscle communication but it also interrupts neural input to the vascular system and altered blood flow may affect the atrophic process. Small cage restraint results in hypokinesia with little or no hypodynamia. Since man and animals in space flight undergo both hypodynamia and hypokinesia, several laboratories set out to develop model systems that would more accurately reflect the results of exposure to microgravity. With a focus on hind limb unloading two systems have emerged as highly utilitarian, namely whole body suspension (WBS) and tail suspension (TS). Additional discussions and illustrations are found in reports by Fitts et al. (1986), Wronski et al. (1987), Musacchia et al. (1989), and Stump et al. (1990). Each system has been used for specific experiments intended to reproduce responses seen during COSMOS and NASA space flights.

Because there has been a notable increase in publications dealing with these two systems only a select few are included. This leads to an apology to those omitted, this is not a denial of their value, but it would exceed the expectations and needs of this brief review.

The WBS system was developed with two features in mind: (1) to mimic the muscle disuse atrophy seen in response to microgravity exposure and (2) to assess some of the physiological parameters of cardiovascular and fluid plus electrolyte changes in head-down (HDT) versus non head-down tilting (N-HDT) in rats. The apparatus for WBS was described in two initial papers (Musacchia et al. 1980 and Deavers et al. 1980). Although we continue to make modifications, the basic design remains unchanged. In addition to providing a rigid support to the vertebral column, the aluminum back brace can be used to hold in position cannulae, recording leads, and other types of instrumentation. Animal weights can be recorded without removing the subject from the harness. The harness and the rat are weighed together with the rat maintained in position and the animal mass is determined by subtracting. One great advantage is the capacity for suspending animals in a normal horizontal position, and the opportunity for changing the angle of HDT and/or producing a head up tilt. Rats and mice have been suspended for days and weeks in HDT and N-HDT positions. Thus, experiments that require hind limb unloading and experiments that require cephalad fluid shifts can be done simultaneously or separately.

The TS method of suspension offers a useful approach to unloading of the hind limb. It is rapid and relatively uncomplicated. TS as well as the WBS have some shortcomings and these too will be discussed. On the positive side, tail suspended rats have provided a great deal of information concerning long bone and skeletal muscle atrophy. Morey-Holten and others have repeatedly demonstrated that bone unloading can be assessed in terms of bone formation and resorption. Numerous laboratories have utilized the TS system for studies of muscle atrophy.

In brief, both WBS and TS unloading of the hind limbs have confirmed results of muscle atrophy and bone loss as seen in rats following space flight in both COSMOS biosatellite and NASA Space Laboratory experiments. The following brief review covers some of the experimental results and offers further justification for the continued use of these and improved revisions of both systems.

Muscle atrophy

Using WBS, both HDT (20 to 25°) and N-HDT systems to unload the hind limbs in rats, we have consistently shown a differential loss in muscle mass: soleus > gastrocnemius > plantaris, and extensor digitorum longus (EDL) (Musacchia et al. 1980, Musacchia et al. 1983). Thus, as long as the hind limbs are unloaded the position of the rat is less relevant in muscle atrophy studies. Comparisons between the soleus, chiefly composed of slow twitch fibers and the EDL, chiefly fast twitch fibers, repeatedly shows greater atrophic response in the load bearing soleus. Suspension experiments for 7 or 14 days are generally comparable to current NASA and COSMOS flight experiments. In our WBS experiments, rats have varied from growing juveniles, about 170 to 200 gm, to adults, about 300 to 400 gm. Additional versatility of our WBS system was demonstrated when the WBS harness was adapted for experiments using mice (Rose et al. 1984, Steffen et al. 1984, Gould and Sonnenfeld).

In 1990, Musacchia et al., showed parallel changes in soleus and EDL from rats exposed to seven days of flight (SLS-3) and seven days of WBS hind limb unloading. The similarities include: reduction in cross sectional fiber area, increase in fiber density and increase in capillary density in atrophying muscles. The
soleus showed the most dramatic changes, the EDL showed some slight changes and the vastus medialis (Musacchia et al unpublished) showed changes comparable to the EDL. Recently Yokogoshi et al. (1990) examined the responses to soleus and gastrocnemius muscles in 10 day WBS rats (180 gm). They reported that muscle weight decreased significantly and that mean fiber diameters and areas decreased. They too argue for the continued use of the WBS in studies that will provide comparable results with microgravity exposed rats.

In the unloaded soleus both the slow and fast twitch fibers showed reduction in size and continued decay through 14 days of unloading and reversal with seven days of recovery. Flynn and Max (1985) also used a body harness system (HTD) to suspend 200 gm rats for 13 days. Their comparison of the soleus and plantaris showed that the highest level of weight loss was chiefly in the slow twitch muscle. The plasticity of muscle is noted in the recovery of both soleus and EDL within seven days after removal from hind limb unloading in the WBS harness (Musacchia et al. 1983). Also, results from our laboratory showed that muscle mass recovery following WBS were comparable to COSMOS biosatellite experiments (Ilyina-Kakueva and Portugalov, 1977). These comparatively parallel results argue for more detailed studies of recovery changes after rats are returned from space flight. Earthside models are providing substantial information that can be used in planning protocols for space flight and this is a major contribution of models for simulation of weightlessness.

The TS system has been highly valuable as a model to assess skeletal muscle atrophy and pathophysiological changes in response to disuse. Much information concerning protein turnover, as well as other biochemical facets, has been provided by Tashler and his associates (Jaspers et al. 1985, Jaspers et al. 1986, Henricksen et al. 1986). Riley et al. (1990) point out that in the TS unloaded processes are differentiated such that protein degradation is chiefly myofibrillar and, in contrast, the cell membrane and intermyofibrillar mitochondria are spared to some degree. Also, using a TS system Henricksen et al. (1986) reported an increase in insulin receptor concentrations which may explain why atrophic soleus muscles show an enhanced response to insulin.

Riley et al (1990) also report that with a week or more of unloading there is a segmental necrosis which effects about 3% of soleus, primarily, type IIa fibers. Ischemic necrosis also affects the highly oxidative type IIa fibers, and it has been reported that blood flow to the hind limb is reduced in suspension (LeBlanc et al. 1985). Thus, there is a reasonable probability that modified blood flow may exacerbate the atrophy during unloading. Another feature that has recently been recognized, and which may be related to central core lesions that occur in soleus of suspended rats, is the plantar flexion response. Riley et al (1990) reports that in TS suspended rats, and in an examination of NASA video tapes of rats in flight STS 41-B, the feet were often plantar flexed. In our laboratory, using WBS rats, plantar flexion is frequently evident. Thus in TS, WBS and weightlessness there is a common postural feature that may be adding to the atrophic response of the soleus and, for that matter, to the less commonly affected EDL.

Bone

Emily Holton - Morey (1979) has been in the forefront in the use of hind limb unloading to demonstrate the loss of long bone mass in the rat. In the growing rat, bone resorption is moderate, and bone weight is chiefly a reflection of bone formation. Results from her laboratory and others (Simmons et al. 1983 and Wronski et al. 1987) clearly show that in terms of skeletal responses the tail suspension system adequately simulates the events that occur under conditions of space flight. Globus et al. (1986) showed that with suspension there is a temporal sequence of events namely, an initial inhibition of bone formation, followed by cessation of accretion of bone weight. In brief, within the first week of unloading, there is an initial inhibition of hind limb long bone growth and vertebral formation, and at about day 10 or 12 bone formation returned toward normal. Thus, at about the two week period there is a resumption of a normal rate of bone formation. They compared changes in the unloading hindquarters with the lack of effect on the weight bearing forelimb. Using radiolabeled incorporation into the bone and tetracycline labeling for histomorphometry they were able to conclude that unloading inhibits both the modeling and remodeling of bone. They argue, and reasonably so, that the hind limb unloaded rat can provide a useful model to assess those factors essential in the regulation of in vivo bone formation.

Roer and Dillaman (1990) used a modified tail suspension system to hind limb unload 28 day old rats for periods of one, two and three weeks. They proposed an anteroposterior gradient response of bone mass loss. Comparing suspended rats to control rats there was a decrease in bone mass in the hind limbs but no significant difference in the forelimbs. They relate that bone perfusion plays a significant role. Their views are supported from the experiments of Hargens et al. (1983) who showed a decrease in blood volume and microvascular pressure and those of LeBlanc et al. (1985) who showed a decrease in blood flow to the hind limbs. Overall these experiments suggest a causative factor between cardiovascular responses and the effects of HTD. In another, and perhaps relatable, series of experiments in our laboratory, we found that during HTD with WBS rats for one week there was a significant increase in blood pressure (MAP, SP and DY) whereas in N-HTD rats the elevations in blood pressure were much less. Also, HTD for a week results in significant increases in muscle capillary density in both flight and WBS rats (Musacchia et al. 1990). We have also reached the conclusion that there are cardiovascular relationships to muscle atrophy, that more attention should be focused on the peripheral circulation of muscle and bone and that additional comparisons be made with flight exposed rats.

Cardiovascular relationships to muscle and bone modification are not yet adequately explored and where additional flight experiments are proposed, serious consideration must be given to recovery studies. These should include earthside WBS and TS experiments in conjunction with flight opportunities.

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Fluid and Electrolyte/Cardiovascular Responses

The HDT animal, whether by WBS or TS, results in a cephalad accumulation of body fluid, and this is accompanied by a modification in excretion of urine and electrolytes. Deavers et al. (1980) reported that during seven days of HDT there was an increase in urine output (diuresis) and increased Na and K excretion (natriuresis and kaliuresis). These changes were rapidly reversed, within a day or two, when the animals were removed from the WBS apparatus and returned to normal ambulatory activities. These authors interpreted their results, in part, as responses to the Gauer-Henry reflex, in which cardiac chambers are enlarged due to increased blood volume. They also demonstrated that N-HDT, WBS rats do not respond in a similar manner and concluded that it is the HDT that results in diuresis, natriuresis and kaliuresis. These and relatable experiments require further exploration.

Hargens et al. (1984) used a unique approach to demonstrate that in TS, HDT rats (30º) showed an increase in subcutaneous tissue fluid pressure in the neck region: from -2.2±0.4 cm H2O in controls to +4.0±1.3 cm H2O in 30º HDT rats after 48 hours, and in post-tilted rats, after 24 hours, the interstitial fluid shift was still slightly elevated -1.1±1.2 cm H2O. Their report suggests that the HDT rat provides a good model for simulation of microgravity responses where cephalad fluid shifts also occur. Fluid shifts coupled with the increased excretion of urine and electrolytes provide additional clues to understanding the responses seen during human and animal exposure to microgravity and during the recovery stages.

Cardiovascular Responses

Head down tilting in the human and the rat also bring about cardiovascular responses that are comparable to changes seen during microgravity exposure. During space flight there is a transient increase in cardiac volume and an increase in cardiac output; such changes are relatable to cephalad fluid shifts (Pottier et al. 1988). Cintron et al. (1990) proposed that during microgravity exposure there is a redistribution of body fluid, with an increase in central blood volume and pressure. In a human model (bed-rest with 5º tilt for 8 hours) used to simulate responses seen during space flight Hargens et al. (1983) reported a fluid shift from the legs with cephalic fluid movement. These changes were related to intracellular fluid loss and tissue dehydration.

Some initial studies HDT rats showed significant cardiovascular responses, including: elevations of mean arterial pressures (MAP) from 100 to 120 mmHg, systolic pressures (SP) from 128 to 150 mmHg and diastolic pressures (DP) from 85 to 105 mmHg (Musacchia and Steffen, 1982 and 1984). Whereas in N-HDT rats, although there were some slightly increased blood pressures, the changes were much less significant. Also, when rats were removed from WBS/HDT and returned to normal ambulatory conditions, the blood pressures rapidly returned to control levels, within 2 to 12 hours. They described this as a transient hypertension. Using TS, Shellock et al. (1985) showed a direct relationship between increased central venous pressures and the degree of tilt (20º and 45º) during a 24 hour period. In considering changes in the peripheral vasculature, Tucker et al. (1987) found transitory elevations in mesenteric and femoral arteries.

Our studies of three day (Musacchia and Steffen, 1982) and seven day (Musacchia and Steffen, 1984) HDT rats (20º) showed that significant elevations in blood pressures during the first few days were followed by what appears to be a sustained elevated response. In general, since restraint and partial immobilization can reasonably be expected to provoke space or stress responses, it is suggested that future investigations focus on at least three phases: the initial period of insult, i.e. first day or two; the period of adaptation, i.e., 7 to 14 days; and the initial days of recovery from suspension and/or microgravity exposure; and that these phases be coupled with presence of stress hormones.

Limitations in Suspension Models

It is reasonable to say that all model systems have some limitations. The WBS system has a limitation for long term experiments when growing rats are studied. As the animal gains weight, the suit must be periodically changed to accommodate the growth. This entails removal from the apparatus, administration of mild anesthetics so that a new denin velcro suit can be fitted to the rat. Thus, in long term studies of growing rats some consideration must be given to the interruption of WBS. Also, it should be recognized that body temperature may be affected. We have noted a slight degree of hypothermia during WBS (Figure 1). Is this a reflection of stress immobilization and/or the Ta of the animal room, i.e., about 24ºC? This must be acknowledged as a possible environmental insult since the neutral temperature zone for rats is about 28ºC. In the TS system there are problems of injury to the skin and development of abscesses. Also, the tail in the rat is essential to temperature regulation. If too much area is covered then the animal can no longer be efficient in temperature radiation. Also, lengthy TS can lead to lordosis. Lately, another complicating feature has come to light. In the use of male rats, TS often leads to retraction of the testes into the abdominal cavity. This does not occur with the WBS probably because the denin suit extends to the lower abdomen and forms a barrier to movement of the suspended testes. Consideration must be given to future investigations when caudal elevation may lead to cryptorchidism and thus bring about hormonal influence on physiological parameters of bone and muscle loss. These and other limitations are relevant in future experiments, particularly those planned in conjunction with space flight projects.

Figure 1.

ACKNOWLEDGEMENTS

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DESIGNING METHODS FOR MUSCULOSKELETAL CONDITIONING IN WEIGHTLESSNESS

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Limited data suggest that decreases in muscle mass and strength of the lower limbs occur in weightlessness (29, 16). In addition, numerous studies employing animal (28) or human (1, 2, 7, 8) models of muscle unloading have simulated similar responses. Because of the magnitude of these adaptations, countermeasures must be developed for future use in space.

Several strategies have been proposed as countermeasures to the physical deconditioning that occurs in microgravity, e.g. artificial gravity (3), pharmacological treatment (20) or lower body negative pressure (14). Although these interventions may be of some benefit to combat muscle deconditioning they have mainly been considered to limit or prevent cardiovascular deconditioning and orthostatic intolerance (20). Electrical muscle stimulation is another proposed (12) time-effective method which would require no voluntary effort. There are reasons to believe, however, that the use of this technique would be painful and hardly admit the performance of simultaneous work tasks and therefore not very likely be accepted by crews. Data is not at hand demonstrating that any of these methods is effective in producing increases in muscle strength or mass at 1–9.

Responses to Physical Exercise

Physical exercise, so far has been the most extensively used countermeasure in space. Unfortunately, these efforts have mainly emphasized aerobic exercise promoting increases in maximal aerobic power and endurance (22). Exercise regimens of aerobic nature, however, will not produce muscle hypertrophy or increased strength. It is therefore not surprising that in-flight cycle ergometry or low-impact treadmill exercise have not been effective to combat muscle atrophy in space (6). Physical training using heavy resistance induce substantial increases in muscle mass and strength (19). When performing such exercise regimens weights are typically raised and thereafter lowered while resisting the weight. These two phases are referred to as concentric and eccentric muscle actions and are integrated parts of daily weight-bearing locomotion (4). Energy cost is much less during eccentric than concentric actions (11). Also, maximum voluntary or involuntary force and force relative to motor unit activation is greater for eccentric than concentric actions (9, 26). It also appears that fatiguability is greater during concentric than eccentric exercise (26). Whereas the limited physical work performed in space comprises mainly concentric muscle actions eccentric muscle activation is virtually absent in flight. Those facts may have important implications for the design of exercise equipment and prescriptions in space.

Physiological Requirements

Athletes aiming at increasing muscle strength and size, perform resistance training using dynamic rather than isometric muscle actions because of its superiority to develop muscle mass (16, 25) and to produce increased strength throughout the full range of motion (10, 21). To achieve near–maximal muscle tension, which probably calls for recruitment of the entire motor unit pool of the muscle, they typically perform a limited number of sets and choose weights resulting in muscle failure within 6–12 repetitions. (19). Furthermore, recent studies have underlined the importance of carrying out coupled concentric and eccentric muscle actions, for optimum training results (5, 10, 15, 17). Exercise regimens using concentric actions only is less effective. Because heavy–resistance exercise, using both concentric and eccentric muscle actions, has been shown to be most effective in inducing increases in muscle strength and mass it appears likely that it will also prevent atrophy and strength loss.

Technical Requirements

Equipment serving in resistance training should meet certain technical requirements. Some of these features are related to the actual exercise routine or the specific muscle group. Intrinsic factors, e.g., actual muscle length and the actual joint lever and one–or multijoint function of the muscle, mode and speed of contraction also impact the external force generating capacity and thus the design of the training equipment for resistance training.

In addition to barbells and dumb–bells weight–stack machines are commonly used to produce resistance. There is a desire, however, to maintain a constant relative resistance throughout the whole range of motion. Exercise machines therefore use advanced lever arm or cam systems to compensate for the biomechanical variations related to joint anatomy and muscle length. An alternative approach to maintain the same relative muscle tension throughout the range of motion and to circumvent problems related to biomechanical leverages is to use an atalowered dynamometer ("isokinetics"). Using such a device force is exerted against a lever arm which moves at a preset controlled velocity. Hence, ankle and knee joint torque, for example, matches the accommodated resistance.

Requirements in Weightlessness

Musculoskeletal conditioning in space should emphasize training of the extensor muscles of the lower body, since these postural muscle groups are most severely affected by microgravity (6, 29).

Lower limb muscles produce impressive forces. Therefore training of these muscles place high demands on the biomechanical solution and materials strength of exercise equipment. For example, a non–athletic male subject performing the supine leg press routine typically use resistance corresponding to 100 up to 200 kg of vertical load. Thus an effective exercise device should be able to offer resistance exceeding twice the body weight.

Technical or physiological requirements discussed so far, apply to ground–based or space–flown equipment. There are, however, certain requirements exclusive for exercise equipment in microgravity. Because of the weightless environment, barbells, dumb–bells or weight–stack machines cannot be used in space. When designing a gravity–independent exercise tool it is therefore necessary to replace the gravitational pull from a lifted weight by an alternative force or power source. In the following, principles and merits of currently available and proposed exercise devices for use in space are discussed.

There are several exercise ergometers available that use dampers or friction to produce resistance. However, those like many isokinetic dynamometers, accomplish concentric resistance only. More recent commercial devices (13, 24) and research prototypes (23, 27), also provide eccentric resistance. Therefore they are potentially more effective as exercise tools. However, electrical power in the order of 2–4 kW is required to supply the eccentric exercise mode. With a reported energy budget for the entire space station in the range of 70 kW and only 10–15 kW available for scientific experiments, regular access to such electrically powered equipment seems remote. Power supply therefore has to be sought elsewhere.
Elastic cords or other spring-loaded equipment provide resistance during both concentric and eccentric muscle actions with no need for external power supply. However, no such device has yet been introduced that offers the load profile necessary for resistive training of the lower limbs. Modified treadmills have been used for squating exercise in space. The vertical loads produced by the accessory bungy cords corresponds to 50–80 kg. This is markedly less than what is typically used in ground-based resistance training and probably not a sufficient stimulus to prevent muscle atrophy and decreases in strength.

A Gravity–Independent Strength Ergometer

To accomplish ground–based evaluation and to allow astronaut familiarization preflight any exercise device should be constructed so that mass–transfer occurs in the horizontal plane.

We have recently developed a device to be used for resistance training in microgravity. It is non–electrically powered, yet gravity–independent and has features similar to traditional equipment known to be effective in producing the desired adaptive responses.

This strength ergometer is constructed for seated leg press (Fig. 1) and uses the fly–wheel principle to produce resistance. By pushing against a footplate the applied force will start unwinding a cord connected to the fly–wheel thereby imparting energy to the fly–wheel. Kinetic energy (Ekin) will increase as a function of the rotational speed (Fig. 2). Once the concentric muscle action is completed the cord will start rewinding and the trainee resists the pull of the fly–wheel by performing eccentric, or braking, muscle actions. The principle is analogous to that of a yo–yo. No external power source is demanded except for the power of the muscles. Resistance is adjusted through speed control by changing diameter of the fly–wheel. The linear force may exceed 300 kg while performing seated maximal bilateral leg press actions. The prototype, crafted in aluminum with fly–wheels in PVC–polymer, has an overall weight of 60 lbs (27 kg).

Any exercise equipment can potentially distort the field of microgravity in a space station or shuttle. This is a major concern for many of the experiments that will be carried out in space. Using a closed mechanical chain, this ergometer, however, can easily be held free–floating from the surrounding walls.

The features of this strength–inertia ergometer has been validated in in–vivo experiments. Muscle activation–patterns, force and power production and energy expenditure was measured in six volunteers who performed repeated exercise bouts. The responses were compared to those produced by exercise using a supine leg press, or an isokinetic knee–extension dynamometer (27). For each bout of exercise 10 maximal voluntary repetitions (isokinetic dynamometer, strength ergometer) or 10 constant load repetitions resulting in failure after 10 repetitions (supine leg press) were performed. Electromyographic (EMG) activity from three aspects of the quadriceps muscle and the calf was recorded by use of surface electrode technique (Fig. 3). Quadriceps and calf activation patterns for concentric and eccentric muscle actions were very similar for exercise using the strength ergometer or the supine leg press. Using the electrical isokinetic dynamometer the quadriceps showed a similar response but the calf muscle showed no activation. When using the strength ergometer the mean force produced averaged 1770 N (180 kg) while mechanical power developed during the concentric muscle actions averaged 128 W. The modest power output was accompanied by very low energy expenditure. Thus oxygen uptake averaged 1.5 l·min⁻¹.

These results and those of earlier studies suggest that resistive exercise can be carried out at a moderate oxygen cost and that eccentric muscle actions are performed at a very low metabolic cost, perhaps only 1/6 of that typical for concentric actions. Hence, the acute exercise responses shown using this dynamometer suggest that it can serve and be effective in resistance training. Because of its mechanical features it could be used in space.
Summary

There is an immediate need to find methods to combat the skeletal muscle deconditioning that occurs in microgravity. Important features to be considered for any ergometer or exercise method to be used as a countermeasure against musculoskeletal deconditioning in space include heavy muscular loading of the postural muscles of the lower limbs and that eccentric and concentric muscle activations can be performed. Those are major requirements to produce optimal gains in strength and muscle mass at t"""" and probably to counteract muscle atrophy and strength loss in microgravity. Resistance exercise with a strength–ergometer, using the fly–wheel principle was carried out at a low oxygen cost and required no external power supply. We have developed a resistance training system employing this ergometer so that force and power production easily can be monitored and calibrated. It is suggested that the features of this ergometer meet the presented requirements for use during long–term space missions.

References


PULMONARY FUNCTION IN MICROGRAVITY

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INTRODUCTION

The lung is exquisitely sensitive to gravity, which induces large gradients in bloodflow, alveolar size, ventilation, and gas exchange. These gradients have been measured by direct techniques. They have been widely invoked to explain many of the gradients in gas concentration seen during exhalation, especially during single breath nitrogen (SBN) washouts although more recently many non-gravitational mechanisms have been postulated. Careful studies using catheters in small animals have established that at least a considerable part of the phase III slope of the SBN washout is non-gravitational (1) and this finding has been re-enforced by an aircraft microgravity study in which phase III slope did not change significantly (2). Since then, there have been a large number of experimental and theoretical studies of gas mixing in the lung where the emphasis has been on non-gravitational mechanisms that produce inhomogeneity (3). There have been questions regarding the origins of cardiogenic oscillations in gas concentration. While the presence of large apical to basal (i.e., remote and peri-cardiac) gradients present an attractive explanation, curious changes in the phase relationships between oscillations of N₂ and CO₂ in the SBN washout during exhalation suggest the presence of non-gravitational mechanisms (4). However, oscillations were reported to virtually disappear during aircraft parabolas (2).

Headward fluid shifts have long been suspected during microgravity, and a long standing hypothesis has related this fluid shift, and postulated enlargement of intrathoracic vascular structures, to a proposed regulatory diuresis and other adaptations to spaceflight. It has even been suggested that pulmonary edema, albeit subclinical, might be a feature of early orbital flight (5).

For these reasons, pulmonary function measurements on orbit were proposed over a decade ago. We can now report the successful outcome of the first major study of pulmonary function on orbit, performed on the SLS-1 mission.

METHODS

We prepared a pulmonary function test system incorporating a NASA respiratory mass spectrometer and experiment computer, and an automated bag-in-box and flowmeter system that we developed to allow crew members to perform the following tests (6):

- quiet breathing for two minutes, allowing measurement of resting ventilation and gas exchange, and a final full exhalation allowing ERV measurement
- intra-breath studies of O₂ and CO₂ gradients during exhalations to RV, from both TLC and FRC (7)
- intra-breath studies of O₂ and CO₂ cardiogenic oscillations after a hyperventilation/breathhold maneuver (2)
- SBN washouts, with an initial argon bolus and inhalations from both RV and FRC
- multibreath nitrogen washouts, with computer controlled tidal volumes of both 700 ml, and 1200 ml (8,9)
- normoxic and hyperoxic single breath carbon monoxide uptake tests (10)
- argon/N₂O rebreathing test with resting lung volume and pulmonary blood flow estimations
- forced expiratory spirometry for ensemble averaged MEFV curve comparisons (11)

The tests were performed in a sequence that separated the foreign gas tests by cabin air breathing, to minimize interactions. The sequence took slightly more than twenty minutes of each subject's time. Syringe, sampling transit time, and gas calibration began and concluded each test day. More frequent gas and transit time calibration was required on days 4 and 5 of the mission when mass spectrometer problems occurred. The tests were performed repeatedly pre-flight, in-flight (days 2, 4, 5 and 9) on the 4 payload crew members, and they were also performed pre-flight and once in-flight (day 4) on the 3 orbiter crew members. The SBN washouts from higher pre-inspiratory volumes, and multibreath nitrogen washouts at 1200 ml tidal volumes were performed in-flight on day 4 only, by the 4 payload crew members. Testing was also performed repeatedly post-flight, over a 6-day period, on both the payload crew (starting on the day of landing) and orbiter crew (starting the day after landing).
Figure 1

Standing SBN data obtained pre-flight. Note the prominent cardiogenic oscillations, especially of argon, which was inhaled as an initial 150 ml bolus. Note the late rise (phase IV) of argon and nitrogen and fall of CO₂ concentration. The CO₂ oscillations are in opposite phase to N₂ and argon. These features all suggest normal apico-basal gradients in gas concentration.

Figure 2

SBN data obtained on day 4 of the SLS-1 mission. Note that the oscillations and terminal inflections seen on the N₂ and argon tracings in Fig. 1 largely persist. There is a slightly reduced N₂ and argon phase IV volume and rise. (some subjects show little or no phase IV for nitrogen, however). Note, however, that the late fall of CO₂ is no longer present and, in fact, CO₂ and N₂ oscillations are in phase at mid exhalation.
RESULTS and DISCUSSION

We have just received the flight data from NASA, and can report our initial findings having completed preliminary analyses of the SBN, rebreathing, and CO uptake tests. We have commenced analyses of the other tests, and the data quality is good. There were a number of problems with both the primary and standby mass spectrometer during the mission, which included both blockage of a sampling system orifice with particles, and intermittent noise and signal instability. Despite these problems, we obtained excellent data on days 2, 4 and 9. Our data was impacted by mass spectrometer problems on day 5, and some may not be recoverable.

We report findings from the 4 payload crew members. We must await, however, data from the orbiter crew and all recoverable data from day 5, before we perform our definitive analyses.

The striking findings are:

Cardiovascular system. Stroke volumes were more than 150% of the pre-flight standing control value (versus approximately 140% for pre-flight supine values), on flight day 2, our first measurement opportunity. They fell progressively, being still approximately 125% of the standing control value on day 9. Post-flight there was the expected initial marked fall in standing stroke volumes to less than 75% of control, and, in fact, the supine stroke volumes were at approximately the pre-flight standing control value. Changes to cardiac output were less striking as heart rates were decreased in flight, especially on day 4.

The D_{CO} remained above 125% of the pre-flight control value throughout the mission. This was greater than was seen in the supine measurements pre- and post-flight. The pre- and post-flight measurements were extremely stable, and even immediately after landing, were very close to pre-flight control values. Hyperoxic D_{CO}s allowed estimation of the membrane diffusing capacity (Dm) and pulmonary capillary blood volume (Vc). In-flight Vc was elevated to slightly less than 125% of the standing control value, and was very close to pre-flight supine measurements. Dm was also elevated in flight, and higher than both pre-flight standing and supine measurements, which were of similar magnitude. The D_{CO} data suggest that the pulmonary blood volume remains elevated on orbit. However, as Dm was always elevated, the specter of early interstitial pulmonary edema (5) seems unlikely, at least at rest, and after the first day on orbit.

Respiratory system. The rebreathing test allowed us to estimate the FRC. The values obtained were intermediate between standing and lying values, consistent with the absence of gravitational standing caudal movement of the diaphragm, and supine cranial movement of the diaphragm.

There was a small ~250 ml reduction in vital capacity, during the first in-flight measurements on the second day on orbit which returned to pre-flight values on days 4 and 9. Reductions of this magnitude have been reported during aircraft parabolas (11,12) and ascribed to an increase in intrathoracic blood volume.

There was an ~25% reduction in the slope of the phase III nitrogen trace, on orbit. We had not obtained data of sufficient quality or quantity to see such a reduction, in the earlier aircraft microgravity study (2). Analysis of the multibreath nitrogen washout, and SBN washouts at more physiological pre-inspiratory volumes, should help define the origin(s) of the non-gravitational component of phase III slope (9).

Interestingly, cardiogenic oscillations of nitrogen, argon, and CO2 which seemed to virtually disappear during aircraft parabolas, were still quite prominent on orbit, at about ~25% of the pre-flight value for argon, and ~50% of the pre-flight value for CO2 and N2 (compare Figs. 1 and 2). Airways closure, as evidenced by phase IV changes, were still very apparent on the argon (bolus) tracings, at comparable lung volumes on orbit. However, resident gas (N2) tracings did not show a consistent phase IV pattern on all subjects.

Summary

We report the successful collection of a large quantity of human resting pulmonary function data on the SLS-1 mission. Preliminary analysis suggests that cardiac stroke volumes are high on orbit, and that an adaptive reduction takes at least several days, and in fact may still be in progress after 9 days on orbit. It also suggests that pulmonary capillary blood volumes are high, and remain high on orbit, but that the pulmonary interstitium is not significantly impacted.

The data further suggest that the known large gravitational gradients of lung function have only a modest influence on single breath tests such as the SBN washout. They account for only ~25% of the phase III slope of nitrogen, on vital capacity SBN washouts. These gradients are only a moderate source of the cardiogenic oscillations seen in argon (bolus gas) and nitrogen (resident gas), on such tests. They may have a greater role in generating the normal CO2 oscillations, as here the phase relationship to argon and nitrogen reverses in microgravity, at least at mid exhalation in those subjects studied to date. Microgravity may become a useful tool in establishing the nature of the non-gravitational mechanisms that can now be seen to play such a large part in the generation of intra-breath gradients and oscillations of expired gas concentration. Analysis of microgravity multibreath nitrogen washouts, single breath washouts from more physiological pre-inspiratory volumes, both using our existing D-2 and SLS-2 missions, should be very fruitful in this regard. It is planned to carry out studies during and after heavy exercise on future missions, and increase the range of studies to include the extent of aerosol deposition, and the regulation of respiration. This seems especially logical now that we have the SLS-1 data as it seems that microgravity per se, is well tolerated by the lung on short duration missions.
REFERENCES


ALTERATIONS IN GLOMERULAR HEMODYNAMICS AND TUBULAR REABSORPTION AFTER 24 HOURS HEAD DOWN-TILT AND FOLLOWING ACUTE RETURN TO ORTHOSTASIS

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INTRODUCTION

Transition from a 1g environment to conditions of microgravity will produce a cephalad fluid shift (1) resulting in redistribution of fluid both in vascular and interstitial spaces. Since most vascular baroreceptors and stretch receptors are located at the heart level or above, this fluid redistribution may generate signals to the kidney similar to fluid volume expansion. Plasma volume expansion has been shown to increase glomerular filtration rate (GFR) both in humans (2) and rats (3). In an effort to mimic the conditions of microgravity experienced during spaceflight, we have used 25° head-down tilt (HDT) in the rat to examine the effects of microgravity on renal function and fluid volume homeostasis (4-6). Previous studies from this laboratory investigating the effects of simulated microgravity on renal function have demonstrated an elevation in whole kidney glomerular filtration rate which was associated with increases in renal plasma flow (5). However, these initial findings did not examine the specific alterations in glomerular hemodynamics in early phases of simulated microgravity.

Since exposure to microgravity will produce a cephalad fluid shift, then return to normal gravity should produce the opposite fluid shift with the attendant response in renal function. Utilizing return to orthostasis after only 24 hour HDT to examine the glomerular hemodynamic and tubular fluid reabsorption response to these fluid shifts should effectively separate the influences of the fluid shift in HDT from cardiovascular deconditioning, which is observed during longer duration spaceflight or simulated microgravity.

The present study utilizes micropuncture techniques to examine the specific changes in the determinants of glomerular ultrafiltration and tubular fluid reabsorption in the proximal tubule and the loop of Henle after 24 hours HDT while remaining in HDT followed by repeated measurements after 1 hour return to orthostasis.

METHODS

Micropuncture experiments were performed on male Munich-Wistar rats weighing 210-270 gm. The animals were maintained at the Veterinary Medical Unit of the San Diego Veterans Affairs Medical Center.

Head-down tilt procedure. Experimental rats (n=7) were positioned in a head-down tilt apparatus (25°) for 24 hours using a procedure described by Morey-Holton and Wronsiki (7) and described in a recent publication from this laboratory (5). The rats were maintained in a HDT position at all times, during surgical preparation for micropuncture and continued through the first period micropuncture measurements. At the end of the first measurement period, the rats were re-positioned on a horizontal axis, a 60 min re-equilibration period was allowed and the micropuncture measurements were repeated.

In the control group of rats (n=8), no HDT maneuvers were performed and the rats were housed in standard cages until the time of the experiment.

Micropuncture studies. On the day of the study, the rats were anesthetized with Inactin (100 mg/kg BW ip) and preparations for micropuncture measurements were as previously described (8). An infusion of [3H] inulin at a rate of 200 μCi/hr was initiated 60 min before the first micropuncture measurements and was continued throughout the remainder of the study as a marker of glomerular ultrafiltration. An infusion of an isotonic solution of NaCl and NaHCO3 was maintained throughout the surgical preparation at a rate of 0.6% BW/hr. An infusion of donor plasma of 1% BW/hr was initiated 60 min before the measurement periods and reduced to 0.15% BW/hr during the measurement periods. All measurements of the determinants of glomerular ultrafiltration were as previously described (8).

Analysis of all samples were performed as previously described in several publications from this laboratory (5,8).

Statistical Analysis. Significance of the data between 24 hr HDT and post-HDT conditions was determined by analysis of variance and paired Student's t test (9). Comparisons between Control and other conditions were analyzed by unpaired t test. All data values are given as the means ± SE.

RESULTS

The effects of 24 hr HDT and return to orthostasis on mean arterial blood pressure (MAP), urine flow (U), glomerular filtration rate (GFR), and renal plasma flow (RPF) are depicted in Table 1.

Table I. Effect of 24 hr HDT on MAP and whole kidney function

<table>
<thead>
<tr>
<th></th>
<th>MAP (mm Hg)</th>
<th>U (ml/min)</th>
<th>GFR (ml/min)</th>
<th>RPF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>112±2</td>
<td>1.2±0.3</td>
<td>0.97±0.05</td>
<td>2.9±1.3</td>
</tr>
<tr>
<td>24 hr HDT</td>
<td>108±2</td>
<td>3.0±2.3*</td>
<td>1.08±0.07</td>
<td>5.3±1.8*</td>
</tr>
<tr>
<td>Post HDT</td>
<td>104±2*</td>
<td>2.9±1.2*</td>
<td>0.92±0.11</td>
<td>3.9±1.6</td>
</tr>
</tbody>
</table>

*P<0.05 Compared to control

Urine flow was increased after 24 hr HDT and remained elevated during the post-HDT measurements. GFR was slightly but not significantly increased after 24 hr HDT compared to Control but decreased during post-HDT (P<0.05). There was an increase in RPF after 24 hr HDT (P<0.05) and decreased during post-HDT (P<0.05) to values not different from control.

Effects of 24 hr HDT and post-HDT recovery on the determinants of glomerular ultrafiltration. The effects of 24 hr HDT and post-HDT on SNGFR are depicted in Fig. 1. The increase in SNGFR from 33±2 to 45±2 ml/min is directly correlated to increases in single nephron plasma flow (SNPF) with SNPF increased in 24 hr HDT and then returned to values not different from control in post-HDT as shown in Fig. 2.

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DISCUSSION

The cephalad fluid shift induced by 24 hr HDT in the rat resulted in increases in both SNGFR and SNPW with parallel increases in whole kidney function. Of the determinants of glomerular ultrafiltration, this increase in SNGFR at 24 hr HDT was best associated with increases in renal function and not alterations in the glomerular hydrostatic pressure gradient or glomerular ultrafiltration coefficient. Even in a small mammal such as the rat, fluid movement induced by 25° HDT will result in alterations in renal function similar to that observed with plasma volume expansion and may find some parallels in the human physiologic response to microgravity. The increased filtered load can potentiate diuresis and nephrogenesis if the compensatory increases in tubular fluid reabsorption are insufficient to prevent increased fluid flow exiting the distal tubule. This appeared to be the case in the present study, with increased distal tubule flow rate at 24 hr HDT and continuing during post-HDT recovery. Although the increase in distal tubule flow rate from 8 to 11 nl/min represents a less than 30% increase in flow, this increase is more than sufficient to increase urine flow to the observed values in this study (Table 1).

One hour orthostasis after 24 hr HDT in the rat was sufficient to restore the determinants of nephron filtration rate and fluid reabsorption in the nephron to values not different from control rats. However, distal tubule flow rate remained increased compared to control and most likely contributed to the continued diuresis.

In summary, short term simulated microgravity in the anesthetized rat will increase renal plasma flow and nephron filtration rate as well as increase fluid flow in the distal tubule, promoting diuresis. One hour return to normal orthostatic position after 24 hr HDT restores most renal function parameters to normal, with the exception of increased distal tubule flow rate and urine flow.

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REFERENCES

EFFECT OF 14 DAY HEAD-DOWN TILT ON RENAL FUNCTION AND VASCULAR AND EXTRACELLULAR FLUID VOLUMES IN THE CONSCIOUS RAT

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INTRODUCTION
The cephalad fluid shift induced by conditions of microgravity or simulated microgravity, such as head-down tilt, has been associated with the observed negative fluid and electrolyte balance that occurs under these situations (1). The initial response to the cephalad fluid shift should stimulate baro and stretch receptors located at the heart or above to decrease adrenergic activity and stimulate production of atrial natriuretic peptide (2). Therefore acute head-down tilt may impact renal function similar to plasma volume expansion with increases in renal plasma flow and glomerular filtration rate (3) with attendant diuresis and natriuresis. However, since head-down tilt or microgravity does not increase total body water or electrolyte content, then the result should be volume depletion over time. This may result in volume redistribution in the various fluid compartments and modify renal function.

The present study obtained measurements in chronically catheterized awake rats positioned in 25° head-down tilt for 14 days followed by a 7 day recovery period to examine changes in vascular and extracellular volumes and alterations in renal function including changes in renal plasma flow and glomerular filtration rate.

METHODS
Experiments were performed on male Wistar rats weighing 210-350 gm. The animals were maintained at the Veterinary Medical Unit of the San Diego Veterans Affairs Medical Center. The rats were familiarized to human handling and trained to stay quietly in a restraining cage with a minimum of three 2-3 hour sessions prior to the chronic cannulation procedure and measurement periods.

Chronic cannulation procedure. After the training was completed the animals were anesthetized with Brevital (67 mg/kg BW, ip) with small doses thereafter as required. Tygon catheters were placed in the left femoral artery and vein (4,5). The vascular catheters were threaded under the skin and exteriorized at the back of the neck as previously described (4). A 14-gauge steel cannula, enclosed in Silastic tubing was implanted in the bladder and plugged with a stainless steel pin covered in Silastic providing the animal to void normally through the urethra, thus reducing the likelihood of bladder infection (4).

Head-down tilt procedure. All rats were positioned in a head-down tilt (HDT) apparatus (25° HDT) for 14 days using a procedure described by Morey-Holton and Wronski (5), slightly modified and described in recent publications from this laboratory (4,5). The rats were maintained in a HDT position at all times during the 14 day head-down tilt procedure.

Conscious rat studies. The first group of rats (n=8) were placed in a restraining cage 6-7 days after the cannulation surgery, and the first of two control measurement periods were performed. The bladder pin was removed to allow collection of urine. The arterial line was connected to a pressure transducer for measurement of mean arterial pressure (MAP) and was also utilized for blood sampling. A solution of PAH and 3H inulin suspended in normal saline was infused at a rate which approximates urinary output (4). After the control, pre-tilt measurements were performed, the rats were placed in head-down tilt and measurements were repeated at 1, 3, 7, 10, and 14 days during HDT. After the day 14 HDT measurements were completed, the rats were returned to a horizontal, 0° tilt, position and measurements repeated at 1 hour, 1, 3, and 7 days post-HDT.

In a second group of chronically catheterized awake rats without the bladder catheter (n=20), plasma and blood volumes were measured using 125I labeled albumin and 51Cr labeled erythrocytes in non-tilt, and 1, 7, and 14 days 25° HDT (n=5 at each time point) as previously described (7).

Analytic methods. Extracellular fluid volume, glomerular filtration rate (GFR), and renal plasma flow (RPF) were calculated as previously described (4). Plasma and blood volumes were also performed and calculated as previously described (7). Urine flow and electrolyte excretion measurements were also performed as in previous studies (4,5).

Statistical Analysis. Significance of the data between pre-tilt and HDT and post-HDT conditions was determined by analysis of variance or Tukey weighted t test (8). All data values are given as the means ± SE.

RESULTS
Body weight in the rats did not change during the duration of the 14 day HDT. During the 7 day post-HDT period, body weight increased by day 7 compared to pre-tilt values. The effect of HDT and post-HDT recovery on MAP are depicted in Figure 1.

MAP increased significantly by day 3 HDT and remained elevated compared to control values throughout the duration of HDT and post-HDT. The effects of 14 day HDT and post-HDT recovery on GFR and RPF are depicted in Figures 2 and 3. GFR increased at day 3 of HDT which is most likely due to the increase in RPF. After day 3 HDT, there were no differences in either GFR or RPF compared to control. Urine flow was also increased at day 3 HDT. However, on a paired basis, on day 14...
HDT, both GFR and RPF decreased after 1 hr return to orthostasis (P<0.05) compared to the same day HDT measurements. The increase in RPF was associated with an early increase in ECF, which occurred on day 1 HDT depicted in Figure 4.

ECF was not different from control during the rest of the HDT period. However, ECF did increase to levels greater than control at day 1 post-HDT and was significantly greater than the previous measurement period (1 hr post-HDT, P<0.05). The alterations in blood volume (BV) during HDT were somewhat different. BV increased slightly, but not significantly at 1 day HDT and was significantly decreased at 7 day and 14 day HDT as shown in Figure 5. These data indicate a dissociation between MAP, ECF and BV with renal function alterations associated with changes in ECF and not MAP or BV.

**DISCUSSION**

Exposure to conditions of microgravity or simulated microgravity can result in moderate fluid shifts from the various fluid compartments with resulting alterations in renal function. In the early phase of HDT, the rats exhibited an increase in extracellular fluid volume of approximately 4% of total body mass (=15% of ECF) without any decrease in body weight. This would indicate a decrease in intracellular fluid volume early in the term of HDT as observed in previous studies (4). The resultant shift of fluid from intracellular to extracellular space most likely contributed to the observed increase in renal plasma flow and glomerular filtration rate which resulted in the early increase in urine flow. After day 3 and throughout the duration of 14 day HDT, GFR, RPF, and ECF remained at values not different from control.

In contrast, blood volume measurements obtained from a second group of rats demonstrated a different pattern of fluid volume alterations. In the early phase of HDT, blood volume increased slightly with a significant decrease at days 7 and 14 during HDT. The decrease in blood volume without significant change in ECF indicates that by day 7 HDT the interstitial compartment of extracellular fluid space had moderately increased in the later period of the 14 day HDT. The shift of extracellular fluid from vascular to interstitial compartments strongly suggest an alteration in normal volume homeostatic mechanisms which are likely to be due to neuro-hormonal changes. Since this decrease in blood volume was not reflected by a similar decrease in ECF, the rats were less volume depleted than suspected from just BV measurements alone. However, the data also indicate that the vascular compartment is probably the most vulnerable to volume depletion during HDT in the rat. In addition, since hematocrit did not change in these rats, red blood cell mass was also decreased by day 7 and more so at day 14 HDT.

None of the fluid shifts correlated well with the changes in mean arterial pressure except for the increase in ECF at day 3 HDT correlating with the increase in MAP at the same time point. After day 3, and for the duration of the study, the MAP was increased while ECF returned to values not different from control and BV decreased indicating a dissociation of blood volume with MAP.

In the post-HDT recovery phase, all parameters examined were not different from control values by day 7 post-HDT except for the continued elevation of MAP. However, in the first post-HDT measurement period, 1 hour after the 14 day HDT measurements were obtained, there were significant decreases in both GFR and RPF compared on a paired basis to the previous measurement period (14 day HDT) without a decrement in ECF indicating a fluid shift towards the lower torso with the resultant effects on the neuro-hormonal signals regulating these aspects of renal function.

In summary, the cephalad fluid shift induced by head-down tilt has a marked effect on the mechanisms that control volume homeostasis, impacting extracellular and intracellular fluid volumes, blood volume, and renal function during the 14 day duration of simulated microgravity and to a smaller degree, during post-HDT recovery in the rat.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

SINGLE HINDLIMB WEIGHT BEARING BY THE RAT DURING SIMULATED MICROGRAVITY

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INTRODUCTION
The suspension of the rat with hindlimbs non-weight bearing has been used to simulate the conditions of microgravity for the study of muscle growth, atrophy and metabolism. Among the changes that are observed for rats during suspension include atrophy or reduced growth rates for anti-gravity muscles (1-3, 5,6), increased glycogen concentrations for atrophied muscles (3,5), and increased responses to insulin for glucose uptake and metabolism (1,3,5). These enhanced responses to insulin may be related to increased insulin receptor densities (1,3) or glucose transporter protein GLUT-4 concentrations (2) which have been observed in atrophied soleus muscles of young rats after suspension. However, it remains uncertain whether the increased muscle responses to insulin are caused by the removal of the weight bearing stimulus from the hindlimbs or from systemic changes in endocrine or metabolic function related to the restraint or position of the rat. The purpose of this study was to compare insulin stimulated 2-deoxyglucose uptake in muscles from non-weight bearing and weight bearing hindlimbs of suspended rats with responses of muscles from control rats. It was hypothesized that atrophied muscles from non-weight bearing rat hindlimbs would exhibit increases in insulin stimulated 2-deoxyglucose uptake and that weight bearing during suspension would prevent both the atrophy and the increased responses to insulin.

METHODS
Adult male Sprague-Dawley rats (250-325g) were assigned to either cage control conditions (CC) or to a 45° head down suspension position (SUS) for 14 days. The SUS animals were positioned so that the right hindlimb (R) was bearing 20% of body mass and the left hindlimb (L) was non-weight bearing. Weight bearing by SUS-R was accomplished using a platform connected to a rod in sleeve, cable, and pulley apparatus to which weight could be added (Figure 1). Knee-ankle-foot angles were determined on days 2, 6 and 12. On day 14, the rats were either killed for the measurement of soleus (SOL), plantaris (PL), gastrocnemius (GAST), and extensor digitorum longus (EDL) muscle masses and glycogen concentrations or surgically prepared for hindlimb perfusion (4). For the hindlimb perfusion experiments, SUS and CC hindlimbs were perfused for 15 minutes with Krebs-Henseleit buffer containing aged rejuvenated human erythrocytes, 4% albumin, 1 mM 2-deoxyglucose, 0.5 mM mannitol, 0.15 mM pyruvate, and either 0, 250, or 24,000 μU.ml⁻¹ insulin. In addition, either 1H 2-deoxyglucose or 14C mannitol tracers were added to the perfusate for the determination of 2-deoxyglucose uptake or extracellular space respectively by the SOL, PL, EDL, and the white gastrocnemius (GW).

FIGURE 1. SUSPENSION WITH SINGLE HINDLIMB WEIGHT BEARING

RESULTS
After the 14-day experiment, the CC rats increased body mass by 19%, whereas the SUS rats exhibited a 9% reduction. Joint angles for SUS-R and CC remained similar (20-30°) throughout the experiment while the SUS-L hindlimbs extended to angles of >150° by day 12. Significant atrophy of the SOL, PL, and GAST muscles was observed in the SUS-L hindlimbs (Table 1). Weight bearing by the SUS-R hindlimbs prevented the atrophy of these muscles, but the PL and GAST muscles did not reach the final mass values of the CC rats. SOL and PL glycogen concentrations were elevated 87 and 39% respectively in the SUS-L hindlimbs, whereas this increase was prevented with weight bearing in the SUS-R muscles.

Extracellular space measured during hindlimb perfusion was significantly higher for the SOL muscles from both the SUS-R and SUS-L hindlimbs (64%) when compared to CC. No significant differences in extracellular space were observed between SUS-L, SUS-R, and CC conditions for the PL, GW, or EDL. In addition, hindlimb perfusion experiments revealed that 2-deoxyglucose uptake at a maximally stimulating insulin concentration (24,000 μU.ml⁻¹) was significantly increased in all the muscles examined for both the SUS-L and SUS-R hindlimbs when compared to CC muscles (Figure 2). 2-Deoxyglucose uptake rates during perfusions at 250 μU.ml⁻¹ insulin were also significantly higher for the SUS-R SOL, EDL, and GW, and the SUS-L SOL and EDL muscles when compared to CC (Figure 2).

TABLE 1. MUSCLE MASS (mg)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>CC</th>
<th>SUS-R</th>
<th>SUS-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOL</td>
<td>111±3</td>
<td>120±2</td>
<td>124±7</td>
<td>79±5*</td>
</tr>
<tr>
<td>PL</td>
<td>311±11</td>
<td>374±6*</td>
<td>324±14</td>
<td>276±7*</td>
</tr>
<tr>
<td>EDL</td>
<td>117±3</td>
<td>127±5*</td>
<td>95±3*</td>
<td>108±3</td>
</tr>
<tr>
<td>GAST</td>
<td>1593±27</td>
<td>1941±34*</td>
<td>1474±85</td>
<td>1254±40*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n=4-13)
*Significantly different from pre-condition
§Significantly different from SUS-R mean (p<0.05)
FIGURE 2. 2-DEOXYGLUCOSE UPTAKE

Values are means ± SE; each data point represents 6-11 muscles. *Significantly different from CC (p<0.05).

DISCUSSION

This study was designed to examine the relationship between muscle weight bearing and the increases in insulin stimulated glucose or glucose analog uptake previously observed in suspended rats (1,3,5). The results indicated that hindlimb weight bearing during suspension (SUS-R) prevented the atrophy of the SOL, PL, and GAST muscles. In addition, the increases in glycogen concentration for the SOL and PL did not occur in the SUS-R hindlimbs. However, weight bearing did not prevent the suspension induced increase in 2-deoxyglucose uptake rates in the presence of insulin for any of the muscles examined or the higher extracellular space values for the SOL. These findings suggest that the removal of weight bearing is not the primary cause of increased hindlimb muscle responses to insulin during suspension, but that a whole body endocrine or metabolic influence may be involved. Supporting this interpretation is the finding that both the GW and EDL exhibited significantly increased 2-deoxyglucose uptake rates in the presence of insulin after suspension when compared to controls. It is unlikely that the removal of weight bearing would be involved with the increased responses of these muscles since they do not play a significant weight bearing role in the rat. Among the endocrine or metabolic effects that may be involved include decreases in body fat percentage and chronic elevations of plasma catecholamine concentrations; both of which have been documented for suspended rats (6). Although these findings argue for a systemic influence, it remains possible that the weight bearing protocol used in this study presented a stimulus to the muscles, unrelated to the suspension, that increased their responses to insulin.

ACKNOWLEDGEMENTS

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REFERENCES

NEUROHUMORAL RESPONSES TO ISCHEMIC HYPERVOLUMIA: A MODEL FOR WEIGHTLESSNESS

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INTRODUCTION

The renin-angiotensin and the renal vasopressin-thirst-aldosterone systems have long been known to play an integral role in the control of body fluid composition. In the last 10 years, atrial natriuretic peptide, a powerful blood volume and salt regulating hormone, has been added to the scheme. The typical hormonal response to an expansion of blood volume includes a decrease in plasma arginine vasopressin (24 h, plasma osmolality and aldosterone (3) whereas atrial natriuretic peptide increases (4). Volume expansion with whole blood (5,6) saline (1) and albumin (4) induces a diuresis and natriuresis. There is a consequent decrease in plasma sodium with volume expansion using saline and albumin. No information is available on plasma electrolyte values for conscious isочemic volume expanded rats.

The majority of the acute volume expansion studies have been confined to the study of 1 or 2 variables and a limited time scale. This is primarily due to the volume of blood that could be obtained from small experimental subjects without compromising physiological changes that would interfere with the hypervolemic changes under investigation. Comprehensive characterization of the compensatory adjustments in the fluid regulatory process in acute volume expansion have not been reported.

The current study investigates the alterations in plasma electrolytes and volume sensitive hormones in response to isochemic blood volume expansion of conscious animals. Blood specimens were analyzed for hematocrit (HCT), plasma sodium (Na), potassium (K), osmolality (OSM), arginine vasopressin (AVP), plasma renin activity (PRA), aldosterone (ALDO) and atrial natriuretic peptide (ANP).

METHODS

Whole blood was drawn, as quickly as possible, from a femoral artery catheter of conscious donor rats, using a heparinized syringe. The blood was immediately infused into the jugular vein of conscious, compatible recipient rats at a rate of 1.5 ml/min using a Harvard compact syringe pump (Model 797; Harvard Apparatus, South Natick, MA). All recipient rats received a volume of donor blood equal to 30% of their estimated control blood volume (estimated as 6.4 ml/kg body weight) (9). Blood samples were drawn from a carotid artery of the recipient rat (immediately post volume expansion, up to, and including 5 days post volume expansion) at specified time points for the measurement of plasma hormone and electrolytes.

The rats were randomly given a code designating the blood collection time prior to the volume expansion procedure. At the designated time, the heparinized saline solution filling the catheter was removed (approximately 0.25ml) and the blood specimens were rapidly drawn in a clean, dry 3ml syringe. The maximum volume of blood drawn at one time from a recipient rat for the hormone and electrolyte determinations was 3ml. If possible, each rat was used twice. A sample (0.75ml) was drawn at a time point soon after volume expansion (i.e., 10, 20, 30, 40 minutes) and then 3ml was drawn at one of the later time points (no less than 24 hours later). Approximately 100ul was used for the hematocrit determination and the remaining portion of the specimen was immediately centrifuged (900 x g) at 4°C. The plasma samples were removed from the red cells and frozen in separate microcentrifuge tubes at -20°C until assayed.

Laboratory Procedures

Hematocrit was determined immediately after collection, using a previously described microhematocrit procedure (10). Plasma sodium and potassium concentrations were determined by flame photometry (11). Plasma osmolality was determined by freezing point depression (Model 110, Fiske Osmometer, Needham, MA) (12). AVP, ALDO, PRA (general renin angiotensin I) and ANP were measured by radioimmunoassay (13,14,15).

Statistical Analysis

Average values of ANP, PRA, ALDO, AVP, NA, K and OSM post volume expansion were compared to baseline values using Student’s t-tests, with Bonferroni adjustment of p-values to control the experiment-wide error rate (for each value) at p = 0.05 (16). Values at each time point were assumed to be independent of baseline values, although this assumption was not valid in all comparisons.

RESULTS

Intravascular volume expansion by 30% with whole blood increased HCT from the control value of 44% to a peak value of 54.4% at 6 hours post volume expansion. The values were still elevated at 5 days post volume expansion (the end of the observation period); however, they were beginning to approach normal levels.

ANP increased dramatically by 156% at 30 minutes PVE. This increase was short-lived and ANP returned to control levels within 40 minutes PVE. ALDO was significantly increased above CTRL at 20, 30, 90 minutes and 6 hours PVE (p<0.01). The ALDO values began to approach CTRL levels by 24 hours PVE. PRA values (Table 1) were significantly decreased below CTRL levels at 30-40 minutes PVE (p<0.05). The PRA levels were not different from CTRL levels at 3 days PVE.

Plasma NA, K and OSM were not significantly different from the control values, at any of time points post volume expansion with the exception of NA at 6 hours PVE (p<0.01). The HCT also peaked at this 6 hour time point (p<0.01).

AVP was not significantly changed at any time point post volume expansion.

DISCUSSION

Gauer and Henry define volume control as "...the continuous adjustment of blood volume to the changing size of the vascular bed so that at all times an adequate fulfull of the blood stream is available to the left ventricle" (17). Many investigators have explored the complex mechanism that governs this adjustment process. However, characterization of the processes from the onset of an imbalance (such as hypervolemia) and an integrated understanding of the operative metabolic pathways, has not been possible to date.

The use of anesthesia in most of the early studies is a problem, since anesthesia itself could cause an aberration of the daptation process making these observations difficult to interpret. Plasma hormone levels are affected by anesthesia (1,18), stress (19) and the method of sample collection (20). One consider the time course of these changes in response to volume expansion. Although fluid exchange processes between the intravascular and extravascular portions of the extracellular fluid space begins to occur in minutes (21), intestinal pressure requires hours (22) and the total body sodium regulation may require days for complete homeostasis (23).

In the current studies, volume expansion using whole blood caused a significant increase in hematocrit at 20 minutes and the hematocrit remained elevated until 24 hours PVE. This is consistent with the results of other volume expansion studies in rats utilizing whole blood (23). This hemococoncentration is probably the result of renal reexcretion of sodium and water and transcapillary extracellular fluid flux.

ANP has been shown to be a potent diuretic, natriuretic and vasodilator that increases immediately after blood volume expansion with saline or blood (4,15,24,25). Volume expansion studies in intact conscious rats using whole blood demonstrated a 26.5% increase in plasma ANP 5 minutes after a 20% volume expansion (25). The current studies support these findings in that there was a significant increase in ANP at 30 minutes PVE.

In agreement with the currently held hypotheses regarding the mechanisms controlling renin release, PRA was significantly decreased by volume expansion. Studies in which central blood volume expansion is induced by head down tilt or water immersion have reported decreased PRA (26). The renin-angiotensin system has been shown to stimulate aldosterone secretion from the adrenal cortex (3). Thus, the paradox in the present studies in that despite a significantly decreased PRA, ALDO was increased. This is contrary to our current understanding of the mechanisms controlling plasma aldosterone secretion, which when blood volume is increased, ALDO secretion is stimulated. Interestingly, increased plasma aldosterone levels have been observed in head-down suspension studies in rats (27) and bedrest studies using human subjects (26,28). These investigators have no explanation for these divergent findings. Other studies suggest that plasma aldosterone is sensitive to small changes in plasma potassium concentrations within the physiologic range. A significant rise in aldosterone has been observed without a statistically significant rise in plasma potassium levels (29). Small changes in plasma potassium could not be detected in the current studies. Increased hypokalemia of aldosterone has also been associated with increased adrenocortical potassium that may be mediated by ACTH (29). Further, ACTH has been shown to stimulate aldosterone secretion (30). Although, the effect of ACTH on aldosterone stimulation is reputedly short lived, the aldosterone response may be modulated synergistically by intracellular and extracellular electrolytes (sodium and/or potassium) or other hormones (corisil, renin, ANP) (30,31,32). Disocclusion of the renin-angiotensin-aldosterone axis has been reported in other studies (33) and observed in space flight (34) and thus warrants further investigation.
Studies by Morris et al. (25) suggest that AVP responds more quickly and dramatically to hemorrhage, whereas, ANP is more responsive to volume expansion. The lack of change in AVP may be due to a number of peptides, including the basal hydration level and dietary sodium intake, the expansion solution and the rate of the expansion.

The current studies are evidence of the complexity of the interactions among and between the renin-angiotensin-aldosterone axis, the renin-arginine vasopressin system and ANP and serves to characterize the volume regulatory hormone and plasma electrolyte response to acute ischemic volume expansion in the conscious animal.

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REFERENCES


A COMPUTER SIMULATION STUDY OF PREADAPTATION OF THE CIRCULATION BY REMOVAL OF DIFFERENT BLOOD VOLUMES TO COUNTERACT CENTRAL FLUID SHIFTS

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INTRODUCTION
Headward fluid shifts in weightlessness are believed to cause a central volume expansion, activating reflexes to reduce the blood volume (BV). This is considered to be an adaptation of the circulation to a new volume setpoint appropriate to the space environment (1, 3). It has previously been shown in two water immersion studies that many of the major physiologic responses to fluid shifts can be attenuated if the BV is reduced by about 15% before the onset of the fluid shift (6, 7). The concept advanced is that it may be possible to preadapt the circulation to the weightless environment so that deleterious physiologic effects secondary to fluid shifts may be effectively counteracted.

This concept was also tested over a 70-day time span using computer simulation (8). Results from this study using computer simulation of the physiologic responses to 6-degree head-down tilt (HDT) support the water immersion studies and suggest that after several hours of HDT, preadaptation of the circulation results in better fluid retention persisting for twenty to thirty days of HDT, producing higher BV, extracellular volume, and total body water. The preadaptation volume used was the difference between the starting blood volume and the equilibrium volume after 70 simulated days of HDT, which was 534 ml in that instance, about 11% of the starting BV of 5024 ml.

In the present study, we modeled the effects of withdrawing different volumes of blood to preadapt the circulation to HDT. Simulated BV reductions of 5%, 11%, and 15% were performed immediately before HDT to help evaluate the optimum BV that might be removed to preadapt an astronaut's circulation to weightlessness.

METHODS
The Guyton Model of Fluid, Electrolyte, and Circulatory Regulation (2) modified for weightless simulation by HDT (5) was used. We further modified it for our long-term simulations of HDT by incorporating an improved model of erythropoiesis and by simplifying the means to simulate blood removal. The modifications to the model and the validations performed have been described previously (8) and in this volume (9). That modified model was also used for this study.

Acute blood volume reductions were simulated by removing blood from the model at the rate of 41.67 ml/min for sufficient time to remove 5%, 11%, and 15% of the starting BV of 5024 ml. The simulated subject was supine while bled, and was placed in HDT immediately after the BV reduction was finished. The timing of the three BV reduction trials was adjusted so that the onset of HDT was identical in all cases.

RESULTS
Data are plotted on a logarithmic time scale so that all phases of the simulation out to 40 days may be clearly distinguished. The onset of BV reduction may be distinguished on each graph as a volume decrease from an initial baseline. The onset of HDT in all groups may be determined by the time of the change from baseline in the "HDT alone" curve. Figs. 1 through 5 show the blood volume, red cell mass, extracellular fluid volume, intracellular fluid volume, and total body water, respectively. A common element in every figure is that the -11% preadaptation produced the least overall change in body fluid volumes after the onset of HDT.

Figure 1. Blood volume

Figure 2. Red cell mass

Figure 3. Extracellular fluid volume
DISCUSSION

There are two ways to view these data. One is to consider the amount of fluctuation in the fluid volumes after HDT: the other is to compare the relative volumes across time. These results suggest that after one day’s exposure to HDT or weightlessness, the highest blood volumes and extracellular fluid volumes might be attained by the largest of the preadaptation BV reductions, in this case -15%. The total body water indicates that this preadaptation volume would produce the best overall after the first day yet the intracellular fluid volume shows an early expansion of that compartment followed by a volume loss exceeding all others. Also the preflight and early inflight blood volume reduction would exceed the volume decrements produced by lesser BV reductions, to the possible detriment of orthostatic tolerance and aerobic capacity in the event of an emergency.

The smallest BV reduction of -5% does provide some improvement in fluid retention, and the physiologic responses to the fluid shifts produced by HDT are attenuated if not minimized. It has the advantage of the least loss of red cell mass of the three treatments, at least until about 10 days of HDT, so it would be least likely of the three treatments to compromise orthostatic tolerance and exercise tolerance.

The concept of BV preadaptation to weightlessness as a method of minimizing the physiologic responses to fluid shifts, however, would appear to be best realized by applying the BV reduction that brings the circulation to its equilibrium value appropriate for the new environment. The data suggest that the fluctuations in fluid balance of all body water compartments would be lowest in weightlessness if the preflight circulation were first brought to its inflight equilibrium volume. It also appears that the extracellular fluid volume would be well maintained for 10 to 20 days of flight, possibly improving postflight orthostatic tolerance for missions typical of today’s Shuttle flights and for anticipated Extended Duration Orbiter missions.

However, these data should not be overinterpreted, and must be viewed with an understanding of their weaknesses. The model is by no means complete or accurate in all respects, but can help to understand physiologic interactions and assist in planning appropriate experiments. In particular, this model simulates the analog of weightlessness, but there is evidence that the physiologic responses to weightlessness are somewhat greater than those encountered in HDT (4). While we do believe that these results have a qualitative validity, they require experimental verification. This also applies to the preadaptation blood volume reductions treated here, -5%, -11%, and -15%; these results do not show that an absolute volume decrement of -11% would be optimum to reduce the physiologic responses to fluid shifts, but rather that the optimum volume to remove from the circulation is that which would be lost eventually in adapting to weightlessness. We do not yet know with confidence what the typical percentage decrease of blood volume is in weightlessness at equilibrium, or how much variation there is between individuals. However, we expect that bracketing the optimum preadaptation BV reduction by ±5% in actual experimentation would produce results resembling those obtained in this simulation.

Our conclusion based on these results is that the optimum volume to remove to preadapt the circulation to weightlessness is that volume which would be lost naturally by slowly adapting to fluid shifts. Such a preadaptation to weightlessness would be expected to minimize the physiologic responses to fluid shifts, thereby reducing the departures from homeostasis and body fluid losses.

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DEPENDENCE OF BLOOD VOLUME CHANGES ON ORAL SODIUM UPTAKE

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INTRODUCTION

Exposure to weightlessness causes changes in fluid and electrolyte balance. The accompanying decrease in blood volume seems to play a decisive role in the orthostatic intolerance regularly seen postflight [4, 14]. Blood volume expansion might therefore contribute to an improvement in orthostatic response. Luft et al. [9] showed that increased sodium intake from 10 to 300 mmol/d resulted in a significant increase of 1.08 ± 0.8 kg SEM in body weight. Sagnella et al. [13] found a similar increase of 1.2 ± 4.0 kg in body weight following an increase of sodium ingestion of 10 to 350 mmol/d. Assuming that the test subjects maintained a similar level of activity throughout these experiments the increased body weight can be concluded due to an increased body water content. This is confirmed by their reported finding of a simultaneous decrease in both hematocrit and plasma protein concentration. These findings indicate that blood volume increased following the increased in sodium ingestion.

The aim of the present study was to determine blood volume change following increased oral sodium intake, under conditions of strictly dietary and environmental system control. Plasma concentration of those hormones involved in regulation of sodium and water metabolism were measured.

MATERIAL AND METHODS

Six healthy men (mean age 23.8 ± 1.4; mean body weight (BW) 76.74 ± 3.76 kg) participated in the study after given their written informed consent. The study period covered 24 days and was subdivided into three identical eight day periods except for variance of dietary sodium intake. During the whole study period the test subjects were housed in a ward at constant room temperature and humidity -with the exception of such time periods when special experiments were scheduled. The first four days of each test procedure were used to adapt to the diet. Subjects were provided 6 meals a day at 7.30 a.m., 10.00 a.m., 1.00 p.m., 4.00 p.m., 7.00 p.m, and 10.00 p.m. Sufficient calories were given to meet individual energy requirements of light muscular work and equaled 130 % of basal metabolic rate (BMR). BMR was calculated by measuring the individual respiratory quotient (RQ). Daily protein intake (1.4 g/kg BW/d) and water intake (42 ml/kg BW/d) were kept at a constant level. Sodium intake was held constant at 2.8 mmol/kg BW/d during the first phase (8 days), corresponding to an average sodium intake of 144 mmol/d to 261 mmol/d in Germany [2]; 5.6 mmol/kg BW/d during the second phase; and 8.4 mmol/kg BW/d during the third phase respectively.

All urine voids were collected throughout the entire study period. Four sampling periods per day (7 a.m., 1 p.m., 7 p.m., 11 p.m.) were used. Urine collected during each period urine was weighted, density measured, and aliquots stored at -20°C. BW was measured by a scale [Sartorius, DVM 5703 MP 8-1] calibrated to ± 0.1 g at the end of the urine sampling periods. Urinary sodium and potassium concentrations were measured by flame photometry. Blood samples were drawn at 7 a.m. of the fifth and seventh day of each study phase using catheters inserted 30 minutes prior to blood withdrawal in order to minimize the influence interventions on plasma hormone concentrations. Samples were immediately separated by centrifugation and the plasma stored at -20°C until analysis.

Commercially available radio immuno assay kits (RIA) were used to measure AVP (Bühllmann Laboratories, Switzerland), Cortisol (Amersham, England), Renin (Pasteur, Paris, France) and Aldosterone (Diagnostic Products Corporation, USA) concentrations. Blood hematocrit was measured by a Coulter Counter, serum proteins by the biuret method and serum electrolytes (Na+, K+) by flame photometry. Labeling of the erythrocytes with 51Cr were used to determine blood volume and erythrocyte half life [10].

Water and sodium balances were calculated for 24 hour periods during the study. Water balance was computed as water intake (water content of solid food + liquid drunk + metabolic water) minus water output (urine and estimated evaporative water loss). The evaporative water loss was determined from weight loss (weight of food and liquid consumed - any change in BW - weight of urine and feces) using the method of Davidson and Passmore [1]. Sodium balance was calculated as the daily difference of sodium intake and the corresponding urinary sodium excretion.

Data are presented as means ± SEM. Data were evaluated by analysis of variance (Friedman test) [7], and if significant the data were subsequently tested using the Mann-Whitney-Wilcoxon test. A one-tailed P value of ≤ 0.025 was taken as significant. The urine flow, urinary sodium excretion and the metabolic balances were statistically tested by paired t-test.

RESULTS

Blood volume measured with the gradual increase in sodium ingestion (2.8 mmol/kg BW/d to 5.6 mmol/kg BW/d and 8.4 mmol/kg BW/d), rising significantly (p = 0.025) from a basic mean value of 5.30 ± 0.15 l on day 5 to a maximum level of 5.90 ± 0.13 l on day 23 (fig. 1).

Figure 1: Blood volume changes (mean ± SEM; n = 6); A: Na+ intake 2.8 mmol/kg BW/d; B: Na+ intake 5.6 mmol/kg BW/d; C: Na+ intake 8.4 mmol/kg BW/d.

This was associated with a significant 7.5 days decrease in the biological half life of the erythrocytes during this period.

Blood volume expansion was accompanied by a significant 59.3 % decrease (p = 0.01) plasma aldosterone concentration. Baseline level on day 5 of
189 pg/ml diminished to a level of 71.3 pg/ml by day 23. Although renin levels on day 23 were lower than that on day 5 the decrease was not significant. Plasma AVP concentration rose a significant 71% (a = 0.025) (Table 1) by the third phase of the study. Mean serum sodium concentration, mean serum osmolality and hematocrit values did not change significantly.

A significantly measured urinary sodium excretion (a = 0.001) paralleled the increased sodium ingestion, beginning on the first day of augmented sodium intake. The increased urinary sodium excretion was not accompanied by an increase in urine flow. Despite increased sodium excretion renal loss did not reach the absolute level of ingestion and a positive sodium balance resulted. No significant change in water balance occurred.

**DISCUSSION**

Findings demonstrated that a stepwise rise in dietary sodium ingestion results in a blood volume increase without change in body weight. Expected changes occurred for plasma aldosterone concentration and plasma AVP. The higher sodium intake resulted in increased urinary sodium excretion without change in urine flow. Sodium is the chief cation of the extracellular fluid and a chief determinant of body fluid volume. In order to maintain osmolality and sodium concentrations within physiological ranges in the extracellular compartment, alterations of sodium concentration are in general counteracted by changes in water content [10]. The increase in blood volume during this study strongly supports this view. The expected increase in body water content based on a total increase of extracellular volume did not occur. These results lead to the following conclusion: A temporary increase in serum osmolality occurred during the first days of transition to the respective higher sodium intake. An additional repeated increase in serum osmolality occurred after each meal. In order to maintain normality of serum osmolality, a constant body weight, an unchanged water balance, an increase in blood volume and a fluid shift from the intracellular to the extracellular fluid compartment had to take place. Additionally the increased serum osmolality caused a fluid shift from the extracellular to the intracellular compartment to compensate for such alterations [7].

The blood samples for the serum sodium concentration, serum osmolality and hormone levels were drawn on days following the period of dietary adaptation and after 8 hours of fasting. This explain why the serum sodium concentration and serum osmolality remained unchanged at the points of measurement.

The slight increase in plasma AVP concentrations and decreased aldosterone concentrations support the position that a serum hyperosmolality had occurred. If the dietary sodium had been given continuously as a single intravenous sodium infusion during the whole study period, the serum sodium concentration would have been raised from 0.38% during the first phase to 0.76% during the second phase and to 1.15% during the third phase. This latter sodium concentration is similar to its concentration in sea water. Therefore reactions during the third phase should be similar to the requested for ingestion of sea water, which lead to an increased extracellular and a decreased intracellular volume.

It is hypothesized that the high sodium intake induces an increase in the extracellular osmolality and a water transfer from the cell to the extracellular fluid compartment. As a result an increase in the intracellular density and a decrease in the cell volume occurs. This includes red cells as well. The findings of a decreased biological half life for red cells in this study indirectly supports this. This is based on findings by Rapoport [11] that red cells are characterized by decreased cell volume and increased density. The hyperosmotic environment in this study would change the morphology of red cells and induce a state similar to the properties of old red cells. This would result in a more rapid disintegration of such cells by the spleen.

To summarize the results the study show, that the augmented sodium ingestion up to an average 644 mmol/d and a simultaneously constant water intake of 3200 ml/d lead to fluid shifting from the intracellular to the extracellular compartment. The fluid phase is an increase in blood volume. This supports the use of oral salt and water loading prior to reentry from space flight and suggests more extension use of this procedure during exposure to weightlessness.

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INTRAMUSCULAR PRESSURE MEASUREMENT AS AN INDEX OF TORQUE DURING DYNAMIC EXERCISE


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INTRODUCTION
One of the most serious problems experienced by astronauts during long-term spaceflight is muscle atrophy (2,9). In order to understand and develop countermeasures for this problem, a simple method for monitoring function of specific muscles in vivo is needed. Electromyography (EMG) has been used as a technique for measuring force output of a specific muscle. Although EMG correlates linearly with contraction force during isometric contraction, it is increasingly unreliable during muscle fatigue and at high workloads (1,4,5). Also, EMG reflects the initiation and maintenance of muscle contraction rather than an intrinsic mechanical property of the muscle. Therefore, EMG may be unsuitable for monitoring specific muscle function during dynamic exercise.

Intramuscular pressure (IMP), the fluid pressure created by a muscle as it contracts within its fascial compartment, correlates linearly with contraction force in specific muscles during isometric exercise (4,6,7). However, at present there are no data available concerning the usefulness of IMP during dynamic exercise. We hypothesized that IMP would give a more direct and accurate index of contraction force than EMG in specific muscles.

METHODS
IMP and surface EMG activity were measured continuously and simultaneously in the tibialis anterior and soleus muscles of nine normal male volunteers (age 28-54), who gave their informed consent. These parameters were measured at rest, during passive movement, and during isometric, concentric, and eccentric exercises. The Lido Active Isokinetic System (Loredan Biomedical, Davis, CA, USA) measured ankle joint position and torque. The protocol was approved by the Human Research Experimental Review Board at NASA Ames Research Center.

IMP was measured using a Teflon catheter with four side holes at the tip (Myopress, Alos Medical Inc., Horby, Sweden). A 2 1/4 inch 16 gauge needle with a plastic sheath was used to insert catheters into muscle under local anesthesia. After penetration of the muscle fascia, the needle was withdrawn into the sheath and bluntly advanced in a direction parallel with the muscle fibers. The catheter was then inserted through the sheath, and the sheath was withdrawn from around the catheter. Catheter position and depth were constant between subjects. Each fluid-filled catheter was connected via pressure tubing to a microcapillary infusion system (8), set at 1.5 ml/hr, and to an Electromedics pressure transducer. Surface EMG electrodes were positioned directly over the catheter insertion site with the ground placed over the tibia. Root mean square EMG values were assessed with a Cadwell 7400 EMG monitor (Kennewick, WA, USA).

The knee of each subject was positioned at a 90° angle to minimize any gastrocnemius contribution to ankle torque. Isometric contractions were performed at 25%, 50%, 75%, and 100% maximal voluntary contraction (MVC) for both plantarflexion and dorsiflexion. Then dynamic plantarflexion and dorsiflexion exercises were performed from the neutral position (90° ankle joint angle between foot and tibia). Five MVCs of each exercise were performed concentrically and eccentrically. Subjects rested for approximately 3 minutes between exercises.

RESULTS
Isometric data obtained in this study agreed well with previous findings of isometric contraction studies (4,6,7). Soleus and tibialis anterior IMP and EMG correlated linearly with ankle joint torque (muscle contraction force) in all 9 subjects.

Concentric Exercise
Because of variability in the strength and muscle mass of our subjects (and resulting differences in IMP and EMG), plotting each subject's IMP or EMG against force output during a MVC yielded a wide range of slopes, regardless of the linearity of each plot (Fig. 1). For this reason, the IMP and EMG data were normalized to percent of maximum for each dynamic exercise.

Linear regression analysis of normalized soleus IMP and EMG against force during concentric plantarflexion yielded coefficients of determination of $r^2=0.937$ and $r^2=0.716$, respectively (Fig. 2A). The coefficients of determination for tibialis anterior IMP and EMG versus force during concentric dorsiflexion were $r^2=0.948$ and $r^2=0.802$, respectively (Fig. 2B).

Eccentric Exercise
Figure 3A shows normalized soleus IMP and EMG increasing with respect to force during eccentric plantarflexion. In this case, IMP correlated well with...
force ($r^2=0.889$) while EMG correlated poorly ($r^2=0.489$). Furthermore, tibialis anterior IMP and EMG versus force during eccentric dorsiflexion were $r^2=0.904$ and $r^2=0.702$, respectively (Fig. 3B).

**DISCUSSION**

Intramuscular pressure values in both the soleus and tibialis anterior correlate well with joint torque during concentric as well as eccentric exercise. EMG also correlates well with joint torque in most subjects during concentric exercise. In the case of eccentric contractions, however, EMG did not appear to correlate as well as IMP with force output. Isometric exercises performed at various loads in our study extend previous findings that both IMP and EMG correlate well with force during isometric exercise (4,5,7).

Although muscle fatigue is cited as the primary reason for the nonlinearity of EMG in relation to contraction force at maximal isometric workloads, this is probably not the case in our protocol, which was designed to avoid fatigue. Instead, the known length-tension properties of skeletal muscle probably explain why IMP may relate better to muscle force production than EMG.

First, skeletal muscle exerts maximal force when activated at its resting length (3). At greater lengths, the number of potential actin/myosin cross bridges decreases; at shorter lengths, steric hindrance of the contractile proteins progressively interferes with continued shortening. IMP is theoretically dependent on tension of muscle fibers at the site of measurement (7), and therefore should be an accurate indicator of force. EMG, however, depends on the size and number of depolarized motor units, which can remain constant with changes in contracting muscle length, and therefore, tension. Second, connective tissue in relaxed skeletal muscle creates tension (and increases IMP) as the muscle is stretched from resting to maximal length. This passive tension contributes to total muscle force during contractions near the upper limit of muscle length, such as eccentric exercise in our study. Because motor units are not activated to generate this tension, EMG can not measure it.

In previous studies, investigators usually chose unipolar intramuscular electrodes to measure EMG for comparison with force or IMP (4). Intradural EMG electrodes record the activity of only a few motor units in the immediate vicinity of the electrode. Because IMP above 30 mm Hg can restrict blood flow in certain areas of skeletal muscle and cause local fatigue, local EMG activity may be altered when the total force output is unchanged. Therefore, surface electrodes were used in an effort to obtain a better overall representation of soleus and tibialis anterior myoelectric activity.

While the IMP and force correlations were nearly linear in each of the 9 subjects, the slopes of these relationships varied greatly between subjects. This is a result of intersubject variability as well as slightly variable catheter insertion site and depth (7). For repeatable assessment of muscle strength and function, IMP measurements must be taken at the same position and depth in a particular muscle. IMP catheterization is a relatively simple andatraumatic procedure. When performed under sterile conditions by trained personnel, risk of complications is minimal. Repeated catheterizations required for long-term assessment, however, may increase the risk of tissue damage or alter local IMP. Advanced technology will minimize this risk with the development of smaller electrical and fiber-optic transducer tipped catheters.

Although more invasive than EMG, IMP provides a more direct and accurate index of muscle contraction force for specific muscles in vivo during both concentric and eccentric exercise. Furthermore, exercise-induced IMP elevation is known to influence both local and systemic hemodynamics. Therefore, IMP may be a more physiologically relevant variable than EMG. IMP may provide a powerful tool for developing exercise hardware and protocols for astronauts and for assessing gait during rehabilitation and training.

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S-116
ECHOCARDIOGRAPHIC EVALUATION OF CARDIAC FUNCTION DURING PARABOLIC FLIGHT

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INTRODUCTION

Significant cardiovascular changes have been described during exposure to weightlessness. A prominent shift in fluid from the lower extremities toward the head and thorax occurs and may contribute to changes in fluid balance, eventually resulting in post-flight orthostatic intolerance. The time course of the fluid shift is not well documented, but its effects on left ventricular (LV) volume and function have been studied. For this report, we evaluated acute changes in LV volume and function during parabolic flight in the sitting position with direct measurement of LV dimensions with echocardiography and determination of LV inflow and outflow velocities with pulsed-wave Doppler. Additionally, we examined changes in common carotid artery flow velocity and dimensions as an indicator of cerebral blood flow. Data collected from the supine position, continuous blood pressure recordings, and right heart velocities await future analysis.

METHODS

Twelve subjects (8 males, 4 females, mean age 34 years) were selected for study after passing flight physicals and after screening echocardiograms demonstrated normal cardiac anatomy and function. All subjects were unmedicated during study, and all gave written informed consent. This study was approved by the NASA and U.S. Army Aeromedical Research Laboratory human use committees. NASA Johnson Space Center's KC-135A aircraft was used to perform parabolic flight profiles in 4 sets of 10 parabolas with 15-30 seconds of a 1G baseline between parabolas (4 flights, n=8), or sets of 10 parabolas without 1G baselines (1 flight, n=4). Two-dimensional echocardiograms and Doppler velocity recordings were obtained with a model 128/XP10 echocardiograph (Acuson Corporation, Mountain View, CA) with subjects in a sitting position (Figure 1). Pulsed-wave Doppler recordings of LV inflow and outflow and carotid artery velocities were performed. Left ventricular and carotid artery diameter and LV cross-sectional area were measured with echocardiography. Respiration, ECG, and head-to-foot gravity (Gz) were recorded simultaneously on VHS videotape and on a separate Vetter™ recorder.

Doppler echocardiographic recordings were analyzed with an off-line computer system (model D-200, Dextra Medical, Inc., Long Beach, CA). Three sequential cardiac cycles were averaged over one respiratory cycle to avoid respiratory influences on velocities. Diastolic filling period and LV ejection time were measured directly from Doppler recordings of the LV inflow and outflow tracts, respectively.

Stroke volume (SV) was calculated from the formula:

\[ SV = CSA \times TVI \]

where CSA was the cross-sectional area of the LV outflow tract measured by echocardiography, and TVI was the time-velocity integral of the LV outflow tract Doppler velocity. The paired t-test was used for statistical comparison of parameters during level flight, climbing (+Gz), early zero gravity (0G) and late zero gravity.

RESULTS

The quality of echocardiographic imaging was good in most subjects during flight. Doppler velocities were adequately recorded from the LV inflow tract in 9, the LV outflow tract in 10, and the right carotid artery in 10 subjects. Left ventricular short-axis diameters and areas could be measured in 5 subjects. The addition of a 15-30 second 1G baseline between parabolas improved the quality and quantity of echocardiographic images by allowing more time for aligning the ultrasound probe. Heart rate with +Gz was significantly higher than during level flight and was also higher than late 0G (Table 1). Peak early LV inflow velocities ("E velocity") rose significantly during 0G without a significant rise in mean velocity, a-wave velocity, or diastolic filling period. No changes were observed in LV outflow velocities, however, the LV ejection time was prolonged from early to late 0G.

Table 1. Left ventricular inflow and outflow velocities

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level</th>
<th>+Gz</th>
<th>Early 0G</th>
<th>Late 0G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>67±15</td>
<td>76±15°</td>
<td>75±15°</td>
<td></td>
</tr>
<tr>
<td>VmM (M/sec)</td>
<td>0.36±0.08</td>
<td>0.44±0.16</td>
<td>0.45±0.17</td>
<td></td>
</tr>
<tr>
<td>V,E (M/sec)</td>
<td>0.71±0.14</td>
<td>0.86±0.16</td>
<td>0.87±0.20</td>
<td></td>
</tr>
<tr>
<td>V,A (M/sec)</td>
<td>0.40±0.05</td>
<td>0.49±0.11</td>
<td>0.50±0.12</td>
<td></td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.8±0.5</td>
<td>1.8±0.5</td>
<td>1.7±0.3</td>
<td></td>
</tr>
<tr>
<td>OTpkr (M/sec)</td>
<td>0.89±0.63</td>
<td>0.87±0.21</td>
<td>0.93±0.20</td>
<td></td>
</tr>
<tr>
<td>OTrm (M/sec)</td>
<td>0.63±0.03</td>
<td>0.58±0.13</td>
<td>0.60±0.13</td>
<td></td>
</tr>
<tr>
<td>DFP (msec)</td>
<td>508±18</td>
<td>458±16</td>
<td>458±16</td>
<td></td>
</tr>
<tr>
<td>LVET (msec)</td>
<td>302±19</td>
<td>296±20</td>
<td>308±20</td>
<td>317±27°</td>
</tr>
</tbody>
</table>

*, p<.05 vs level; †, p<.05 vs +Gz; ‡, p<.05 vs early 0G; VmM=mean inflow velocity; V,E=peak inflow early velocity; V,A=peak inflow a-wave velocity; OTpkr=peak LV outflow velocity; OTrm=mean LV outflow velocity; DFP=diastolic filling period; LVET=LV ejection time.

No significant changes were found in LV dimensions or short-axis area during 0G (Table 2). Stroke volume was higher at late 0G compared to early 0G, and although cardiac output rose, it did not meet statistical significance (p=.08).
Table 2. Left ventricular dimensions and output

<table>
<thead>
<tr>
<th>Parameter</th>
<th>+Gz</th>
<th>Early 0G</th>
<th>Late 0G</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV (cc/beat)</td>
<td>66±15</td>
<td>63±16</td>
<td>71±19*</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>5.1±1.3</td>
<td>4.7±1.4</td>
<td>5.4±1.4</td>
</tr>
<tr>
<td>Ded (cm)</td>
<td>4.7±5</td>
<td>4.4±3</td>
<td>4.5±3</td>
</tr>
<tr>
<td>Des (cm)</td>
<td>3.0±3</td>
<td>2.8±5</td>
<td>2.9±4</td>
</tr>
<tr>
<td>Aod (cm²)</td>
<td>16±1</td>
<td>16±2</td>
<td>16±2</td>
</tr>
<tr>
<td>Aes (cm³)</td>
<td>7.3±1.1</td>
<td>6.6±1.4</td>
<td>6.9±1.4</td>
</tr>
</tbody>
</table>

Abbreviations as for Table 1. SV=stroke volume; CO=cardiac output; Ded=end-diastolic diameter; Des=end-systolic diameter; Aod=end-systolic diastolic size; Aes=end-systolic short-axis area.

Right carotid artery dimensions remained constant between +Gz and early 0G, however, a rise in peak systolic velocity was observed from early to late 0G (Table 3). Additionally, a rise in systolic flow time (ET) was seen between +Gz and both early and late 0G.

Table 3. Carotid artery dimensions and velocities

<table>
<thead>
<tr>
<th>Parameter</th>
<th>+Gz</th>
<th>Early 0G</th>
<th>Late 0G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ded (mm)</td>
<td>44±11</td>
<td>45±10</td>
<td>--</td>
</tr>
<tr>
<td>Des (mm)</td>
<td>56±7</td>
<td>60±7</td>
<td>--</td>
</tr>
<tr>
<td>Vpk (M/sec)</td>
<td>1.1±.3</td>
<td>1.0±.2</td>
<td>1.1±.24</td>
</tr>
<tr>
<td>Vmn (M/sec)</td>
<td>62±.16</td>
<td>57±.11</td>
<td>.61±.12</td>
</tr>
<tr>
<td>ET (msec)</td>
<td>251±20</td>
<td>270±24</td>
<td>291±15</td>
</tr>
</tbody>
</table>

Abbreviations as for Table 1. Vpk=peak systolic velocity; Vmn=mean systolic velocity; ET=ejection time.

DISCUSSION

This study demonstrates the utility of noninvasive methods for the measurement of changes in cardiovascular function during acute exposure to microgravity. Doppler echocardiography can be successfully performed during parabolic flight in most subjects and can provide a direct assessment of cardiac flow under changing gravitational states. The quality of echocardiographic recordings was highly dependent on the use of prescreened subjects and experienced echocardiographers. The addition of a 1G baseline between parabolas aided data acquisition by providing sufficient time to change transducer locations and allowed cardiovascular function to return to baseline.

We observed a significant increase in heart rate during acceleration followed by a decline during 0G. Similar observations have been reported, and probably reflect alterations in autonomic tone. A significant rise in transmural E velocity was found on transition from +Gz to early and late 0G, possibly reflecting an increase in preload. Prolongation of LVET was consistent with an increase in preload. No variations in left ventricular dimensions were observed, which may reflect alterations in afterload or contractility in addition to preload. Stroke volume increased in 0G with a trend toward higher cardiac output. Considering the hydrostatic effects on carotid artery pressure, we had expected increased carotid artery dimensions and even increased carotid flow during 0G. This was not confirmed in the measurements, partly due to technical limitations and possibly due to feedback regulation of cerebral flow in 0G.

Several limitations should be considered in reviewing these observations. Our sample size was small, comprising a maximum of 10 subjects per parameter measured; an insufficient number of subjects were analyzed for LV and carotid artery dimensions at level flight. The parabolic flight profile we used also has significant limitations. This profile results in a sudden transition from nearly +2Gz to 0G without an interval at 1G. The time at 0G is brief, averaging 20-30 seconds, and data collection using hand-held transducers is challenging. The brief exposure to microgravity also raises a concern that regulatory mechanisms may not reach a steady state during the period of data collection.

In summary, we have demonstrated the feasibility of Doppler echocardiography for evaluation of cardiovascular function during parabolic flight. Our data confirm the changes in LV function due to increased venous return and altered hydrostatics upon the transition to microgravity.

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Army or the Department of Defense.

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DATA ACQUISITION SYSTEM FOR THE ARTIFICIAL GRAVITY SIMULATOR (AGS)

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Introduction

A 100% gradient centrifuge has been developed for human use as a countermass of the physiological effects of the microgravity environment in space. Centrifugal forces generated while spinning on the centrifuge simulate a gravitational field in the human body. This centrifuge has been termed the artificial gravity simulator (AGS). It can accommodate up to four subjects at a time.

This paper deals not with the centrifuge but with the physiological data acquisition system which has been assembled for making observations on the human body’s response to the simulated gravity. Physiological observations focus on but are not limited to the cardiovascular system.

In the planning phase, taking physiological measurements on four subjects while rotating on the AGS presented challenging problems. Control of data acquisition, display, analysis and storage was to be done by computer located off the rotating platform. Where possible, the data acquisition system was to utilize presently available instruments and duplication be avoided. This meant that there was to be one set only of measuring instruments for all four beds. The software for data acquisition was to be commercially available. Decisions had to be made for selecting measuring instruments, mode of data transmission, and computer hardware and software.

Overview of Instrumentation

![Schematic of the AGS Data Collection System](image)

Fig. 1 presents a schematic overview of the data acquisition system’s hardware. Central to the system is the masterswitch which was especially developed for this system. This switching device has a Masterswitching Net Working Unit which, by means of a common serial (RS232) and parallel (Centronic) interface, interconnects the computer, the peripheral devices and the subjects. A masterlink utility program is used for setting the transfer path and flow of data within two 24-channel relay boards. Under computer program control the masterswitch can be instructed to select a subject, select and activate specific measuring devices, and transmit desired signals from the subject to the measuring devices. Data is then transmitted from the measuring devices to the computer or oscilloscope by radiotelemetry or slip-rings located on the central rotating shaft of the centrifuge.

A cardiac monitor which measures electrical impedance of the thorax is used to determine the thoracic fluid index (TFI), ejection velocity index (EVI), stroke volume (SV), heart rate (HR), cardiac output (CO) and the ventricular ejection time (VET). Blood pressure is obtained by an automatic blood pressure device which has an air-pump for rate controlled cuff inflation. Detection of Korotkoff sounds employs two matched piezoelectric crystals which enable extraneous noise subtraction. Oxygen saturation in the blood going to the head is obtained by pulse oximetry through an infrared sensor placed on an earlobe. During subject selection, the ECG signal from each subject is transmitted to the ECG telemetry transmitter through the master switch. A multichannel oscilloscope is used for monitoring these signals.

Values for electrical impedance of the total body, leg and trunk can be obtained with a multi-frequency bio-impedance analyzer. The analyzer has an internal microprocessor for signal conversion and digital signal processing. The conversion and signal frequencies can be set manually or by computer. Data is read on the instrument display or by computer. The selection of surface electrodes for impedance of total body or a body segment is made by the AGS computer through the masterswitch.

Sleep studies while rotating are an objective of this project. The ECG, EEG, EOG and HRG signals are transmitted by a multichannel transmitter which employs standard sub-carrier frequency generators (ICG) and voltage control oscillators (VCO). The receiver uses frequency discriminators for demodulation. Analog signals are directly monitored on the multichannel oscilloscope and can be recorded on a slip-chart recorder, or magnetic tape, or by computer directly into the CPU by a 16-channel A/D converter. Due to cost constraints only one telemetry system is used at this time.

A special wiring harness for the non-invasive sensors was made for each bed. Electronic connections are established through 36 pin centronic connectors. A connecting hose for blood pressure inflation is part of each harness assembly. During the study each subject may wear as many as 18 electrodes, an IR transducer on the earlobe, a sensor for systolic and diastolic pressure and a pressure cuff. A separate harness for the four channel biotelemetry transmitter to record sleep parameters adds additional electrodes.

There are 18 slip-rings located on the central rotating shaft. Six rings transport AC power to the beds, the masterswitch and to the measuring instruments. Four slip-rings are used for transmitting commands to the GPIB instruments and bringing data to the CPU. The remaining rings are for analog signal transmission to the multichannel oscilloscope display.

The 1-EEE 488/4 bus which interfaces the GPIB instruments with the computer has four ports which can be programmed for baud rate, stop bits, data
bits, parity and hand shaking. To minimize the number of slip-rings assigned for data communication only one port is used. The computer presently used is a HPC 386 having a 25 MHz 80386 microprocessor and a 25 MHz 80387 numeric co-processor. This computer has 4 MB RAM and a 100 MB hard disk.

Data Acquisition Software

The AGS data acquisition system makes use of the Asyst GPIB software package developed by Asyst Technologies Inc. This package is a menu driven data acquisition software which enables the user to acquire data from instruments controlled by GPIB. Data may be acquired, manipulated, analyzed and displayed in tabular or graphical form by interactive or programmable routines. Since the present project requires repetitive experiments, programmable routines were developed for data acquisition, display and storage. Interactive processing of the data involves recall of stored data files, printouts. All data are permanently stored on floppy disks for further analysis at a later time.

The computer routines select and initialize communication with a bed and measuring instruments, set up variable registries, instruct the computer to acquire data from each instrument (discarding that data and then file the data on the hard disk. A set of routines has been developed for data acquisition from each bed. These sets of routines can then be initiated in any desired sequence to form a program of data acquisition from only those beds being used in a particular experiment. The automatic data acquisition can be interrupted by the computer operator and data acquisition from a particular bed selected. Automatic data acquisition can be then resumed.

Results

Several experiments have been conducted on a group of subjects. The experiments have differed in rpm and total rotation time in order to test the human cardiovascular response to the AGS.

Figure 2 is a sample of computer tabulated data for cardiac output (CO), thoracic fluid index (TFI), heart rate (HR), and stroke volume (SV). The number in parenthesis indicates that these data were acquired from bed number one. Time of data acquisition is shown as the number of seconds since midnight. Additional data acquired but not shown in this table are ejection velocity index (EVI), and systolic and diastolic blood pressures.

Observations were made at rest (30 min.), during rotation (30 min. at 28 rpm) and during 30 min. after rotation. Part of the data is presented graphically in figures 3 through 6.

Changes in heart rate during rotation and recovery are shown in fig. 3. Figures 4, 5 and 6 show respectively stroke volume (SV), thoracic fluid index (TFI), and the cardiac output (CO) for the same periods of time.
Summary
A physiological data acquisition system has been developed to study the human response to the stress caused by the AGS. This system meets most of the expectations of the experimental design. Once initiated the computer controlled sequential switching among individuals and measuring devices, and the process of data acquisition, display and storage, continues automatically. The automatic mode can be over-ridden and data on a specific individual acquired. The data can be transferred to floppy disks for permanent storage or later retrieval and analysis.

References

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SHORT RADIUS CENTRIFUGE AS A METHOD IN LONG-TERM SPACE FLIGHTS

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INTRODUCTION

Manned space missions, their increased durations have demonstrated that the lack of terrestrial gravity results in the development of negative physiological effects which are integrally defined as a "deconditioning" state of the body. In respect to weightlessness, this concept schematically depends on the following reasons:

1. Reduced weight-bearing of musculoskeletal system both due to removing of weight effects on the supporting structures and unloading of antigravitational muscles.
2. Blood redistribution to cause negative fluid-electrolyte balance and a change in regulation of vascular tone (venous tone in particular).
3. As regards biophysical processes, the weightlessness looks as if it removed internal elastic strains of the bodily organs and tissues. There disappears the sensation of body weight. This leads to a rearrangement of the control mechanisms at the expense of altering the centripetal system effects.

The above-mentioned generalized concepts only stress the complex combination of physical and biological regularities relating to the body responses to weightlessness effects. Nevertheless, it can be stated that the gravitational component of an environment, being, as it were, an "latent" factor under Earth conditions, at the cost of its constant value plays an important role in producing the regulatory mechanisms responsible for maintaining a required level of adaptational reactions of the body (1-5).

Therefore, further improvement of the countermeasures to prevent negative effects of weightlessness is still a task of high priority in the field of space medicine.

The most adequate prophylactic means for long-duration space missions is a generation of artificial gravity (6).

At present, there proposed two ways of producing artificial gravity: rotation of space system around the center of masses (7) and use of the short-radius centrifuge (SRC) aboard space station (8). The main difference between these ways resides in the fact that, when in use, SRC produces not constant but periodic G-loads. In this case, along the longitudinal axis of the body there occurs G-gradients ranged from "0" G at the head level to a desired magnitude of its value at the feet level.

In the literature, there is evidence for experiments on SRC which are primarily concerned with studying some physiological reactions of tolerance to the rotations and to subsequent orthostatic effects (8-12). The experiments on Cosmos-782 and Cosmos-936 indicated that rotations on an onboard 1G centrifuge produce on the biological objects the same effect as the Earth gravity does (13).

All the above merits attention in order that studying of gravitational effects using SRC can be regarded as an important scientific trend directed toward problem of artificial gravity. In this case, it is necessary to stress the significance of clinico-experimental validity of physiological contribution which is determined by gravitational component reproduced on SRC.

This paper was aimed at studying human +Gz acceleration tolerance after simulated weightlessness and applying on SRC of artificial gravity ranged from 1 to 2 G.

METHODS

The 7.25 m radius centrifuge experiments have been done to evaluate +3 Gz acceleration tolerance for 5 min before and after exposure to simulated weightlessness.

"Dry" immersion was used as a model of weightlessness (Fig.1).

Figure 1. Simulation of microgravity effects using a "dry" immersion test

The 2 m radius centrifuge has produced +Gz accelerations of 0.8; 1.2 and 1.6 G. Resulting values of the accelerations with consideration for earth gravity were 1.3; 1.6 and 1.9 G respectively. Time of an acceleration exposure was 40 - 60 min, periodicity was 2 - 3 times a day (Fig.2).

The 1st series of studies has been done to evaluate +3 Gz acceleration tolerance before and after 3-day immersion and use of SRC without combination with other prophylactic means.

The 2nd series of experiments also assessed +3 Gz acceleration tolerance after 3-day immersion and SRC combined with water and salt supplements (WSS). WSS was used 1 hour prior to the rotation of SRC in amounts of 5 ml of water and 0.09 g of NaCl per 1 kg of body weight (14,15).

The 3rd series of experiments has studied +3 Gz acceleration tolerance before, during and after 28-day immersion.
RESULTS

Three sets of experiments have been performed (Fig. 3).

<table>
<thead>
<tr>
<th>Experimental Period</th>
<th>Length of Immersion</th>
<th>Experimental Design</th>
<th>Short-Radius Centrifuge</th>
<th>Water and Salt Supplements</th>
<th>Bicycle Acceleration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3 days</td>
<td>Pre-Imersion</td>
<td>+3 Gz</td>
<td>+3 Gz</td>
<td>BE exercises</td>
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<tr>
<td>II</td>
<td>3 days</td>
<td>Pre-Immersion</td>
<td>+3 Gz</td>
<td>+3 Gz</td>
<td>BE exercises</td>
</tr>
<tr>
<td>III</td>
<td>28 days</td>
<td>Pre-Immersion</td>
<td>+3 Gz</td>
<td>+3 Gz</td>
<td>BE exercises</td>
</tr>
</tbody>
</table>

During immersion SRC runs and bicycle ergometer (BE) exercises have been applied. The regimen of physical exercises was 500 kg-m/min for 10 min. 3 times during a 60-min period of SRC rotation. Such exposures were performed 2 times a day according to a special cyclogram.

In all experiments with +3 Gz acceleration exposures, the Nebb leads electrocardiogram, photoplethysmogram of ear lobe vessels to evaluate the blood flow status in a carotid arteries basin, time of sensorimotor responses to light cues using of the perimeter established in the measurements taken during centrifugation.

A threshold of human +3 Gz acceleration tolerance was determined on the basis of an occurrence of visual disturbances or their precursors - a decrease in aural pulsogram amplitude up to isoelectric line, increased time of sensorimotor responses to light cues lasting more than a second. Experiments were also terminated upon development of prognostically unfavorable disorders of cardiac rhythm - grouped, polytopic and multiple extrasystoles.

Figure 2. The general view of the short-radius centrifuge.

Figure 3. Experimental design.

decreased and was 237 ± 16 s (minus 21%).

After immersion and use of + 0.8, 1.2 and 1.6 Gz on SRC the acceleration exposure time was 246 ± 34 s; 278 ± 14 s and 299 ± 12 s and was shorter respectively by 18.7 and 1 % as compared to baseline (Fig. 4).

Figure 4. Changes in human tolerance to +3 Gz (Δ %) after 3-day immersion and prophylactic rotation on short-radius centrifuge (SRC).

The results obtained point to the principal possibility to use the low-level G accelerations on SRC as a prophylactic means to prevent decreasing body G-tolerance in simulated weightless environment.

Of particular importance is also the question concerning the possibility to use the SRC in combination with other countermeasures and an effectiveness level of such exposures.

Based on existing evidence that the microgravity - induced effects of body hypohydration play an important role in the genesis of decreasing an orthostatic tolerance of the cosmonauts after landing an evaluation of the combined use of low-level accelerations and water-salt supplements.

Data on +3 Gz acceleration tolerance after applying SRC and water and salt supplements during a 3-day immersion are presented in Fig. 5.

Figure 5. Changes in human tolerance to +3 Gz acceleration (Δ %) after 3-day immersion and use as a prophylactic means of short-radius centrifuge (SRC) and water and salt supplement (WSS).
After immersion without applying SRC the exposure time to +3 Gz accelerations decreased by 21 % and was 237 ± 16 s. In case of using on SRC of +Gz accelerations of 0.8 and 1.2 G in combination with water and salt supplements the tolerance to +3 Gz accelerations was 252 ± 24 s and 298 ± 11 s (by 13 and 4 % lower than the baseline value) respectively. Under conditions mentioned the exposure times to +3 Gz accelerations were respectively longer by 5 and 3 % than when used only SRC which is indicative of an effective combined use of SRC and water and salt supplements.

The muscular deconditioning occurring in weightlessness is known to be one of main factors of a decreased G-tolerance of the cosmonauts. Because of this, it was necessary to assess an effectiveness of combined use of SRC and physical exercise in the simulated weightless environment.

As illustrated in Fig. 5, prior to starting 28-day immersion the tolerance to +3 Gz accelerations was 298 ± 2 s. Seven days after immersion without using prophylactic means a marked tendency toward a decreased tolerance to +3 Gz accelerations to 130 ± 87 s (by 56 %) was noted. After terminating the immersion and the use of SRC combined with ergometer exercise the exposure time to +3 Gz acceleration was 273 ± 15 s and was only 8 % lower as compared to baseline.

Figure 6. Changes in human tolerance to +3 Gz acceleration during 28-day immersion without and with the use of short-radius centrifuge (SRC) and bicycle ergometer (BE) exercise tests.

The materials presented point to the fact that the exposure to low-level accelerations in long term exercise during long-term simulated weightlessness had also positive effect on subsequent human tolerance to the accelerations.

DISCUSSION

The positive prophylactic effect of the SRC results from the fact that the artificial G-exposures create conditions for musculoskeletal loading, maintain bone weight-bearing, produce hydrostatic gradient of blood pressure in the large vessels of the body, support afferent stimulation of CNS (Fig. 7).

Figure 7. Physiological mechanisms of prophylactic effects of +Gz accelerations produced by SRC in simulated microgravity.

Undoubtedly, an increase in secreting antidiuretic hormones, renin, catecholamines, a decrease in diuresis and excretion of salts which appeared to result in an evaluation of circulating blood volumes also played its positive role (15-18).

However, prior to applying SRC as a prophylactic device in extended manned space missions the additional ground-based and in-flight studies are needed to be done. The main purpose of these investigations is the development of adequate profiles of rotation on SRC in combination with other countermeasures.

CONCLUSION

Applying the artificial G-accelerations on SRC in the range of 1 to 2 G without use of other countermeasures and combined with water and salt supplements or ergometer exercise in simulated microgravity had a distinct positive effects on the subsequent tolerance to + Gz accelerations.

The findings obtained supports the possibility to apply SRC as a prophylactic device in extended space flight.

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PROGNOSTICATION OF FLIER’S +Gz TOLERANCE ON THE BASE OF STATIC MUSCULAR STRENGTH ENDURANCE

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INTRODUCTION

Transition of military pilots on new high performance aircrafts in modern tactical aviation has considerably increased demands to their tolerance in air combat maneuvering. In this relation there may play very important role the development of special functional load test, which would allow to medical examiner reliably extrapolate and appreciate the flyer’s individual +Gz tolerance to high level of flight accelerations instead of cost human centrifuge expert investigation.

The data presented in available literature demonstrate rather low correlation of hypoxic hypoxia endurance test, orthostatic load test, lower body negative pressure, treadmill physical exhaustion test and veloergometric functional probe etc. with +Gz tolerance limits (1, 2, 3).

At the same time in numerous studies there was established, that individual human +Gz tolerance to a large extent depends on protective anti-\( G_z \) straining maneuvers, involving both special muscular and breathing procedures.

We have found, that under effect of high \(+G_z\) accelerations about 8-9 \( G \) and with duration up to 30 s. the pilot leg muscles effort on pedals, while performing anti-\( G \) straining maneuver would attain pressure about 260-280 kg-power. So, the individual pilot \(+G_z\) tolerance limit is determined by his ability to produce and maintain for some tens of seconds the isometric strengthening efforts of leg and abdominal wall muscles. Supporting on our own experience and long term observations we have proposed a special method of individual human \(+G_z\) tolerance limit prognostication, which is based on a static isometric muscular effort gauge recording. We have designated this functional load test as statocergometric one.

METHODS

The test is being performed in static conditions on special stand "Statoc ergometer", which we have constructed and built ourselves (Fig. 1).
observe the muscular efforts of two legs and abdomen during anti-G straining maneuver, in kg-power.

The test has been conducted in following way. After applying of EOG sensors and leads the tested person has fixed himself in a seat motionless. Before to start the functional load test, the angle of knee joint flexion has been arranged equal about 120 degrees. After the 5-minute rest duration and recording of initial (control)data the experted person, following the command of aviation medical officer, exerts the staged growing lower body muscles efforts at steps 120, 160, 200, 240 and 280 kg power, maintaining each stepped effort during 30 seconds. The test has been completed up to full indicated level or stopped at the subject's demand due to physical exhaustion and fatigue or to revelation of other objective or subjective signs, which contraindicate the continuation of functional lower body straining test in process of isometric exertion endurance investigation. In process of test performance and on 1, 3 and 5 minutes after its termination there has been recorded the EOG and blood pressure.

In experiments took participation 20 healthy persons, all male subjects, age 19-22, which have had rich experience in high +Gz human centrifugation training. There have been compared the
cardiovascular reactions of test subjects to the effect of high level $+g_z$ stress, statoergometric and veloergometric functional load tests.

RESULTS and DISCUSSION

As can be seen from graphic data on Figure 2 during veloergometric load test the cardiac output of subjects has increased more than 3 times. This phenomenon is accompanied by relative augmentation of stroke volume (average 59%) and substantial decrease of total peripheral resistance (average 65%), which is very characteristic for such dynamic load test. Arterial pressure has changed to some lesser degree, mainly at expense of moderate increase (average 37%) in systolic pressure and decrease of diastolic (average 25%) against the non-significant shift of mean arterial pressure.

As distinct from above the cardiovascular reactions to statoergometric functional load test are characterized by qualitatively different features and similar to those, which were observed during high $+g_z$ acceleration runs in centrifugational tests. As might be seen from materials, shown in Fig. 2, the most striking peculiarity of static isometric functional load is a marked (average 48-62%) increase in all arterial pressure parameters - systolic and diastolic.

![Figure 2. Comparison of hemodynamic reactions under influence of $+g_z$ centrifugation, statoergometric and veloergometric functional load tests.](image)

Graphic designations:

HR - heart rate
SBP - systolic blood pressure
DBP - diastolic blood pressure
TPR - total peripheral resistance
CO - cardiac output
SV - stroke volume

All data are referred to initial level, which is equal to 100, and expressed in per cents.

Incidentally the arterial pressure increment (more than 1, 5 times) is stipulated predominantly by rise of total peripheral resistance, because the cardiac output against a background of high tachycardia during isometric load changes in a small degree owe to pronounced (nearly 2 times) decrease of stroke volume.

Thus, under the dynamic muscular
effort heart works in conditions of low total peripheral resistance and augmented load by volume readjustment, while under the isometric physical strengthening, it pumps in less favorable conditions owing to output blood flow resistance from the heart.

In connection with this the reactions of cardio-vascular system to high +Gz stress are analogous, though more manifested in comparison to static isometric physical exertion. Just right during +9 Gz acceleration run there has been registered more prominent tachycardia, increased level of blood pressure and total peripheral resistance compared to static isometric lower body muscular contraction.

The qualitative differences were revealed only in systolic arterial pressure of ear lobe vessels. The main cause for such disparities is hydrostatic blood column, which affects the regional blood redistribution under influence of +Gz acceleration. Such redistribution, as it is known, leads to venous return decrease, pooling the blood in lower parts of human body and reduction of arterial pressure on the head level. As opposed to it during static isometric muscular effort there occurs increase of blood pressure at head level, which is a mechanism of protective effect of static leg and abdomen muscles strengthening during hypergravitational field action.

It might be concluded from data cited above, that judging by character of hemodynamic reactions, the static ergometric load test quite definitely imitated the effects of acceleration force. It is clear, that such test may be used as predictor criterion of individual subject +Gz tolerance limit.

The further studies have detailed the close correlational interaction (R=0.89) between static physical work capability and +Gz tolerance limit during single centrifugation run in extent from 4 to 8 +Gz with duration up to 30 s without antigravity suit.

The special investigations were conducted, which involved 75 military flyers, who mastered the MiG-29 aircraft and made air combat maneuvering sorties. We have studied the validity of static ergometric load test for prediction pilot +Gz tolerance test to high level of acceleration stress.

In our centrifuge experimentation tests the +Gz tolerance limit was appreciated by maximum +Gz, demonstrated by flyer in absence of visual disturbances.

The estimation of results in staticergometric load tests was performed on a base of pilot physical work capability and physiological reactions, using a three-staged scale: excellent, good and satisfactory.

The results of investigations have indicated rather reliable and high correlation of pilot acceleration...
tolerance in flight and statoergometric load endurance value.

The best mark of statoergometric test with probability 0.75 corresponds to highest $G_z$ tolerance level $-8.5\, G$ and more, the good mark - to $7.5-8\, G$ and satisfactory - to $7\, G$ and less.

The excellent mark of statoergometric appraisal test suggests a good athletic status of flyer, his preparedness to perform high $+G_z$ air combat maneuvers. The flying personnel with good and satisfactory marks according to results of statoergometric functional load test needs before admitting to flight duties on high performant aircraft of additional course in physical training, aimed to develop the force and static endurance qualities in leg and abdominal muscles.

The substantial and important prognostical role should be attributed to cardiac rhythm disturbances and intracardiac conductivity alterations in flyers during the functional probe exertion. As a rule, such changes indicate the hidden myocardial hypoxic deficiency and may have certain prognostical significance for real flight conditions.

It should be noted too, that in estimation of the efficiency for some rehabilitational and medico-prophylactical measures as well as special physical training of pilots, one must take into account, that increase of test mark only on one point approximately corresponds to flyer's $+G_z$ tolerance gain equal to $+1\, G_z$.

Thus, proposed statoergometric functional load test might be used in flying practice for individual flyer $+G_z$ -tolerance limit prediction under effect of high $G$-forces and for appreciation of his physical training level, effectiveness of restorative measures and prophylactic means.

REFERENCES


Two of the major cardiovascular problems associated with high sustained +Gz are maintenance of venous return to the heart and the maintenance of blood pressure and blood flow to the brain. Eye-level blood pressure (ELBP) is known to decrease by 22-25 mm Hg/G as a result of the inertial load on the eye-to-heart hydrostatic column of blood (9). Thus, the brain is at a disadvantage when the body is in the upright position (1 +Gz), where, if mean blood pressure at heart level is 100 mm Hg then brain level blood pressure will be around 78 mm Hg, assuming a 30 cm eye-to-heart distance. As +Gz progresses from 1 +Gz to 5 +Gz then ELBP will decrease from 78 mm Hg to -10 mm Hg, resulting in a G-induced loss of consciousness (G-LOC), assuming no interventions. During +Gz, venous return generally decreases significantly, whereas with adequate protective equipment and the anti-G straining maneuver (AGSM) adequate brain level blood pressure and flow can be maintained. Recent swine data demonstrate that with adequate venous return support (impaired G-suit) brain level blood pressure and flow can be maintained with significantly reduced straining effort (2).

Currently, +Gz protection is provided by the standard 5 bladder G-suit, which has changed very little in design over the past 45 years, and the anti-G straining maneuver (AGSM) which is a very fatiguing physical maneuver. The inflated G-suit provides average relaxed +Gz protection of 5.4 G (8), while the AGSM, singularly, or in combination with the G-suit can provide variable +Gz protection to 9 +Gz (5), or greater, depending upon the efficiency and effectiveness of the AGSM and the physical condition of the performer.

Figure 2 illustrates the importance of a strong and rhythmic AGSM during a sustained 8 +Gz exposure in man. ELBP was obtained from a catheter placed through the nasopharynx into the esophagus, placed at mid-chest level and connected to an external transducer. Note that when EP decreased during the expiration/inspiration period, between strains, ELBP fell precipitously below zero and then rose sharply when EP rose during the strain. At about midway through the +Gz exposure the subject was instructed to let up on the strain to lose approximately 25% of their peripheral vision to insure that they did not strain more than necessary. Note the reduction in EP at mid-exposure.

Venous return into the thorax can only occur when venous pressure at the venous inlets to the thorax is greater than intrathoracic pressure. There is generally a positive abdominal-thoracic venous pressure gradient, which is amplified by G-suit inflation (3,9). However, the gradient decreases dramatically during the strain, explaining the sharp reduction in venous return through the diaphragm at that time (3). Upon initiation of the AGSM venous flow abruptly becomes negative, gradually increases throughout the strain and then increases sharply during the
no strain period (expiration/inspiration), and becomes greatest just prior to the next strain (Fig 3). If the AGSM is maintained too long venous return will be further decreased, as in the Valsalva maneuver. Cardiac output will be dramatically reduced and ELBP will decrease, even though BP is normally elevated by the AGSM. On the other hand, if the time between strains is extended beyond several seconds the extended loss of ELBP and blood flow to the brain will result in loss of vision and/or consciousness (G-LOC) if not corrected. Thus it is extremely important that the AGSM be cycled every 3-5 sec (Fig 2) to allow venous return during expiration/inspiration between strains, followed by the generation of pressure during the strain.

More recent simultaneous pulmonary artery and aortic blood flow data from the swine demonstrates that during the AGSM output from the left ventricle increases relative to the right ventricle and during the no strain period the opposite occurs. Thus it appears that during expiration/inspiration (no strain period) a bolus of blood is moved through the vena cava and right ventricle into the lung but not into the left ventricle, whereas, during the strain the increased intrathoracic pressure forces blood out of the lung vasculature and through the left ventricle into the systemic circulation while slowing venous return into the vena cava and right ventricle.

Fig 3. Influence of the AGSM, measured as esophageal pressure (EP), on other measured parameters, especially venous flow through the diaphragm. Note that at initiation of the straining maneuver (a) flow decreased dramatically and remained low until relaxation of the strain (b) when it increased and was greatest just prior to the next strain. See Fig 1 for legend. From: Burns, et al (3).

The transfer of developed intrathoracic pressure into the cardiovascular system during the AGSM is demonstrated in Fig 4 which illustrates the influence of the AGSM on left and right ventricular pressure (LVP and RVP), central venous pressure (CVP) and EP. LVP, RVP and CVP were obtained from high fidelity solid state transducers while EP was obtained from an air

filled latex balloon catheter. Note the close similarity between the different waveforms. Also note that the sharp rises in LVP, RVP and CVP are the result, almost exclusively, of extravascular intrathoracic pressure generation by the AGSM and thus transmural vascular pressures are minimally affected.

Fig 4. Influence of the AGSM (EP) on left and right ventricular pressure, central venous pressure and aortic blood flow during an 8 +Gz exposure.

The linear relationship between the augmentation of ELBP in response to developed EP in the swine at 3.5 and 7 +Gz is nicely demonstrated in Fig 5.

Because of the fatiguing effort involved in performance of the AGSM and the continuing loss of pilots and aircraft to G-LOC it has become very important to develop additional techniques and/or equipment for G protection. A very successful G-protective technique, adopted from altitude protection, is pressure breathing during G (PBG). This technique provides balanced pressure (chest counterpressure) to the lungs at a maximum of 60 mm Hg at 9 +Gz. It has been demonstrated that increased intrathoracic pressure from pressure breathing (PB) is transmitted into the intrathoracic vascular system on a 1:1 basis. Thus, blood pressure can be expected to rise by 60 mm Hg, with an associated rise in G tolerance. The most significant benefit of PBG has been a reduction in fatigue demonstrated by extended time at G (1,10). An additional benefit of PBG has been the ease of obtaining inspiratory air since it is available under pressure, even
though, theoretically, the transthoracic pressure should be zero due to the chest counterpressure garment. However, the counterpressure garment appears to be slightly undercompensated, which is advantageous to the user.

Fig 6 illustrates the immediate effect in the swine of PB of 60 mm Hg without increased acceleration and without G-suit inflation. Mean data from 12 swine demonstrates a reduction of stroke volume and cardiac output from both ventricles and an increase in heart rate of approximately -30%, -22% and 14%, respectively. The increase in mean aortic blood pressure (AP) was only about 1/2 of mask pressure because of the absence of G-suit inflation. The percentage of transfer of intrathoracic pressure to the cardiovascular system is dependent upon the amount of counterpressure (i.e., legs, torso, arms, etc.), and can be as high as 125% of mask pressure (4).

It can be seen from these data that the AGSM and PBG are similar in that they both produce intra-thoracic pressure which augments cardiovascular pressures, most importantly head-level blood pressure for brain perfusion. The basic differences between these two pressure sources is that the AGSM is an active, fatiguing process which pressurizes the lung parenchyma from without, whereas, PBG is a passive process which pressurizes the lung parenchyma from within through the trachea.

Another recent improvement in G-protective technology has been the extended coverage G-suit (ECGS), also known as the advanced technology G-suit (ATAGS). This suit covers the entire legs and feet with a interconnected abdominal bladder. The suit has been demonstrated to provide a 60% improvement in G-time tolerance on the centrifuge (7) and a prototype has been well received by test pilots during preliminary flight trials (6).

In summary, the G-suit and the AGSM are an integral part of high-G survival. There is a continuing need to develop improved G-protective equipment and/or techniques for augmentation of venous return to the heart and for reduction of AGSM effort.

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METABOLIC BASES OF +Gz DURATION TOLERANCE

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INTRODUCTION

Interest in and understanding the effects of high sustained G (HSG) on humans has greatly increased during the past twenty years. High performance aircraft can exceed the capability of the anti-G protection systems (Burton, 1990) and expose aircrew to high G intensity (i.e., greater than +7Gz) for sustained periods of time (i.e., greater than 15 sec). From the outset, the primary focus of human research on +Gz tolerance to HSG was the cardiovascular system (Burton, 1989a; Burton et al., 1974; Leverett et al., 1972) because of the belief that the principal limitations to exposure to HSG are related to visual impairment and altered cardiac rhythm.

Efforts have been directed toward increasing +Gz tolerance through the use of mechanical aids (e.g., anti-G suit) or physiologic countermeasures (e.g., anti-G straining maneuver, AGSM) to counteract the hydrostatic pressures caused by the accelerative forces and to maintain adequate blood flow to the brain. The simulated aerial combat maneuver (SACM) was developed to provide a G-probe on a human-use centrifuge which was continuous, repetitive, and without a predetermined duration (Burton and Shaftstall, 1980). The SACM has become the principal research method for measuring +Gz duration tolerance (Burton, 1990).

The AGSM includes both the M-1 and L-1 straining maneuvers which, in turn, require tension of skeletal muscles (Burns and Baldin, 1988; Burton et al., 1974). Because the AGSM sometimes requires maximal isometric muscular tension activity, fatigue often limits tolerance to HSG and the SACM (Burton, 1989b; Burton and Shaftstall, 1980; Miller et al., 1959). This has led to attempts to identify the metabolic bases (i.e., anaerobic and aerobic) for +Gz duration tolerance. In particular, an anaerobic (i.e., strength training) exercise has been suggested as useful in increasing SACM tolerance (Epperson et al., 1982; 1985; Tesch et al., 1983) because heavy-resistance training has been shown to increase muscle energy reserves of glycolysis and phosphagens (MacDougall et al., 1977). However, muscular endurance usually is limited by local weakness and pain resulting from intramuscular lactate accumulation which inhibits glycolytic enzymes. Further, circulation may be occluded when muscular contractions are about 68% of maximum isometric force (Start and Holmes, 1963). Thus, endurance time depends upon the rate of energy use, residual blood flow to muscles, local stores of glycogen and phosphagens at the beginning of contractions, and diffusion of lactates and other metabolites away from the muscle fibers.

On the other hand, SACM duration tolerance does not appear to be influenced by aerobic capacity (Epperson et al., 1982; 1985), although it is known that moderate levels of hypoxia can impair work performance at 1G (Astrand and Rodell, 1986). Increased aerobic condition (i.e., VO2 max) does not appear to enhance +Gz tolerance (Whinnery and Parnell, 1987). Supplemening or reducing inspired oxygen (FIO2) should have little effect if this is true. Further, successful G riders appear to have higher resting systolic blood pressure (Whinnery, 1979) and lower aerobic power and capacity (Baldin et al., 1985). Information regarding the role of aerobic metabolism on human G-duration tolerance is necessary to determine the importance of supplemental oxygen for operational use. Accordingly, a study was initiated to determine the effects of different FIO2 on +Gz duration tolerance. Preliminary results are reported here.

MATERIALS AND METHODS

Subjects: Six male subjects from the Armstrong Laboratory acceleration panel all received the same hypoxia and acceleration profile training prior to data collection.

Physical Testing: Because the physical training state may account for interindivudual responses to hypoxia, all subjects' aerobic (Bruce treadmill protocol) and anaerobic (Wingate Anaerobic Test, WATS) fitness were assessed prior to data collection on the cehol and hyperoxic stage. In addition, all subjects breathed 12% oxygen at 1G through an aviator's oxygen mask (Model MBU-20/P) with positive pressure to provide the opportunity for subjects to experience hypoxia and to obtain baseline blood oxygen saturation (SaO2) data. Mask fit was pressure tested for leaks to assure there was no dilution of inspired gas mixtures.

Table 1. HYPOXIA CONDITIONS AND EQUIVALENT ALTITUDES.

<table>
<thead>
<tr>
<th>FIO2 (%)</th>
<th>PO2 (torr)</th>
<th>Equivalent altitude (m/ft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>457</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>155</td>
<td>168 [550]</td>
</tr>
<tr>
<td>0.18</td>
<td>138</td>
<td>1.219 [4000]</td>
</tr>
<tr>
<td>0.16</td>
<td>123</td>
<td>2.130 [6990]</td>
</tr>
<tr>
<td>0.14</td>
<td>109</td>
<td>3.048 [10000]</td>
</tr>
<tr>
<td>0.12</td>
<td>92</td>
<td>4.267 [14000]</td>
</tr>
</tbody>
</table>

1 Based on sea level pressure of 760 torr (U. S. Standard Atmosphere, 1952)

Breathing Gas Mixtures: Tanked gas mixtures of 12, 14, 16, 18, 20, and 60% (Table 1) oxygen were used. The first five mixtures simulated altitudes ranging from 4267 m (14,000 ft) down to sea level. The hyperoxic gas mixture of 60% was used because significant increases in acceleration aletecastas are observed above that level (Haswell et al., 1986). The percent oxygen in the tanked breathing gas was verified by mass spectrometry (Perkin-Elmer, Model 1100, Medical Gas Analyzer) after each exposure. Each subject was exposed to the various O2 concentrations, in series and in semi-random order, and each was unaware of the percent oxygen he was breathing on any given day.

Measurements: Heart rate and rhythm from standard EKG
leads were observed on a video display on a real-time basis and recorded (Gould, Model 2802S) for further analysis. Blood oxygen saturation was monitored continuously during the entire experimental period using an ear oximeter (Hewlett-Packard, Model 47201A) and recorded. The finger prick method was used to obtain pre- and post-SACM blood samples from which lactate levels were measured according to the procedure described elsewhere (Hohorst et al., 1959).

**Experimental Procedure:** On each day of data collection, the subject donned the EKG electrodes and the standard USAF anti-G suit (Model CSU-13B/P) with standard operational ALAR high flow anti-G valve and was seated in the Armstrong Laboratory (Brooks AFB) Centrifuge gondola in an upright F-15 Aces II seat. The EKG leads and ear oximeter were accessed to the data acquisition system and the pre-G checkout completed; a finger prick provided blood (i.e., 0.2 ml) for determination of pre-G (Control) lactates while the subject breathed ambient air. The subject then began breathing the gas mixture selected for that day for 5 min at 1G or until the \( \text{SaO}_2 \) remained stable for 1 min; this was followed by another finger prick. The acceleration profile consisted of, in series, a rapid onset run (GOR, 6 G·sec\(^{-1}\)) of +3G\(_2\) for 30 sec with anti-G suit inflated, gradual onset run (GOR, 0.1 G·sec\(^{-1}\)) with anti-G suit uninflated, and the simulated aerial combat maneuver (SACM) of +4.5 to +7.0G\(_2\) with alternating 15 sec plateaus (Burton and Shaftstall, 1980). The GOR was terminated when the subject experienced 100% peripheral light loss (PLL) and 50% central light loss (CLL); the SACM was terminated when voluntary fatigue or light loss criteria were reached, when \( \text{SaO}_2 \) reached 60%, or for reasons determined by the medical monitor. Duration time for voluntary fatigue has been validated as a reproducible end point which represents an individual's SACM G tolerance (Burns and Baldwin, 1988; Burton and Shaftstall, 1980; Epperson et al., 1982). Between each run, the subject rested for 2 min period or until his \( \text{SaO}_2 \) had stabilized for 1 min. Prior to the SACM, another finger prick blood sample was obtained. Finger prick blood samples also were obtained at 1, 3, and 9 min post-SACM. Three minutes post-G is the optimum time for peak blood lactate levels following the SACM (Tamir et al., 1988; Wiegman et al., 1989).

**Statistical Analyses:** Unpaired t-test was used to determine statistical significance (i.e., \( p < 0.05 \)) between groups.

**RESULTS AND DISCUSSION**

All subjects in this study displayed age (26.8 ± 3.2 yr), height (173.3 ± 3.2 cm), weight (75.5 ± 2.5 kg), aerobic capacity (47.8 ± 3.5 ml·kg\(^{-1}\)·min\(^{-1}\)), and anaerobic capacity (627.8 ± 15.2, Mean Power, W) characteristics similar to those previously reported (Tamir et al., 1988; Tesch et al., 1983; Wiegman et al., 1989) of subjects exposed to SACM (Table 2).

<table>
<thead>
<tr>
<th>Reported Wiegman Tesch et al. Herein et al., 1989</th>
<th>1983</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26.8 ± 1.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.3 ± 3.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.5 ± 2.5</td>
</tr>
<tr>
<td>Aerobic Capacity (ml·kg(^{-1})·min(^{-1}))</td>
<td>47.8 ± 3.5</td>
</tr>
<tr>
<td>Anaerobic Capacity (Mean Power, W)</td>
<td>627.8 ± 15.2</td>
</tr>
</tbody>
</table>

Preliminary results (Table 3) revealed that SACM duration time of 248 sec for our subjects at F\( \text{O}_2 \) of 20% was not quantitatively different from most previous reports (Epperson et al., 1982; Tesch et al., 1983; Tesch and Baldwin, 1984; Wiegman et al., 1989). There appears to be a positive relationship between +G\(_2\) duration and inspired (12 to 20%) oxygen (Table 3). Although fatigue and light loss were the end points of interest, some SACM runs were terminated because of protocol (e.g., \( \text{SaO}_2 \) below 60%) or medically (e.g., premature ventricular contractions) related reasons. From limited data available to date, it appears that SACM runs involving the

<table>
<thead>
<tr>
<th>( F\text{O}_2 ) (%)</th>
<th>( n^1 )</th>
<th>SACM Duration Time (Sec)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>3</td>
<td>105 ± 23</td>
<td>Reported herein</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>155 ± 33</td>
<td>Reported herein</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>122 ± 42</td>
<td>Reported herein</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>184 ± 47</td>
<td>Reported herein</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>248 ± 22</td>
<td>Reported herein</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>201 ± 19</td>
<td>Epperson et al., '82</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>245 ± 35</td>
<td>Tesch et al., '83</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>242 ± 37</td>
<td>Tesch &amp; Baldwin, '84</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>250 ± 97</td>
<td>Wiegman et al., '89</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>261 ± 20</td>
<td>Reported herein</td>
</tr>
</tbody>
</table>

\(^1\) Number of subjects terminating the SACM due to fatigue or light loss criteria. Remaining subjects terminated SACM due to other reasons (e.g., \( \text{SaO}_2 \) below 60%)

lower inspired \( \text{O}_2 \) mixtures (e.g., 12%, 14%) were terminated because of light loss criteria; higher inspired \( \text{O}_2 \) mixtures (e.g., 20%, 60%) were terminated because of fatigue.

It is known that \( \text{SaO}_2 \) rapidly decreases during sustained +G\(_2\) exposures (Burns and Baldwin, 1988; Burns et al., 1974; Besch et al., 1978), and decreases in \( F\text{O}_2 \) appear to exacerbate this reduction. Because the lung appears to be the body organ most directly susceptible to HSO, oxygen delivery to the tissues is vulnerable and subjects may experience symptoms of hypoxia. It is

<table>
<thead>
<tr>
<th>( F\text{O}_2 ) (%)</th>
<th>( n^1 )</th>
<th>Lactates (mmol/L)</th>
<th>Duration Time (Sec)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>6</td>
<td>2.91 ± 0.62</td>
<td>3</td>
<td>105 ± 23</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>4.06 ± 0.55</td>
<td>4</td>
<td>248 ± 22</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>4.73 ± 0.96</td>
<td>4</td>
<td>112 ± 18</td>
</tr>
<tr>
<td>20</td>
<td>18</td>
<td>5.3 ± 1.3</td>
<td>11</td>
<td>245 ± 35</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>4.77 ± 1.50</td>
<td>10</td>
<td>250 ± 97</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>4.20 ± 0.84</td>
<td>5</td>
<td>261 ± 20</td>
</tr>
</tbody>
</table>

\(^1\) Number of subjects generally accepted that the main effects of +G\(_2\) on the pulmonary system are airway closure, altered ventilation perfusion, and intrapulmonary shunting of blood resulting in venous admixture, and atelectasis (Glaister, 1970). Although increases in \( F\text{O}_2 \) reduce and sometimes eliminate the reduction in \( \text{SaO}_2 \) (Haswell et al., 1986), no changes in the +G\(_2\) duration at \( \text{O}_2 \) levels between 20% and 60% were
detected in our study. Thus, our data suggest hyperoxia does not appear to enhance +Gz duration.

Peak blood lactate values (Table 4) for subjects breathing 20% O2 were similar to previous reports (Tamir et al., 1988; Tesch et al., 1983; Wiegman et al., 1989). No changes in peak lactates were detected between subjects breathing 20% and 60% O2 but they were reduced (P<0.05) in the 12% O2 group. Although a shorter SACM duration time was also detected in the 12% O2 group compared to the 20% (P<0.005) and 60% (P<0.025) O2 groups (Table 4), no significant difference in peak lactates was detected between the 20% and 60% O2 groups.

A positive relationship was detected between SACM duration times and peak blood lactates regardless of the level of inspired O2. This finding appears to confirm a previous report (Tesch et al., 1983) of a positive correlation between blood lactate levels and SACM duration. However, when we analyzed lactate data from individuals breathing either 12%, 20% or 60% oxygen, we could not detect a significant correlation between SACM duration and peak blood lactate levels within any of the groups.

Limited SaO2 data are available from subjects who breathed 12% O2 during 20-min periods off the centrifuge at 1G compared to their breathing 12% O2 during comparable periods—incuding the SACM—on the human-use centrifuge. For the pre-SACM period on the centrifuge, it was observed that the temporal decrease in SaO2 was nearly identical to that obtained from subjects breathing 12% O2 seated in a laboratory at 1G. Further, the decrease appeared to stabilize at about 72%. However, when the same subjects who breathed 12% O2 were exposed to the SACM, a further decrease was detected that also appeared to be asymptotic, and extrapolation of that curve suggests SaO2 stabilizes at a value somewhat lower than 72%. These data also suggest a portion of the observed SaO2 decrement was due to the reduced FIO2 and a portion of the +Gz stress per se. Further data collection and analysis are needed before a more detailed assessment can be made.

Table 5. RELAXED G TOLERANCE AND BLOOD OXYGEN SATURATION (MEAN ± SE) AT PEAK G FOR SUBJECTS (n=6) BREATHING DIFFERENT CONCENTRATIONS OF OXYGEN

<table>
<thead>
<tr>
<th>FiO2 (%)</th>
<th>G-Tolerance (G)</th>
<th>Time on tanked (Gas min)</th>
<th>SaO2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5.51 ± 0.38</td>
<td>10.88 ± 1.00</td>
<td>76.4 ± 2.7</td>
</tr>
<tr>
<td>14</td>
<td>5.69 ± 0.45</td>
<td>10.00 ± 0.51</td>
<td>83.7 ± 1.9</td>
</tr>
<tr>
<td>16</td>
<td>5.56 ± 0.40</td>
<td>10.76 ± 0.42</td>
<td>88.7 ± 0.9</td>
</tr>
<tr>
<td>18</td>
<td>6.07 ± 0.29</td>
<td>10.10 ± 0.40</td>
<td>89.2 ± 0.6</td>
</tr>
<tr>
<td>20</td>
<td>5.68 ± 0.37</td>
<td>10.11 ± 0.30</td>
<td>90.5 ± 1.1</td>
</tr>
<tr>
<td>60</td>
<td>5.62 ± 0.50</td>
<td>9.96 ± 0.21</td>
<td>95.1 ± 2.9</td>
</tr>
</tbody>
</table>

From other data (Table 5) we were unable to detect any significant relationship between relaxed +Gz tolerance and percent of inspired oxygen which, at 12% O2, represents breathing air at an equivalent altitude of 4267 m (14,000 ft, Table 1). This is not surprising because relaxed +Gz tolerance is directly related to eye-level arterial pressure which is determined by heart-level arterial pressure (Burton, 1986). The eye-level arterial pressure is determined by the hydrostatic pressure, height of the hydrostatic column, and ambient accelerative inertial force (G). Because seatback angle remained constant for all our subjects, only the +Gz intensity could influence relaxed +Gz tolerance. Nonetheless, it is interesting to note that our findings are in agreement with a previous report (Gauer, 1950) that relaxed +Gz tolerance is constant to about 4,000 m (13,100 ft) and that a higher altitude is required to show a decrease in blackout tolerance.

CONCLUSIONS

It has been reported that physical training improves both aerobic capacity (Cooper and Leverett, 1966) and anaerobic power (Astrand and Rodell, 1986) and that several weeks of resistance training enhances tolerance to the SACM profile (Epperson et al., 1982). We also know that SACM duration time has been increased by between 39% (Tesch et al., 1983) and 53% (Epperson et al., 1985) utilizing weight training regimens. It is also known that WATS, a cycle ergometer test to measure muscular power, provides a valid laboratory test of ability to endure SACM exposure (Wiegman et al., 1989).

From our preliminary data, it appears that +Gz duration is directly related to SaO2. Further, the temporal decrease in SaO2 from reduced FIO2 not only is exacerbated by but also is additive to the effects of +Gz. Although we were unable to detect any evidence that breathing 60% O2 enhances +Gz duration compared to breathing 20% O2, a more definitive relationship between aerobic metabolism and +Gz duration awaits further investigation. On the other hand, the magnitude of observed peak lactate levels suggests that the metabolic basis for tolerance to HSG is primarily anaerobic capacity and ability of the body to utilize that capacity (Burton, 1989b).

REFERENCES


Enhanced Aerobic Capacity and Increased Acceleration Stress: Is Their Interaction a Problem for Pilots?

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Brooks Air Force Base, Texas

Introduction
To permit or to restrict high performance aircraft (HPA) pilots from engaging in activities that enhance aerobic capacity is the question. Hippocrates wrote “Eating alone will not keep man well; he must also take exercise.” The issue of exercise and its relationship to the HPA pilot does not lead to such an obvious conclusion. There is a growing body of scientific evidence that strongly supports engagement in endurance enhancing activities. This evidence suggests aerobically conditioned individuals are more physically capable, have better physical and mental health, and have more productive lifestyle characteristics when compared to their sedentary peers. A concern expressed within the aeromedical community is that HPA pilots are a special subpopulation and the training benefits manifested in the general population may be a liability to successful tolerance of sustained acceleration.

The purpose of this report is to summarize the benefits derived from participation in endurance enhancing activities and to review the existing evidence regarding the interaction between aerobic capacity and sustained acceleration tolerance.

Definitions
Before discussing the physiological effects and health and lifestyle benefits of a regular aerobic exercise program, I believe it would be beneficial to describe some terms related to this topic that have caused some to be confused in their understanding of this issue. First, there are two basic types of exercise: 1) exercise obtaining a majority of its energy from glycolytic (anaerobic) metabolic sources; these are generally work bouts of very short duration requiring near maximal effort (strength, speed and power), and 2) exercise using the oxidative metabolic pathway to provide energy for work; these activities are generally, but not necessarily, of longer duration and require a lower percentage of the individuals maximal capacity to perform. It is important to recognize that cardiorespiratory (aerobic) capacity is related to, but is not synonymous with anaerobic capacity. Unfortunately much of the aeromedical literature has chosen a single term “Physical Conditioning or Fitness” to identify both types of exercise. This sharing of a common descriptive term has muddled our understanding of the influence each exercise type has on acceleration tolerance. I will emphasize the “aerobic” or endurance exercise activities and will not address issues related to so-called “anaerobic” or resistance type exercises. It is important, also, to appreciate that for any given task both glycolytic and oxidative energy sources are being used. It is rare for either energy pathway to work in isolation to accomplish a specific task.

Finally, we must define the term “physical fitness”. This term is an obscure concept and is often associated exclusively with cardiorespiratory or aerobic capacity. However, cardiorespiratory capacity is only one of four essential elements comprising a “truly fit” or the “physical fitness” of an individual. Muscular strength, flexibility, and motor coordination must be considered, in addition to cardiorespiratory capacity, when describing the “physical fitness” of an individual.

Physiological Responses
Aerobic training elicits numerous adaptations resulting from a complex set of central and peripheral mechanisms in humans. In fact, most physiological functions and structures are in one way or another influenced by endurance training. Complete description of the changes caused by aerobic training is beyond the intent of this report. Several excellent resources describe the combined and individual system adaptations to aerobic exercise (5, 9, 20, 24, 25). In this section, I shall present a brief summary of the cardiovascular adaptations thought to have an impact on the pilot’s ability to sustain acceleration stress.

Principal cardiovascular changes produced by endurance training in healthy individuals include increases in maximal oxygen uptake, stroke volume, cardiac output, systemic vascular conductance and total and regional oxygen extraction. In addition to these functional changes, modulation of the autonomic input to the heart and circulating blood volume are altered in trained versus untrained individuals. Changes in dimension and function of the heart, in combination with increased blood volume, would appear to provide the trained individual greater maximal cardiac output in exhaustive work such as that performed during +Gz stress. It is interesting to note that aerobic conditioning improves cardiac pump performance against an increased afterload (12). This effect would be a distinct advantage during sustained +Gz where afterload is intentionally increased.

Increases in skeletal muscle capillary density, in association with increases in biochemical and morphological changes, promote greater oxygen extraction, and could be an advantage for the trained pilot encountering moderate acceleration stresses. The advantage afforded these individuals would be found in their ability to rely for longer time intervals on oxidative metabolic pathways to provide energy for ATP resynthesis. However, the hypothesis that increased capillary bed capacity, especially in the leg muscles, may provide a greater reservoir for blood pooling during sustained G is untested.

Normal heart rate response to exercise is modulated by a combination of parasympathetic withdrawal and sympathetic excitation (23). Parasympathetic influence is the dominant factor at low work levels, whereas, sympathetic activity becomes increasingly important as the heart rate reaches near 100 beats per minute (24). A characteristic of endurance trained individuals is a sinus bradycardia at rest and lower heart rates at any absolute level of submaximal oxygen uptake; maximal heart rate is generally unchanged following conditioning. Some have expressed a concern that highly aerobic conditioned pilots may be susceptible to an inappropriate slowing of the heart during the offset of acceleration stress. It is hypothesized that loss of consciousness during the offset of acceleration stress may be the result of decreased sympathetic activation coincident with the decreasing straining effort. This may result in the potential for a rapid footward shift in fluids due to opening of the large vascular beds in the legs since sympathetic nerve activity to the heart parallels that to
various organ systems, including vasoconstricted muscular beds (24).

In summary, physiological responses to aerobic training are envisaged to have a positive effect on an individual’s ability to perform during sustained acceleration stress. However, there remain unanswered questions regarding which, if any, adaptations to aerobic training may manifest as a problem for some HPA pilots.

Health and Lifestyle Responses

Exercise (almost exclusively aerobic) is rapidly becoming the primary method of intervention in comprehensive health promotion programs. A 1979 survey (> 250 employees) indicated only 2.5% of sampled corporations had a formal health promotion program (19). A similar survey (> 50 employees) in 1987 found this number increased to 63%, and of these programs approximately 80% included exercise as a major component (16). The reason for this interest is clear; exercise is very effective in improving health and decreasing disease. Corporations recognize the monetary benefits of exercise programs. Benefits range from lower employee health care costs to enhanced employee productivity manifested by reduced absenteeism, and employee turnover. Improved morale and attitude have also been identified in exercise program participants (8).

Again, discussion of many health and lifestyle benefits derived by participation in a habitual regimen of aerobic activity is not possible within the limits of this report. Several excellent resources are available for the interested reader (1, 7, 8). To appreciate the wide impact exercise has on the health and well-being of the individual, it is important to look beyond the benefits most frequently associated with regular aerobic exercise. In addition to the management of coronary heart disease, other major health conditions positively influenced by aerobic exercise include atherosclerosis, hypertension, diabetes, obesity, osteoporosis, back pain, chronic obstructed pulmonary disease, cancer, immune function and mental health (7, 8).

Blair and colleagues in a landmark report (4) described all-cause and cause-specific mortality by physical fitness categories (aerobic capacity) for >13,000 men and women observed over 8 years. They concluded there was a strong, graded, and consistent inverse relationship between fitness and mortality in men and women. They also state that the high prevalence of sedentary habits and low physical fitness capacity produce a high attributable risk. This risk constitutes an important public health problem and deserves remedial attention.

In summary, there is strong persuasive evidence suggesting individuals engaged in an aerobic exercise program gain meaningful health and lifestyle benefits, both tangible and intangible. The responsibility for achieving and maintaining health rests with the individual; however, it appears prudent for the aeromedical community to act in a proactive role assisting individuals in sharing in the full benefits derived from these programs, to the extent the exercise program does not interfere with duty performance.

Aerobic Training and the High Performance Jet Pilot

The relationship between an individual’s aerobic capacity and the ability to sustain increased levels of G stress is controversial. There is widespread belief in the aeromedical community that this relationship is inverse in nature. The foundation for this hypothesis was based upon evidence that may not be appropriate for this unique type of stress (18, 26). Research evaluating this issue are few, and due to design limitations (i.e., definition of exercise parameters, controlling important sources of error, etc.), provide much less than a lucid understanding of the interaction of these physical attributes.

The earliest remark regarding the influence of aerobic fitness on +Gz tolerance was by Wessel (33). She reported that subjects with low fitness test scores consistently grayed out. She also mentions that some subjects classified in the “good fitness group grayed out easily.” This study employed a multi-component training regimen including both strength and endurance; however, a moderate positive relationship between G tolerance and over all fitness for strenuous work was concluded. Jumonville (17), two years later, reported the influence on G tolerance of an exhaustive aerobic exercise bout immediately before exposure to +5 Gz after six and one-half weeks of physical conditioning. He concluded that the strenuous acute exercise bout decreased G tolerance. However, he stated that subjects with high fitness scores had greater G tolerance, and subjects with low scores tended to have less G tolerance. Jumonville recommended pilots plan their physical training programs not to immediately precede flight. While this recommendation appears sensible, additional data must be gathered to clarify this issue further.

Meehan and Jacobs (21) in a later study, also concluded G tolerance is apparently unaffected by a considerable change in an individual’s physical performance capabilities as measured by the Harvard Step Test and a strength performance test battery. Of interest in this study was the absence of a significant correlation between G tolerance and prestress, or resting, blood pressure. This finding is important since lower resting blood pressure, generally accepted as a byproduct of endurance training, is frequently offered as a reason endurance trained individuals might have reduced tolerances.

Cooper and Leverett (13) assessed the influence endurance training (running) had on relaxed, peak G tolerance. They reported neither an increase nor a decrease in tolerance could be correlated with endurance (aerobic capacity) — measured by maximal treadmill ergometry (control, 41.4 ml kg-1 min-1 vs. trained 52.1 ml kg-1 min-1). The authors measured several physiological variables in the two groups and noted typical endurance training responses were evident in the runners. They postulated, while some of the training-induced changes (e.g., lower resting heart rate) may theoretically decrease +Gz tolerance, other changes (i.e., improved muscle tone and venous return, increased cardiac output during work, etc.) may enhance G tolerance or, at a minimum, counter the speculated negative changes. The authors’ hypothesized that if the trained subjects had been permitted to strain, they would have demonstrated greater tolerance than the controls. This theory was not evaluated.

Klein et al. (18) compared physically untrained subjects with highly trained individuals (VO2 = 43.9 ml kg-1 min-1, 64.9 ml kg-1 min-1, respectively) and found that for gradual onsets (0.07 G s-1) the two groups displayed nearly identical tolerance characteristics. Of interest in this study was nearly identical blood pressure response to an orthostatic challenge (tilt) by the two groups. This point is important since it suggests aerobically conditioned individuals may not be as susceptible to an inappropriate baroreceptor reflex during G-stress as some postulate. Whether or not orthostatically intolerant individuals are also intolerant to G-stress has not yet been evaluated. The results of
Klein et al. (18) and Jacobs and Meehan (21) suggest this relationship may not be as direct as some propose. Epperson et al. (14) investigated the influence of aerobic and anaerobic training on G-endurance tolerance — measured during a 4.5 -7.0 +Gz simulated air combat (SACM) centrifuge profile. They concluded the aerobically trained group performed as well as the group of control subjects (SACM time; 226 ± 33 s, 242 ± 39 s, respectively). The improved pre- to post-condition SACM tolerance times for these two subject groups were nearly identical (25%), despite the nearly 1% per week increase in aerobic capacity found in the trained subjects. However, the resistance trained subjects displayed improved G-endurance tolerance (411 ± 67 s, approximately 75% over initial level) over both the aerobic and control groups.

Pirquin et al. (22) concluded from an evaluation of the VO2max in Belgian F-16 pilots that those with average aerobic capacity should improve their tolerance and those with exceptional aerobic capacity should limit their training. Unfortunately, the authors did not report the G-tolerance data that provided the basis for this statement.

Banta and Grissett (3) discussed their review of cardiopulmonary fitness in 111 Naval aviators, wherein 84 were involved in flight training and 27 were HPA aviators. Comparing cardiopulmonary fitness with other psychophysical components of flight, the authors' concluded cardiopulmonary fitness had a strong favorable effect. They suggest that fitness induced advantages may include enhanced flight task performance.

No meaningful difference in +Gz tolerance was reported (32) in a study comparing peak G-performance characteristics in a group of aerobically trained subjects (VO2peak = range 43 to 71 ml kg-1 min-1) to data gathered on all healthy subjects undergoing +Gz testing on the Armstrong Laboratory Centrifuge from 1978-81 (no description of VO2peak). In fact, mean tolerance values for the aerobic group were slightly better than those obtained from the data repository (GOR: 4.61 ± 0.72, 4.65 ± 0.89 +Gz; ROR: 3.40 ±0.52, 3.34 ± 0.45 +Gz, respectively).

Finally, a study exposed exercise conditioned (run/jog 20 miles/week) and less-conditioned (no specific exercise program) subjects to a series of peak-G profiles. The purpose of the study was to compare the dynamic reflex heart-rate responses (change in heart-rate) during three phases of G exposure; rest to peak +Gz, rest to onset of +Gz, and rate of change per unit of +Gz. The authors concluded that aerobic conditioning was not associated with a reduced dynamic heart rate response to +Gz stress.

Two issues of potential concern related to aerobic fitness and acceleration exposure were not addressed in the above literature. These include the potential for increased susceptibility to motion sickness in trained persons, and the possibility for a greater incidence of cardiac rate and rhythm irregularities during G-stress in highly fit riders.

Increased motion sickness susceptibility in endurance trained centrifuge riders was first noted by Whinnery and Parnell (32); endurance trained riders had a higher incidence of emesis when compared with untrained riders. Subsequently, Banta et al. (3) reported a moderate relationship (r = -0.51) between fitness and a vestibular disorientation test. The low correlation coefficient implies there may be a relationship present; however, the lion's share is not explained by fitness alone. The authors suggest several alternative contributing variables to the model including conditioned alertness, motion sickness history, and altered levels of stress hormones. Cheung et al. (11) in a longitudinal study reported a meaningful increase in susceptibility to motion sickness following a physical training regimen. Although these studies suggest a potential motion illness sensitivity in trained people, there may be a bias in the conclusions introduced by the testing procedures. Endurance trained individuals, especially runners, are conditioned to expect sensory inputs in a characteristic way and sudden unexpected changes, spin tests, may accentuate motion sensory discord (2). Furthermore, conditioning induced motion illness may not be an operational problem, since highly sensitive individuals are selected out during training leading to a HPA assignment. Individuals with minor sensitivity to motion illness can easily manage this condition with desensitization training.

The second issue involves the potential for some aerobically conditioned subjects to exhibit cardiac rate and rhythm disturbances when exposed to +Gz stress. There is the potential in some individuals, both conditioned and sedentary, to exhibit electrocardiographic disturbances during and immediately following acceleration stress (27, 28, 29, 30, 31, 32). Also, some conduction disturbances may be enhanced by excessive endurance training; however, not all aerobically trained individuals are G-sensitive. If excessive aerobic training does contribute in potentiating dysrhythmic activity, individual training regimens could be throttled to a threshold level that will not compromise his mission as a HPA pilot. Additional experimentation is necessary to identify potentially susceptible individuals and to define, on an individual basis, the cause, and provide solutions for reducing their susceptibility.

The objective of this report was to summarize the benefits derived from participation in activities that enhance cardiorespiratory capacity and to review what is known of the interaction between increased levels of aerobic capacity and sustained acceleration stress. The evidence presented suggests aerobically conditioned individuals have a clear advantage in physiologic, health, and lifestyle characteristics. Also, enhanced aerobic capacity does not appear to affect +Gz tolerance adversely; although susceptibility to dysrhythmias and motion sickness have been identified in some individuals. With the evidence weighing heavy on the side of participation, the question is raised for counsel regarding how much chronic exercise is necessary to achieve and maintain the benefits of conditioning. The American College of Sports Medicine (1) describes the quantity and quality of training necessary to provide the stimulus for the health benefits of chronic exercise. Guidance offered to HPA pilots in a joint U. S. Navy/Air Force report (10) is similar; however, some suggest it is too conservative. Within the limits of the existing counsel on this issue, HPA aircrew should be encouraged to be active in physical conditioning activities that include both endurance and strength development.

In the Forward to a text dedicated to the physiological effects of inactivity (6), Bourne states "...physiologically the most dangerous activity to indulge in is inactivity." While this statement is principally directed toward space flight, it has important relevance for sedentary aircrew as well. The extensive evidence proving aerobically conditioned individuals are more physically capable, have better physical and mental health, and have more productive lifestyle characteristics when compared to their more sluggish counterparts suggests prudent participation in exercise can be an important adjunct to successful performance in the very demanding arena of high performance flight.
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BRAIN BIOCHEMICAL FACTORS RELATED
TO G-LOC

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Introduction
In the last decade there has been renewed interest in
gravity induced loss of consciousness (G-LOC) experienced by
pilots of high performance aircraft (Burton and Whinnery, 1985; Werchan, 1991 b). G-LOC is characterized by
approximately 15 sec of absolute mental and physical
incapacitation followed by a brief period of relative incapacitation
after exposure to +5 Gz or greater (Whinnery, et al., 1987).
In theory, there are similarities between the physiologic cause(s)
of G-LOC and other forms of loss of consciousness (LOC), most
notable is the critical reduction in cerebral blood flow (CBF).
However, other factors such as mechanical stress imposed by
rapid acceleration and deceleration may contribute to G-LOC (Werchan et al., 1991 a). It should be emphasized that the
biochemical and physiological steps leading from the reduction
in CBF to the cessation of brain activity (isoelectric EEG) or
LOC are numerous, complicated and ill-defined (Hansen, 1985).

The aim of this study was to find a suitable animal
model(s) to systematically identify the mechanism of G-LOC.
Rodents have been selected due to the abundance of tools and
techniques available to study cerebral ischemia. Compared to
humans, rodents possess a comparable systemic blood pressure,
but the hydrostatic column leading to the brain is substantially
reduced due to a relatively short eye to heart distance. Due to
this reason rats have a sustained +Gz tolerance (>1 min) to +10
Gz exposure on the Armstrong Laboratory Human Centrifuge
(Werchan et al., 1988). Therefore to simulate the G-LOC
experienced by human subjects and pilots a small animal
centrifuge (SAC) was fabricated with a high +Gz (+80 Gz) and
rapid onset/offset capability (+20 Gz/sec).

We hypothesized that since high +Gz exposure
causes a reduction in CBF a condition of cerebral ischemia can
result. In this report, to test our hypothesis, we describe the use
of SAC to expose rats and mice to high +Gz and the comparison
of the results with two well known static models of global
ischemia in rats: four vessel occlusion (4VOCC) and aortic
transection (AT). For technical reasons we speculate that no
single model will be sufficient to allow investigations into all
aspects of the G-LOC mechanism. For example, studies of
regional single cell electrophysiology, membrane ion flux etc.,
are currently impossible to accomplish in the dynamic
environment of the SAC. Therefore, it is expected that all
models used in the present study will complement each other in
the investigation of the G-LOC mechanism.

It is our philosophy that the mechanism of G-LOC
must be acquired first to effectively evaluate future physiologic
and/or pharmacologic efforts to minimize the risk of G-LOC
from occurring and/or to minimize the recovery time following
a G-LOC episode in pilots of high performance aircraft.

Methods:

4VOCC model: Male Sprague Dawley rats (250-400 g, n=6 in
each group) were anesthetized by intramuscular injection of
Ketamine HCl (35 mg/kg) and Xyloazine (5 mg/kg). They then
underwent surgery to electrosuturerize the vertebral arteries and
to place silk ligatures around both carotid arteries as
described by Pulsinelli and Brierly, 1979. Biparietal EEG
electrodes were implanted by using small screws and cranial
plastic cement (See SAC section). Ischemia was induced by
tightening both ligatures. Experimental rats were exposed to
either 15 or 30 sec of ischemia. Control rats received the same
surgical protocol without ischemia. At the end of each exposure
brain samples were collected by freeze fixation (See below) and
stored at -70°C.

Aortic transection (AT) model: This model is a slight
modification of the method described by Winn, et al., 1979, and
is the least complicated of the four models. Three groups of
anesthetized rats (n=6 in each group) underwent surgery for
EEG electrode implantation. An abdominal incision followed by
blunt dissection of the descending aorta and inferior vena
cava just distal to the left kidney was performed. A stainless
steel ligature was placed around both vessels and the two ends
drawn through the point of end of an 18 g needle. Ischemia (15
and 30 sec) was induced by pulling both ends of the wire through
the needle. At the end of ischemic periods brain samples were
collected as in 4VOCC.

Small Animal Centrifuge (SAC): The SAC, is 5" in diameter
and has a 21" head to axis ratio. It is powered by a 15 h. p. 3
phase regenerative DC drive motor and is capable of +1 to 85
Gz, with an onset/offset rate of +20 Gz/sec. The operation of
SAC is fully automated and controlled by customized software
(Zenith 248 computer) linked to an optical encoding sensor.
SAC is equipped with a video camera, syringe pumps, pressure
transducers and amplifiers for monitoring EEG and ECG. Eight
channels of physiological data can be fed directly into a Macintosh
II fx computer for online or subsequent EEG analysis. SAC is
equipped with two types of sample collection devices: (a) freeze
fixation and (b) microwave fixation. Both devices allow sample
collection while the centrifuge is running and following
deceleration.

The freeze fixation method was modified from the
one described by Veech et al., 1973. Compressed air (35 psi) is
injected via a stainless steel probe which quickly penetrates into
the rat cranium. Within 1 sec the brain is homogenized, in situ,
by compressed air and exits the skull through a second probe and
collected as a frozen wafer between two aluminum disks pre-
cooled in liquid nitrogen.

The second fixation system consists of a 10-kW,
2450-MHz microwave unit situated outside the SAC. A WR340
waveguide extends from the microwave to SAC and down the
center shaft. The waveguide exits the shaft and travels down the
arm to a microwave applicator located on the end. A rotary joint
above the centrifuge allows the waveguide, to spin in concert
with the SAC. Generally a microwave pulse of 650 millisecond
is sufficient to elevate brain temperature to the point of denaturing
all enzyme activity (Medina, et al., 1975). The 2450-MHz
microwave unit is best suited for the delivery of energy to small
rodents such as the mouse (Stavinoha, et al., 1973). For this
reason mice were utilized exclusively on the SAC in conjunction
with microwave fixation.
Rats were anesthetized with halothane and a mid line incision was made to expose the cranium. Biparietal lateral EEG electrodes were screwed in place by using two 0-80 stainless steel screws. An additional screw, which serves as the ground, is placed slightly cranial to the bregma suture and lateral to midsagittal suture. All screws and attached wires were completely imbedded in a mound of cranial plastic cement and the wound site was closed. Fully awake rats were placed in a plexiglass holder and clamped to the centrifuge arm such that the head was facing towards the center shaft of the centrifuge (+Gz orientation). One group of rats were exposed to five 30 sec exposures at +15 to 25 Gz with 3 min rest period between each run. Four additional groups of rats (n=6 in each group) were exposed to a single +25 Gz exposure and brain samples were collected after 15 or 30 sec during the run or 1 and 3 min after deceleration by freeze fixation. Fully awake mice were subjected to a single +35 Gz exposure and brain samples were collected by microwave fixation at the same time points as in rats. Control mice were subjected to a 30 sec exposure at +0.5 Gz.

Assay of Metabolites: The frozen brain tissue (0.2g) was homogenized in 2 ml of 7% cold perchloric acid and neutralized with KOH as previously described (Shahed, et al., 1979) and lactate was measured enzymatically (Hohorst, et al., 1959)

HPLC analysis of creatine phosphate (C-P) and adenosine triphosphate (ATP): An aliquot of neutralized supernatant was filtered (0.45 μ) and analyzed by ion pair reversed phase high pressure liquid chromatography (HPLC) using a Waters NOVA PAK C18 column. A linear gradient of mobile phase consisting of 50 mM potassium phosphate containing 3 mM tetra butyl ammonium phosphate (TBAP) pH 5.8, and mobile phase b in addition, containing 15 % acetonitrile was used.

Statistics: The data was analyzed by one way analysis of variance (ANOVA) and Dunnett followup test for multiple comparisons. All data is presented as mean ± SD of 5-6 animals.

Results:

The main objective of this research was to examine LOC and overall energy status of the brain in rodents exposed to high +Gz in SAC and other models of global ischemia. Figure 1 depicts typical rat EEG recordings during five high +Gz runs ranging from +15-25 Gz. Isoelectric EEG was observed between 15 to 20 sec at exposures higher than +20 Gz. Based on EEG recordings during +Gz exposures in baboons (Burns et al., 1991), G-LOC, in the present study, was designated as the timepoint when EEG becomes isoelectric. The EEG remained isoelectric for approximately 10 sec, and fully recovered 15 to 20 sec after the run. Table 1 shows the average time to LOC in rats and mice exposed to high +Gz and rats during 30 sec ischemia induced by either 4VOCC or aortic transection (EEG recordings not shown). The average time to LOC under these conditions was not significantly different.

Table 1: Average Time to LOC in 4 Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Time to LOC (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4VOCC</td>
<td>10.71 ± 3.3</td>
</tr>
<tr>
<td>AT</td>
<td>18.17 ± 5.5</td>
</tr>
<tr>
<td>SAC (Rat)</td>
<td>14.46 ± 2.9</td>
</tr>
<tr>
<td>SAC (Mice)</td>
<td>17.64 ± 3.3</td>
</tr>
</tbody>
</table>

Mean ± SD

Discussion:

When investigating LOC three questions commonly arise, 1. What causes LOC ?, 2. Why does LOC occur; i.e. is LOC physiologic or pathophysiological phenomenon and 3. What is the mechanism of LOC ?

A comprehensive review (Manalis et al., 1990) of clinical classification and causes of syncope or LOC divide the findings into 3 major categories: cardiovascular, non-cardiovascular and unexplained. In most cases LOC appears to be the result of either insufficient or abnormal supply of blood or substrate to the brain. LOC is evident usually within 15 sec of such an insult. The results of this investigation show that LOC in either rats or mice occurs also within 10-18 sec of either a global ischemic insult or a single high +Gz exposure (Table 1). This suggests that during +Gz exposure CBF is critically reduced.

It is unclear if LOC is a physiologic or an early pathophysiologic manifestation of clinical disorders. Teleological considerations argue that LOC may be the brain's way to minimize the hydrostatic blood column by placing the body in a horizontal plane. Additionally, LOC will concomitantly...
It is well known that brain metabolism and CBF are tightly coupled. Therefore, the measurement of both of these parameters are important while investigating the mechanism of LOC or G-LOC. Our multi-model approach is designed to address both. Since, at present, measurement of CBF is difficult in SAC model (Werchan and Forster 1990), the metabolic responses obtained in this model can be compared to those obtained in models with known decrement in CBF (4VOCC and AT).

Each of the models used here have some advantage over the others for e.g. 4VOCC potentially could be used to allow multiple episodes of acute global ischemia (by inserting a balloon occluder in one of the carotid arteries) which may simulate the combat profiles of a high performance aircraft. However, this method lacks the mechanical stress involved in rapid acceleration and it involves a complicated surgical procedure. Similarly, the AT model is relatively simple but it can not be used for repeated episodes of ischemia and reperfusion. Additionally, it is likely that certain aspects of G-LOC can not be measured in SAC model but may be practical in the two static models described here, thus enabling us to characterize all steps of the mechanism of G-LOC.

The SAC model is best suited to investigate the mechanism of G-LOC, because it can most closely simulate the air combat profiles experienced by the pilots of high performance aircraft, due to its rapid acceleration and deceleration and because it allows both physiological (EEG, ECG, blood pressure) and biochemical measurements. One of most unique feature of the SAC model is tissue fixation devices: freeze fixation and microwave fixation. The two systems described here offer both redundancy (two species) as well as flexibility. Both of these devices can be used while the SAC is still running and the tissue can be fixed within 0.5 to 1 sec. An advantage of the freeze fixation is that it does not denature the tissue and thus can be used to measure both metabolites and enzyme activity etc. The disadvantage of this system is that since the tissue is homogenized, regional response to high +Gz stress in brain can not be determined. In contrast, the microwave device fixes the brain in less than 1 sec but the brain remains anatomically intact and thus allows us to study regional responses to high +Gz stress. The tissue can also be used for sectioning and histological examination to study changes in brain morphology under high +Gz stress. The disadvantage of this method is the small size of mouse. This increases the challenge of physiological type instrumentation. Additionally, most of this instrumentation can not be placed in a microwave field.

The time course of changes in lactate, C-P and ATP (Figure 2, 3, and 4) and time to LOC (Table 1) are similar in all models. This suggests that +Gz exposure induces a critical reduction in CBF, probably within 1 or 2 sec, as is known to occur in 4VOCC and AT models. A significant accumulation of lactate within 15 sec of ischemia or +Gz exposure may signify a switch to anaerobic glycolysis which is expected if the blood flow to the brain is interrupted. Similarly, the rapid decrease in the concentration of C-P to maintain brain ATP level shows that oxidative production of ATP is not sufficient. It is notable that during the time course used in this investigation neither C-P or ATP were completely depleted. Thus it is not yet clear if the significant reduction in C-P and ATP are causative factors of G-LOC or if they initiate factors that directly cause G-LOC e.g. neurotransmitter release or ion channel activation etc. Also in support of this theory, it is noted that EEG recovery routinely occurs within 20 sec following +Gz exposure, however, the brain energy state is still depressed even 1 min post G-LOC. It
is possible that there may be local pools of ATP necessary to maintain membrane function which are preferentially depleted and are not fully reflected in global changes in ATP as measured here (Whittingham, 1990). Also since more than 50% of total energy consumption by the brain is utilized for maintaining ionic membrane gradients, G-LOC or LOC may be a way to conserve energy.

In summary, the results show that SAC is the most suitable model to study physiological and metabolic ramifications of high +Gz exposure. Since the metabolic changes observed in the SAC model are identical to those observed in ischemia models, it is concluded that +Gz exposure causes global cerebral ischemia. Future research will be focused on pharmacologic and dietary perturbations aimed at modifying the time course of metabolic changes and G-LOC. If we can successfully alter the time course of energy depletion and, in turn, alter the pattern of G-LOC then a cause and effect relationship could be better established.

The animals involved in this study were procured, maintained and used in accordance with the Animal welfare act and the "Guide for the care and use of laboratory animals" prepared by the Institute of Laboratory Animal Resources National Research Council. Armstrong Laboratory is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

References:

AEROMEDICAL PROBLEMS IN THE HIGH-G ENVIRONMENT: A LOOK AT THE FUTURE

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The importance of research thrusts in acceleration (+G\(_\text{z}\)) medicine and physiology is multi-faceted for understanding problems in: 1) clinical medicine, as exemplified by establishing a unified field theory for alterations in consciousness due to ischemia, hypoxia, disease, trauma, and toxic insults; 2) space medicine, as exemplified by a stated theme of the life sciences research and development activities of the National Aeronautics and Space Administration to acquire fundamental knowledge about life's origin and evolution under a gravitation field (What better way to understand how we developed in a gravitational field than to carefully observe how the body continues to react to a higher +G\(_\text{z}\)-field, the problems it encounters, and the mechanisms it employs to protect itself in this stress environment.); and 3) aviation medicine, as exemplified by ultimately solving the fighter aviation medicine problem associated with +G\(_\text{z}\)-induced loss of consciousness (G-LOC). These medical thrusts are in addition to the basic scientific research goals of simply understanding basic acceleration physiology. Fighter aviation medical research in high-G, particularly the scope of research involving G-LOC, is uniquely beneficial for gaining an understanding of the basic problems under investigation in each of these areas of medicine and physiology (1).

It is a valuable exercise to predict the future goals of acceleration medical research by reviewing what is lacking from the past (2), since it is what we currently lack in knowledge that will guide us toward what we shall endeavor to discover. Strong evidence exists to unquestioningly demonstrate that early acceleration researchers failed to approach G-LOC as an operational fighter aviation medical problem, instead considering it as a traditional clinical medicine symptom. This approach is evident from the development of visual "assays" which used greyout, tunnel vision, and blackout to the complete exclusion of G-LOC (3). These assays virtually eliminated experimental observation of G-LOC in the laboratory and pushed it into the closet until we began to approach G-LOC as an operational problem in the mid-1970's (4,5). We subsequently began efforts to understand the neurophysiologic basis of G-LOC (6,7,8). The lack of understanding +G\(_\text{z}\)-induced neurophysiologic alterations, including G-LOC, was extremely detrimental, resulting in: 1) aeromedical standards that continued to disqualify aircrew for simply exceeding tolerance to +G\(_\text{z}\)-stress, something that will happen in everyone if the +G\(_\text{z}\)-level is high enough; and 2) a state of being completely unprepared for the introduction of advanced aircraft (F-15, F-16, F-18 into the operational inventories with subsequent losses of life and multi-million dollar aircraft. It is unfortunate that developing visual loss "assays" combined with not recognizing G-LOC as the operational problem was so devastating. The future will therefore be charged with the requirement to fill the void that was left by not investigating G-LOC and must include answers to the following failures to:

1) formally define G-LOC (the loss, unconsciousness, and recovery),
2) develop and describe methods to measure G-LOC and related phenomena,
3) accurately describe how the G-LOC measurements reported are made,
4) provide an accurate description of the exposure envelope (onset, duration, offset, and equipment/protective techniques) used in G-LOC exposures,
5) accurately differentiate between visual systems (blackout) and brain symptoms (G-LOC, unconsciousness) — by using terms such as anti-blackout suits,
6) recognize G-LOC as a problem instead of simply a symptom,
7) convince aviators of the operational importance, characteristics, and significance of the G-LOC problem,
8) predict the potential for an increased risk of G-LOC as aircraft performance and maneuverability increased, along with failure to recognize that more rapid inflation of the anti-G suit would be needed as aircraft maneuverability increased,
9) develop a complete and accurate description of G-time tolerance (this resulted in inappropriate utilization and misunderstanding of the existing G-time tolerance curve),
10) develop centrifuge profiles (simulated aerial combat maneuvers (SACM)/aerial combat environment simulation (ACES)) that simulate inflight G-stress (reliance on the use of a narrow...
range of centrifuge profiles that do not affect the central nervous system (CNS) or cardiovascular system (CVS) the same as ACES/SACM),
11) investigate the CNS aspects of +Gz-stress by exclusively concentrating on cardiovascular physiology,
12) understand the importance and need to investigate G-LOC by instituting a bioassay technique utilizing blackout to the exclusion of G-LOC in acceleration research (this reduced the observation of G-LOC to an almost non-existent state),
13) establish the safety of conducting research in healthy humans using G-LOC as an endpoint or the safe limits of exposure to the transient +Gz-induced ischemia/hypoxia required to produce unconsciousness or the associated symptoms of the G-LOC syndrome,
14) conduct deliberate G-LOC research,
15) provide a detailed qualitative and statistically sound quantitative description of all psychophysiologic symptoms and sequelae associated with +Gz-induced ischemia/hypoxia of the eye and brain (CNS),
16) establish the effects (if any) of CNS ischemia/hypoxia on the CVS or other body systems,
17) develop G-LOC theory that would have focused research concerning +Gz-induced ischemia,
18) establish the relationships of various aerospace stress etiologies of alterations of consciousness and thereby develop a unified field theory of loss of consciousness.

Based on this lack of information, theoretical formulation, and long-term research program focus, current acceleration scientists have sought to remedy these inadequacies as rapidly as possible. Our current endeavors to thoroughly assault G-LOC include:

1) establishment of an advanced definition of G-LOC which separates the loss of consciousness from unconsciousness and recovery and recognizes the loss of integrated central nervous system (CNS) function as the basis of G-LOC (9,10,11),
2) development for the first time G-LOC theory which establishes G-LOC as a normal physiologic protective mechanism (8,12),
3) careful experimental observation of symptoms that have prompted description of the G-LOC syndrome as a normal symptom complex which results from +Gz-induced ischemia/hypoxia (13,14,23),
4) recommendation of appropriate modifications to the aeromedical standards to reduce the potential for unnecessary aircrew disqualification from G-LOC (and cardiac dysrhythmias) and therefore increase the probability for fighter pilots reporting of the in-flight G-LOC problem (15),
5) enhancement of the ability to investigate mishaps and document G-LOC as a causal factor (16,17),
6) recognition of G-LOC syndrome symptoms as resulting from regional ischemic differential within the CNS allowing linkage of neurophysiology with neuroanatomy as has been done in clinical neurology for centuries (18),
7) development of a G-LOC mechanism that is compatible with a survival and protective mechanism starting at the whole organism level and extending down to the neuronal level which is not based on energy exhaustion but on neuronal membrane stabilization and energy conservation (32),
8) establishment of a framework for describing tolerance (anatomic, level, cardiovascular, neurologic, and duration) to +Gz-stress which, as a complex combination, forms the basis of operational +Gz-tolerance (19,20),
9) development of an understanding of +Gz-time tolerance that clarifies the inadequacies of existing curves and describes the needs for developing much needed operationally applicable tolerance metrics and curves (21,22),
10) provision of a unified cardiovascular/neurophysiologic response template that focuses where anti-G protective equipment/techniques actually fit into a G-LOC protection mechanism (12,24),
11) development of a theoretical description of G-LOC in terms of neuroanatomy and neurophysiology where the state of consciousness (and unconsciousness) is dependent on the brain stem structures and the content of consciousness (and unconsciousness) is dependent on the higher CNS structures including the cerebral cortex (10),
12) establishment of +Gz-exposure envelopes for safely investigating G-LOC in normal humans (25,27),
13) initiation of techniques aimed at extending neurologic tolerance to +Gz-stress and reducing incapacitation should G-LOC occur (28,29,30).
Although some acceleration technicians remain blindly tied to using modification of cockpit geometry (25), it is evident that operational aircrews do not yet favor such a configuration optimal for their overall mission. Effective acceleration protection alone cannot drive the entire tactical fighter mission. Review of our recent Persian Gulf experiences reveal that even in a conflict with minimal air-to-air combat engagements, surface-to-air missile capability can require maximal sustained, high-G maneuvering to successfully negate the threat and survive. Mastery over the adverse effects of \( +G_z \)-stress will therefore remain a priority for future manned tactical weapon systems. As we move into the future it will remain important not to repeat the evident mistakes of the past (2).

Understanding the neurophysiological basis of consciousness and its loss has a scope that extends from the roots of our human evolution, through the essence of our being, and provides the closest glimpse we mortals can gain of the process of death which terminates our terrestrial being. G-LOC research, therefore, has broad importance, not only for aviation, but also for the space medicine quest for fundamental knowledge concerning evolution in a gravitational environment, and the clinical medicine goals of improving tolerance to CNS ischemia and improving neurological recovery from a variety of pathologic processes that alter conscious function. A final goal of acceleration medicine subspecialists will be to integrate the spectrum of scientific disciplines that share the neurological circuitry responsible for consciousness, postural motor control, memory, dreaming, convulsive activity, and visual function; ultimately bringing them all to bear on the G-LOC problem.

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POSITIVE PRESSURE BREATHING FOR G:
EVOLUTION AND PROMISE

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INTRODUCTION:

The adverse effects of positive G (headward, or +Gz acceleration) on pilots of high performance aircraft are attributable to the increased inertia of blood during high Gz (2,8,17). This can so reduce perfusion pressure at the eye and brain that ischemia resulting in visual disturbances, black-out, and loss of consciousness will occur when the exposure satisfies the intensity and duration criteria of Figure 1.

Measures which increase total peripheral resistance (TPR), and would otherwise increase systemic arterial pressure under normogravic conditions, are useful in attenuating the G-induced pressure drop at head level (15,16). These include generalized muscular straining and the application of pressure to the legs and lower abdomen by pneumatic or hydrostatic means (16,17). When these are coupled with the direct elevation of blood pressure afforded by a Valsalva-like respiratory strain, as with the M-1 or L-1 maneuver, consciousness can be sustained for periods as long as 45 seconds at levels as high as 9 Gz (2). These means have thus remained the primary source of acceleration protection from WW II to the present day. They effectively raise the curve in Figure 1 by a distance proportional to their intensity, and for a duration limited by fatigue.

![Figure 1. G-time tolerance relationship adapted from the work of Stoll, 1956 (14).](image)

Aviators can enter the incapacitating zone of this relationship inadvertently, without benefit of immediate strain, or by a miscalculation of stamina. Investigation has thus frequently sought to reduce the conscious attention and physical effort required to sustain vision or consciousness at high G. One promising approach has been to let the oxygen regulator assume the work of a respiratory strain by applying positive breathing pressure during Gz, a practice now termed PBG (Pressure Breathing for G). In 1973 Shubrooks (13) demonstrated that breathing pressures on the order of 30 mmHg provided protection equivalent to a respiratory strain during what we now view as moderate (maximum 7 G) exposures. Although flight tested soon thereafter by the RAF (6), the technique was never further developed because aircraft of the time did not require it.

By the late 1970s, however, the remarkable maneuvering capabilities of F-15 and F-16 aircraft led us to reinvestigate the protective effectiveness of PBG, this time at higher pressures (50 to 70 mmHg). This interval was selected because the practice of pressure breathing at altitude (PBA), which had long been used to prevent hypoxia above above 40,000 feet, left us heir to experience and equipment permitting ready exploration of that range (3, 4). Early investigation was structured to allow comparison of different design approaches: We evaluated ensembles of British, Canadian and Swedish origin which differed chiefly in the extent of torso counterpressure they provided (11). Increases in relaxed G tolerance were noted with all ensembles, and the endurance (time to peripheral light loss) of straining subjects exposed to alternating ten-second periods of 4.5 and 7 G was significantly increased by the use of PBG (11,12).

This spawned work which sought both to better explore the limits of this technique and develop it in a form acceptable for flight tests: Burns and Baldlin (1) using a PBG ensemble incorporating an improved Canadian torso garment were able to achieve a two-fold increase in endurance on an even more arduous profile (alternating ten-second periods of 5 and 9 G). So impressive was this effect that PBG advanced to flight tests as a part of the Tactical Life Support System (TLSS), a technology demonstration program for ideas potentially incorporated in the next generation of fighter aircraft (10).

TLSS combined the improved Canadian torso garment and an extended-coverage 5-bladder G-suit into a single integrated garment which provided endurance enhancement similar to that reported by Burns and Baldlin. Flight tests in both F-15 and F-16 aircraft quickly brought enthusiastic aircrew acceptance of PBG, but poor acceptance of the garment integration concept (5,7). Thus in 1988 we were tasked to develop a modular PBG system, one using existing conventional flying gear and an optional torso overgarment, for retrofit to the F-16 fleet. The development of that system, specifically the growth potential signalled by our criteria study, is the subject of the following:

METHOD

Our task was to obtain the acceleration protection afforded by the integrated TLSS using a modular system, one in which fiscal constraints required that we make maximum use of existing life support equipment. To this end we initially structured a simple three-way comparison between TLSS and two candidate modular systems which differed solely in the extent of
torso bladder coverage. But additional ensembles were added as it became apparent that substitution of a new anti-G suit, and possibly a better breathing regulator, might offer increases in performance. Equipment combinations comprising the total of five ensembles compared in this study are described in Table I.

**TABLE 1. CANDIDATE EQUIPMENT COMBINATIONS**

<table>
<thead>
<tr>
<th>Ensemble Number</th>
<th>Torso Garment</th>
<th>Anti-G Suit</th>
<th>Breathing Regulator</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt;---- Integrated ----&gt; TLSS</td>
<td>Modified CRU-73</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>MLSS</td>
<td>CSU-13/P</td>
<td>Modified CRU-73</td>
</tr>
<tr>
<td>III</td>
<td>EMLSS</td>
<td>CSU-13/P</td>
<td>Modified CRU-73</td>
</tr>
<tr>
<td>IV</td>
<td>EMLSS</td>
<td>ATAGS</td>
<td>Modified CRU-73</td>
</tr>
<tr>
<td>V</td>
<td>EMLSS</td>
<td>ATAGS</td>
<td>NGL Derived</td>
</tr>
</tbody>
</table>

*All ensembles used the TLSS oxygen mask and helmet.

Combination I, the integrated TLSS garment and PBG-modified CRU-73 regulator, was the system originally tested in F-16 aircraft and the standard against which we planned to compare the modular combinations. Combination II used an externalized version of the TLSS torso bladder modified to be worn over the flight suit (MLSS), in conjunction with a standard CSU-13/P 5-bladder anti-G suit. Combination III substituted an extended torso overgarment (EMLSS), derived as a means of reducing the number of sizes in which the garment might have to be produced. Combination IV substituted a full-coverage anti-G suit, now known as ATAGS (Advanced Technology Anti-G Suit) for the 5-bladder CSU-13/P; this added far more extensive and uniform lower body pressurization, to include the feet. Combination V retained this configuration and substituted a low resistance breathing regulator derived from an earlier NGL (Normalair Garrett Ltd, Yoevil, UK) design. Both regulators provided PBG onset at 4 G and increased linearly at 12 mmHg/G to a maximum of 60 mmHg at 9 G. Their respective flow properties were evaluated on a laboratory breathing simulator (Krug International, San Antonio, TX) and are reported below. All ensembles used an Alar Products (Kent, OH) high-flow anti-G valve readjusted from its normal factory setting to provide the TLSS inflation schedule (1.5 psi/G to a maximum of 11 psi at 9 G).

These ensembles were tested on the USAFSAM human centrifuge by a panel of six volunteer subjects who were both experienced in PBG and routinely exposed to high G. Prior to beginning the collection of data each rode a laboratory PBG system twice weekly to exhaustion on an alternating 5/9 G profile derived from that of Burns and Baldin (1), as modified by the use of a higher (4G/sec) onset rate. After a two week conditioning period data collection proceeded with the same twice weekly exposure frequency, while the various combinations were introduced randomly, to distribute the influence of any remaining conditioning artifact.

The F-16 seat-back angle (33 degrees) and rudder pedal position was used in all tests. ECG was monitored for both medical safety reasons and heart rate effects to be reported elsewhere. The protective effectiveness of a given ensemble was rated solely on the endurance it conferred on the alternating 5/9 G profile. The selection of a given combination for further development was additionally tempered by the ease with which it might be adapted for later production and operational use.

**RESULTS**

Endurances recorded from the five equipment combinations of Table I are expressed in Figure 2. Those ensembles most closely related by design, specifically Combination I (the TLSS comparison standard) and Combinations II and III (related externalized torso garment designs) vary slightly in their average values, but not significantly. ANOVA and paired-t tests of these three groups suggest that their differences are reflective of sampling error.

Combination IV, the ensemble formed by substituting the full-coverage ATAGS lower garment for the conventional CSU-13/P anti-G suit, displayed a much higher average endurance than the above ensembles. The effect of this substitution, which coupled the ATAGS with the "extended" externalized torso garment (EMLSS) was to increase endurance by more than 50% over Combination III (the same ensemble with a conventional G-suit).

Combination V gave the best overall result. This ensemble used the same clothing items as number IV, but substituted the NGL-derived low-resistance breathing regulator for the modified CRU-73 used in all other combinations. The result was a nearly 20% increase in endurance over that attained by Combination IV. An ANOVA describing the influence of changes to Combination III (changes which resulted in IV and V) was significant to the .01 level. Internal paired-t tests subsequently identified specific components attributable to ATAGS (p<.001) and the breathing regulator (p<.01) respectively.

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Figure 2. Average endurances for the five equipment combi-
nations described in Table 1. Mean values appear above each bar with standard errors in parentheses. Stippling identifies increments of statistically significant change in paired-t tests.

Breathing regulator performance is reported in Figure 3. Note that the suction required to extract a given flow rate is lower for the NGL regulator than for the modified CRU-73. This is increasingly the case as higher and higher flows are encountered.

![Figure 3. Comparison of the pressure/flow characteristics resulting from use of the modified CRU-73 (*) and NGL-derived (+) breathing regulators in this study.](image)

**DISCUSSION**

Our objective in this work was more developmental than investigative: We set out not to further demonstrate the protective advantage afforded by PBG, an effect amply proven by several previous studies (1,10,13), but rather to learn if the way we proposed to extract and modularize the PBG subsystem of TLSS in any way eroded its effectiveness. It apparently did not: We failed to note functionally or statistically significant differences between the endurance values for Combinations II and III, and what can be properly called the TLSS control in Combination I. The values of all three are within the range of those reported for 50 to 70 mmHg PBG on similar endurance profiles (1).

This finding permitted the selection between Combinations II and III to include the more pragmatic considerations of engineering development and provisioning: Combination III was selected for further development because use of the larger EMLSS torso garment promised to simplify the garment's sizing tariff and assure a lower G-garment interface with less chance of gapping. Desirable features common to both ensembles included the prospect of using the conventional CSU-13/P anti-G suit already in service with the USAF, the ability to use an adaptation of the existing high-flow anti-G valve, and the use of a modified inventory breathing regulator. Combination III subsequently entered its engineering development phase under the program name of COMBAT EDGE. In addition to meeting our performance requirements, that ensemble, as modified into IV by the introduction of ATAGS, and V by further adding a low resistance regulator, demonstrated both the surprising gains which could be accrued from improving features of hitherto uncertain value, as well as the ease with which such change might later be integrated.

Improvements afforded by addition of the full-coverage ATAGS garment were the most striking. Early anti-G garments had been evaluated only in relatively brief exposures (15,16) and in the absence of PBG. Their effectiveness in that context most likely reflected an indirect pressor effect secondary to inflation-related increases in TPR (a phenomenon also demonstrable at one G), but one potentially fading during protracted exposures if significant quantities of venous blood are allowed to pool in unsupported areas of the lower extremities. A uniform pressure garment like the ATAGS could be expected to provide more even support, reduce or eliminate related pooling, and return more blood centrally to support the cardiac output. Thus the protective requirements of brief exposures, as used to develop the traditional 5-bladder suit, probably resulted in a garment optimized to give arterial support, while the ability of ATAGS to sustain much longer exposures may reflect the importance of limiting venous capacitance. A similar explanation accounts for the need to provide lower body counterpressure during extended periods of normogravice pressure breathing (3,4). Impedance plethysmography, which has been used to measure G-induced limb volume changes in non-PBG studies (9), might prove useful in better understanding and optimizing the advantage conferred by a uniform pressure garment in the present case.

Increases in endurance conferred by the low-resistance breathing regulator also invite further investigation. They provide a first-ever observation that breathing resistance can measurably affect G-tolerance, and additionally imply that efficiency of the full coverage garment was not the sole determinant of endurance in the preceding ensemble. A potential source of advantage in low-resistance pressure breathing is reduction of the inspiratory effort (and thus negative-going pressure) required with each inhalation. Large excursions would provide corresponding reductions to intrathoracic and thus arterial pressure, while small excursions would have a lesser effect. A low resistance system might thus support a higher average arterial pressure. This in turn could reduce the intensity of strain required to assure continued perfusion of the eye and brain, and thus confer additional endurance. Figure 3 demonstrates that substitution of the NGL-derived regulator for the modified CRU-73 in our breathing system substantially reduced the negative pressure required to extract a given flow. This is proof of a condition essential to the foregoing explanation, but not the explanation itself. Investigation of the temporal relationships conferred by different breathing patterns, and the the work of breathing under high G, are needed to assure a fuller understanding.

This study demonstrates that the combined influences of introducing a full-coverage anti-G suit, and a low resistance breathing regulator, nearly doubled the protective capability of a PBG system already conferring twice the endurance of existing operational anti-G equipment. Thus equipped with knowledge of what to change, and how, we plan to proceed with introduction of the currently available COMBAT EDGE.
system (derived from Combination III), while continuing work to assure the understanding, development and operational introduction of ATAGS and an optimized breathing regulator.

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PROTECTING THE PILOT DURING HIGH-G LOADING

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HISTORICAL PERSPECTIVE

In 1972, the F-15 began a new era in high-performance aircraft. This aircraft which has since been followed by the F-16, F-18, MiG-29 and others, has G-level capabilities of 9G-sustained, an increase of 2G above the G capability of F-4 type fighter aircraft. Considering that 2G is only a 30% increase in G capabilities, it may seem curious to some people, including scientists, why it has taken two decades to develop and field G-protection systems that adequately protect aircrew during sustained exposure to 9G. But, in fact, that 2G increase was in reality a 100% increase in G-protection requirements.

The average relaxed human G-level tolerance is 4G plus another G for the anti-G suit offering a total tolerance of 5G. Above this level, aircrew must strain their muscles and perform a forced expiratory maneuver against a closed (M-1) or partially closed glottis (L-1) called the anti-G straining maneuver (AGSM); if done correctly, the AGSM can elevate G-level tolerance by 4G. Earlier "pre-F-15" aircraft were capable of 7G-sustained, i.e., 2G above the relaxed G-suit tolerance. Consequently, in order to tolerate the 9-G capability of the F-15, the AGSM effort required of the pilot was doubled.

Therefore, beginning in 1972-1973, pilots were flying the F-15 with 5-6G protection levels (e.g., anti-G suit and an underdeveloped AGSM) that had been conceived in World War II (WW II) for aircraft with reciprocating engines. It should not be surprising that the AGSM had not been developed to a level of 4G protection that was possible with proper training; nor should it be surprising that the anti-G suit was capable of increasing G-level tolerance of only 1 to 1.5G. Simply, greater G-level tolerance increases were not required to fly those WW II aircraft to their maximum capabilities. In fact, the G environment had not been defined completely; acceleration effects were not well understood, nor had the technology to determine G-duration tolerances been developed. And, too, the deadly syndrome known as G-induced loss of consciousness (G-LOC) had become hidden behind the blackout phenomenon that is in itself, not dangerous (9). This interesting situation occurred because G-level tolerances were measured using only light-loss criteria (grayout and blackout) totally ignoring the potentially more dangerous G-LOC problem.

During the last 2 decades, many technical advances have been developed that are now in use by aircrew; i.e., high-flow anti-G valve, AGSM centrifuge training, G-LOC awareness, and physical conditioning primarily aimed at increasing muscular strength and anaerobic capacity. In the wings of the operational theater is positive pressure breathing during G (PGB) that, combined with a greatly improved uniform coverage anti-G suit requiring only a moderate AGSM, will allow pilots to fly 9G aircraft properly protected. How these improvements came about in the laboratory will now be reviewed in chronological order.

From 1950-1970, the potential of the AGSM as an anti-G method capable of increasing G-level tolerances by an average of 45G was not realized; proper teaching techniques had not yet been developed. A prototype teaching method using the human-use centrifuge at Brooks Air Force Base, Texas was developed early in 1971 using F-4 pilots in Red-Flag training at Nellis Air Force Base, Nevada. This method was refined over several years and finally adopted by the Tactical Air Command (TAC) and by other NATO nations in the early to mid 1980s. Earlier implementation was tried, but was unsuccessful because the G-LOC problem had not yet been identified (13).

In the early to mid 1970s, the basic physiologic effects of anti-G suits began to become known, but were not completely determined until the G environment had been better defined (6, 10). However at this time an animal model, the miniature swine was developed as a human-analogue model for physiologic research during high sustained G (HSG) (3). This model has been used extensively in G-physiologic research defining the hazards of the high-G environment by physiologic effects of the anti-G suit (2, 7).

An early life support deficiency that was identified during the flight tests of the F-15 was the slow inflation of the anti-G suit. Once our laboratory was apprised of this situation in 1975, we had developed several improved anti-G valves that could be installed operationally by the following year. The completely mechanical high-flow ready-pressure anti-G valve that reduced suit-inflation time by 75% was satisfactorily flight tested in 1976 (11), but because of production problems, the ready-pressure feature was not adopted. However, the high-flow capability was made operational and subsequent laboratory testing has verified its ability to provide sufficiently rapid anti-G suit inflation (5).

Perhaps the most profound advancement in G-tolerance research in the 1970s was the development of a method to measure G-duration tolerance that related to the operational aerial combat maneuver (ACM) called the simulated ACM (SACM). This G profile, that was a repeated 4.5G for 15 sec to 7G for 15 sec cycle which continued until the subject became fatigued; it identified for the first time G-duration tolerance, the other dimension of the G environment (Fig. 1). It is impossible to develop adequate protection techniques against hazardous environments when the hazards have not been adequately quantified; i.e., the environment must be sufficiently defined.
Once the G environment became characterized and a method developed to measure the duration dimension, several G-protection advances were rapidly made: (1) the importance of muscular strength in performing the AGSM was identified; (2) the development of the uniform pressure anti-G suit, and (3) the development of assisted positive pressure breathing as an anti-G method (PBG) (12, 15, 16). The basic research accomplishments for adequate pilot protection at 9G were now in place in the late 1970s, but not yet operational.

But at this time (1978-1979), operational requirements had not been identified by the user command since G-LOC had not yet been recognized as an operational problem. It was only after G-LOC was found to be a physiologic problem in the laboratory, and not a pathologic entity, that its hazard to flying became obvious (18). Without this rediscovery that G-LOC was a physiologic deficiency causing aircrew incapacitation for 30 sec or more, previously identified in 1940, but forgotten until now, operational requirements for improved anti-G methods would probably not have been recognized in the late 1970s, but probably much later (17, 19).

FUTURE RESEARCH REQUIREMENTS

The near-term advanced operational anti-G systems consist of a more complete coverage anti-G suit coupled with assisted PBG and pressurized with an adequate system. A pilot who has considerable muscle strength and anaerobic capacity and who is well-trained in performing the AGSM on the centrifuge will be capable of operating current 9G fighter aircraft to their maximum capability. Is, then, our research in this HSG environment complete? I must answer that question with a resounding no. Then, what are the current problems, and how are they being addressed with research?

First, let’s examine current requirements for operational 9G aircraft using current seat configurations with 15-30 degree seat-back angles. G-LOC will remain a problem as long as pilots are exposed to HSG as they sit upright in the cockpit (9). Although our present and near-term anti-G systems with G training reduces its incidence, the G-LOC threat remains. This hazard can only be completely removed by reducing the eye-heart vertical distance (called h) with pronation or supination at levels that allow pilots to pull 9G sustained relaxed without using any G-protection methods; e.g., 65-degree seat-back angle (supination) (1). Presently, because of our limited knowledge in that area and the understanding that these seat configurations will require major cockpit design changes of unknown dimensions, a drastic reduction of h is not yet feasible.

Without changing present seat back angles, we must concentrate our efforts in three major areas: (a) developing specific physical conditioning requirements to increase AGSM capabilities, (b) understanding the physiologic bases of G-LOC, and (c) developing pilot G-selection methods with G training. The first and last areas are complementary and with present knowledge, we can make major advancements against G-LOC. Dr Khomenko of the Soviet Air Force recently made an excellent presentation at the fall meeting of the International Union of Physiological Sciences, Commission on Gravitational Physiology (should be published in this issue of The Physiologist) that describes physical conditioning requirements for their pilots. I suggest that you read his article entitled “Prognostication of pilot high +G tolerance on the basis of static muscular strength endurance” (14). These stringent physical requirements that he has found highly correlated with HSG tolerance, in themselves provide aircrew high-G tolerance selection. This use of lower body muscular strength as a selection criteria is particularly valid for the Soviet Air Force since Soviet pilots perform a type of AGSM that emphasizes lower body muscular contractions and much less respiratory effort.

The physiologic basis of G-LOC remains a mystery, but several of us (acceleration scientists) believe that once we understand the exact physiologic mechanism, we will be able to either lengthen the time of consciousness in association with a critical reduction in blood flow to the brain or reduce the length of incapacitation caused by G-LOC. Either increased consciousness and/or reduced incapacitation duration could be beneficial in limiting the hazards of G-LOC.

Until we know the specific high-G requirements of future fighter aircraft, we should explore the possibilities of increasing G-level tolerances up to 12G using the advanced anti-G system of PBG with the uniform coverage anti-G suit and a reclined seat back angle of 55 degrees -- an angle that is possible with current cockpit design. The G-tolerance model that we have developed indicating a moderate synergism between known G-protection systems (PBG, anti-G suit, and AGSM) and seat-back angles is illustrated in Figure 2 (4). If this effect is validated in the laboratory on the centrifuge, then 12G tolerances become possible with current anti-G systems incorporating a 55 degree seat-back angle.
Over the past two decades, for pilots of high performance aircraft, laboratory acceleration research has produced: (1) a training program for AGSM; (2) a muscular strength regimen that enhances G protection; (3) an improved anti-G valve; (4) a PBG system that increases G tolerance, and (5) an improved anti-G suit that also increases G tolerance. We have also determined the importance of G tolerance in pilot selection criteria.

Acceleration scientists actively involved in these research programs over the last two decades can be proud of their achievements; they have saved pilots' lives.

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ADVANCED +Gz PROTECTION SYSTEMS AND THEIR PHYSIOLOGIC BASES

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INTRODUCTION

This paper discusses the known physiologic bases of advanced methods for protecting against the effects of headward acceleration (+Gz). The topic is preceded by brief descriptions of the physiologic effects of +Gz and of current anti-G systems.

PHYSIOLOGIC EFFECTS OF +Gz EXPOSURE

Rapid pull-out from a dive and tight turns in tactical aircraft produce high levels of +Gz with downward inertial forces acting on the body. These forces can cause unconsciousness which results from the cerebral / neurological consequences of +Gz effects on the cardiovascular system. Although inadequate venous return of more dense blood was originally suspected, the problem is now believed to lie on the arterial side because: (i) blood pressure at heart level is relatively stable; (ii) after several seconds at +Gz, blood pressure increases; and (iii) the decrease in blood pressure at head level is proportional to +Gz level (48).

Figure 1 illustrates the effect of different +Gz levels on systolic arterial blood pressure at different levels of the cardiovascular system according to a simple hydrostatic model superimposed on the cardiac component of intravascular pressure. The difference in blood pressure between head and feet in the upright individual increases with increasing +Gz level.

The adverse consequences of +Gz exposure result from the changes in blood pressure at heart level. With increasing +Gz level, blood pressure decreases. At approximately +3 Gz, vision becomes impaired. Retinal perfusion decreases because blood pressure falls below the pressure exerted on the retina by the eyeball (25). +Gz-induced loss of consciousness occurs at approximately +5.5 Gz (11) to inadequate cerebral perfusion.

Man is most susceptible to the effects of +Gz in the first several seconds of exposure, before cardiovascular reflexes can react to increase blood pressure at head level. That the body can tolerate +4 Gz at all is due to 4 main events:

(i) Venous return is adequate because the pressure gradient between peripheral and central venous circulations is maintained by gravity-related increases in intra-abdominal pressure acting on the splanchnic vasculature (39,40). As a result, systolic blood pressure at heart level only decreases by 4 mm Hg +Gz. The diastolic pressure remains unchanged (26).

(ii) With near constant heart-level blood pressure, the hydrostatic gradient of approximately 30 cm from the heart to the head can still be overcome.

(iii) Even at very low arterial pressures, an arterial-venous pressure differential in cerebral beds is maintained due to negative pressure on the venous side (20).

(iv) Pericardial pressure gradients provide the heart with some protection against the effects of +Gz (1).

For very short periods of time, man is able to tolerate +Gz levels which would eventually produce unconsciousness. Oxygen stores in nervous tissue are believed to provide up to 5-7 sec of normal function even though cerebral perfusion may be interrupted (44, 50). This can be a problem at +Gz onset rates greater than 1 +Gz/sec as unconsciousness can occur without visual warning signs.

CURRENT +Gz PROTECTION SYSTEM

To improve +Gz tolerance, anti-G systems must improve or maintain blood pressure at head level. This can be accomplished by increasing arterial blood pressure at heart level or by decreasing the hydrostatic distance between the heart and head. Postural alterations which reduce the heart-head distance are effective +Gz protective measures (14), but this discussion will only consider the near-upright pilot since this is the position of aviators of current and next generation tactical aircraft.

Anti-G Suits

Fighter pilots currently use anti-G suits and anti-G straining maneuvers (AGSMs) to reduce the effects of +Gz. The G-suit, changed little from the early designs of the 1940's, uses five interconnected bladders, one over the abdomen, and one over each thigh and lower leg. It is pressurized at 1.5 psi +Gz beginning at +2 Gz and confers approximately 1 +Gz of protection.

The main effect of the G-suit is an increase in head-level blood pressure (47, 48, 49). In short duration +Gz exposures of up to 10 sec, the hypertensive effect is produced by increases in peripheral vascular resistance (29) which is evident as an increased heart-level blood pressure. The lower limb vasculature and/or the splanchnic vessels could be the site of the increased resistance. Intra-abdominal pressure increases with G-suit inflation (49) and this helps increase arterial filling pressure (43). The pressurized abdominal bladder of the G-suit also prevents the descent of the diaphragm that normally occurs during +Gz. This confers two benefits (40):

(i) it increases the vertical height of the abdominal contents, resulting in greater hydrostatic pressure against abdominal vessels during +Gz;

(ii) by maintaining diaphragm position, it prevents any further increase in the heart-head vertical distance which would occur if the abdominal contents and lower chest wall were not supported.

1 Tolerance - the capacity to accept the effects of +Gz to a predetermined alteration of some specified physiological function(s); there are +Gz-intensity and/or +Gz-duration components to +Gz tolerance.
The abdominal bladder is the most important component of the G-suit. When used alone, the abdominal bladder increases relaxed +Gz tolerance by 0.6 +Gz. Tolerance only increases by 0.2 +Gz when just the leg bladders are used. However, the combined use of the various parts appears to potentiate their individual effects, as +Gz tolerance then increases by 1.2 +Gz. (49). The premier importance of the abdominal bladder for relaxed +Gz tolerance has been verified (7).

**Anti-G Straining Maneuvers**

Anti-G straining maneuvers of various forms have also been known since the early 1940's. These generally consist of intense muscular contractions of the chest wall (Valsalva maneuver) exerting an expiratory effort against a closed or partially-open glottis. This is coupled with equal forceful tussling of the arms and legs. If the closed glottis method is used, the maneuver is maintained for 3-4 sec, followed by rapid expiration, then rapid and deep inspiration, and initiation of another straining effort. The high intra-thoracic pressure is transmitted directly to the heart and the great vessels (18, 41). If intra-abdominal pressure does not maintain a pressure gradient from abdomen to chest, blood pressure rapidly decreases (3, 40). But the risk of this effect during AGSM is reduced when a G-suit is used (49) due to the increased intra-abdominal pressure effects.

The peripheral muscular tussling component probably contributes to the blood pressure increases with AGSM. The extreme hypertension of high-intensity, resistance exercise is regarded to be the result of mechanical compression of vasculature and powerful pressor responses (30) with increases in total peripheral resistance and possibly cardiac output (32). Similar to the functioning of the G-suit, the cardiac output would then be distributed to a reduced arterial bed.

**ADVANCED +Gz PROTECTION SYSTEMS**

Compared to the average +Gz tolerance level of +3.7 Gz in relaxed and unprotected subjects, pressurized G-suits, and G-suits combined with straining maneuvers increase mean +Gz tolerance to +5.4 and +8.8 Gz, respectively (33). However, an increase in the number of incidents and fatalities in the current generation of tactical aircraft due to their advanced +Gz capability has renewed efforts to devise better +Gz protective systems.

**Improved G-suits**

Our knowledge of the design requirements for improved G-suits has progressed little since WWII when several acceleration laboratories, particularly in the US, had produced G-suits which provided better +Gz protection than current issue G-suits. These superior G-suits, however, were rejected for operations because they were complicated, cumbersome, and uncomfortable, and because their performance actually exceeded what was necessary at that time. It is important to consider this early work.

(i) Knowing that the problems with +Gz exposure affected the arterial system more dramatically than the venous side, efforts concentrated on increasing blood pressure at heart level. Arterial Occlusion Suits were produced to increase vascular resistance. These suits consisted of an abdominal bladder and a cuff on each upper thigh. Inflicted to arterial occlusive pressures, e.g. 325 mm Hg at +5 Gz, these provided an average of 2.1 +Gz of protection above the normal tolerance point (45). The addition of arm cuffs increased the protection to 2.9 +Gz. Ischemic pain made these suits unacceptable.

(ii) Most of the G-suits used by NATO countries today are minor variants of the USAF G-3A G-suit. In contrast, the G-4A incorporated larger bladders, and more extensive and better fitting fabric pressure on the body surface into a coverall garment. The G-4A provided approximately 2.0 +Gz of protection, double that afforded by the G-3A (31).

The Full-Pressure-Half-Suit is like the lower half of a full pressure altitude suit. It applies uniform pneumatic pressure to all body surfaces below the chest and provides approximately 2.4 (28) to 3.2 +Gz of protection to visual symptoms (10). In a comparison with the G-4A overall, the 0.9 +Gz extra protection provided by the Full-Pressure-Half-Suit was due to improved blood pressure at eye level (43). This was attributed to increased peripheral arterial resistance and venous return. A possible benefit due to greater reduction in the heart-head distance was ruled out.

Several types of G-suits applying more uniform pressure to the lower body compared to the standard suit have been investigated recently by the USAF (8, 21, 22, 23). According to impedance plethysmographic measurements, the latest USAF uniform pressure G-suit displaces more blood from the calves and thighs into the abdomen than does the standard suit (23).

With counter-pressure exerted to the entire body surface below the umbilicus except for the ankles and toes, Prior of the RAF has observed that +Gz tolerance compared to the unprotected condition is increased by 2.9 +Gz, a 1.3 +Gz increase above that provided by the standard G-suit (36). Gluteal region coverage was speculated as the main reason for the improvements.

G-suits with greater pressure in the lower leg bladders and less pressure in the abdominal bladder (48), or suits inflated from the bottom bladders upwards (6), offer no better +Gz protection than single pressure suits.

The anti-G valve is an important component of +Gz protective systems because G-suit performance also depends on receiving the correct pressure at the appropriate time. How fast the G-suit should be inflated is currently unresolved. If the rate of G-suit pressurization is improved so that the delay is reduced to only 0.5 sec, significant improvements in +Gz tolerance over the standard conditions have been recorded (13). In contrast, a mean delay of 2.0 sec in pressurization with no deterioration in protection has also been observed (5). Although cerebral blood pressure decreases with +Gz onset, the cerebral ischemic reserve time could offer a buffer period before +Gz protective systems must be fully activated.

**Positive Pressure Breathing**

The AGSM effectively increases blood pressure, but it requires concentration and proper instruction to ensure it is performed correctly, disturbs respiration, and is fatiguing due to the muscular effort. Two physical characteristics are associated with the AGSM. Tolerance of high +Gz levels requires high intra-thoracic pressures, therefore muscle contraction will be of high intensity. Sustained aerial combat maneuvers require repeated AGSM, therefore endurance capacity is important. The procedure of positive pressure breathing during +Gz (PBG) has the potential to reduce much of the physical stress of performing the AGSM.

PBG uses a modified breathing regulator to increase the pressure of gas delivered to the oxygen mask and lungs. As with the AGSM, the increased airway pressure from PBG is transmitted to the left ventricle and thoracic systemic arteries (16). The benefits and optimum use of pressure breathing have been well studied in relation to reducing the hypoxia from exposure to the very low barometric pressures of altitude (16). The hypertension with pressure breathing and the potential role for +Gz protection were recognized as early as 1944 (24).

Interest in PBG was revived in the early 1970’s with investigations conducted in the US and UK (27, 42). Pressure levels up to 40 mm Hg did not alter +Gz tolerance compared to tests using straining maneuvers, but the subjects reported less fatigue with PBG. Arterial blood pressure was also more stable with PBG. During the AGSM, inspiration between strains causes intra-thoracic pressure to drop with subsequent decreases in heart-level blood pressure. Because PBG provides consistent levels of airway pressure, the respiratory-related fluctuations in blood pressure are reduced and head-level pressure is more sustained.

3 Intra-thoracic pressure is loosely defined as the pressure around the heart, i.e. intra-pulmonary pressure.
Positive pressure breathing increases lung volume in relaxed individuals. End-expiratory lung volume is determined by the balance of forces (38) tending to deflate the lungs (i.e., static recoil of respiratory system), and forces tending to inflate the lungs (e.g., pressure breathing). PBG at approximately 20 mm Hg would move the respiratory system to near total lung capacity.

Due to the inflating effect, exhalation is more difficult with pressure breathing. Normally passive exhalation now requires expiratory muscle contraction, the intensity of which will be proportional to the airway pressure. The effort required for expiration and the sensation of lung overdistension are reduced by garments which encompass the thorax* and contain a bladder for pressurization (16). The standard procedure applies pressure to the outside of the chest at the same level as the airway pressure.

At low pressure levels, thoracic counter-pressure garments make PBG easier. They also allow greater pressures to be used. The maximum pressure breathing level without thoracic counter-pressure is generally regarded as 30 mm Hg. With pressure breathing equipment limited to a "partial-pressure ensemble" (self-tightening oro-nasal mask, helmet, thoracic garment, and G-suit), 80-90 mm Hg airway pressure will probably be the maximum tolerable. While these are certainly high pressures, the risk of burst lung syndrome is remote in healthy lungs because thoracic counter-pressure garments control the pressure gradient between the chest wall surface and alveolar space.

**PBG Schedules**

Design engineers of PBG regulators must know at what +Gz level PBG should begin, how much pressure is required at different +Gz levels, and the maximum pressure output required from the regulator. The requirements of the PBG schedule have only received attention recently. On a physiologic basis, PBG should begin at +Gz levels causing a deterioration in physiologic function. For example, if visual impairment is not to exceed the loss of peripheral vision, then PBG should begin between +3-Gz. PBG could start at a lower +Gz level should pilots require verification of the proper functioning of the PBG system.

As AGSM intensity increases with increased +Gz, so too must the PBG level. An example of PBG proportional to the +Gz level is shown in Figure 2. If head-level blood pressure is to be maintained at some specified level, e.g., equivalent to the pressure normally obtained at +3 Gz, heart-level pressure must increase enough to translate the BP-height line to the point where head-level pressure is the same as the pressure at +3 Gz. Since head-level blood pressure decreases by approximately 22 mm Hg / +Gz in average man, the PBG schedule would be expected to at least match this requirement if it is to be the sole source of intra-thoracic pressure increase. Therefore Figure 2 shows 86 mm Hg PBG restoring head-level blood pressure at +7 Gz to the +3 Gz equivalent. Any deficiency in the amount of pressure supplied to the airways must be made up by supplemental AGSM.

The amount of PBG delivered at different +Gz levels is being addressed from two perspectives: (i) subjective satisfaction with the protection provided by the level of PBG given; and (ii) measurement of +Gz tolerance with different levels of PBG. Subjective ratings of PBG schedules during in-flight trials (2, 12, 17, 19, and USAF Tactical Life Support System trials) and in the centrifuge (35, 37) indicate PBG beginning at approximately +3.4 Gz and increasing at approximately 5-15 mm Hg/ +Gz is superior to the standard G-suit and AGSM system at conferring +Gz protection. In contrast, measurements of +Gz tolerance show that greater PBG levels are required to produce protective benefits. Average PBG schedule requirements of approximately 18 mm Hg/ +Gz (16), 26 mm Hg/ +Gz (9), and 32 mm Hg/ +Gz (34) have been reported. PBG requirements significantly greater than 22 mm Hg/ +Gz show that the blood pressure increase for increases in intra-pulmonary pressure can be less than unity (16).

An important question that will need to be answered is: once the required intra-thoracic pressure increase for each +Gz level is known, should that pressure be produced entirely by the regulator while the pilot remain reasonably relaxed, or should a portion of the intra-thoracic pressure increase come from AGSM? The only PBG system that is operational, albeit in the very early stages, is the Combat Edge programme of USAF. It produces 12 mm Hg/ +Gz beginning at +4 Gz and has been well-received by aircrew.

Although the maximum tolerable airway pressure is approximately 90 mm Hg with partial pressure ensembles, maximum pressures in the 60-70 mm Hg range will be more acceptable to aircrew. With such levels of PBG, what is the maximum +Gz-intensity tolerance that could be expected? This will depend greatly on the maximum intra-thoracic pressure that can be developed. Recent experiments using limited-coverage thoracic garments have shown that the maximum intra-thoracic pressure from the simultaneous use of PBG and maximum AGSM is not different than the intra-thoracic pressure from maximum AGSM alone. The average of these pressures was approximately 140 mm Hg (4).

Although PBG is unlikely to increase +Gz-intensity tolerance above levels attained with AGSM, PBG would be of value during repeated exposures to moderate +Gz levels and in replacing poorly-performed AGSMs. Ineffective AGSMs could occur from lack of knowledge or practice in proper AGSM techniques, the inability to execute a strong AGSM due to muscular fatigue, or from concentration being diverted elsewhere, such as aircraft control.

**FINAL REMARKS**

What improvement in +Gz tolerance can be expected from the combination of full coverage G-suits and PBG? Relaxed subjects reached +8.3 Gz with these counter-measures compared to +3.6 Gz without +Gz protection (38). Of this increase, +2.9 +Gz of protection was due to the full coverage G-suit. Therefore, we would expect most pilots to be able to conduct flying tasks at +10 Gz when a well-executed AGSM is added to PBG and greater coverage G-suits. Significant improvements in +Gz-duration tolerance will also be realized.

Carbon dioxide and some pharmacologic agents have also conferred hypertensive effects, but these will not be reliable components of any +Gz protective system. Should +Gz protective

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*S. Such garments vary from those coverage the entire trunk (called jerkins) to those just covering the rib cage (called pressure vests or waistcoats).

†PBG schedule = 9 mm Hg/ +Gz beginning at +2 Gz, with 60 mm Hg cabling.
systems fail, or reach their limit during flight, unconsciousness-avoidance monitors which measure some standard level of physiologic function such as head-level blood flow, could intervene to reduce the aircraft's +Gz level. We must also be cautious about how far we are willing to push +Gz tolerance. The body has limitations (46), and if exceeded, serious consequences could result without having reached the +Gz tolerance endpoint.

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COMPUTER MODELLING THE EFFECTS OF GRAVITY ON THE CARDIOVASCULAR SYSTEM

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Introduction

The effects of gravity on the circulation are manifold, some of them obvious, some come only into light under extreme gravitational influences. In general, physiology of the cardiovascular system is described concentrated on the supine subject. This is the position of patients in hospital and of experimental animals on the operation table. We tend to view the upright position simply as an extrapolation of this 'supine physiology'. However, space flight has made us reconsider this simple paradigm and so has high performance flying.

For the present study we developed a computer model for the rapid adaptation of the cardiovascular system to changes in gravitation. This model was designed to help us understand the dynamic changes in blood pressure and heart rate that are observed when a test subject is passively tilted from supine to upright and vice versa. We put data from the physiological literature together to describe the supposed underlying phenomena in mathematical form. This exercise shows to what extent our understanding of the circulation is sufficiently complete. The problems that we encountered, uncover basic lacks in our understanding of cardiovascular regulation.

Elements for a cardiovascular model.

Figure 1 shows the basic elements that were incorporated in our model. The model has a double circulation, the right heart emptying into a compliant pulmonary circulation, the left heart emptying into a less compliant systemic circulation. The systemic circulation provides blood to a resistant peripheral bed from which it is drained to a highly compliant venous reservoir. Regulation is brought about by the baroreflex that is signalling blood pressure in the systemic circulation. Feedback is to heart rate via a fast parasympathetic and a slower sympathetic influence. The sympathetics, moreover, influence vascular ('peripheral') resistance.

Since we want to use the model for the simulation of cardiovascular responses to tilt maneuvers, we have to point out where gravity might play a role. First we may point to the position of the (carotid sinus) baroreceptors. In the supine position these receptors observe blood pressure as it exists at heart level. Upright, however, the pressure at the receptors is some 25 mmHg lower than at the heart. Even when the pressure at heart level does not change, it still is lowered at the level of the receptors, leading to a correction signal for heart rate and peripheral resistance. Next, we must consider the filling of the ventricles from their respective venous reservoirs. The right heart will, temporarily, receive less blood when we move to upright, the left heart, on the other hand, will undergo an increased filling from the lungs, when blood is redistributed under the influence of gravity. After a few heart beats this situation is altered: now the pulmonary reservoir has been emptied and is undergoing the effects of decreased venous return. So, as a provisional conclusion, we expect gravity to act on the baroreceptors and the venous filling of the left and right ventricles. The model outlined in figure 1 was put into mathematical form.

Mathematical formulation of the model

The elements of the model, of course, resemble those in many other physiological models of the circulation. The mathematical implementation, however, makes this model special: rather than by continuous differential equations we modelled the properties of the cardiovascular system by beat-to-beat difference equations. This model is an extension of the model that we developed in earlier research projects (1) to explain the spontaneous variability in recordings of blood pressure and heart rate. Since we want the model to describe fast changes in blood pressure and heart rate, emphasis is placed on the short-term characteristics. The cardiovascular system is characterized in beat-to-beat numbers, that are updated each heart beat. This
approach has the advantage of being computationally simple (a spreadsheet program can do much of the work) and it avoids the necessity to derive time-continuous formulas for phenomena that are less important for the present aim. In the steady-state situation the numbers for successive beats become equal.

For the present review we will not go into a full display of all the formulas that were designed to construct the mathematical model. Let it be sufficient to outline the main considerations:

1. end-diastolic pressures are computed for the different compartments in figure 1 from the filling in the previous beat, plus the inflow minus the outflow in the present beat, divided by the compliance of the compartment.
2. stroke volumes for right and left heart are determined by venous filling pressure (Starling’s law) and the duration of the previous interval (restitution phenomenon).
3. systolic pressure in the systemic circulation is computed from the diastolic pressure plus stroke volume divided by systemic compliance (neglecting ‘water hammer’ effects and wave reflections that may contribute to the actual systolic pressure).
4. values for heart period and peripheral resistance are computed taking the pressure input signal to the baroreceptors into account for the current beat and a number of previous beats.

Experiments and simulations by the model

Tilt experiments have been performed using a fast tilt table (effective tilt times of minimally 1 second). The resulting transients in blood pressure and heart rate from such experiments are shown in figure 2 (dotted lines).

Superimposed in dotted lines are the results of simulations for tilt-up and tilt-down, using the same parameter settings for both maneuvers. It is obvious that the model is capable of simulating the tilt-down responses quite well. However, tilt-up cannot be simulated without an undershoot in blood pressure. This is a logical consequence of the position of the baroreceptors in the system: in order to give a feedback response they have to ‘see’ a blood pressure drop.

This reasoning leads to the search for a ‘predicting’ element in the cardiovascular system. Since a shortening in cardiac output will present itself, first, as a relative shortening in venous return, we located this predictive element in the so-called low-pressure receptors, that signal the pressure in the right atrium and the pulmonary circulation. In animal studies the action of such low-pressure receptors on the systemic circulation has been investigated in detail (2).

However, the function of these receptors for normal physiological functioning in humans is still subject to debate (3). With the incorporation of a cardiopulmonary reflex (CPMR) we were able to simulate both tilt-up and tilt-down responses quite well, as is shown in figure 3. Roughly speaking, we supposed the CPMR to have reflex effects parallel to those of the baroreflex, i.e. decreasing pressure on the atrial side leads to increases in heart rate and systemic blood pressure. For increasing atrial pressures the reverse (although less strongly) is supposed to occur.

Conclusions

An important finding from this study is the probable insufficiency of a ‘baroreflex-only’ system to explain the events that lead to the well-maintained upright position. Extension of the model with a predictive element, signalling a reduced venous return was found necessary. The cardiopulmonary - or low-pressure receptors are ideally suited to fulfill this task.
Another (unforeseen) outcome is the characteristic course of the tilt-down transients in heart rate and blood pressure. These can be well explained by the redistribution of blood in the lungs at the very first moments of tilting, followed by increased venous return and blood pressure overshoot. This maneuver gave very reproducible responses, comparable to those in the well-known Valsalva-manueouver. For practical purposes the tilt-down maneuver may be preferable as an autonomic control test, since it is not dependent on test-subject cooperation.

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Fluid loading is a countermeasure currently in routine use to improve the g-tolerance of crewmembers during reentry and return of Space Shuttle flights. However, its effectiveness diminishes with mission duration. Countermeasures that will be effective on long-duration flights are needed and are presently under development. This paper discusses the application of computer simulation in the analysis of the effects of countermeasures for reentry orthostatic intolerance. The results suggest improvements upon the fluid loading countermeasure currently in use.

INTRODUCTION

The physiological response to weightlessness exposure covers a wide spectrum of significant and complex changes in body fluid volumes, including a redistribution of fluids and electrolytes among the major compartments of the body (1). In particular, there is a shift of intravascular and extravascular fluids from the lower extremities to the trunk upon entering weightlessness, as reflected by head congestion and a decrease in leg volume (6). Such fluid redistributions are evidently interpreted as increases in blood volume by the centrally-located volume-sensing elements. The reflex response is a compensatory loss of fluids and electrolytes. A significant result of these adjustments is a decrease in the total circulating blood volume (1), which has been measured in numerous ground-based and spaceflight studies (6). Thus, it is a major factor of cardiovascular adaptation to weightlessness and similar one-g conditions. Another factor that has been examined more recently in both ground-based and flight studies (3,4) is the sensitivity of the baroreceptor-heart rate reflex. Data from these studies on autonomic control mediated by carotid sinuses strongly indicate a reduction of as much as 30 percent in the sensitivity of high pressure baroreceptors. Such reduction in baroreceptor reflex gain in combination with a loss of blood volume can significantly alter the response of the cardiovascular system to an orthostatic stress. Computer simulation studies (15) show a high degree of interaction between these two parameters leading to far lower levels of orthostatic tolerance than those that would be obtained if their effects were additive.

The decrease in orthostatic tolerance is a concern in Space Shuttle flights because of the headward accelerations (+G1) to which the crew are exposed during reentry. The headward g-force is up to two times that of normal acceleration due to gravity at sea level and may reduce cerebral perfusion pressures and flows. The changes are magnified by the loss of blood volume and decrease in baroreceptor sensitivity caused by weightlessness exposure. In certain circumstances, they may reach such critical proportions as to seriously affect crew performance at a time when performance decrements can be least accommodated or tolerated. Also, post-flight orthostatic intolerance could hinder crewmembers’ ability to escape in an emergency. These problems underscore the need for development of effective countermeasures for orthostatic intolerance as NASA moves forward with its planned Extended Duration Orbiter (EDO) flights, which will be longer in duration than the current Shuttle flights.

A countermeasure currently in routine use to improve the g-tolerance of Shuttle crewmembers during reentry is fluid loading. The aim is to replace fluids and electrolytes lost during the course of the space flight and thus reverse some of the adaptive changes that occurred in weightlessness, especially changes in cardiovascular parameters such as the central venous pressure. The effects of fluid loading are transitory, and therefore, it is generally administered just a few hours prior to reentry in order to achieve peak beneficial effects during the reentry phase. Although it produces varying results among different individuals, it does afford some degree of protection against orthostatic intolerance. Data from bed rest and acceleration studies (7,9) and Shuttle flights (2) provide some guidance of its effectiveness as a countermeasure to cardiovascular deconditioning.

However, the effectiveness of fluid loading diminishes with mission duration (2). Countermeasures that will be effective on long-duration flights are needed for successful implementation of EDO missions. Some new countermeasures and possible combinations of them are currently being investigated in ground-based studies, and others have been proposed. These include prolonged exposure to lower body negative pressure (LBNP), preadaptation with blood volume reduction, and treatment with fluid retaining drugs such as Florinef and analogs of vasopressin.

Evidently, the countermeasures, their combinations, and protocol variations are numerous. It would be highly useful to have some means of evaluating the effectiveness of potential countermeasures...
theoretically prior to experimental verification. One possible approach is to use computer simulation with mathematical models. This paper presents such an approach to analyze the effects of countermeasures for reentry orthostatic intolerance.

**THE MATHEMATICAL MODEL**

The Guyton model of fluid and electrolyte regulation (8) is used in this analysis. It involves a number of subsystems and incorporates many known neural, hormonal, and hemodynamic feedback control mechanisms as well as adaptation effects in the heart, blood vessels, and pressure receptors for control of long-term blood pressure disturbances. Thus, it is comprehensive in scope and is capable of predicting long-term circulatory events under a variety of stress and stimuli. The original model developed by Guyton et al. (8) was modified at NASA to simulate the observed circulatory response to hypograv-ic stress (11), by including lower body fluid compartments, colliders, orthostatic mechanisms, and gravity-dependent effects. The modified version has been used in a number of simulation studies to analyze fluid and electrolyte alterations in zero-g, head-down bed rest and water immersion (13).

**SIMULATION RESULTS**

**Fluid Loading.** Weightless conditions were simulated by placing the model in a head-down tilt position of -6 degrees. The physiological parameters were allowed to attain steady levels relative to preflight values by running the model for a period of 3 days of simulation time. Simulation results indicated this period to be adequate to define new steady levels in the cardiovascular parameters of interest. The reentry g-profile from the first Shuttle flight (STS-1) with a peak g-force of approximately 5 g was used to simulate reentry g-stress. The time course of parameter changes was tracked from the beginning of reentry to touchdown (TD) and 25 minutes thereafter under one-g conditions. The simulation runs provided responses of circulatory, fluid and electrolyte, and hormonal systems, both with and without pre-reentry fluid loading. The salt concentration and the ingestion time (prior to reentry) of the fluid load were varied and their influence on the response changes was examined. The volume of fluid load was kept at one liter in all simulation runs. This is close to the dose of one quart recommended to the Shuttle astronauts.

Fig. 1 is an example of the simulation run showing the effect of increasing the salt content of the ingested fluid. A hypertonic fluid load shows the maximum beneficial effect in terms of minimizing the cardiovascular variables toward preflight levels. The time course of changes for different salt concentrations are similar in most instances.

The ingestion time normally used by Shuttle astronauts is approximately two hours prior to reentry for reasons of operational convenience. Simulations on the effect of varying the time of ingestion showed that moving the fluid intake closer to reentry (closer to two hours) would have the effect of moving the responses closer to preflight levels, although the improvement was only marginal. On the other hand, increasing this time interval had the opposite effect and the differences were more pronounced. In the hypotonic case, the peak changes (from preflight levels) were progressively less as the time of ingestion was increased. Just prior to reentry time; whereas, the hypotonic case showed much less sensitivity to ingestion time. The results emphasize the relative ineffectiveness of hypotonic fluids as countermeasures for orthostatic intolerance. In contrast, a hypertonic solution taken just prior to reentry will provide the optimum benefit in improving the cardiovascular status during and following reentry. This is currently being tested in Shuttle flight experiments.

**Preadaptation Blood Volume Reduction.** In order to validate the model for analysis of this countermeasure, simulated data on plasma and red blood cell changes that occur in head-down bed rest were compared with experimental data from a 10-day head-down bed rest study specifically designed as a ground-based simulation of the Spacelab-I mission (14). The comparison was not initially satisfactory and pointed to the need for modifying the model. The major modification introduced was the replacement of the subsystem on erythropoiesis regulation by a more detailed representation (12). Minor parameter adjustments were made to obtain a new equilibrium under conditions of head-down tilt with losses of red cell mass and total blood volume close to experimentally observed values (top panel in Fig. 3).

As further validation of the model with the above modifications, it was used to simulate the response to a 15% of blood volume in 18 minutes. Fig. 2 shows a comparison of the simulated hematocrit with experimental measurements at the same bleed rate from two normal subjects (unpublished data, Simanokok).

The validated model was then used to test the hypothesis that blood volume reduction would counteract fluid shifts. The model was first run in the head-down tilt position (-6 degrees) until a new equilibrium was established. The difference between the initial and final equilibrium blood volumes was calculated. This amount (approximately 15% of the initial value) was removed to preadapt the circulation before initiating head-down bed rest in the second simulation. The results from the two simulations were compared. Fig. 3 shows some of the results with and without preadaptation blood volume reduction. The protective effect of the countermeasure is clearly evident from a comparison of the graphs in these figures. After 10 hours of bed rest, the blood volume is better maintained up to 30 days with the countermeasure.
FIG. 1. Effect of varying the salt concentration of the fluid load on cardiovascular response during reentry. Ingestion time is 2 hours prior to reentry.

FIG. 2. Validation of the model for response to hemorrhage (15% of blood volume in 18 minutes).

FIG. 3. Circulatory response with and without preadaptation blood volume reduction.

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DISCUSSION

The loss of fluids and electrolytes plays a key role in spaceflight-induced cardiovascular deconditioning and the resultant reduction in orthostatic tolerance. The best evidence for this is the demonstrated effectiveness of fluid and electrolyte replacement as a countermeasure to improve orthostatic tolerance. Fluid loading has its limitations, however. Since the loss of fluids is not the only causative factor behind cardiovascular deconditioning due to weightlessness, compensation for fluid loss alone cannot be expected to reverse completely the adaptive manifestations of weightlessness exposure.

As mentioned earlier, fluid loading is an operational requirement for Shuttle astronauts. The recommended fluid load is one quart of water with salt tablets to make it equivalent to isotonic saline. Since the amount and the time of ingestion (approximately two hours prior to reentry) are largely dictated by operational constraints, any improvement in the beneficial countermeasure effect of fluid loading may have to be achieved through modifications of the fluid contents. In this study, we have considered altering the salt content, and our simulation results favor increasing the salt concentration of the fluid load. There is also experimental evidence from ground-based studies (5,7) to support this contention.

With regard to preadaptation blood volume reduction, the simulation results support the hypothesis that it would counteract some of the major effects of fluid shifts. These results are in agreement with water immersion studies (14). The most unambiguous potential benefit of this countermeasure is that postflight orthostatic tolerance might be improved by the enhanced fluid retention for missions up to 20 days in duration.

The studies presented here are two examples of simulations that have been performed using the Guyton model. They exemplify the value of mathematical modeling and computer simulation as a tool in physiological experimentation.

CONCLUSIONS

- Simulation results on fluid loading show that a hypertonic solution administered just prior to reentry would provide the maximum beneficial effect.
- Simulation results on blood volume reduction prior to a headward fluid shift support the hypothesis that it would counteract some of the major effects of the fluid shift.
- The study demonstrates the usefulness of modeling and simulation in addressing questions of physiological importance to Shuttle operations.

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1 Introduction

The development of high performance aircraft capable of providing substantial positive accelerations (+Gz) has created the need for a better understanding of the adverse physiological responses that can affect a pilot's judgement. This adversity ranges from the less severe temporary loss of peripheral vision to unconsciousness and, in very severe cases, permanent damage to heart tissue. For safety reasons, there is considerable interest in the cardiovascular system since it plays a vital role in the human physiological changes resulting from exposure to sustained +Gz accelerations (see [3, 6, 14]). Under these high +Gz loading conditions the heart tissue is highly stressed. Therefore, the possibility of permanent cardiac tissue damage due to these high +Gz forces has attracted considerable attention (for example [4, 13, 14, 19]). In previous studies, experimental investigation has been the principal method to study the adverse physiological changes associated with exposure to moderate-to-high +Gz accelerations. However, the approach adopted in the present work focuses on the development of a computational model in the hope of providing additional insight into the effects of +Gz stresses on the human heart.

This paper gives the theoretical foundation on which a computational model for the determination of the stress / strain state of the human ventricle myocardium during sustained +Gz acceleration is based. The model utilizes the finite element technique where the effects of finite displacements, large strains, non-linear nearly incompressible material behaviour, and the irregular shape of the heart are accounted for. Emphasis has been placed on the incorporation of a realistic constitutive relation into the finite element formulation. For a further description of the finite element modeling considerations of the human heart see Moore et al. (1991)[17]. When experiments cannot be justified, the computational model can provide valuable quantitative (gross distortion and predicted stress) data on the effects of +Gz induced stresses in humans. Ultimately, the goal is to provide some form of cardiac risk assessment for pilots of high performance aircraft.

2 Theoretical Development

2.1 Kinematics of Deformation

In order to analyse the motion of a deformable body either a Lagrangian (referential) or Eulerian (spatial/current) reference configuration may be used [15] (see Figure 1). In this study, the so-called Updated Lagrangian spatial reference is adopted. For this approach, the reference configuration, denoted by B0, moves with changes in geometry. Alternatively, for the Total Lagrangian method all variables are referred to the initial configuration, B0. These formulations are mathematically equivalent, however, either may possess some computational advantages over the other depending on the problem under consideration.

The deformation of the original body can be represented by the mapping χ: B0 → B, such that:

\[ x_i = \chi_i(X_\alpha, t) \]  

where \( X_\alpha \in B^2 \), \( x_i \in B^3 \), and \( \alpha, i \in \{1, 2, 3\} \), are the Cartesian coordinate system indices. For a quasi-static deformation process, Eq. (1) becomes:

\[ x_i = \chi_i(X_\alpha) \]

The deformation gradient which is a measure of the deformation of the original body, B0, is a second order (two point) tensor with components:

\[ F_{\alpha\beta} = \frac{\partial x_i}{\partial X_\alpha} \]

Also, note that \( F_{\alpha\beta} \) is non-singular and from mass conservation:

\[ J = \det F_{\alpha\beta} = \frac{\rho^0}{\rho} > 0 \]

where \( \rho \) and \( \rho^0 \) are the densities in the spatial and reference configurations respectively. In addition, for an incompressible material behaviour the isochoric deformation process is subject to the constraint:

\[ J = \det F_{\alpha\beta} = 1 \]

![Figure 1: Incremental description of deformation.](image)

2.2 Strain Measures

Defining Green’s–Lagrangian deformation tensor by:

\[ C_{\alpha\beta} = F_{\alpha\gamma} F_{\beta\gamma} \]

the positive definite tensor has principal invariants:

\[ I_1 = tr C \]
\[ I_2 = \frac{1}{2} [(tr C)^2 - tr C^2] \]
\[ I_3 = \det C \quad (Incompressibility : I_3 = 1) \]

where \( tr \) is the trace. One can express Green’s–Lagrangian strain in terms of Green’s deformation tensor as:

\[ E_{\alpha\beta} = \frac{1}{2} (C_{\alpha\beta} - \delta_{\alpha\beta}) \]

where \( \delta_{\alpha\beta} \) is the Kronecker delta function.

Alternatively, the strain measure in terms of the spatial configuration can be expressed as:
\[ e_{ij} = \frac{1}{2} (\delta_{ij} - F_{ai}^{-1} F_{aj}^{-1}) \]  

where \( F_{ai}^{-1} = \partial X_a / \partial x_i \).

From Eqs. (7) and (8), it is evident that the relationship between Lagrangian and Eulerian strain measure is given by:

\[ E_{a\beta} = F_{ia} e_{ij} F_{j\beta} \]  

2.3 A Strain Energy Function for Cardiac Tissue

It is vital that any computational model developed uses reliable data for the mechanical properties of the heart muscle. In essence the heart wall behaves as a non-linear anisotropic composite material [20]. Recently, a number of three-dimensional anisotropic constitutive relationships for passive cardiac tissue, undergoing finite strain, have been proposed by Humphery et al. [11, 12]. These relationships use uni-axial and bi-axial experimental stress-strain data to provide information for selecting best fit parameters in constructing a strain energy function, \( W \). However, due to the lack of a reliable constitutive relationship for modeling the anisotropic viscoelastic material behavior of the human heart [7], an isotropic, homogeneous, (uniform properties) non-linear elastic behavior proposed by Demiray and Vito [5] has been incorporated in the computational model. This strain energy function takes the form:

\[ W(I_1) = a [e^{b(I_1 - 3)} - 1] \]  

where \( a \) and \( b \) are material constants derived from experimental data. The constants \( a \) and \( b \) represent the best fit parameters for passive cardiac tissue data obtained.

Since this strain energy function applies to soft biological tissue, it can also be applied to the modeling of the pericardial sac, which is a conical fibrous membrane surrounding the heart and proximal portions of the cardiac vessel. The inclusion of the pericardial sac in the analysis ensures that the correct boundary conditions are realised; further enabling accurate geometric modeling.

2.4 Stress–Deformation Relationships:

The stress–deformation relationship for 2nd Piola–Kirchhoff (Lagrangian) stress tensor which refers to the original configuration, \( B^j \), is given by [18]:

\[ S_{a\beta} = \frac{\partial W(E)}{\partial E_{a\beta}} - p B_{a\beta} \]  

and defining \( B_{a\beta} = C_{a\beta} \). In terms of the spatial configuration, \( B^j \):

\[ \sigma_{ij} = F_{ia} \frac{\partial W(E)}{\partial F_{aj}} - p \delta_{ij} \]  

The introduction of \( p \), an arbitrary hydrostatic pressure (or Lagrangian multiplier), is used to maintain the incompressibility constraint. In addition, one may note that the 2nd Piola–Kirchhoff stress is related to the Cauchy–(Eulerian) stress tensor by:

\[ \sigma_{ij} = J^{-1} F_{ia} S_{a\beta} F_{j\beta} \quad \text{or} \quad S_{a\beta} = J F_{ai}^{-1} \sigma_{ij} F_{j\beta} \]  

The stress–deformation relations cast in the form of Eqs. (11) and (12) give rise to a mixed finite element formulation, that is, both displacement and pressure \((u, p)\) are taken as independent unknowns. However, the elimination of \( p \) from the system of equations can be accomplished by relaxing the incompressibility constraint, \( I_3 = 1 \), using a penalty function [9]. This approach has been adopted herein. By selecting:

\[ p = p_o - \frac{\lambda}{2} \ln(I_3) \]  

where \( \lambda \) is a penalty parameter, the number of system equations can be drastically reduced while maintaining (near) incompressibility. In addition, in order to satisfy the initial condition \( x_{i|t=0} = \chi_i(X_o, 0) = X_o \) such that \( S_{a\beta|t=0} = 0 \) yields \( p_o = \frac{\partial W(E)|t=0}{\partial E_{a\beta}|t=0} \).

2.5 Equations of Motion

Consider the Eulerian equations of motion

\[ \frac{\partial \sigma_{ij}}{\partial t} + \rho b_i = \rho \ddot{u}_i \]  

and the surface traction on part of the boundary, \( \partial B_T \):

\[ T_i = \sigma_{ij} n_j = T_i(x_k, t), \quad x_k \in \partial B_T \]  

where \( n_i \) is an outward normal to the surface, so that \( \partial B = \partial B_u \cup \partial B_T \). With the following initial conditions:

\[ u_i(X_o, 0) = \psi_i(X_o) \quad \dot{u}_i(X_o, 0) = \ddot{u}_i(X_o) \]  

For the special case of a body at rest, Eq.(15) is referred to as the equilibrium equations with the right hand side being zero.

One may consider the variational form of the equilibrium equations with the virtual displacements \( \delta u \) rather than velocities, so that:

\[ \int_B \left( \frac{\partial \sigma_{ij}}{\partial x_j} + \rho b_i \right) \delta u_i \, d\Omega = 0 \]  

Now, applying the divergence theorem to Eq.(19) one obtains the virtual strain energy:

\[ \int_{V.W.\text{body~forces}} \delta u_i \rho b_i \, d\Omega + \int_{V.W.\text{surface~tractions}} \delta u_i \sigma_{ij} n_j \, d\Gamma - \int_{V.W.\text{strain~energy}} \delta \epsilon_{ij} \sigma_{ij} \, d\Omega = 0 \]  

where \( \delta \epsilon_{ij} \) is the strain increment tensor and is defined as:

\[ \delta \epsilon_{ij} = \frac{1}{2} (\frac{\partial \delta u_i}{\partial x_j} + \frac{\partial \delta u_j}{\partial x_i}) \]  

The finite element formulation presented herein is based on these fundamental non-linear continuum equations (equilibrium and constitutive) which are ultimately expressed in a linearized form for numerical implementation at each incremental step. Hence, producing a system of equations which are solved repeatedly using a Newton-Raphson type procedure. A detailed description of the finite element formulation used is given in references [1, 8, 16]. However, a statement of the variational form of the incremental equations is given for completeness:

\[ \int_{\partial B} c_{ijkl} \Delta u_k \delta \epsilon_{ij} \, d\Omega + \int_{\partial B} \sigma_{ij} \delta \eta_{ij} \, d\Omega = \int_{\partial B} T_i \delta \Delta u_i \, d\Gamma + \int_{\partial B} \rho b_i \delta \Delta u_i \, d\Omega - \int_{\partial B} \sigma_{ij} \delta \Delta \epsilon_{ij} \, d\Omega \]  

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where $\Delta u_i$ is the incremental displacements, $\Delta \varepsilon_{ij} = \Delta u_i + \Delta u_j$ is the linear and quadratic terms of the strain tensor corresponding to $\Delta u_i$, and $c_{ijkl}$ is a fourth order material response tensor in the current configuration, $B'$. This approach the non-linear system of equations are solved iteratively as a set of linear equations in each iteration. Thus a discrete load history of the responses is obtained as the load is increased in small steps.

2.6 Stress and Strain Rates

In order to ensure that certain physical quantities are independent of the kinematics, relationships in terms of stress and strain rates are used. That is, the quantities are unaffected by an observer transform $x^* = c(t) + Q(t)x$ where $Q$ is a proper orthogonal tensor and $c$ is a translation. The term objective [18] is used to describe a tensor which is independent of the kinematics: (ie. for a second order tensor $F_{ij} = Q_{ij}Q_{jm}F_{bm}$) One such frame invariant rate used for the constitutive law in the reference configuration is the second Piola Kirchoff stress and Green's strain rate tensors. In rate form:

$$\dot{\sigma}_{\alpha\beta} = C_{\alpha\beta\gamma\delta} \dot{E}_{\gamma\delta}$$

(23)

where $C_{\alpha\beta\gamma\delta}$ is a fourth order material response tensor in the reference configuration and $\dot{}$ denotes time differentiation. The rate form of $S$ given in Eq. (11) becomes:

$$\dot{S}_{\alpha\beta} = \frac{\partial W(E)}{\partial E_{\alpha\beta}} \dot{E}_{\alpha\beta} - p \dot{B}_{\alpha\beta} - \dot{p} B_{\alpha\beta}$$

(24)

After some manipulation the above result may be expressed alternatively as:

$$\dot{S}_{\alpha\beta} = \left[ \frac{\partial W(E)}{\partial E_{\alpha\beta}} + p(B_{\alpha\gamma}B_{\beta\gamma} + B_{\alpha\delta}B_{\beta\delta}) + \lambda B_{\alpha\delta}B_{\beta\delta} \right] \dot{E}_{\alpha\beta}$$

(25)

where the term inside the square brackets is equivalent to $C_{\alpha\beta\gamma\delta}$ in Eq. (23). In addition, the incompressibility constraint $\text{div} \ u = 0$ must hold from mass conservation.

However, for the Updated Lagrangian form of Eqs. (23–25) an alternative rate form is required. One such objective Eulerian rate is the Truesdell stress rate. Where the Truesdell–Rate Equation of the Cauchy–Eulerian stress is given by:

$$\sigma^{T}_{ij} = c_{ijkl} \dot{\varepsilon}_{kl}$$

(26)

In addition, $\sigma^{T}_{ij}$ can be related to the rate of change of the Lagrangian stress tensor by:

$$\sigma^{T}_{ij} = J^{-1} F_{\alpha\beta} \dot{S}_{\alpha\beta} F_{ij}$$

(27)

Now, taking the time derivative of Eq. (13) and substituting into Eq. (27) for $\dot{S}_{\alpha\beta}$ yields:

$$\dot{\sigma}_{ij}^{T} = \dot{\sigma}_{ij} - \Gamma_{i} \dot{\sigma}_{kj} - \sigma_{ik} \Gamma_{jk} + \sigma_{ij} \dot{\varepsilon}_{kk}$$

(28)

where the quantity $\Gamma_{ij}$ is the velocity gradient $\Gamma_{ij} = \partial u_i / \partial x_j$. In addition, the velocity gradient can be decomposed into a deformation-rate tensor, $\dot{\varepsilon}_{ij} = \frac{1}{2}(\Gamma_{ij} + \Gamma_{ji})$ and spin-rate tensor, $\omega_{ij} = \frac{1}{2}(\Gamma_{ij} - \Gamma_{ji})$. Further, for an incompressible material the trace of $\dot{\varepsilon}$, which is a measure of the dilatational part of the strain-rate, is zero.

Using Eq. (9) and Eqs. (25–27) the instantaneous material response tensor in the current configuration becomes:

$$c_{ijkl} = J^{-1}[F_{i\alpha} F_{j\beta} F_{k\gamma} F_{l\delta} \frac{\partial W(E)}{\partial E_{\alpha\beta}} + p(\delta_{ki}\delta_{lj} + \delta_{kl}\delta_{ij}) + \lambda \delta_{ij}\delta_{kl}]$$

(29)

Now, applying the strain energy function for soft biological tissue, as given in Eq. (10) with $J = 1$, yields:

$$c_{ijkl} = 4ab^2 e^{2bE_{\text{mm}} F_{i\alpha} F_{j\beta} F_{k\gamma} F_{l\delta} + p(\delta_{ki}\delta_{lj} + \delta_{kl}\delta_{ij}) + \lambda \delta_{ij}\delta_{kl}}$$

(30)

where $p_2 = 2ab$ with pressure-rate $\dot{p} = -\lambda$. In the stress point algorithm implementation of the finite element formulation, the pressure is increased via a time-discretization of the pressure rate equation $p$:

$$p_{n+1} = p_n + \lambda \int_{t_n}^{t_{n+1}} \Gamma_{ii} \frac{d \Omega}{\Omega}$$

(31)

this is performed over each element.

3 Geometric Modeling Considerations

An accurate description of the human heart requires the use of elements capable of conforming to the irregular geometry. For this particular problem, such elements are best developed based on a 3D continuum formulation. Further, these elements must be able to handle arbitrary large displacements and rotations while maintaining kinematically admissible displacement fields at element interfaces. Two such element types satisfying these conditions have been selected for use, making them ideal for problems involving geometric and material non-linearities (see Figure 2). The first is a 20-node isoparametric solid stress element [1], which is used to model the left and right ventricles. The second is an 8-node degenerated 3D continuum based shell element [2], which is used to model the pericardial sac, a conical fibrous membrane surrounding the heart.

4 Myocardium Test Case

In order to verify any finite element code developed, numerous benchmark tests are required. One such test used in the verification of the algorithm involves the comparison of experimentally obtained equiaxial myocardial data with theoretical and finite element predictions. The biaxial

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Figure 2: Continuum based isoparametric (a) 20-node stress solid and (b) degenerated 8-node shell elements used in (c) modeling the human heart.
data was obtained from the work of Yin et al. (1987) [21] where a thin slice (1–2 mm) of myocardium (4x4 cm) was subjected to an equibiaxial tension. The constants a and b for the strain energy function were chosen from the Best fit parameters for stress-strain data given by Humphrey et al. (1987) [10], (where $E_{11}/E_{22} \approx 1$, specimen II) and are $a = 1.951 \text{ g/cm}^2$ and $b = 14.39$. The FE mesh consisted of (2x2) 20-noded isoparametric elements. A comparison of the FE predicted stresses and experimental Cauchy stress data is given in Figure 3. For this particular test case, values for a and b selected, best predict the behaviour of the cross-fiber experimental data. These results are in good agreement, which shows some promise. However, it should be noted that anisotropic and viscoelastic behaviour can play an important role in the interaction of stress and deformation and can significantly effect the constants a and b.

Figure 3: Equibiaxial myocardial data comparison with theoretical and computed FE predicted responses [Results obtained using best fit parameters $a = 1.951 \text{ g/cm}^2$ and $b = 14.39$. Experimental data taken from Yin et al. (1987)]

5 Conclusions

This paper gives the theoretical foundation on which a computational model for the determination of the stress/strain state of the human ventricle myocardium during sustained $+G_z$ acceleration is based. The model utilizes the finite element technique where the effects of finite displacements, large strains, non-linear nearly incompressible material behaviour, and the irregular shape of the heart are accounted for. Emphasis has been placed on the incorporation of a realistic constitutive relationship into the finite element formulation. Preliminary results indicate that reasonable gross fiber-deformation responses can be computed with a prudent choice of constants in the strain energy function employed, even though, cardiac tissue may exhibit complex anisotropic deformation dependencies.

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The Role of Subclinical Cardiovascular Diseases in High-G Flying: A Mathematical Modeling Approach

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INTRODUCTION

Early mathematical models of the cardiovascular system (CVS) has emphasized the simulation of normal physiology. It is only later that models have been used to study abnormal physiology involving, for example, G-stress or, to a lesser extent, cardiovascular diseases. One of the newer challenges in medical physiology is to understand the role of combined stress. Of particular interest to us is the combined stress of subclinical cardiovascular diseases and acceleration. In this paper, we will report on our preliminary attempts to use mathematical models to help understand some of these issues. The particular cardiovascular disease (disorder) we will address is mitral valve prolapse (MVP).

BACKGROUND

The pumping function of the heart is mainly regulated by the mitral apparatus consisting of the mitral leaflets, the annulus, the chordae tendineae, the papillary muscles (PM), and the heart wall. The proper functioning of these components together are essential for the heart to deliver the right volume of blood at the right pressure. The exact mechanism governing the closure of mitral valve (MV) is however not clear and is controversial. It is generally believed that the MV closure is due to two concurrent actions: (1) the downward movement of the ventricular wall, due to blood rushing into the LV under the small diastolic pressure gradient, inducing a similar movement of the PM, and (2) the contraction of the PM itself due to a well-timed action potential excitation. The combined action pulls down on the chordae tendineae which in turn draw the valve cups toward apposition. Other factors however are also believed to be involved in MV closure. These include annular contraction, decreased filling, and eddies formation behind the leaflets. Because some of these factors could feasibly be modified by acceleration, thereby leading to improper closure of MV and resulting in mitral regurgitation (MR), it is important to re-examine the mechanisms involved in MV closure, especially in conjunction with acceleration (and mitral valve prolapse to be discussed next).

Mitral valve prolapse is a disorder in which the mitral valve protrudes into the left atrium during systole. Although it is mostly asymptomatic, MVP is frequently diagnosed. In fact, four to five percent of the population has it and is mostly congenital. While the natural history of MVP is undefined, its prognosis is generally good. MVP may not be accompanied by mitral regurgitation, but because valvular connective tissue can change without inflammation, nonrheumatic MR is not uncommon. Infrequent complications associated with MVP are: sudden death, cerebral ischemia, rupture of chordae, arrhythmias, emboli, bacterial endocarditis, mitral annular calcification, and progressive mitral regurgitation. It is believed to be the most common cause of stroke under age 40. Embolization resulting from pieces of thrombotic material formed in association with coagulation around the abnormal mitral valve. It is also believed that MVP with MR is functionally insignificant because of compensatory mechanisms, but these together with coronary artery diseases may lead to left ventricular dysfunction. There are documented cases of asymptomatic people with MVP undergoing spontaneous idiopathic chordae rupture (SRC) leading to sudden severe hemodynamic deterioration. It is natural to conjecture that certain MVP patients are more vulnerable to SRC when subjected to abnormal strain. This is not uncommon in runners. In patients with MVP, the possible increase in vulnerability to SRC or simply to mitral valve closure dysfunction (even without SRC) due to possible adverse effects of acceleration on factors governing mitral valve closure, leads one to conjecture that certain MVP patients may have, in certain cases, a lower G-tolerance. It has been reported [6] that rapid onset rate may affect MVP patients more in terms of G-tolerance.

We would like to answer the following two closely related questions: (1) Assuming for the moment that acceleration indeed induces MR in MVP patients who otherwise do not have MR, what are the effects of MR on G-tolerance as measured by opthalmic pressure? and (2) Are MVP patients (again assuming without MR) more susceptible to developing MR under acceleration than non-MVP people? This paper shows how we use mathematical models to address these questions. While we will not be able to answer them to our complete satisfaction using mathematical models, they (the models) do provide us with valuable insights.

SOME RELEVANT MODELS

We need basically two types of models. To address the G-tolerance problem (Question 1), we need an overall model that can relate cardiac performance to opthalmic pressure under acceleration. To address the MVP problem (Question 2), we need a detail model of the left heart so that we can analyze fluid flow through the mitral valve under acceleration. While the use of mathematical models to study cardiovascular diseases is not new and in fact quite active, for example, numerical studies on flow past stenoses or action potential propagation modeling to understand the genesis of ECG or cardiac arrhythmia, the use of mathematical models to study the effects of the combined stress of cardiovascular diseases and high acceleration on physiological systems performance is practically non-existence. In the following, we will review some relevant models and describe how we have extended them to study our combined stress problems.

Realistic mathematical studies of MR are few. MR was studied by Pater-Berg [4] using an analog model wherein he modeled MR quite naturally by setting up a backward path from left ventricle (LV) to left atrium (LA) during systole.
G-Models are models that simulate the overall CVS and at the same time take into account the effects of acceleration. There have been quite a few G-models constructed over the years, but the early ones understandably tended to be too simplistic (too few compartments, for example). There are three later G-models each with over 20 compartments that are typical of the current crop of G-models. They are the Hardy-Collins model, a modified Croston model, and the Jaron model. The Hardy-Collins model [2] was developed in the late 70's and early 80's. It is one of the earliest G-models that consider gas transport and exchange. One difficulty with this model, at least in its original form, is its inability (for numerical stability reasons) to simulate even moderately high G. The modified Croston model was an extension of [1] by Krug International for NASA to study Lower Body Negative Pressure. Unlike Hardy-Collins model, it can also simulate high-G in the centrifuge. However, it does not consider gas transport and exchange. The model developed by Jaron and his coworkers [3] for the Navy and the Air Force is one of the few models that consider anti-G measures: L1/M1 straining maneuvers, anti-G suits, and, in the latest version, positive pressure breathing. Extension of this model to include gas transport and exchange is currently underway.

An MR-G model is a model that considers the combined stress of mitral regurgitation and acceleration. It is an easy matter to take any G-model and extend it to include MR in a way similar to what Pater-Berg have done. We have done so using the modified Croston's model. This model choice was made because it was the most reliable G-model available to us at the time of this study. We will only briefly discuss this model and refer interested readers to the original paper [Croston, 1970] for details of the basic model. As all CVS modelers know, the most critical features in the CVS to model are the heart pump and various regulatory mechanisms. In the modified Croston model, Sugawa's time dependent elastance model for the heart is used. The model's regulatory mechanisms involve several controlling and controlled variables. The controlled variables are the heart period, the heart contractility (amplitude of elastance), selected lower body resistances, pulmonary resistances, selected lower body compliances, and a reference pressure. The controlling variables are moving averages of mean lower carotid pressure, of mean ascending aortic pressure, and of mean cardiac output. The relationships between controlling and controlled variables are typically piecewise linear.

In our MR-G model we assume the degree of MR is a linear function of acceleration above 1G. We envision the case in which a person has MVP with no MR under normal (1G) condition, but develops MR as acceleration stress starts. This is a "what if" worst-case-scenario study in which a mathematical modeling excels. Assuming the resistance to flow through the mitral valve is inversely proportional to the square of its effective cross-sectional area, and defining the percent MR, denoted by $P_{cMR}$, as the ratio of the effective cross-sectional area of the mitral valve for the back flow (regurgitation) to that for the forward (normal) flow, the relation between the resistance of mitral back flow ($RB$) and that of the forward flow ($RF$) is given by $RB = RF / P_{cMR}^2$. Thus, if the percent MR is 10%, then the resistance to back flow is one hundred times that of the forward flow. The percent MR is assumed to be G-dependent and is given by $P_{cMR}(g) = P_{cMR}G * (g - 1)$, where $P_{cMR}G$ is a parameter measuring the percent MR per G above 1G, and g is the existing acceleration stress.

There are many models of the left ventricle. To study the dynamics and performance of the MV, we need a fluid mechanical model of the left heart. The only model of this type that we are aware of is the MV model originally developed by Peskin [5]. The main assumptions of his model are that the heart is submerged in a pool of blood, that it is neutrally buoyant, and the heart wall is made up of hundreds of muscle stripes each obeying Hill's dynamics and being excited at appropriate time in the heart cycle. For simplicity, the left heart is modeled in 2-dimensional plane and the time period of simulation is restricted only to diastole. The hemodynamics inside the heart including the opening and closing of the MV can be determined by solving the Navier-Stokes equations (which for this problem has a singular forcing term in the momentum equations and a non-zero source in the continuity equation) coupled with a large system of ordinary differential equations for dynamics of the heart walls, leaflets, and the muscle stripes. We refer interested readers to the original paper [5] for further details.

There is no model of the heart that we know of that studies the direct effects of G on cardiac flow or ventricular functions. May be this is because the hydrostatic column involved is relatively short and so the effects are negligible. For small to moderate acceleration, this may be true. For higher G, say above 9G, this may no longer be the case. In most models the G-effects on the heart are taken into account by modifying a stiffness constant associated with the heart or by altering the heart-rate based on G-induced mean pressure changes sensed by baroreceptors or chemoreceptors.

To understand the direct influence of G on blood flow in the left heart, we extended Peskin MV model to a MV-G model which includes the effects of G. This involved modeling the great vessels attached to the heart. (Otherwise, the heart will float away under the influence of acceleration.) This in turn is accomplished by setting up in the fluid field imaginary posts to which the heart is tied, using springs. By varying the spring constants, we can control how much we allow the heart to shift in the fluid.

**NUMERICAL EXPERIMENTS**

We report here some of the numerical experiments we have done. Experiments EXP-GOR and EXP-ROR below were done using model MR-G and experiments EXP-VORTEX and EXP-FLOW were done using model MV-G.

**EXP-GOR.** Here we study the effects of MR when acceleration goes from 1G to 4.5G in 15 sec, stays at this (highest) level for 15 sec, and returns to 1G in another 15 sec. This corresponds to an onset rate of .233 G/sec. We studied MR for six different PctMRs: from 0% to 50% at 10% increments. These are the PctMR values achieved at the plateau (4.5G) of the G-profile.

**EXP-ROR.** This study is identical to EXP-GOR except for a higher on-set rate of .933G/sec. This amounts to changing the G-profile so that acceleration goes from 1G to 4.5G in 3.75 sec instead of 15 sec.

**EXP-VORTEX.** Here we investigate the effects of three different accelerations (approximately -1.7, 0.0, and 3.0 Gs) on vortex formation and apex-to-annulus distance, as these are some of the factors that may affect proper mitral valve closure. Incidentally and unfortunately, we have to limit
ourselves to these relatively low levels of acceleration because numerical instabilities tended to creep into our current numerical algorithm in our model whenever the magnitude of acceleration became large.

**EXP-FLOW.** Before we even address the question regarding conditions under which MVP without MR can lead to MR under G, it is important that we first assess the effects of acceleration on mitral flow (cc/sec) in general. We have done so using the three different accelerations in the previous experiment.

### RESULTS AND DISCUSSION

**EXP-GOR.** The results of this study is shown in Fig. 1. As expected the G-tolerance as measured by mean carotid pressure (PMC) decreases as the severity of MR as measured by PctMR increases. Moreover, the relationship between minimum PMC and PctMR is almost linear (Fig. 3) for PctMR from 10% to 80% with a slope of -0.714 (mmHg/PctMR). The rate of change of minimum PMC from 0% PctMR to 10% PctMR is on the average -0.26 (mmHg/PctMR). This data indicates that minimum PMC is relatively insensitive to small degrees (below 10%) of MR. While more calibrations of the model and/or refinement of the model will be made, we believe this general relationship will still hold.

![Fig. 1: MEAN CAROTID PRESSURES (OSR=0.233)](image1)

**EXP-ROR.** Because of the more severe onset rate (.93 G/sec), the mean carotid pressure, as expected, has dropped much lower than that in the milder onset rate (.23 G/sec) case (Fig. 2). However, except for a uniform downward shift, the relationship between PMC and PctMR still holds (Fig 3).

![Fig. 2: MEAN CAROTID PRESSURES (OSR=0.923)](image2)

**EXP-VORTEX.** Figures 4 and 5 show the streamlines of blood flow in (and around) the left heart approximately 350 ms into diastole for acceleration levels of -1.7 and +3.0 Gs respectively. While these streamlines plots are clearly different, vortices in the neighborhood of the leaflets are still visible and are qualitatively similar for the two case. The vortices here are not as distinctive as those shown in Pe Skin’s original (0 Gs) model. This may be due to the difference in the heart geometry employed and the introduction of imaginary posts into the MV-G model to simulate great vessels. Nevertheless, the MV closure mechanisms as measured by vortices formation and apex-to-annulus distance do not seem to have been affected significantly by acceleration stress, at least not at the levels considered. Additional research on numerical algorithms as well as improved modeling of the mitral valve (to simulate the complete cardiac cycle) will be needed to allow us to address more severe acceleration stress properly.

![Fig. 4: STREAMLINES and LEFT HEART: -1.7Gs, 350 ms](image3)
CONCLUSIONS

This study is necessarily exploratory, as models are still and will continue to be evolving. However, as models become more and more refined, they will be more able to provide better hypotheses and deeper insights.

The MR-G model has demonstrated that it can acquire qualitative and quantitative information about the combined effects of cardiovascular diseases (MR) and G-stress on aircrews hemodynamics. The qualitative results regarding the effects of MR on PMC is not unexpected. The quantitative results seem to imply that a small degree of MR (up to 10%) is hemodynamically not significant.

The study using the MV-G model has suggested that mitral flow may be modified by moderate increases in acceleration. An increase in mitral back flow during early-systole induces an additional strain on the mitral valve. This may have important implications to MVP patients, although more studies will be needed. On the other hand, the mitral valve closure mechanisms studied, vortices formation and apex-to-annulus distance, did not show significant changes in the moderate range of accelerations considered. Research on numerical algorithms and further development of model will be needed to study these and other mechanisms under more severe acceleration stress.

References


THE LUNG AT HIGH G; MAJOR FACTORS IN MODELLING

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Many models of the lungs and lung function exist at present. Unfortunately, none of them is suitable for the description of the gas exchange efficiency of the lungs under conditions of increased gravitational acceleration (high G). Because models in general are valid only for the conditions for which they were developed, and because the extant lung models were constructed using 1 G data, the effects of G cannot be separated from other influences. Because models tend to be applicable only for a specific purpose and because the purpose of the models currently available is either the detailed exploration of one aspect of lung function or an overview for teaching, the needs of those investigating high G have not been met. Furthermore, the computing power needed to combine multiple detailed models for a comprehensive picture that is not overly dependent on untested approximations is only now becoming available.

AIMS OF MODEL

The lung model we are proposing would allow the user to select the conditions of G load, the medium surrounding the body, the mouth pressure and the pattern of muscular effort. The model results would be the expected arterial blood gases for a given ventilation. The vertical distribution of ventilation, perfusion, gas and blood volumes and alveolar gas tensions would also be available from the model. Vascular motor tone in response to local alveolar gases would be an integral part of the model. The input values of cardiac output, blood pressures and mixed venous blood gas partial pressures would be obtained from empirical measurements as a function of the conditions.

ORGANIZATION OF MODEL

The lungs are particularly susceptible to the effects of G because they contain two fluids, air and blood, of two very different densities and, unlike all other organs, are not surrounded by a fluid of similar density but must support their own weight. The expansion of a region thus depends on the gravitational forces acting on the region, and all properties that are functions of volume vary with vertical position in the lung. No lumped model can be applied to the lungs overall. Therefore, a piecewise model of n horizontal slices is proposed in which each slice has uniform properties. Ventilation, perfusion and the resultant gas exchange is to be considered in each segment, and the end-capillary blood gases, ventilation and perfusion of all slices is to be combined to obtain the overall lung function.

Lung properties are not uniform throughout a horizontal slice. However, the average for a horizontal level is the initial goal of this model. Both computational constraints and the availability of data limit our ability to define the variables in more than one axis.

REGIONAL VENTILATORY MECHANICS

Ventilatory mechanics are affected by G, and regional mechanical properties by vertical position. The chest wall pressure - volume relation is a function of G because the chest wall affects its recoil pressure. The lung pressure - volume relation is a function of both G and vertical position because the weight of the lung region affects its recoil pressure and the effective lung tissue density changes with the amount of blood contained within the tissue. The blood content of a region is a function of both G and position. The pressure - flow - volume relation for a lung region is a function of vertical distance because of the effect of path length from the mouth to the region and because the regional volume and alveolar pressure, themselves functions of the pressure - volume relation, affect the airway diameter and the regional flow.

The pressure - volume relation of the lungs for a set of conditions of G, volume, surrounding pressure and blood distribution may be obtained from a detailed force balance model as has been done at 1 G [2,4,5,7,10,11]. The balance of effective density, surface
tension forces, tissue traction and transpulmonary pressure can be applied to small elements of lung tissue, with the weight of the heart and mediastinum included on the surface of the appropriate elements. However, because the pressure - volume characteristics must be calculated for all lung gas and blood volume distributions that occur during modelling, because these change between iterations, and because only the average over a horizontal slice would then be employed, the full finite element model is impractical at present. An analytical model like that of de Wilde et al. [4] is a more workable solution, even though the effects of the weight of the heart and mediastinum cannot be included in full. The appropriate human tissue characteristics must be known to obtain a reasonably detailed picture of the pressure - volume relation for each set of conditions and G. The pressure - flow - volume relation is well characterized, but appropriate values of path length and diameter must be used.

REGIONAL PERFUSION

Regional perfusion of the lung is affected by vertical position and G. The hydrostatic column from the heart is an important influence on capillary inlet and outlet pressures. The regional blood volume depends on the distension of the capillaries which is a function of the transcapillary pressure and the wall stiffness. Mean regional transit time depends on the distension of the capillaries, on the number of recruited capillaries, on the capillary impedance to blood flow, and on the cardiac output. Vascular wall stiffness and impedance are functions of regional lung gas volume and of smooth muscle tone. Local smooth muscle tone is affected by regional partial pressures of oxygen and carbon dioxide, that is, by the regional gas exchange. However, once all of the appropriate values are obtained, number of models exist for the calculation of the blood volume and flow [3,6].

REGIONAL GAS EXCHANGE

Regional gas exchange depends on the regional ventilation to perfusion ratio, both factors of which are affected by G and position, as has been discussed. The mean contact time and possible pendelluft caused by unequal regional ventilatory time constants also must be considered.

SOLUTION PROCEDURE

Because the interplay among the different components of blood volume, perfusion, gas volume and ventilation is highly interactive, a solution procedure that is iterative in several loops is necessary. The solution proceeds from the bottom to the top of the lung.

FRC conditions -- First slice.

A first guess of the pressure - volume relation in the most dependent slice at functional residual capacity (FRC), where alveolar pressure equals mouth pressure, can be made initially, from which the perfusion and blood volume distribution can be calculated using an initial guess of vascular muscle tone. This distribution can be fed back into the pressure - volume relation to correct it, and the updated blood distribution can be computed. This iteration for starting values must be repeated until it converges to an internally consistent initial approximation.

Breathing -- First slice.

Once the approximate conditions at FRC are established, the simulation of breathing can be begun. The muscle pressure for the first time step is applied to the most dependent slice. Regional flow, volume, and alveolar pressure are calculated, and regional perfusion and blood volume adjusted as necessary. The entire breathing cycle is constructed for one horizontal slice, blood transit time is computed and gas exchange in that slice is calculated. The vascular tone is adjusted based on the resultant alveolar gas composition [9], the perfusion and blood volume are adjusted, and the calculations are repeated until the values for the unit become stable.

Other slices.

The subsequent vertical sections can use the vascular tone from the slices immediately below as their initial estimates. The regional FRCs are calculated based on the new estimates of blood volume. The uppermost perfused region is the one in which the regional perfusion, when added to the sum of the perfusion in regions below it, brings the total to the cardiac output.

Overall results.

When all horizontal slices have been calculated, the overall
results can be computed. Arterial blood gases are the perfusion-weighted average of the blood gases from each slice, while alveolar gases are the ventilation-weighted average of the alveolar compositions. Total ventilation is the total of all the regional ventilations. Total perfusion is fixed to equal the cardiac output.

MATCHING TO DATA
All model results must be based on and compared with experimental results. We have human data obtained at 0, 1, 2 and 3 G giving ventilation, end-tidal gases, ventilatory responses, cardiac output, mixed venous gas partial pressures and FRC at rest and during exercise [8]. At 1, 2 and 3 G we also have these ventilatory and cardiovascular variables during water immersion [1]. These data will give us a set of values of cardiac output and mixed venous gases for different G loads and allow us to check the resultant alveolar gases for the appropriate ventilation. If the total ventilation is less than the ventilation measured empirically under the given conditions of G, the amplitude of the muscle pressure swings must be augmented. If the ventilation is in line with the experimental values but the arterial gases would have been expected to have stimulated ventilation, the possibility that greater ventilatory effort was not possible could be considered.

Not all data that are needed for the construction of the model are available. Our laboratory is currently preparing to collect data on regional pulmonary blood volumes at elevated G. Human perfusion parameters and vasoconstrictor response data must be obtained, as must more detailed information about the chest wall and the regional patterns of application of muscular forces during breathing. However, we possess sufficient data for a "good guess" for development of the model framework. More accurate values will be available in time, from our laboratory and from those of other investigators. This information should improve the model and increase our understanding of the function of the lungs at high G.

REFERENCES
Mathematical Modeling of the Cardiovascular System To Study Acceleration Stress

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INTRODUCTION: Flight maneuvers in new high performance aircraft can generate acceleration forces which exceed the tolerance limits of the pilot. High acceleration in the +z direction results in forces applied to the systemic circulation which diminish blood flow to the cerebral circulation and cause blood pooling in the lower extremities. The eyes and the brain become ischemic, leading to loss of vision and consciousness. In addition, it is believed that Gz stress hampers lung function and reduces coronary flow.

Presently there are at least four techniques known to enhance Gz tolerance: straining maneuvers, use of an anti-G suit, changing the subject's orientation (seat-back angle), and positive pressure breathing. The first two of these methods are commonly employed in military aircraft. The latter two are still in experimental stages. None has been scientifically optimized.

One reason for our lack of knowledge of the physiologic mechanisms involved in high G stress and of the best means of G protection is the difficulty in performing experimental studies. Such experiments usually require use of the human centrifuge and are hampered by centrifuge availability, restrictions on the use of invasive instrumentation, extensive time and cost, and the wide variation of results among subjects.

A model of the cardiovascular system, capable of simulating G stress effects, can be a valuable tool in helping to direct experimental efforts along more efficient and productive lines. Insight provided by modeling can indicate which physiologic effects are most critical. Tests of G protection systems on the model can quickly suggest approaches likely to be most beneficial. Model tests combined with experimental studies can lead to optimization of these systems.

THE MODEL: We have developed a non-linear, multielement model of the cardiovascular system which can calculate blood pressures and flows at any point in the cardiovascular system. It includes the effects of forces caused by acceleration, as well as effects of several G protection modes including the four mentioned above [2, 5]. The model’s predictions compare well with experimental data and it has recently been used to design an improved G-suit inflation mode [4, 5]. A block diagram of the model is given in Figure 1. Its hydraulic component consists of right and left heart models, multielement models of the systemic arterial and venous systems, lumped models of important peripheral beds, and a multielement pulmonary model. A detailed coronary model is under development. Hydrostatic effects of G are included in each of these models. Provision is also made for the coupling of external pressures such as provided by an anti-G suit, positive pressure breathing, straining maneuvers, etc., to appropriate model elements. The control component of the model includes heart rate and venous tone controls which are dependent on carotid pressure. The simulation of gas exchange is currently under development. This will involve a model of oxygen transport in the lungs and oxygen utilization in the cerebral and coronary circulations.

The left and right heart models are the time-varying elastance type, based on the work of Suga and Sagawa [6]. In these models, the elastance of the ventricle is low during diastole, allowing filling. During systole, the elastance follows a function of time which increases ventricular pressure and causes ejection. It is important in G stress models that a closed loop circulation exists and ventricular output pressure and flow is a function of venous return.

Figure 1. Block Diagram of Cardiovascular Model

The multielement systemic arterial and venous models each contain 24 connecting segments. Each segment is modeled as shown in Figure 2. In the figure, Ls stands for the inertance of the blood in segment n, and Rsn is the resistance to flow (Qn) through that segment. Both of these values are non-linear functions of vessel radius and are recomputed for each segment at each computer time step. Initial values were obtained from the literature. Cn stands for vessel wall compliance and R2n accounts for losses due to wall motion. In most locations, Cn is a non-linear function of segment volume. This is necessary since under G, drastic changes in volume and pressure can occur, well beyond what could be considered a linear range. The pressure sources of Figure 2 are used to represent hydrostatic pressure effects of G (Pgn), and external pressure applied to the vessel wall (PEn). The latter can include effects of a G-suit, PPB, straining, etc.

Figure 2. Systemic Arterial or Venous Segment
Nine peripheral circulation beds are included in the simulation. Each is represented by a lumped model such as given in Figure 3. These connect at each end to corresponding arterial and venous segments. The pressure source, $P_{En}$ again represents external pressure applied to this peripheral bed area.

![Diagram of peripheral circulation](image)

**Figure 3. Peripheral Segment**

Two physiologic control mechanisms of importance to $G$ stress studies are incorporated in the model: heart rate control and venous tone control. Both are sensitive to changes of pressure at the carotid sinus. The heart rate control algorithm was derived from a model given by Katona [3]. Venous tone control based on carotid pressure is adapted from a model given by Green and Miller [1].

A new pulmonary model is currently under development. This model will consist of several zones, one of which is given in Figure 4. Each zone will represent a lung region at a different level (pressure sources $P_{An}$ represent the hydrostatic $G$ effect). Pulmonary arteries and the venous return supplying each zone are assumed to be at heart level. Each section is represented by a compliance element and a flow resistance. The pressure source in the capillary segment represents alveolar pressure. The other pressure sources represent intrathoracic pressure. These pressures, will be functions of muscular straining, positive pressure breathing, and other external pressure sources.

![Diagram of pulmonary circulation zones](image)

**Figure 4. One zone of the pulmonary circulation**

**MODEL RESULTS:** The model's prediction for eye-level blood pressure during a typical $G$ profile run used on the human centrifuge is given in the center panel of Figure 5. The horizontal lines at 50 and 20 mmHg represent the approximate values of peak pressure below which there is loss of peripheral vision (50 mmHg) and loss of central vision (20 mmHg). Note that the increases in heart rate and venous tone function to bring the pressure back after an initial drop, but not enough to restore even partial vision. The bottom panel of Figure 5 illustrates the effect of the same $G$ profile with the added protection of an anti-$G$ suit. The model predicts that the standard $G$-suit will provide approximately 1G of additional protection. This prediction is consistent with reports in the literature [7].

![Diagram of pulmonary pressure](image)

**Figure 5**

Model predictions of ophthalmic pressure for an unprotected subject (center panel) and a subject protected by an anti-$G$ suit (bottom panel). The $G$ profile is given in the top panel.

**CONCLUSIONS:** To be applicable to the study of the cardiovascular effects of $G$ stress, a model must incorporate several features. It must provide a closed loop circulatory path, include distributed hydrostatic pressure effects of $G$, allow for non-linearities in vessel parameters, include the important physiologic control mechanisms active during $G$ stress, and include the application of $G$ protection techniques. The model described here has these properties and has become a useful tool in the analysis of observed $G$ stress effects and in the improvement of $G$ protection devices.

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**REFERENCES:**
The mathematical models of the vestibular system developed 30–50 years ago are based on a description of the vestibular apparatus as a system with concentrated parameters [Steinhaum, Dohman, de Vries]. Actually, the originators of a given type of models approached not a mathematical modeling of the dynamics of real vestibular structures but a qualitative mathematical description of their operation neglecting them. Functioning based on the experimental observations. It should be noted that owing to scientific intuition and high skill of the investigators, the developed models proved to be quite useful and in many respects have governed the progress of the ideas of the vestibular system function. The mathematical models are based on the experimental facts concerning anatomic structure and primarily spatial structure of vestibular apparatus. Therefore, the mathematical modeling of vestibular system compartments as the systems with distributed parameters is a success in progress [2–7].

Torus full of homogeneous incompressible Newtonian liquid (endolymp) cross-section of which is covered by elastic body (cupula) was used as a structural element in the semicircular canal. According to the latest observations, the ratio of cupula thickness h, to its height a low value, therefore cupula was presented as a plate. Also, the surface tension at cupula-endolymp interface resulted from an integration of cupula substance with the semicircular canal wall conditioned by adhesive properties of cupula has been taken into consideration. Since the relation of radius of canal cross-section, r, to its radius of curvature R is a low value, it was believed that only longitudinal component of endolymp velocity vector \( \mathbf{v} \) is nonzero. Based on these approximations the equation describing the dynamics of cupula takes the form

\[
\frac{\partial^2 \zeta}{\partial t^2} = -\Delta \zeta + \frac{1}{\rho c} \left( T_v \mathbf{v} + \frac{1}{2} \mathbf{v} \cdot \nabla \mathbf{v} \right) + f, \tag{1}
\]

where \( T_v = \mathcal{D} [P(1+h)]^{-1} \), \( \rho \), \( \zeta \), \( T_v \) - surface tension coefficient, \( \rho \) - dynamic viscosity of endolymp, \( E \) - Joung's modulus, \( - \mathbf{P} \) - Poisson coefficient of cupula, \( f = \mathbf{A}_0 \mathbf{v}(t) \), \( \mathbf{A}_0 \) - component of angular acceleration vector perpendicular to canal plane, \( L \) - length of canal full of endolymp, \( \mathbf{A}_0 = \frac{\mathbf{u}}{\rho c} \), \( \rho \) - density of endolymp.

The solution of equation (1) was obtained at following starting and marginal conditions:

\[ u(t=0) = 0, \quad \frac{\partial u}{\partial t}(t=0) = 0, \quad \mathbf{P}(r = 0, t) = 0, \quad \mathbf{A}_0(r = 0, t) = 0 \]

The results of comparing the model calculations with experimental data [8,9] are as follows: 1) values of time constants of semicircular canal system were equal to \( T_v = 0.4 \), \( T_v = 3 \times 10^{-3} \) s, respectively, natural frequency \( \omega_0 = 4.3 \) Hz; 2) Young's modulus of cupula substance \( E = 10^9 \) dyn/cm\(^2\); 3) comparison with experimental data [8,9] pointed to dependence of elasticity factor of semicircular canal system on the rate of external action making it possible to assume that cupula is a viscous-elastic hard body. Viscous-elasticity of cupula widens a frequency range within which the semicircular canals display an integrating effect and function under conditions of phase delay from external stimulus in direction of low frequency region which increases the semicircular canal tolerance in various dynamic conditions.

The developed model made it possible to determine new approach on the differences in densities between cupula and endolymp on the dynamics of semicircular canal (effect of difference in densities, EDD). The analysis of equation solution (1) based on the EDD and experimental data [2] demonstrated that 1) the magnitude of a density difference in the order of \( 10^{-5} \) g/cm\(^3\) is sufficient to occur the EDD; 2) availability of the EDD makes the sensitivity of semicircular canals in response to the linear acceleration effects. A possibility of occurring the cupula equilibrium positions depending on the parameters of external exposures which makes possible explaining some known experimental facts, is shown [10]. The parametric dependence of cupula dynamics on the characteristics of dynamic effects leading in particular to an occurrence of \( n \)-fold frequencies of cupula oscillations, has been found; 3) it is demonstrated that during periodic stimulations the intracranial pressure changes causing alternations of intralabyrinthine pressure \( P \) may lead to false calculations.

As the basic model of the distributed parameters system to describe the principles of otolith organs functioning, an otolith membrane model has been developed to approximate a generalized plane tense state including an adjacent endolymp. In this case, the equation describing an otolith membrane displacements field takes the form

\[ \mathbf{u} = \mathbf{u}_0 + \mathbf{u}_1 + \mathbf{u}_2 \]

where \( \mathbf{u}_0 \) - external force vector, \( \mathbf{u}_1 \) - membrane component of angular acceleration vector, \( \mathbf{u}_2 \) - intralabyrinthine pressure \( P \) is external vector, \( \mathbf{u} = \mathbf{u}_0 + \mathbf{u}_1 + \mathbf{u}_2 \) - is displacement vector, \( \mathbf{u}_1 = \mathbf{u}_1 \mathbf{n} \) - is external acceleration vector, \( \mathbf{n} = \mathbf{r} \times \mathbf{A}_0 \). The analysis of possible marginal conditions demonstrated that for the soft otolith membranes \( (E < 10^7 \) dyn/cm\(^2\) ) they may be written as:

\[ \mathbf{u}_1 = \mathbf{u}_2 \mathbf{n} \mathbf{r} \mathbf{c} \]

where \( k \) is the elasticity coefficient of marginal fixation, \( \mathbf{r} \) is component of the normal of boundary contour of the otolith membrane.
Comparison of solution with the available observations [14] has led to the following conclusions: Young's modulus $E$ of soft otolithic membrane has an order of $10^3$ dyn/cm$^2$; 2) displacements of various portions of the otolith are different and depend on configuration and orientation with respect to external force. Because of this, there occurs a change of impulsation rate even of those cells the morphologic polarization vector of which is perpendicular to acting force. The calculated ratio of displacement projections in perpendicular and parallel directions of the force about the vector of morphologic polarization is in good agreement with experimental data [15]; 3) intralabyrinthine pressure can be responsible for an original heterogenous deformation of hairs of sensory cells at rest which is a possible cause of the experimental disorientation and impulsation rate of uniform nerve fibers which innervate different areas of receptors lauer. Pressure change occurring under various conditions may lead to alterations in initial deformation of otolithic membrane and affect an adequacy of information on external exposure.

Approaching to a description of the otolith membrane as a distributed parameters system also made it possible to evaluate natural frequencies of transverse oscillations of the otolith membrane. Semi-infinite ideal liquid in which a thin plate is submerged has been regarded as an evaluation model. The potential of velocities $\varphi(r, t)$, determined from the equation

$$\frac{\partial \varphi}{\partial t} = \frac{\partial^2 \varphi}{\partial z^2} + \frac{\partial^2 \varphi}{\partial r^2}$$

within following operating limits:

$$\Delta r / 2z (z = 0) = \alpha (r_0 - r) 3\alpha = \alpha,$$

where $u_0 = u_0 (r, t)$ is a transverse deflection of otolithic membrane.

The calculated value of a lowest proper frequency of the system under consideration has a $0.1 \text{Hz}$ order and correlates with the frequency levels responsible for probable development of motion sickness symptoms [10].

The data obtained point to the fact that the real structure of vestibular apparatus limits the range of system sensitivity within which its compartments are responsible for perceiving of specific exposure parameters. For instance, there exist limitations on the external actions at which the semicircular canal is an integrating system and the tilt of cupula edge is proportional to angular velocity. This range (let it be termed physiologic), corresponds to the possible evolution - selected, usual motions and cannot coincide with the requirements placed by the dynamics of modern transportation facilities. The physical range of exposure is wider than a physiologic one, the lower limit of these exposures is bounded by perception thresholds, and an upper limit exists - the irreversible changes in vestibular structures, therefore, under real conditions at the output of semicircular canals and otoliths, there could appear an information which is "physiologically senseless" or inconsistent with external exposures.

An approach realized in the described models provides a means for their improvement as new evidence on the structure of vestibular system and physical processes occurring inside it is accumulated. The immediate tasks of the models are the consideration of homogeneous distribution of otoconia masses and types of receptor cells over otolithic membrane. It is much more complicated thing to take into account the fact that all the processes occurring in the regions of vestibular system are of mechanoelectrical origin and take place in electroconductive media. At present, these two elements in the models are separated. Limiting the latest observations as a base, their integration is the next task in the field of modeling of vestibular apparatus as the system with distributed parameters.

References
**INCREASED INTRACRANIAL PRESSURE IN HUMANS DURING SIMULATED MICROGRAVITY**

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**INTRODUCTION**

Headaches and space motion sickness commonly reported during exposure to microgravity may be a consequence of or exacerbated by increased intracranial pressure, ICP (14,15). Headaches also occur in subjects during simulated microgravity (6° head-down tilt, HDT) and may be a result of edema caused by elevated capillary blood pressure and flow in blood vessels above the heart (1,9,13), both of which may elevate ICP. Due to the encasement of the brain and its vasculature within the cranial vault, any increase in volumes of intracranial blood or cerebrospinal fluid (CSF) may also increase ICP. Recent studies document perivasular edema, neural degeneration (4), and increased ICP (5) in rhesus monkeys exposed to 6° HDT. The invasive nature of ICP techniques has prevented basic studies involving the measurement of this variable in humans. The purpose of our study, therefore, was to examine alterations of ICP during acute 6° HDT in humans using a noninvasive tympanic membrane displacement (TMD) technique (6).

**METHODS**

*Subjects-* Informed consent was obtained from six healthy male subjects, ages 22 to 46, who were selected to participate in this study.

*Technique-* The TMD technique is based on the principle that movement of the tympanic membrane (TM), induced by the stapedial reflex, produces volume displacements in nanoliters (nl) that can be measured with a special computer-based instrument (6,12). The TM is attached to the ossicles of the middle ear. The third ossicle, the stapes, is fastened to the oval window of the inner ear via the footplate and to the stapedial muscle of the tympanum. The stapedial muscle contracts in response to loud auditory stimuli (3) and alters the geometry of the ossicles in a manner which depends on the resting position of the stapes footplate within the oval window. The resting position of the footplate in turn depends on the pressure of the perilymphatic fluid of the cochlea (10,11,12). The perilymphatic fluid pressure is essentially equal to ICP due to a direct connection created by the cochlear aqueduct (7,10,11,12). Upon contraction of the stapedial muscle, changes in ICP relate to TM inward or outward displacements depending on whether the ICP (and perilymphatic pressure) has increased or decreased, respectively (11,12).

TMD was thus measured by a volume displacement transducer probe attached to a head set. The probe was tightly sealed into the external auditory meatus. Computer-based instrumentation quantified and measured the mean TMD volume during each stapedial muscle stimulation (6).

*Protocol-* Subjects were instrumented with the TMD device in the control upright-sitting posture and underwent the following protocol: 90° upright-sitting, 0° supine, 6° HDT, 15° HDT, 6° HDT, 0° supine, and upright-sitting recovery. A 1000 Hz, 110 dB sound pressure level auditory stimulus was given 10 times every 7 seconds for a 500 milliseconds duration near the end of 10 min for each posture. A pilot study indicated that 10 min was sufficient time for the TMD to reach a stable waveform, i.e. a stable ICP.

*Analysis-* Mean TM displacements at each posture were averaged for 6 subjects and the results were analyzed statistically with repeated measures ANOVA followed by post-hoc paired t-tests. Statistical significance was set at p < 0.05.

**RESULTS**

Compared to upright-seated posture, 0° supine, 6° HDT, and 15° HDT produced TMD changes of 317 ± 112, 403 ± 114, and 474 ± 112 nl (means ± S.E.), respectively (Fig. 1). Furthermore, postural transitions from 0° supine to 6° HDT and from 6° to 15° HDT generated significant TMD changes (p < 0.05). There was no hysteresis when postural transitions to HDT were compared to reciprocal transitions toward upright seated posture.

![Tympanic membrane displacement](image-url)

**DISCUSSION**

Our results indicate that simulated microgravity (HDT) increases ICP based on the sigmoidal relationship that exists between TMD and ICP (8). For the group as a whole, our stimulus intensity corresponds to 20-25 dB above reflex threshold (10), and at this level our mean displacement in the sitting position, +194 nl, compares favorably with other investigations, 170 to 210 nl (8). TMD of +194 nl corresponds to an ICP of about 1.5 mm Hg.
In 6° HDT posture, ICP pressure of 17 mm Hg was supported by Dr. as indicated by TMD, is greater than that found in patients with clinically confirmed raised ICP of 14 mm Hg (8,11). Increasing the angle to 15° HDT generated a further increase in ICP as expected from the elevation of hydrostatic pressure in cerebral circulation and CSF.

Evaluation of ICP by the TMD technique depends upon a normal middle ear, intact stapedial muscle reflex contraction, and a patent cochlear aqueduct. The magnitude of the stapedial reflex and TMD decrease with increasing age (10). Although the technique does not provide absolute ICP measurements, mean TMD allows interpolation of ICP to already obtained normal and abnormal ICP values (8).

ICP is potentially a critical parameter for understanding physiological changes during actual and simulated microgravity. Increased ICP (i.e., increased perilymphatic pressure) may affect vestibular apparatus function, and may thereby cause or exacerbate space motion sickness (15). This condition is experienced by many astronauts during initial exposure to microgravity and adversely impacts crew performance during shuttle flights (14,15).

During HDT, increased blood pressure in the head elevates capillary pressure, which may be responsible for facial edema (9), vascular distension, and increased ICP. If ICP is sufficiently high, it may reduce brain blood flow and deprive brain tissue of oxygen and metabolites, causing decreased performance or headaches in microgravity. A detailed understanding of the relationship between cerebral hemodynamics and ICP changes in microgravity requires further studies during prolonged HDT and actual microgravity.

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CEREBRAL BLOOD FLOW VELOCITY
INCREASES WITH
ACUTE HEAD-DOWN TILT OF HUMANS

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INTRODUCTION

Leg volume decreases during simulated and actual microgravity (6,13) due to passive shifts of blood and interstitial fluid from the lower body. Microgravity, as simulated by head-down tilt (HDT), also increases capillary pressure and cutaneous blood flow in tissues of the head (2,11). In the brain, dilatation of vessels and perivascular edema were demonstrated by a morphological study in monkeys exposed to HDT for 7 and 19 days (8). These observations suggest that the cephalad shift of fluids due to microgravity may alter cerebral circulation.

Blood flow velocity in human cerebral arteries can be measured noninvasively by means of transcranial Doppler (TCD) velocimetry (1). Using this technique, Bondar and co-workers (4) recently measured blood flow velocity in the middle cerebral artery (MCA) during parabolic flights (about 25 seconds of microgravity). They observed statistically significant differences in mean and peak frequencies, systolic/diastolic ratios, and Doppler power between 2 Gz and microgravity (10-2 Gz). However, there is no current literature on cerebral blood flow velocity during microgravity as compared to that in normal physiological conditions (upright posture in +1 Gz). The purpose of the present study was to examine the effect of acute HDT on blood flow velocity in the MCA of humans. We hypothesized that HDT increases MCA blood flow velocity as compared with that velocity during upright sitting posture.

METHODS

Experiments were carried out on six healthy male subjects (height: 173 ± 3.1 cm, age: 22-47 years) who gave their informed consent. At the beginning of the experiment, each subject was asked to sit upright (90°) with feet hanging over the side of an electric tilt table. Then the subjects were placed supine (0°), tilted head-down at 6° and 15°, and returned to 6°, 0°, and then upright sitting. Tilt speed was approximately 2°/sec. Subjects were kept at each posture for 5 minutes.

Blood flow velocities in the right MCA were measured using 2 MHz pulse transcranial Doppler (Transpect, Medasonics Inc., Fremont, CA). The Doppler signal was obtained through a temporal window using a probe attached to a headband. The sampling depth (50 - 65 mm) was adjusted so that the trunk of the MCA was insonated. Mean blood flow velocities were averaged during the last 20 seconds in each posture and expressed as a percentage of the baseline control value (initial sitting position).

Statistical analyses were performed using one-way analysis of variance followed by post-hoc test. A difference in means was considered significant when p<0.05.

RESULTS

In our six volunteers, MCA blood flow velocity increased significantly to 111 ± 4% (p<0.05), 114 ± 3% (p<0.01), and 109 ± 3% (p<0.05) at 0° supine, 6° HDT and 15° HDT, respectively, compared to velocity during initial upright posture (Fig. 1). Mean flow velocity during the second period of 6° HDT (111 ± 5%) was not significantly different (p=0.077) from the baseline value because velocity decreased in one of the subjects. There was no significant difference in velocities between 6° and 15° HDT during either increasing or decreasing tilt. After returning to 90° seated posture, flow velocity (104 ± 3%) was not significantly different from the control baseline level.

![Figure 1: Blood flow velocity in right middle cerebral artery with posture. Velocities at initial sitting posture were taken as 100% for each subject. Asterisks indicate a significant difference compared to the control (sitting). * p<0.05, ** p<0.01. All values represent means ± SEM.](image_url)

DISCUSSION

Transcranial Doppler (TCD) is a useful noninvasive technique to measure blood flow velocity continuously in cerebral arteries (1). Blood flow through a vessel is equal to the product of flow velocity and cross-sectional area of the vessel. Although changes in the cross-sectional area cannot be measured by TCD, this technique provides a useful estimate of blood flow under conditions in which changes in vessel diameter are relatively small. Huber and Handa (7) reported that the dilator effects of hypercapnia, hypertonic glucose solution, and papaverine on MCA in humans (internal diameter of 1.5 - 2.5 mm) are much less than those on the smaller arteries which have diameters ranging from 0.5 to 1.0 mm. Diameter alterations of large cerebral arteries in cat during changes of blood pressure are also relatively small (5). In the present experiment, therefore, TCD signals were obtained from the trunk of MCA in order to minimize the influence of altered MCA diameter on the measurements.
Head-down tilt (HDT) is an effective experimental model to simulate microgravity (6,10). Exposure to microgravity alters many hemodynamic parameters. However, these changes vary as a function of the time after onset of microgravity. Central venous pressure increases within the first 15 minutes of HDT in both humans (9,10) and animals (12). Various investigators demonstrated that much of the cardiovascular adaptation to simulated microgravity is accomplished within the first 12 hours (3,10,11). Thus, the present study was designed to determine the initial responses of cerebral blood flow to HDT. Our results reveal that blood flow velocity in MCA increases by 14% at 6° HDT during 5 minutes and returns toward control levels after HDT. The increase in flow velocity can be explained either by increased blood flow rate through the MCA, reduction of MCA diameter, or both. Elevation of intracranial arterial pressure due to the hydrostatic effects of HDT may increase cerebral blood flow rate. On the other hand, increased local blood pressure may also induce autoregulation in the form of arterial smooth muscle contraction. As mentioned before, however, diameter alterations in the trunk of MCA due to autoregulation are probably small. Thus, the increase of blood flow velocity may indeed represent increased arterial blood flow.

In conclusion, simulated microgravity due to acute HDT increases blood flow velocity in the MCA of humans. This increase of blood flow velocity may promote capillary blood flow and transcapillary filtration within the brain. Thus, edema formation and associated increased intracranial pressure may be a possible mechanism for the headache, altered performance, and space motion sickness that are commonly observed in astronauts during microgravity.

ACKNOWLEDGEMENTS

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REFERENCES


Gas exchange and cardiovascular responses to tilting during graded exercise

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INTRODUCTION

Blood volume redistribution and the change in the hydrostatic pressure caused by sudden postural change are great stimuli for the cardiovascular and respiratory regulating systems. In our previous experiment using parabolic flight, the sudden elimination of the hydrostatic force during mild exercise resulted in a transient increase in the mean arterial pressure at carotid sinus (MAP(carotid)), and reflexly heart rate (HR) decreased with delay time of 4 seconds, unexpectedly long. Also unexpected result was that the reflex decrease and increase in HR at the high-gravity (G) → μG, and μG → high-G were asymmetrical.

For the respiratory regulating system, previous parabolic flight studies showed controversial results. Our study showed marked transient changes in the respiratory pattern, and the study by Palva et al., showed no change in the respiratory pattern during the parabolic flight. The solution of this controversy is crucial for the concept of cardio-dynamic mechanisms for the exercise hyperpnea, that is, the inspiration would be influenced by the pulmonary blood flow, or the pulmonary CO₂ flow.

In the present study, using tilting during graded exercise, we examined 1) the baroreflex sensitivity at different work intensities, 2) the baroreflex sensitivity during exercise in the relationship with fitness, and 3) transient changes in respiratory pattern depending on the G-changes during exercise.

METHODS

Subjects  Ten healthy male volunteers participated in this study. All subjects were non-athletic with normal daily physical activity, and used no medication. All experiments were performed in the morning. Equipment  A electric-braked cycle ergometer was settled on a tilt-table. The tilt maneuver from supine to 80° upright or vice versa took 2.5 sec to be completed.

Subject breathed in room air through a flowmeter and a mouthpiece with a nose-clip.

Protocol

Measurements  HR was obtained from ECG leads, using a beat-to-beat HR meter with a linear analog output. MAP(carotid) was calculated by hydrostatically compensated continuous arterial pressure measured using Finapres. Expired flow was continuously measured by calibrated turbine flowmeter connected to the mouthpiece.

Computation  From the continuous calibrated data, beat-by-beat or breath-by-breath values were computed. Individual mean responses  The computed values aligned at 50 Hz were synchronized by the tilting phase and, the data from three identical repeated tilts were averaged. The synchronizing point was middle of postural transitions. Group mean responses  Group mean responses were computed from the ten individual mean response curves by the same procedure as for the computation of individual mean responses. Open-loop analysis at the postural changes  The time course of changes in MAP(carotid) along 15 sec after the beginning of the transition was chosen as stimulus of the carotid sinus baroreflex, and the time course of changes in HR with the same duration but with time shift was regarded as a responses. The best fit linear line of MAP(carotid) and the properly time-shifted HR was determined by a least-square method, and the slope was regarded as the open-loop baroreflex gain.

RESULTS

Group mean responses of HR, MAP-(carotid), minute ventilation, tidal volume, and breath rate are shown in Fig. 2.

The delay time of HR responses to the changes in MAP(carotid) was about 4 seconds and not significantly different with different work loads at the upright→supine postural change. On the other hand, at the reverse postural change, it became slower as work load increased (Table 1).

The open-loop baroreflex gain did not significantly change with the increase in work load at the upright→supine postural change. At the reverse postural change, it became less as work load increased (Fig. 3).

The open-loop baroreflex gain at the upright→supine postural change at 50 and 100 W was significantly correlated.
with the relative work load indicated by %WCI (percent of the physical capacity at HR=170) (correlation coefficient (R) = -0.78 and -0.84, at 50 and 100 W, respectively).

Subjects with lower fitness indicated by WCI had stronger gain of the baroreflex at 50 and 100 W (R=0.67 and 0.73 at 50 and 100 W, respectively).

**DISCUSSION**

In the present study, we concluded that 1) the baroreflex at upright→supine postural change had 4 sec delay of HR as at the 1.8 G→μG transition in parabolic flight, 2) the sensitivity of the baroreflex at upright→supine postural change was stronger in lower fit subjects at 50 and 100 W, 3) the sensitivity of the baroreflex at upright→supine postural change was the strongest at moderate relative work intensities, 4) the HR response at supine→upright postural change was nearly symmetrical to the reverse change only at the lowest work intensities, and 5) G-dependent changes in respiratory pattern were re-identified in tilting during exercise.

The delay time of 4 sec seems too slow to be explained by ordinary baroreflex with vagal efferents. On the other hand, the responses of HR seems too distinct to be explained by that with sympathetic efferents. The responses might represent the higher system to regulate the vagal-sympathetic balance against postural change. Acetylcholine released from vagal endings is known to be more effective to decrease HR in an existance of sympathetic activity. This fact may explain the relationship between the baroreflex gain and relative work intensity.

**REFERENCES**

PRESENCE OF ATRIAL NATRIURETIC FACTOR IN SALIVA: COMPARISON OF PLASMA AND SALIVARY CONCENTRATIONS DURING A HEAD DOWN TILT.

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INTRODUCTION.

Many studies on the early cardiovascular and hormonal modifications induced by space flight or simulated weightlessness have been published (1,2,5,7). However, in space physiology, it is difficult to obtain blood from astronauts during flight. This also the case for athletes during exercise. Thus, we decided to measure the Atrial Natriuretic Factor (ANF) in saliva. Saliva analysis represents many advantages: stress may cause elevation of many hormones, salivary sampling avoids the pain and apprehension sometimes associated with venepuncture. Dynamic tests of endocrine function often require multiple samples; saliva sampling avoids the need for repeated venepuncture. The easy, stress-free, non-invasive collection procedure greatly facilitates the study of normal subjects or patients (1,3). Our aim was to:

1) demonstrate the presence of ANF in saliva.
2) examine the relation between concentrations in serum and saliva during the first hours of a head-down tilt (-6°) (HDT), a situation which is now well known to increase ANF (6).

MATERIALS AND METHODS.

Test design:

Five healthy male volunteers, aged 30-40 years, participated in this study. The details of the protocol were explained to each subject and their informed consent was obtained. The protocol had the approval of the National Ethics Committee. On the experimental day, a catheter was inserted into an antecubital vein 1 hour before the first blood sampling. Room temperature was maintained at 20-21°C. Blood and saliva samples were taken before (1h) and after 1h30, 4h, 9h30, 13h30, 23h30, 27h30, 29h30, 31h30 and 33h30 of HDT at -6°.

Collection of samples:

Blood was collected by venepuncture from an antecubital vein into heparinized tubes. The separated serum was frozen at -20°C until assayed.

Unstimulated whole saliva was collected with Salivate (Sarstedt Numbrecht, Germany). Briefly, the cotton was placed under the tongue, then centrifuged and frozen. The generally applied technique of saliva preparation before assay is freezing, thawing and centrifugation, which produces a clear, easily pipettable supernatant.

Freezing and thawing of saliva, however, results in precipitation of globular proteins: centrifugation leads to a loss of only protein content (10,11,13).

Sample preparation:

The saliva samples were thawed at room temperature. After thorough mixing, each sample was centrifuged for 10 min at 1500 g, which yielded a clear saliva supernatant. Molecules < 8000 Da were excluded with membrane cellulose ester (Centri/Pre), so potential blood contamination in saliva did not interfere with the analysis.

Assay Procedure:

One ml of saliva and plasma was extracted by C18 Sep-Pak Cartridges (Waters Associates, Milford, CA) and measured by a specific radioimmunoassay. To demonstrate parallelism of dilution curves generated from human saliva extracts with the assay of standard curve, dilutions of extracts (25 μl, 50 μl, 100 μl) were made in assay buffer. Recovery of ANF was studied after addition of radiolabelled (Amersham Inc.) or "cold" synthetic ANF (Boehringer Mannheim) to the saliva and buffer. Different levels of radioactivity ranging from 2000 to 50000 cpm were added to 1 or 2 ml of plasma and extracted on Sep-Pak Cartridges (4). At each step of the extraction the radioactivity was measured. For recovery of "cold" ANF, 3, 12.5, and 25 pg of synthetic ANF were added to saliva, extraction on Sep-Pak Cartridges, lyophilized and assayed. ANF immunoreactive was a gift of Dr J. Gutkowska (IRCM, Montreal). Reproducibility was established by multiple measurements of ANF concentration in the saliva extracts from the same saliva in the same assay (within-assay variation) and extractions of the same plasma on separate consecutive assays (between-assay variation).

Analysis of data:

For Figure 1 (bottom right), data are given as mean ± SEM. Statistical differences between means were analyzed by Mann-Whitney's test. Differences were considered significant at p < 0.05. Correlation coefficients were calculated with Spearman's rank test.

RESULTS

ANF was detected in the saliva with a specific RIA. Mean individual recovery of cold ANF was 72 ± 4% (n = 32), and 96 ± 2% for radiolabelled ANF. In another experiment (data not shown), saliva samples were directly subjected to the RIA, which was performed as described above, except that 100 μl of plasma were directly incubated, but no parallelism was observed.

Figure 1 shows the data found in saliva and plasma concentrations in five subjects after a head down tilt (-6°) of 33h30. An increase in saliva and plasma concentrations was noted after 1h30 of HDT. This increase persisted up to 9h30. There was a good correlation between plasma and saliva in five subjects (r = 0.4 to 0.92).

DISCUSSION

The findings clearly indicate that ANF is present in human saliva. Parallelism curves were obtained, thus confirming the validity of the assay system used.

It is the first time that ANF has been described in saliva, but steroid hormones are usually measured in saliva (Cortisol, DHEA). Recently, a RIA for substance P in saliva was described (12). Extraction is therefore an important step in saliva ANF determination. The reliable measurement of ANF by radioimmunoassay requires extraction in order to eliminate the non - specific interference of saliva proteins as well as to concentrate the saliva because of the low level of saliva ANF.
Fig. 1: Modifications of plasma and saliva ANF concentrations during a head down tilt (45°). Results are expressed as mean ± SEM for each parameter (n=5 subjects).

A good correlation between the plasma and saliva is very important. The level of ANF in saliva may be significantly affected by a marked fluid shift from the lower to the upper half of the body. The findings in plasma ANF are not surprising, as we obtained the same results in other experiments (6).

For steroids, there appear to be two possible mechanisms by which plasma components may enter the saliva (apart from active transport): intracellular diffusion or ultrafiltration (13). Probably this is also the case for ANF. The concentration in whole saliva of ANF represents 10% of the plasma concentration.

The realization and the clinical application of many hormones in saliva are now possible. This is specially interesting for ANF (2). This will require however the development of more sensitive and specific assays to detect the very low levels involved for other hormones, principally, those for which saliva concentration reflects a useful biological, medical and physiological parameter (12, 14).

This methodology will be used during the Soviet French Space Flight planned for 1992 (ANTARES PROJECT) and in exercise physiology, but further studies are necessary to determine whether a modification of flow rate can affect, for example, the ANF concentration in saliva.

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REFERENCES
BLOOD PRESSURE, BLOOD VOLUME REGULATING HORMONE AND ELECTROLYTE RESPONSES AFTER A 28-DAY CONFINEMENT PERIOD IN A HYPERBARIC CHAMBER AT 1.5 ATA.

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INTRODUCTION
Prolonged bed rest induces hormonal changes which are linked to modifications of extracellular fluid as in microgravity (4). Confinement and inactivity induce large physiological and psychological modifications (7). Thus, some of the hormonal modifications described in bed rest studies could be related to confinement, inactivity and stress. Our aim was to assess some volume regulating hormones (Renin, Aldosterone, Arginine Vasopressin, Atrial Natriuretic Factor) to see their modifications during a 28-day confinement and compare these results with others obtained during a 28-day Head Down Bed Rest (HDBR) experiment (3). Electrolytes (sodium and potassium) and creatinine were also assessed.

METHODS
This isolation and confinement experiment, called ISEMSI 90 (Isolation Study for the European Manned Space Infrastructure), was carried at the Norwegian Underwater Technological Center (NUTEC) for the European Space Agency (ESA).

Six healthy male subjects were confined in a hyperbaric chamber (4 rooms for a total volume of 85 m$^3$) for a 28-day period, at a simulated depth of 5 meters. The compression to 150 kPa (1.5 ATA) was made using pure nitrogen and the ppO2 was set at 21 kPa.

Blood pressure (systolic and diastolic) and cardiac frequency were measured in the morning after wake-up, and a 10 min. rest period in sitting position, just before blood collection. Blood samples (40 ml) were taken twice during control period (PRE 1-2) before confinement, once each week during confinement (D2 to D27) and twice after confinement during recovery (POST 1-2). Measurements of hematocrit, hemoglobin concentration, electrolytes and creatinine plasma concentrations were done immediately after blood collection inside the hyperbaric chamber. Hormonal assays in plasma were done using radioimmunoassay for Active Renin, Aldosterone, Arginine Vasopressin (AVP) and Atrial Natriuretic Factor (ANF).

Urines were collected during the whole period with a day/night separation to measure diuresis and to assess electrolyte and creatinine concentrations.

All results are expressed as mean value ± SEM. For statistical analysis, we used variance analysis followed by Fischer test. The statistical significance level was p < 0.05.

RESULTS
Regarding plasma concentrations, we can notice first that there was an initial fall of sodium and potassium at the beginning of the confinement and that these concentrations increased during confinement (Figure 1). Creatinine presented all along a marked increase (p<0.01) with a return to basal values just after outcome (Fig. 1).

In Figure 2, we can see the variations of daily diuresis. During the first 3 weeks, diuresis was lower than the total confinement mean value. Gunga (5) demonstrated also that diuresis was under a circaseptan rhythm during these 3 weeks.

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CONFINEMENT:

Figure 1: Time course variations of plasma sodium, potassium and creatinine during a 28-day confinement (results are presented as mean ± SEM)

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CONFINEMENT:

Figure 2: Time course of diuresis variations during a 28-day confinement (results are presented as mean ± SEM and white bars for Saturdays and Sundays).

Blood pressure (systolic and diastolic) is presented in Figure 3. Systolic Blood Pressure (SBP) showed a significant increase at the beginning of the confinement (p < 0.01 at D2), until the second week and decreased after that. In contrast, heart rate did not change significantly during the whole period.

Blood volume regulating hormones (Renin, Aldosterone and AVP) (Figure 4), showed a similar significant increase. They increased at the beginning of the confinement...
with the same pattern, stayed nearly constant during this period and returned to basal values after the outcome. ANF is not presented here because there were no significant variations observed during this confinement study.

DISCUSSION

The aim of this ESA study was to simulate space conditions, not for microgravity but for confinement. In this respect, the hyperbaric rooms were considered as a space station and all the problems raised by this situation were studied: communications with the "outside world", psychosocial reactions, effect of confinement and relative inactivity and physiological reactions of the subjects under these special conditions.

After the original decrease of plasma sodium and potassium concentrations, we measured an increase of sodium concentration all along the confinement period. This is the response to Renin-Angiotensin-Aldosterone System (RAAS) activation. The increase of potassium during the same period is more paradoxical in regard to aldosterone elevation and the fact that urinary excretion of potassium did not change. This could be related to a decrease of extracellular fluid volume (hematocrit was increasing at the same time) or a potassium loss from muscular cells due to a relative inactivity. The increase of plasma creatinine could be related also to the decrease of extracellular fluid volume and a dehydration state (2) but creatinine is also a metabolite of striated muscle and so it could be a consequence of inactivity.

The results of blood pressure measurements fall in line with the data concerning defense reactions (6) and psychosocial stress reactions (1) in an abnormal restricted area. So the blood pressure elevation seen at the beginning of the confinement is certainly due to the individual high stress level of the subjects but also to a direct vasoconstrictor effect of the RAAS.

Concerning blood volume regulating hormones, the significant increases of renin, aldosterone and AVP are related in part to an activation of the sympathetic nervous system. Renin and aldosterone variations are also in relation with the earlier fall of sodium level. Plasma osmolality is one of the factors linked to AVP production at the beginning of this confinement period.

One of our primary goals in this study was to compare the results obtained in such confinement conditions with others obtained during a head down bed rest of same duration (3). The first finding is that the hormones (renin, aldosterone and AVP) show the same pattern of response. So, we have to consider this "confinement effect" in the interpretation of simulated weightlessness studies.

ACKNOWLEDGEMENTS

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WATER INTAKE, URINE VOLUME, Ca INTAKE, AND URINE Ca DURING 10 DAYS BED-REST IN YOUNG WOMAN

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(Introduction)

As being at the bed-rest for short term in the young subjects, the water intake should be depressed, because the diuretic is probably accelerated, and then the total body water and body weight is decreased by the water loss. Also, Ca in urine should be increased, with increasing urinary volume. This study has been performed to investigate the relationship between water intake and urinary volume, and between Ca intake and urinary Ca during 10 days bed-rest in young woman.

(Method)

5 female students participated as the subjects in this study. Before and after the bed-rest, the body weight and the fat volume estimated by skinfold of upper arm back were measured. During the bed-rest, also, we measured the hypo-glossal temperature, arterial blood pressure and heart rate at raising point 7:00-8:00 in every morning. At raising point in the morning of the 1st, 3rd and 10th day, 10cc venous blood was sampled at their empty stomach, which have not intake any food over 10 hours. Urine excreted was stored for 24 hours from A.M. 8:00 to next A.M. 8:00. Laboratory analysis for blood were measured plasma volume estimated by hematocrit and hemoglobin, plasma Ca, plasma Na, vasopressin, angiotensin II and cortisol, and for urine were urinary volume and urinary Ca. Everyday during bed-rest, water included in food and drunk were measured in each subject. Depending on investigating food and volume, also, Ca and Na intake were decided in the subject.

(Result)

The averaged age in the subjects was 20 years old. The body height was 164cm. The body weight before 10 days bed-rest was 56kg. Fat volume 10kg and peak VO₂ 2.881/min.

These characteristics of the subjects were little higher than in the averaged levels of in Japanese same age women.

The heart rate at the 3rd, 4th, and 5th day of bed-rest were about 70bpm, which were little bit higher than as comparing with HR given at other days, despite of no significant. The systolic arterial pressure were kept about 102 mmHg for the first 4 days of bed-rest, and then became little bit lower below 100mmHg. The diastolic arterial pressure was variable in a degree for the first 4 days, and then became almost constant at about 64mmHg.

According the impression of the subjects, they had some pain on the back and felt some psychologic stress at the 3rd and 4th day of bedrest. As the bad feeling days were over gone, however, they submitted to the bed rest situation. The averaged hypo-glossal temperature was about 36.6℃ at the 1st and 2nd days, and then became little bit lower. In women the hypo-glossal temperature should be influenced by the basal temperature, but there were no subjects with menstruation during the bed rest period.

The averaged body weight was 56.3kg before bed-rest and 55.4kg after bed-rest. The decrement in the weight by bed-rest was 0.9kg and 1.5%, but no significant difference between the two body weights. The averaged lean body mass was 46.2kg before bed-rest and 45.4kg after bed-rest.

In contrast to lean body mass, fat volumes before and after bed-rest were similarly about 10kg. That is, the decrement in the body weight should be caused decrease in the lean body mass by 10 days bed-rest.

Figure 1. shows the averaged time courses of water intake, urine volume, and %Δ plasma volume in the 5 subjects during 10 days bed-rest.
The upper part of the figure shows the water intake. From the 1st to the 3rd day of bed-rest, the water intake was about 1900 cc per day, which was not different from it at the 1st day. The water intake at the 4th day significantly decreased from it at the 1st day and once increased at the 5th, and then further gradually decreased. The averaged urinary volume was about 1400 cc per a day at 1st day, and slowly significantly decreasing at the 2nd day, maintained the bottom for next 3 days. The bottom were about 700 cc per a day. The urinary volume was then altered to increase and reached again 1500 cc at the 10th day.

The down part of the figure shows the averaged time courses of %Δ plasma volume at the 3rd and 10th days against the initial value given before bed-rest. The decrement in %Δ plasma volume was -9% at the 3rd day, and then was coming back to the initial value to the 10th day. Water intake was gradually decreased as going bed-rest days, while urinary volume was reduced for the first 4 days and then altered to increase. As the result, water intake was about only 100 ml greater than urinary volume at the 10th day of bed-rest.

The vasopressin was 3 times higher and angiotensin II 30% higher at the 3rd day than the initial values. In contrast, cortisol was 16% lower than the initial level.

The Na intake for first 2 days were about 5000 mg/day. Na intake was then gradually decreased in spite of temporary increase in it at the 3rd day. This tendency was similar to the water intake.

Although plasma Na at 1st day was 142 mEq/l, it was significantly decreased to 130 mEq/l at the 3rd day. And then at the 10 days it was almost returned to the first day's value.

Figure 2 shows the averaged time courses of Ca intake, urinary Ca and blood Ca in the 5 subjects during 10 days bed-rest. The upper part of the figure shows the Ca intake. Ca intake was about 850 mg for the first 2 days of bed-rest and then significantly decreased in the range 520 mg to 750 mg except at the 8th day.

The middle part of the figure shows the urinary Ca. The urinary Ca at 1st day was 87 mg/day, which was about 10% of Ca intake. After that, in spite of respective decreasing Ca intake, the urinary Ca was gradually increased with increasing urinary volume. The down part of the figure shows the blood Ca. The plasma Ca at the 3rd day was significantly decreased from the initial value and then came to return at the 10th day.

(Conclusion)

The results presented by 10-days bed-rest study in young woman suggest that urinary volume and urinary Ca are gradually increased as going pass the 4th day, while water intake and Ca intake are decreased. The decrement of the water intake and the increment of the urinary volume should bring to the negative water balance as a result, and the increase in Ca loss might be related to the increase in the urinal volume.

(Reference)

INTRODUCTION

In the previous study, it has been demonstrated that LBNP tolerance capacity was decreased for short term bed rest such as 6 hours or 5 degree head-down tilting bed rest in young women. The results suggest that the tolerance capacity was related to Lean Body Mass and VO\textsubscript{max}. However, whether the two variables are primary factors of the capacity is not clear. To make clear the problem, in this study, cardiovascular responses to LBNP has been investigated after bed-rest, and further after 4 weeks of post bed rest with and without moderate physical training in young women.

SUBJECTS AND METHODS

Five healthy females, age 19-21 (mean 19.6) years old, body height 160-167 (mean 164.0) cm and weight 49.3-65.5 (mean 50.26) kg, volunteered to participate in this study. Details of protocol were explained to each individual and their informed consent was obtained. The subjects were divided into two groups. Subjects in Training group (n=3) performed exercise by bicycle ergometer (50 watts, 50 minutes exercise time per a day, 5 times per week of frequency) for 4 weeks after 10 day’s bed rest. Subjects in Non-training group (n=2) spent on usual life activity after 10 day’s bed rest. All subjects underwent the lower body negative pressure (LBNP) test, the maximal oxygen uptake test by bicycle ergometer just before and after 10 days bed rest, and after 4 weeks of post bed rest. LBNP was applied the following -10 and -20mmHg for one minutes, -30mmHg for 3min., -40, -50, -55, and -60mmHg for 5 min. in the supine position. The criterions for the LBNP tolerance were the same as NASA’s one except arterial pressure. The criterions of arterial pressure were below 80mmHg systolic pressure or below 20mmHg pulse pressure. The measurements were heart rate (HR), arterial blood pressure (ABP), oxygen uptake (VO\textsubscript{2}) and ultra sound echocardiography (UCG). HR was recorded continuously by ECG. ABP was measured by auscultation method in the left upper arm and UCG was calculated from ultra sound echocardiogram.

RESULTS AND CONSIDERATION

As seen in Figures 1, 2, the results are presented as changes of individuals values at each stage. All of them are shown the percent changes after 10 day’s bed rest and after 4 weeks of the post bed rest from the control level given before bed rest. Fig. 1 shows the changes in LBNP tolerance time pressure, VO\textsubscript{max} and VO\textsubscript{max}/LBM. In every subjects, tolerance time after bed rest was decreased. The averaged decrease was 45% lower than the control. After 4 weeks although the average of training group was increased 10% higher than the control, non-training group wasn’t restored until control. In the tolerance pressure of LBNP, values of four subjects were decreased after bed rest. The tolerable pressure of LBNP was -40mmHg in all subjects after 10 day’s bed rest. According to previous study (1986 at Tokyo) the tolerance of cardiovascular functions against LBNP in women are broken at an average -44mmHg LBNP, in which the LBNP was gradually enhanced by NASA procedure after 6 hrs rest in 5 degree head down tilting. In all subjects, VO\textsubscript{max} were reduced by 10 day’s bed rest.

Fig. 1 Changes (% of before bed rest) of the LBNP tolerance, VO\textsubscript{max} and VO\textsubscript{max}/LBM.

But the values was only 5% except one. In the training group, VO\textsubscript{max} increase the averaged 14% from the control. But non-training group, the values weren’t restored to the control. Maximal oxygen uptake per Lean Body Mass was almost same as maximal oxygen uptake.

Fig. 2 shows the change in LVEDd at rest and -40mmHg LBNP. The average of LVEDd decreased 9% against the control after bedrest. Even after 4 weeks of the post bed rest, both of training and non training group, LVEDd didn’t completely return to the control. The
average of LVEDd at -40mmHgLBNP reduced 19% from the control. The average of training group were almost restored to the control. But the average of non-training group still lower 12% than the control.

\[ \Delta \% \text{LVEDd} \]

\[ \text{REST} \quad -40 \text{mmHg LBNP} \]

- Training Group at post Bed Rest
- Non-Training Group

Fig.2 Changes(%) of before bed rest)of LVEDd at each stage.

Figs 3, 4. shows the relationships between percent change in measurements from each control value and percent change in LBNP tolerance time from the control. Fig. 3 shows the relationship between \( \Delta \% \text{VO}_{\text{max}} \) and \( \Delta \% \text{LBNP} \) tolerance time and \( \Delta \% \text{VO}_{\text{max}} / \text{LBM} \) and \( \Delta \% \text{LBNP} \) tolerance time. Both of the relationships were significantly correlated (\( p < 0.05 \)).

\[ \Delta \% \text{VO}_{\text{max}} \text{ vs } \Delta \% \text{LBNP} \text{ Tolerance Time} \]

\[ Y = 0.297X + 7.629 \]

\( r = 0.9525 \)

\( p < 0.05 \)

\[ \% \Delta \text{LBM vs } \% \Delta \text{LBNP} \text{ Tolerance Time} \]

\[ Y = 0.25X + 5.741 \]

\( r = 0.8565 \)

\( p < 0.05 \)

Fig.3 The relationships between \( \Delta \% \text{VO}_{\text{max}} \) and \( \Delta \% \text{Tolerance time} \), and between \( \Delta \% \text{VO}_{\text{max}} / \text{LBM} \) and \( \Delta \% \text{Tolerance time} \).

Fig 4 shows the relationship between LVEDd at -40mmHgLBNP and LBNP tolerance time. There was significantly correlated (\( p < 0.01 \)). These relationship suggest that the tolerable capacity against -40mmHg LBNP after 10 day's bed rest might be decreased with increasing in LVEDd and VO_{max}. Especially keeping of LVEDd against LBNP may be one of the most important factors.

Summary

The tolerable capacity against LBNP was decreased by bed rest and recoverd by physical training. The tolerable capacity was related to VO_{max} and LVEDd.

However, the LBNP tolerance capacity should be primarily limited by factors involving venous return in LVEDd, because VO_{max} must be controled by the factors.

Reference


Effects of moderate physical training after 10 days horizontal bed-rest on peakVO2 and cardiorespiratory functions during submaximal supine and sitting exercise in young subjects

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Introduction
In young subjects, although physiologic changes due to continuous bed-rest (BR) have been investigated, the mechanisms involved in cardio-respiratory functions before and after BR are not completely clear. Whether moderate physical training program given at post-BR responds to returning VO2\textsubscript{max} and cardio-respiratory functions during submaximal exercise to the pre-BR levels more than simple resumption of usual physical activities after BR is also no general agreement.

In the present study, young subjects were investigated peakVO2 and cardio-respiratory functions during submaximal supine and sitting exercise before and after 10 days horizontal BR and then the effects of moderate upright exercise training for 4 weeks after post-BR on the variables.

Subjects
Eight young sedentary students (19-24 yrs) who were 5 female and 3 male volunteers had been BR subjects (BRG) and two similar students were participated as control subjects (CG) with usual physical activity. BRG were further divided into two groups, exercise training group (TG) after post-BR and untraining group (UTG).

Protocols and Conditions
At 4 weeks before experiment, the subjects were selected and explained for purpose and details of the study and risks which were possibly considered BR period was for 10 days. Except going to stool with a wheel chair derived by a helper, the subjects were not allowed to make upright position for the BR period.

Fastening basal blood collection were carried out on the 1st day, 3rd day and 11th day during BR, and on the 28th of post BR.

Rest and exercise tests were performed on the previous day to starting BR, the 11th day of BR and the 28th of post-BR. Following determining physiologic values in supine rest, lower body negative pressure (LBNP) tolerance test was done by means of blood suction to the lower body.

Peak oxygen uptake (peakVO\textsubscript{2}) in upright position was determined by using a bicycle ergometer, of which the load was gradually ramped with increasing rate of 20 watts/sec per minute until all-out. Submaximal exercise tests were performed in upright sitting and supine by using a bicycle ergometer developed and named as "ergo-SSR" by us, which can any time freely and continuously change in load and posture. Exercise was 100 watts load for 10 minutes period, of which the first 5 minutes was in sitting and the latter in supine. Physiologic variables were measured for the last 1 minute during each exercise.

Physical exercise training after post-BR was programmed for TG, of which the load was 50 watts corresponded to 40-60%VO2\textsubscript{max} in individual subject, the time for 50 minutes, the frequency 5 times/week and the period 4 weeks.

Measuring items and methods
Basal blood collected at rising point in the morning was for plasma volume (PV) to estimate from hematocrit (Ht) and hemoglobin (Hb) (3).

In the tests of resting and LBPN, heart rate (HR) by standard EKG, blood pressure in the left upper-arm by auscultatory method, cardiac output (CO) and stroke volume (SV) by ultrasound cardiology (UCG), oxygen uptake (VO\textsubscript{2}) and ventilation (VE) by mean of a metabolic analyzing system, forearm blood flow (FFB) by rubber strain gauge plethysmography, and left ventricular end diastolic diameter (LVDD) by UCG were measured, respectively. In maximal and submaximal exercise tests, HR, VO\textsubscript{2}, VE and blood pressure were measured by the same methods as mentioned above, while CO was measured of acetylene-argon gas rebreathing at each stage.

Statistical Analysis
Comparing mean values used student's t-test. The levels of statistical significant difference between two mean values and significant correlation between two variables together were p < 0.05.

Results
Peak VO\textsubscript{2} in BRG was averaged 2.76 ± 0.80 l/min

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Figure 1}
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\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Figure 2}
\end{figure}

before BR, but reduced significantly to 2.53 ± 0.31 l/min (p < 0.01) after bed-rest, of which the %Δ was -10.1%. After post-BR, peakVO\textsubscript{2} was returned and rather increased 9.1% more than the control value given before BR in TG (p < 0.001), while it did not
The peak HR was a little bit higher after BR (196 bpm) than the control (194 bpm), but not significant. After post-BR there were no differences from the controls in TG and also UTG. The same tendency was observed on peak SAP.

As shown in figure 1, VEDd at the rest before BR in BR was averaged 49.5 ± 4.2 mm before BR and was reduced to 45.0 ± 3.4 mm (-9.0%, p < 0.01) after BR. At -40 mmHg LBNP, also, it was decreased from average 40.7 ± 4.6 mm before BR to 34.1 ± 6.2 mm after BR (-17.5%). After control, VEDd at rest and -40 mmHg LBNP were still slightly lower than the control levels in UTG while they were returned to in TG.

SV at the rest in BR was averaged 89.6 ± 20.2 ml before BR and significantly decreased to 50.2 ± 17.3 ml (p < 0.001). After -40 mmHg LBNP SV was reduced to 69.2 ± 13.8 ml (p < 0.05) corresponding to -22.2 ± 7.9% from it before BR, and then to 41.0 ± 17.7 ml (p < 0.01) by -40 mmHg LBNP. After post-BR SV was returned to the control level or rather more increased at rest and -40 mmHg LBNP in TG while in UTG it did not come back at rest and was significantly lower than the control (56.8 ± 4.4 ml, p < 0.05) at the LBNP.

The control PV given before BR in BR was 60.0 ± 5.4 ml/dl and then significantly decreased to 54.2 ± 9.1 ml/dl after BR (p < 0.05) corresponding to -6.4% decrement. After post-BR it was returned to the control and TG and UTG respectively. The relationship between %Δ PV and % Δ peakVO2 was significantly correlated after BR (p < 0.01), but not after post-BR, although it represented significant correlation (p < 0.01) as using all data given at the both stages in BR (Figure 2).

The control VO2 during exercise before BR in BR were averaged 1.27 ± 0.0451/min in supine and 1.30 ± 0.0631/min in sitting. The control values were kept almost the same levels after BR and post-BR in TG and UTG no matter of the exercise positions. The control VE in BR was averaged 31.6 ± 2.54 l/min in supine, and 33.5 ± 4.221/min in sitting. VE after BR was significantly increased to 35.3 ± 8.13 l/min (11.1%, p < 0.01) in supine and 36.6 ± 5.261/min (10.4%, p < 0.01) in sitting from the controls. After post-BR in the both of supine or sitting, VE became lower in TG and still maintained higher level in UTG as compared with the controls. During 100 vs exercise, the control SV in BR was averaged 72.8 ± 23.5 ml in supine and 70.7 ± 22.3 ml in sitting, but after BR it was reduced to 65.5 ± 19.5 ml (-7.7%) in supine and significantly 65.9 ± 20.0 ml (6.2%, p < 0.05) in sitting. After post-BR it was almost returned to the control in TG or UTG in supine, but still significantly lower in UTG in sitting (-5.2%, p < 0.01). The control HR was averaged 149.4 ± 26.4 bpm in supine and 151.0 ± 25.4 bpm in sitting. It was then significantly increased to the former 163.5 ± 24.4 (10.1%, p < 0.05) and the later 162.1 ± 25.6 bpm (7.7%, p < 0.01) after BR. However, the HR were returned to the controls after post-BR.

Consideration

[Effect of bed-rest]: A short period of BR such as 10 days in healthy young men should make VO2max significantly increase while the control obtained before BR (1). In the present study, similar decrease in peak VO2 after 10 days BR, 10.1%, was represented in 19 to 24 years old students. The decrease is probably due to a synergistic effect with each other of changes in physiologic functions. When the decreases in no only VO2 but also lean body mass (LBM) are considered likely to reflect the decrease in muscle mass, the primary determinant should be the decrease in circulatory blood volume and thus SV with accelerating water loss due to BR. This hypothesis should be supported by the presented results which were significantly decreased in PV and LBM (-720 g, p < 0.05) and significant relationship between %Δ PV and % Δ peak VO2 after BR, because they showed decreasing venous return probably caused by decreasing circulating blood volume and thus failing to keep CO during maximal upright exercise. Failing to maintain venous return during exercise usually results in sympathetic activation and the consequent circulatory regulation after BR should also strongly promote hastening the failure to keep CO, as considered by greater %Δ SV and %Δ LVEDd against -40 mmHg LBNP after BR than before BR. It was contradictory from the previous results (2) that VO2 during sub-maximal sitting exercise was not changed after BR, and VE was significantly increased with increasing in HR, 10.1% in supine, and 7.7% in sitting, and decreasing in SV, -7.7% in supine and -6.2% in sitting. The contradiction was resulted by that the subjects in the present study were much younger than the previous study (2). Due to young subjects HR should be sufficiently increased to compensate for maintaining CO despite even the decrease in SV, and in sitting, which makes to play to keep VO2. The increased VE during sub-maximal supine and sitting exercise following BR appeared to be independent of deconditioning effect due to BR on VO2 and CO, but respiratory efficiency represented by VO2/VE should be reduced by BR.

[Effect of training]: Programming a moderate upright bicycle exercise training to TG for 4 weeks, the facts that peak VO2 was returned to and further increased more than BR, while it in UTG was still 3.5% lower, and that after BR, PV and LBM in TG and UTG together were returned to the controls, and that the relationship between %Δ peak VO2 and %Δ PV declined to negligible after post-BR should suggest that the functional mechanism of venous return during upright exercise was restored and thus CO was returned to the control before BR by the training but not by usual physical activity for 4 weeks after post-BR. The effect of moderate exercise training on improving venous return after BR should be represented rather than keeping circulating blood volume than muscular pump, because in the present study muscular strengths and muscle mass in the lower body parts were similarly returned to the controls in UTG and TG.

After 4 weeks of post-BR, despite even remaining the same VO2 as the control values during supramaximal supine and sitting exercise, the facts that VE, HR, and SV were not returned to the controls in supine and also sitting in UTG tell us that restoring the respiratory and cardiac efficiencies deconditioned by BR could be hastened more by moderate exercise training rather than by usual life activity.

In conclusion, the present study showed in the young subjects (1) that the decrease of 10.1% in peak VO2 after 10 days BR in upright exercise should be caused to decrease in CO due to decreasing venous return against gravitational stress (2) however, returning to the initial peak VO2 after 4 weeks of post-BR should be hastened by moderate upright exercise training, whereas it was not returned by usual life activity, and (3) that VO2 and CO during sub-maximal supine and sitting exercise should not be affected by 10 days BR and also by the moderate exercise training (4) but HR and SV during the exercise should be improved the deconditioned levels and become further better than the initial values by the training.

Reference

INTERSTITIAL SPACE DYNAMICS: SIMULATED RESPONSE TO LBNP

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INTRODUCTION
There exists a basic understanding of the effects of Lower Body Negative Pressure (LBNP) on interstitial fluid dynamics. A more detailed in-depth knowledge is however still prevented by the difficulties inherent in the measurement of the relevant parameters. The use of computational models to support interpretation of experimental data can increase the scientific outcome of experiments.

A computer model was developed that reflects current knowledge on interstitial space dynamics. It is based on previous work of Guyton (1), Ikeda (3), and Wiederheilm (4). The model is part of a more global one that includes addition to capillary and interstitial space dynamics and the lymphatic system—also considers the heart, lungs, kidney, aorta, cava, and pulmonary artery and veins. The behaviour of each compartment is described by a set of properties (compliance, contractility, resistance, pressure, volume, flow and protein concentration). The model simulation runs under LISP on a Xerox 1186 machine.

BACKGROUND
Fig. 1 shows the volume changes induced by LBNP in the calf of five test subjects. The pressure profile (15 min at -20 hPa and 5 min at -40 hPa) is also depicted. During the first pressure level there is a simple linear volume increase. During the second pressure level two components that have different steepness can be recognized. Application of differential negative pressure causes primarily a venous distension through an increase in transmural pressure. The resulting sudden intravascular fluid displacement at any pressure step causes the rapid increase in total limb volume seen at the beginning of the -40 hPa step. A similar steeper increase at the beginning of the -20 hPa step cannot be found at calf level. Tissue distensibility varies along the longitudinal axis of the legs inducing a kind of a filling gradient along the lower limbs as schematically depicted in Fig. 2. Thus, the plethysmographic measurement does not reflect any abrupt change due to venous filling during the -20 hPa pressure step at the level of the calf sample volume (CSV). The steep increase becomes evident at the -40 hPa step. The volume increase recorded during the first step, and the second component of the volume increase at -40 hPa reflect interstitial fluid increase. This interstitial component of limb volume increase deserves our further attention.

In the following, results of a model simulation of the LBNP effects on the interstitial space are discussed and compared with actual plethysmographic data.

METHODS
The interstitial space is modeled as consisting of a free fluid and a gel phase. Volume (V) and Protein Concentration (C) of the interstitial space as a whole (Vt, Ct) and of its free fluid (Vf, Cf) and gel (Vg, Cg) components were computed. An LBNP test was simulated that consisted of two steps: 15 minutes at -20 hPa, and 5 minutes at -40 hPa. It was assumed that externally applied pressure was completely and instantaneously transmitted to the interstitial spaces of the exposed tissues.

Leg volume data were obtained from five test subjects by means of a plethysmographic device during actual performance of the same protocol. The changes in leg volume were compared with the simulated changes in Vt. For each test subject, and each LBNP step, a simple linear regression was fitted between the Vt values—that served as independent variable—and the experimental data. For the -40 hPa step regression, only the slower component of the volume change was considered.

RESULTS
Time course of Vt is depicted in figure 3. At the end of the -20 hPa step, Vt increased 8% and 13.3% at the end of the -40 hPa step. Fluid that remained in the interstitial spaces expanded Vg by 2.5% during the -20 hPa step and 5.7% during the -40 hPa step whereas Vg increased 122.7% at the end of the -20 hPa and 169.7% at the end of the -40 hPa step.
DISCUSSION

Comparison of simulated and measured volume data shows that the model is able to predict volume changes in the interstitial spaces of the regions exposed to LBNP and that these changes explain most of the leg volume increase caused by actual LBNP application. Since the change in $V_t$ is the sum of the changes that occurred in $V_p$ and $V_{ff}$, and the latter are fully explained by the models of capillary fluid transfer involved in the simulation, it is possible to develop a chain of inferences that provide insight into the dynamics of the changes. The used models represent the available knowledge on the dynamics of the interstitial compartment. Their response allows for a sequestration of protein in the interstitial space, a fact that contributes to explain the observed long term effects of LBNP on total body fluids (2).

The use of models and their computational simulation becomes a powerful tool that supports the investigator in recalling the available knowledge on a determined field for in-depth analysis of experimental data.

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PERIPHERAL HEMODYNAMIC ASSESSMENT during LBNP for the EVALUATION of the VASCULAR DECONDITIONING induced by a Long term HDT.

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I - INTRODUCTION: During spaceflights, both an increase in the left ventricle diastolic volume (LVDV) and in the cardiac output (CO) are observed at the beginning of the flight. These parameters recover within some hours or some days depending on the subject and tend to stay below the basal value during the rest of the flight (2,3,4,12,18,19). No deterioration of the heart contractility is noticed. After the flight, the volume of the cardiac chambers decreases, the heart rate significantly increases and many astronauts suffer from orthostatic intolerance. The cardiovascular disadaptation syndrome is associated to a disturbance of the lower limb vasomotor regulation and a cardiac deconditioning. During zero g ground simulations (H.D.T.), cardiac variations of the same kind have been observed but of weaker amplitude than in flight (6,7,9,11,13,14,16,17,20). Different countermeasures (LBNP, fluid loading, isotonic or isometric exercise...), to minimize the effects of weightlessness on the cardiovascular system and to reduce the cardiovascular deconditioning occurring after the flight, or following simulated weightlessness have been proposed (1,5,8,10,12,13,14,15,16,21). The LBNP (lower body negative pressure test) has also been used to evaluate the orthostatic response of pilots and astronauts (10,15). When performed in zero g or during a H.D.T., the LBNP induces a fluid shift from the cephalad part of the body towards the limbs. This transfer of liquid partially simulates the effects of the hydrostatic pressure on one g environment, and induces an adapted cardiac response (1,14,16). Over long term flights, the LBNP test, and isotonic or isometric exercises have been used intensively as a countermeasure. As a result a significant reduction of the cardiovascular deconditioning has been observed on the astronauts. Recently (2) the efficiency of LBNP in reducing the orthostatic intolerance has been established during a 30 day HDT. In the control group (without countermeasures), the volemia decreased and the peripheral vascular tone (cerebral, renal, femoral, vascular resistance) lowered significantly. In the LBNP group, both volemia and peripheral vascular tone remained constant and higher than the basal pre HDT value. After the HDT, most of the control subjects suffered from orthostatic intolerance during the tilt test and showed evident signs of vascular deconditioning at rest (blood pressure drop, heart rate acceleration...).That was not the case in the LBNP group. As we know that long term HDT induces vascular deconditioning, LBNP is actually efficient in reducing the vascular disadaptation, we defined our new objective: Detect and quantify the vascular deconditioning before the end of the HDT, in order to predict the vascular response to the tilt test after HDT. To achieve this goal, we assessed, the intra-cerebral and the femoral flows during a "LBNP test" (-20 to -50 mmHg), on adapted, & non adapted subjects. This test induces, during a short period (15 mn), a progressive fluid shift towards the lower limbs, and triggers a vascular response similar to those observed during orthostatic tolerance test (tilt table - squat up test) or when returning from space to one g gravity.

II - MATERIAL and METHOD:
II - 1: Population: 6 healthy volunteers participated in the 28 day HDT. In the control group, the 3 subjects stayed at rest during the 30 days of the HDT. In the countermeasure group, the 3 subjects were submitted since HDT day 8, to daily exercise sessions, and since HDT day 15, to LBNP sessions. The exercise sessions consisted, in maximal, and sub maximal isotonic, and isokinetic training, on both legs and postural muscle of the trunk. The LBNP session consisted in a 15' LBNP of -30 mmHg, 1 time a day, one day another, during the third week, and daily during the fourth week. All the 6 subjects were submitted to the orthostatic tolerance test "LBNP test" 3 times: pre, in +15d, & post HDT. During this test, the depression changed from -20 to -50 mmHg by four steps of 3 mm each.

II - 2: Hemodynamic parameters: The cerebral and femoral flows were monitored during the pre, in & post HDT LBNP orthostatic tolerance test (LBNP test). The cerebral flow was assessed by transcranial Doppler recording of the middle cerebral artery (MCA). The transcranial probe was fixed on the skull at the level of the temporal window, using an adjustable head set, the transducer facing the middle cerebral artery. For the femoral flow investigation we used a continuous Doppler flat sensor fixed on the thigh facing the common femoral artery. (Preoriented sensor stuck on the skin). The blood flow volume and the vascular resistance changes, occurring in the cerebral and the lower limb vascular beds, were calculated from the Doppler waveforms. Simultaneously, the heart rate and the blood pressure were measured. Because the sensors are in a fixed position, the angle between the Doppler beam and the vessel axis remains constant. Therefore, since we assumed that the main trunk of the middle cerebral artery and the femoral artery diameters did not change significantly, the mean Doppler frequency or velocity, was considered to change as the blood flow volume (3,18,19). The variation of the MCA blood flow (Qc) and the femoral one (Qf) were expressed in percentage of the pre LBNP basal value. The peripheral resistance changes were evaluated by using a resistance index calculated from the maximum Doppler frequency curve. The index used for the intracranial circulation is based on the evaluation of the end diastolic frequency. As the resistance increase, the diastolic component decreases. The index writes Re=(S-D)/S, with S and D for the systolic and the diastolic frequencies (18). The vascular resistance in the lower limbs were assessed through using another index based on the measurement of the diastolic reverse flow amplitude. As the vascular resistance increase, the reverse flow increases as well as the vascular resistance index. The index writes Rf=D/S, with S the systolic and D the diastolic reverse flow amplitude (18).

III - RESULTS and DISCUSSION: Results are presented on figures 1 to 3. Each point represents the variation of one Doppler parameter, expressed in percent of the pre LBNP basal value, and averaged over the 3 subjects (3 controls or 3 countermeasures).

III - 1 - Femoral circulation: On all the 6 subjects, during each LBNP Test (pre, in +15d, & post HDT), we observed the same kind of response (fig 1): The heart rate increased progressively from the first level of the depression (-20 mmHg) until the end of the test (-50 mmHg). The maximal variation ranged from 15 to 40 % depending on the subject. By the same time the systolic femoral blood flow decreased also progressively during the test. The maximal decrease was comparable on all subjects and ranges between -40% to -60%. The vascular resistance increased progressively and reached a maximum of about +20 to +60% of the basal value. The heart rate and the systolic flow variations were not significantly different between the two groups and during the pre, in +15d & post HDT LBNP tests. Nevertheless, the vascular resistance response during the LBNP tests were different according to the date of the LBNP tests and within the two groups. At HDT day +15, we observed on the two populations a vasodilation response of lower amplitude when compared to the response obtained during the pre HDT LBNP test (p<0.05) (fig 2 and 3). After HDT, the vascular resistance response to the LBNP test remained lower than at pre HDT-LBNP test (as at HDT day 15) in the control group; on
the other hand, in the countermeasure group, the amplitude of the vascular resistance response was higher than during the 15d HDT-LBNP test (p < 0.05), and the pre HDT-LBNP test.

III - 2 - Cerebral hemodynamic: The MCA cerebral flow slightly decreased during the LBNP test but not significantly. The cerebral vascular resistance decreased by -10% to -16% during the test. The haemodynamic parameters showed similar changes in the two groups and during the 3 LBNP tests (pre, in +15d & post HDT). These variations show that regulation of cerebral circulation is different, and how this circulation is preserved. Nevertheless, after the end of the LBNP test, the MCA vascular resistance did not recover immediately in the control group, the cerebral resistance remaining lower than the pre LBNP value.

On the 6 subjects investigated at rest during the HDT, we observed the same cardiac changes as already observed on 12 subjects over a previous 30 day HDT (2). The left cardiac chamber volume, the cardiac output, and the volemia decreased during the HDT on the control group; however these parameters remained higher than (the pre HDT) basal value in the countermeasure group.

At the end of the HDT, the control subjects showed evident signs of deconditioning (elevated heart rate-orthostatic hypotension-decrease work capacity) and most of them suffered from orthostatic intolerance during the tilt table test. On the contrary, the countermeasure subjects did not show orthostatic intolerance signs and underwent the tilt table test without any problem.

Finally, the decreased vascular reactivity (at the lower limb level) in the two groups, when staying at rest during the first two weeks of the HDT, or being submitted only to exercise the second week, is in favor of a loss or a decrease of the arterial vasomotor response to the fluid shift toward the legs induced by the LBNP. Finally the normalization of this vascular reactivity after a program of daily countermeasure applied from HDT day 8 or 15, to the end of the HDT, confirms the efficiency of the muscle exercise and the LBNP in preserving the vasomotor reflex in the lower limbs.

These preliminary results must be confirmed on a larger number of subjects, but it seems that the monitoring of the lower limbs arterial hemodynamics could be helpful to detect the vascular deconditioning in flight, and to predict the reaction of the cardiovascular system when returning to one g. These information may be of interest for the management of the countermeasure program during space flights.

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Figure 1: Femoral flow response to LBNP according to the depressurization level, (from -20 to -50mmHg) during one of the HDT "LBNP test" (20mm). HR: heart rate - Qf: femoral systolic flow - RF: lower limb vascular resistance index.

Figure 2: Control group. Lower limb vascular resistance index (RF) changes according to the depressurization level, during the pre, in +15day, & post HDT "LBNP test". The vascular resistance response at +15 days, & post HDT, is lower than at pre HDT-LBNP test (p<0.05).

Figure 3: Countermeasure group. Lower limb vascular resistance index (RF) changes according to the depressurization level, during the pre, in +15day, & post HDT "LBNP test". The vascular resistance response at +15 days, is lower than pre HDT (p<0.05) as in control group. Post HDT the vascular resistance response is significantly higher than at +15 days(p<0.05).

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MATHMATICAI. MODELING OF HUMAN CARDIOVASCULAR RESPONSE TO LBNP

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INTRODUCTION

The physiological processes known to be involved in the elicitation of the cardiovascular response to head-up tilt or lower body negative pressure (LBNP) are the same (1, 6), and include: blood distribution, left ventricular filling, coupling between left ventricle and peripheral circulation, and baroreflex control of arterial pressure. It is not clear, however, whether adjustments in these processes are sufficient to explain cardiovascular response to LBNP or tilt as experimentally observed. In order to examine this question, we have developed a mathematical model of the cardiovascular system aimed at simulating the response to LBNP. We hypothesise that the cardiovascular response is the consequence of the adjustments in the four processes listed above. Methodology for developing the model is to develop a sub-model for each process, and then combine the sub-models to form an overall model of the cardiovascular system.

METHODS

Blood Volume Distribution: We consider that blood is stored in two venous compartments, the upper body and the lower body. A negative pressure applied to the lower body results in movement of blood from upper to lower body. Let 6CVP, 6VUP, and 6VLO denote respectively the changes in central venous pressure and blood volumes of upper and lower body from their control values when LBNP pressure is 0 mmHg. These changes are related by:

6CVP = 6VUP / CUP

6CVP - LBNP = a1 tan(6VLOK/a2)

6VUP + 6VLO = 0

where CUP is the compliance of upper body compartment, and a1 and a2 are constants that represent the nonlinear compliance of lower body compartment (6). Values for CUP, a1 and a2 are 140 ml/mmHg, 17 mmHg, and 605 ml respectively.

Left Ventricular Filling: The decrease of CVP results in a decrease of the left ventricle end-diastolic volume (LVEDV). The relationship between CVP and LVEDV is the end-diastolic function of the left ventricle and is modeled by an exponential curve (3):

CVP = P0 + a.(eβ-LVEDV - 1)

where a, b, and P0 are constants related to the overall shape of the curve. Values of a, b, and P0 are 0.063 mmHg, 0.044 ml^-1, and -0.34 mmHg respectively.

Coupling Between Left Ventricle and Peripheral Circulation: The mean arterial pressure (MAP) and the stroke volume (SV) generated by the left ventricle mainly depend on its filling volume (LVEDV), on its isotropic and chronotropic states, and on the resistive properties of peripheral circulation (8). Following Suga et al. (7) and Sunagawa et al. (8), we use the following equation for the end-systolic pressure-volume relationship:

MAP = Emax . (LVEDV - SV - Vo) (5)

where Emax and Vo are constants which define the level of contractility of the left ventricle. Values of Emax and Vo are respectively 2 mmHg/ml and 32 ml (4). Resistive properties of peripheral circulation are expressed by the relation:

MAP = SV. HR . TPR (6)

where HR and TPR refer to heart rate and total peripheral resistance. Value of TPR depends on the resistances of the different local vascular beds.

Baroreflex Control of Arterial Pressure: We can summarise the modulation of heart rate and of local vascular resistances through arterial and cardiopulmonary baroreflexes by the equation:

6Y/6X = K 6X

where X is MAP for arterial baroreflexes and CVP for cardiopulmonary baroreflexes, and Y is the hemodynamic parameter modulated by the baroreflex. 6X and 6Y are the changes in X and Y from their values at 0 mmHg LBNP. K is the gain associated with the reflex. We consider that arterial baroreflexes modulate only HR (2), splanchic (2), and renal (9) vascular resistances, and that cardiopulmonary baroreflexes modulate vascular resistances of upper and lower limbs (2, 10). The value of K is -5 6/mmHg of MAP for modulation of HR, -28 6/mmHg of MAP for modulation of splanchic or renal vascular resistances, and -13 6/mmHg of CVP for cardiopulmonary baroreflex modulation of limbs vascular resistances.

Eqs. (1-7) constitute the overall model. For a given level of LBNP, solution of Eqs. (1-3) gives 6CVP. Eq. (4) is used to calculate LVEDV for a given value of CVP. Simultaneous solution of the remaining equations yields the changes in the values of the various hemodynamic variables.

RESULTS

The model was used for simulation of cardiovascular response to an LBNP ramp test from 0 to -40 mmHg. Fig. 1 shows the changes in MAP, CVP, HR, FVR, CO, LVEDV as simulated by the model and as reported by Bloomquist and Stone (1) from experiments on humans. There is a good agreement between simulated and experimental results.
DISCUSSION

Methodology to develop the model was to include in the model only the processes that are thought to be directly involved in the cardiovascular response to LBNP. This resulted in a very simple model depending on only two nonlinear equations (Eqs. (2) and (4)) and on 13 parameters. If the model had failed to accurately simulate experimental data, it would have meant that processes other than the ones included in the model are important in the cardiovascular response to LBNP. The satisfactory comparison of model and experimental results is an indication that no other mechanism of importance is involved in mediating the response to LBNP. For example, the model did not include any modulation of cardiac contractility by baroreflexes. This confirms the experimental results of Nixon et al. (5) who did not find any significant increase of left ventricular contractility during LBNP exposure up to -40 mmHg.

The parameters which influence the cardiovascular response to LBNP include leg compliance, left ventricular end-diastolic compliance, and gains of baroreflexes. Through an analysis of sensitivity of the response to changes in these parameters, the model is able to evaluate their relative contributions to the total response. This is the aim of further research based on our model. Other future efforts will focus on expanding the model to enable simulation of time-dependent cardiovascular response to head-up tilt and to +Gz acceleration.

ACKNOWLEDGEMENTS

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Long-term (18.5-21 days) space flight onboard biosatellites has impaired the general state and motor responses in the animals. The greatest changes have been observed in the rats stayed in a weightless (W group). The animals were flabby; the most part of time were laying prone; when moving they walked in unsteady steps with the limbs planted widely apart, frequently moved crawling. The have been no typical movements and postures associated with orientating in the rats when they explore the surroundings. In the rats exposed to artificial gravity (A group) the less pronounced changes have been noted. The animals were more active, have overcome obstacles, come to the hind limbs, their gait was almost normal [1]. The physical state of the animals has been judged by the static tolerance determined from the limiting which the animals could stay on the pole. Immediately postflight, the static tolerance was dropped in the flight groups animals approximately three-fold and in the animals of synchronous control group (S) and rotating on the ground-based short-radius centrifuge (SRC) there has been a two-fold decline as opposed to baseline and values typical of the vivarium control rats (V).

Throughout the subsequent investigations, the more higher levels of tolerances have been noted in the A group animals as compared to those W group. Remoting of static tolerances to their original values in these animals has occurred on day 6 postflight while in the W group animals this parameter remained low up to a 24th investigative day [3]

It is important to emphasize that the restoring rate of static tolerances in the A group animals and those from the S group was almost the same.

Fig.1. Cinegram of turning over reaction of S group rats at R+0.

The equilibrium function of animals has been evaluated from their ability to keep on a narrow horizontal bar. Immediately after the flight, this function was impaired but to a lesser extent in the A group rats. Typical of the W group animals was inactive behaviour on the bar, in losing equilibrium the rats did not even attempt to restore it. The A groups animals have actively attempted to keep equilibrium and when the rats failed to do this they have hanged in an upside down position on the bar clutching at it with the limbs [1].

The semicircular canal function has been judged by the changed characteristics of nystagmic reflex in response to a number of ever-increasing angular accelerations.

Throughout the experiments there was no significant difference in latent periods, durations, beats number and rate of nystagmus between W group, S group and V group animals.
Completely different regularities have been found during an assessment of nystagmic reactions in the A group and SRC group animals [4]. During an investigation conducted at the 2nd postflight day these animals exhibited a marked tendency for an increase in latent periods as well as a significant decrease in heart rate and rate of nystagmus both in comparison to the baseline level and the values typical of S and V group animals. By the postflight days 7-11, the animals from various experimental groups displayed no differences in nystagmic reaction manifestations.

Otolithic system function has been evaluated on the basis of latent period of lifting reaction (LPLR) occurring during a progressive downward motion of the animal. The 4-5 hours postflight, electromyography of oculomotor and gastrocnemius muscles did not reveal changes in the LPLR of W group rats. At the same time, there was a significant increase in the LPLR determined by the mechanical component of the muscle reaction while in A group rats the LPLR remained unchanged. Throughout the investigations, i.e. artificial gravity eliminated negative effect of weightlessness [2].

The turning over reflex persisted in W group animals at 4-5 hours after the flight; they experienced only the difficulty in postturning over stabilization of the body and associated particular imperfection of landing which was clearly noted in eye-closed rats (Fig. 1). Turning over of A group rats has persisted but the eye-closed rats did not make attempt to turn over and at their backs (Fig. 2). The behavior patterns of SRC group animals were similar. In two animals turning over was absent on day 10 too.

In conclusion, the following facts should be emphasized: long-term weightlessness does not alter functioning the receptors and centers of semicircular canals and otoliths, or alternatively these changes are so negligible and short-term that on the 2nd postflight day they are undetectable. A long-term stay of the animals in conditions artificial gravity does not change the otolith function but decreases the sensitivity and reactivity of the semicircular canals. Finally, under conditions of artificial gravity the role of vision in spatial orientation judged by lacking the turning over reflex in the centrifuged rats on eliminating visual control is greatly increased.

References


Fig. 2. Cinegram of turning over reaction of W group (A) and A group (B) rats at 4-5 hrs after landing of "Cosmos-936".
+Gx - TOLERANCE FOLLOWING ONE-YEAR REAL AND SIMULATED MICROGRAVITY

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INTRODUCTION

One of the key goals of manned spaceflight medical support is to predict cosmonauts' G-tolerance to G-loads following prolonged stay in weightlessness.

The available data (1) indicate a reduction of cosmonauts' G-tolerance after short-term weightlessness. The levels of G-load are apprehended more severe than real by 1-2 G and induce marked sinus tachycardia and tachypnoe.

Cosmonauts' tolerance to G-loads after prolonged, up to a year stay in weightlessness, has not been investigated so far.

It is also remains unknown whether the model of durable weightlessness permits to predict alterations in G-tolerance on the stage of spacecraft descent after long-term weightlessness.

In view of this it seemed worthwhile to make analysis of the available data about cosmonauts' tolerance to +Gx-loads following prolonged space flights and to compare the results of this analysis with those obtained after simulated weightlessness of the same duration.

METHODS

Tolerance to +Gx-loads was evaluated in 8 cosmonauts on the conclusive stage of 125-, 237-, 241-, and 366-day orbital flights and in 3 subjects (12 runs) following 120-, 240-, and 360-day antioorhostatic hypokinesia.

The values of G-loads in real conditions were in the range from 3.6 to 4.7 G, in experimental conditions - up to 8.0 G. The seat back was inclined at 60 to the acceleration vector with such angle the longitudinal component of +Gx loads averaged 18% of the resulting forces (1).

In real and simulated conditions the complex of prophylactic means against adverse effects of weightlessness on the human organism was used on all stages of the investigations. In all cases, except for the 237-day space flight, elastic uninflated anti-G suit was employed. Traditional for G-loads studies complex of physiological parameters was measured in all investigations. Their values were calculated for maximal peak loads. After hypokinesia physiological changes were also examined during the onset of the centrifuge at 4.8 G.

RESULTS

On the whole tolerance to G-loads after various periods of actual and simulated weightlessness was recognized satisfactory. Physiological responses in both types of conditions had similar directions. There were complaints of a sense of burden on the chest, impeded breathing, observations of sinus tachycardia and tachypnoe, in several cases vision disorders and extrasystolic arrhythmia were found (Fig.1).

However, vision disorders and arrhythmia after hypokinesia were registered only at 8.0 G rather than at 4.8 G.

In comparison with the control data the tension of physiological systems increased in response to G-loads following both real and simulated weightlessness reflecting decreased G-tolerance in these conditions. At the same time the use of anti-G means prevented further reduction in G-tolerance, observed after 120-day hypokinesia (or 125-day space flight), with prolongation of modelled or real weightlessness to 200 (or 237-241) and then up to 360 (or 366) days.

Physiological responses to G-loads after weightlessness were more expressed than to the equal G-loads after hypokinesia of the same duration. For example, the subjective evaluation of G-loads by

![Figure 1. The main physiological changes under +Gx-loads following weightlessness and hypokinesia with the use of prophylactic means.](image-url)
cosmonauts on the final stage of their flights was by 1–2 G and in several cases by 3–4 G greater than real values. In experimental investigations after hypokinesia no such observations were made. Vision disorders during descent also occurred at less values of loads (4.1±0.1 G) than after hypokinesia (8.0±0.45 G) (Fig. 2).

**Figure 2.** Values of +Gx-loads which induced vision disorders during centrifugation or space flight (Mm)

Sinus tachycardia and tachypnoea at G-loads in the interval between 3.6 and 4.7 G after spaceflight weightlessness were, as a rule, more severe than at similar loads (4.8 G) after hypokinesia of the same duration (Fig. 3, 4).

**Figure 3.** +Gx induced changes in heart rate after equal periods of weightlessness and hypokinesia with the use of prophylactic means (mean M).

The tension of physiological systems at abovementioned loads after long-term spaceflight weightlessness (up to one year) was approximately similar to that at 8.0 G after hypokinesia of the same duration.

**DISCUSSION**

The results of these investigations showed decreased tolerance to G-loads after various periods of real and simulated weightlessness in comparison with the control. It appears to be related to the development of deconditioning of the main physiological systems of the human body (cardio-vascular and neuromuscular) and reduced volumes of circulating blood due to water-electrolyte shifts (1). The investigations also demonstrated that employment of prophylactic measures and means for anti-G protection prevents further progress of physiological reactions to +Gx-loads with increasing of man’s stay in real or modelled weightlessness up to a year. This fact is likely to result from positive effects of prophylactic means and anti-G protection.

Analysis of the obtained data made it possible to identify similarities and distinctions in human G-tolerance after actual and simulated weightlessness of the same duration. The directions of physiological reactions were the same under these two types of conditions. The distinction consisted in greater tension of the physiological systems in response to G-loads after weightlessness in comparison with equivalent G-loads after hypokinesia of equal duration.

The results of these investigations also showed that applying significantly greater G-loads in experimental conditions than in real we can predict physiological effects of +Gx-loads on the final stages of a spaceflight lasting up to a year.

**CONCLUSIONS**

Man’s tolerance to +Gx-loads following prolonged stay in actual or modelled weightlessness conditions with the use of a complex of means of prophylactics and anti-G protection was satisfactory though it was worse if compared with control data.

The tension of physiological systems under equivalent G-loads was greater in real flights than in experimental conditions.

To predict human G-tolerance at the end of long-term space flights it is advisable to apply higher G-loads that real after simulated weightlessness of the same duration.

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INTRODUCTION

The importance of nutrition in the adaptation of humans to microgravity was recognized long before the first space flight. In spite of findings to date, nutrition remains a primary concern for a number of reasons arising from health, biological or operational considerations.

Energy balance depends upon the equilibrium between energy input, resulting from food intake, and energy expenditure, involving several processes.

Weight loss is usually associated with an energy deficit. A negative energy balance exists when energy intake is less than energy expenditure. The deficit is compensated for by tissue catabolism, principally of fat, but also of protein.

Body weight loss as well as a modification in body composition has been universally observed in spaceflight. The weight loss, averaging around three to four percent of preflight weight, occurs early in the flight. Total weight loss is independent of mission duration [1,2,6,8].

While some research concerning energy expenditure during spaceflight has been done, the influence of microgravity on eating behavior remains practically unknown.

Different crews have provided anecdotal evidence for altered responses to taste. A possible factor which has been hypothesized to cause taste shifts in weightlessness is reduced stimulation of taste buds as a result of changes in convective activity in zero gravity [7].

A reduction in appetite has also been reported. A feeling of fullness in the abdomen, nausea and preoccupation with critical mission tasks are among the reasons most commonly reported by astronauts [3,4].

In the present study, part of our current research program on energy metabolism and nutritional behavior of human subjects in spaceflight, or in chronic recumbency, our specific objectives are:

- to measure energy intake and macro-nutrient composition of the diet.
- to score motivation to eat.
- to determine any changes in food preferences.

METHODS

Six healthy male volunteers (age 34 ± 4.7 years), without special knowledge about nutrition, participated in this study.

After a 7 day ambulatory control period, subjects remained in continuous head-down (-6°) bed rest for 28 days, followed by a 5 day ambulatory recovery period.

During the last three weeks of the bed rest period, three subjects performed physical exercise and L.B.N.P. as a countermeasure against musculoskeletal and cardiovascular deconditioning. More detailed information on these countermeasures has been described elsewhere [5].

In our experiment, energy intake was measured by weighing each meal or snack presented to the subjects. At the end of the meal, food left over by each subject was weighed again. The macronutrient composition of the diet was determined from manufacturer's data.

To rate motivation to eat, subjects were asked about their hunger and their post-ingestion satiety over the preceding 24 hours. A rating scale was used to rank their responses.

Eventual changes in food preferences were determined in two different ways: a) subjects selected four items they would like to eat from a check-list consisting of 40 commercially available foods items; b) on test days, subjects tasted and rated four different foods: high protein (ham); high-fat (cheese); high carbohydrate, not sweet (cracker); and high carbohydrate and sweet (jam).

All measurements was performed on days 4 and 7 of the control period, on days 1, 3, 7, 14, 21, and 28 of the bed rest period, and days 1 and 4 of the recovery period.

Analysis of variance were used to compare both groups, with and without countermeasures. Each subject served as his own control for comparison of data from the control, bed rest and recovery periods.

RESULTS

Energy intake: In both groups of subjects, with and without countermeasures, a significant decrease (p <0.05) in energy intake was observed during the bed rest period. Mean reduction was of the order of 414 kcal per day compared to the control period. However, by day 4 of the recovery period, prior energy intake was restored.

Macronutrient composition of diet: In subjects without countermeasures, the composition of diet remained stable. Intake of protein, lipids and carbohydrates decreased along with total energy intake. In contrast, subjects who performed countermeasures maintained their control period level of protein intake. During the bed rest period, the
difference in protein intake between subjects with and without countermeasures was highly significant (p<0.05).

Motivation to eat: During the bed rest period, subjects executing countermeasures felt more hungry than the non-active subjects. However, the difference failed to reach statistical significance.

Food preferences: Regardless of the period or the physical activity of the subjects, no significant differences appeared in the pleasantness ratings of the tested foods or in the foods items chosen from the food preference checklist.

DISCUSSION

The present study shows that voluntary eating behavior of individuals can be affected by chronic recumbency.

A consistent finding is the confirmation of decreased energy intake during the bed rest period independent of the use of countermeasures. This reduction in energy intake is directly correlated to the loss of body weight observed during the bed rest period.

The main result of this study is the maintenance of the control period amount in protein intake in subjects who executed countermeasures compared to the non-active subjects.

Although, subjects performing countermeasures seemed to feel hungrier than the non-active subjects, in both cases no significant differences appeared in their food preferences. This finding suggests that the observed changes could have their origin the metabolic effects of chronic recumbency and not a change in voluntary eating behavior.

However, considering the low number of subjects it is not possible at present to claim these findings as definitive. In our present research program we anticipate additional studies concerning body composition and energy metabolism in order to evaluate the metabolic significance of changes observed in the present study.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the management skills of Ms. M.M. Faurat in coordinating the entire bed rest experiment and the significant contributions of Ms. P. Vasseur in executing this experiment.

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EXPERIMENT "CHLAMYDOMONAS" ABOARD BIOSATELITE "COSMOS-2044"

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INTRODUCTION

The experiment on the cultures of unicellular eucariotic organisms, carried out with the help of clinostates and centrifuges and also in the conditions of space flight, revealed an equivocal results. In space experiments with Chlorella the delay of sporulation, increasing of spore formation with number of daughter cells not multiple to four, the autosporation not equal in size (1,10), increasing of cell sizes were shown to be reliable, growth rate and dry biomass output did not changed as to compare with the control (4,5). There were demonstrated no in the cellular and subcellular morphology and dynamics of development in the experiments on rapid clinostates (7,8). There were no changes in fine structure after the space flight (6), or they were not significant (4,5). The experiments on infusoria Paramecium and Tetrahymena showed the acceleration of culture growth, increasing of cell size and ratio of cell volume to protein content (3,9). The main problem of this experiment was the studying of fine structure of the culture of Chlamydomonas reinhardtii CALU 495+ in the conditions of space flight.

METHODS

The material was sown on the surface of 8% agar-agar media three days before the launching. Petry dishes were installed on the wall of illuminated aquarium. The flight was 14 days long. After the processing of the information, received by the telemetry channels, the control experiment was carried out, precisely repeating the cultivating conditions (temperature and illumination). After the landing the chilled material was carried to IBMP laboratory in Moscow, washed off the agar and fixed with glutaraldehyde-osmium. The ratio of organell area to the area of cytoplasm on the ultra-thin sections was calculated to elucidate whether the ratio of organell volume to cytoplasm one changes. For the cytometrical measuring the casual cell sections near there diameter were used. The large number of analyzed cells (85 control sections and 80 - experimental) allows to make the reliable conclusions. The statistic processing was carried out with the help of the program of image analyzer IBAC.

RESULTS

The cell vital ability was determined according to the number of colonies, grown up on the solid nutrient medium after the sowing of suspension of certain concentration. The number of colonies in the flight and in the control experiment was approximately the same. The light optical observations showed, that 16% of cells in experiment possessed flagella, while in the control only 0.8%. The number of deformed cells in flight was 17%, in control experiment - 30%. It leads to the proposal, that in culture after two-week flight there were no changes on the stage of active growth than in the control experiment. The results of cell measurement are presented in Table 1 (the standard error is given in brackets).

Table 1. SIZE OF CELLS AFTER COSMOS FLIGHT STUDIES

<table>
<thead>
<tr>
<th>Flight</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, μ</td>
<td>10.7 (0.1)</td>
<td>8.8 (0.1)</td>
</tr>
<tr>
<td>Width, μ</td>
<td>9.4 (0.1)</td>
<td>8.5 (0.1)</td>
</tr>
<tr>
<td>Volume, μ³</td>
<td>539 (19)</td>
<td>368 (12)</td>
</tr>
</tbody>
</table>

The cell sizes in the experiment exceeded in the control in all studied parameters. The experiment data showed the increasing of cell sizes on all the stages of life cycle. The average ratios of organell-cytoplasm areas are given in Table 2 (the standard error is given in brackets).

Table 2. THE RATIOS OF ORGANELL-CYTOPLASM AREAS IN CELLS IN COSMOS FLIGHT STUDIES

<table>
<thead>
<tr>
<th>Flight</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroplast</td>
<td>0.577 (0.016)</td>
<td>0.567 (0.012)</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.040 (0.009)</td>
<td>0.038 (0.002)</td>
</tr>
<tr>
<td>Nuclei</td>
<td>0.096 (0.007)</td>
<td>0.086 (0.007)</td>
</tr>
<tr>
<td>Pyrenoid</td>
<td>0.051 (0.006)</td>
<td>0.051 (0.006)</td>
</tr>
</tbody>
</table>

As the data presented demonstrate, there are no reliable differences in this parameters in the flight and in the control experiment. The increasing of cell size in the space flight is due to the proportional increasing of organell and cytoplasm volume. There are no changes in the structure and mutual arrangement of organelles. The cells under microgravity have greater variability of nuclei and mitochondria area than in the control experiment. In the experiment with Tetrahymena the increasing of variability of DNA content in macronuclei was observed (3) which is in agreement with our data.

DISCUSSION

The prolongation of active growth phase and the increasing of cell size in weightlessness were stated not only for Chlamydomonas, but also for Chlorella (4,5), Paramecium (9), Tetrahymena (3). These affects may be caused just by microgravity, because in conditions of hypergravity the delaying of culture growth was observed, the cell division phases, occurred earlier, the cell size decreased (3,2). The studied unicellular organisms are of different phylogenetical lines, but posses the common laws of growth in the conditions of space flight. The mechanisms of weightlessness action on the cell are probable to the common also. The increasing of Paramecium cell size was connected with increasing of water content in the cells, with the increasing of membrane permeability (9). It is confirmed indirectly by the absence of changes of dry weight during the increasing of cell size in Chlorella, the decreasing of the ratio of protein content to the whole volume of Paramecium and Tetrahymena cells (2,3,8).
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NORADRENERGIC AND CHOLINERGIC INNERVATION OF SPLEEN IN THE RATS DEVELOPED UNDER CHANGED GRAVITY

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INTRODUCTION

The investigation of noradrenergic and cholinergic innervation of the spleen in rats developed under different gravity levels - microgravity, hypergravity and Earth gravity - is necessary to understand autonomic nervous system involvement in regulatory mechanisms of spleen function in weightlessness. In the present study by means of morphological and histochemical methods noradrenergic and cholinergic nerve fibres, white pulp volume density, lymphoid nodule square, T-zone and B-zone squares were investigated in the spleen of 60-day and 75-day rats developed pre- and postnatally under 2-G gravity and also under 1-G after rat transition from 2-G gravity into Earth gravity (2).

METHODS

60-day (PI group) and 75-day (P2 group) Wistar rats with masses of 142 g and 181 g respectively, grown under 2-G gravity at constant rotation of centrifuge (with a stop at 15-20 min), and 60-day vivarium rats (V group) with a mass of 148 g were sacrificed 40 sec after the centrifuge stop. 60-day rats grown under 2-G gravity were sacrificed also 15 days after transition from 2-G gravity into Earth gravity (P4 group). The one part of rat spleen, immersed into 10% gelatin solution, was frozen at -150°C in Freon-12 precooled by liquid nitrogen, and in spleen sections with 15 μm thickness, prepared in a cryostat at -18°C, the localization of noradrenergic and cholinergic nerve fibres was developed respectively by histofluorescent glyoxylic acid method and by histochemical method for acetylcholinesterase activity. The volume density of nerve fibres within spleen structures was analysed by stereological method with the help of eyepiece reticle with test point of 500 and was expressed as percentage of test points located over nerve fibres. The catecholamine fluorescence intensity in noradrenergic fibres was measured by means of fluorescence microscope Lumam-I3 at 483 nm. The sections of the second part of rat spleen, fixed in Carnoy solution and embedded in paraffin, were stained by azure II-eosin and hematoxylin-eosin for identification of T- and B-lymphocytes. In these sections the white pulp volume density was measured by stereological method; the greatest and the least diameters of lymphoid nodules and T-zones were measured also with following calculation of the lymphoid nodules, T-zone, B-zone squares. Each group of rats consists of 6 animals.

RESULTS AND DISCUSSION

A volume density of noradrenergic fibres in a trabecularis (AT), a-pulparis (AP) and a.centralis (AC) areas of the rat spleen was 8.53%, 3.06% and 1.09% respectively; a volume density of cholinergic fibres in the same areas was 10.24%, 5.07% and 0.85% respectively with predominance of cholinergic innervation over noradrenergic, with the exception of AC areas, located within lymphoid nodule T-zones, where inverse ratio has taken place (Fig. 1).

The 60-day rats developed under 2-G gravity had a volume density of noradrenergic fibres in AT, AP and AC areas of the spleen 2.34, 1.84 and 1.91 times as less respectively (Fig. 1) and decrease of catecholamine content in the fibres of the same areas (Table 1) in comparison with V rats. It is known, noradrenergic system in a spleen inhibits T-lymphocyte proliferation (2) and macrophage activity (4), and, probably cholinergic system in a spleen, like a cholinergic system in a thymus (1), has activating influence on the same processes. The decrease of volume density of noradrenergic fibres in AC area of PI rat spleen has to follow by decrease of inhibitory influence of noradrenergic system on T-lymphocyte proliferation. The last conclusion obviously is supported by the fact of intensive white pulp proliferation expressed as white pulp volume density increase (plus 186%) (Fig. 2) and lymphoid nodule T-zone increase (plus 50%) (Fig. 1) in comparison with V rats.

The continue of rat postnatal development under 2-G gravity till 75-th day produced in AC area the further decrease of noradrenergic fibers volume density and predominance of cholinergic fibres. As result the inhibitory influence of noradrenergic system on T-lymphocyte proliferation has become much weaker. This assumption is supported by further increase of white pulp volume density (Fig. 2) and T-zone square (Fig. 1).

15 days after transition from 2-G gravity to Earth gravity in the rats developed till 50-th day under 2 G the catecholamine content in nervous fibres (Table 1) and the predominance of noradrenergic innervation over cholinergic one in AC area - typical for V rats - were restored (Fig. 1). But, presumably, the time was not enough to realise the increased inhibitory effect of noradrenergic system on T-lymphocyte proliferation because the white pulp volume density did not decrease in comparison with PI rats (Fig. 2) and lymphoid nodule T-zone increased also.
Table 1. Catecholamine Content in Noradrenergic Nerve Fibres of Spleen
(FLUORESCENCE INTENSITY, RELATIVE UNITS)

<table>
<thead>
<tr>
<th>Rat group</th>
<th>a.trabecularis area</th>
<th>a.pulparis area</th>
<th>a.centraris area</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>22.1±2.5</td>
<td>20.9±2.8</td>
<td>20.1±1.9</td>
</tr>
<tr>
<td>P1 (60 d 2 G)</td>
<td>9.8±0.8*</td>
<td>7.7±0.9*</td>
<td>6.5±1.1*</td>
</tr>
<tr>
<td>P2 (75 d 2 G)</td>
<td>10.1±3.6*</td>
<td>8.2±2.8*</td>
<td>15.3±2.6*</td>
</tr>
<tr>
<td>P4 (60 d 2 G +15 d 1 G)</td>
<td>18.3±3.1</td>
<td>16.5±2.9</td>
<td>21.2±2.1</td>
</tr>
</tbody>
</table>

* = significant in comparison with V.

Fig. 1. Nerve fibre volume density in a.centraris area and lymphoid nodule zone square in the spleen of V, P1, P2, P4 rats. ■ cholinergic fibres (test point percentage); □ noradrenergic fibres (test point percentage); T - T-zone(μm², in round numbers); B - B-zone (μm², in round numbers).

Fig. 2. Volume density of spleen white pulp in V, P1, P2, P4 rats.

Although that increase is less than in P2 rats (Fig. 1).

On the basis of data obtained it would be reasonable to suppose that changes of white pulp volume density and T-zone square in the spleen after transition of rats from one gravity level to other depend on ratio of inhibitory noradrenergic and activating cholinergic influences.

References
MORPHOLOGY AND HISTOCHEMISTRY OF SPINAL CORD AND SOLEUS MUSCLE IN RATS GROWN UNDER HYPERGRAVITY

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INTRODUCTION

The study of the spinal cord structures and anti-gravitation muscles of animals grown under hypergravity is important for understanding the mechanisms at the basis of mammalian motor adaptation to altered gravity conditions. In this connection the spinal cord at the level of lumbar enlargement and the soleus muscle in the rats pre- and postnatal development of which took place under 2-G gravity during continuous centrifuge rotation (1), are studied by morphological and histochemical methods.

METHODS

Pre- and postnatal development of Wistar male rats took place under 2-G gravity during continuous (with a stop every day for 15-20 minutes) rotation on centrifuge with radius of 1.41 m at the speed of 33 r.p.m. 60- and 75-day-old animals grown under 2-G with mass of 142 g and 181 g respectively, were sacrificed 40 seconds after the centrifuge stop, and 60-day old ones were also sacrificed on the 2nd and 15th day after their transition from 2-G gravity to 1-G conditions. A fragment of the lumbar enlargement of the spinal cord (L5-S1) was fixed in Carnius's liquid and embedded in paraffin. Tissue sections 7 mm thick stained with gallocyanin were used to measure by a television microscope at x2000 (for nucleolus x2500) in 40 motoneurons of anterior horns, the large and small diameters of body, nucleus and nucleolus of neuron and then volumes of body (Vb), nucleus (Vn) and nucleolus (Vnl) were computed. The number of perineuronal glia (PG) cells per one motoneuron was also counted, as well as number of "hyperchrome" motoneurons (HCM). The second LESC fragment was frozen at -150°C in freon-12 cooled by liquid nitrogen, and in tissue sections 12 mm thick obtained in a cryostat at -20°C the activity of succinated dehydrogenase (SDH) and acetylcholinesterase (AChE) was developed by histochemical methods. The intensity of histochemical reaction in motoneurons bodies was measured densitometrically at 460 nm and enzyme activity was expressed in optical density units (O.D.U.). The muscular fibres of red and intermediate type were identified in histological sections of soleus muscle by means of histochemical demonstration of myozin ATPase activity and their per cent content was calculated. The spinal cord and soleus muscle of 6 animals were studied in each group of rats.

RESULTS AND DISCUSSION

In 60-day old rats, pre- and postnatal development of which took place under 2-G gravity (group P1), the number of "hyperchrome" motoneurons, indicating an extreme functional stress of these nervous cells, in anterior horns of LESC was sharply increased as compared with vivarium control (VC) rats; volume of body, nucleus and nucleolus in motoneuron is grown and the AChE activity is increased, as well as the number of PG cells (Table 1), which indicates a higher level of functional activity of motoneurons. An increased content of slow, tonic, intermediate type muscular fibers (IMF) and a considerably lower content of fast, red muscular fibers (RMF) was found in the soleus muscle of the same rats (Table 2) as compared with VC rats. This fact indicates existence of high static load on anti-gravitation muscle during development under hypergravity.

On the second day after transition of rats from 2-G conditions to Earth's gravity (group P3) in comparison with P1 rats the volume of body and nucleus and AChE activity in motoneurons were decreased, as well as amount of PG cells; in soleus muscle the tendency to increase IMF content and decrease of IMF content was appeared.

On the 15th day after transition of the rats from 2-G gravity to Earth's gravity (group P4) the volume of body, nucleus of motoneurons, number of PG cells and SDH and AChE activities were increased sharply as compared with those of animals of groups P1 and P3 (Table 1), and a further increase of RMF content (Table 2) took place in the soleus muscle, which displays an increase of motor activity of animals after their transition to 1-G gravity.

An extension of postnatal development under 2-G gravity from 60 up to 75 days (group P2) did not cause essential changes of motoneurons parameters, excluding a decrease of the number of "hyperchrome" motoneurons.

Thus, the morphometric and histochemical results of the study show that the pre- and postnatal development of rats under 2-G gravity is characterized by a higher functional activity of LESC motoneurons and a considerable predominance of IMF in the soleus muscle, which represents a higher static load under hypergravity. Transition of rats from 2-G gravity to 1-G gravity evidently causes a decrease of functional activity of motoneurons during the first days after transition as result of the static load...
diminution. The further stay of animals grown under hypergravity under 1-G gravity conditions is accompanied by an increase of functional activity of motor-neurons and an RMF content increase in soleus muscle, reflecting a growth of the animals' motor activity.

### TABLE 1. MORPHOMETRIC AND HISTOCHEMICAL ANALYSIS OF MOTONEURONS AND GLIA IN THE SPINAL CORD OF THE RATS GROWN AT 2-G GRAVITY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups of animals</th>
<th>VC 2G 60 days</th>
<th>P1 2G 60 days</th>
<th>P3 2G 60 days</th>
<th>P4 2G 60 days</th>
<th>P2 2G 75 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_B$, $\mu m^3$</td>
<td>11927±297</td>
<td>14921±922</td>
<td>12669±437 $^+$</td>
<td>17930±724 $^{+2, +3}$</td>
<td>13935±591 $^+$</td>
<td></td>
</tr>
<tr>
<td>$V_n$, $\mu m^3$</td>
<td>1887±33</td>
<td>2175±63 $^+$</td>
<td>2080±54</td>
<td>2473±56 $^{+2, +3}$</td>
<td>2125±86 $^+$</td>
<td></td>
</tr>
<tr>
<td>$V_{nl}$, $\mu m^3$</td>
<td>41.0±0.8</td>
<td>43.7±0.5 $^+$</td>
<td>36.5±0.7 $^{+2}$</td>
<td>41.0±1.2 $^{+3}$</td>
<td>40.1±1.1 $^{+2}$</td>
<td></td>
</tr>
<tr>
<td>PG, number</td>
<td>1.7±0.07</td>
<td>2.2±0.09 $^+$</td>
<td>1.7±0.06 $^{+2}$</td>
<td>2.7±0.1 $^{+2, +3}$</td>
<td>2.1±0.08</td>
<td></td>
</tr>
<tr>
<td>SDH, O.D.U</td>
<td>0.211±0.07</td>
<td>0.264±0.01</td>
<td>0.222±0.09</td>
<td>0.311±0.01 $^{+2}$</td>
<td>0.270±0.02</td>
<td></td>
</tr>
<tr>
<td>ACHBE, O.D.U</td>
<td>0.275±0.01</td>
<td>0.384±0.02 $^+$</td>
<td>0.279±0.02 $^{+2}$</td>
<td>0.392±0.01 $^{+3}$</td>
<td>0.375±0.01 $^+$</td>
<td></td>
</tr>
<tr>
<td>HCHM, %</td>
<td>3.6±0.6</td>
<td>18.2±1.2 $^+$</td>
<td>14.0±1.4 $^{+2}$</td>
<td>8.6±0.8 $^{+2}$</td>
<td>13.0±0.8 $^+$</td>
<td></td>
</tr>
</tbody>
</table>

+ - significant differences in comparison with VC; 2+ - significant differences in comparison with P1; 3+ - significant differences in comparison with P3.

### TABLE 2. MUSCLE FIBER CONTENT IN SOLLEUS MUSCLE IN THE RATS GROWN AT 2-G GRAVITY

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>RED muscle fiber, %</th>
<th>Intermediate muscle fiber, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC</td>
<td>36.66±2.65</td>
<td>63.36±2.64</td>
</tr>
<tr>
<td>P1</td>
<td>10.38±2.51 $^+$</td>
<td>89.62±2.48 $^+$</td>
</tr>
<tr>
<td>P3</td>
<td>14.24±2.80</td>
<td>85.76±3.00</td>
</tr>
<tr>
<td>P4</td>
<td>22.64±3.30 $^+$</td>
<td>77.36±3.70 $^+$</td>
</tr>
</tbody>
</table>

+ - significant differences in comparison with VC and P1.

### REFERENCES

NEURON-GLIA-CAPILLARY SYSTEM IN SPINAL CORD OF RATS AFTER 14-DAY SPACE FLIGHT

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INTRODUCTION
Morphometrical and cytochemical study of motoneurons in the lumbar enlargements of spinal cords of rats flown aboard "Cosmos-605" (1), "Cosmos-782" (3) and "Cosmos-936" (2) biosatellites revealed changes indicating decreased functional activities of these nervous cells after 18-22-day weightlessness. To estimate an effect of a more short-term weightlessness upon the state of motoneurons, as well as upon glia cells and capillaries, spinal cord of rats flown for 14 days aboard biosatellites "Cosmos-1887" and "Cosmos-2044" and also rats tail-suspended for 14 days was studied by means of morphological and histochemical methods.

METHODS
Segments of cervical (CESC) and lumbar (LESC) enlargements of the spinal cord of rats were fixed in Carna liquid and embedded in paraffin. On 7 mm thick sections stained with galloycyanin: 1) the RNA content in motoneurons was visually estimated; 2) by means of the television microscope at x2000 (for nucleolus x2500) in 40 motoneurons large and small diameters of body, nucleus and nucleolus were measured with subsequent calculations of body (Vb), nucleus (Vn) and nucleolus (Vn) volumes; 3) number of perineuronal glia (PG) cells per one motoneuron was counted. A part of the spinal cord was frozen in freon-12 at -150°C, and in 12 mm thick sections obtained in cryostat at -20°C, the activity of acetylcholinesterase (ACHE), alkaline phosphatase (AP) and cytochromoxidase (CC) was developed by histochemical reactions. The intensity of histochemical reactions was measured densitometrically and enzyme activity was expressed in optical density units (O.D.U.). The number of "active" capillaries (AC) in which AP activity was detected was measured stereologically and was expressed per microscopic field (n/mf). The spinal cord of 5 animals was studied in each group of rats.

RESULTS
In rats exposed for 14 days to microgravity and 9-11 hours to Earth gravity after microgravity ("Cosmos-2044") there were: 1) a decrease of the RNA content in motoneuron bodies of CESC and LESC; 2) an absence of RNA in the nuclei of motoneurons; 3) a decrease (by 12%) of nucleolus volume and CO activity (by 22%) were detected; 4) a trend to the ACHE activity decrease was revealed; 5) a decrease of the PG number (by 33%) (Table 1). With the extension of the period of rats' stay under the Earth gravity up to 48-51 hours after 14-day weightlessness ("Cosmos-1887") the RNA appears in dendrites of LESC motoneurons, the volumes of nucleus increases (by 11%), as well as that of nucleolus (by 11%), and AC number is increased (by 25%) (Table 2). However the RNA content in motoneuron bodies and PG number remained decreased. In the CESC after 14-day space flight and 8-11 hours of stay under Earth gravity after landing ("Cosmos-2044") there was a decrease of nucleolus volume (by 17%) and the ACHE activity in motoneurons and the AC number (by 26%), whereas the PG number was increased (by 45%) (Table 1). 14-day microgravity suspension did not induce morphological changes in LESC, except CO activity decrease; but in CESC the increase of motoneuron body, nucleus and nucleolus volumes and PG number were found (Table 1).

DISCUSSION
Absence of RNA in motoneuron dendrites in the flight rats ("Cosmos-2044") suggests a sharp decline of afferent impulsion, incoming to dendrites in weightlessness. At the same time a decrease of nucleolus volume, found in the same motoneurons, reflects a decrease of the level of basic biosynthetic processes in these nerve cells and finally indicates a decrease of their functional activity in weightlessness. This is also indicated by a ACHE activity decrease, more marked in the LESC. The CO activity decrease was revealed in flight rats and synchronous ground-based experiment rats, and therefore it is not proving one. A decrease of PG number in LESC of the flight rats indicates, evidently a decrease of the role of these cells for maintenance of neuron metabolism. The AP is localized in the capillaries endothelium and is participating in the active transport of metabolites through vascular walls. Therefore a decrease of the AP number in LESC of the flight rats ("Cosmos-2044") reflects a decrease of the number of actively functioning capillaries in weightlessness. The RNA appearance in motoneurons dendrites, increases PG number, increases nucleolus of motoneurons and an increase of the PG number in the LESC of rats in 48 hours after 14-day weightlessness ("Cosmos-1887") indicates the process of restoration of afferent impulsion incoming to dendrites, the increase of motoneurons functional activity and reflects the increase of muscular activity required for an animal to realize its motor activity under Earth gravity after weightlessness.

The spinal cord study in tail-suspended rats showed, that 14-day tail-suspension does not induce morphological
changes in LESC, like a 14-day space flight, while in CECS the morphological changes are arised indicating the increase of motoneuron function activity connected apparently with increased muscular load of forelimbs.

Thus the morphological and histoc
dematic data: 1) indicate a decrease of af
ferent impulsion incoming to dendrites of the spinal cord motoneurons in weight-
lessness; 2) confirm the earlier obtained
data on the LESC motoneurons hypofunction in weightlessness and indicate the deve-
lopment of this phenomenon in the CECS and
LESC already on the 14th day of weight-
lessness; evidently in result of a decrease
of impulsion incoming to dendrites; 3) indicate the restoration of the level of afferent impulsion incoming to den-
drites and an increase of functional activ-
ity of motoneurons under Earth gravity after weightlessness; 4) represent the PG
and AC participation in the motoneuronal
adaptation to functioning in weightless-
ness and under Earth gravity after weight-
lessness.

### TABLE 1. MORPHOLOGICAL AND HISTOCHEMICAL ANALYSIS OF MOTONEURONS, GLIA CELLS AND CAPILLARIES IN THE SPINAL CORD OF THE RATS FLOWN ABOARD BIOSATEL- LITE "COSMOS-2044"

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
</tr>
<tr>
<td>$V_B$, $\mu$m$^3$</td>
<td>$10733\pm554$</td>
</tr>
<tr>
<td>$V_n$, $\mu$m$^3$</td>
<td>$1811\pm62$</td>
</tr>
<tr>
<td>$V_{n1}$, $\mu$m$^3$</td>
<td>$38.0\pm1.3$</td>
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<tr>
<td>ACHE, O.D.U.</td>
<td>$0.289\pm0.04$</td>
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<td>PG, number</td>
<td>$1.2\pm0.11$</td>
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<td>AC, n/mf</td>
<td>$17.2\pm0.7$</td>
</tr>
<tr>
<td>$V_B$, $\mu$m$^3$</td>
<td>$16056\pm557$</td>
</tr>
<tr>
<td>$V_n$, $\mu$m$^3$</td>
<td>$2025\pm108$</td>
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<tr>
<td>$V_{n1}$, $\mu$m$^3$</td>
<td>$40.2\pm0.96$</td>
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<tr>
<td>ACHE, O.D.U.</td>
<td>$0.346\pm0.02$</td>
</tr>
<tr>
<td>CO, O.D.U.</td>
<td>$0.447\pm0.013$</td>
</tr>
<tr>
<td>PG, number</td>
<td>$2.5\pm0.27$</td>
</tr>
<tr>
<td>AC, n/mf</td>
<td>$13.3\pm0.5$</td>
</tr>
</tbody>
</table>

V - vivarium rats; S - synchronous ground-based experiment rats; P - flight rats; suspension - tail-suspended rats. + - significant differences in comparison with V; 2+ - significant differences in comparison with V and S.

### TABLE 2. MORPHOLOGICAL AND HISTOCHEMICAL ANALYSIS OF MOTONEURONS, GLIA CELLS AND CAPILLARIES IN SPINAL CORD LUMBAR ENLARGEMENT OF THE RATS FLOWN ABOARD BIOSATELLITE "COSMOS-1887"

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups of animals</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
</tr>
<tr>
<td>$V_B$, $\mu$m$^3$</td>
<td>$16064\pm1998$</td>
</tr>
<tr>
<td>$V_n$, $\mu$m$^3$</td>
<td>$1520\pm143$</td>
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<tr>
<td>$V_{n1}$, $\mu$m$^3$</td>
<td>$35.4\pm1.4$</td>
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<tr>
<td>PG, number</td>
<td>$2.5\pm0.18$</td>
</tr>
<tr>
<td>AC, n/mf</td>
<td>$15.4\pm0.28$</td>
</tr>
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</table>

REFERENCES
2. GORBUNOVA, A.V. AND V.V. PORTUGALOV. Effect of artificial gravity in space-
flight on the protein and RNA content in motoneurons of anterior horns of
RESULTS OF IMMUNOLOGICAL EXPERIMENTS ABOARD THE COSMOS BIOSATELLITES AND PROBLEMS IN SPACE IMMUNOLOGY

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INTRODUCTION

Studies have demonstrated a number of alterations in the immune systems of cosmonauts after extended periods aboard the Salyut-6, Salyut-7 and Mir orbital space stations (1), as well as in astronauts after flights on the Space Shuttle (2). There is a notable reduction in the ability of human T-lymphocytes to be activated. It has been shown that cells from cosmonauts after long orbital flight (from two to twelve months) had diminished phytohemagglutinin (PHA) reactivity of T-cells, functional activity of natural killer cells and T-helper cells, and suppressed production of immune mediators, principally interleukin-2 (3). A detailed quantitative analysis of immunocompetent cells in rats flown on Cosmos biosatellite flights 1884 and 2044 showed an increase in the splenic concentration of a certain population of lymphocytes (4). Research in animals (rats) and in isolated immunocompetent cells opens the way for a broad variety of investigations into the effects of zero gravity on the immune system.

The goal of the immunological experiments carried out on the Cosmos series of biosatellites was the elaboration of mechanisms that disrupt the immune system during flight. This paper presents data on several functional characteristics of lymphocytes in rats exposed to zero gravity.

METHODS

Male specific pathogen free rats of Czechoslovak-Wistar origin were flown on the Cosmos 1667, 1887, and 2044 biosatellite flights for 7 or 14 days. Flight, housing, feeding, age and weight conditions were as reported previously (5). After sacrifice, one-third of the spleen and two inguinal lymph nodes of each rat were dissociated into individual cells and suspended into supplemented RPMI-1640 medium. Bone marrow cells were extruded sterilely from the left femur of each rat. Cells from synovohous and vivarium control rats (5) were obtained in the same fashion as cells from flight rats. Cells from 5 different animals were included in each test group. Lymphocytes were tested for proliferation (incorporation of $^3$H-thymidine) and interleukin-2 (IL-2) production. Lymph node cells were also cultured with phorbol ester and calcium ionophore. Natural killer cell cytotoxic activity against two different tumor cell targets (YAC-1 and K-562) was also determined. In this case, $5 \times 10^6$ target cells were labeled with $^3$H-thymidine (10 $\mu$Ci in 3 ml of RPMI-1640 medium supplemented with 1 mg/ml of bovine pancreatic ribonuclease (6). After incubation (37°C with 5% CO$_2$ for 14 hr), the level of radioactive counts was determined using a liquid scintillation counter. IL-2 containing culture medium were assayed for the level of IL-2 production by determining the level of $^3$H-thymidine incorporation into CTLL cells, and IL-2 dependent cell line.

RESULTS

Incorporation of $^3$H-thymidine in cultures of splenocytes challenged with PHA was decreased equivalently after seven days and fourteen days of flight; this decrease was not observed in synchronous control rats and experimental controls with head-down tilt (Figure 1). An analogous effect was observed upon mitogen stimulation of bone marrow cells. Production of IL-2, as tested by activation of cell proliferation in cell line CTLL, was notably decreased in the flight group of rats (Figure 2). Cytotoxic activity of natural killer cells in the spleen and bone marrow was also suppressed significantly in rats in the flight group (Figure 3). The decrease in activity of natural killer cells in bone marrow occurred against a background of increased levels of T-lymphocytes, natural killer cells and a number of other subtypes (activated T-lymphocytes with IL-2 receptors, T-helpers) (4). In contrast to the spleen and bone marrow, functional changes were absent in cells from lymph nodes of the flight rats. T-cells were shown to be sensitive to activation by mitogens (concanavalin-A and PHA) after flight, just as B-cells continued to proliferate in the presence of Escherichia coli lipopolysaccharide. A deficit in production of IL-2 by T-cells was not observed.

DISCUSSION

These results from experiments on rats exposed to flights of one to two weeks duration show a definitive decrease of the following basic functional characteristics of lymphocytes in the spleen and bone marrow: T-lymphocyte activation (blastogenesis), activation of natural killer cells, and production of interleukin-2. Analogous changes were, as a rule, also seen in cosmonauts after extended space flights. Such alterations, seen in cells from both people and rats after exposure to zero-gravity conditions, support the use of data and materials derived from experiments in rats as a model of space flight-induced immunodeficiency. A detailed study...
The present results show a decrease in the functional effector population of splenic lymphocytes by virtue of their concentration in that organ. This implies that this observation, made for the first time in experiments aboard the Cosmos biosatellite, follows a regular pattern demonstrated not long ago in investigations at the molecular level (7,8). The authors showed that in cosmonauts who served aboard space stations Salyut-7 and Mir, there was generally a sharp decline in production of lymphocytic interleukin-2 as measured by the functional activity of this cytokine. At the same time, a quantitative analysis using the ELISA assay showed just the opposite result, namely, an increase in IL-2. It was hypothesized that perhaps a compensatory change in the production of substrate caused a loss of biologic activity. In this work a similar change was observed at the cellular level. It is also possible that such an observation is related to immunodeficiency secondary to some other cause.

Another interesting observation is that in lymph nodes, in contrast to the spleen, the activation of lymphocytes was not decreased after a fourteen-day flight on biosatellite Cosmos 2044. B-cells were seen to remain proliferative in the presence of lipopolysaccharide from E. coli and there was no deficit in the production of IL-2 by T-cells. Production of IL-1 by accessory cellular elements also showed no change. The difference in reactions of splenocytes and lymph node cells to flight suggests new research possibilities directed toward elucidating the role of stress hormones and other biological products in the circulation during stress and increased gravity.

ACKNOWLEDGEMENTS
We thank Dr. A. Kaplansky and the Cosmos Dissection Team for their extraordinary efforts in obtaining samples for us.

REFERENCES
EFFECT OF SPACE FLIGHT ON IMMUNE RESPONSES: BONE MARROW CELL RESPONSE TO COLONY STIMULATING FACTOR AND LEUKOCYTE SUBSETS

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INTRODUCTION

For the past several years, alterations in immunological parameters have been reported after space flight (1-3). These changes have included alterations in functional immune responses as well as alterations in the distribution of leukocyte subpopulations (1-3). The purpose of the studies we carried out on the Cosmos 1887 and Cosmos 2044 biosatellites was to define further the immunological parameters affected by space flight. The Cosmos 2044 flight permitted a similar, but not identical, set of experiments to be carried out as was carried out on the Cosmos 1887 flight. In addition, direct comparison of the effects of antiorthostatic suspension with the effects of space flight on immunological parameters was carried out during the Cosmos 2044 flight. These studies included the determination of the ability of bone marrow cells to respond to monocyte or granulocyte-monocyte colony stimulating factor (M-CSF or GM-CSF) and the distribution of immunologically important leukocytes as determined by flow cytometry.

METHODS

Male specific pathogen free rats of Czechoslovak-Wistar origin were flown for 12.5 (Cosmos 1887) or 14 days (Cosmos 2044). Flight, housing, feeding recovery, age, weight and sacrifice conditions were as reported for the entire missions (4,5). Procedures were similar after both missions. After sacrifice, bone marrow cells were extruded steriley from the left femur of each of 5 rats. One-third of the spleen of each rat was dissociated into individual cells and placed into supplemented RPMI-1640 medium (4). All samples were held at 4°C and transported from the recovery site to Moscow where analytical work began approximately 30 hr after harvest of the tissue. Additional rats were suspended antiorthostatically by the tail for the same time period as the Cosmos 2044 flight. Cells from synchronous control, vivarium control, and antiorthostatically suspended rats were obtained in the same fashion. M-CSF was a gift of Dr. Robert N. Moore, University of Tennessee, and GM-CSF was a gift of Dr. Steven Gillis, Immunex Corp, Seattle, WA. For CSF assays, the CSF was incorporated into a semi-solid agar layer and the number of colonies of 50 or greater bone marrow cells formed was determined after 5-7 days of incubation (4). For the leukocyte subpopulation determination, spleen and bone marrow cells were stained with a variety of antibodies directed against cell surface markers obtained from WAKO or Accurate Scientific, maintained at 4°C and transported to Louisville for flow cytometric analysis (4).

RESULTS

Bone marrow cells from both flight and suspended rats had a severely reduced capacity to respond to either M-CSF (biosatellite Cosmos 1887) or GM-CSF (biosatellite Cosmos 2044) by forming colonies of cells on soft agar. This was in comparison to cells from both synchronous and vivarium control rats.

For both flights, a higher percentage of spleen cells expressed CD4 and CD8 markers in the flight as compared to synchronous and vivarium control cells. Only after the Cosmos 1887 flight, a higher percentage of cells expressing interleukin-2 receptors was observed.

For the bone marrow cells, a complete analysis was possible only after the Cosmos 2044 flight. For the Cosmos 1887 flight, the only change observed after flight compared to cells from synchronous control rats was an increase in the percentage of cells carrying surface immunoglobulin in the myelogenous bone marrow cell population. After the Cosmos 2044 flight, increases in the percentage of cells expressing CD4, interleukin-2 receptors, and the target of anti-asialo GM-1 antibody (primarily natural killer cells) in the lymphoid population were observed after flight compared to cells from synchronous and vivarium controls. In the myelogenous population, only the percentage of cells staining with CD4 was increased after flight.

Cells from suspended rats showed no changes in the percentages of cells expressing markers that had no correlation with the changes induced after space flight.

DISCUSSION

The current studies provide data that indicate that both functional immune responses and the distribution of leukocyte subpopulations can be altered by space flight. This both confirms previous reports that suggested this possibility (1-3) and extends the functions and subpopulations examined. The Cosmos 2044 biosatellite flight had an advantage over the Cosmos 1887 biosatellite flight. In the Cosmos 1887 flight, the biosatellite landed off-
course and there was a serious delay in sacrifice after landing, perhaps allowing some re-adaptation to gravity (4). There was no such delay after the Cosmos 2044 flight.

The ability of bone marrow cells to respond to M-CSF or GM-CSF was impaired after both flights. This suggests that space flight could impair the ability of bone marrow cells to divide, differentiate, mature and then carry out immune functions.

The results of the leukocyte subpopulation distribution analysis were consistent in both flights, but not identical. Differences in the subpopulation analysis could have been due to changes in flight and recovery conditions. In any cases, these changes in subpopulation distribution could contribute to the wide variety of changes in functional immune responses observed after space flight, including alterations in lymphocyte blastogenesis and cytokine production (1-3).

The suspension study also suggests that antithorostatic suspension is a useful model for modeling the effects of space flight on functional immune responses such as activity of CSF and other cytokines or cytokine production. Antithorostatic suspension does not appear to be a useful model for the effects of space flight on leukocyte subpopulation redistribution.

ACKNOWLEDGEMENTS

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DYNAMICS OF STRUCTURAL CHANGES IN SKELETAL MUSCLE NEUROMUSCULAR JUNCTIONS OF RATS UNDER THE INFLUENCE OF THE SPACE FLIGHT FACTORS

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INTRODUCTION

As reported previously (1), neuro-muscular junction (NMJ) responded to a damage by the plastic reconstruction, which included a set of rather standard structure reactions. Pathological process specifics was a result of the standard reactions combination, their intensity and appearance time. Moreover, NMJ responds structurally as a whole. From this point of view it was interesting to study the synaptic apparatus of muscles after the action of damaging factor (or factors), which animal organism did not meet in evolution and the reaction to which was not programmed. This was important from practical point of view as well, because space flight factors and main of them - weightlessness - influence substantially the neuro-muscular system.

METHODS

Ultrastructural analysis was performed postflight on skeletal muscle of rats orbited for 7 and 13 days aboard the "Cosmos" biocentres 1667 and 1687. Motor end plates were studied in antigravity soleus muscle (SM), only partly weight-bearing gastrocnemius muscle (GM) and continuously contracted diaphragma muscle (DM). The latter is typical cross-striped muscle, in spite of some structural peculiarities. They were compared with their muscles of the synchronous and vivarium control rats.

Specimens of the muscles were fixed by immersion in buffered solution of formal-sucrose, and postfixed in 1% osmium tetroxide; ultrathin sections were stained with uranyl acetate and lead citrate.

RESULTS

Atrophic-dystrophic changes developed in muscle fibers of the soleus muscle of the rat orbited for 7 and 13 days. They were very commonly observed in all structures and organelles of myofibers: myofibrilles, nuclei, mitochondria, sarcoplasmic reticulum.

The structural reconstruction took place in neuromuscular junctions. As a rule, junctions on the preserved muscle fibers consisted of few axon terminals (AT). A quantity of synaptic vesicles (SV) in the terminals was variable, as in normal conditions. Large ribbon-like mitochondria were commonly preserved. Marked decrease of SV amount, clearance of axoplasma, lysis of organelles and vacuole formation were found in some motor nerve terminals. The invasion by processes of the hypertrophied Schwann cells (SC) between TAs and postjunctional membrane took place (Fig. 1). At advanced stages of TAs degeneration Schwann cells revealed phagocytosis activity. Commonly, there was no destruction in subsynaptic region.

Nevertheless, growing axones were found in extracellular area. Besides, there was little difference between the state of neuro-muscular junctions after 7 and 13 days space flight, in spite of pathological process progress in muscle fibers. Neuro-muscular junctions were determined on fragments of muscle fibers even after the breakdown of the latter. The terminal sprouting was determined in these cases. All these data are suggestive of regeneration process, which took place simultaneously with degenerative one.

The main result of the process was the diminishing of synaptic contact area in soleus muscle, related to the destruction of presynaptic structures. The present findings demonstrated that some motor end plates exhibited either total or partial denervation. The morphological changes were similar to those seen after axotomy.

In muscle fibers of gastrocnemius muscle early atrophic changes were found only. The synaptic zone was expanded and included 7-9 small TAs. Increased quantity of SV were found. They were round and occupied practically all free volume of terminals. In some junctions synaptic folds were shortened and deformed, cholinoreceptor zone was indistinct. In another - synaptic folds were deep, anastomosed, with broadened cholinoreceptor zone. These are morphological signs of neurosecretion process activation.

Two types of degenerative changes were detected in TAs, which were defined as vacuole and fragmentary degenerations (Fig. 2). They were connected probably with different muscle fibers types. Growing axones in synaptic zone suggested the simultaneous regenerative process. Schwann cell activation was absent. In sole-plate of NMJs characteristic spiral multilayer structures appeared (Fig. 2, 3). We believe they are some kind of sarcoplasmic reticulum modification. They were found more often after 13-days space flight. The structural reconstruction of synaptic apparatus in GM was adaptive-compensatory one.

Figure 1. SC processes (arrow) interposed between TA lacked SV and postsynaptic membrane.
continuously worked muscle were signs of synaptic reconstruction: expansion of the synaptic zone due to increase of TA number. Postsynaptic membrane formed numerous hypertrophied synaptic folds with atypical discretely increased cholinoreceptor zone in some junctions (Fig. 4). These data suggested the synaptic function activation as well. However, denervated motor end-plates were found very seldom.

DISCUSSION
Thus, ultrastructural changes were found in NMJs of all studied muscles. The severity and character of the changes differed in muscles with different functions and depended on flight duration. They were most pronounced in the SM and least - in DM (2). Undoubtedly the appearance of the alterations in DM was of great interest.

Atrophic-dystrophic process was determined in SM (2, 3). Probably this was connected not only with the influence of weightlessness, but also with disturbing of neuro-muscular interactions due to alterations in synaptic apparatus of the muscle.

The enlargement of synaptic contact zone in GM seems to be inadequate. However, we considered the reconstruction of synaptic apparatus in this muscle as adaptive-compensatory process, which strengthened neurotrophic influences. Probably, this explains the delay in the developing of atrophy process in the muscle. Synaptic region of muscle fiber is most reactive and in the same time most stable to an injury. This provides trophic substances need for the structure regeneration.

Specific structure reactions were determined in neuromuscular junctions of the muscle. However, they could be not only the result of weightlessness, but a general disturbing of metabolism as well, especially calcium metabolism.

Destructive-regenerative process took place in synaptic apparatus of skeletal muscle of flight rats, as in other forms of neuro-muscular pathology. The changes, found at these stages of the process were reversible.

REFERENCES
THE POSSIBLE MECHANISM OF CONJUGATION BETWEEN MYOFIBRILLAR AND CELLULAR
BIOMECHANICS IN MUSCLE AND ITS ALTERATION UNDER WEIGHTLESSNESS

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It is known that physiological and biomechanical properties of skeletal muscle are affected by the pattern of muscle activity. Although these changes must be analysed on the level of integer reprogrammation of muscle cell some factors can be singled out as of major significance. One is transformation of isomyosin spectrum during adaptation to the extremal conditions. Lately we have shown on the ground based models of weightlessness [1], and then in the rats exposing aboard the "Cosmon" biosatel-lites [2,3], that properties and composition of contractile and regulatory proteins from postural and fast moving muscles can change in opposite directions. The similar results were observed by other authors after muscle inactivation by different ways [4,5]. In most cases the changes were strongly expressed in slow muscle fibres which displayed high sensitive reaction to the weightlessness. On the other hand, the changes in isometric tension generation, usually have the same trend for fast and slow muscles [6,7].

This discrepancy is difficult to explain without an understanding of underlying mechanisms of conjugation between the contractile activity of myofibrillar apparatus and biomechanics of the muscle cell. It is generally accepted that speed of muscle shortening relates with the myosin ATPase activity [8] whereas the force generation by sarcomere is determined by the number of active cycling myosin bridges [9,10]. In this connection, changes in tension of isometric contraction are usually interpreted in terms of alterations of rate constants in cross-bridge cycle [11].

![Diagram](https://via.placeholder.com/150)

Figure 1. Metamorphoses of time courses of SPP (solid lines) and Mg²⁺-ATPase (dotted lines) of actomyosin under different conditions. Ct – contraction, Rlx – relaxation, RCF – rigor complexes formation.

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Recently we have presented another approach to this problem based on studies of the complex formation, so-called superprecipitation (SPP), and Mg$_2^+$-ATPase reaction in actomyosin solutions under physiological ionic strengths.

It has been shown that active striction of actomyosin particles takes place only in one (I) of two (Fig. 1) "classic" mechanisms of SPP [12]. In the II mechanism SPP is due to the rigorous complexes formation and exhaust stocks of ATP. Changes in experimental conditions induce gradual transitions between two alternative mechanisms of SPP (and Mg$_2^+$-ATPase) via the mixing, two-stage mechanism which reflects a superposition of two main mechanisms [13,14]. These findings lead us to conclusion that the shifting in equilibrium between two matter mechanisms of SPP is due to the mutual transitions of their "carriers", i.e. two types of actomyosin complexes [12,13]. It appears that transitions can be induced by different factors (Fig. 2) and are characterized with low free energy ($\Delta C^0$ -4.9kJ/mole [13]) what makes it possible their realization at body temperatures.

Based on these data we have submitted the two states model for force regulation in muscle cell functioning via an alteration of the alignment of "active" (participating in contraction), and "passive" (easily dissociating) acto-myosin complexes (Fig. 2). Our present investigations reveal good correlation between the changes in maximal tension of contraction, prophesied by this scheme, and real changes observed on single skinned muscle fibres (unpublished data). The same conclusion follows also from the comparison of these findings with the existing data of mentioned factors action upon the force generation in muscle [16]. Moreover, thermodynamic parameters for temperature induced transitions I II states of actomyosin coincided with parameters evaluated for temperature-dependent transitions of glycerinated fibres from the low isometric force generating state in the state with high force [13,16].

$$\uparrow pH, [ATP], \mu; \downarrow [Ca^{2+}], t^\circ C$$

![Diagram](image)

Figure 2. Hypothetical two states model of actomyosin complex. The effects of mentioned factors have been observed experimentally [12-15]. ↑ and ↓ are rising and lowering respectively.

These facts favour the view that transition of acto-myosin complex into "active" (in our term, I) state in vivo can lead to the rising of generated tension. Of course, we can not deny the way of force modulation
through an alteration of kinetics of cross-bridge cycle [11], however this mechanism may be essential perhaps, in respect of active state only.

The dynamics of transitions between two states of actomyosin can be traced experimentally by dependences of chosen physico-chemical factors upon the extent of SPP for two mechanisms [13-15]. The typical dependences of this type and two allowable variants of their alteration are demonstrated in the Fig.3.

The first type of modulation of transitions is due to the change in cooperativity, but not in the fullness of transition (Fig.3A). This effect must be ascribed to the predominance of different (slow and fast) myosin isoforms in actomyosin preparations from m.Soleus and Extensor Digitorum Longus [17]. From this it is clear that changes in isomyosin spectrum can govern the cooperativity of transitions between two states of actomyosin complex. Such mechanism may secure increasing in the rate of enhancement of tension of glycerainated fibres from m.Soleus and m.Tritoepe in rats flown aboard the "Cosmos" biosatellites [6,7] for account of transformation its isoprotein spectrum in a manner of that for fast muscles [3,18].

The second type of modulations couples with the changes in the fullness of transition (Fig.3B). It takes place under dramatic changes in conditions, or after "rough" processing of protein (partial denaturation, limited proteolysis, etc.). For example degradation of tropomyosin-troponine complex during space flight and so, declining of Ca-sensitivity of actomyosin [2,3,18], according to our scheme, must shift an equilibrium to-

Figure 3. Two kinds of modifications of transitions between two mechanisms of SPP. Dependences of extent of SPP for two mechanisms of this reaction: A (by [19]) from substrate concentration for natural actomyosin from EDL (solid lines) and m. Soleus (dotted lines), and B (by [15]) from pH for actomyosin from m. Quadriceps at relatively high (solid lines) and low (dotted lines) ionic strengths.
wards the "passive" state of actomyosin (it can be increased by leakage of Ca\(^{2+}\)). That can lead to the lowering of maximal tension which have been observed in postural Soleus and Triceps muscles under weightlessness conditions [6,7].

Thus, proposed regulatory mechanism and submitted scheme give reasonable explanation for discrepancy in speed and force alterations under weight unloading of muscles. It should be noted that the more exhaustive analysis requires an account of contribution of series of intracellular medium factors which can be changed at these conditions (e.g., K\(^+\)-balance, water-saline homeostasis, macroergic phosphates content, etc.). That is another essential link of function of myofibrillar proteins and contractility of muscle. The submitted model makes it possible such approach [19] and, thereby, may be helpful for an understanding of underlying mechanisms of conjugation between the changes in biomechanical traits of the muscle cell and the properties of contractile apparatus.

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INFLUENCE OF WEIGHTLESSNESS ON ERYTHROID SYSTEM IN MONKEYS


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INTRODUCTION

One of the important and undecided problems of space medicine is a diminishing of erythrocytic mass in cosmonauts (3). Relative to this problem complex studies, including cytological, biochemical, radiometric investigations of blood and bone marrow in primates exposed in weightlessness, are very important for study of developing of anemic syndrome in weightlessness.

METHODS

The results of investigations in 4 primates exposed in weightlessness during two week space flights on board biosatellites "Cosmos 1887 and 2044" are united. The next parameters were determined: plasma volume (with 131 albumine), content of erythrocytes in vessel bed (with complex Tc peroxidase and tin-pyrophosphate), erythrocytes life's duration and erythropoietic function of bone marrow (with i.v. marker of erythrocytes by 51 Cr and 59 Fe in indicator doses), lipid and phospholipid composition of erythrocytic membranes by thin-layer chromatography method, metabolic parameters of erythrocytes by spectrophotometry method. Na+, K+-ATPase by dephosphorilution of ATP, Na+ and K+ content in cell by flame photometry, erythrocytic membrane resistance by acid erythropgrams method, surfacial architectonic of red cells with electron scanning microscopy, cytological composition of bone marrow with the help of 500 cells and smears of sternum's puncture. All these investigation were performed before the flight and in different dates after landing.

RESULTS AND DISCUSSION

Biochemical studies, performed right after biosatellite "Cosmos-2044" landing and in 2 days after biosatellite "Cosmos-1887" landing have showed a considerable change in morphfunctional condition of circulating erythrocytes' membrane: there was changed a lipid and phospholipid content (increasing of cholesterol's ethers, lysophosphatidilcholine and reducing of phosphatidylethanolamine), indicative of change membrane's phase condition; there was noted a raising the membrane's permeability that confirms by inducing K+ and Na+ ATPase activity and by increasing Na+ cell's level.

Noted changes in structural composition erythrocytic membrane led to the rising of hardness and fragility and decreasing its resistance to hemolytic factors that confirmed by more rapid hemolysis occurrence.

Destructive cell's changes were affirmed with data about 2 times decreasing of reconstructed form of glutathione content.

All of this was reflected in superficial erythrocytic architectonic. On the 2nd of readaptation period after biosatellite "Cosmos-2044" flight there was noted somewhat increasing in echinocytes cells of the 1 and 2 deformation stages, which were characterized by buige of discs and tuberculum appearance on their surface. Shown changes are responsible for a rising of erythrocytic pool with shorter circulation period of cells. Direct testing with using in vivo radionuclide Sr performed on day 2 after the "Cosmos-2044" biosatellite flight showed that erythrocytic life period was shortened by more 40%.

Erectrogenic's system indexes study revealed 1.5 times rising of ATP, 3 and 5 times rising of lactate and 2 times increasing of hexokinase. So markedly expressed activation of energy metabolism in erythrocytes was caused apparently by gravitation stress, which developed during a transition from weightlessness to Earth gravity (2). At the same time it should be taken into consideration that more higher metabolic level is so characteristic for cells, having more short period of their life (1). Judge by noted changes a part of these cells present in erythrocytic population on day 0 and 2 after landing.

Erythropoietic function of bone marrow in this period was studied in one monkey on day 0 after "Cosmos-1887" biosatellite flight and in two animals on day 2 after "Cosmos-2044" flight. It was stated that time of biged plasma clearance from i.v. injected 111In radionuclide increased in first monkey by 52% and in two others by 10 and 18%. These changes may be due to a number of reasons: a decreased transferrin synthesis and its lowered blood level, a disturbance of mechanism of its binding to iron, a decreased ability of the bone marrow to uptake this type of iron. The existence of one of stated above reasons and, moreover, their sum caused an reducing of hemoglobin production and transferring of erythroid system on more low functioning level, which caused, in turn, a reducing of erythrocytic production in bone marrow: output of marked erythrocytes in circulation was decreased by 25-40%, that was reflected in circulating erythrocyte volume (by 16,3% in first monkey, and 6,2 and 7,8% in the others).

In earlier adaptive period there was revealed an increased value of hematocrite, which could be explained by erythrocytic circulation volume. It was shown that in primates after landing in space flight a hydration level decreased, mostly by diminishing its extracellular fluid.

On day 6 after landing "Cosmos-2044" biosatellite there was made a cytological study of sternum punctured bone marrow. For this readaptation period hemopoietic cells
have passed bone marrow developing period, including proliferate and mature pools. Cytological content of bone marrow presented itself by population of cells, formed in conditions of developing a new homeostasis, answering to increased demands of system.

General myelogram and partial erythroblastogram revealed marked signs of erythroid production activation, manifested in increasing of cells - early generation of erythrocyocytes in 26-62% and in increasing of erythroid production level that was confirmed by twice rising of bone marrow reticulocytes and their precursor-cells.

Rejuvenation of circulating erythrocytes population confirms by the data about increasing of synthetic processes activity in venous red cells. on day 8 after the flight. So, on activity G6PDH - key ferment of pentose phosphate way of glucose metabolism, providing a synthetic processes by substrata by 34% and 89%, content of lactate was 3 and 6 time increased, content of reconstructed form of glutathione was normalized. In this period a hematocrit index returned to normal level.

To day 30 of readaption period there was noted a normalization of erythropoiesis with reconstruction of cytological content of bone marrow and normalization of metabolic processes in cell; at the same time some changes in morphobiochemical membrane's condition remained.

So, changes revealed in different links of erythroid system are adaptive character and directed on a new homeostasis forming which is adequate to space flight conditions or to Earth gravity readaptation. There are 2 processes which take part in reduction of circulating erythrocytes pool:
- the first is a destructive, connected with changes in morphofunctional membranes condition and the second - is systemic decreasing of erythropoiesis led to reducing of red cells life period and to reducing their production by bone marrow. At the same time primate's bone marrow during two weeks space flight reserves a high potentiality of physiological regeneration permitting to normalize erythroid blood production during the month.

REFERENCES
Erythrocyte functional status and adrenoreactivity in monkeys after space flight and control experiment.

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INTRODUCTION.

Prolonged exposure to real and simulated weightlessness results in marked structural and functional changes in erythrocyte membranes, primarily adaptive in character. In the development of adaptational responses and providing conditions necessary for vital functioning of the body under extremal circumstances an important role is played by the hormonal mediator elements of the sympatoadrenal system (SAS), an important part of which are B-adrenoreceptors bearing the initial effect of the altered environment, and responding to them most quickly.

MATERIAL AND METHODS.

In connection with this in monkeys exposed on board Cosmos 2044(SF) and in a control experiment (C), an investigation of structural and functional properties including adrenoreceptor ones, was performed simultaneously with a study of hormonal and mediator elements of SAS which was evaluated by the excretion of catecholamines (CA), epinephrine (E), norepinephrine (NE), dopamine (D) and doxyphenylalanine (DOPA). These parameters were measured in 24-hour urine using spectrofluorometric techniques. The status of erythrocyte membrane was evaluated by lipid and phospholipid spectra (using thin-layer chromatography), transport ATP-aze activity (using ATP dephosphorylation), intracellular Na+ and K+ ion content (by flame photometry), parameters of binding of a DSP-6 (p-toluidine sulphonate-4-(p-dimethylanisosteryl) -1 - gelseperidine) to the membrane.

B-adrenoreactivity was studied using antihemolytic test parameters characterizing the interaction of propranolol (a B-receptor blocker) in increasing concentrations with the membrane adrenoreceptors.

RESULTS AND DISCUSSION

Results of the study reveal changes both in the metabolic status of the cell and in the structural and functional membrane status. Thus, on day 0 after completion of space flight both monkeys showed a fall of cellular membrane resistivity (a leftward shift of the erythrogram), an increased velocity of hemolysis, a decreased time before 100% hemolysis was achieved. Later, on day 8, there was a rightward shift of the erythrogram, on day 14 some normalization was seen, on day 30 a marked leftward shift occurred. The observed character of changes confirms the existence of shifts in structural and functional membrane status. Thus a study of membrane lipid and phospholipid spectra in monkeys after the flight revealed a marked increase of cholesterol esters (CSE) with a relatively constant levels of phospholipids (PL) and free cholesterol (FC), which led to an increased ratio of FC+CSE to PL (fig 1.).

The changes of control experimental parameters were of a similar nature. The increase of CSE in the membrane is known to cause an increase in membrane permeability, which is related to the structure and character of incorporation of these molecules into the lipid layer. The increased FC+CSE/PL ratio itself indicates a change of lipid bilayer structure, its phasal status. An increase of membrane microviscosity, its rigidity was registered also using a fluorescent probe. This was associated with a study of the concentration of the membrane-bound probe (R), binding constant (K), concentration of binding sites (N); a calculation of the KN coefficient characterizing the effectivity of binding and the ratio of


FIG.2. Parameters of binding DSP-6 with membrane(flight-14d)
intensiveness of fluorescence of the probe with a wave length of 360 nm (binding within the lipid layer) and 740 nm on its surface. It was shown that immediately after flight (on day 0) all the parameters of binding of the probe to the membrane lowered (fig.2).

The most marked changes were noted in the control experiment. However in this case the parameters studied normalized already by day 7, while after the flight a tendency to normalization of the parameters studied was noted only by day 3 (fig. 2).

The noted changes in the parameters of probe binding to the membrane, together with the data characterizing the intensiveness of probe fluorescence with varying wavelengths indicate an increase of membrane viscosity, decreased hydrofobity, and a lowered effective negative charge.

Changes of membrane phase status, modifications of lipid bilayer, lead to a molecular resetting of the membrane protein components, in particular, of transport ATP-aze activity and B-adrenoreceptor sensitivity. After the flight and the ground-based C experiment, there was a marked fall in Na and K-ATP-aze activity, the decrease of Na, K-pump intensity led to a decrease of intracellular K content. More marked changes were noted in Zhaconya after F on days 0 to B; the layer suggesting a disturbance of cellular membrane permeability.

Erythrocyte membrane has functionally active B-adrenoreceptors and responds to agonistic effects by an increase in the magnitude of hypotonic hemolysis, a decrease of erythrocyte deforming capability. This effect is prevented by B-adrenoreceptor blocker propranolol which also has a marked anti-hemolysis effect. In certain concentrations this effect is dose-related, which allowed, on the basis of plotting and mathematical analysis of the curve, to calculate the following parameters of interaction between propranolol and erythrocyte membrane: Hill’s coefficient, dissociation constant, minimal propranolol dose inducing the effect and the maximal dose; cooperation index, membrane-stabilizing effect for an average concentration.

Earlier animal experiments have shown a unidirectional change of adrenosensitivity of various tissues and parameters of anti-hemolysis test. From this it was concluded that by the erythrocyte membrane response to propranolol the body adrenoreactivity on the whole can be evaluated in vivo.

Immediately after the flight both monkeys showed an increased B-adrenoreceptor sensitivity, judging by an increased affinity to the given blocker, a lower sensitivity threshold, widening of the range of active concentrations. The lowering of Hill’s coefficient is also indirect evidence of a decrease of the number of adrenoreceptors. It is believed that during stress desynthesis may form with a decreased number of adrenoreceptors and diminished affinity to KA.

Results of a study of KA excretion did not reveal an activation of the stress realizing system of the hormonal element at the E level and of the mediator element at the NE level, characteristic of extremal circumstances (fig.3). A simultaneous activation of these two elements was seen on day 7. However on day 0 there was a significant mobilization of KA and DA resources, as well as DOPA, suggesting a strained state of the SAS. A lower E and NE excretion can probably be explained by higher requirements of the body for these bioregulators and a quickening of their metabolism.

![Graph](https://via.placeholder.com/150)

**FIG.3. Extention of E, NE, DA, DOPA (flight-14d)**

It is also possible that the resonance of the cathecholaminergic system to extremal circumstances depends upon the initial status of the SAS. In monkey (Zabiyaka) with a higher KA excretion during the baseline study there was an increased SAS responsiveness immediately after SF and C experiments, with a further manifestation of an insufficiency of reserves of E and NE synthesis. A markedly multidirectional nature of SAS changes during the control experiment was probably the reason for varying changes of the anti-hemolytic test, which may be the consequence of individual membrane sensitivity and of a less marked influence of experimental conditions.

Thus, the results obtained show that early readaptation after SF, HDT and to lesser extent the control experiment, leads to a noticeable strain of SAS. As a natural consequence of the physical and chemical membrane status the structure of its lipid bilayer, the stability and a decrease of the effective negative charge, the activity of membrane-bound enzymes. Of importance is the confirmation of conclusions which we arrived at earlier for humans concerning the close relationship between SAS and the membrane status, in particular its adrenoreceptor element.
ELEMENTAL BONE COMPOSITION OF THE RATS FLOWN IN "COSMOS 2044" BIOSATELLITE

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INTRODUCTION

It has been established in the experiments with the rats exposed in the biosatellite that the lifting of the weight and static loads in the conditions of weightlessness results in the development of osteoporosis (2), a decrease in the bone strength properties (7) and in inhibiting the process of the bone posttraumatic regeneration (3). It is supposed that the processes of the bone tissue mineralization are broken at the same time. The purpose of the present investigation was to study the content of the macro- and microelements which were closely connected in metabolism both with the bone mineral and organic components in the structurally different skeleton sections of the rats in which the healing process of the bone fracture took place in the conditions of the space flight.

METHODS

The method of the neutron activation analysis having permitted to determine the content of 16 elements in 5 different fragments of the bone tissue including the organic and mineral parts was used. The investigation was carried out with 40 male rats in the half of which the experimental fracture of the fibula was made in the its central parts 2 days before the space flight or "tail suspension" in the antorthostatic position during the 14-day (3). The following was investigated in the traumatized animals: the middle part of the diaphysis, proximal proximal metaphysis and caput in the humerus, fibial metaphyseal part and proximal part of the fibula separated in the fracture line. The analogue bones of the animals kept in the conditions of the vivarium and spaceship model (synchronous control) served as a control. The specimans were centrifuged to remove the bone marrow and dried to the constant weight at a temperature of 105 C. The investigation was carried out with the use of the fast neutron reactor. The concentration of elements was calculated per 1 g dry substance.

RESULTS AND DISCUSSION

The comparison of the fibular element composition in the operated and intact rats kept in the conditions of the vivarium has shown that 17 day bone callus (Fig.1) had a decreased concentration in the majority of the investigated elements: by 25% in Ca, 20% in Cr, 14% in P, 10% in Ba, 20% in K and Rb, but concentration of J increased as many as two times. A temporary loss of the elements for this period of time corresponds to the phase of the ossification beginning and posttraumatic change of the bone regenerate and takes place not only in the bone callus, but also in the other parts of the skeleton (6). The demineralisation effect of the trauma should tell in a certain way on the element composition of the other bone samples of the traumatized animals.

The concentration of Ba decreased (by 12-29%) and the concentration of Br and Co increased (by 2-4 times and 10-55%, respectively) in all the specimens of the flight rats (Fig.2). A decrease in Ba testifies to the violation of the ossification process in the bone matrix (6, 7). The Co accumulation in the bones is probably connected with a decrease in the utilization in the synthesis of vitamin B12 in the composition of which it is contained in the organism that well correlates with the inhibition of erythropoiesis under weightlessness (1, 4). A considerable increase in the concentration of Br and, in some fragments of J connected in metabolism with the thyroid gland hormones (5,6,9) supposes the violation of its functional activity and, probably, is specified by the stress genetical factors of the content to which a still more expressed accumulation of these elements in the rat bones of the synchronous control testifies. The disbalance of the Br and J accumulation in some individual specimens of the flight and tail suspended rats indicates the absence of the complete analogous effects.
of the space flight and used model of the antigravitational hypokinesia. As for the rerearrangement the concentrations of Ca, P and Sr included in considerable quantities in the composition of the hydroxyapatite crystals. Their decrease was noted only in the tibial metaphysial part and made up 15, 15 and 9,5% in the indicated succession. In the other fragments their concentrations increased: Ca in the caput of the humerus by 23%, P in the diaphysis of the humerus and in the traumatized fibula by 25 and 12%, respectively, Sr in the traumatized fibula by 9%. For Mg, Na and Zn being of the obligatory components of the bone tissue their decrease was noted only in some individual fragments. The highest loss of all the macro- and a part of microelements was marked in the tibial metaphysial part characterized by the highest level of metabolism: the concentration of 10 elements from the 16 investigated ones decreased by 10–30% (Fig. 2.4) The Ba, Zn and J contents decreased in the bone callus of the flight rats. Since Ba and Zn participate in the ossification processes (6) and J exists in a considerable quantity in the decreased content of these elements in the callus testifies to the violation of the mineralization processes of the synthesized bone matrix in the conditions of the flight. The changes of the element composition in the non-traumatized fragments of the bone tissue were less expressed in the “tail suspended” rats and a delay in the mineralization of the bone regenerate was greater than in the flight ones.

The rat bone tissue of the synchronous control was characterized by a greater mineral saturation, especially, Br and J. Thus, the factors of the space flight exert a modifying influence on the processes of the bone posttraumatic regeneration.

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METABOLIC STUDY IN PRIMATES EXPOSED TO MICROGRAVITY ON BOARD "COSMOS" BIOSATELLITES

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INTRODUCTION

In spite of a large body of metabolic studies in man and animals during space flight and after its finishing, this problem remains actual for space medicine and biology. Changes in cardio-vascular system, reduction of functional loading on sympathomuscle system, which were noted in man and animals during long- and relatively, short lasting stay in weightlessness and hypokinesy leads to metabolic changes that in complex may be the cause of lowering of reserve possibilities of living organism.

Experimental data received after finishing space flights of different duration revealed a loss of body mass, decrease of blood volumes and extracellular fluid, changes in activity of energetic and plastic metabolism, markedly changes in mineral metabolism and systems of its regulation.

One of the important facts is a loss of Ca in organism. In this situation a development of negative Ca balance takes place not only for reason its kidneys excruction increasing but also as a result of reducing its absorption velocity in the intestine

METHODS

In complex experiments on board "Cosmos" biosatellites the objective of metabolic primates studies was to investigate biochemical indices of homeostasis in blood and urine samples after 5, 7, 13 and 31-day space flight to appreciate weightlessness's influence and transition to Earth gravity on organism.

In accordance with these tasks in pre-flight period there were tested more than 40 primates, which had been prepared for space flight's programme, to receive background points, and 8 animals exposed to microgravity on board "Cosmos-1514, 1667, 1887 and 2044" biosatellites. To each flight corresponded a ground based control experiment performed in a mock-up of the biosatellite.

Water-electrolyte metabolism's indices were studied in restrained animals during 3 "metabolic days" that made it possible to collect fecal and urine samples.

During metabolic studies there were registered next indices: body mass, amount of food and water intake, ammonia, urine and excrements, water balance, food assimilation and V excruction.

In blood serum, received from cubital vein, in urine, excrements and food samples by spectrophotometry method studied Na, K, Ca, Mg and P concentration.

In blood serum and urine by RTA methods measured concentration of aldosterone and cortisol-hormones regulating water-saline homeostasis.

Using "Tevonit PA-1000" unit alaninaminotransferase (ALT), aspartataminotransferase (AST), lactatdehydrogenase (LDH), creatinokinase (CPK), glutamatedehydogenase (GIDH), amilase and cholinesterases were measured in blood serum. Moreover, there were studied concentration of glucose, indicies of nuclear metabolism (common protein, albumine, creatinine, carbamide) and lipid metabolism (cholesterine, triglycerides, and lipase activity).

RESULTS

Body mass dynamics in primates exposed on 4 biosatellites showed, that in "acute" adaptation period in some animals there was noted reduction of food intake, but in further animals animals got used to a new situation they had a good appetite and their weight was practically unchanged. It must be noted, that in monkey Rion (in 5-day space flight on board Cosmos-1514 biosatellite) which suffered with motion sickness all day and as a result this animal had loss of body mass about 7.8%. It should be mentioned specially that in monkey Krossa, beginning with day 2, there was a defect in a food supplying system, as a result of which the animal did not receive food during the remaining period and had a considerable loss of body mass and markedly changes in water metabolism's indicies.

In analysis of results performed on board "Cosmos-1667, 1887 and 2044" biosatellites revealed that over the each experiment a total diurnal amount of consumed liquid, including water containing in paste-like diet, was about 350 - 315 ml. Water balance (that is liquid excretion) in post-flight period reduced, but was positive in character. In post-flight period an excruction of common nitrogen was slightly increased. During ground-based control experiment water balance had a average value +20,6 ml; that is noted post-flight changes were due not only to weightlessness's influence but also to presence of animals in mock-up of biosatellite.

After finishing of space flights of different duration there was noted that excretion of electrolytes by kidneys changed not equally (Fig. 1, 3, 4).
It may be admitted that increasing Ca concentration in blood and its excrition by kidneys during space flight were due to distrophic processes in muscles and resorption processes in bone tissue, which develop in weightlessness. Electrolyte metabolism's disorders may be due to changes in hormonal status of organism.

In blood samples taken on 0-day after "Cosmos-1667" flight there were not noted markedly changes in aldosterone secretion level; at the same time there was noted twice increasing of cortisol concentration. This high level of cortisol excretion (in 3 - 5 times in comparison with pre-flight values) was noted at the end of the first week of post-flight period.

It is likely, that process of readaptation to Earth gravity is accompanied with a strain of hormonal system, resembling stress-reaction.

1. Results of electrolyte metabolism study and blood biochemistry indicies, characterising energetic and plastic metabolism in monkeys, have shown a moderation of this reaction.

Absence of stress condition right after the flight may be confirmed by unchanged values (as compared with pre-flight period) of glucose, cholesterol, triglycerides and lipase activity.

Study of protein metabolism indicies right after flight showed not significant, but equal in all experiments decreasing of common protein concentration due to reduction, mainly, of albumin fraction (Fig.6).

To day 7 after the flight common protein concentration was normalized, but albumin value even in 30 days of readaptation period was slightly lower than in background point.

Dynamics of creatinine and carbamide have shown on figure7 and confirmed the absence of markedly changes in protein metabolism after space flight.
After finishing "Cosmos-1887 and 2044" experiments in all four animals there was noted that KFX activity increased in 2.5-3 times.

AST activity in monkeys Dryoma and Erosha in comparison with pre-flight investigation was increased in 2.5-3 times; in monkeys Zhakonya and Zabiya it was increased in 30% accordingly.

ALT activity in monkeys Zhakonya and Zabiya after flight was not changed, but in Dryoma it was increased in 75% and in Erosha in 7 times.

Previously received data about blood fermental activity changes in monkeys under influence of different factor of space flight, which may be designed on the earth, permit to explain noted changes by reason of radial (for AST and LDG) and pressing (for KFX) accelerations which accompany the landing of biosatellite.

More markedly changes in monkeys Dryoma and Erosha can be explained by difficulties in biosatellite landing process. Thus, the changes, noted in animals' blood after two week space flight, in minerals and protein metabolism as the changes in fermental activity were functional in character and returned to baseline level within two weeks after completion of the space flight.

The exception is the fact of persistent Cox excitation, when weightlessness intensifies the effect of restrict animal's activity during their presence in fixating primatological chair.

In monkeys Dryoma and Erosha exposed on board biosatellite "Cosmos-1887" in two days after landing there was noted a slightly increasing of carbamide concentration, what could be explained by the fact that for this period animals did not receive a food.

In monkeys Zhakonya and Zabiya carbamide and creatinine concentration in 0-day after flight differed slightly from background values, that could give an evidence about preponderation of anabolic processes over catabolic ones.

An important integrative indices of tissue metabolism condition are the fermental activity data in blood serum.

The majority of tested ferments did not change their activity after space flight.

Nevertheless, on figure 8, there is shown that LDG activity in monkeys Dryoma and Erosha in two days after landing was in 2.5 times higher than in pre-flight period.

Figure 7. Creatinine and carbamide blood concentration in monkeys Dryoma, Erosha, Zhakonya and Zabiya.
B. p. - background point

Figure 8. Blood fermental activity in monkeys Dryoma, Erosha, Zhakonya and Zabiya.
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The Physiologist, Vol. 35, No. 1, Suppl., 1992
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INTRODUCTION

Thirty years of manned flights has confirmed our concept of the feasibility of prolonged active existence of humans under weightlessness and provided a great amount of material on the peculiarities of adaptation to this conditions. The investigations of adaptive mechanisms were based on a profound study of metabolism and systems controlling it as shown by responses of inter- and intrasystemic homeostasis to space flight factors. The analysis of data from 5 prime missions to the "Saljut-5" OS of 75-to 185-day duration (10 cosmonauts) and 10.7 -to 14-day visiting missions (17 cosmonauts) was the first attempt to generalize the results of biochemical studies and to compare the responses of cosmonauts to short-term and long-term missions (1,2). This revealed significant differences in the nature of metabolic responses and changes in metabolic control depending on flight duration. Biochemical alterations noted after short-term flights fitted into the symptomatology of acute phase of moderately marked stress-responses, characterized by activation of the sympathoadrenal system, as well as of the hypothalal-adrenal one, adequate activation of correcting mechanisms and quick normalization of homeostasis. Data obtained after prolonged flights revealed more profound alterations of metabolic and hormonal status. Later this was confirmed by the results of observation of 24 cosmonauts who flew on board "Saljut-7" OS and were exposed to weightlessness for 8 to 237 days. Variational statistics was applied using bifactorial dispersion analysis ( 3 ). Factor 1 evaluated the contribution of space flight effect and factor 2 evaluated individual variability of parameters in the subjects. The results of analysis support the hypothesis that the nature of shifts in human blood biochemistry differs between short and long-term flights.

However, both short and long-term flights produce changes mostly in biochemical parameters characterizing the status of energy metabolism, namely blood levels of carbohydrate and lipid metabolic substrate, activity of enzymes involved in tissue metabolism of these compounds. Post-flight changes may result from both flight effects and individual variability of blood biochemistry in humans. We also studied blood biochemistry of cosmonauts who made space flights on "Mir" stations in 1986 - 1990 to confirm and extend previous observations.

MATERIALS AND METHODS

Study was performed in the cosmonauts who made orbital flights of 125, 131, 151, 160, 165, 175, 179, 241, 326, 366 days in duration. Morning blood samples were drawn from an antecubital vein of fasting cosmonauts before flight ( usually -30 ) and twice after recovery (1, 47). Biochemical analysis of blood serum was carried out by routine methods on analyzer Technicon RA-1000.

RESULTS AND DISCUSSION

Due to the central position of energy metabolism special attention should be paid to this problem. Stationary and transitory blood level of carbohydrate and lipid substrates is an objective characteristics of the status of energy metabolism. Data obtained by American authors during three "Skylab" missions (4) show that blood glucose and insulin concentrations during flight gradually decrease. Post flight, however, a moderate hyperglycemia is frequently observed; in our opinion it results from a stressor activation of the sympathoadrenal system, invariably expressed during the acute period of readaptation. As a consequence of this hyperglycemia, insulin secretion grows. During a study of cosmonauts of "Mir" prime crews, an increased glucose level on day +1 was noted in 15 subjects (table ). 11 of them showed a moderate hyperglycemia ( maximal values 7.4 to 8.5 mmol/l ) after missions of 160-, 131-, and 366-day duration. Normalization occurred as a rule within the first week post-flight. 10 cosmonauts showed an increased blood lactate concentration which was higher than the physiological standard in 4 cases ( 125-, 131-, 160-, 366-day missions). A growth of blood lactate is also in accord with the concept of a quick mobilization of carbohydrate resources during the acute phase of stress resulting from activation of glycolysis and glycogenolysis. No data on fatty substrates content in human blood during space flight have yet been obtained; results of post-flight studies are greatly affected by individual variability of these parameters. This is well demonstrated by the results of factor analysis( 3 ). The significant lowering of triglyceride content in the cosmonauts...
blood after 7- or 8-month flights was only in 44.4% of cases determined by space flight effects: 64% of cases could be accounted for by variability of the parameter.

Results of determining the parameters characterizing lipid metabolism in "Mir" crewmembers comply with the conclusion of a marked lability and variability of this type of metabolism. Half of the cosmonauts studied showed signs of activation of lipolysis and lipomobilization. The opposite type of lipid metabolism with a markedly lowered level of blood fatty substrates is characteristic of the other half of subjects. It is believed that this is the type of lipid metabolism established during weightlessness.

An important element in the study of specific biochemical responses and metabolism as a whole is the investigation of activity of blood enzymes. In the studies which have been performed during flights little attention has been paid to investigating blood serum enzymes whose activity is an integral criterion of the status of specific metabolic processes in the body.

The most characteristic type of disorientation observed after prolonged missions is a lowering of other cycle dehydrogenase activity (MDH, ICODH), as well as that of glycogenolysis enzymes (LDH). Also, an increased activity of enzymes of gluconeogenesis from aminoacid precursors (GOT, GPT) and substrate phosphorylation (CPK).

A decrease of MDH activity was seen in 14 "Mir" crewmembers (table). On four of them it was lower than normal standards (125-, 325-, 366-day flights).

In 10 cases of 16 there was a decreased LDH activity. In 2 cosmonauts (166- and 326-day missions) the values were below normal, however by day 7 the fermentemia was completely normalized.

CPK activity was markedly increased in 12 "Mir" cosmonauts; in 10 of them it exceeded the normal values. In all cases hyperfermentemia is caused by the activation of muscular isoenzyme: 7 cosmonauts simultaneously showed a marked increase of the myocardial isoform.

A higher level of blood GOT and GPT activity was noted in 9 and 8 cosmonauts respectively; in half of the cases GOT values were beyond normal limits. The highest level of the relatively stable up to day 7 - GPT hyperfermentemia was noted after 160- and 125-day missions.

A characteristic peculiarity of protein/nitrogen metabolism during space flight as shown by Soviet and American authors (5,6) are decreases of aminoacid blood pool and elevated aminoaciduria with an increase in the ratio essential/nonessential urinary aminoacids. There was also a decrease of creatinine clearance and uric acid excretion.

As a rule, those changes of protein/nitrogen metabolism are secondary and due to such factors characteristic of weightlessness as body mass loss, muscle atrophy and increased protein proteolysis in muscles and partly bones, as well as to acute stressor effects. Only slight changes in blood total protein concentration were usually seen postflight.

A marked increase in blood urea content was seen only in 3 cosmonauts after 131- and 241-day flights. Normalization of this parameter occurred on day 7. Blood uric acid level was decreased in the majority of "Mir" cosmonauts (10 of 12). In all cases by day 7 there was no complete recovery of the initial level.

CONCLUSION

Results of biochemical studies in "Mir" cosmonauts demonstrate good correspondence with main concepts on the effect of space flight factors upon metabolism in humans.

An important result is a certain individuality of responses and the absence of correlation with flight duration for 4-month to 1-year missions.

REFERENCES


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LIPID PEROXIDATION AND THE SYSTEM OF ANTIOXIDANT PROTECTION IN RATS AFTER SPACE FLIGHTS OF VARYING DURATION

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The process of intensified lipid peroxidation (LPO) is a universal non-specific element of patogenetic mechanisms in the realization of various external extremal effects. The level of LPO in organs and tissues is determined by the balance between the processes of free radical oxidation and antioxidant protection. Control of LPO intensiveness as well as reparation of free-radical damages are associated with the expenditure of macrometabolic, metabolically significant cofactors, and plastic substances. An important feature of LPO is its capability to reflect the extent and depth of an unfavorable effect and the feasibility of its being compensated by the body.

METHODS

After space flights on board "Cosmos" biosatellites of 7-, 13-, and 14-day duration in liver, myocardial, and skeletal muscle homogenates, using methods described earlier, the content of primary LPO products was determined: conjugated dienes (CD), an intermediate product - malondialdehyde (MDA), end products - Schiff bases (SB); the main lipid antioxidant - tocopherol (TP); the activity of antioxidant enzymes (AOE) - superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR), and catalase (CAT). In blood plasma - the concentration of LPO products, TP, and the level of total antioxidant activity (AOA) were determined. Investigations in each experiment were performed on three groups of rats: flight, vivarium control, and synchronous control for which all space flight factors were simulated except weightlessness. The number of animals for each group was 5 to 14. The data obtained were processed by variation statistics using Student's t-test.

RESULTS AND DISCUSSION

In the liver of the rats after a 7-day space flight there was a generalised intensification of LPO as shown by an increased content of all LPO products studied (Fig. 1) and disbalance of antioxidant enzyme activity (Fig. 2). However, the increased TP content reflects the fact that adaptive potentialities of the body were maintained during the early postflight period. Taking into account the equal growth of SB concentrations in the flight and synchronous control groups, one cannot be absolutely sure whether the phenomenon observed results from the effect of a battery of space flight factors upon the body, or it formed during landing and in the postflight period.

After 13- and 14-day flights the hepatic SB levels in the animals lowered while the concentrations of other LPO products were either unchanged or lowered. This was associated with a higher AOE activity. This changes indicates an enzymatic inactivation of early stages of free radical processes in the liver. The identical directions of changes in the flight and synchronous control groups suggest that the differences found were caused by stress-factors accompanying the landing of biosatellites. The greater magnitude of changes in flight groups is, probably, related to a readaptation effect on return to the Earth after 2 weeks of weightlessness.

Skeletal muscles of the rats after 7- and 14-day flights did not show any significant changes of the parameters studied (Fig. 3). After a 13-day flight there was an increase of CD content with a lowering of MDA concentration and SOD activity (Fig. 4). The absence of changes in the concentration of SB - the end LPO product - indicates a short-term activation of the process, most probably during readaptation to terrestrial conditions.

On all figures values not significantly different from control are shown as 100%.
Fig. 4.
The activity of antioxidant enzymes in rat's skeletal muscle after space flights.

Because of the emergency biosatellite landing after a 13-day flight, the rats were sacrificed only 2 days after landing. Therefore the changes observed formed most probably during readaptation of the animal's muscular system to normal gravity.

In the myocardium of flight rats from all 3 experimental groups changes had the same direction: with unchanged or lowered content of LPO products, TP concentration (Fig. 5) and SOD activity (Fig. 6) rose markedly; after a 14-day flight there was also a rise in catalase activity. This was, in our view, a consequence of the compensatory response of the system of antioxidant protection to slightly intensified free radical processes during the post-flight period related to increased myocardial loading on return to normal gravity.

Investigation of LPO parameters in blood plasma (Fig. 7), giving an integral characteristic of the process indicates also a tension of the antioxidant body systems in the animals during readaptation to terrestrial conditions. An increase of total AOA in flight rats with no rise in the content of the end LPO products - SB - indicates a short duration of the process and its complete compensation.

The results of study of LPO and antioxidant protection systems in rats after three space flights of varying duration indicate a compensation of the process in tissues and blood plasma. The tension of antioxidant systems and the absence of a rise in the content of LPO end-products proves reflect the response of the body to the final stage of space flight and acute phase of gravitational stress during readaptation to terrestrial conditions.

Stress of varying ethology is known to be accompanied by LPO activation with the magnitude of which is, to a great extent, determined by the magnitude and duration of the stressful effect. The data obtained suggest that the battery of factors of the orbital phase of space flight, primarily weightlessness, does not have a marked stressful effect, at least during the first 14 days.

REFERENCES
INTRODUCTION

A large body of information concerning the varied circulatory responses in cosmonauts during exposure to microgravity indicates a significant role of the so-called individual peculiarities of the organism. For example, cerebral circulation can, during the first 2 or 3 days of the flight, both increase and decrease (1). To what extent does such dynamics of regional circulation depend on the parameters of systemic hemodynamics, the latter in turn depending on the nature of body fluid shifts, first of all blood shifts, in the cranial direction, leading to changes of central blood volume?

This paper considers the possibility of realization of such dependence by evaluating central and peripheral resistances in monkeys exposed on board a number of 'Cosmos' biosatellites.

METHODS

Studies were performed on monkeys exposed on board "Cosmos-1514, 1667 and 2044". The stroke volume value (SV) was calculated by two methods: rheopetisography using the Kubick formula, and by the time of blood ejection from the heart using a linear regression equation.

A comparison of SV values obtained by these methods in monkeys during the flight experiment revealed marked differences in the dynamics of these values. It can be supposed that the reason for this differences is a significant change of hematocrit in monkeys during the flight. Admitting, on the other hand, that during the control experiment hematocrit values did not change noticeably, for each monkey a SV linear regression equation was obtained basing on control experimental data and on ejection time, which was used to determine SV basing on control and in-flight ejection time.

The same rheopetisographic technique also gives an idea of changes in the amount of thoracic fluid and when changes of specific blood resistance are taken into consideration, also of thoracic blood volume dynamics, i.e. central blood volume (CBV) dynamics. Changes of specific blood resistance were determined in relation to SV calculated basing on ejection time and on Kubicek, which logically results from the supposition that hematocrit values are the reason for the differences in SV dynamics. Arterial pressure (AP) and linear flow velocity (LBFV) were measured by extravascular sensors (pressure sensor and ultrasound Doppler sensor) implanted on the common carotid artery 1.5 months preflight. Brain pO2 was measured by the polarographic technique: active platinum electrodes covered by a polyethylene film were implanted into the middle caudal part of the frontal cortex.

RESULTS

From figure 1 it can be seen, to what extent body fluids shifting in the cranial direction during microgravity (mainly the intravascular component) influence the amount of blood in the thorax and central circulation characteristics.

![Figure 1. Diurnal dynamics of central circulation parameters in monkeys in flight and control experiments (in %).](image)

Flight experiment.

Control experiment. - - - - -

A - Heart rate (HR)
B - Stroke volume (SV)
C - Cardiac output (CO)
D - Central blood volume (CBV)

During the control experiment thoracic blood volume in monkeys deviated mostly not more than 10% from the initial level.

In flight, the same monkeys show quite different blood volume dynamics. Verny showed central blood volume changes which were most consistent with theoretical concepts of microgravity-induced effects upon body fluids. During flight day 1 CBV progressively increases, exceeding on day 2 140%, after a fall, there is a second wave of its increase, so that throughout the flight CBV was significantly higher than control values.

Bion also showed an increase of CBV however, in contrast to Verny, it is maintained only on day 1. After that it returns to the initial level and a value corresponding to the control experiment.

Finally, in Gorky CBV which was initially much higher than the control level, on flight day one not only failed to increase, but even decreased.
it remained markedly higher than the control level. After the second wave of growth which appeared on flight days 2 to 3, CRV normalized. The reason for this marked growth of CRV in the baseline state is unclear.

The observed differences in the magnitude and even direction of CRV alterations, particularly on flight day 1, had their effect upon central circulation parameters. Thus, Rion and Verry on flight day 1 in parallel with an increase of CRV, also showed an increase of SV. This suggests a role of preload, i.e. volume loading, in the realization of cardiac contractions.

At the same time in Gordy variations of the initially high CRV had no effect upon SV dynamics: this parameter throughout the flight remained lower than the corresponding level during the control experiment. In all monkeys during the flight CO changes were similar to those of SV.

Therefore, the data obtained show an important role of individual variations in the response to microgravity-induced effects. The dynamics of thoracic blood volume during the flight can be considered as one of the factors determining the nature of initial central circulatory responses during microgravity.

To what extent can individual peculiarities be reflected in the dynamics of peripheral circulation in monkeys during the flight?

In particular, on the nature of circulation in the common carotid artery bed? Figure 2 shows the dynamics of AP, LBVF and RPPR (regional peripheral blood resistance) in Rion and Gordy during the flight.

![Figure 2](image-url)

Figure 2. Dynamics of arterial blood pressure, linear blood velocity and regional peripheral resistance in monkeys Rion and Gordy during space flight.

3-point-averaging --- --- ---
12-point-averaging --- --- ---

Rion, during the first hours of exposure to microgravity, showed a marked decrease of LBVF, an increase of RPPR and AP. In Gordy AP did not change, LBVF on the contrary, increased; this resulted in a lowered RPPR in the vascular bed of the common carotid artery.

The general direction of AP and LBVF changes in monkeys during flight also varied. As compared to the control period, in Rion AP decreased LBVF remained at a higher level; whereas in Gordy there was a clear tendency to a growth of AP with a lowered LBVF.

Blood flow dynamics in the vascular bed of the common carotid artery and in the central circulation were unrelated. In Gordy, with unchanged values for SV and CO, blood flow to the head increased; Rion, on the contrary, despite a marked growth of central circulation parameters, showed even a decreased blood flow to the head.

A comparison of the magnitude hemodynamic alterations and diurnal variations with the animal's well-being the level of feeding motivation, the effectiveness of implementing instrumental reflex programs shows that during the early period of adaptation to microgravity, its direct effect, as a physical factor causing cranial body fluid shifts upon the circulatory system is less marked than its mediated effect due to a discordance in the activity of various functional systems and their newly appearing coordination which undoubtedly depends on the initial function status of the body systems.

As far as PO dynamics in the frontal brain cortex is concerned, its magnitude increased by 27% on flight day 1, remaining at this level on day 2 (Fig.3).

![Figure 3](image-url)

Figure 3. Diurnal dynamic of fore-brain PO tension in monkey Zbakonya in flight and control experiments.

Flight experiment. --- --- ---
Control experiment. --- --- ---

* = p < 0.05
** = p < 0.01

During the subsequent 3 days, 24-hour mean PO values continued to increase (140, 170 and 203% of the pre-flight values respectively). The observed shifts of the oxygen homeostasis were equally due to higher PO values both in the day time and at night.

Subsequently, there was a tendency to a lowering of cerebral PO, however even during the last registration on flight day 11, its 24-hour means were lower than the pre-flight level.
During the ground based control experiment, in contrast to flight, PO_2 in the brain cortex not only failed to increase, but even decreased slightly.

Strong evidence has been obtained indicating a relation between frequency of oscillation of slow non-electric processes in the brain and changes of the functional status of neuronal (glial) populations (2). The curves of PO_2 variation in the frontal brain cortex we obtained were analyzed in the frequency range from 0.2 to 0.01 Hz, which gave an idea of the dynamics and ratio of waves with periods of 5 to 100 sec. Three wave groups were identified, with periods: 1st - 5 to 25 sec, 2nd - 35 to 50 sec, and 3rd - 75 to 100 sec.

The analysis of spectrum power curves calculated for the baseline period, all flight days, and control experiments showed that during the baseline period waves with periods of 33 to 50 sec slightly dominated (42%), whereas the percentage of waves with larger and smaller periods was approximately equal: 31 and 27 per cent respectively. On the whole, during the flight the probability of group 1 waves did not change; however the percentage of group 2 waves lowered, and the probability of group 3 waves markedly increased. The shift of the spectrum to waves with larger oscillation periods shows a lowered functional activity in the described brain segment, as well as less intensive metabolic processes in this segment.

The higher PO_2 level in the frontal brain cortex of monkeys during the 2 week space flight may be due to the combination of the observed decrease in the intensity of metabolism in the brain segment discussed, with a discordance (caused by the hyponoradrenergic syndrome) which developed in flight (3) of the neurogenic mechanism of control of local cerebral blood flow.

REFERENCES
THE EVALUATION OF BIOLOGICAL EFFICIENCY OF ELECTROMAGNETIC FIELDS GENERATED BY IMPLANTED RADIOTELEMERIC TRANSMITTERS USED IN SPACE RESEARCH ON ANIMALS


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ABSTRACT. The study was carried out in 50 male rats abdominally implanted with biotelemetric systems (BTS) or mock-up. The animals were provided with 12/12 light/dark schedule during 6-week experiment. The electromagnetic field (EMF) frequency was 455 kHz, magnetic induction near transducer was about 10-2 mT. Circadian rhythm of the body temperature and locomotor activity was controlled in course of the experiment. The latter was finished, some tissues and the blood of the animals have been sampled to test corticosterone, testosterone, T3, T4 level in serum with radioimmunoassay and membrane permeability for Ca++. Na+, K+-ATPase activity and charge changes in liver microsomes was detected. The probable way of the EMF influence on whole body are discussed.

Electric and magnetic fields (EMF) are to be evaluated among the factors affects the living systems in space environments. One of the aspects of this problem is the possibility of developing the biological effects from EMF generated by onboard hardware including biotelemetric systems (BTS) to monitor the physiological parameters. The recent experimental findings dealing with biological effectiveness of weak (approximately 10^-2-10^-3 mT) EMFs have contributed to conducting animal studies to examine biological effects of electromagnetic impulses generated by implanted BTS similar to those used in space experiments.

Fifty male Wistar rats originally weighting 250 g have been investigated. The animals were housed in individual cases and were provided with 12/12 light/dark schedule and controlled environments. The locomotor activity and body temperature of the rats have been recorded using two types of abdominally implanted BTS of extended ellipsoid in shape and 8x18 mm in size. One of BTS (active, ABTS) was battery-powered and generated continuous radio signals. The second type of BTS contained microcircuit excited by an impulse from external EMF (pulse duration of 0.2 s; magnetic induction near case - 10^-2-10^-3 mT; frequency of 455 kHz; interpulse interval of 300 s).

The animals were divided in 5 groups: 20 vivarium control rats, 10 BTS mock-up implanted rats, 5 BTS implanted rats exposed to external EMF, 5 ABTS implanted rats and 10 BTS mock-up implanted rats exposed to external EMF.

Ca++-transport in skeletal muscle and myocardium homogenates with the aid of ion-selective electrode, Na+, K+-ATPase activity with malachite green method and total charge of membrane vesicles using lipophilic synthetic ions (tetraphenolphosphonium - TPP, tetraphenylborate - TPB) have been detected. Serum levels of corticosterone, testosterone and thyroid hormones were tested with radioimmunoassay. Circadian rhythm of the body temperature and locomotor activity were studied by means of Fourier and periodogram analysis. The rates of calcium ion transport in the homogenates of femoral skeletal muscle and myocardium showed no significant differences between experimental groups. Measurements of total charge of sarcoplasmic reticulum (SR) membranes from the same types of muscles exhibited an increase of negative charge in SR from rats subjected to EMF exposure (data not shown). This effect can probably be accompanied by the changes in excitation-contraction process and activity of ion-transporting ATPases.

A decrease of Na+, K+-ATPase specific activity in hepatocyte membranes of rats exposed to EMF have been revealed (Fig. 1). Inhibition of ATPase activity agrees with an increase in total negative charge of liver microsomes from the animals of the same groups (Fig. 2). These changes can probably affect total level of an locomotor activity of the animals.

Na-K-ATPase activity in liver membranes

![Figure 1](image1.png)

Binding of TPP (-) to liver membranes

![Figure 2](image2.png)
Judging from the hormone contents in the serum, the considerable changes occurred in the implanted mock-up animals: their blood corticosterone level was diminished by 70% as opposed to that of vivarium control animals (Fig. 3). An additional exposure to the external EMF decreased the hormone level in the BTS animals and, by contrast, increased it in those animals with implanted mock-ups. Two latter groups exhibited approximately the same hormone level (about 50% of baselines).

**Figure 3**

Thyroxin measurements (Fig. 4) revealed the tendency for an increase of its level in the implanted BTS rats and a significant elevation in the implanted mock-up rats. EMF exposure decreased the thyroxin level in both groups. The dynamic of the thyroxin changes well agree with the changes in an activity of Na⁺,K⁺-ATPase and in the charge of liver microsomes.

**Figure 4**

The more pronounced changes have been noted in the triiodothyronine level, there was its significant increase as compared with the controls in all experimental animals (data not shown). The testosterone level changed insignificantly exhibiting the trend toward a decrease only in the mock-up implanted rats (data not shown).

Analysis of the biorhythms of locomotor activity and body temperature of the animals revealed some influence of the external EMF on the structure of these rhythms: the EMF exposure enhanced the significance of ultradian component of the circadian periodicity, in particular, those, having 6-hour periods.

Thus, the results obtained made it possible to establish the definite signs of the rat body response to the abdominally implanted BTS and evidently to system-generated electromagnetic fields. There are three ways of realizing the appropriate influences: through blood supply, through stimulation of abdominal cavity receptors and by a direct field exposure to the closely placed organs - kidneys, adrenals, liver and myocardium. The thyroid gland could possibly be involved in the response mediated through the nervous and endocrine systems.

The revealed changes in the membranes and hormone levels are sufficient characteristics of biological efficiency of used EMF. This study should be continued, however, the data obtained make us to place particular emphasis upon the phenomena accompanying the use of implanted systems including the sources of electromagnetic fields in the automated experiments.
ELECTROMAGNETIC AND MAGNETIC FIELDS AS ACTIVE ENVIRONMENTAL FACTORS IN BIOSATELLITE

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INTRODUCTION

There are two major peculiarities of magnetic and electromagnetic fields (EMF) surrounding of biological objects onboard: the changes in the level and dynamic of geomagnetic field (GMF) and the existence of EMF generated by different equipment. The main research concept of this problem is based on the idea that electromagnetic factor on one side and microgravity on the other together could influence same regulatory points of metabolism and biological reactions during space flight. During almost all biological experiments onboard one have combined action of microgravity and EMF on the objects. The particular case of this problem are biosatellite experiments. Therefore, this preliminary study examined the intensity and probable biological efficiency of EMF generated by different onboard equipment in biosatellite "Cosmos 2044".

METHODS

The electric and magnetic component of EMF from "Cosmos 2044" working dummy was measured by high-density registrators in the frequency range of 90 MHz-300 MHz. The measurements were performed two times at the one meter distance of opened and locked satellite had magnetic switched off (background) and on all inside equipment.

The magnetic induction generated by monkey's head turning velocity transducer was measured by millitesla meter F4354/1.

RESULTS

There are a lot of transducers, registrators, amplifiers, electromotors, biotelemetric systems (BTS) etc. in the inner volume (~4 m³) of biosatellite. This equipment is the source of constant and variable magnetic fields. The metal shell and construction of satellite create the complicated structure of absorbance, reflection and emanation of EMF in working volume with observed biological objects. As it is shown on Fig. 1 and 2, the level of EMF-signal increasing 10–30 times during the work of all biosatellite systems. The maximal EMF fluxes (58–335 μV/m) was registered at frequencies 19.9 and 2.1 MHz (Fig. 2). Head turning velocity transducer generated the gradient of magnetic induction (maximal value 1μT) in the space where monkey's head is placed (data not shown). The transducer used near at 50 mm near body surface and animal's head experiencing magnetic field 10-fold higher than EMF.

DISCUSSION

As it was shown earlier (1-3) the low level magnetic and electromagnetic fields can affect cell proliferation, cellular membrane processes, protein synthesis and functional stability of ion-transporting enzymes. Lerchi et al. (4) also showed that inversion of GMF (140 μT) influenced the function of pineal gland. On the other hand our measurements demonstrated the existence of such EMF in the inner volume of biosatellite. So, it is important to pay attention to the contribution of EMF to biological effects (especially cellular and subcellular level) of the space flight conditions. These effects sometimes mainly attributed to microgravity but the role of EMF is still unknown. However, detailed investigation of the EMF action on organisms in space flight is needed.

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