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

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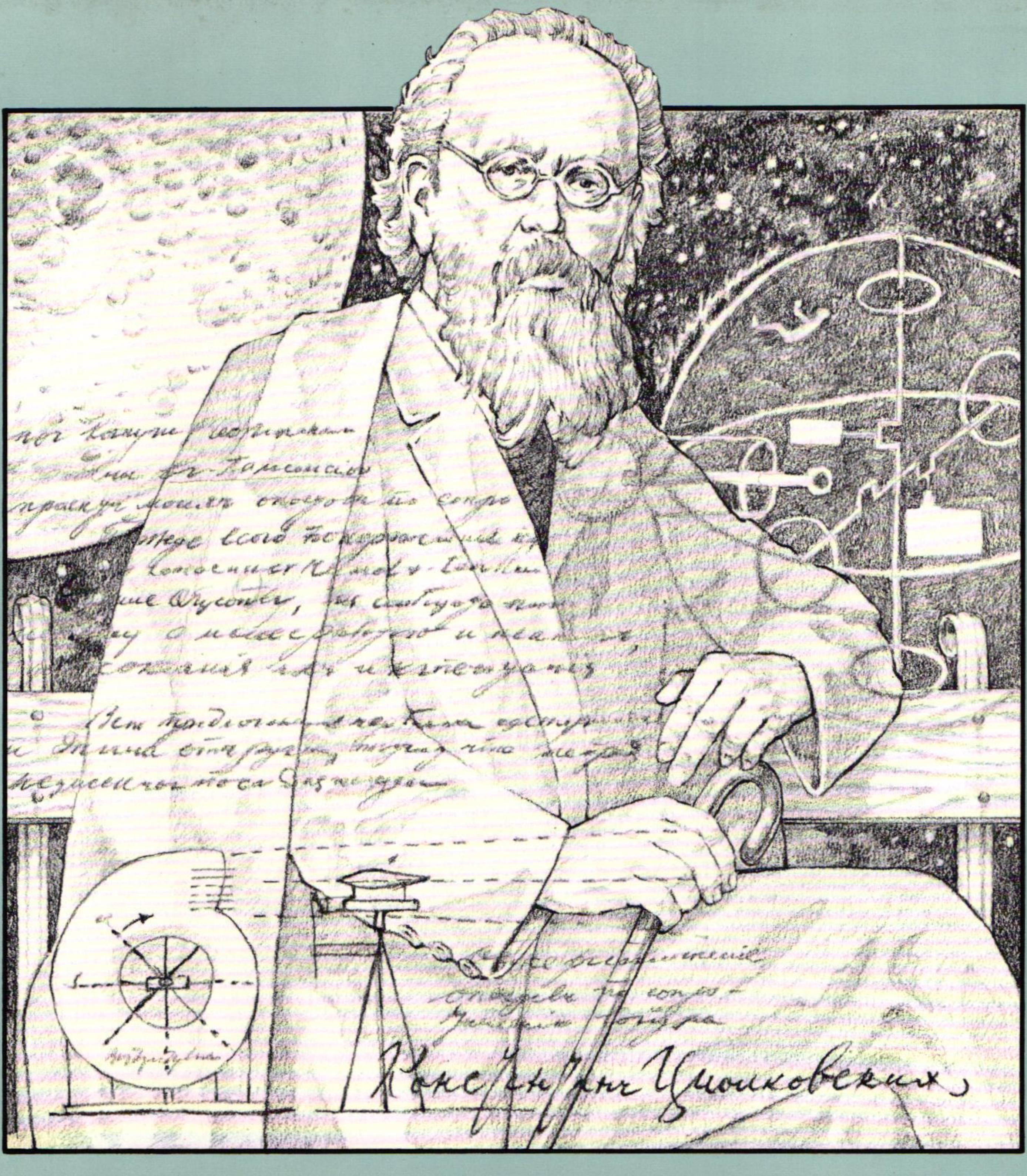

DEDICATION

Forward on, always forward on.
The Universe belongs to man.
K.E. Tsiolkovsky

The Fifth Annual Proceedings of the Commission are dedicated to Konstantin E. Tsiolkovsky in recognition of his founding role in astronautics and significant contribution to gravitational physiology.

Konstantin E. Tsiokovsky was born in a village of Izhevskoye, not far from the city of Ryazan, Russia, 17 September 1857 to the family of a forester. When he was a little after ten, he contracted scarlet fever and, as a result, lost hearing almost completely. Deafness influenced his life in a large measure. "It made me suffer every minute of my association with other people. It made me engrossed in myself and strive to do domething outstanding to win the approval of others" - he wrote later. His deafness interfered with his lessons but the young boy yearned for knowledge and developed a specific system of lessons. He did not only read textbooks but carried out many experiments, often using instruments designed by himself. The scope of his scientific interests grew continuously, becoming really amazing - mathematics and engineering, astronomy and geology, biology and medicine. In 1879 K.E. Tsiolkovsky passed examinations as an external student and obtained a certificate of education from the Ryazan high school which authorized him to teach mathematics in junior school. In 1892 he became a school-teacher of arithmetics and geometry in the city of Kaluga where he lived until his death, 19 September 1935.

The entire life of K.E. Tsiolkovsky is an excellent example of selfless devotion to science and passionate drive to use scientific achievements for the benefit of mankind. He wrote about himself: "My biography is a collection of work and trifles of everyday life. My only attraction is research, new findings, the rest bores me".

What fascinated him in particular was the idea of going beyond the Earth. In 1883 he completed a specific treatise in the form of a diary "The Free Space" in which he, for the first time, formulated his ideas about man's penetration into outer space. In 1896 he started a profound theoretical study of the problem of space flight. In his two publications "The Study of the Outer Space by Rockets" and "Beyond the Planet Earth" the scientist presented a well-documented substantiation of a rocket to be used as a space vehicle. Tsiolkovsky was the first to show that the rocket is a flying vehicle whose dynamic characteristics can be made adequate to meet the biological and physiological requirements of living organisms.

Biomedical problems of astronautics occupied an important place in the scholar's activities. In 1876-78 he carried out experiments on centrifuged insects and chicks and demonstrated that "the fivefold increase of their weight produced no harmful effect on them". In 1882 Tsiolkovsky prepared (but did not publish) the book "Mechanics of an Animal Body", which discussed the effect of increased or decreased gravity on the human body.

To get a better understanding of what man and animals may face in the weightless state, Tsiolkovsky felt it important to identify the role of the constant effect of Earth gravity on the living body. He put forth the hypothesis that the anatomy and physiology of animals and plants can be modified as a result of their prolonged exposure to a weightless environment. In 1895 he advanced the idea to generate artificial gravity by rotating the space cabin.

Even today we feel amazed by the intuition and foresight of K.E. Tsiolkovsky. At the turn of this century he proposed two methods for simulating the weightless state on the ground: free fall of man in an enclosure (chamber, lift, etc.) and immersion into a fluid. He was the first to speak about a closed life-support system in a space rocket.

The great thinker, Tsiolkovsky not only laid the foundation of astronautics but also developed a logical concept of the future of the humanity. He believed that man's space-oriented activities would go beyond the geocentric limitations of the human society and make accessible unprecedented facilities for social progress and complete harmony of man and his environment in the Universe.

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

26–29 July 1983
Moscow, USSR

International Union of Physiological Sciences
Commission on Gravitational Physiology

Proceedings of the Fifth Annual Meeting of the IUPS Commission on Gravitational Physiology 26–29 July 1983, Moscow, USSR

The Fifth Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences was held in Moscow on 26–29 July 1983 at the kind invitation of the USSR Academy of Sciences and the Ministry of Health. The meeting started with a symposium lasting a day and a half during which 22 invited papers on gravitational research in plants and animals were presented. The symposium was followed by two days of open sessions in which 45 voluntary papers were given, and the meeting was concluded with a half-day of 2 round table discussions of motor and vestibular function and of cardiovascular function in altered gravity states. The number of registered participants in the meeting was 116.

The Commission also organized a half-day symposium on gravitational physiology as part of the IUPS Congress held in Sydney, Australia, on 28 August–3 September 1983. The symposium comprised 6 invited papers by speakers from Australia, the USA, and the USSR.

The following Proceedings of the Commission on Gravitational Physiology include the symposium and voluntary papers from the Moscow meeting, as well as the symposium papers from the Sydney meeting, and are presented by arrangement with the American Physiological Society. In the interest of timeliness and economy they are published without full editorial review. Financial support for the preparation of the Proceedings has been provided by the USA National Aeronautics and Space Administration, for which the Commission is grateful.

Travel support for many of the participants was generously provided by the National Aeronautics and Space Administration, the European Space Agency, and the American Physiological Society. We also express appreciation to the USSR Academy of Sciences and Ministry of Health for their gracious hospitality and provision of excellent facilities for the meeting.

The Sixth Annual Meeting of the IUPS Commission on Gravitational Physiology will be held in Lausanne, Switzerland on 18–21 September 1984 at the kind invitation of the Institut de Biologie et de Physiologie Végétales, Université de Lausanne. A Preliminary Announcement giving details follows, and a welcome to participate is extended to all interested scientists.

H. Bjurstedt, Sweden, *Chairman*
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PRELIMINARY ANNOUNCEMENT

IUPS Commission on Gravitational Physiology—Sixth Annual Meeting 18–21 September 1984 Lausanne, Switzerland

The Sixth Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences is being planned for Lausanne, Switzerland, on 18–21 September 1984. The meeting will be held at the invitation of the Institut de Biologie et de Physiologie Végétales, Université de Lausanne.

The Commission Meeting will comprise open sessions for slide presentations of voluntary papers dealing with the effects on physiological systems of humans, animals, and plants of changes in magnitude or direction of the force environment. Included are the effects of the weightlessness during space flight, acute and chronic acceleration, vibration, and the various forms of simulated weightlessness. Also included is consideration of the evolutionary consequences of gravity and the role of gravity in the manifestations of scale effects in animals and plants. The Commission Meeting will also include a one-day symposium by invited speakers on several topics in gravitational physiology.

It is planned to publish the Proceedings of the Sixth Annual Meeting in *The Physiologist*. As previously, the Proceedings will contain the voluntary papers and symposium papers presented at the Meeting.

Your participation in the Commission Meeting is welcomed. If you are interested in the particulars, please contact Dr. Orr E. Reynolds, Commission Business Officer, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814, USA, by 15 February 1984.

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OPENING REMARKS

E. I. Vorobyov
USSR Vice-Minister of Health,
Chairman of the USSR Organizing Committee

Distinguished Colleagues,

In behalf of the USSR Ministry of Health I feel pleased and honored to welcome you on the occasion of the opening of the Vth Annual Meeting of the IUPS Commission on Gravitational Physiology. This Meeting is attended by well-known scientists from 14 countries. The Program of the plenary and sectional sessions includes over 70 papers.

At present gravitational physiology is in the focus of scientists from many countries. However, there are still many white spots in our understanding of gravity effects on biological objects, there is no single theory interpreting the relationship between a living body and gravity. It is interesting to study intimate mechanisms of the primary biological effect of the gravitational factor. It would be of great theoretical and practical importance if the possibility of a direct effect of zero-g on the cell and subcellular structures of gravity-oriented and nonoriented organisms can be proved or disproved.

Gravity is a constant environmental factor for man, animals and plants on the Earth. Every living being has adapted to the effects of an invariable gravity. It is hoped that an investigation of pathways and mechanisms of the phylogenetic adaptation to the Earth gravity may make a significant contribution to the theory of evolution, general principles of adaptation and exobiology.

Today gravitational physiology plays an important part as an applied science. The spectacular achievements in aviation and astronautics make it necessary to support the life and work of man under altered gravity conditions, varying from several g's to zero-g. This practical problem can be solved only on the basis of fundamental investigations.

The space flight is characterized by gravitational effects of opposite polarity: at powered stages hypergravity develops and in orbital flight hypogravity persists. This large scope of gravitational effects is of key interest for physiologists. Particular attention should be given to the vestibular function. The theoretical and practical aspects of this problem are obvious but our achievements in the clarification of vestibulo-autonomic disorders in zero-g are so far very modest.

When emphasizing the significance of space studies, it is important to bear in mind the limitations they may impose on gravitational physiology.

Until now each space flight remains a unique event and often the data obtained are insufficient to ensure statistically significant results. This problem is further aggravated by the fact that in space flight man, animals and plants are exposed not only to acceleration and zero-g but also to many other environmental effects that can hardly be controlled. Some of these factors may produce a biological effect of greater magnitude than that of acceleration or zero-g. In this context, experimental investigations on the ground are very important.

Increased gravity effects can be easily reproduced with the aid of centrifuges of different configuration. Ground-based studies have shown that from the biological point of view the gravitational environment is very important. It is also interesting to elucidate the relations between hyper- and hypogravity and to determine whether the results of long-term centrifugation can be extrapolated to real weightlessness.

As known, prolonged weightlessness cannot be reproduced on the ground. Therefore, various models simulating the major physiological effects of weightlessness are widely used. They include water immersion, clinostatic and antiorthostatic hypokinesia. It is hoped that a critical discussion of the adequacy of these models and their limitations may help solve many problems associated with zero-g effects on the human body.

The investigations of certain aspects of gravitational physiology are closely related to clinical and preventive medicine. They, in particular, extend our ideas about the adaptation of the human body to environmental effects, enlarge our knowledge about the mechanisms underlying the functioning of different physiological systems, and contribute to the proper understanding of hypokinetic and hypodynamic effects.

The importance of gravitational physiology is obvious, hence the great interest of the life sciences community in it. From this point of view, the annual meetings held by the IUPS Commission on Gravitational Physiology are very important. At these meetings scientists have every possibility to discuss the results of their research, to share their ideas and to outline further investigations.

It is beyond doubt that the joint efforts aimed at determining the contribution of gravity to the fundamental biological processes may help answer many questions associated with man's conquest of outer space and with the role of gravity in the origin and evolution of life on the Earth.

In conclusion, may I express my gratitude to you for attending this Meeting and wish you interesting and stimulating discussions. Although the program is packed, I do hope that our guests will be able to do sightseeing in and around Moscow.

This Meeting has been convened by the IUPS Commission on Gravitational Physiology. I am sure all the participants will join me in extending my thanks to the Commission members and its Chairman Prof. H. Bjurstedt for their efforts associated with the preparation of this Meeting.

H. Bjurstedt
Chairman, IUPS Commission on Gravitational Physiology
Department of Environmental Physiology
Karolinska Institutet, Stockholm, Sweden

As Chairman of the IUPS International Commission on Gravitational Physiology I take great pleasure in welcoming you to another of the Commission's Annual Meetings, the fifth of its kind. Out latest meeting was held in San Diego, Calif., in conjunction with the 1982 Fall Meeting of the American Physiological Society at their kind invitation. During this Commission Meeting, the Soviet participants conveyed an invitation from Professor Oleg Gazenko, Member of our Commission, to hold the Fifth Annual Commission Meeting in Moscow to be co-sponsored by the Soviet Academy of Sciences and Ministry of Health. Clearly, this indicated a desire to further consolidate the international cooperation already established in gravitational physiology through our previous Meetings, and the invitation was promptly and gratefully accepted.

As our Fifth Annual Meeting now materializes I could not continue my remarks without expressing, on behalf of the Members of our Commission, my distress over the death of Rodolfo Margaria on 29 January this year. This deprives gravitational physiology of one of its pioneers. Margaria was born in Chatillon in Northern Italy in 1901. During his professional career he held the chairs in physiology at the Medical Schools in Ferrara and Pavia, and was subsequently appointed the chairman of the Institute of Physiology at the Medical School in Milan, where he remained until his retirement in 1972. He held other appointments outside the Academe, such as in aviation medicine and sports physiology. Margaria made important contributions to gravitational physiology; among other achievements in this field he was able to predict the mechanics of human locomotion in the subgravitational field of the moon 5 years before the first astronaut tested his ability in practice. He served as IUPS representative to COSPAR from 1965 to 1974. He played a central role in the formation of our Commission. To acknowledge Margaria's distinguished achievements the Proceedings of the Second Annual Meeting of our Commission was dedicated to him. I am certain that he would have been delighted to witness the progress of gravitational physiology as manifested by this Meeting. His spirit will live on in the endeavors of our Commission - may he rest in peace.

The printed program of this Meeting outlines the activities for the coming 4 days. Apart from the present Symposium of invited papers, there will be 4 workshops and 2 round tables dealing with various aspects of gravitational physiology. The Commission is pleased to acknowledge that the total number of submitted papers for the Symposium and workshops is 70, which means a steady increase of the scientific contributions to our Annual Meetings. The presentations and discussions deal with many aspects of the biological significance of gravity.

Normal gravity as well as increased gravitational fields can be used as a tool to study normal physiological adaptive mechanisms in both plants, animals and humans. The importance of gravity interacting with other environmental factors to control the physiology and morphology of organisms is still largely unknown. We are now able to manipulate the force of gravity from its norm down to the zero-G state. In due course, we will thus be able to find answers to questions whether e.g. fertilization and early development can proceed normally without the otherwise constant and prevasive action of the normal force envirnoment. What is the role of gravity in reproduction, maturation and evolution? Many of the presentations here will deal with these and related questions, and it is only natural that interest will center around the role of weightlessness and its physiological effects. However, some of the presentations here will deal with the other extreme, the physiological effects of increased gravitational force and the remarkable ability of the human organism to withstand sustained, extreme G forces.

It is proper on this occasion to acknowledge the effort put forth by Professor Gazenko and his staff in the local organizing committee toward making this Meeting a success. We especially appreciate the thoughtfulness spent on the organization of the scientific program and various workshops, which I am sure will greatly contribute to facilitating the scientific exchange. Last but not least, much appreciated evidence of the gracious hospitality shown to us by the local organizers are the excellent meeting premises, interpretation facilities and other arrangements made for our convenience.

The Proceedings of this Fifth Annual Meeting of our Commission will be published in The Physiologist by arrangement with the American Physiological Society. Financial support for the preparations of the Proceedings has been kindly provided by the US National Aeronautics and Space Administration. So far we have been able to publish the Proceedings of all our previous Annual Meetings within a few months of the event, and we will do our best to maintain this good tradition.

I would like to conclude these remarks by extending, on behalf of the Commission, my sincere thanks to all speakers and their colleagues who have expended time, money and effort in the preparation of their papers. We feel that this Annual Meeting has an excellent representation of the many apsects that form the substance of gravitational physiology. It is our hope that all of you who have come to Moscow on this occasion, will find this Meeting as enjoyable as I am sure it will be for those who are here as representatives of different organizations, be they sponsoring, supporting or otherwise cooperating.

PIONEERING IN GRAVITATIONAL PHYSIOLOGY

GERALD A. SOFFEN
DIRECTOR OF LIFE SCIENCES

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, DC USA

We are at the frontier!

Gravitational physiology is in its infancy. This is essentially because of two facts. The first is inaccessibility to weightlessness until recently. To understand the influence of the force of g on biological systems, it is necessary to be able to alter and remove the force. In the last few years we have begun to perform some experiments in various g fields. We are now gaining the laboratory needed for understanding.

The other problem is that the influence of g on biology is often very subtle; sometimes concealed or mitigated by compensating physiological processes. To observe these changes either at the whole organism level or at the cellular level, requires highly refined techniques and carefully planned experiments. These techniques are just now being developed. We are still in the exploratory phase. Single definitive experiments are rare. Understandably, this is the normal course of a science at its beginning.

What do we know?

Gravity affects biology at almost all levels with the possible exception of the chemical and biochemical levels which are dominated more by ionic forces, osmotic pressure, diffusion rates, surface tension, hydrogen bonding, and similar strong ultramicroscopic influences. For anything at the cell organelle level or larger, the g vector is expected to have some influence.

Biologists have long been aware of the "obvious signs" of response to gravity. Just a century ago Darwin extensively used the term geotropism, apogeotropism, and diageotropism, and devoted the entire Chapter X of his classic Power of Movement in Plants to this subject. The importance of the vestibular system in the vertebrates as an organ of balance goes back to the anatomists of the last century. Response to the direction of gravity in some lower animals was established long ago. However, today most fundamental questions still remain unanswered.

From simple calculations, it can be shown that structures at the micron size range or above may be influenced by the force of g in the conventional time dimension of living creatures. Particles in liquids at the colloidal size and below are more influenced by brownian movement, which is why they remain in suspension. An interesting idea is that minimal cell size is determined by the ability to sense 1 g. In metazoa most cells are between 10-50 microns. Does 1 g dictate this size range? In the biological world there are many events taking place on structures larger than one micron. This is the realm of growth, and structure of tissue, and of organ function. This size of structure influences hydrodynamics, balance, motion, extension and retraction, behavior, and social patterns of all larger organisms.

Using centrifuges, clinostats, inverted preparations, linear devices, water immension, free fall, and in some cases short and long-term spaceflight, we now have some general ideas about the influence of gravity on the physiology of life, and what questions should be investigated. When considering the question of gravitational physiology, there are two motivations, one is practical or applied, and the other more fundamental or basically scientific.

The applied field deals mostly with the health of the human crew-members or their food and nutrition in spaceflight. To illustrate how these fundamental and applied problems are interlinked, I would like to consider a unique but not impractical problem. We have flown over a hundred people in space at various levels of micro g, and performed thousands of experiments at hyper g for varying periods of time. The human is not fragile. He is well-engineered to endure spaceflight, adjusts to the changes, the fluid shifts, central nervous system accommodation, metabolic adjustment, and perhaps even accomplishes mineral loss surpression. We have seen relatively long periods (211 days) at low g levels. However, beyond a few g for a short period, our system cannot adjust to high g forces without extensive countermeasures. It is clear that a person traveling near or at the planet Jupiter could be in serious trouble. This raises the question of the limits to the size of organisms that can exist as related to the g of their particular planet.

We have redwood trees that grow to almost a hundred meters. Are they limited in their size by the g of the Earth? This is still an unanswered question. Is the size of animals limited by the force of g? We certainly know of very large aquatic animals, and have evidence of the giant land dwelling reptiles of the past, the dinosaurs. The size of these creatures was limited mostly by temperature and food supply. But what are the limitations imposed by gravity in permitting an animal to bear its weight and retain its mobility and nutrition. This area of evolutionary biology as determined by gravity, has been a fertile area of study, enhanced by field observations, but its solution is limited; conventional laboratory experimentation is not too useful.

Presently we are entering a phase of extended travel at hypo g for humans in low Earth orbit, and only brief periods of hyper g during launch and landing. Even on an extensive trip to land humans on Mars or its satellites, the crew will be mostly exposed to hypo g. A trip to Mars, which is likely to be done by the end of this century, will likely take three years, one year of travel in each direction, and one year at the planet, while the celestial mechanics are aligned for the return trip. The gravity on Mars is .4g, between the gravity of the moon and the Earth, so there will be no unusual g force that we have not experienced. However the much longer duration required than any we have experienced could result in unforeseen physiological problems. With the Soviet data from the recent 211-day mission, it certainly appears plausible that such a trip will be made in the next 20 years. With the Viking data, we now know how to land on Mars. The technical problems of this mission may be now within our grasp. Will there be some nation or group of nations, that siezes the opportunity?

In the next century we will see humans travel throughout the solar system. Extensive travel in space at hyper g velocity will have its own time of investigation, and the practical problems will be different from today.

In the experiments and observations that have been made in plants and animals we know that below the level of the whole organism there are considerable changes. In plants the major change is in the growth cycle. Some changes in leaf epinasty have been observed in weightlessness, but not sufficient to suggest interference with the normal function of the plants' physiology. During development there are extraordinary changes in the formation of roots, stem length and diameter, and internodal elongation. Leaf size, flower, and fruit demensions are all likely to be influenced by a change in g during their growth periods. Experiments have been done that suggest some differences among the plant species, but not enough to understand the underlying mechanisms.

During last year, the US has flown some plants on STS-3. Pine seedlings, oats, and mung bean seedlings were flown for several days to observe lignification (Coles). Generally the experiment showed that plants can be grown in space, but with some effects on their morphology. There was a 4-25% reduction in lignin content. Two enzymes that are employed in lignin biosynthesis, phenylalanine amonium lyase and peroxidase were both reduced. Fortunately the centrifuge offers a way of simulating the hyper g condition.

The role of lignin in protein synthesis may be important if plants are to become an important amino acid source for extended flight.

An experiment called the HEFLEX (Helianthus Flight Experiment) is planned for an upcoming flight (Brown). An engineering test was performed on STS-3. This will be used to study the reduction of circumnutation in weightlessness. The study is to validate the clinostat as a tool for this kind of work.

In the laboratory we have learned some of the dynamics of the processes of response to the g stimulus. R. Bandursky has determined that the curving growth of a plant can occur in as short as 5-10 minutes in zea mays, and reach its maximum rate in one hour. Feldman has recently shown that the roots of corn seedlings grown in the dark are not responsive to changes in gravity, however, brief flashes of light will trigger the bending process. The biochemical processes of plants do not act independent of one another.

The mediator of this gravity sensitivity are becomingcorn, has been studying the difference between the effects of auxin and that of abscisic acid. Apparently the auxin effect is gravitropic, but not the abscisic acid. Further studies by this investigator showed that there are asymetric gradients of protons and calcium ions that parallel the growth patterns of geotropism. This implication of calcium gradient in the root tip as the mediator is an important idea, and suggests new methods of simulating weightlessness. In another laboratory, that of Hayes, it has been shown that the blade of the leaf is the part that senses gravity. She further showed that auxin regulates and affects the pulvinus of the leaf by regulating the ethylene production. Auxin-induced ethylene synthesis appears to be the link between blade perception and leaf angle response to gravity.

The amyloplasts which exist is special sensitive cells called statocysts have been studied by Galston and also by Leopold. Dr. Galston has succeeded in isolating the cells in a viable condition and studied their fall as vertically mounted under the microscopic. Dr. Leopold has determined that the amyloplasts have a net negative surface charge. Using ion techniques and fluorescent indicators, he determined that the calcium is bound to the charge shell around the amyloplasts. Here again calcium is linked as the mediating ion. Leopold theorized that the settling of the amyloplasts redistributes the calcium which may be the basic sensing mechanism.

Peter Kaufman is studying the tonoplast. This is a vacuole membrane. It surrounds the starch statolith at the bottom of the statocyte. He has established a direct proof of the interaction between the free floating statoliths and a fixed cellular component. He is also studying the extensive activity of the statolith on the upper and lower side of the pulvinus. The lower side has a higher esterase activity consistent with the patterns of gravity response.

Lastly, Hoshizaki has succeeded in a seed-to-seed demonstration of a plant (arabidopsis) grown on a clinostat. He learned that CO2 and moisture control are critical for the success of this experiment. Viability of the seeds are currently being tested.

Other investigators, Picard and also Salisbury have shown that in addition to g force other stimulators are needed for successful natural plant physiology. Friction and flexure are mechanical stresses that also can change the pattern of growth in stems; this may be due to a change in ethylene production. Dr. Picard's theory of gravity stimulation of cells is that there are deformational shifts in the macromolecules that control permeability of the membrane to the calcium ion. This would result in calcium migration into the cytosol against an actively controlled concentration gradient. As the calcium concentration rises, it binds to a modulalor protein--called calmodulin. This has two effects, it affects the transport of indolacetic acid out of the cell and activates a protein that stimulates ethylene production of the surrounding cells. The result is an activation of auxin production at one place, and a suppression at another place which produces the asymetry.

In the animal kingdom, we have been examining a few lower ordered organisms, such as spiders and insects, for sensitivity to gravity but most work is done on mammalian preparations. Finch is studying the lyriform organ on the leg of the spider which appears to be g sensitive. This investigator is studying changes in the heart rate following centrifugation. Current theory suggests that the spider's gravity response system operates by sensing the strain in their exoskeleton. It is suggested that there are reflex mechanisms of the heart which respond to the g stimulus, producing a blood pressure change in the legs. This allows the spider to maintain postural homeostasis.

Fruit flies have been studied on the Cosmos flight and recently bees were flown in the Shuttle. A curious conclusion by Miguel, comparing the flight of the two different insects, suggests that the bees were unable to fly in weightlessness, whereas the fruit flies attempted to fly and through their poorly controlled flight, they suffered injury due to the impact against the experiment housing unit.

Several investigators are using a rat suspension model developed by Morey-Holton of Ames Research Center. In this preparation the forepaws are permitted to bear weight but not the hind paws. The animal is suspended by various devices and certain organ systems, bone, muscle, blood vascular, etc. are studied. There is some dispute over the rat as a suitable model for the study of changes in men and primates. There are anatomical differences that may cause the systems to react differently. Nevertheless, since animals will be flown in space for other experimental purposes, it is important to establish their baseline, independent of those results that will be gathered on humans and other primates.

Bickle has done elaborate studies using this model, and shown that calcium loss is controllable by dietary intake. This has been corroborated by Halloran. Bickle followed the time course of bone loss for several weeks and compared the suspended animals with controlled organisms. Using this same preparation, and studying the histology, Doty has determined that the surface osteoblasts and fibroblasts is the site most affected by the non-weight-bearing condition. Electron micrographs reveal a reduction in the communicating junctions between adjacent cells. The periosteal fibroblasts appear to be absorbing the collagen fibers of the periosteum changing their microanatomy. This could mean that variations in mechanical stress affects the entire muscleloskeletal system.

Dunn, using this same model, to study haemopoeisis has found transient changes in hemoconcentration, reduced production of red blood cells, decrease in the average size of the cells, reduced blood volume, anemia, and a postsuspension increase in the number of white blood cells. He notes that these changes are all compatible with data from human spaceflight. Dunn believes that this antiorthostatic hypokinetic rat is a useful tool in which to study the hematological effects of weightlessness.

Drs. Pace and Smith have devoted years to better understanding the scaling laws that govern metabolic rates. They have studied a variety of organisms including rats, guinea pigs, hamsters, and rabbits and established that gravitational loading very likely influences the scaling of metabotic rates. They postulate that in weightlessness this will be considerably less that the classical .75 power.

Dr. Muriel Ross has been studying the otoconia of the saccule and utricle. The classical view is that the dense crystals add mass to the otoconial membrane, making the sensory cells more sensitive to linear acceleration. Dr. Ross' eloquent ultra high resolution micrographs have shown a very elaborate structure in the otoconia. They appear to be highly ordered composites of organic and inorganic materials quite different from pure calcite. The crystals may exhibit a piezoelectric effect by altering the adjacent field during linear acceleration. If this is the case, they may not actually move over the underlying receptor area.

We are beginning to see some results in the study of gravitational effect on embryology. Keefe, who is well-versed in the individual control timed-events of the developing rodent embyro has been studying embryos at hyper g, especially the developing central nervous system. He anticipates that at less than 1 g the alterations in the cardiovascular system are likely to affect the formation of the pheripheral and central integrating elements of the vestibular system. In the upcoming Cosmos flight this is going to be examined.

The achievement in manned spaceflight has been magnificent. Both the United States and the Soviet Union have demonstrated the human's adaptability to the weightlessness of the space environment for long periods, and for a wide variety of people. The most dramatic short-term influences on the human are the malaise and illness in the first few days resulting from motion in space, and the changes in the cardiovascular system.

The cardiovascular changes are the direct result of the translocation of fluids from the lower extremities. In lower gravity the blood is pooled in the upper torso. This apparent central hypervolemia is adjusted by the body by increase in kidney function and a reduced total blood volume. During subsequent flight duration there appears to be no significant physiological problems from this reduction of blood volume. There is no evidence omyocardium.

Upon re-entry, the abrupt changes back to 1 g results in a hypovolumia and a subsequent orthostatic intolerance sometimes called cardiovascular "deconditioning." This temporary instability can include tachycardia, changes in arterial blood pressure, narrow pulse pressure and syncope. The results can be partially compensated by intake of isotonic fluids prior to entry. This practice appears to eleviate those problems.

The problems dealing with the vestibular system are more manifest. We have observed some degree of illness in half of our crewmembers during the first few days of spaceflight. This condition we have come to call space adaptation syndrome. It consists of several symptoms which may include anorexia, palor, nausea, malaise, sweating, headache, and vomiting. Not all ill crewmembers experience all of these symptoms. Based on our ground tests, we find very poor correlation which allow us to predict who will develop symptoms in space flight. One common idea is that it is accompanied by head movement and postural changes. By the third or fourth day in flight all symptoms appear to be gone and accommodation is complete. The current theories center around a sensory conflict within the central nervous system. Incoming signals from the visual, proprioceptive, postural, and vestibular systems are not properly reconciled within the brain, and the body exhibits a characteristic "rejection" at a primitive level. Some neurophysiologists believe that fluid shifts or anatomical assymetry contribute to the changes and imbalance. We now know the problem is common. We do not understand the physiological basis.

Another direct observation in human space flight is the loss of bone mineral. Weight bearing bones lose mineral at something like 1% per month. It is still unclear if this can be controlled by dietary intake. Excercise has been tried as a countermeasure, and appears promising, but as stated before, we do not understand the process, and so our measurements are still unrefined. We also do not know if it continues or is self-limiting.

Other changes in the human crewmember include muscle loss, hematological and immununogical changes, ionic and fluid shifts. These are less debilitating, especially in short-term flights; however, when flight duration is extended beyond today's record 211-day flight, it would not be surprising to see changes in the physiology that are either more serious or, worse perhaps, not reversible.

Lastly, something must be said about radiation. Inside the protection of the Van Allen belts we feel quite safe from the invisible effects of radiation. But radiation hazard still remains the most serious hazard for interplanetary spaceflight. The problem is mostly damage from high energy high cross sectional particles known as HZE. Shielding against this radiation is very limited onboard today's class of spacecraft. We have little understanding about the nature of tissue damage by these large nuclei. The only way that we can simulate this kind of energy is in large accelerators.

<u>What do we need to know?</u>

Starting from the most basic question we need to know the answers to these general questions. As is always true, each question leads to another question:

1. What are the fundamental sensing systems of gravity, e.g., hair cells, starch grains, membrane deflection, piezoelectric effects?
2. How do these sensors work?
3. How do they mediate their signals to the rest of the organism?
4. How do these systems develop embryologically?
5. How did they arise evolutionarly?
6. What are the physiological (or anatomical) consequences of changes in g, on what time scale, and to what magnitude?
7. How does the organism compensate for the changes to hpo and hyper g?
8. Are the changes reversible?
9. What countermeasures can be employed or developed for the safety of human spaceflight?
10. Are there changes in g that can be beneficial to living organisms? (We tend to think in terms of the negative aspects.)

<u>What is NASA's future program?</u>

From my perspective, almost all of NASA's program is linked to gravitational physiology. Most of our research work has been done in ground-based laboratories. In recent years, with access to spaceflight limited only to the Soviet Cosmos missions, we have used the time to develop our ground-based laboratories, our theories, and our techniques. Models and methods have been strongly supported.

With the advent of the successful Shuttle, we can now resume direct experimentation in space flight. The early Shuttle missions have been limited, they have been mainly operational test flights, but now we have an opportunity for an increase in biological and biomedical experimentation. This fall (1983) the European Spacelab 1 will be flown which will include a rich complement of life sciences investigations. On this Spacelab series Spacelab 2 has some plant experiments and some investigations in biochemistry. Spacelab 3 (1984) is about 50% committed to life sciences. It will carry a full complement of rats and primates. Our philosophy is to learn to fly normal animals and assure ourselves of good baseline physiological data before any experimentation. Spacelab 3 will have 24 rats and 4 squirrel monkeys with enough monitoring to study the controlled normal animals.

With Spacelab 4, we plan to have our first biomedical mission. This will consist of 24 investigations in neurophysiology, cardiovascular, pulmonary, renal, endocrine, haemotology, immunology, muscle, calcium metabolism, and biorythms in animals and humans, some plant experiments, and an experiment in amphibian embryology.

Our plan is to develop a standard space biomedical laboratory that can be flown on the Shuttle at about 18-month intervals. The equipment will be developed as a standard core used from mission to mission. We will invite world-wide participation to assemble the best physiological teams of scientists possible. Our present Spacelab 4 mission includes three foreign investigations.

We are discovering a capability to do some limited scientific experimentation in the mid-deck of the Shuttle orbiter. The crew have shown a good deal of interest and cooperation in carrying out some relatively simple vestibular and visual experiments even on the past three missions. On the next mission, STS-8, Dr. William Thornton, M.D., will carry out a rather extensive set of these tests.

NASA appreciates our participation in the Soviet Cosmos missions. It has been the backbone of our flight program. We also appreciate participation in the upcoming German D-1 mission. US scientists collaborating with their ESA colleagues will be conducting important neurophysiological investigations using a human sled developed by ESA. Our colleagues of ESA are in the process of developing several other opportunities that we hope to share. France and Japan have shown a very active interest in life sciences in space, and my intention is to help and stimulate all efforts. Our experience tells us that the more Life scientists we can recruit, the more we may catch up with the problems, and the faster we can understand our subject.

The NASA research program has recently been organized into two branches, one in Space Medicine and the other in Space Biology. This has been in response to a strong directive towards a "more permanent human presence in space." NASA is now planning a strong effort for a space station as the port of flight between Earth and low-earth orbit. The Shuttle will be used mostly as a transportation system, while the space station will be used for long-term experimental observations and possibly for commercial processing.

Life sciences will play a strong role in establishing that permanent presence. This will require much more knowledge about the physiology of spaceflight. In addition, there is the very important frontier of performance and human capability that must be explored. We must learn to make maximum use of the precious opportunity of experimenting and working in microgravity. The human passengers are our richest source of data.

The present US strategy has three classes of human passengers. Some individuals will make a single trip on a single mission. These are experimental specialists who are unique either in making an observation or recognizing an unexpected result. They may make a brief trip of a few days or weeks. Aside from the medical personnel, we will learn least from this group. They will be highly variable, with poor medical histories, and little opportunity for followup. Their training is likely to be only a little longer than their exposure in space. Eventually these passengers may be quite common. The importance of this group will be in their variability and their cooperative spirit in revealing their experiences.

A second class of crewmember will be those having many exposures. Both the Soviets and the US have people who have flown numerous missions. The long-term exposure and the stress of launch and landing will become easier and more familiar. These crewmembers will have the occupational records upon which we will depend for statistics. We have little knowledge of accumulated effects from multiple voyages.

Lastly are those who will have very long duration in space. The USSR's remarkable 211-day mission is solid evidence that the limits to human flight are still unknown. That bold adventure is reminescent of the 15th Century European explorers who sailed for weeks, then months, and finally years as the globe was revealed. There will be space travelers who are on missions for years before the end of this century!

From here to there

How do we move from the present program to the more ambitious one needed for a space station? In order to deal with the space adaptation syndrome in a timely way, NASA has taken a bold step by developing an "Institute" devoted essentially to that problem. The concept is to develop a plan to attack, much as one would in solving an engineering problem, by organizing! We realize that to some scientists, especially those who have worked so long on this very different problem, this may sound simplistic. Nevertheless, the virtue is in assuring ourselves that we are applying all possible means of solution to the problem.

So far, our plan as emerging has five fronts. The first is in selection. We will have a group of people who will explore correlation, new kind of tests, statistical approaches or whatever may seem appropriate for better prediction. This will not be used as a selection criteria. We find that to obtain cooperation of the astronauts, they must not see the research as a threat to their career.

The second front will be searching for better drugs. With the recent advances in pharamacology, it is very likely that we can improve our treatment, especially the side effects problems, by development of better drugs. Most of our advisors believe that this is the most promising route. The US pharmaceutical industry will be recruited to this purpose.

A third front involves the use of psychological techniques for surpressing the effects. Biofeedback is one such method; autogenic practices are becoming more common these days in controlling sympathetic and parasympathetic physiological responses, such as controlling blood pressure. The US Air Force has used these techniques successfully in overcoming air sickness in training pilots. One of the astronauts will be trained in this technique on an upcoming flight.

A fourth front will make use of tests or experimental procedures performed in flight. While the schedule of the crewmembers is usually heavily occupied, there is often time left for performing short-term observations or tests. Taken together these represent a great opportunity for us to learn rapidly.

The last front (one used when all else fails), is to understand the basic physiology. The problem of motion sickness is not new. It is a very difficult problem! We may be very fortunate, and it may be tractable. Or it may resist solution until we completely understand the nervous system, which is a long time indeed. We anticipate that with this new effort we will certainly see an acceleration in our understanding.

Our strategy is to apply strong steady pressure, attract the world's best talent, continue to refine the questions, continue to gather data, build a set of milestones for tests, answers and opportunities. From this Institute, this problem will become a focal point for a constant stream of seminars, conferences, new lines of endeavor, tests of new ideas, rapid reporting of flight information, and it will be raised in the consienceness of many life scientists as an important practical problem that needs rapid solution.

Our other biomedical problems will be studied in the style more familiar to us, as we have in the past. The other problems associated with cardiovascular changes, fluid shifts, muscle loss, bone demineralization, blood alteration, radiation protection have a longer term of solution. The obvious urgency associated with space adaptation syndrome is because of loss of precious time during the short-term missions.

One last area of space medicine that deserves attention is that of performance and capability. Presently we are examining the effects of spaceflight on work/rest cycles, circadian rhythms, and psychomotor performance. Our program explores various aged people, and the effects of nutrition, motivation, and group composition. This area is regarded as extremely important, because of the long time for establishing conclusive results, and the difficulties of experimentation. In the near future we plan to develop a plan of action to better organize and assess our needs as we approach the long-term permanent presence in space.

The biological sciences

The NASA Biological Science Branch includes four programs: Space Biology; a program to develop a controlled life support system; Global Biology and Exobiology. These seemingly dissimilar efforts are all tied by a common idea. They deal with life at different scales, but all are influenced in some way by gravitational physiology.

The questions posed here are:

1. How did life originate and evolve on an evolving planet?
2. How has life adapted to the chemical and physical conditions of nature?
3. How does life alter the environment?

The unifying principle is that the physical and chemical laws that govern the universe also govern the dynamics of life. We can study life and its environment at the global or at the organismal level. Common to both of these is the life requirement for energy, environmental forces which include gravity, and the principles of natural selection and evolution.

As part of this we include the study of prelife at the cosmic scale and the formation of the biogenic elements. Factors like mass, density, distance from a star are critical parameters for any planet's potential for biogenisis. These dictate the composition of the primitive atmosphere, the surface elements, the available energy, and possible subsequent organic chemical reactions that initiate the process. Once life was started, evolution and diversification of species were dominated by the environmental factors. Life on Earth can only be understood as an integral part of the whole environment.

On the global scale gravity plays a critical role in the various biogeochemical cycles. It determines turnover rates for some nutrients, it determines the rate at which our atmosphere can escape. Our oceans are only here because Earth has a critical mass that holds on to our atmosphere. There is a dynamic interaction between the biota and the non-biological competent of the Earth. The modification of the environment occurs as a direct consequence of life; and conversely the environment determines the nature of life on the Earth. This complex feedback system may someday be understood.

At the organism scale emphasis is placed on understanding how living systems adapted to various evolutionary forces, especially gravity. Understanding how gravity shaped the development, form, and function of life requires our knowledge of its perception and response. Since Earth has maintained a relatively constant gravitational force of 1 g throughout its history, our access to space is a powerful approach to manipulate or effectively remove the g vector as an environmental parameter and thereby investigate the effects on organelles, individual organisms, and populations.

Only 100 years ago, Konstantin Tsiolkovsky, the genius and father of Russian rocketry, wrote: "The earth is the cradle of humanity, but mankind will not stay in the cradle forever."

We are out of the cradle,
We have fallen down a few times,
We are sometimes crying, and crawling
  pretty well.
Now it is time to stand up and take our
  first steps.
The new height will give us new and
  better vision.
Our confidence will suddenly improve.
But most of all, our intense curiosity
  will perceive a whole new view of the
  world and the cosmos.

HORMONAL AND METABOLIC RESPONSES TO SIMULATED WEIGHTLESSNESS

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It is presently well known by all authors that periods of weightlessness cause important metabolic and hormonal modifications in man (1,18); this is the most important consequence of the redistribution of blood mass and of the organic fluids towards the thoracic and the cephalic regions from the lower part of the body. An improved understanding of these phenomena should allow longer stays in weightlessness.

On earth, it is impossible to recreate the absence of gravity : however, certain conditions such as prolonged horizontal or antiarthostatic bedrest or immersion can allow us to obtain, but to a lesser degree, a similar redistribution of the organic fluids.

The first studies in bedrest were performed between 1920 and 1929, in the United States by CAMPBELL (3) and CUTHBERSON (7) who were investigating the effects of bedrest on muscles, bone and nitrogenous compounds. These works were interrupted and only restarted by American teams after 1940. The first Russian publications appeared after 1963 : they were done by CHAZOV (5) about problems of anticoagulation capacities and by KAKURIN (15) about the effects of 20 days of hypokinesia on the motor, circulatory and respiratory systems. Because of the simplicity of this methodology, the bedrest studies have become frequent (table 1). Over the past 15 years, a new experimental methodology has appeared : it consists in placing the subjects not only in prolonged bedrest but also in antiorthostatic position (head-down) ; the inclinations warying from -2° to -15° according to the authors (table 1).

As to immersion, and we will only mention the immersion experiments with the head above water, the for runner was the Professor Otto GAUER (9) who studied the neuro-endocrin control of plasma volume and who described the reflex which carrier his name (table 1).

The aim of this present report is to do a synthesis of the hormonal and metabolic responses to simulated weightlessness and more specifically of certain parameters which participate in the regulation of the blood volume : diuresis, hematocrit, Na+, Plasma Renin Activity, aldosterone, antidiuretic hormone.

Hormonal and metabolic responses

1. Diuresis and natriuresis

The majority of authors described a significant increase in the diuresis during the bedrest experiments (11,13). That diuretic fluid loss appears during the 48 first hours after the beginning of the experiment. Concerning water immersion (8,10,20), the diuretic responses is immediate : GRIGORIEV (14) described the persistence of this phenomenon during a prolonged water immersion (56 days) experiment. At the same time of this diuretic fluid, there is a decrease in the interstitial volume during the first days of horizontal or antiorthostatic bedrest studies.

It is classical to observe a significant increase in the Na+ excretion during the experiments of bedrest or immersion (8,10,11,13,20). This increase appears classically during the first days of the experiments, however a large number of authors observed a negative Na+ balance throughout

Experimental methodology	Bedrest	Orthostatism	Water immersion
Number of subjects	About 1500	About 300	About 300
Duration	24 hours ↕ 210 days	24 hours ↕ 180 days	1 hour ↕ 56 days
Principal teams	Sandler, Kakurin, Greenleaf, Parin, Vasilyev, Leach	Nixon, Volicer, Blomquist, Grigoriev, Güell, Convertino, Krotov, Kakurin	Gauer, Graveline, Epstein, Grigoriev, Skipka

Table 1. Hormonal and metabolic studies during weightlessness simulation experiments.

2 or 3 weeks of bedrest studies. This increase in Na+ excretion is a response to the hypovolemia provoked by the loss of fluid via the kidney.

2. Hematocrit

Blood analysis showed a significant increase of hematocrit level in the two types of experiments (bedrest and immersion) during the first 48 hours. This increase varies from 2.5 % to 10 % (21). Increased hematocrit evidences a decrease in plasma volume (11).

This increased of the hematocrit is more significant during immersion experiments than bedrest studies (19).

3. Hormonal changes

Concerning hormonal system implicated in the blood volume regulation (Plasma Renin Activity, aldosterone, antiduiretic hormone), this results seem more heterogeneous principally during bedrest experiments (table 2).

Authors	Experimental methodology	Duration of the experiment	Principal results
Volicer (21)	O—| (-5°) 8 subjects	24 hours	Aldosterone : NS P.R.A. : NS
Chavarri (4)	O—| 8 subjects	24 hours	P.R.A. ↑ Aldosterone ↑ (after 8 hours)
Nixon (17)	O—| 8 subjects	24 hours	P.R.A. ↓ Aldosterone ↓ (after 12 hours)
Blomquist (2)	O—| (-5°) 8 subjects	24 hours	P.R.A. Aldosterone } NS A.D.H.
Chobanian (6)	O—| 6 subjects	2-3 weeks	Aldosterone } NS P.R.A.
Keil (16)	O—|	21 days	P.R.A. ↑ A.D.H. ↓
Güell (12)	O—| (-4°) 4 subjects	7 days	Aldosterone ↑ P.R.A. ↑ (3-4 days)
Greenleaf (9)	O—|	2-3 weeks	P.R.A. ↑ A.D.H. ↑

Normally it is observed a decrease of the plasma aldosterone and plasma Renin Activity during the first hours of bedrest experiments, but others authors described an increase of these values after 8 or 12 hours. Some publications describe any significant variation.

According to us, we have described in the 1st phase, a tendency to the decrease of the aldosterone in plasma and Plasma Renin Activity, following by a return to the basal values and finally at about the 3-4 day a significant increase.

According to the variations of antidiuretic hormone during bedrest studies, we have noted in literature important divergences. Immersion resulted in a significant decrease of plasma renin activity, plasma aldosterone and A.D.H. : the phenomena begin within 30 minutes after the beginning of the study.

Discussion

As we have seen it appears that there are important differencies among the different authors specially concerning the hormonal variations. We believe that this diversity of results is due to lack of standardisation in the past was making it extremely difficult to compare and therefore to make optimal use of the many bedrest experiments already carried-out. The protocol varied as regards subjects (age, sex, training), control values, duration, measurement times, external temperature, water temperature ...

We think that are of the most problem is the control value : the question is : what is the best basal estate ? Several interesting ideas on controls were put forward, during the Workshop held in Toulouse (March 1982), including :

- Experience had shown that when a control group was confined to the facility, but allowed to walk around, the results showed that slight deconditioning took place in the control group, due to the confinement. Control by a confined group, correctly age-matched, was feasible.
- Use of the volunteer as his own control by comparison with measurements taken in the erect position as being the most natural.
- An expensive, but possible control method would be to repeat the experiments with the same subjects after a period of (say) two months, with the subjects in a different position (horizontal or inclining upwards).

In conclusion, we can see, that after studying about 2000 subjects in horizontal or anti-orthostatic bedrest or also immersed in swimming-pools, all the problems are not resolved We think that the study of the following systems with standardized protocols should allow us to reach a better knowledge of the regulation of blood volume: β endorphine system, kinine-kallicrein system and natriuretic factor.

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WEIGHTLESSNESS INDUCED CHANGES IN HUMAN CARDIO-RESPIRATORY SYSTEM

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ABSTRACT

The possible diagram of the main genetic relations in the human cardio-respiratory system determining fainting reaction on orthostatic exposure has been developed in this paper. This diagram takes into account orthostatic test results obtained after zero-g simulation experiments and space flights. Stability of feedback loops, presented at the diagram, has been considered qualitatively.

Experimental Background

Water immersion, combined with anti-orthostatic hypodynamia with tilt angle -6° at night time, was used for the simulation of physiological zero-g effects. The orthostatic tolerance was studied after the 7-day simulated weightlessness. Ventilation and pulmonary gas-exchange shifts were continuously observed during the whole period of orthostatic test (head-up 75° tilt), being most noticeable in case of orthostatic fainting. It makes to think of their possible role in the orthostatic intolerance.

These studies have shown that postural faintness develops on the background of the rapid and considerable systemic blood pressure decrease, the appearance of relative bradicardia alongside with the comperatively constant cardiac output, the moderate pulmonary blood pressure reduction and proved alveolar ventilation ($\dot{V}_A$) increase. This last factor caused markable diviation in the O_2 and CO_2 arterial blood tensions (P_aO_2, P_aCO_2).

Postural hyperventilation, defined as the $\dot{V}_A/\dot{Q}$ increase, has been pronounced during the first minutes of the orthostatic test when $\dot{Q}$ diminished while $\dot{V}_A$ was comperatively constant, and during the last minutes before the fainting development when the respiratory frequency and pulmonary ventilation ($\dot{V}_E$) considerably increased. These data testify to to the prominent respiratory centre activation and the cerebral vasoconstrictive centre depressia on the eve of faintness. Meanwhile, with the persons that have good orthostatic endurance the P_aCO_2 increase and stabilization have taken place after the initial moderate hyperventilation period though V_E and tidal volume remained increased throughout the whole orthotest.

The transcutaneous observation of P_aO_2 obtained before and postflight in Soviet-Mongol, Soviet-French international expeditions and the 75-day flight on the Solyut-6 orbital stations also may prove the hyperventilation development after the head-up tilt. In a postflight period the described reations were individually variable but on the average more prominent.

The orthostatic faintness mechanisms have remained unclear. Along with it the lack of O_2 brain supply is commonly recognized as the direct cause of faintness. As far as in our studies the arterial blood oxygenation level has remained rather high during the whole orthotest the decrease of cerebral blood flow should be considered as the main reason of fainting.

FEEDBACK DIAGRAM

At the block-scheme (Fig. I) the most important physiological effects directly or indirectly influencing the cerebral blood flow are shown. The correlations between the variables are marked with arrows. Signs ± signify the positive and negative effects of the relationship influence on a physiological parameter. In other words, a correlation sign notes whether the conditioned relation is direct (+ correlation) or inverse (- correlation).

The presence of a negative feedback in control systems is known to lead to stability and that of the positive feedback to a loss of system stability. This makes us to suppose the orthostatic faintness to be the revealiation of such cardio-respiratory system parameters changes which cause the predomination of positive feedback relations over negative ones in some loop. And as a result, the given functional state of the system becomes unstable.

In all cases of orthostatic fainting the syncope has happened on the background of the rapid and noticiable average systemic arterial pressure (P_a) decrease, that leads to the abrupt cerebral blood flow ($\dot{Q}_b$) diminishing. In such a case as it can be seen on the scheme a blood circulation control loop with a positive feedback, the so-called "vicious cycle", may occur.

That is, the considerable cerebral tissue PO$_2$ (P_bO_2) decrease, caused by the $\dot{Q}_b$ diminishing, lessens the vasoconstrictive centre activity (VCC) which results in the peripheral arterial vessels tone weakening and peripheral vessel resistance (R_t) downfall; all these phenomena cause the peripheral blood flow increase, the P_a decrease and further $\dot{Q}_b$ downfall. As far as all mentioned correlations are positive, or direct, taken together they form the positive feedback loop that makes cerebral blood circulation unstable.

However, in case of good orthostatic endurance this instability is fully compensated with the stabilising effect of the two negative feedbacks: in a baroreceptor (BR) control loop, regulating R_t in accordance with P_a deviations, and in a local humoral control loop, regulating cerebral vessel resistance (R_b).

The feedback effect in a baroreceptor loop, regulating **R_t**, depends upon VCC, that is why it would decrease if insufficient cerebral oxygenation occurred. Thus, while orthofainting development, the negative feedback can be oppressed by the above-mentioned positive feedback.

The decrease of the $\dot{Q}_b$ local control stabilizing effect during an orthotest might be conditioned by the fact that the arterial hypocapnia and respiratory alcalosis prevent the **R_b** diminishing.

Actually, there are cirtain data about the P_aCO_2 decrease direct constrictory effect on the cerebral arterial vessels. They have been obtained at hyperventilation and hypercapnia /I,2/.

As it is seen on the block-scheme in consequence of the direct P_aCO_2 effect on the R_b a positive feedback occurs, embracing the $\dot{Q}_b$ local control loop and respiratory centre (RC) control loops regulating pulmonary ventilation. Under the arterial hypocapnia this positive feedback will prevail over the two negative feedbacks which ensure these loops stability. It must result in the cardio-respiratory system destability evoking the $\dot{Q}_b$ progressing downfall and simultaneous $\dot{V}_E$ growth.

Hence, the cerebral hypoxia evoked by the orthostatic faintness depressing the vaso-constrictive centre creates the positive feedback in the cerebral blood flow control loop and simultaneously leads to the weakening of the negative feedback in a baroreceptor control loop. Under such circumstances the arterial hypocapnia lessening the stabilizing negative feedback effects in the local cerebral blood flow and $\dot{V}_E$ chemoreceptor regulating loops can lead to a loss of the cerebral blood circulation stability, hence to the systemic arterial pressure and cerebral blood flow progressing downfall and, as a result, to the postural faintness.

It should be emphesized that the principal point of the stated orthostatic faintness mechanisms is the existence of P_aCO_2 direct effect on cerebral arterial vessels. It must be confirmed by special experiments.

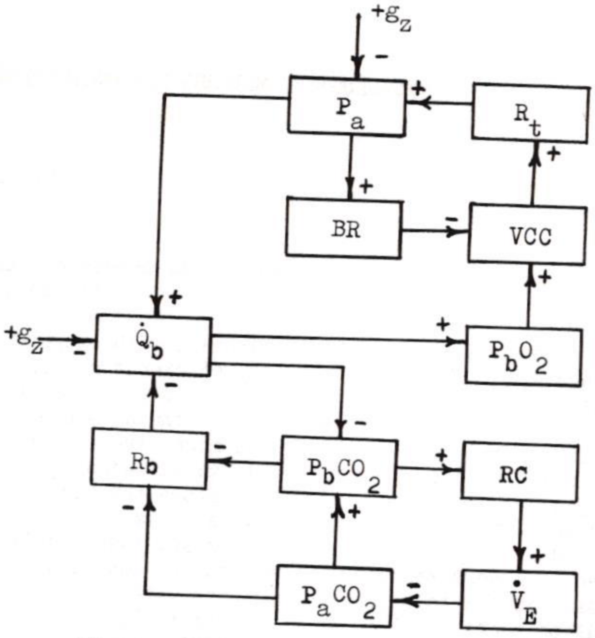

Figure I. Diagram of the possible orthostatic fainting mechanisms.
$+g_z$ - gravity acceleration at orthotest.

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PHYSIOLOGICAL METHODS FOR PROTECTION AGAINST HIGH SUSTAINED G_Z ACCELERATION

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Modern high performance aircraft with extreme maneuverability and possibility of executing very steep turns may expose the pilot to high sustained G_Z-acceleration. The onset rate of the acceleration may also be very high, and might thereby reduce and even abolish the early visual warning signs, gray-out and black-out, before the baroreceptor compensatory mechanisms have time to respond with a blood pressure increase, thus resulting in a very rapid G-induced loss of consciousness. Such a loss of consciousness with incapacitation times of 15 to 20 s in a fast flying aircraft may be disastrous to the pilot and the aircraft. Deadly accidents due to G-induced loss of consciousness when flying high performance aircraft are currently occurring. The G-tolerance of the pilot may therefore be a limiting factor for making a full use of a high performance aircraft. Even pilots flying in aerobatic competitions or air shows may be exposed to the risks of G-induced loss of consciousness. This safety hazard is due to the very quick transitions from negative to positive G_Z-accelerations during such flights, where the baroreceptor response also may be too slow. The research in different methods to protect the pilot against the adverse hemodynamic effects of high sustained and rapid onset G-acceleration has therefore gained much interest as well as methods to increase the G-tolerance.

Until now selection of pilots has aimed mainly at excluding those with diseases, deformities and behaviour disturbances or those not performing very well in psychomotor tests. But when the physical ability and endurance of the pilot to perform the straining maneuvers necessary to maintain or even to increase his G-tolerance may be crucial for the mission, the selection process might need to include even other constitutional factors. The relaxed G-tolerance may be higher in short, fat, older and hypertensive pilots (1), while the active G-tolerance, which includes the very tiresome performance of straining maneuvers, might be better in physically fit young pilots. To create test programs to select those individuals with good ability and endurance to perform the G-tolerance increasing straining maneuvers with a high anaerobic capacity must therefore be of great value. So far preliminary results of muscle histochemical tests, as done by muscle biopsies, have not revealed any differences in fiber types or capillary densities in pilots with good or poor G-tolerance (2). The G-tolerance was then measured as the duration of simulated aerial combat maneuvers until volitional exhaustion in human centrifuge tests. Other muscle function tests or anaerobic capacity tests might be more promising in the selection of pilots with high G-tolerance or with the capacity to get a high G--tolerance.

Reduction of negative factors that influence the G-tolerance is also protecting against high sustained or rapid onset G_Z-acceleration. Heat stress with peripheral vasodilation is a factor that reduces G-tolerance as well as the dehydration with diminished plasma volume that might accompany the heat stress. Cooling systems of both body core and peripheral parts of the body to protect against the warm environment should therefore be important, especially if the pilot has to wear protective equipment against chemical or bacteriological agents, where heat stress is a problem. The rehydration of a dehydrated pilot is also important to maintain G-tolerance. The effect of rehydration seems to be about the same if water or a low concentration sugar solution is taken (3), but it appears to be important that a sufficient amount of liquid is given. A liquid filled stomach also seems to be beneficial for the G-tolerance. Avoidance of hypoglycemia is essential to maintain a good G-tolerance as well as avoidance of the possible reactive hypoglycemia after a big and fast sugar intake should be (4). Hypocapnia through a hyperventilation may cause cerebral vasoconstriction and peripheral vasodilation and a mild hypoxia may also cause a peripheral vasodilation, all of which might be deleterious to the G-tolerance and should therefore be avoided. Other factors that negatively influence G-tolerance are alcoholic intake, hang-over, medication, general fatigue, infection etc and should be taken into consideration still more nowadays, when the G-tolerance is so crucial and when even a small decrement may be deleterious to the flight safety.

If hyperventilation causing a hypocapnia may enhance the risk of a G-induced loss of consciousness or reduces the G-tolerance, the reversed situation is valid when adding up to 5% of carbon dioxide to the breathing gas (5). This hypercapnia may induce a peripheral vasoconstriction that actually will increase the G-tolerance. The problem with this G-protecting method is the experience of dyspnoea and possible decrements in mental and psychomotor performance. Another way of inducing peripheral vasoconstriction as a G-protecting method is the use of vasoconstrictor drugs. The drawbacks are its side-effects. New vasoconstrictor drugs with less side-effects might, however, be tested in the future and show up to be of value to increase G-tolerance.

The most effective, but the most unlikely way of G-protection is by immersion. Immersion to the heart level by a water suit was tested by the Germans already in the 30ths with some success (5). The weight of the water, however, reduced its further use in aeroplanes. The pulmonary arteriovenous shunting was, however, not prevented by this partial immersion. With total immersion the situation is much better (6). In the 50ths totally immersed mice were exposed to 1 300 G for 60 s and survived (7). And rat foetuses may survive impacts corresponding to 10 000 G when the mother is floating in water (8). And unicellar water living animals may survive up to more than 200 000 G for up to 4 hours in water (7). The problem with normally air-breathing higher animals immersed in water is the breathing resistance with the liquid in the lungs and the oxygenation of the blood. If the liquid has the same density as saline, blood and other tissues, the relative movements of the heart, lungs, and other tissues and blood would be prevented and blood pressure fall and pulmonary arteriovenous shunting minimized. Dogs breathing oxygenated liquid fluorocarbon with a density of 1.8 with a great oxygen solution capacity in a whole-body immersion respirator have been exposed to 7 G without pulmonary atelectasis, arteriovenous shunting or downward displacement of the heart (6). Even if this method should be very effective to protect against high G-forces, the practical application in human pilots is far away.

Another very effective way of increasing G-tolerance is supine body position or at least increased seat back angle. With more than 75° seat back angle the G-tolerance is doubled (9, 10). The Pelvis and Legs Elevating Seat (PALE) also increases the G-tolerance substantially and G-levels up to 15 G are acceptable (11). Due to technical difficulties with for instance the escape system, the instrument display and also with vision the PALE system or supine body position is not introduced yet. But if high performance aircraft will fly with higher G-loads than today these systems probably have to be used. The diminished blood column distance between heart and eye/brain is a method used already by the pilots when taking a crouching position during G-maneuvers and it increases the G-tolerance somewhat (12).

The since long time used anti-G-suit renders the pilot a good G-protection and it has been improved with preinflation, and with faster, more physiological, filling (13, 14). There are reports of that sequential inflation of the anti-G-suit is beneficial to the pulmonary capillary blood flow (15), but the effect on G-tolerance seems to be more disputable.

Increasing the blood pressure during G-exposure by Valsalva and muscle straining maneuvers, the so called M_1 or L_1 straining maneuvers, is also very effective to protect against high G, when performed correct (10, 16). When they are performed too slow the result may be a blood pressure decrease, due to reduced venous return, finally resulting in a loss of consciousness. When performed too fast, they may cause hyperventilation with hypocapnia which also will result in a decreased G-tolerance. Therefore they have to be performed with straining in about 3 s and with a rapid inhalation phase and combined with peripheral muscle straining. Indoctrination and training of these maneuvers, preferably in the controlled and surveyed situation in a human centrifuge is therefore essential. Training at 1 G level may, however, cause too high blood pressures and might possibly be dangerous. The possibility of a rupture of the round window in the middle ear during the Valsalva maneuver should probably not be taken too seriously.

The combining of the mentioned techniques may increase the G-tolerance still further from a combination of additive, synergistic and overlapping mechanisms (17).

Positive pressure breathing (PPB) increases the arterial blood pressure, the pressures in the heart and lung vessels proportionally to the raised airway pressure (18). Higher values of PPB decrease the stroke volume and cardiac output, however, why it has to be combined with the use of an anti-G-suit. At 1 G the blood pressure may raise to more than 27 kPa (200 mm Hg) with PPB of 9.3 kPa (70 mm Hg). With this high airway pressure the subject has to be protected against the risk of overinflation and lung rupture by counterpressure to the thorax, so called assisted PPB, which also is less fatiguing. This blood pressure increase during PPB has been tested on the G-tolerance, as measured as the duration of simulated aerial combat maneuvers (ACM) with repeated 15 s periods at 7 G until volitional exhaustion. With only 30 mm Hg (4 kPa) of PPB an about 20% increase in ACM-time has been demonstrated if the PPB was combined with faster filling and ready pressure in the anti-G-suit (19). Results from others with unassisted 30 mm Hg PPB are, however, more dubious (20). If the 30 mm Hg PPB is assisted with counterpressure to the thorax, however, which is not so fatiguing, a 40% increase in G-tolerance has been found at the USAF School of Aerospace Medicine (20). If assisted PPB with airway pressure at 50 mm Hg (6.7 kPa), a more than 100% increase in G-tolerance at very high G-levels with an ACM-profile with 10 s periods at 5 and 9 G has been registered at the USAF School of Aerospace Medicine (21). With 70 mm Hg (9.3 kPa) assisted PPB the increase with the same G-profile up to 9 G was 70%. There seems to be an optimum effect of PPB on the G-tolerance when the benefits of the blood pressure increase is greater than the disadvantage of the stroke volume decrease with the use of an anti-G-suit. The optimum seems to be with about 50 mm Hg assisted positive pressure breathing. This has also been confirmed at the Aviation Medicine Institute in Poland (22). PPB at 1 G is very strenous and discomfortable but at higher G-levels the subjects hardly know if they have PPB or not. PPB has also the advantage over using the M_1 straining maneuvers in that the short episodes of gray-out during the inhalation that may appear with the M_1 straining maneuvers is eliminated with PPB.

Another use of PPB to protect indirectly against high sustained G_z-acceleration is to avoid the G-induced pulmonary atelectasis. With both high sustained G and not so high G-loads there might be G-induced pulmonary atelectasis with lung volume reductions up to 50% or more in some individuals when they are breathing more than about 70% oxygen. As during oxygen breathing during immersion in divers (23), those pulmonary atelectases during G-loads may be diminished substantially by applying PPB or M_1 straining maneuvers (24).

Another way of increasing G-tolerance has been shown to be muscle strength training. In contrast to aerobic training such as ordinary running, or marathon-running activity, a muscle strength training program, such as weight-lifting, has been shown to increase the ACM-time G-tolerance (25, 26). This has been claimed to be due to neuromuscular adaptation and increased endurance to perform the M_1 or L_1 muscle straining maneuvers.

As very high intraabdominal pressures could be registered during the performance of the muscle straining maneuvers at high G-loads, training of just the abdominal muscles should possibly be of value to increase the G-tolerance. In two studies made at about the same time it was, however, shown that abdominal muscle training only is not sufficient to increase ACM endurance G-tolerance (27, 28). Apparently more muscles have to be involved to increase the endurance to do the M_1 or L_1 muscle straining maneuvers in order to increase the G-tolerance.

In summary, the G-tolerance of the pilot flying high performance aircraft is crucial and could be a limiting factor. Several methods to increase the G-tolerance are available. Excluding the highly effective but impractical immersion and supine body position, a carefully training to do the M_1 or L_1 straining maneuvers, technological development of the anti-G-suit systems, extreme assisted positive pressure breathing and muscle strength training program, seem to be of great value to increase G--tolerance and to protect against high sustained G_z-acceleration.

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HEART RATE RESPONSE DURING +Gz OVERLOAD ON THE HUMAN CENTRIFUGE AND DURING MAXIMUM BICYCLE ERGOMETER LOAD[1]

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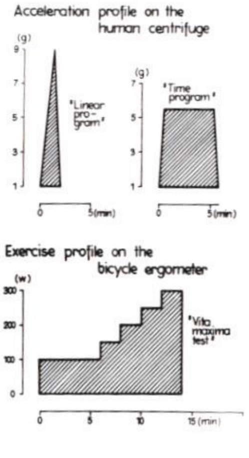
INTRODUCTION

The determination of performance and of strain in aircrew members under different load conditions requires, among other things, task-orientated function and performance diagnostics with regard to aeromedical opinion. In this connection the frequent use of heart rate for evaluating the strain in the working subject gave rise to the analysis of heart rate response with regard to measurable load variables in different function diagnostic tests.

MATERIALS AND METHODS

In the present analysis results from investigations on the human centrifuge and on the bicycle ergometer, carried out within the scope of aeromedical opinion, were involved.

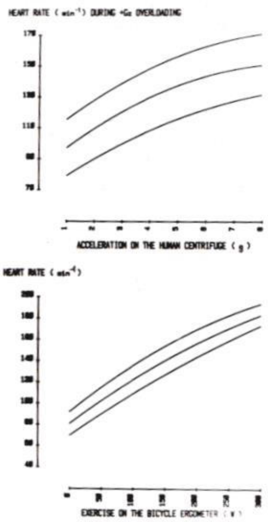


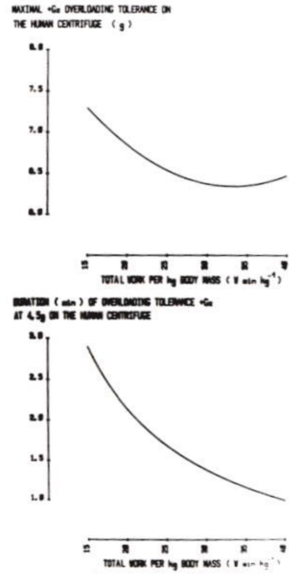
The load structure of the test conditions is shown in Figure 1 and demonstrates, that the increase of acceleration during centrifuge tests (3), using the so-called "linear program", occurs linearly at an acceleration gradient of 0.1 $g \cdot s^{-1}$ up to a peak value of 9 g and, when using the so-called "time program" according to a linear increase in overload of 0.2 $g \cdot s^{-1}$ up to a value of 4.5 g, a constant acceleration at the last-mentioned value for a maximum duration of 5 minutes is maintained. The bicycle ergometer load is increased in stages until vita maxima, beginning at 100 Watt for a duration of 6 minutes and then continuously enhancing it every two minutes by 50 Watt until subjective exhaustion or until break-off for medical reasons.

The heart rate at each measuring moment was calculated from the duration of every five cardiac periods.

RESULTS

The statistical mean value ($\bar{x} \pm s$) of heart rate at the described load configuration is shown in Figure 2. It can be seen, that the heart rate during +Gz overload on the centrifuge, when using the "linear program", increases from an initial value of about 97 ± 18 beats per minute to about 150 ± 20 beats per minute at an acceleration level of 8 g. In the same group of subjects, as shown in the upper part of Figure 2, also the mean heart rate during gradually increasing load on the bicycle ergometer, which could be identified from the measured values during the first minute at each load level, was presented. The average initial value of heart rate of about 83 ± 12 beats per minute was significantly below the pre-load value on the centrifuge. The average maximum heart rate, however, reached a value of 182 ± 10 beats per minute near the load limit of about 300 Watt. The higher values of heart rate before the onset of overload on the centrifuge may be regarded as an indication of increased anticipation strain. On the other hand, a comparison of the average heart rates measured before breaking off the compared loadings, which showed 148 ± 22.8 and 180.8 ± 11.0 beats per minute, respectively, indicated, that the heart rate reached a 22 % higher value near the load limit during ergometrical exercise. Under the "linear program" on the centrifuge the averaged maximum heart rate during +Gz overload was still





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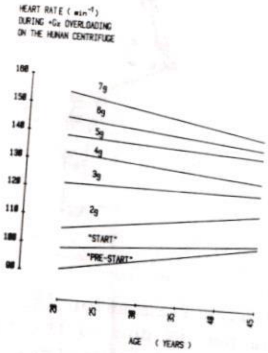
[1] The authors thank M. Wojtkowiak (Institute of Aviation Medicine, Warsaw, Poland), G. Pönisch, F. Vietor, and P. Spranger (Institute of Aviation Medicine, Königsbrück, GDR) for their participation in carrying out the investigations.

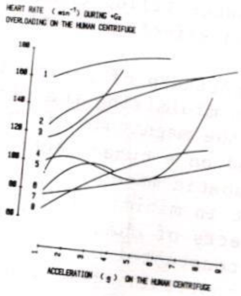
lower and showed 140.9 ± 18.5 beats per minute.

In addition to this Figure 3 represents the mathematical statistical relation of both the maximum +Gz tolerance and the +Gz tolerance time at an overload level of 4.5 g with total work per kg of body mass during vita maxima testing on the bicycle ergometer. Essentially, for both a negative relation between ergometrical performance and +Gz tolerance on the centrifuge was found within the measuring range of the subject group.

According to the well-known general dependence of heart rate on age, this age trend is described by linear functions in Figure 4. This figure shows, that the heart rate anticipatorily increases before and at the onset of +Gz overload with growing experience of life and professional experience, whereas the heart rates at higher overload levels decrease with growing age.



The dynamics of the individual heart rates during the +Gz tolerance tests with the "linear program" on the centrifuge, illustrated in Figure 5 by selected examples, allows the discussion of different psychophysical performance conditions of +Gz tolerance. The figure illustrates, that there exists not only a very great inter-individual variability concerning the absolute level of heart rate before onset of acceleration, during increasing acceleration, and at acceleration break-off, but also a strong inter-individual variation concerning the dynamics of heart rates. Thus, besides delayed adjustment responses to increasing acceleration (curves 2, 5, and 8) also linear slopes (curve 7), overproportional slopes (curve 3), and nondelayed adjustment responses of heart rates (curves 4 and 6) can be observed. A systematic correlation between the individual regulating quality of the heart rates as well as their absolute levels during +Gz overload and the values for maximum +Gz tolerance on the human centrifuge is not given. Because of the well-known multi-factor condition of +Gz tolerance some of the factors shall be mentioned only as examples: The positive dependence on age, on flight experience, and on body mass as well as on ergometrical performance are overlapping. Of particular interest are the high overload tolerance of a former, considerably obese fighter pilot with very poor endurance capacity (curve 2) and the low overload tolerance of a former fighter pilot of the same age with a substantially better endurance capacity (curve 3).



DISCUSSION

According to the information presented in the literature a direct systematic comparison of heart rate responses during +Gz overload on the centrifuge, during +Gz overload in real flight, and during bicycle ergometer load or under other physical strain conditions cannot be made.

In addition to the efficiency of anti-G suits, of muscular tension as well as of special respiratory techniques and so on, a major reason for the differing data is the efficiency of various acceleration gradients (1). But also the effects of constitutional characteristics (2), of the state of health and training condition, of age and adaptation during the training process as well as the regulative characteristics of functional systems should be mentioned.

CONCLUSIONS

1. The heart rates observed during ergometrical vita maxima tests are generally higher than the maximum heart rates during +Gz overload on the human centrifuge, which, in turn, show differences depending on the centrifuge acceleration profiles.

2. With identical +Gz overload profile there is a great inter-individual variability of heart rate as to its absolute value, its increase induced by increasing overload, and its regulative characteristics.

3. The results substantiate, that heart rate can be used as an indicator of strain only when the individual response pattern for the load types and structures concerned is known.

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Interserosal Forces, the Pressure Environment of the Central Circulations and Natures Internal "G suit". (Part I)

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The fact that the abdominal contents behave like a hydrostatic system, i.e., as if the organs were immersed in a bucket of water the upper surface of which is at the diaphragm (1) so that venous return to the heart is well maintained during exposures to $+G_z$ acceleration, has been documented on the Mayo human centrifuge (2).

In spite of this fact, it would be expected that the flaccid anatomical structure of the heart would make it very ill suited for maintaining its normal pumping function during large changes in the weight of its component parts which occur with large changes in the magnitude of the force environment.

Finding of large, 10 to 20 torr, average increases in right atrial pressures in 6 healthy humans at the onset and duration of 10-minute exposures to forward ($+G_x$) acceleration (3) (Fig 1), suggested that serious impairment of cardiac function may occur during the high $+G_x$ segments of the launch and reentry phases of space flight.

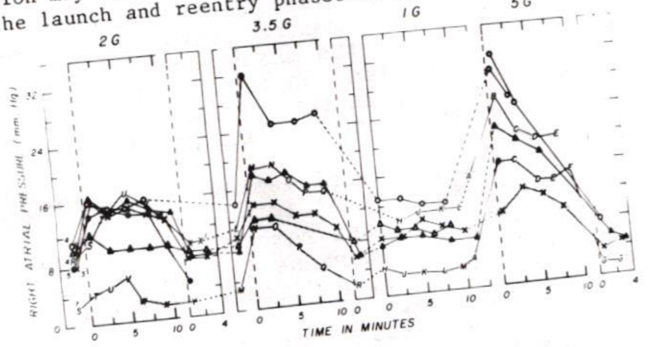

Figure 1. Reproduced with permission (3).

However, the hemodynamic significance of increases in intracardiac pressures is indeterminate without measurement of simultaneous changes in intrapericardial pressure, knowledge of which is mandatory for estimation of the actual effective right atrial distending pressure, i.e., myocardial transmural pressure.

Realization of this need coupled with the constant finding of quite large decreases in arterial oxygen saturation and infrequent instances of acute mediastinal emphysema during exposures to forward acceleration (4) prompted development of techniques for measurement of both intrapericardial and pleural pressures in dogs and chimpanzees studied without thoracotomy during changes in body position and the force environment (5-7).

Pericardial pressures at non-dependent sites in the pericardial space decreased progressively with increasing levels of forward acceleration while concomitantly pressures at the most dependent site increased to highly positive values (40-50 torr) at 7 G_x (Fig 2). If closely similar increases in dependent intra-atrial pressures did not occur concomitantly with these very high levels of pericardial pressures, collapse of the myocardial walls in dependent regions of the atrial chambers would surely occur.

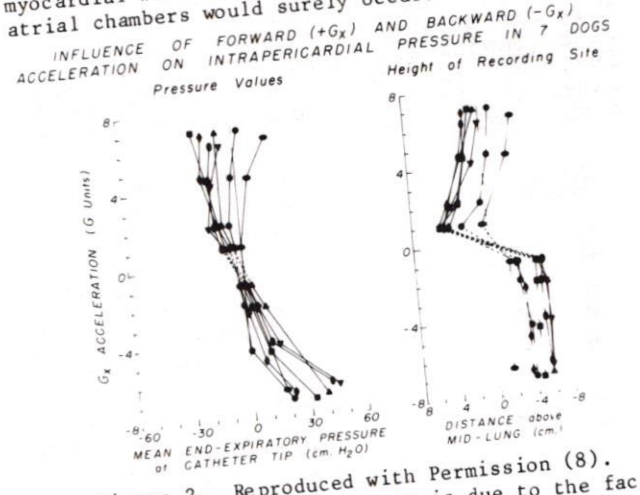

Figure 2. Reproduced with Permission (8).

That this does not happen is due to the fact that intracardiac pressures behave hydrostatically, i.e., at 1 G they change about 1 cm of water per cm of vertical height within any given cardiac chamber. Furthermore, this change in pressure per cm of vertical height is in exact proportion to the magnitude of the force environment.

Figure 3 indicates that this is indeed the case. The fact that left and right atrial transmural pressures shown in the right panels were essentially constant independent of the level of acceleration and vertical position in the thorax is convincing proof that pericardial pressure does indeed behave like a water-filled chamber and thus provides what is in effect an ideal "G suit" for the heart (8).

In addition to the importance of pericardial and peritoneal pressures in minimizing the effects of large changes in the magnitude of the force environment generated on a human centrifuge, or as occurs in aerobatic maneuvers and in space flight, their effect in minimizing the every day orthostatic effects of changes in body position in the normally constant 1 G environment of planet earth is also important. Pericardial pressure values recorded at multiple sites extending from the most dependent to the most superior regions of the hearts in 6 dogs studied without thoracotomy in the head-up and head-down positions (Fig 4) indicate that pericardial pressures change 1 cm of water per 1 cm change in vertical height as would be expected in a water-filled hydrostatic system (Fig 5) (9,10).

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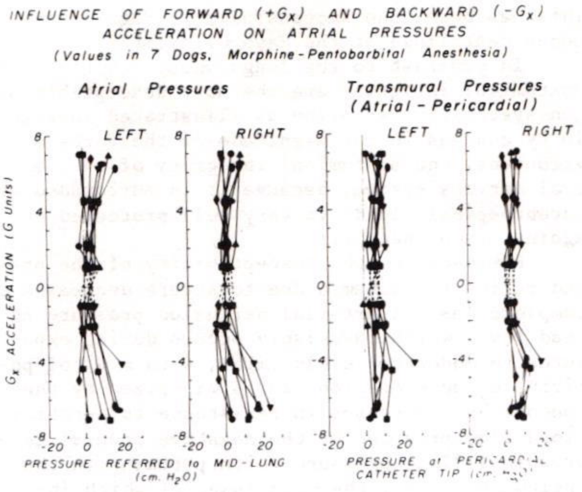

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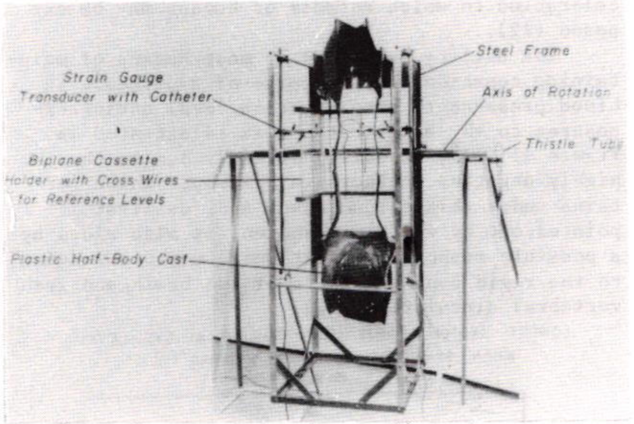

Figure 4. Apparatus for studies of orthostatic effects of body position (10).

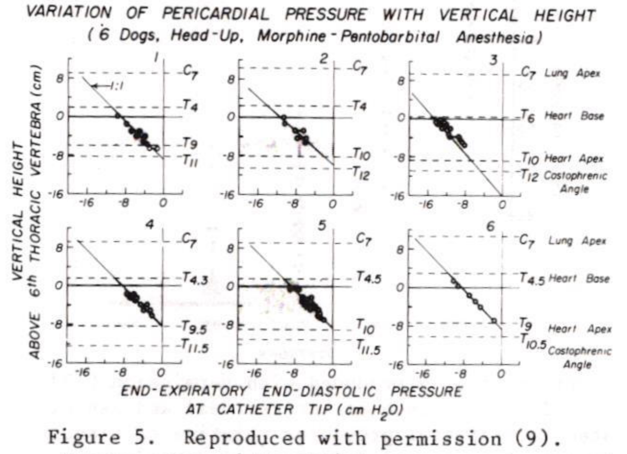

Figure 5. Reproduced with permission (9).

Average mean right atrial pressures decreased to about -10 cm of water when these dogs were tilted to the head-up position. However, pericardial pressures decreased simultaneously by the same amount so no systematic change in right atrial transmural pressures occurred with these sudden changes from the horizontal to the vertical head-up or head-down positions (Fig 6).

Left atrial transmural pressures were also relatively unchanged in all three body positions. This degree of constancy of atrial pressures with vertical head-up and head-down tilt positions indicates that anesthetized dogs when well supported by a form-fitting cast (Fig 4) have a surprisingly high degree of resistance, particularly for an animal whose normal body position is horizontal, to orthostatic decreases in cardiac output and associated arterial hypotension (9,10).

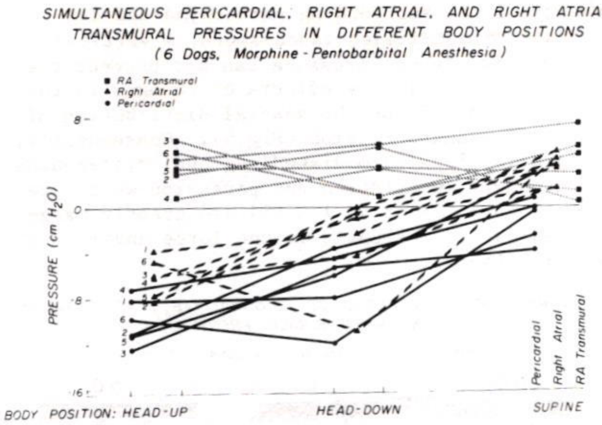

Figure 6. Reproduced with permission (9).

The fact that pressures recorded simultaneously at various sites in the pericardial space and at juxtaposed sites in the pleural space are not significantly different indicates that pleural pressures near the heart are closely similar to pericardial pressures at the same vertical height in the thorax and therefore that there must be a 1 to 1 hydrostatic pressure gradient in the pleural liquid pressure at least in the region of the heart (Fig 7) (8,9).

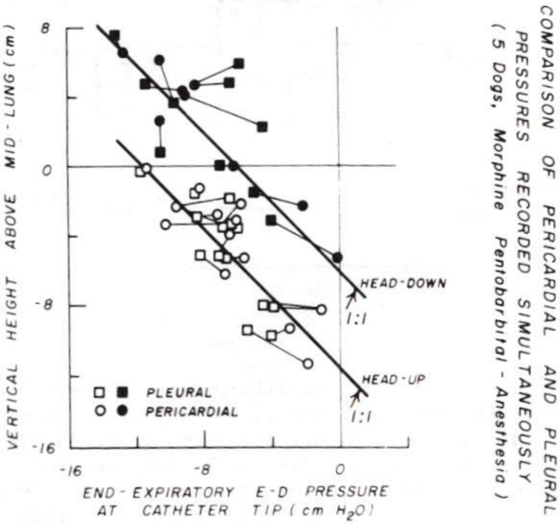

Figure 7. Reproduced with permission (9).

It should be remembered, however, that the relationship between pressures measured in a liquid film between serosal surfaces and the forces acting on these surfaces is uncertain if the thickness of the interposed liquid is not sufficient to preclude the presence of significant intermolecular forces such as adhesion between these surfaces (11,12). It is of interest in this regard that recent studies using new, independent techniques indicate the presence of overall vertical gradients in interpleural expansile forces similar to or even greater than the gradients in liquid pleural and pericardial pressure values reported herein (13,14).

However, because of the large difference between the practically zero specific gravity of the relatively weightless gaseous alveolar contents and the slightly greater than 1 specific gravity of the surrounding parenchyma and its abundant, blood-filled vasculature, a vertical gradient in pleural pressure can not prevent the large disproportionate effects of changes in the force environment on the spatial distribution of ventilation and perfusion (Fig 8). Consequently, the gravitationally-mediated regional differences in pulmonary ventilation and perfusion which are known to be present at 1 G (5) are greatly exaggerated during increases in the force environment (Fig 9) (15-19).

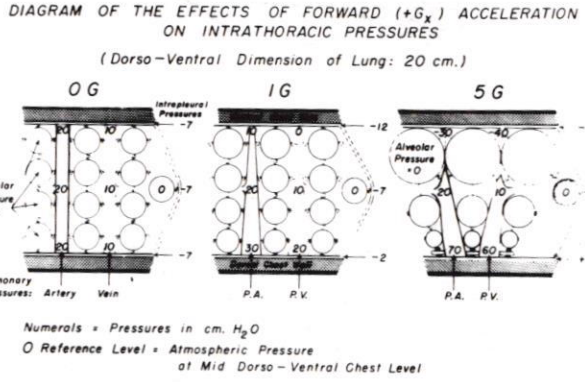

Figure 8. Reproduced with permission (5).

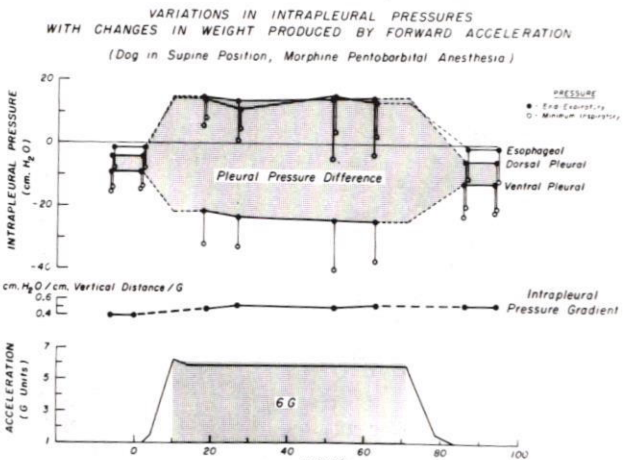

Figure 9. Reproduced with permission (4,15).

Furthermore, because of the very large intrapulmonary pressure imbalances which occur at high levels of acceleration due to the differential displacements of the essentially weightless alveolar gases relative to pulmonary blood and tissue, instances of rupture of the anatomically fragile lung parenchyma during high G exposures have been reported in man and animals (Fig 10) (5,15,19).

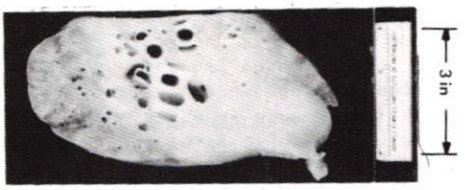

Figure 10. Section of chimpanzee lung after fatal exposure to 40 G in a water immersion capsule. Reproduced with permission from E.L. Beckman et al (20).

These potentially very dangerous effects on the lungs can not be prevented except by the impractical stratagem of replacing the respired gas by a very high oxygen content liquid with a specific gravity closely similar to blood and tissue. This has been done successfully in dogs on the human centrifuge at the Mayo Clinic (21).

In contrast to the lungs whose function and anatomical integrity are the most susceptible organ system to disruption as illustrated in Figure 10 by changes in the magnitude of the force environment, the anatomical integrity of the central nervous system, because it is surrounded by cerebrospinal fluid, is very well protected against such changes.

Furthermore, the susceptability of the brain and retina to ischemia due to severe decreases or complete loss of arterial perfusion pressure at head level which invariably occurs during exposures to moderate levels (i.e., 4 to 6 G) of positive G_z acceleration (2) is minimized by the concomitant decreases in resistance to cerebral blood flow produced by the negative cranial cerebrospinal fluid pressure which pertains in the upright position, the magnitude, of which increases proportionately with the level of $+G_z$ acceleration to which animals or humans may be exposed (22).

The ability exhibited by most humans of maintaining consciousness in spite of a decrease in blood pressure to zero at brain level during exposures to $+G_z$ acceleration, as illustrated in Figure 11 (23), is quite certainly due to the highly negative intracranial pressure which pertains under this circumstance and, as first pointed out by von Diringshofen, is also aided by a possible siphoning effect which may occur due to the rigid bony enclosure of the brain and its vertebral circulation (24).

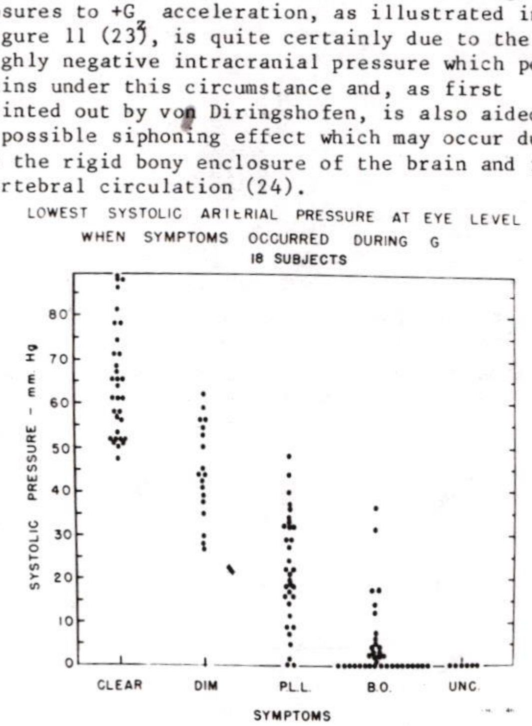

Figure 11. Reproduced with permission (23).

The importance of interserosal and associated interstitial pressures relative to tissue perfusion is exemplified in the eyes in which, because of their positive intraocular pressure and, furthermore, because they are outside of the cranial vault, retinal perfusion is not facilitated by the highly negative intracranial pressure during $+G_z$ acceleration as is the case for the brain. The fact that application of negative pressure to the eyes (Fig 12) abolishes the loss of vision that normally occurs at levels of positive acceleration 1 to 2 G less than the level which causes loss of consciousness (25,26) confirms the importance of interserosal and interstitial pressures as determinants of tissue perfusion particularly during changes in the force environment.

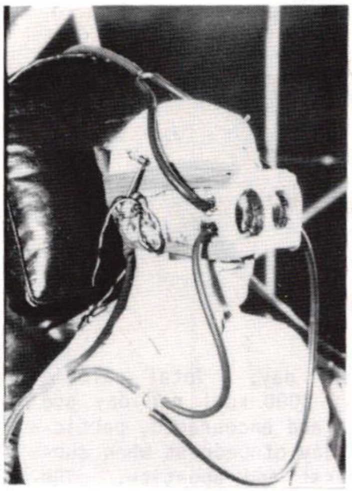

Figure 12. Apparatus for increasing or decreasing $+G_z$ blackout threshold by negative or positive pressure respectively on eyeballs. Reproduced with permission of E.H. Lambert (25,26).

These and other considerations are the basis for our belief that the thoracic hyperemia and resulting cardiovascular deconditioning which occur in the near zero G force environment of outer space could be prevented by concomitant exposure of the head and airways to a moderate degree (about 10 to 20 torr) of positive pressure (27).

However, unequivocal confirmation of this belief and related considerations will require quantitative knowledge of the amounts and regional distributions of pulmonary gas and blood volumes and also blood volume-myocardial dynamics of the cardiac chambers in astronauts using the non-invasive vivisection technic made possible by a miniaturized model of the high fidelity synchronous volumetric scanning tomographic, D.S.R., system currently operational in the Mayo Biodynamics Research Unit (Fig 13) (28,29).

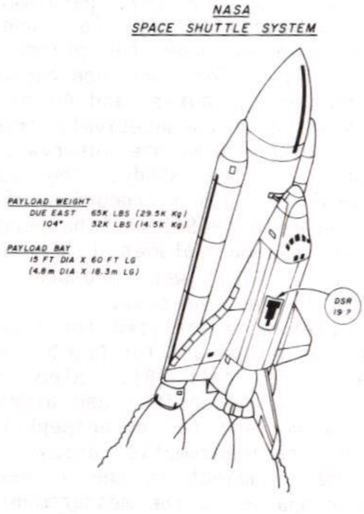

Figure 13. Reproduced with permission (16,30).

It is a reasonable prediction that the currently evolving state-of-art of X-ray to electrons transducer devices and associated computer based image display and analysis systems (30,31) will make incorporation of such a system in a space laboratory feasible by the end of this decade.

It is a promising and exciting future for the life sciences in space!

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CURRENT CONCEPTS OF SPACE FLIGHT INDUCED CHANGES IN HORMONAL CONTROL OF FLUID AND ELECTROLYTE METABOLISM

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A systematic analysis of body fluid and renal dynamics during simulated space flight (head-down bedrest) was undertaken to increase understanding of the physiologic effects of acute cephalad fluid shifts. The earliest effects were increases in central venous pressure and decreases in plasma aldosterone, epinephrine and norepinephrine and glomerular filtration rate, 2 h after the beginning of bedrest. Decreases in plasma angiotensin I at 6 h may have resulted from the increased effective pressure and decreased sympathetic activity seen earlier in bedrest. The early decrease in aldosterone and ADH is thought to contribute to an increase, by 6 h, in urinary excretion of salt and water. Fluid and electrolyte losses occur during space flight, and analysis of body fluids from Space Shuttle crewmembers has indicated that conservation of these substances is begun almost immediately upon cessation of weightlessness. Operational medicine measures to counteract dehydration and electrolyte loss resulted in a less extreme physiologic response to the flight.

INTRODUCTION

In 1970, we proposed a hypothesis to explain fluid, electrolyte, and hormonal changes which occurred in US astronauts during weightlessness (1). While adaptive changes late in the missions (>4 days) are well documented, early changes leading up to adaptation have to be surmised. To study early adaptation in a systematic manner, we have undertaken a series of experiments to record early responses of renal function and fluid and electrolyte balance in a series of 6° head-down bedrest studies to simulate adaptation to weightlessness. Head-down bedrest has been reported (2,3,4) to produce fluid and electrolyte changes and cardiovascular responses similar to those observed in space flight (5). However, renal measurements have heretofore not been included in head-down bedrest studies of fluid, electrolyte, endocrine and cardiovascular changes. Results of this and other studies have been used by NASA to devise measures which alleviate fluid and electrolyte deficiency in returning Space Shuttle crewmen. The effects of one such countermeasure are described in this report.

METHODS

Twenty-eight healthy adult subjects participated in the bedrest study. The mean age of the 18 males was 27 years and the mean age of the 10 females was 27 years. Throughout the study, the diet contained 130 mEq of sodium and 70 mEq of potassium per day. Total caloric intake was approximately 2000 kcal per day and fluids were unrestricted and encouraged, particularly during the first day of bedrest when subjects tend to lose thirst and appetite. The basic experimental design consisted of a 3-day ambulatory control period, a 60-hour period of 6° head-down bedrest beginning at 10 a.m., and a 3-day recovery period. The 3-day period prior to bedrest served as a period of adjustment to the diet and provided baseline determinations of renal function, endocrine and biochemical values and body fluid volumes. Renal function tests were initiated during the ambulatory control period and were repeated on each day of bedrest, at the same time each day. For one group of subjects this time was when bedrest began, and for two other groups it was 2 or 6 hours after the start of bedrest.

Single injections of markers were used for estimating glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and plasma volume. Inutest (a polysaccharide similar to inulin) was used for GFR (6), para-aminohippurate (PAH) was used for ERPF (6), and ^{125}I-human serum albumin was used for plasma volume determination (7). The two non-radioactive markers (150 mg per kg Inutest and 40 mg per kg PAH) were administered consecutively from pre-loaded syringes over a 5-minute interval. During the course of the study, the subjects received a total of 10.5 microcuries of ^{125}I-labeled serum albumin (RISA) for the purpose of estimating four plasma volumes (7). Central venous pressure (CVP) was measured by a catheter placed in the vena cava.

Blood samples were analyzed for Inutest by the microanthrone method and for PAH by the procedure of Waugh and Beall (8). Blood samples were analyzed for angiotensin I and aldosterone by radioimmunoassay and for norepinephrine and epinephrine by radioenzymatic assay. Data obtained for each subject at the 6° head-down position were compared to the measurements made for that subject at the same time of day during the control period.

Approximations of GFR and ERPF were calculated by the method of Rosenbaum et al. (6). The approximate rates were corrected for excretion of the tracer by using the approximations to estimate the amount of tracer excreted during the injection period. After the injected dosage was reduced by estimating the amount excreted, a new clearance rate was calculated from the "effective" dosage. By sequential application of this iterative procedure, a value of the clearance rate was obtained which did not change by more than a few tenths of a percent with successive rounds of approximation. This value was then normalized to a body surface area of 1.73 m^2 (6).

The Space Shuttle (STS) has so far completed seven missions of 50 hours to several days. In the first three Shuttle flights the crewmen experienced no intervention in their preparation for return to Earth. In Shuttle flights 4 through 7, the crew were given the support of ingested salt and water (approximately 1000 cc physiologic saline) to help accomplish readaptation to Earth's gravity. Blood and urine specimens were obtained from Space Shuttle crewmembers on three days before flight and immediately after landing (9). Postflight measurements were compared to mean preflight measurements for each crewmember, and analysis of variance was used to reveal significant differences between the crewmembers who received extra salt and water and those who did not.

RESULTS

Statistical analyses showed no difference between males and females; therefore, no distinction was made between sexes in the presentation of the results.

Central venous pressure exhibited a biphasic pattern (Fig. 1). It was elevated at 2 h into head-down bedrest, but within 5 h it decreased to 42% below control and it was still reduced at 6 h when the test was terminated. GFR, also shown in Fig. 1, was depressed at 2 h and had returned to control levels by 8-9 h. The same figure shows that ERPF also had a biphasic pattern; it had decreased by 4 h but before 8 h had surpassed the control level. Plasma aldosterone (Fig. 1) had decreased by 3 h but was elevated above control levels by 9 h. Angiotensin I, which is a measure of renin activity, decreased by 6 h but had increased to exceed the pre-bedrest level by 9 h (Fig. 1). Plasma norepinephrine and epinephrine (Fig. 2) decreased after 2 h or less of bedrest.

After 24 h of bedrest, renal blood flow and glomerular filtration rate were back to control values, and the norepinephrine level was still decreased (-15.9 ± 7.2%). A measurable decrease (5.7 ± 1.2%) in plasma volume was recorded at this time.

The changes in electrolyte and hormone concentrations in the blood and urine of Space Shuttle crewmembers are summarized in Table 1. Plasma sodium was close to preflight levels, but urinary sodium was much lower after space flight, especially in crewmembers who did not ingest the saline solution. Plasma and urinary potassium were lower after landing than before

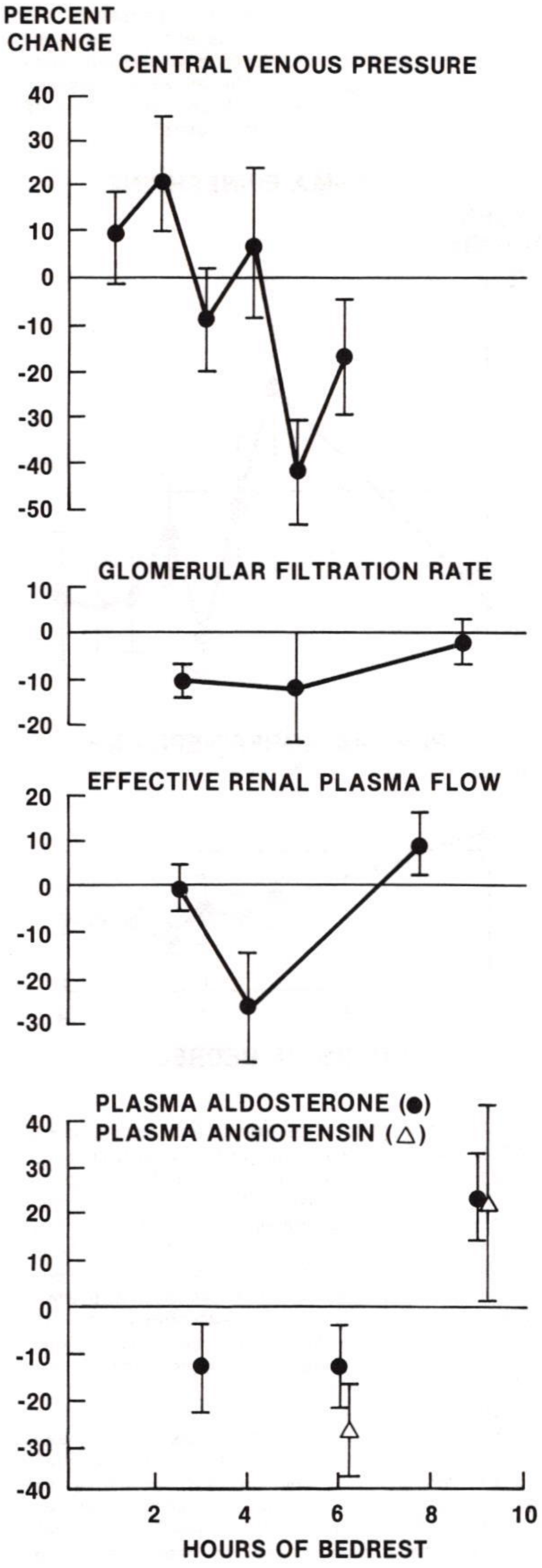
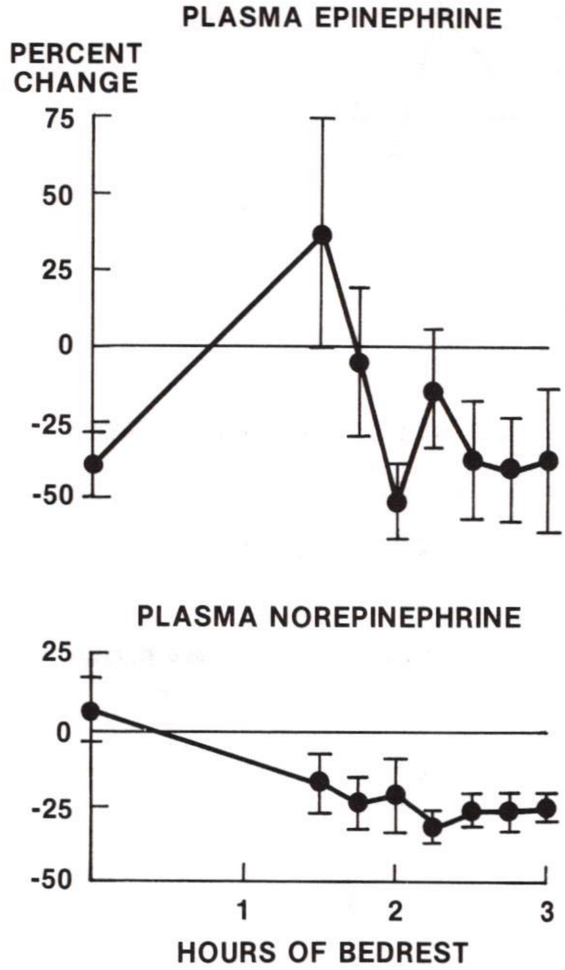


Fig. 1. Time course of changes in several parameters in the first few hours after head-down bedrest began. Each point represents the mean percent change for 4-12 subjects, and bars represent standard error. For all parameters except CVP, each measurement during bedrest was compared to the average measurement for that subject taken at the same time of day on the two days preceding bedrest. Since CVP was measured on only one day, measurements for other time points were compared to measurements made at the beginning of bedrest (0 hours) for each subject. For GFR and ERPF, each time point represents the midpoint of the duration of the clearance test.

Fig. 2. Time course of changes in plasma epinephrine and norepinephrine in the first 3 h of head-down bedrest. Each point represents the mean percent change for 11 subjects, and bars represent standard error. Each measurement during bedrest was compared to the average measurement for that subject taken at the same time of day on the two days preceding bedrest.



flight, but the decreases were less in crewmembers of STS 4-7. Plasma and urinary aldosterone increased substantially as a result of space flight in crewmembers of STS 1-3, but in the saline-supplemented crewmembers plasma aldosterone was significantly ($p<.05$) lower, close to preflight levels. The supplemented crewmembers had less than half the increase in plasma angiotensin shown by the crewmembers of STS 1-3. Urinary ADH, on the other hand, was about the same in the two groups of crewmembers.

DISCUSSION

Results of the head-down bedrest study have prompted us to propose that there are several phases of response to the initial exposure to bedrest and by analogy exposure to microgravity. These begin with the elevated central venous pressure [while there is reported to be no change in cardiac output (3)], decreased sympathetic activity, decreased glomerular filtration rate and a decrease in the plasma levels of hormones which affect the renal tubule [ADH (3) and aldosterone]. By 4 h into bedrest, central venous pressure has returned to the baseline value while ERPF has decreased. Plasma ADH, angiotensin and aldosterone remain decreased (3). After 6 h of bedrest, central venous pressure has decreased to below the control value, and plasma angiotensin and aldosterone are below control. By 8 h, renal blood flow has increased and glomerular filtration rate and plasma antidiuretic hormone and aldosterone are probably approaching baseline values (9). One hour later, however, plasma aldosterone and angiotensin are elevated while other parameters are close to normal. At twenty-four hours others have reported that central venous pressure remains low, cardiac output is at control levels, plasma volume has decreased and plasma angiotensin, aldosterone and antidiuretic hormone have increased (3,10).

The 6° head-down bedrest seems to serve as a ground-based analog for the period immediately following entry into microgravity, a time for which data from space flight are sparse. The removal or reduction of hydrostatic pressure in the legs during head-down bedrest or space flight results in shifts of blood and tissue fluid from the lower body to the central circulation. This central hypervolemia activates autonomic, hormonal, and renal responses which change blood flow, blood pressures, and central blood volumes toward more acceptable levels.

In the well-hydrated subject, the kidneys can be considered to be the principal means of acute fluid regulation. They work in concert with central thirst and appetite sensations. The kidneys apparently are responsible for reducing plasma volume to levels appropriate to hypogravity. Reduced fluid intake, the only other mechanism for reduction of plasma volume, has been observed in space flight and in numerous bedrest studies, but it is not desirable because of the extent of dehydration required before plasma volume decreases.

The mechanism of subsequent increase in ERPF cannot be ascertained from these studies. Several factors extrinsic to the kidney are known to increase ERPF; e.g., pyrogens, increased protein intake, and decreased sympathetic tone. Of these factors decreased sympathetic tone appears to be the most likely mechanism in this situation.

We propose the following mechanism for the alterations in renal function during the first 24 h of head-down bedrest: At the start of bedrest, an increase in central venous pressure and thus increased right heart preload causes no measurable change in cardiac output or in ERPF. The CVP increase may or may not be reflected in the measured decrease in GFR, but subsequent decreased sympathetic activity causes a decrease in local angiotensin activity by 6 h and results in a decrease in both afferent and efferent arteriolar resistance. The early decrease in aldosterone and ADH (3) resulting from central mechanisms causes decreased tubular reabsorption of sodium and water (3), which is eventually responsible for the decreased plasma volume at 24 h.

The observations of Nixon et al. (3) indicate that fluid redistribution occurs rapidly and the period of increased central venous pressure lasts only a few hours. The flight data indicate that some endocrine and electrolyte

Table 1. Analyses of Body Fluids of Space Shuttle Astronauts

	Percent Change (Postflight Value Compared to Preflight Mean)	
	STS 1-3 n=7 Mean ± SE	STS 4-7 n=14 Mean ± SE
Serum or plasma parameters		
Sodium	-1.1 ± 0.5	-0.3 ± 0.3
Potassium	-11.2 ± 2.1	-3.1 ± 2.3
Aldosterone	47.3 ± 18.1	-2.8 ± 9.0
Angiotensin I	272.7 ± 84.0	113.8 ± 36.7
Urine parameters		
Sodium	-60.2 ± 17.9	-35.7 ± 7.4
Potassium	-23.1 ± 5.6	-9.3 ± 14.6
Aldosterone	171 ± 64.4	29.9 ± 21.5
ADH	6.2 ± 21.7	10.6 ± 17.9

changes which might be expected to occur with the fluid shifts continue long after central venous pressure is believed to have returned to normal.

Although analysis of urine and blood samples taken from Skylab astronauts during flight indicates early losses of electrolytes (5), analysis of data from returning Space Shuttle crewmembers indicates that electrolyte conservation has begun by the time the first samples are taken immediately after landing. The crewmembers are nevertheless potassium-depleted at this time, probably as the result of increased aldosterone secretion, and are somewhat dehydrated. This is partially corrected by the addition of one liter of water containing 150 meq of sodium chloride prior to reentry. Use of this countermeasure appears to have promoted a less extreme physiologic response to grounding after the flight, and NASA cardiologists have concluded that it results in a significant improvement in a crewman's ability to withstand the cardiovascular stress from quiet standing during the first few hours after return to gravity (11).

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REGULATION OF MAN'S HYDRATION STATUS DURING GRAVITY-INDUCED BLOOD REDISTRIBUTION

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It is known that changes in functioning blood circulation apparatus associated with blood redistribution in the body occupy one of the first places in a complex of functional shifts observed under conditions of a head-down tilting (1). The marked blood shifts toward the upper part of the body, especially during an acute adaptation of the body to head-down position, lead to changes in blood circulation and mediately to shifts of fluid-electrolyte homeostasis (2) which cannot be indifferent to general resistance in response to the influence of extreme factors. As shown by previous experience, solution of these questions is quite successfully accomplished by implementation of a set of special measures to protect the body against the influence of adverse factors of head-down tilting.

In this case, tactics of their use is determined by specificity and objectives of the studies; by the state and individual peculiarities of the test subjects.

In order to make a more active and purposeful correction of the functions mentioned, a more fundamental study of available preventive measures, clarification of mechanism of their influences and a search for the ways to increase their effectiveness, seem to be needed.

The purpose of this paper was to estimate quantitatively the available means and methods to protect blood-circulation homeostasis and hydration status of the body; to search for the new ways of correcting the functions mentioned; to select and optimize tactics of their use under simulation conditions.

Work has been carried out in these stages. During first stage, an effect of intake of water supplement (WS) of 20 ml/kg and water-salt supplement (WSS) containing 20 ml/kg water and 0.11 g/kg NaCl during regular motor activity on a hydration status of man has quantitatively been evluated. The supplements listed were given in three stages in 3 hours (after dinner, supper and before going to bed).

The 20 healthy male test subjects participated in the studies. Test subjects consumed the controlled diet and water rations. Volumes of extracellular fluid space and media composing it - vascular and interstitial fluids, have been determined. In the experiment, the nuclear-physical, biochemical and radioimmune techniques have been used. Plasma volumes were determined with the aid of radionucleid ^{131}I -albumin, an extracellular fluid - from the stable Br folliwng the method of X-ray-fluorescent analysis. Interstitial fluid volume was determined by means of a calculation method.

A body fluids content has been assessed before, in 1,3,6,12 and 18 hrs after supplements intake. The findings of the studies performed indicated that in an hour after water intake a statistically significant increase of extracellular fluid space volume against an elevated diuresis has been recorded. The fluid content in a vessel and interstitial space during this period exhibited tendency toward a slight increment. Further observations demonstrated a clear tendency toward decreasing vascular fluid and increasing interstitial fluid volume with maximum changes - 4.1% by 12 hrs of observations. The highest level of diuresis was observed after 2nd intake of water supplement. By 18 hrs of observation, all the shifts noted did not differ from the initial levels.

An WSS intake following the same pattern resulted in a more significant increase of a hydration level of the body. The WSS content by 12 hrs of observation (after 2 intakes of supplement) increased with respect to initial level up to 5.1% (p 0.05), both at the cost of vascular component (approximately 4.0%) and interstitial fluid (5.5%). Diuresis reached the highest level at the last hours of observation. In 18 hrs the volumes mentioned still exceeded their baseline volumes by 2-3%.

The studies performed indicated that use of measures mentioned exceeded a hydration level of the body and, to a greater extent, due to interstitial fluid. Use of WSS proved to be more effective. This conclusion is in a complete accord with the data from the hormonal status studies. Arginine-vasopressin activity during WS intake was not practically changed, as WSS resulted in a significant rise (p 0.01) of a renin level in the blood.

During the 2nd stage, an influence of adiuretine (AD) on a hydration status was studied. This medicine is effective in a clinical use and has no side effects (3).

For this purpose, a number of studies, in which 20 test subjects performed their regular motor activity, have been conducted to investigate:

- Influence (AD) on hydration status of the body;
- Influence of WS on the background of AD uptake;
- Combined effect of AD and WSS.

Adiuretine uptake caused an increase of vascular fluid over 6 hrs with a maximum effect (up to 7%) over 3 hrs of observation.

An insignificant increase in interstitial fluid induced by AD (maximum effect - 6 hrs) was also noted. By 12 hrs of observation, the mentioned changes in body fluid compartments practically disappeared.

WS intake against the AD use caused a more significant rise of plasma volume (up to 10%) and interstitial fluid.

It should be noted that fluid retention effect still observed in 18 hrs (up to 6%) after its consumption.

A combined use of WSS and AD has led to a more marked fluid retention in the body and to an decreased urinary sodium and potassium excretion. An increase of vascular fluid (up to 10%) by 24 hrs after taking medicine and by 18 hrs after beginning of WSS uptake.

Thus, adding AD to the mentioned combinations permitted to increase body fluids content and to control quite actively its excretion. The study of a renal function against the mentioned effects by renography did not reveal any changes in their functional state.

An analysis of data obtained showed also that the combinations listed and particularly AD-WSS combination have quite a long-term effect which gives certain advantages on correcting hydration status. Correction procedures were tested during the 3rd phase of studies in a 7-day head-down tilting experiment. An angle tilting head end of a bed was -15°. Seven healthy men participated in the experiment. The objective of the experiment is: with the aid of head-down tilting to cause a decrease in postural stability due to falling a body fluids level; to evaluate these shifts on day 5 of head-down tilting and to perform correction of a hydration level (on day 6) with subsequent assessment of influence of this correction on day 7 of head-down tilting.

On day 5 of head-down tilting, a statistically significant decrease of extracellular fluid content (about 8%) both due to interstitial fluid (7.5%) and plasma (10.5%) was recorded.

Use of ADH-WSS combination on day 6 of head-down tilting increased markedly water content of WSS, interstitial and vascular fluids. Deficit of content of the mentioned fluids (relative to a baseline level) reduced on day 7 up to 1-2%.

The balance studies showed that on day 1 of head-down tilting the fluid and sodium excretion by kidneys increased against a significant decrease in plasma renin activity and aldosterone content. By day 5 of head-down tilting the changes noted practically reached the baseline level.

The first intake of AD+WSS on day 6 did not change the diuresis magnitude and somewhat increased natriuresis (from 0.15 to 0.18 meqv/min). After the 2nd intake of WSS there was a decrease in plasma renin activity from 1.91 to 0.96 pg/me/hr and aldosterone from 62.1 to 44 pg/ml. An intake of the 3rd supplement caused insignificant increase in diuresis, natriuresis and calciuresis. Plasma renin activity during this period decreased up to 0.73 pg/ml/hr. Head-up tilting (70° - 10 min) performed on day 7 the test subjects withstood well. Abovementioned features of postural instability disappeared practically completely.

The radiographic method of recording blood redistribution in the body used during head-down tilting (on day 7 of head-down tilting) clearly revealed a marked decrease of a degree of hemocirculation shifts.

Thus, addition of AD to a set of traditional correction ways permitted to change more actively and purposefully a hydration level of the body during head-down tilting.

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SYSTOLIC TIME INTERVALS IN HEAD DOWN TILTING (-85°)

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ABSTRACT

Heart rate (HR), systolic time intervals (STI) and Q-T interval were studied in a head-down (HD) tilting of -85° in a group of lo young subjects averaging 27 years. This postural change is accompanied by a significant shortening of pre-ejection period (PEP), due to the increased venous return and enddiastolic volume. HR, left ventricular ejection time (LVET) and Q-T values remained practically unchanged. STI were determined by poligraphic noninvasive method including ear densitogram.

INTRODUCTION

STI may be measured by noninvasive methods (7, 15). The value of this methods has ben tested by comparative studies with invasive methods (1, 8, 12). The most important information obtained from STI concern the isovolumetric phase, expressing the force of myocardial contraction. The shorther is this interval, the greater is the force of contraction, in accord with the force-velocity relation. Nevertheless it is difficult to determine the proper isovolumetric phase by noninvasive methods. It is included in the pre-ejection period (PEP) wich contains an initial phase corresponding to the electromecanical delay, conventionally apreciated to 38 ms (6). The value of PEP is however highly informative for the isovolumetric phase, keeping in mind the short time due to ventricular activation that precede pressure increasing and especially its relative invariability. It is available a rich material concerning the modifications of STI in cardiac pathology (ischemic cardiopathy, arterial hypertension, valvular lesions) as well as in different physiological circumstances, as exercice (3, 4, 14) or postural changes (10), studied by noninvasive poligraphic methods. STI were investigated also in cosmonauts(2).

The force of contraction may be modified in two ways, by extrinsic mechanism, in accord with the Frank-Starling relation, and by intrinsic mechanism, which depends on the metabolic condition of myocardium regulated by sympatho-adrenergic stimulation, as in exercice. The extrinsic mechanism, depending on enddiastolic volume, is enhanced by increased venous return in central cardiovascular areas, as in immersion,-Gz acceleration, changing stand in supine position or the last in antiortostatic. The aim of this study was to investigat STI after head-down tilting of -85° wich induces a marked increase of the venous return in central areas of the cardiovascular system.

MATERIAL AND METHOD

Ten young men, mean age 27 years, were investigated by changing on a tilting table the supine into an anti -ortostatic (-85°) head-down (HD) position.

HR, PEP and LVET were derived of ECG and arterial pulse wave. The ECG was recorded by means of a V5 lead and the arterial pulse wave by means of an ear densitogram (DEN) (3, 11) utilizing a photoelectric transducer. The method permits an artifact-free recording of ECG and DEN.

The ECG and DEN signals were stored on magnetic tape for subsequent computer analysis (Haemodynamic Analyser M 5810), enabling an automatic measurement of STI as HR, PEP, LVET and the ratio PEP/LVET. The measured data are displayed in alphanumerical form on TV tube.

In order to obtain a time profile of HR, PEP and Q-T, these variables were converted in to amplitude modulated pulses in a special analogue processor.

The subjects were kept on the tilting table in supine position for 5 min. after a previous resting period of 15 min.in the same position on a cough. An automatic smooth and uniform movement to an antiortostatic (AOS) (-85°) position was accomplished in 17 sec. The subjects remained 30 sec in this head-down position. In an other delay of 17 sec, the subject regained the supine position and remained thus for 5 min.

The signals recording started in the last minute of the supine position on the tilting table and then uninterrupted the whole experiment.

The systolic and diastolic systemic arterial pressure were measured in the supine, in HD and the last supine position.

RESULTS

During AOS, HR changed inconstantly, increasing or decreasing, PEP was shortened ($p<0.001$) and LVET was inconsistently modified. QT has the same variation as LVET. The PEP/LVET ratio shortened during the pasive head-down tilting test ($p<0.001$) After recovery in supine position the values return approximatly to the initials. (Table 1). The systolic arterial systemic tension do not change but the diastolic presented slightly decreased values.

Posture	Supine	AOS	Supine
HR p/min.	81 ± 7	83 ± 7	77 ± 8
		$p>0.2$	$p>0.05$
PEP ms	96 ± 10	81 ± 10	96 ± 12
		$p<0.001$	$p<0.001$
LVET ms	286 ± 15	287 ± 17	288 ± 16
		$p>0.2$	$p>0.2$
PEP/LVET	0.33±0.04	0.27±0.05	0.33±0.05
		$p>0.001$	$p<0.001$
QT ms	324 ± 22	321 ± 25	321 ± 24
		$p>0.5$	$p>0.5$

Table 1. Heart rate, systolic intervals and Q-T in -85° antiortostatism

COMMENTS

The decreased duration of PEP in AOS expresses an increased force of contraction that is due to increased venous return and corresponding to the enddiastolic volume of the left ventricle, in accord with the Frank-Starling relation. HD tilting produced a slight decrease in diastolic pressure that may devance the aortic sigmoid aperture and contribute to the decrease of PEP. In accord with others, the slight decrease of the diastolic pressure and the contribution of the devanced sigmoid aperture may be considered as unimportant for the PEP decrease. The AOS tilting do not influences characteristically the HR.

A clear pulse arterial wave may be obtained recording the ear densitograme, that served as a second parameter for non-invasive measurement od STI. We confirmed thus our previous tehnical experience (9).

The increased force of contraction is independent from heart rate, confirming this way others investigations in wich pharmacological means (atropine), or cardiac pacing were used (13). This supports the idea that the decreased PEP is produced by extrinsic factor represented by the increased enddiastolic volume and not by a sympathoadrenergic reaction.

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VISUAL-VESTIBULAR INTERACTION IN ROLL: PSYCHOPHYSICS AND PHYSIOLOGY

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INTRODUCTION

The phenomenon of visual-vestibular interaction has first been described by Mach (1). Its detailed psychophysics have later on been studied in great extent by Fischer and Kornmüller (2) and Dichgans and Brandt (3). The work of these authors in summary indicates that vision considerably contributes to ego-motion perception about and along all of the six axes of freedom and that it supplements and dominates vestibular function in the low frequency (below 0.1 Hz) and constant velocity range. It was a surprise to find that the integrative interaction of these two sensory inputs occurs already at the level of the second order neuron within the vestibular nuclei and is similarly observed in all vertebrates so far tested with the exception of the frog (see reviews by Dichgans and Brandt (3) and Henn et al. (4)). The two inputs together are integrated with weaker contributions from the auditory and somatosensory system, most probably also at the level of the brain stem, and together constitute dynamic (motion) and static (position) spatial orientation in terms of perception and motor control.

The purpose of this paper is to briefly summarize what is known about visual-vestibular interaction in roll to point out its pecularities due to the one G gravity pull acting in this plane and to hint to the possible alterations of sensation as well as motor behavior in zero gravity.

Visually induced sensation of roll motion, displacement of apparent vertical, and postural consequences

The sensation of continuous roll motion about the x-axis of the head (roll vection) may be elicited in a stationary observer while viewing a large disk or a hemispheric dome rotating around his line of sight (5, 6). The induced motion sensation is opposite to the direction of pattern motion. Subjects report the pardoxical perception of limited displacement despite a continuous sensation of self-motion. The constraint on displacement is probably due to the inputs from otoliths and somatosensory graviceptors, conflicting with the visual effect. The apparent position consequently may be conceptualized as the result of a compromise weighing the different and in part contradictory sensory inputs for gravitational orientation. This interpretation is supported by the finding that the visually induced tilt markedly increases when the otoliths are placed in a less favorable position by lateral head tilt (7). In this situation, the otolith signal possesses a proportionately lower sensory weight and accuracy. Conversely, both roll and pitch displacements are reduced if the head is tilted 25^o forward - so as to put the dominant plane of the utricular otoliths in an optimal earth horizontal position, a fact that may vote for their predominant role in visual-graviceptive interaction (8). In roll, the potentiation of the net visual effect on apparent vertical in an inclined head position is maximal if the visual stimulus is moving opposite to the head tilt. This asymmetry has been interpreted as the functional hypertrophy of a biologically adaptive mechanism (7) since lateral head tilt causes opposite relative motion of the visual surround. Another possible consequence of the conflict during roll-vection around a horizontal axis is the considerably lower saturation velocity (6) at which the actually rotating stimulus display looks stationary and all the motion is seemingly taken on by the subject. In yaw where gravitation does not interfere, the perception of motion occurs without limitations on displacement.

Induced displacement can be measured in terms of tilt angles of apparent visual and postural vertical from gravitational upright. These are indicated by the adjustments subjects perform in the attempt to compensate for an apparent inclination of both (5). Visual effects on posture have been measured in terms of the apparent upright of a moving-base airplane trainer, the position of which was to be adjusted while moving patterns were projected onto its windows (5), or measuring postural sway by means of a platform while the subject stands erect and views a rolling visual display. The fact that the tilt effect during rotation of the visible surround is common to both visual and postural orientation supports the explanation that a shift occurs in the internal representation of the gravity vector. This explanation is further supported by the fact that a real displacement of the gravitational vector, such as in a human centrifuge, results in a corresponding change in perceived visual and postural orientations. Tilts of visual vertical measured during the roll vection range up to 40° and saturate at an average of 15° at 30 - 40°/s stimulus velocity. The gain of visually induced displacement greatly varies among subjects. The angle of induced tilt with sinusoidal roll-motion stimuli linearly increases with the logarithm of the stimulus oscillation amplitude (9). Such a system integrating stimulus velocity over time could subserve the visual feedback stabilizing posture. In normal life body sway causes a relative counterrotation of the visual surround. The consequent visually induced tilt perception adds to otolith information and thereby assists postural rightening at least below frequencies of 0.1 Hz (10, 9). - That spinal motoneurons are tonically driven by a moving visual display has been demonstrated in the cat which shows a direction specific modulation of monosynaptic hind limb reflexes in response to a large optokinetic stimulus moving in roll (11).

The important characteristics of the stimulus

Visually induced self-motion perception requires movement of a large portion of the seen environment (12). Perceived tilt during roll-vection almost linearly increases with a logarithmic augmentation of the total area moving (6, measured in steradians). The threshold total area for induction of roll-vection and apparent tilt is below 1 % if this area is split into four or more equally sized portions randomly distributed within the visual field. Tilt is saturated if 30 % of the visual field (scattered into 120 visual objects) are subtended by moving contrasts. This density effect seems to indicate that a large number of retinal elements has to be simultaneously stimulated as to cause the maximal effect. Using moving stimuli equal in area it was shown that the peripheral retina dominates visually induced vection and spatial orientation (12, 6), whereas central vision dominates pattern perception and object motion detection. In the laboratory situation of an opposite motion stimulation of the center and periphery spatial orientation relies on the peripheral stimulus whereas pattern-motion perception and optokinetic nystagmus depend on the central stimulus. This experiment illustrates that both mechanisms of motion perception may work in parallel as they frequently do in real life, enabling man to perceive motion of objects while experiencing active locomotion. Pattern contrast and average luminosity of the moving pattern are not critical for visual-vestibular interaction within wide limits (13). Stationary contrasts if simultaneously presented to the subject inhibit vection (14). If one considers their spatial arrangement in depth, background information is of much greater significance than is foreground information (14). Thus, visually induced self-motion and spatial orientation rely mainly on the information from the seen periphery, both of the retinal periphery and periphery in depth. Self-motion is perceived when sizable portions of the depth and/or the retinal periphery are in motion, since this is the inevitable consequence of movement of the body in space, even if the foreground is stationary with respect to the observer as in passive movement in a vehicle.

Possible alterations of visual-vestibular interaction in zero-gravity

If the above mentioned concept about gravity limiting the apparent displacement of visual and postural vertical during roll vection is true one may assume the following alterations in space. The assumptions will be tested in the forthcoming Space-Laboratory Mission. Subjects will wear a helmet within which they view a rolling stimulus displayed on a miniaturized television screen through a wide angle lens. Limitations on postural displacement will be provided by the fact that subjects wear shoes that by means of suction cups are fixed to the measuring platform. Though despite the absence of gravity there are somatosensory cues about body position in relation to space lab coordinates while posture is examined.

Static orientation and ego-motion sensation while locomoting within the space laboratory should predominantly rely on vision. The orientaton of visual contours per se should be important for static orientation, but relative motion caused by locomotion or postural sway should dominate dynamic spatial orientation. - On earth, the visual stabilization of posture requires motion detection and is absent with stroboscopic illumination at a frequency below 8 Hz (15). Roll-vection in space should be accompagnied by the sensation of continuous instead of limited displacement just as it does in the prone position on earth. Saturation velocity of roll vection in space should exceed the 10°/s measured on earth (6) and may reach the 100°/s measured in yaw (12). The gain of the visual effect upon posture as demonstrated through the application of a rolling stimulus should increase because of the lack of a conflicting otolith signal. It will be interesting to study the time course of the probably occurring alteration of the gain in space and after return to earth the setting of a target cross. A target cross projected into the center of the moving field, if at all, should minimally deviate from the real orientation of the body axes. It might also be interesting to investigate the perceived orientation of the longitudinal body axis in relation to the coordinates of the space laboratory by having the subject adjust the target cross such as to seem parallel to the lateral walls of the lab actually not seen by the subject while wearing the helmet. Finally, static head tilt towards one shoulder should not augment the visual effect, as it does on earth, since there is no otolith input the comparative weight of which can be altered by altering head orientation in relation to gravity. If there is a residual modulation this would indicate the additional contribution of neck receptors to the internal computation of spatial coordinates in relation to which perceived and postural orientation are organized.

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VESTIBULAR DYSFUNCTION IN COSMONAUTS DURING ADAPTATION TO ZERO-G AND READAPTATION TO 1 G

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It is obvious today that vestibular dysfunctions often attend an early period of adaptation to zero-g and readaptation to 1 g. They manifest as illusionary sensations of spatial body position, vertigo and autonomic disorders of varying degree.

We have analyzed symptoms of illusionary reactions and motion sickness in 36 cosmonauts (12 members of long-term and 24 members of short-term flights). Inflight 80% of them experienced illusionary reactions. In most cases (about 70%) the reactions were associated with spatial body position. Almost half of the crewmembers showed autonomic disorders. Motion sickness symptoms were usually provoked by motor activity and sometimes by optic stimulation during visual observations through the viewport.

Upon return to the Earth the cosmonauts exhibited new manifestations of vestibular dysfunctions, which often depended on the flight time. For instance, motion sickness symptoms were found in 27.3% of crewmembers of the 4-, 7- and 14-day flights and in 91.7% of long-duration crewmembers (i.e. 11 of 12 cosmonauts). During an acute period of adaptation, particularly after long-term flights, the following disorders were seen: otolith asymmetry, reduced threshold sensitivity, altered reactivity of semicircular canals and canal-otolith interaction, as well as disturbances in the perception of spatial coordinates. Optokinetic stimulation was associated with frequency-amplitude changes in optokinetic nystagmus, dysrhythmia, and significant asymmetry in responses to exposures of different signs (Fig. 1). During the first 2-3 days postflight four cosmonauts showed distinct spontaneous and positional nystagmus. (Fig. 2). In addition to the nystagmus, some cosmonauts exhibited the phenomenon of floating eyeballs, i.e. the eyes moved sideways when they tried to fix the gaze at an object located in front of them. Many crewmembers showed vestibulo-autonomic reactions of the I or II grade during cupulometric and Vojacek tests that were never seen preflight. More than half of the cosmonauts exhibited lowered, as compared to the preflight level, autonomic tolerance in response to 6-min optokinetic stimulation.

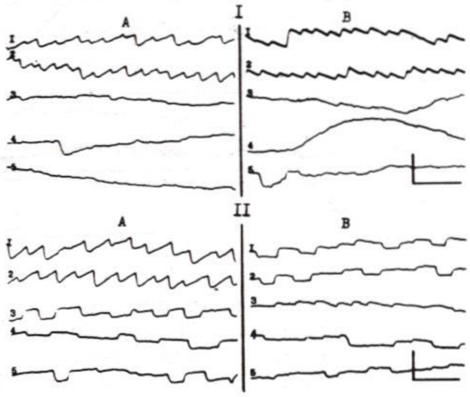

Fig. 1. Optokinetic nystagmus in the Commander (I) and Flight-Engineer (II) of Soyuz T-4 - Salyut-6 in response to black-white stripes moving to the left (A) and to the right (B) at a rate of 40 (1), 60 (2), 80 (3), 100 (4), and 120 (5) stripes/min immediately after 75-day flight. Calibration: 20°, 1 s.

Noteworthy is distinct otolith asymmetry during an acute period of readaptation which was measured by the eyeball counter-rolling reaction according to Fischer (4). Another important finding was a direct correlation between motion sickness frequency and otolith asymmetry. Asymmetry was observed in every cosmonaut who showed overt symptoms of motion sickness after long-term space flights. Only one crewmember had distinct otolith asymmetry and no motion sickness (Fig. 3).

It should be emphasized that long-term missions caused more marked sensory and autonomic disorders during readaptation as compared to short-term flights. It should also be noted that motion sickness and other vestibular dysfunctions were less pronounced both inflight and postflight when the cosmonauts made their second or third missions.

What are the factors responsible for vestibular dysfunction in orbital flight and upon return to the Earth? Presumably, they are: 1) so-called sensory conflict due to mismatch of the sensory systems controlling spatial perception (5), 2) disorder of canal-otolith interaction (2), and 3) interlabyrinthine asymmetry (1, 3).

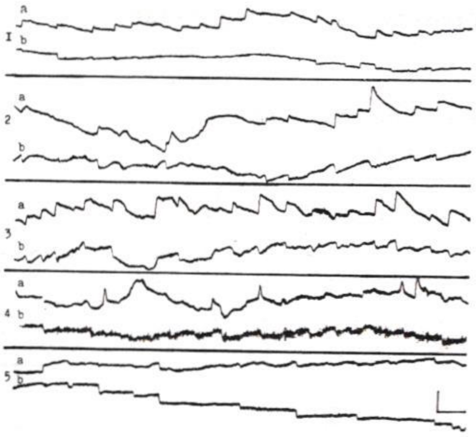

Fig. 2. Spontaneous and positional nystagmus in the Soyuz T-6-Salyut-7 Commander. I - Electrooculogram in the sitting position, 2 - in the supine position with the head lifted, 3 - on the right side, 4 - on the left side, 5 - in the supine position with the head thrown back. a - EOG - in the vertical and b - EOG in the horizontal lead, in the dark with eyes open. Calibration: 200 μV, 1 s.

According to the last hypothesis, the otolith organs of both sides are normally associated with some functional asymmetry which at 1 g is offset by central compensation and in zero-g becomes perceptible. Simulation flights revealed vestibular dysfunction symptoms manifested as vestibular asymmetry. Prolonged head-down tilt studies demonstrated overt otolith asymmetry. It also developed in response to Coriolis - cross-coupled acceleration (Fig. 4). Nevertheless, motion sickness tolerance was significantly lower in the case of the presence than in the absence of initial otolith asymmetry. Similar results were obtained when interlabyrinthine asymmetry was generated by direct descending current of 5 mA being passed through one labyrinth. In this situation tolerance to Coriolis - cross-coupled acceleration provoking motion sickness decreased more than twofold.

Thus, our examinations give evidence that the vestibular function may be modified in space flight. They also demonstrate that the changes are transient and that the vestibular system may adapt to prolonged space flights.

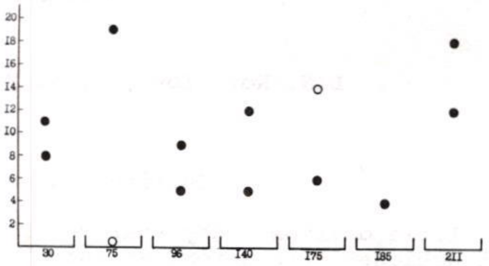

Fig. 3. Frequency of otolith asymmetry and motion sickness after long-term space flights. Full circes show cases of motion sickness and open circles - no motion sickness. Abscissa - flight time, days, ordinate - asymmetry of eyeball counter-rolling, deg.

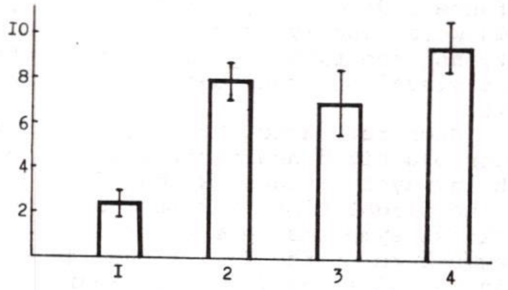

Fig. 4. Otolith asymmetry measured by eyeball counter-rolling in healthy test subjects in the norm (1), immediately after 182-day head-down tilt (2), after experimental motion sickness induced by Coriolis - cross-coupled acceleration (3) and after long-term flight at R + 0 (4).

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THE VALIDITY OF AN ANIMAL MODEL FOR EXPERIMENTS RELATED TO WEIGHTLESSNESS

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ABSTRACT

Animal evolution has witnessed morphological and physiological adaptations to gravitational forces. In the rat, hind limb muscles can be used to illustrate a range of load bearing functions: soleus> gastrocnemius =plantaris> extensor digitorum longus (EDL). A harness suspension apparatus is used to induce hypokinesia and hypodynamia (H&H) and to simulate responses comparable to those seen in weightlessness (i.e., COSMOS experiments). After one and two weeks of suspension H&H there is muscle atrophy with a loss in muscle mass, the result of loss in muscle protein. Concommitantly, there is a decrease in RNA, but not in DNA content. The effects are greatest in the soleus and least in the EDL. These recent findings, in concert with our earlier reports of increased nitrogenous excretion, suggest that both decreased protein synthesis and increased protein catabolism are characteristic of muscle atrophy. Recovery is seen in terms of reversal of these effects after removal from suspension.

INTRODUCTION

In the course of evolution, adaptations are clearly evident in a variety of physiological functions, including locomotion, body mass support and behavioral posturing. In the hind limb of the quadruped, various muscles relate to such functions. The soleus, a slow-twitch, red fiber muscle, is credited with postural and loading (i.e. support) functions. These and locomotor functions are also credited to the gastrocnemius and the plantaris, composed of a mixture of fiber types. In contrast, the extensor digitorum longus (EDL) a fast-twitch muscle, has no load bearing functions. With this spectrum of muscle types, studies can readily question functional attributes relating to gravitational influence, i.e., load bearing and non-load bearing functions. Since most hypotheses concerning muscle responses to microgravity or hypogravity (i.e., weightlessness) can only be tested in space, we are faced with the problem of experimental limitations. Three animal systems are often used to assess physiological and biochemical responses to hypogravitational environments: small cage restraint, hind limb immobilization (plaster casting, joint pinning) and suspension systems (dorsal bonding, harness and tail fastened suspension). Drawing conclusions from comparisons between these systems must be done with caution. The indiscriminate use of terms, without adherance to basic and widely acceptable definitions, may lead to confusion as the literature grows. Unless there is significant opinion to the contrary, it is proposed(Oganov et al 1980a, Musacchia et al 1980a, Deavers et al,1980b, Musacchia and Steffen 1982) to view the suspended, hind limb unloaded, rat as both hypokinetic and hypodynamic(in either the head-down tilted or non-tilted position). The weightless flight animal is also both hypokinetic and hypodynamic. In both subjects, there is a reduction in limb movements and muscle loading. In small cage restraint subjects are chiefly hypokinetic but not hypodynamic, since the limbs are positioned to bear weight (Federov et al 1981). With limb casting techniques, muscle positioning (contracted or extended) is crucial (Booth 1978). In this system one may be dealing with both hypokinesia and hypodynamia. A brief summary of comparisons is presented in Table 1.

Our approach to the problem of simulated weightlessness has been to utilize a suspension system which allows the rat to freely contract and extend the hind limbs while eliminating all opportunities for load bearing during periods of one and two weeks. The harness suspension system (Musacchia et al 1980, Deavers et al 1980) differs from other systems, which include: skin bonding (adhesion) to a rigid surface (Morey 1979), tail-fastened suspension (Novikov and Ilyin 1981 and Morey-Holton and Wronski 1981) and dorsal sutured steel rod (Templeton et al 1983). The chief advantage of our harness system is the opportunity to alter the head-down tilt, the ready enlargement of the suit as the animal grows, and the elimination of tissue insult due to irritation, infection or invasive stress. A major difference between our experiments and Cosmos flight experiments is the animal subject. We use male Sprague-Dawley rats (170-190gm) whereas in the Cosmos biosatellites, more mature male Wistar SPF rats (212gm) were used.

Our protocol has been sufficient to cause marked atrophy in some muscles and yet permit the occurence of recovery phenomenom after removal from suspension. We use hind limb skeletal muscles because they provide good representation of load-bearing functions (soleus), partial load bearing function (gastrocnemius and plantaris) and non-load bearing (EDL). Also, these muscles have been selected for numerous studies in small cage restraint, plaster cast immobilization and weightlessness, viz., in the Cosmos series of flight experiments. The four muscles selected have been shown (Musacchia et al 1980, 1983, and Musacchia and Deavers,1981) to atrophy differentially in response to non-load bearing suspension (soleus> gastrocnemius=plantaris>EDL). Recovery phenomena have also been assessed after removal from suspension.

In the present experiments, we focused on the relationships between muscle atrophy and protein, RNA and DNA contents during one and two two weeks of H&H as well as during week of recovery. It was hypothesized that the various degrees of atrophy in hind limb muscles are related to differential changes in protein, RNA and DNA.

TABLE 1

Comparison of Animal Skeletal Muscle Responses

CAST IMMOBILIZATION: HYPOKINESIA & HYPODYNAMIA	
atrophy	Booth:1978
decreased synthesis of protein	Booth & Seider:1979
SMALL CAGE RESTRAINT: HYPOKINESIA	
atrophy	Baranski, et al:1971
atrophy	Bykov and Smirnov:1970
atrophy	Szilagyi, et al:1981
decreased synthesis of protein, DNA, RNA	Federov, et al:1982
increased fatigability	Oganov, et al:1980
SUSPENSION: HYPOKINESIA & HYPODYNAMIA	
increased fatigability	Fell, et al:1982
atrophy, increased nitrogen excretion	Musacchia,et al:1980-83
decreased synthesis of protein	Feller, et al:1981
decreased protein and RNA content	This study:1983
decreased synthesis of amino acids	Tischler & Jaspers:1982
increased glucocorticoid receptor levels	Steffen & Musacchia: 1982
WEIGHTLESSNESS (COSMOS): HYPOKINESIA & HYPODYNAMIA	
increased fatigability	Oganov:1980
decreased protein content	Gayevskaya, et al:1979 a&b
WEIGHTLESSNESS + 1-g CENTRIFUGATION	
neutralization of 0-g effects, and evidence of muscle plasticity	Oganov:1980

MATERIALS AND METHODS

Male Sprague-Dawley rats (170-190g) were suspended in denim/velcro harnesses for one and two weeks as previously described (Musacchia et al, 1980). Some animals were sacrificed by cervical dislocation while others were permitted to recover from suspension H&H during one week in metabolism cages. With each one and two week suspension experiment, a group of weight matched controls were sacrificed for comparison. Whole muscles were rapidly excised, trimmed of excess fat and connective tissue, and frozen in liquid nitrogen. Protein was analyzed with a modified Lowry procedure (Schacterle and Pollack, 1973) following tissue homogenization in trichloroacetic acid and resolubilization of protein in 0.1 N NaOH. RNA was analyzed by the method of Schneider (1945) while DNA was estimated by the method of Burton (1956).

Statistical analyses (significance at 95% confidence level) were performed using the Student t test comparing various experimental groups: suspended, recovered, and weight matched controls.

RESULTS AND DISCUSSION

Data from the present experiments are summarized in Table 2. The soleus muscle showed the most pronounced loss in protein and RNA following one and two weeks of H&H, and the most notable recovery. The decrease was greatest after one week. The EDL showed the least change in both parameters. The DNA content in all muscles remained relatively unchanged in response to H&H. The fact that the DNA content remains unchanged argues for the veiw that during one and two weeks of unloading the hind limb muscles did not lose mass in terms of cell numbers. Rather, the loss of muscle mass can be seen as a loss of cellular protein. We have used the protein/DNA and the RNA/DNA relationship as appropriate indicators of tissue responses. Changes in protein/DNA suggest differential effects on cell size,while changes in RNA/DNA reflect differential effects on protein synthetic capacity. Protein and RNA contents reverse toward control levels following removal from suspension.

The question of alteration in protein synthesis in response to exposure to weightlessness and the examination of the suspension system for comparison has remained relatively unexplored. Some initial deductions may be made in light of experimental results after exposure to weightlessness. Gayevskaya et al (1979a, 1979b) reported a marked decrease in both contractile and sarcoplasmic proteins (about 50%) in soleus one day after 20.5 days of weightlessness. On day 26 postflight, there was a normalization of these functionally active protein. More recently, Feller et al (1981) examined protein synthesis by incorporation of ^{14}C-leucine in muscles of suspended H+H rats. They concluded that there was a severe reduction (about 50%) of the rate of synthesis by the fifth day of suspension. Booth and Sieder (1979) have reported a rapid (6hr) reduction in muscle protein synthesis in casted limbs. Thus, the limited information from model systems suggest that initial associations can be made with weightlessness exposed subjects.

Evidence for increased protein breakdown during suspension H&H has been limited. Musacchia et al (1980, 1983) reported increased excretion of nitrogenous end products. Feller et al (1981), however, were unable to demonstrate an increase in protein catabolism.

In order to justify the use of the suspension system to simulate exposure to weightlessness, the skeletal muscle changes must be comparable. The alterations in hind limb muscles of rats used in Cosmos flights have been assessed during post flight recovery (Ilyina-Kakeuva, et al 1976, and Ilyina-Kakeuva and Portulgalov, 1977, Gayevskaya et al, 1979 a,b). The alterations in hind limb muscles of rats used in our harness system not only reflect those which must have occured during weightlessness but also show comparable recovery changes. We conclude from our results that antigravity muscle mass changes in suspension H&H result from both decreased protein synthesis and increased protein break down. We propose that similar changes occur in hypokinesia and hypodynamia of weightlessness.

Table 1. Protein (P), DNA, and RNA content of hindlimb muscles of rats: suspended 1 and 2 week (1 wk and 2 wk) hypokinetic and hypodynamic (WKH), one week post-suspension recovery (R) and weight-matched controls (WMC)

SOLEUS	PROTEIN (mg)	DNA (mg)	RNA (mg)	P/DNA	RNA/DNA
1 WKH (14)	6.5 ± .45^{1+}	0.06 ± .01	0.08 ± .01^{+}	1.16 ± .09^{+}	1.51 ± .11^{+}
2 WKH (10)	5.5 ± .58^{++}	0.06 ± .01	0.07 ± .01^{++}	1.02 ± .12^{++}	1.39 ± .11^{++}
1 WKH + R (11)	10.3 ± 0.61**	0.07 ± .01	0.16 ± .02**	1.67 ± .19**	2.52 ± .25**
2 WKH + R (11)	9.6 ± .68***	0.06 ± .01	0.14 ± .01***	1.66 ± .13***	2.42 ± .15***
WMC (28)	9.3 ± 0.38	0.06 ± 0.01	0.14 ± .01	1.54 ± 0.06	2.24 ± 0.19

GASTROCNEMIUS	PROTEIN (mg)	DNA (mg)	RNA (mg)	P/DNA	RNA/DNA
1 WKH (18)	77.6 ± 3.85^{+}	0.34 ± 0.02	0.63 ± 0.06^{+}	2.32 ± 0.13	1.99 ± 0.16
2 WKH (12)	87.1 ± 4.02	0.35 ± 0.02	1.00 ± 0.06*	2.58 ± 0.17	3.04 ± .030*
1 WKH + R (11)	151.2 ± 6.73**	0.39 ± 0.03	1.95 ± 0.29**	4.18 ± 0.37**	5.50 ± 1.11**
2 WKH + R (11)	147.6 ± 6.04***	0.39 ± 0.04	2.05 ± 0.11***	4.40 ± 0.55***	6.15 ± 0.76***
WMC (28)	100.9 ± 4.2	0.39 ± 0.01	1.02 ± 0.09	2.65 ± 0.15	2.65 ± 0.27

PLANTARIS	PROTEIN (mg)	DNA (mg)	RNA (mg)	P/DNA	RNA/DNA
1 WKH (10)	15.5 ± 2.0	0.11 ± 0.01	0.19 ± 0.03^{+}	1.48 ± 0.13	1.72 ± 0.16^{+}
2 WKH (11)	18.9 ± 1.06	0.11 ± 0.01	0.29 ± 0.03	1.88 ± 0.13*	2.25 ± 0.33*
1 WKH + R (8)	25.9 ± 0.86**	0.12 ± 0.01	0.55 ± 0.02**	2.23 ± 0.09**	4.73 ± 0.29**
2 WKH + R (11)	29.6 ± 0.75***	0.12 ± 0.01	0.49 ± 0.04***	2.47 ± 0.13***	3.98 ± 0.29***
WMC (13)	20.0 ± 1.05	0.11 ± 0.01	0.30 ± 0.03	1.76 ± 0.07	2.69 ± 0.29

EDL	PROTEIN (mg)	DNA (mg)	RNA (mg)	P/DNA	RNA/DNA
1 WKH (17)	12.9 ± 0.7	0.05 ± 0.01	0.12 ± 0.01	3.16 ± 0.37	2.83 ± 0.14
2 WKH (12)	14.0 ± 0.87^{++}	0.04 ± 0.01	0.15 ± 0.01	3.33 ± 0.19	3.56 ± 0.28*
1 WKH + R (11)	17.3 ± 0.99**	0.04 ± 0.01	0.21 ± 0.02**	4.00 ± 0.36	4.92 ± 0.47**
2 WKH + R (11)	18.7 ± 1.04***	0.04 ± 0.01	0.23 ± 0.02***	4.46 ± 0.31***	5.35 ± 0.49***
WMC (27)	12.0 ± 0.4	0.04 ± 0.01	0.13 ± 0.01	2.88 ± 0.15	3.08 ± 0.19

[1] Mean ± S.E.M. (N)

1 WKH vs 2 WKH*

1 WKH vs 1 WKH + R**

2 WKH vs 2 WKH + R***

WMC vs 1 WKH^{+}

WMC vs 2 WKH^{++}

Since the evidence for adequate simulation models continues to grow, it is argued that the validity of animal models for weightlessness is well founded. It is our contention that suspension systems can be further extended to examine both functional aspects of atrophy due to weightlessness and mechanisms involved in muscle alterations. Evolutionary considerations of animal adapations to the forces of gravity can now be explored with the advent of the Shuttle/Space Laboratory and future Cosmos flights.

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SUBCELLULAR INVESTIGATION OF THE INFLUENCE OF REAL AND MODULATED WEIGHTLESSNESS UPON PERFORMANCE AND REGENERATION PROCESSES IN MUSCULAR TISSUE

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ABSTRACT

Investigations were carried out in animals flown into space in Biosputniks or undergoing various periods of hypokinesia. This observation resulted in disturbances in the mitochondria and endoplasmic reticulum of myofibryls. Similar changes occurred in animals both under weightlessness and hypokinesia. The regeneration process during hypokinesia is discussed. Evaluation of the influence of the environment on the muscle system was done on the basis of electrophysiological and stereological studies of cell structures as well as diffractometric analysis of the mitochondria.

Our investigations concern observations carried out on rats subjected over various periods of time to a hypokinetic environment or to 21-day flights in Biosputniks 782 and 936. The study is part of the Intercosmos Program. Estimation of the influence of changed environmental conditions upon the muscular system was based on electrophysiological studies as well as on qualitative and quantitative ultrastructural examinations, with the use of diffractometry of mitochondrial profiles. In animals subjected to space flight in the Biosputniks slight changes in musculus /m./ quadriceps and musculus gastrocnemius were found /1/.

The sarcomere volumes and the lengths of separate disks /ZIA/ did not undergo any changes either in red fibres or in white ones. Disturbances in triglyceride metabolism with numerous fatty granules between sarcomeres were also revealed. These changes may have been the effect of the oxidation-reduction process in the muscle cell resulting from hemodynamics changes and lack of sufficient oxygen supply. These suggestions were confirmed by slight degenerative changes in mitochondria, although the relative volume of these structures in the cell did not show any changes. However, a more thorough study of these **external** elements of mitochondria allowed to detect some subtle changes in these structures /7/. Thus it has been proved that in weightlessness conditions the surface membrane of mitochondria becomes thinner. This results in a decrease in the ratio between the surface and the internal membrane areas, which may suggest a slight change in the shape of the mitochondria; moreover, an increase in relative mitochondrial matrix volume, accompanied by a diminution in relative inner compartment volume, was observed. These results may suggest disturbances in mitochondria and changes in their energy state. For, according to Hackenbruc on the basis of observations of the state of internal mitochondrial components one may conclude as to the energetic activity of these structures.

It should be stressed that a more objective estimation of mitochondrial performance can be achieved through electronograms diffractometry of these structures. This method developed at our Institute, has been employed for mitochondria estimation in animals subjected to hypokinetic conditions /6/.

In animals undergoing space flight a decrease in relative volume of smooth sarcoplasmic reticulum was noted in both kinds of fibres, which may indicate electrolytic disturbances /Mainly Ca^+/ in the muscle cell.These disturbances may be connected with an insufficient number of impulses for inducing muscle contraction. This fact is reflected in investigation results of neuromuscular junctions. Accordingly, it has been observed that under conditions of weightlessness a decrease in relative synaptic vesicle volume in motor plates takes place, causing in turn a diminution of acetylcholine liberation, which results in depolymerization of the cell membrane. It is worth stressing that the sensitivity of different muscles under the same environmental conditions varies. M.soleus,e.g., seems to react more drastically to gravitation changes than m.quadriceps and m.gastrocnemius /2,3/. According to some scientists,there is a correlation between the percentage of muscle fibres and their sensitivity. Unlike the previously discussed muscles, soleus contains about 90% of red fibres /type I/. Weightlessness causes sarcomere volume diminution in this muscle, which can be interpreted as atrophic changes. A diminution of the mitochondrial volume falling to both one sarcoplasm unit and to one sarcomere /Table I/ also occurs.

TABLE I. MEAN SARCOMERE VOLUME /μm^3/ AND RELATIVE VOLUME OF MYOFIBRILS /$Vv_s\%$ ± SE/ AND MITOCHONDRIA /$Vv_M\%$ ± SE/ IN SOLEUS MUSCLE

GROUP	SARCOMERE	MYOFIBRIL		MITOCHONDRIA	
		$Vv_s\%$ ± SE	p	$Vv_M\%$ ± SE	p
CONTROL	2.07±0.22	66.28±1.03		8.34±0.61	
BIOSPUTNIK 936	1.20±0.02	69.09±1.42	<0.01	6.84±0.50	<0.02

The number of mitochondrial profiles decreases, whereas the accumulation of glycogen grains increases /Fig.1/. The electronographic images of mitochondria are also changed,this being manifested by their swelling,vacuolization or even decay. It can be supposed that disturbances in the weightlessness period concern oxidation processes in this muscle.

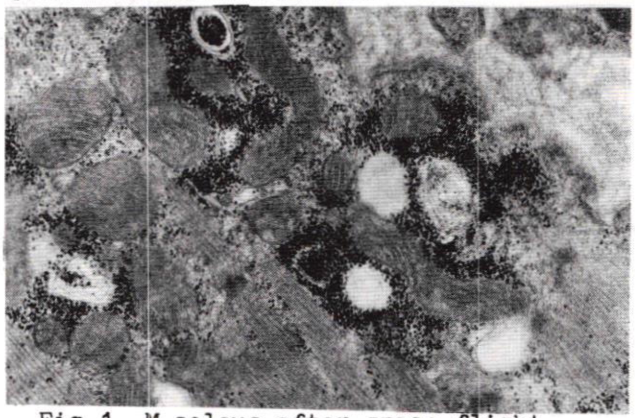

Fig.1. M.soleus after space flight demonstrated accumulation of glycogen and degeneration of mitochondria

In neuromuscular junctions of the soleus more drastic changes than in m. quadriceps and m.gastrocnemius were found /4/. They were of similar type,i.e., a diminution of relative volume and number of mitochondria and synaptic vesicles occurred /Table II/. The fact seems interesting that weightlessness also results in discrete heart muscle changes /4/. Our observation indicates a diminution of smooth endoplasmatic reticulum relative volume, and of mitochondria, and a slight acumulation of glycogen, which may testify some changes in transmission and transport of ions through the cell membrane, and slight hemodynamic disturbances.

TABLE II. RELATIVE VOLUME /Vv% ± SE/ AND SURFACE AREA PER VOLUME UNIT /Sv $\mu m^2/\mu m^3$ ±SE/FOR STRUCTURES OF THE AXONAL ENDINGS OF THE NEUROMUSCULAR JUNCTIONS IN THE SOLEUS MUSCLE

STRUCTURES	CONTROL Vv%±SE	BIOSPUTNIK 936 Vv%±SE	
MITOCHONDRIA	12.4±2.6	27.6±3.8	<0.01
SYNAPTIC VESICLES	15.2±1.4	6.6±1.1	<0.01

The presented investigation results illustrate changes after a relatively short period of weightlessness, i.e. 21 days. We are mainly concerned with the effects of progressively prolonged stay of living organisms in space. Therefore, physiological observations at the cell level can often be carried out through model tests only. The weightlessness equivalent on Earth is hypokinesia. In EMG /5/ of rats under prolonged immobility a **marked increase** of the percentage of **multiphase potentials** with unchanged amplitude was observed in some of them, whereas others showed changes characterized by a shortened registered potentials duration and a lowered amplitude.Automatic analysis of the muscular potentials spectrum /8/ revealed changes in bioelectric performance of m.soleus,in the other muscles no changes were found.Deviations in the electromyographic power spectrum image of the m.soleus during walking occurred after three weeks of hypokinesia, and in standing position were observed after 4 weeks of immobility.In this group of animals important changes at the cell level were also observed,and the degree of their intensity was proportional to the duration of immobilization /5/.Thus 6 months hypokinesis was manifested by atrophy of muscle fibres and their sarcomeres.Mitochondria were degenerated and scarce.Drastic changes were noted if after 6 months immobilization the animals were forced to physical effort.The main changes appeared to consist to myofibral apparatus defects. Vast areas of fibreless sarcoplasm were observed.Scarce as they were,smooth endoplasmatic reticulum channels profiles were profoundly swollen and dilated,mitochondria being often swollen,with few crests vacuolized in the peripheral areas.It should be added that in rats affected with effort after a period of immobility,mitochondrial changes were observed not only in skeletal muscles mitochondria,but also in the heart muscle /4/.Apart from ulstrastructural changes,a lowered succinic and dehydrogenase activity in the mitochondria was noted,and ECG results pointed to features of heart muscle anoxia /5/.

In the light of the above observations the question of the anticipated course of muscle fibres regeneration under disturbed gravitation conditions seems interesting. Hence the model experiment on regeneration in animals under hypokinetic conditions.

The observation was based on animals subjected to m.soleus denervation.It was found that muscle fibres degenerative changes were more clearly marked,and the regeneration process was slower than in the control group of animals.Thus after 36 days following muscle denervation,the muscle fibre diametre was smaller as compared with the control group or the baseline values.In the investigated muscle of the tested animals a diminution of endoplasmatic reticulum volume and glycogen grains concentrations were observed.

It was also concluded that the dynamics of the denervated muscle changes in mitochondria is differeat in animals subjected to hypodynamic conditions /Fig.2/. For instance,under laboratory conditions,in seven days following denervation the gap between crest centres increased rapidly /I/.This could be connected with swelling and vacuolization of mitochondria.

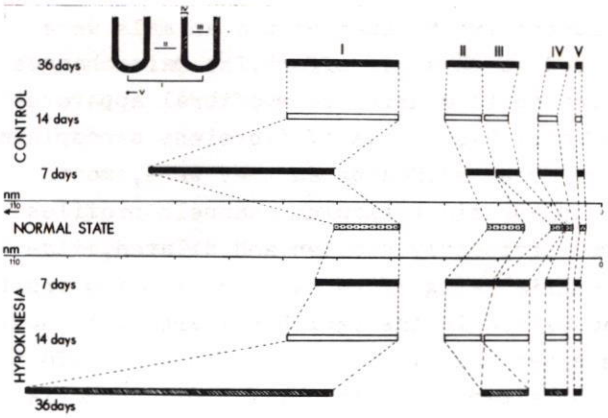

Fig.2 Changes in mitochondria of m.soleus in subsequent days following denervation.

These forms of mitochondria probably undergo decay, as in 14 or 16 days the values in question decrease.This is an inversible process, and it is near to normal.However,under hypokinetic conditions this process takes a different course.In each time interval a growing gap between crest centres is observed.

The other parametres,such as: intercrestal distances/II/,crest thickness /III/,internal membrane thickness /IV/, increase much more under conditions of hypokinesia than in the control.

Therefore,the diffractometric method allowed to establish objective numerical data concerning the changes and the observations made it possible to become acquainted with the mechanisms of the disturbances caused by changes in gravitation at the subcellular level. These point to gradation of changes dependent on the composition and performance of muscle fibrils.Thus muscleswith prevalence of red fibres were more sensitive,whereas the heart muscle was more resistant to gravitation change effects. Disturbances induced by hypokinesia were similar in character to disturbances resulting from weightlessness conditions,although they were always more intensive than after a similar period of weightlessness.Physical effort at early stages of re-adaptation lead to a greater intensity of the changes. However,the problem of regeneration requires further Biosputnik program investigation.

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EXAMINATION OF EFFECT OF HYPOKINESIS ON STATE OF GASTROINTESTINAL TRACT IN RAT

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ABSTRACT

For estimation functional state of gastric mucosa, transmucosal potential difference /PD/ and mucosal ion flux were measured by the authors. On the basis of PD decrease and H^+ rediffusion increase it is stated, that hypokinesis injuries the gastric mucosal barrier, that have also been reinforced by histological examinations. It was stated, that barrier-function damage - elicited by hypokinesis - increases ulcer sensitivity both in reserpinous and distensional ulcer models. The radiographs prove that already from the first days of hypokinesis, the motility of stomach increases, while motility of small intestine decreases. All the investigations indicate, that the most expressed impairment emerges in the 4 weeks group.

INTRODUCTION

Today it is already widely accepted that hypokinesis is one of the adequate modes of modelling weightlessness. In present experiments we have turned our attention to gastrointestinal tract.

METHODS AND RESULTS

We used Lati: CFY male rats with 220 g average weight, that were exposed to hypokinesis - lasting for 1-2-4-8 weeks employing a special stock and they were given standard ratfood /1/.

For estimation functional state of gastric mucosa we measured transmucosal potential difference /PD/ and mucosal ion flux /2/.

As it can be seen in Figura 1, transmucosal PD decrease is significant in hypokinetic groups from the first week onward, compared to the controls. Most expressed decrease can be noticed in the 4 weeks group, while PD increase appears in the 8 weeks group. In addation to the examination of gastric mucosal barrier we measured the change of mucosal ion flux.

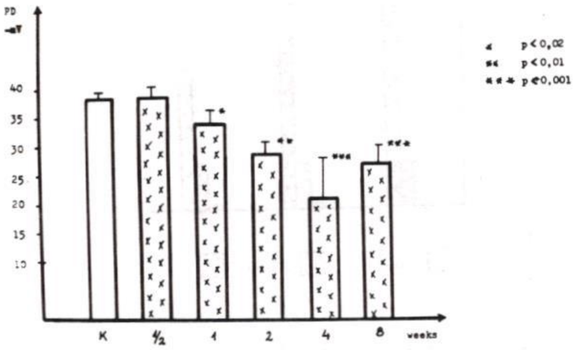

Figure 1. Effect of hypokinesis on gastric mucosa in rat.

In our experiment we experienced that rediffusion of H^+ significantly increased in all tested groups, in addition considerable amount of Na^+, K^+ and Cl^- appeared in the lumen. With passing of time it was accompanied by an increasing dilution. According to the above it can be stated that hypokinesis affects adversely the gastric mucosal barrier.

Simultaneously with PD and ion flux changes we examined photo- and electron microscopic changes of gastric mucosa and liver. The external cells become swollen and the intercellular space grows wider. Later erosion of external cells and at the end their necrosis and desquamation can be detected. In the liver cells lysosomes accumulate.

In the further study it was researched that is the ulcer-sensitivity increased by a barrier-function damage caused by hypokinesis. In support of this on the one hand the effect of hypokinesis on gastric ulcer - induced by reserpine - and on the other hand on distensional ulcer model have been examined /3, 4, 5/.

In the reserpinous experiment 3 tested groups were established:
I. Control-without treatment
II. Reserpine
III. Hypokinesis /2-4-8 weeks/ + reserpine

The members of group II and III received intramuscularly 5 mg reserpine/kg body weight, and they were starved for

24 hours /they received water ad libidum/. After this the stomachs of the animals were removed. Following the irrigation of the stomach, the mucosal lesion was macroscopically evaluated and scored. Within a group, the total point divided by the number of the animals produces the ulcer-index /U_i/. Moreover the ulcer-frequency /U_f/ was counted.

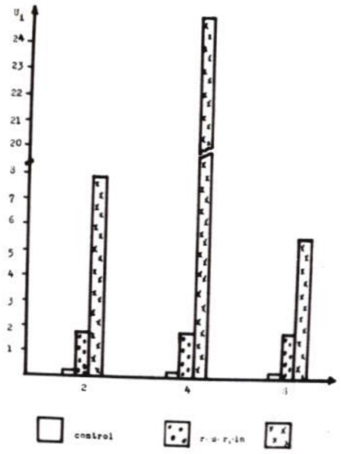

Figure 2. Effect of hypokinesis on ulcer-index in reserpinous ulcer pattern.

As shown in Figure 2, the U_i of hypokinesis + reserpine group significantly increased compared to all control groups. The maximum of U_i was noticed in the 4th week, while decrease is obvious in the 8th week.

Considering the U_f there is a significant difference between the U_f of hypokinesis + reserpine and control groups. The difference is in favour of the former. The U_f of 4 weeks group is 100 %.

The effect of hypokinesis on distensional ulcer model was examined as follows: passing the hypokinetic time the stomach was removed, exposed and irrigated and the mucosa was macroscopically tested.

As shown in Figure 3, the increase of U_i compared to the control is quite expressive. It can be noticed already in the 1 and 2 weeks groups, but it is the most significant in the 4 weeks group. After 8 weeks of hypokinesis the U_i practically corresponds to the control.

Later we examined what changes appear in gastric mucosal motility of the rats, exposed to hypokinesis. Motility testing was followed radiologically, after the introduction of 2 ml/100 g b.w. Novobarium via catheter. The radiographs were taken of the animals in the 1st, 2nd, 3rd, 6th, 9th and 12th hours, following the administration.

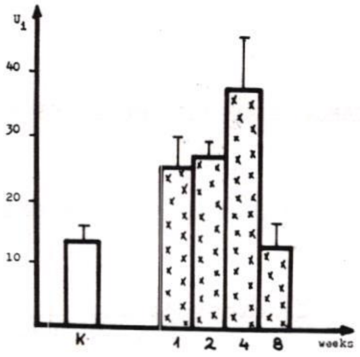

Figure 3. Effect of hypokinesis on distensional ulcer pattern in rat.

The results indicate that under the influence of hypokinesis the defecation of stomach increases, while the defecation of ileum decreases. The change develops during the first days of hypokinesis, and it still exists in the 8th week.

RESULTS

Our results prove, that hypokinesis brings about expressive damages in functional state of gastrointestinal tract. The morphological examinations verifies - in addition to the noticed gastrointestinal changes - even the significant damage of the liver. The noticed functional changes decrease the resistance of gastric mucosa and increase the ulcer sensitivity. On the basis of noticed changes, the damage of nutritional and absorptional processes are also probable. These investigations are the main task of our next experiments.

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THE ROLE OF CHRONIC ACCELERATION IN GRAVITATIONAL PHYSIOLOGY

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Gravitational physiology is a recently organized field of environmental physiology that is concerned with the effects of gravity and gravity-like acceleration fields upon organisms. Although the recent interest in gravitational physiology has been greatly stimulated by space exploration the roots of the subject extend back to Galileo [5].

Research in environmental physiology generally depends upon arranging a suitable change in an environmental factor and observing the effects of the modified environment upon exposed organisms. With such treatment the experimenter will observe a characteristic sequence of adverse changes, called stresses, and adaptive responses that will vary depending upon the nature of the environmental modification. There are problems in applying these general procedures in gravitational physiology, since there is no way presently known to attenuate or otherwise modify the field of gravity. Consequently, much of the animal research in gravitational physiology has depended upon modifying the effect of earth gravity, for example, with buoyancy produced by immersion, mass loading or unloading, reorienting the gravitational load by recumbency, and free fall for very short periods -- such as 500 msec [11]. Since the effect of gravity is proportional to body size, gravitational effects also have been inferred from studies of anatomic and physiological characteristics in a series of organisms with varying body size (scale effects).

It is possible to simulate changes in gravity by summing gravity and inertial fields, which are produced by motion. Inertial fields develop with a change in velocity -- either as a change in rate of motion without change in direction, linear acceleration, or by a change in direction without a change in rate of motion, radial or centripetal acceleration. Inertial fields developed by linear acceleration are inherently too time-restricted to be useful for most studies. For example, if a field 2½ times as strong as earth's gravity is established by linear acceleration, exposed objects will go into earth orbit in about 8 minutes, and achieve the speed of light in 8 months. So, only inertial fields produced by rotation (centrifugation) will be useful in chronic acceleration experimentation.

Objects that are restrained in an acceleration field develop a load, called weight, that tends to deform them and determines the work required to move the object against the field. Unrestrained objects will merely move under the influence of the field, and develop no load -- a condition sometimes described as free fall. The load that develops in restrained objects can be evaluated by Newton's laws of motion:

$$W = k\ m\ a$$

where: W is the load;
m is the mass of the object;
a is the acceleration field; and,
k is an arbitrary constant.

Two systems of units have developed depending upon the value of the constant, k. In the absolute system, used by physicists, k has the value of unity -- so mass and weight are in different terms. In the gravitational system, used by engineers, k is the reciprocal of the earth's gravitational constant (1/g). The latter convention is particularly useful to gravitational physiology, providing a terminology commonly used for evaluating gravitational field strength (G):

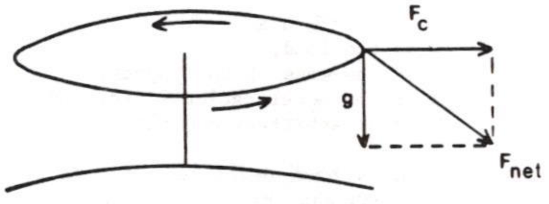
$$W = (1/g)\ m\ a;$$
$$W/m = a/g = G$$

G is a dimensionless unit that expresses the weight-to-mass ratio, or the acceleration field as multiples of the earth's gravitational constant. An altered weight:mass ratio is the physical basis for gravitational physiology.

Two general procedures have developed for the summing of inertial and gravitational fields. In one of these, the rotation is around the earth, and in the plane of a great circle (Fig. 1). If a complete rotation takes about 90 minutes at an elevation of 170 km, the inertial field will be about 0.95 G. If one calculates the strength of earth gravity at this altitude (from Newton's Law of Universal Gravitation), it will be found to be 95% its surface value (0.95 G). So gravity and the inertial field will be equal, but in opposite directions, and their vector sum will be zero. At the center of mass of the orbiting vehicle, a condition of weightlessness will exist, even though the field of gravity has 95% of its earth-surface value.

In another method of summing inertial fields and gravity, the plane of rotation is arranged tangential to the earth surface, with a centrifuge (Fig. 2). The physical relationships of such rotatory motion were first described by Huygens [21], a contemporary of Galileo and Newton, and he also coined the term "centrifugal force."

Figure 1. The orbital rate and altitude of a satellite establish a centrifugal field which is equal and oriented 180° to the local gravity field. If these two forces on the satellite are unequal, it will move to an orbit where they become equal.



$$F_{net} = \sqrt{F_c^2 + g^2}$$

Figure 2. Centrifuges develop an inertial field that is perpendicular to earth gravity. The net acceleration field is readily evaluated with the Pythagorean theorem. Animal centrifuges characteristically have cages mounted with 1° of freedom, which orient in operation so that the net acceleration field is perpendicular to the cage floor. Consequently, the net field has the same orientation, with respect to contained animals, as does earth gravity for static animals.

The validity of combining inertial fields and gravity requires some examination, since inertia and gravitation are different physical phenomena. The relationship between inertial mass and gravitational mass was investigated by Eötvös, a Hungarian physicist, in 1889, finding that any difference between them was less than one part in 10^9. This information was formalized by Einstein in 1904 as the Principle of Equivalence -- which was the point of departure for his General Theory of Relativity. This proposes that the effects of acceleration fields are indistinguishable, irrespective of their physical bases, such as gravitation or inertia. Consequently results obtained on an animal centrifuge can be considered to be the same as would be found on a more massive planet that has an equivalent field of gravity, and the results obtained in earth-orbit can be assumed to be the same as those from a gravity-free condition. The assumption that gravity has no effect in a net-weightless situation may be tested eventually by comparing results from animals in earth-orbit and in moon orbit. Since gravity will be 6-fold greater in earth-orbit, a similarity of results from these two weightless conditions would constitute a biological confirmation of the Principle of Equivalence.

Experiments with animals on centrifuges extend back over a century to the pioneering studies of Tsiolkovsky in Russia and Salathé in France. From the many animal centrifugation studies conducted since then, it has become apparent that the response of animals depends upon the duration and intensity of the treatment as well as some characteristics of the subjects -- such as body size, posture and orientation to the field. Short-term exposures (hours/days) generally produce a physiological stress, and the animals are debilitated. However, over longer periods (weeks/months) the surviving animals will become physiologically adapted and function without signs of distress. These prolonged treatments, which allow the animals to return to a steady state, have been designated as chronic acceleration, and briefer treatments, characterized by a labile physiology, have been distinguished as acute acceleration. The modern concept of utilizing protracted centrifugation in studying the physiological effects of earth gravity was first proposed by E. C. Dodds, an English nutritionist, in 1950 [4], and the first results of such studies were reported by B. H. C. Matthews, of Cambridge, in 1953 [12]. Since then, numerous studies of chronic acceleration have been reported, and several reviews are available [13, 16,22]. A discussion of the technical aspects of the procedure also is available [17].

Animals exposed to chronic acceleration exhibit a variety of anatomic and physiological modifications and many of these can be attributed directly to the greater G-load of the animal, an increased weight-to-mass ratio. Gravitational load increases the physical work requirement for posture and equivalent movement in the greater acceleration field, and this is apparent in the greater maintenance requirements of centrifuging animals, including energy metabolism [10,15,18]. Gravitational load also increases the muscular requirements for postural maintenance, and this becomes manifest in the selective hypertrophy of antigravity muscles, which increases the extensor:flexor muscle mass ratio [1]. The effect of gravitational load upon muscles proceeds slowly, requiring perhaps a year for completion in chickens, whereas the effect increasing energy metabolism is produced rapidly.

Some of the physiological responses observed in chronically accelerated animals are not readily attributable to the increased gravitational load. Foremost among these is the marked decrease in body fat which has been observed in all chronically accelerated animals except monkeys [14,20]. For example, chickens physiologically adapted to a field of 3 G may have a body fat content of 2% whereas earth gravity controls have 23% body fat. This is not the result of an inability to acquire feed nor of any metabolic insufficiency, because losses in body substance during a superimposed fast are readily recovered upon realimentation. Consequently, these decreases in body mass and body fat are regulated phenomena. Another response not readily related to the increased load of chronic acceleration is an increase in plasma protein in some species. In chickens this is arithmetically related to field strength, and at 3 G plasma protein concentration is increased 45% [2]. Such physiological responses to chronic acceleration indicate that the effect of the gravity field is transduced by some gravitationally sensitive tissue. This also implies that there are gravitationally sensitive organs that are not yet identified as such. It has been proposed that the brain may function as a gravity receptor [7,19].

Where chronic acceleration treatments are repeated at several field intensities, the degree of the observed physiological modification is generally related to field strength, and these can be rationalized by a regression of the degree of physiological response upon field strength. This analysis will yield an equation, which may be arithmetic, exponential or some other mathematical form. Characteristically the equation will have two coefficients: one of these is a proportionality coefficient, relating the two parameters, and the other is an intercept value, a mathematical prediction of the effect of weightlessness. Implicit in such a procedure is the concept that the effect of gravitational fields is continuous, so that the changes produced by fields weaker than earth gravity are related to the changes produced by stronger fields. There is some support for this concept. All observations obtained by various methods in gravitational physiology indicate that the independent variable is the acceleration field strength -- which, of course, is a continuous function. Consequently, the physiological responses, which are dependent variables, should be continuous, except where there are qualitative changes in the field effect. For example, some threshold field may be required for a particular physiological response. Above that threshold, the character of the physiological response will be proportional to field strength. However, in lesser fields any changes in that physiological process would not have the same relationship to changes in field intensity -- and the kinetics established in the gravitationally-effective range would no longer apply. Likewise, some gravitationally-sensitive processes may have maximal limits and become nonresponsive in greater fields. For example, the severe decalcification in unloaded bone, encountered in bed rest and in weightlessness, has no counterpart in hyperdynamic environments, where any reported changes in calcification have been minor. This indicates that the gravitational influence on bone density reaches a maximum in fields of near earth-gravity strength.

At present there is not sufficient equivalent information for a reasonable test of the validity of a continuity-of-effect concept; however, for some phenomena it does appear to apply. A comparison of plasma volumes in animals that are chronically accelerated or exposed to orbital weightlessness indicate a continuity of the gravitational effect above and below earth gravity [2]. Red cell mass also increases in chronically accelerated animals [2], and the kinetics are sharply hyperbolic, becoming maximal between 1.5 and 2 G, where the process appears to be saturated. This does not represent a limit of erythropoiesis, which is capable of much greater production under a hypoxic stimulus. In astronauts exposed to 60 days of weightlessness, red cell masses decrease about 12% [9], so there also may be a continuity of gravitational effect on red cell mass. The changes in plasma volumes are rational in terms of the Henry-Gauer reflex, however there is no basis in current understanding for a gravitational influence upon body red cell mass.

The occurrence of thresholds for some gravitational effects can be anticipated. Periods of weightlessness, up to 45 seconds, can be produced in high performance aircraft by flying a ballistic trajectory -- the "parabolic maneuver." In some experiments a small centrifuge (0.9 m radius) has been carried on aircraft and operated during periods of flight-weightlessness, producing net fields between weightlessness and 1 G. In rabbits and dogs the leg electromyogram was observed to decrease in fields less than 1 G [23]. Proprioceptive reflexes adjust very rapidly in a decreased gravitational field, the leg stretch-reflexes being lost within 500 msec of weightlessness [11]. In mice and rats, a normal posture and locomotion is lost in fields less than 0.28 to 0.30 G -- and it was concluded that 0.3 G was the minimum field compatible with normal orientation and locomotion [24]. A similar result obtained with chickens counterbalanced at earth gravity. When 60% of the normal leg loading was removed, animals lost normal posture and were severely stressed [3]. This indicates that chickens require a field of at least 0.4 G for maintenance of posture and locomotion. It might appear that larger animals can function in lesser fields since the Apollo astronauts maintained a normal posture and competent locomotion on the moon surface, 0.16 G. However, the astronauts, who had a mean body mass of 76 kg [8], were mass-loaded with 172 kg (PLSS and suit [7]), and their leg loading on the moon was equivalent to an 0.35 G field for an unencumbered astronaut. So, it appears that 0.3 to 0.4 G may be a general threshold for maintenance of the posture and locomotion that is characteristic of terrestrial existence. Since the chronic loss of these functions should have a broad influence on the animal's physiology, 0.3 to 0.4 G may be a general threshold for functions characteristic at earth gravity and greater fields. The loss of such proprioceptive function in lesser fields may be a factor in the space sickness that has been encountered in weightlessness.

Ultimately, colonies on the moon (0.16 G) and Mars (0.4 G) will provide fields for gravitational studies between weightlessness and earth gravity. However, current examination of the physiological effects of fields intermediate between weightlessness and earth gravity will require placing centrifuges on orbiting satellites, summing three acceleration fields. Such a centrifuge, operating at 1G, will also provide an essential control for a satellite environment. A similarity of results from a satellite 1G-centrifuge and from simultaneous earth-surface observations will provide assurance that the principal factor in the satellite environment is the altered acceleration field. It is commonly understood that nongravitational factors in the centrifuge environment may also contribute to the results of chronic acceleration experiments [17]. These factors include rotatory rate, variability of field intensity including head-to-foot gradients, changes in gas diffusion resulting from a local increase in gas density and machine effects -- noise, vibration, etc. In terrestrial situations, limitations from the nonacceleration factors are avoided by using large diameter centrifuges and the use of controls -- such as animals exposed to the machine factors without a significant change in the acceleration field. However, satellite constraints may not permit these usual procedures for on-board centrifuges. Developing suitable satellite centrifuges will require a much better understanding of the technology than now exists. Development of such information would be an additional benefit derived from a vigorously pursued program of chronic acceleration research.

There also are substantial theoretical benefits from the application of chronic acceleration. Without the use of centrifuges, current observations will be limited to weightlessness and earth gravity. Since it is not possible to generalize from two points of observation, chronic acceleration techniques will be necessary for the development of scientific generalizations in gravitational physiology. Consequently, substantial scientific benefit would be obtained by the addition of centrifugation studies (on satellites and earth surface) to experiments conducted in earth-orbit.

In order to understand the physiological consequences of earth gravity, observations in fields greater than 1G will be as important as those in fields of lesser intensity. A full understanding of the effects of earth gravity must be the ultimate objective of Gravitational Physiology, and this will be of great general importance, since it is the one common factor in all terrestrial phenomena. Although we still may not be able to modulate earth gravity, understanding its role will be of great assistance to the solution of problems or understanding of processes where it is a factor.

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THERMONEUTRAL ZONE AND SCALING OF METABOLIC RATE ON BODY MASS IN SMALL MAMMALS

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It has been well known for many years that the metabolic rate of mammals may be increased as much as 2- to 3-fold under low ambient temperature conditions (5). It is also well established that there is a temperature range, known as the thermoneutral zone, within which the metabolic rate is at a minimum. Below the thermoneutral zone, body temperature is maintained by an increase in heat production rate, as in shivering, and by a decrease in heat loss from the body surface through mechanisms such as skin vasoconstriction, piloerection and behavioral modification. Above the thermoneutral zone, body temperature is maintained by increased evaporative heat loss through sweating, panting, or wetting of the body surface by licking, all of which entail an increased metabolic cost.

We have previously described (3) a 4-species animal model suitable for experimental study of the effect of change in gravitational loading on the scale relationship between metabolic rate (MR) and total body mass (TBM). The model comprises 6 metabolically mature animals of each of the 4 common laboratory mammals, hamster, rat, guinea pig and rabbit, which provides a body mass range from 0.1 kg to 4 kg. Measurement of individual metabolic rates in the animals under standard conditions reproduces the classic Kleiber allometric relationship for mammals

$$\mathrm{MR, kcal\cdot hr^{-1}} = 2.82(\mathrm{TBM, kg})^{0.756};\quad r = 0.998.$$

Ideally, the metabolic rate measurements should be made at thermoneutrality, but the thermoneutral zone for the rat has been the subject of some argument (2,4,7), and is poorly defined for the hamster, guinea pig, or rabbit. Accordingly, we have carried out a study of the effect of ambient temperature on metabolic rate in 6 male animals, 8-10 months of age, of each of the 4 species in the ambient temperature range 20°-36°C. The animals were obtained from commercial sources and maintained in individual cages in an animal colony room kept at 25° ± 1°C.

On the day of an experiment an animal was transferred from his home cage into a Thermonetics Corporation, San Diego CA, gradient layer calorimeter located in a controlled environment room set at a particular temperature with 50% relative humidity. The continuously ventilated calorimeter chamber used for the hamsters, rats and guinea pigs measured 15 x 15 x 30 cm, and that used for the rabbits measured 30 x 30 x 60 cm. The apparatus measures sensible heat loss from the animal by means of thermopiles in the chamber wall and in the entrance and exit air streams. Latent heat loss is measured by means of a Benzinger-type condensing platemeter. The animal remained in the calorimeter chamber for 4 hr, and the mean sensible and latent heat loss rates during the final hour were summated to yield a value for metabolic rate. At the end of the 4-hr period the animal was returned to his home cage, and was not placed in the calorimeter again for at least 4 days. Each animal was measured at 5 or 6 different ambient temperatures in the course of the study.

The metabolic rate measurements are summarized in Fig. 1. Each point represents the mean and standard error values for 6 animals at the ambient temperature indicated. It may be seen that the minimum metabolic rate occurred in the vicinity of 29°C for the hamster, guinea pig and rabbit, whereas for the rat it was near 27°C. Analysis of variance revealed that the metabolic rates both below and above these temperatures were significantly greater than the minimum values at $P < .05$.

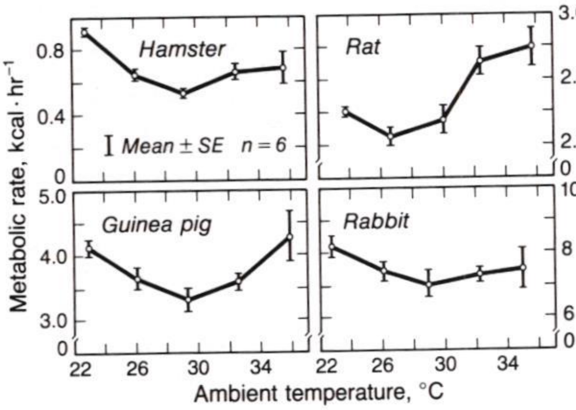

Fig. 1. Metabolic rate as a function of ambient temperature.

In order to facilitate comparison between species, the metabolic intensity ($\mathrm{kcal\cdot hr^{-1}\cdot kg^{-1}}$) was computed for each animal at the various ambient temperatures and expressed as a percentage of the minimum metabolic intensity observed. The results are shown in Fig. 2.

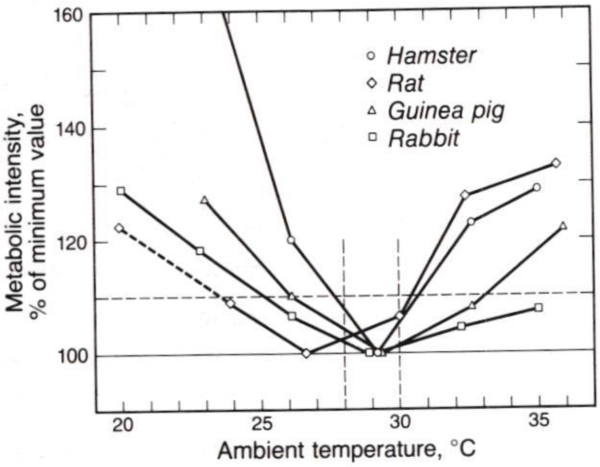

Fig. 2. Metabolic intensity as a percentage of the minimum metabolic rate.

It is evident that the ambient temperature bandwidth about the minimum, in which the metabolic intensity increases by no more than 5% is about 2-5°C, depending upon the species. Ideally, the standard metabolism of hamsters should be measured at 29° ± 1°C, while that of rats could be measured at 27° ± 2°C. Both guinea pigs and rabbits could be measured at 29° ± 2°C.

However, it would be desirable in some scale effect studies, such as in spaceflight experiments to measure the metabolic rate of animals of the 4-species model at nearly the same time and at a common ambient temperature. Hence, a reasonable compromise appears to be the ambient temperature at 29° ± 1°C, which yields metabolic intensities well within 10% of the minimum values for all 4 species.

As a test of this compromise we computed interpolated values for metabolic rate at 29°C for each of the 24 animals of the 4 species studied, and derived the allometric relationship between metabolic rate and total body mass by logarithmic least squares fit with the following result

$$MR_{29°C}, \mathrm{kcal \cdot hr^{-1}} = 2.91(\mathrm{TBM,kg})^{0.766}; \; r = 0.979.$$

The equation parameters are not significantly different from those for the Kleiber relationship.

On the other hand, when the interpolated values for metabolic rate at an ambient temperature of 24°C were used, the allometric relationship became

$$MR_{24°C}, \mathrm{kcal \cdot hr^{-1}} = 3.51(\mathrm{TBM,kg})^{0.676}; \; r = 0.987.$$

In this instance the equation parameters are significantly different by analysis of covariance (6) from both our 29°C data and the Kleiber relationship. The positioning constant is elevated because of the higher metabolic rates at the lower ambient temperature, and the exponent is lessened because the metabolic rate of the smaller animals was increased proportionately more at the lower temperature than was that of the larger animals. Hence, it may be concluded that the condition of thermoneutrality is important for metabolic scale effect studies, and that the thermoneutral zone for the species considered here is a narrow one.

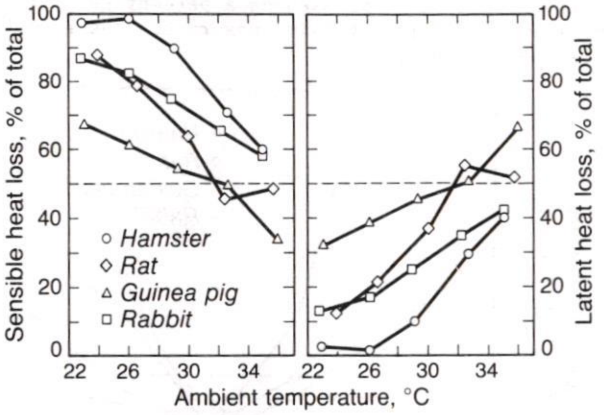

Fig. 3. Sensible heat loss (upper graph) and latent heat loss (lower graph) as percentages of total body heat loss.

Our measurements also permitted partitioning of total body heat output into sensible heat loss by radiation, conduction and convection, and into latent heat loss by evaporation of water from the body surface, as a function of ambient temperature at 50% relative humidity. The results are shown in Fig. 3, where it may be seen that sensible heat loss is predominant in all 4 species at the lower ambient temperatures, as might be expected, and that latent heat loss does not become predominant until the thermoneutral zone is exceeded. Furthermore, it is evident that a species difference exists with respect to partitioning of heat loss. The hamster and the rabbit consistently lose relatively more body heat by sensible loss over the ambient temperature range studied than do the rat and guinea pig. The hamster and rabbit evolved as desert mammals, and perhaps have been selected through evolution to minimize body water loss by maximizing sensible heat loss from the body.

However, as ambient temperature exceeds body temperature, latent heat loss accounts entirely for total body heat loss. Eventually, a limiting ambient temperature is reached beyond which body temperature can no longer be maintained and death ensues. The limiting ambient temperature is a function of the relative humidity of the ambient air and of the capacity of the animal to provide water for evaporation to the body surface. Adolf (1) has described the conditions representative of survival of 50% of a group of animals of various species for an exposure period of 3 hr at various combinations of ambient temperature and relative humidity. For 50% relative humidity the lethal temperature for 50% of the animals, or LT-50, is 38°C for the mouse, 39°C for the rat, 41.5°C for guinea pig and rabbit, and 46°C for man, cat and dog. Thus, the LT-50 for our 4-species model appears to be about 10°C above the thermoneutrality zone.

ACKNOWLEDGEMENT

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THE REGULARITIES OF RELATIONSHIPS BETWEEN STRUCTURE AND FUNCTION UNDER DIFFERENT FUNCTIONAL LOADS (HOMEOSTASIS AND HOMEOMORPHOSIS)

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INTRODUCTION

The effect of hypergravitation and hypogravitation on the structures and functions of the organism is a part of the more general problem of relationships between functional loads, on the one hand, and functional and structural responses of the organism, on the other hand. According to the presently accepted views, a function is subjected to a direct control, a variation of functional loads produces directly adaptive alterations of functions. The latter causes secondary functionally dependent changes in the structure of the organ (1-12). This implies that with increasing functional loads, a hyperfunction of the organ arises which then leads to its hypertrophy and hyperplasia. Decreasing functional loads on the background of hypofunction results in hypotrophy and hypoplasia of the respective structures.

The processes of the structural-functional changes of any organ in a wider sense may be considered as adaptive homeostasis, because in this way a relationship between structure and function is maintained at a certain level.

However, in late 70-ties we obtained several data suggesting that a control over structures may be achieved independently of functions in addition to function-dependent control of the structure(9). The regulation of the structure is being realized by special mechanisms whose nature is still uncertain. Such a type of homeostasis, in contrast to homeorhesis (a term "homeorhesis" was proposed by C.Waddington for designation of the functional homeostasis), we called homeomorphosis or homeostasis of structures (8).

Revealing homeomorphosis in an experiment is rather difficult since only in a few cases, created experimental conditions could provide an independent control over the changes in structure and function. In this respect, the small intestine is an adequate and fruitful object.

In this report, some general regularities of the structural-functional changes in the mucosa are considered in terms of relationships between the structure and function of the rat small intestinal mucosa under different functional loads. For this purpose, we used a convenient and easily interpretable model of hypo- and hyperfunctioning intestinal tube.

MATERIALS AND METHODS

The experiments were performed on male Wistar rats with initial weight of 180-200 g given a standard ration.

For a provision of increased or decreased functional loads on definite parts of the small intestine and modification of qualitative characteristics of these loads, we carried out 10 types of surgical operations which are schematically represented in Fig.1. Each group of the operated animals included 7-8 rats. The small intestine was subdivided into 7 segments. The first of them is the duodenum and subsequent six are jejunal and ileal segments, equal in lenght.

Sham-operated rats of the same sex, age and weight, referred to as control, underwent a laparotomy (I) followed by a digital manipulation of the small intestine. The next 6 types of operation included a resection of distal (II) and proximal (III) parts of the small intestine; an isolation of distal (IV) and proximal (V) parts when the corresponding intestinal segments are in the body but excluded from digestion; a bypass of distal (VI) and proximal (VII) parts when the small intestinal segment is isolated from food passage but the chyme from this segment enters more distal part of the gastro-intestinal tract.

To study the role of direct contact effects of the chyme factors, a special operation was made (VIII) when the 7th segment of the isolated part of the small intestine (4-7 segments) was anastomosed with proximal jejunum. Moreover, a transposition of proximal and distal parts of the small intestine (IX) was accomplished which increased functional load on the distal parts and decreased the load on the proximal parts of this organ. Finally, a transplantation of the biliary-pancreatic ducts to the beginning of the bypass segment (X) providing a release of the secret of the digestive glands into non-functioning segments of the small intestine, as well as some other operations not included in the present report were performed.

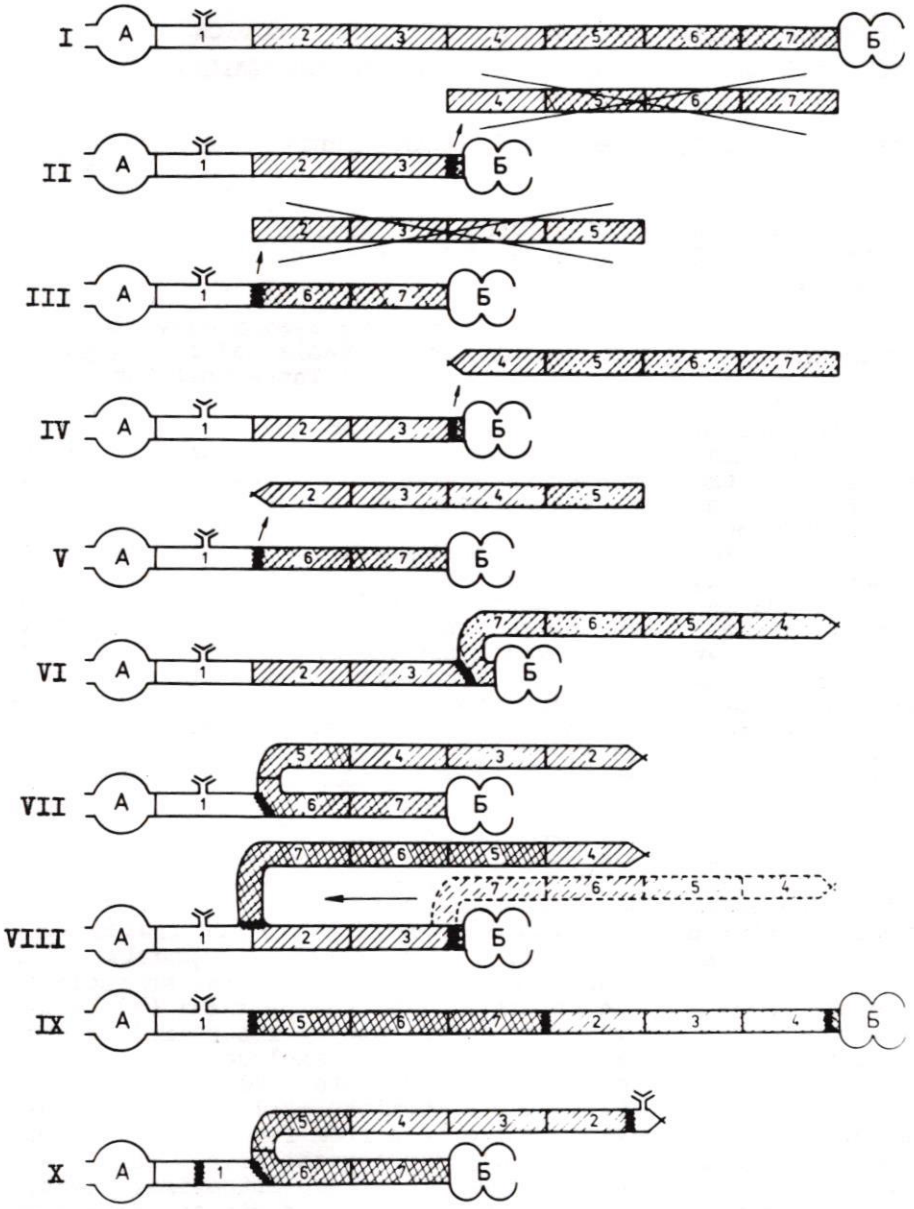

Fig. 1. A scheme of operations in studying the structural and functional characteristics of the small intestine. A - the stomach, Б - the colon. 1 - the duodenum, 2-7 - segments of the jejunum and ileum. Other explanations see in the text.

We estimated the mucosal mass, light and electron microscopic characteristics of the mucosa as well as sucrase (EC 3.2.1.48) and alkaline phosphatase (EC 3.1.3.1) activities of the enterocytes in each of the 7 segments of the rat small intestine in 7, 15, 30 and 120 days after the above mentioned surgical interventions. Technical details have been described previously (9). The data obtained were estimated by the Student and Fisher method.

RESULTS

This report presents the results from the studies on the structural changes of hypo- and hyperfunctioning segments of the rat small intestine at different intervals after III, V and VII types of operation. For a resection, isolation and bypass we used a segment which is in immediate contact with the duodenum and equal in lenght to two-thirds of the jejunum and ileum (2-5 segments on Fig.1). These data were compared with the results of studies on the same parameters in the control (I).

Fig. 2 demonstrates changes in mucosal mass of the rat duodenum at different times after the operations in per cent to the control. As can be seen, after the resection (type III) a hypertrophy of the duodenum was markedly expressed; this finding can readily be explained by an enhancement of function and function-dependent hypertrophy (1-12). The comparison of the consequences of the isolation (type V) and the bypass (type VII) operations is of great interest for understanding of the effects of the isolated segments

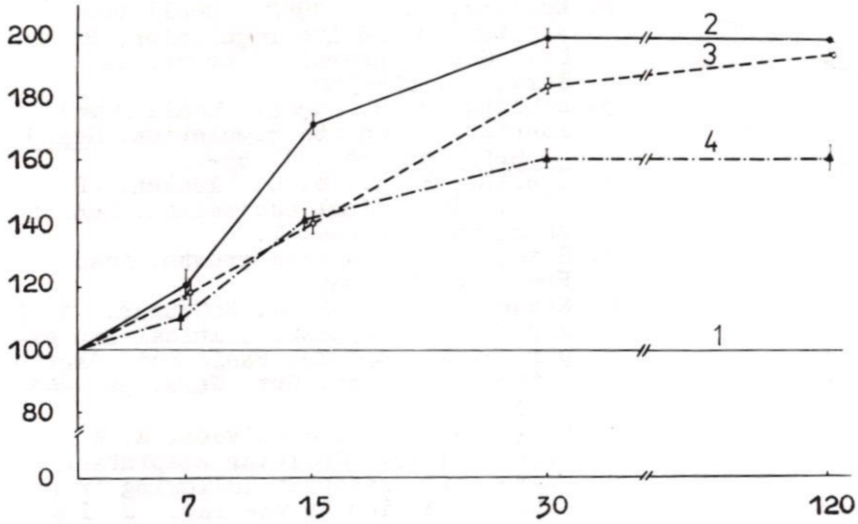

Fig. 2. Changes in mucosal mass of the rat duodenum after laparotomy (1), resection (2), isolation (3) and bypass (4) of the proximal part of the small intestine. Abscissa: time after operation (in days); Ordinate: the mucosal mass of the duodenum in per cent to the control taken as 100.

of the small intestine on its functioning parts. In fact, in the first case, a connection between the isolated part of the small intestine and the organism is being realized only through blood vessels and mesenteric nerves, whereas in the second case, apart from these ways the isolated segment remains an entry of the chyme through a shunt into the functioning parts of the digestive apparatus. As can be seen (Fig. 2), as early as in 15 days after the bypass the effect of this operation on the mucosal mass of the duodenum becomes practically indistinguishable from that of the resection. However, later in 4 months following the operation, the effect of the bypass intestine is maintained, in contrast to the isolation (when the effect of "presence" disappears). This indicates that compensatory hyperplasia of the duodenum depends not only on increased loads per unit of mucosal area but also on the number of enterocyte populations.

The changes in the total mucosal mass of 7 segments of the small intestine at different intervals after the isolation and the bypass are shown in Fig.3.

The analysis of the time dynamics for a compensatory process (from the 1st week to 4 months) reveals that after both operations a progressive hypertrophy of the mucosa takes place in the functioning segments (curve 3) as well as its atrophy in the segments excluded from digestion (curve 2). However, a summation of the mass of functioning and non-functioning segments of the small intestine has made possible to conclude that throughout the entire period of observations the total mass of the small intestinal mucosa remains almost unchanged (curve 1).

CONCLUSION

It is believed that the fit of the structure to function is a measure of its perfection and, therefore, a concept of autonomic regulation of the structure independently on the function seems to be paradoxal and unconvincing. Really, it is easy to understand the physiological role of the mucosal hypertrophy and hypotrophy developed in the small intestine in response to either increasing or decreasing dietary loads.

The mucosal hyper- and hypoplasia arising, as a consequence, from hyper- and hypofunctions in various segments of the rat small intestine, may be characterized as a function-dependent regulation of the structure. Along with this, Fig.3 shows that there exists a mechanism by which the total mucosal mass of the functioning and non-functioning parts of the small intestine is maintained at a constant level, close to that of mucosal mass in control animals during all the periods of development of an adaptive-compensatory reaction in different type operations. Such a homeostasis of the mucosal mass can not be interpreted as an adaptation of the structure to enhanced or deteriorated function. Rather, this phenomenon should be considered as a maintenance of the steady-state structure independent of either increase or decrease in functional loads. It is the circumstance that served as a basis for an introduction, as was above noted, of the concept of homeomorphosis (8).

It may, probably, be suggested that the function-dependent structural changes provide a possibility for their adaptation to the existing conditions of functioning. Homeomorphosis reflects genetically predetermined adaptation of the structure to a certain level of functioning. The vital importance of homeomorphosis may be illustrated by a number of examples from biology. In particular, the absence of marked mucosal atrophy in the gastro-intestinal tract during a prolonged fasting, widespread in the nature, is an essential

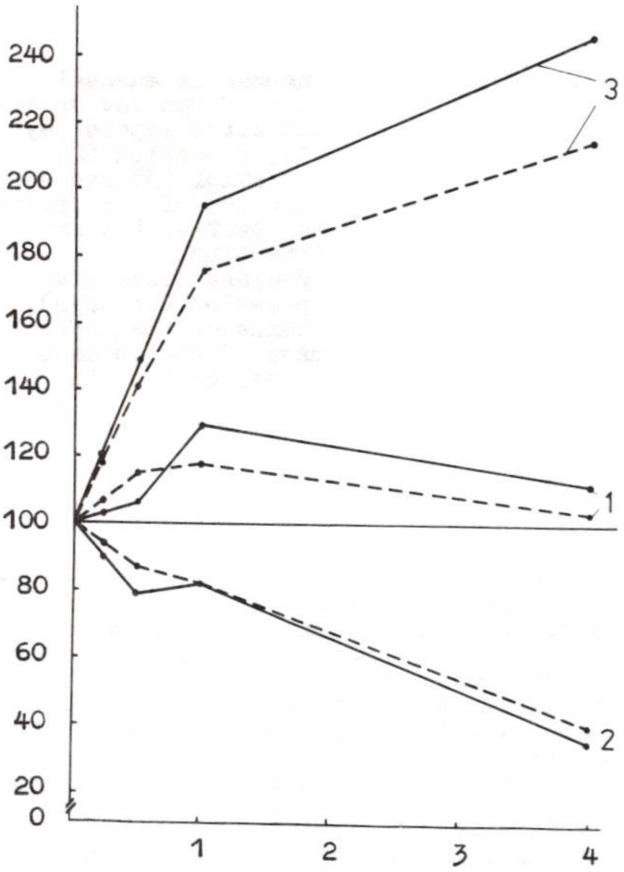

Fig. 3. Changes in the mucosal mass of the entire rat small intestine after an operations. Solid line - isolation, dashed line - bypass of the proximal part of the small intestine.
Ordinate: the mucosal mass of the small intestine in per cent to the control taken as 100; Abscissa: time after an operation (in months). 1 - the total mucosal mass of functioning and non-functioning segments, 2 - the total mass of non-functioning and 3 - the total mass of functioning segments of the small intestine.

prerequisite for a survival of individual organisms and whole populations after a renewal of feeding (8).

A relationship between the function-dependent and function-independent systems of regulation of structures may differ in distinct animal species, at varying functional states, at different ages etc.

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EVOLUTIONARY AND PHYSIOLOGICAL ADAPTATION TO GRAVITY

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ABSTRACT. The genetic, morphological and physiological status of present-day organisms is a result of the effect of gravity that plays three different but closely related roles, viz. as a creator and transformer of the abiotic environment, as a factor of natural selection, and as a physiological stimulus producing mechanical stresses. The gravity effects in these different roles are addressed to various structures, realized via various mechanisms, and actualized on an incomparable time scale.

Since the early space flights investigations of effects of weightlessness are under way. Traditionally, these investigations are divided into two types -- physiological and biological. This division appears to be purely conventional at first sight but actually it has an important meaning because the investigations employ different methods and, this being more significant, have different goals.

Life sciences are known to consist of how-biology, what-biology, and why-biology. How-biology is in the scope of physiologists, who describe the experimental purpose, object and results as a search for mechanisms used by a living organism to modify its state or behavior in response to environmental effects. This is termed physiological adaptation. Questions put to living systems and their answers at the physiological level are always viewed as exhustive. It is a common error because they are adequate only within the physiological scale of time. This is an answer that is satisfactory today.

Wthat-biology deals with the phenomenology of vital processes. It raises no questions concerning mechanisms of changes and treats so-called elementary biological processes. All data obtained are expressed as integral numbers. In physiological sciences even raw data are some transformation that converts measured data into numbers. There is an instrument between a researcher and a phenomenon to be measured.

What-biology is a starting point for why-biology. Its findings help to determine the evolutionary strategy of morphological and physiological transformations in living organisms in response to a continuous effect of zero-g or to get related information about the evolutionary role of gravity.

The simplest result of the fact that elementary biological events develop in zero-g without obstruction is that growth dynamics in zero-g, as well as on the Earth, can be described by a universal biological sigmoid curve. A sigmoid curve was plotted to describe the growth of bacteria, paramecia, animal cell culture, plant seedlings in zero-g.

In order to account for the normal development of biological processes in zero-g, we have offered a hypothesis that claims that living organisms are adapted to a gravity-produced environment with a vectoral physical arrangement or, simply speaking, top and bottom. Therefore gravity adaptation in the physiological (but not the evolutionary) sense is an indirect parameter of at least second-order. Moreover, adaptation observed in nature mainly has a phylogenetic meaning. Adaptations emerge in the course of a unitary continuous process whose duration is equal to that of biological evolution on the Earth or at least to the duration of biological evolution that began with cell emergence, i.e. no less than 3.8×10^9 years ago. The mechanism of adaptation is identical to that of any evolutionary change: development of incidental mutational events and selection of specimens with respect to their adaptive values in the next generation. The morphology, physiology and etology of many living organisms are obviously adapted to a gravity environment and whereas zero-g has no effect on elementary (and not only elementary) biological processes, this is an illusionary contradiction.

The morphology and physiology of living organisms are under the influence of both electromagnetic and gravitational forces. The great difference in the strength of electromagnetic and gravitational forces shows that each of them has its own scope of action. Since the structure of atoms and molecules, including biomolecules, is controlled by electromagnetic forces which are at that 39 orders of magnitude larger than gravitational forces, it is clear that those latter (altered gravity and hypogravity) are unable to affect cell structures such as DNA, proteins, lipids. They are also unable to modify biomolecule compounds that constitute cell organelles such as nuclei, plastids, mitochondria, membranes. Beyond the gravity effect is the cell itself whose size is strictly limited by nature. Being unable to interfere with the structure of biomolecules, gravity has generated an adequate substrate in the form of Earth's crust, oceans and atmosphere. Hence the importance of gravity of the first order for vital processes. About 3.8 x 10^9 years ago when primitive cells appeared the polar organization of the environment initiated morpho-genetic evolution that gave rise to dorso-ventral polarity.

A typical feature of thermodynamically nonequilibrium systems is that the primary structural unit of an organism, i.e. the cell, must be small or the organism must devide on small structural units. Due to energy dissipation that maintains and increases the internal order, the content higly organized matter in the cell-grows. However, this process cannot be infinitely long. As the mass and volume of the matter increase, its surface/volume ratio decreases. The matter and energy are exchanged and maintained in the steady-state via membranes that separate living systems and their environment. As long as the surface/volume ratio decreases, the balance becomes, gradually but inevitably unfavourable to life. Some regions of the living system, especially those located in-depth, reach a steady state and an old order disappears. Any system that is bound to exist and develop needs a mechanism that separates it into subsystems or isolated systems. The development of this mechanism, i.e. the mechanism of cell division, appears to be one of the early inventions of evolution. This is a universal rule - all living organisms are either single cells of about 10 mcm or complexes of such cells.

A viable organism must be a very small open part of the Universe that has a semipermeable surface and a division mechanism. To diminish their entropy, living organisms utilize processes of energy dissipation that work on a space scale. This law is important for space biology. It implies that the elementary structure of living matter, i.e. a cell, irrespective of the time and place of its location, must have a definite size with an average diameter of about 10 mcm. Any exception is to be thermodynamically justified.

With respect to gravity the basic difference between small and large animals is that the shape of those latter develops via natural selection obeying technical specifications dictated by gravity. It appears that in the case of tiny organisms gravity does not act as a factor of natural selection. For them gravity is only an evolutionary factor of the first order, i.e. the one that generates and transforms an environment. For large animals the parameters that relate gravity with the shape and physiology are determined by natural selection. Natural selection alone is capable of producing forms that provide optimal adaptation of the specimens having these forms. Gravity as one of the abiotic constituents of natural selection should be considered as a factor of form development of the second order. This factor is of crucial importance for large animals. In the final analysis, this very factor specifies the maximum size of at least terrestrial animals. In contrast to unicellular organisms whose maximum size obeys laws of thermodynamics and therefore is independent of space and time, the upper limit of multicellular terrestrial animals is controlled by the mass of the planet and the duration of evolutionary history.

Disregarding gravity, it is impossible to understand correctly the diversity and arrangement of forms in the organic world. Gravity can be defined as a technical specification in the framework of which natural selection produces species that meet these specifications. For large animals gravity may be the principal specification.

A study of the third-order gravity meaning is within the scope of physiology. The physiological significance of gravity is the least important. Gravitational variations cause minor and, as a rule, reversible changes that are most distinct in large terrestrial organisms. However, in space biology and medicine this line of research occupies a leading position. Most biomedical experiments are physiology-oriented. Gravitational physiology studies may influence the fulfillment of manned space missions. In physiological studies gravity effects involve their perception; in man this perception is reinforced by consciousness; working hypothesis related to gravitational physiology can be readily verified by experiment.

Physiological adaptation should be regarded as a conformist agreement between an organism and its environment. The agreement has been reached in the course of evolutionary development. It allows the existence and sometime the progressive development of the organism. This agreement normally provides for organisms the existence in a varying environment, particularly if the variations are periodical and their range is not too large. The organism can adapt to an unexpected environmental change with greater difficulty and usually cannot adapt at all to an erratic change. Such changes lead to the death of the organism or to the death of the species if they proceed on a global scale. The fact that , in weightlessness, elementary biological events may occur, certain animal and plant species may develop through several generations and mammalians can easily adapt to the state gives evidence that weightlessness can be referred to the category of insignificant nonspecific environmental changes.

Ronald Fischer once remarked that an insignificant and nonspecific change in the environment has an absolutely equal opportunity to increase or to decrease the adaptive capability of an organism. The probability that this capability will grow is inversely proportional to the magnitude of the environmental change. In weightlessness the adaptive property of a living organism, man including, will not necessarily deteriorate. Theory predicts that in weightlessness physiological adaptation of certain organisms may well improve.

The genetic, morphological and physiological status of present-day organisms results from the effects of gravity that perform three different although closely related roles, viz. the rule of the creator and transformer of an abiotic environment, the role of an important factor in natural selection, and the role of a factor of environmental physiology that causes stable mechanical stresses.

The fact that elementary biological processes may develop in the weightless state is the condition which allows manned flights whose duration will be comparable with that of man's active life. In such long-duration flights the major limitations and difficulties will be associated with psychological, hygienic and engineering problems. From the biological point of view the way to outer space is opened for man.

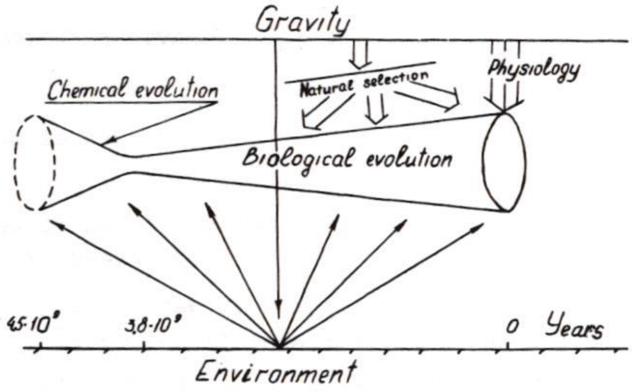

Fig. 1 Scheme of the triple role of gravity. According to the scheme, the physiological role of gravity is the smallest.

GRAVIPERCEPTION IN PLANT CELLS

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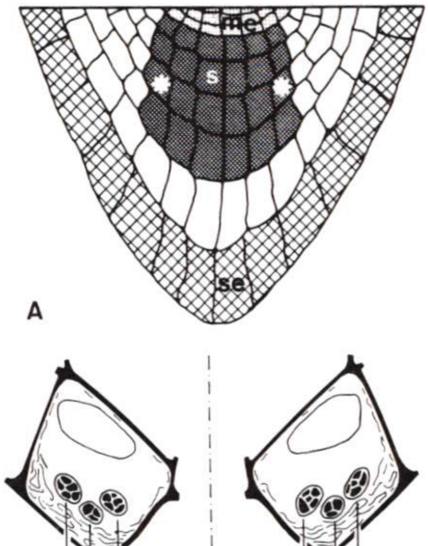
Plants are capable to grow in defined directions in a gravitational field. Gravity is perceived (transformed into a physiological signal: perception), which is the precondition for an orienting growth reaction (response). Radicles of garden cress (Lepidium sativum L.), when placed horizontally, respond with a downward curvature, caused by differential growth of the physical upper and lower flank of the elongation zone, respectively. In case of roots the graviresponse of the elongation zone is spatially separated from the site of graviperception, which is localized in the statenchyma of the root cap (for details compare Volkmann and Sievers 1979). The root cap of the garden cress consists of longitudinally arranged cell rows (meristem cells, statocytes, secretion cells), neighbouring cells form stories of the same degree of differentiation. The outer shape of the root cap approaches a rotation paraboloid. Single statocytes thus are arranged in radial symmetry (Fig. 1).

This symmetry of the statenchyma, in combination with the ultrastructural polarity of the statocytes (Fig.1B: the nucleus is situated near the proximal cell wall, starch-containing amyloplasts sediment on a complex of rough endoplasmic reticulum



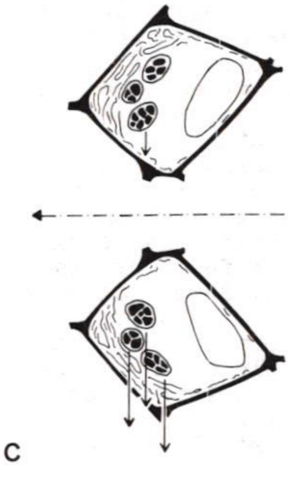


FIG.1A. Diagram of a longitudinal section through the root cap of Lepidium with basal meristem (me), statenchyma (s) and secretion cells (se). Two corresponding statocytes, shown in Fig. 1B, are indicated by asterisks. FIG.1B illustrates the pressure exerted by the amyloplasts on the distal ER complex (arrows) during vertical growth of the root. FIG.1C shows the same statocytes during horizontal exposure of the root. Amyloplasts now exert a differential pressure (arrows) in statocytes of the upper and lower flank, respectively.

(rER) at the distal cell pole) is regarded as the basis for the perception of gravity (Sievers and Volkmann 1972).

As shown by germination of the seedlings on fast and slow rotating clinostats, the polarity of the statocytes develops independent of the gravity vector. As demonstrated earlier, most convincingly by destarching (Iversen 1969), the amyloplasts serve as statoliths, which sediment according to the direction of the gravity vector. During vertical downward growth all amyloplasts in the statocytes symmetrically lie on the distal ER complex (Fig. 1B). When the root is placed horizontally, amyloplasts sediment in the direction of the gravity vector, which leads to a loss of amyloplast-ER-contact in statocytes of the upper flank of the statenchyma, whereas in statocytes of the lower flank the contact is maintained. It is assumed that this differential contact (Fig. 1C) is an essential part of graviperception and causes at last gravitropic bending of the root (response).

That the ultrastructural polarity of the statocytes, referred to in this scheme, indeed is a necessary precondition for graviperception was shown by experiments with centrifuge (Sievers and Heyder-Caspers 1983) and clinostat (Hensel and Sievers 1980):

Centrifugation of roots (20 min at 1000g) causes a distribution of organelles along a density gradient: amyloplasts and the nucleus sediment at the most distal (i.e. centrifugal) part of the statocytes, the ER is shifted proximally to the anticlinal cell walls (Fig.2). Restoration of the original polarity is achieved within minutes range after ceasing centrifugation and placing the roots in 1-g-environment. The ability to perceive gravity is strictly correlated to this restitution of polarity as the time lag of graviresponse after centrifugation coincides with the time needed for restitution of polarity (Sievers and Heyder-Caspers 1983).

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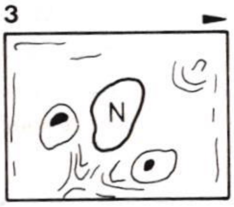

FIG. 2. Statocyte from a root after centrifugation at 1000 g for 20 min (N=nucleus).

FIG. 3. Statocyte from a root after rotation on a horizontal clinostat for 20 h at 2 rpm. Arrowheads indicate direction of the root tip.

Prolonged rotation of previously normal grown roots on a horizontal clinostat causes a number of events (loss of cell polarity, destarching of the amyloplasts, confluence of lipid droplets; Fig. 3), which finally end up in autolysis of the statocytes. The continuous rotation has to be regarded as an overstimulation, which causes self-destruction of the sensitive tissue. Cells from other root tissues remain unaltered (Hensel and Sievers 1980).

As a conclusion from these experiments it can be stated that (1) graviperception is restricted to the statocytes, (2) the cell polarity is a necessary precondition for graviperception and (3) the interaction of amyloplasts and ER serves in stimulus transduction.

In order to characterize the nature of the endogenous signal, electrophysiological measurements were executed. An electric current flow around the root of garden cress was measured with a vibrating electrode (Behrens et al. 1982). During vertical growth an outward flow of positive ions (probably protons) occurs in the elongation zone, while in meristem and root cap the current flow is directed inward (Fig. 4A). When the root is placed horizontally, this symmetrical pattern changes within 30 s; at the physical upper flank of the root cap the current flow is directed acropetally and basipetally at the lower flank. This can be interpreted as a current flow directed outward at the upper and inward at the lower flank, respectively (Fig. 4B).

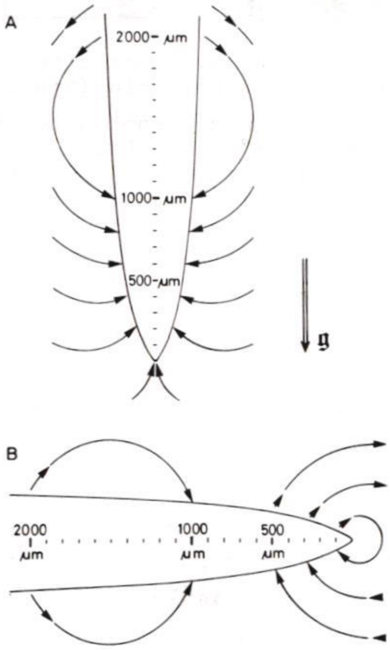

FIG. 4. Hypothetical pattern of current flow around the root tip of Lepidium (cap up to 200 μm, meristem 200-800 μm, elongation zone extending in basipetal direction). The indicated electric field lines are based upon single measurements with the vibrating electrode. FIG. 4A. Symmetrical pattern during vertical growth. FIG. 4B. Three minutes after horizontal exposure of the root, this pattern becomes asymmetrical in the root cap and the distal part of the meristem, while it remains symmetrical in the elongation zone. Graph by courtesy of H.M. Behrens.

As these alterations precede the first visible graviresponse, an involvement of endogenous current flows in transduction of the physical stimulus seems likely. During normal vertical growth, a current of 30 nA flows through the root, directed from the root cap to the elongation zone (Behrens et al. 1982). During gravistimulation, a differential flow of ions could occur, originating from the interaction of amyloplasts and the endomembrane system of the statocytes, and could be transfered to the surrounding medium via plasmamembrane and apoplast.

This was confirmed by measuring the membrane potential of the statocytes. During normal vertical growth, the membrane potential is about -120 mV. After tilting the root to 45°, this resting potential is depolarized to -80 mV in statocytes of the lower flank and slightly hyperpolarized to -135 mV in statocytes of the upper flank (Sievers and Hensel 1982).

As the ER is regarded to act in stimulus transduction, it was worthwhile to test, whether isolated ER-vesicles are capable of transporting ions selectively. In a first attempt, the Ca^{2+} transport was measured (Buckhout 1983). Vesicles from root tips of garden cress were isolated and, by morphometric analysis on electron micrographs from this fraction, determined to consist of about 80% rER. This fraction was further characterized by an enrichment of cytochrome-c-reductase, a marker enzyme of the ER. ATP-dependent Ca^{2+} accumulation with oxalate as a Ca^{2+} trap in these rER vesicles indicates that ER membranes are capable of active Ca^{2+} transport.

In combination with the plasmalemma, which maintains an intracellular Ca^{2+} concentration several orders of magnitude lower than the environment, ER membranes of statocytes regulate a cytoplasmic Ca^{2+} concentration by accumulating or releasing Ca^{2+}, respectively. This would be in accordance with the function of the sarcoplasmic reticulum from muscle cells.

At present the comprehension of these data into a hypothesis remains speculative. The following scheme, however, is suggested: The interaction of amyloplasts and ER during gravistimulation could trigger a Ca^{2+} release from the ER by membrane-bound Ca^{2+} pumps. An altered Ca^{2+} concentration in the cytoplasm of statocytes could account for the rapid depolarization of the plasmamembrane, which altogether might be steps of graviperception.

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SKIN AND MUSCLE VASCULAR RESISTANCE DURING TILT AND NECK SUCTION

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INTRODUCTION

During regulation of the arterial blood pressure the baroreceptors are active adapting the local peripheral vascular resistances to the cardiac output. However, very littel is known as to the relative contribution of the different vascular beds, how important a role they play or if at all they contribute. In the present investigation the carotid sinus baroreceptors were stimulated by neck suction which will increase the transmural pressure in the carotid sinus. This is sensed by the baroreceptors as an increased arterial pressure. Thus afferent nervous impulses are started which via the vasomotor center evoke efferent impulses to the arterioles apt to decrease the total peripheral vascular resistance. We, therefore, measured arterial pressure, heart rate and skin- and muscle blood flows during neck suction in order to investigated if skin and muscle vascular resistances are regulated via arterial baroreceptors.

METHODS

Four young healthy males served as subjects. They were all familiar with human physiological experiments from their own experience and gave their informed consent.

During the experiment the subject was lying down on his back on a tilt table, so that the situation in horizontal position could be compared to that of increased sympathetic nervous activity during a gravitational stress as during tilt (Skagen and Bonde-Petersen 1982).

The arterial pressure was measured by a Statham pressure transducer from a short catheter in the right radial artery in three of the subjects while a forth had the arterial pressure measured by an arm cuff.

Skin- and muscle blood flows (SBF and MBF) were measured in both underarms by the local isotope clearance technique using 133-Xenon and external counting (Kety 1949, Lassen et al. 1964). About 100 uCi 133-Xe solution in isotone saline was injected in the skin overlying the distal end of radius on the left arm and a similar dose was injected into the dorsal muscle group of the right underarm.

The reason why arms were used for measuring the reaction in skin and muscle is that they could be kept at heart level during tilt, so that the same relative hydrostatic pressure would prevail as in the horizontal position (Henriksen 1977)

The subcutaneous 133-Xenon injection was given 45 min and the intramuscular injection 15 min before the experiment respectively in order to let the injection trauma subside, because this is known to increase flow.

Electrocardiogram was monitored on a strip chart and later used for the calculation of heart rate (HR).

Suction on the neck was produced by fitting a hood over neck and head tightening the breast plate of this hood by a plastic bag and paper tape. The subject was allowed to breathe from the surroundings through a short tube. This arrangement also allowed measurements of cardiac output during neck suction by a rebreathing method using a mixture of acetylene, argon, and oxygen in nitrogen, and a mass spectrometry for gas analyses (Bonde-Petersen et al 1981)

A mercury manometer was used to regulate the pressure to -20 mmHg when suction was applied from a vacuum cleaner with variable pumping speed.

Measurements were repeated during 0 and 45 degrees tilt, which is known to decrease SBF and MBF due to the increased sympathetic nervous activity.

After 5 min of control measurements in the horizontal position a negative neck suction pressure of -20 mmHg was applied for 5 min and followed by a second control period of 5 min. The subject was then tiltet to 45 degrees angle. After the tilt the subject was taken back into the horizontal position and for an additional period of 5 min control measurements were performed in order to secure that the resting results from before the experiment could be reproduced.

The radioactive counts were fed into a minicomputer stored on floppy discs and later processed to obtain clearance values. These are given in relative units.

RESULTS

The results are indicated in Fig. 1. The upper panel shows mean arterial pressures (MAP) and heart rates (HR). Head and neck suction did not change MAP significantly, while heart rate increased both during horizontal and tilted position. The expected increase in heart rate was observed during tilt at 45 degrees.

The lower panel shows SBF and skin vascular resistance (SVR), calculated as MAP divided by SBF. The results during tilt showed most clearly that there was no reaction in SVR due to the neck suction. In the horizontal position there was a relative large scatter, which was mainly due to the variation in one persons flow results. If this subject was omitted, there was no systematic variation in SVR due to neck suction, and the expected reduction in SBF was seen as a consequence of tilt.

The lowermost panel shows the mean values of MBF and muscle vascular resistance (MVR). It is evident that neck suction increased MBF. In the horizontal position the increase was 74% and in the tilted position the increase amounted to as much as 123%. MVR followed the opposite trend.

Until now we only measured cardiac output in one subject who did not change his cardiac output as a consequence of neck suction. However, we found the expected decrease in cardiac output due to tilt alone.

DISCUSSION AND CONCLUSION

The present results indicate that the muscle vascular beds are controlled not only by the general sympathetic vasoconstrictor tone but also by the high pressure baroreceptors as part of the arterial blood pressure regulating mechanisms. The SVR does not participate in this regulation, even if they react by vasoconstriction to increased sympathetic drive during an increased gravitational stress. Other studies by us have shown that the SVR is in fact regulated by a baroreceptor activity and we have pointed to the low pressure barorecptors as being responsible for this regulation (Skagen 1983). This hypothesis concurs with the present results.

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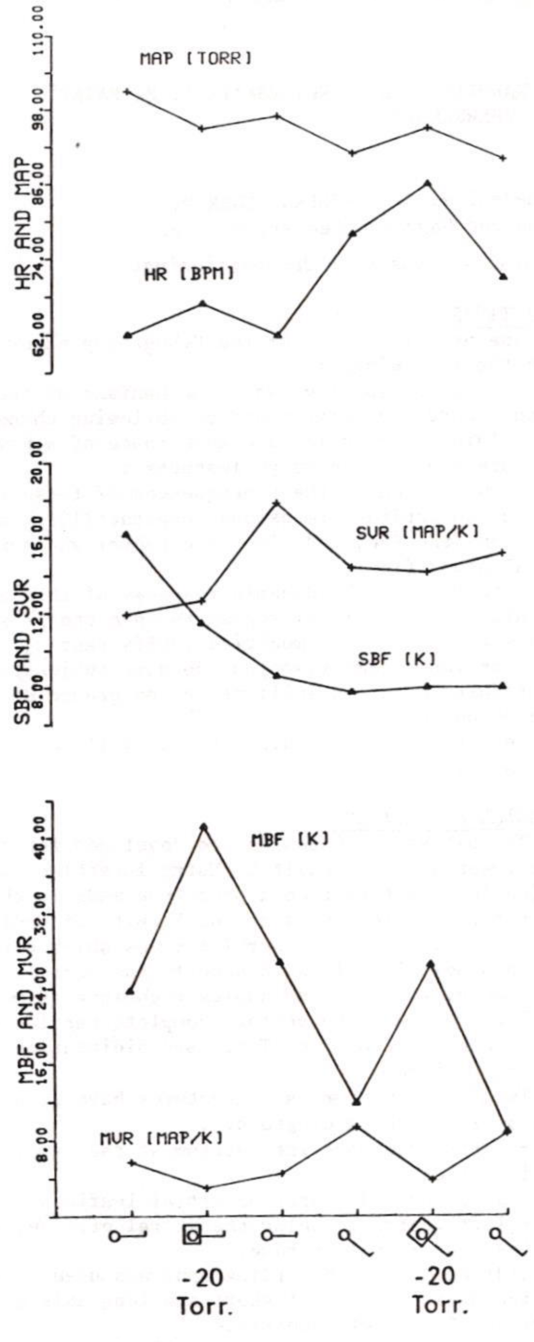

Fig. 1. Mean arterial pressure (MAP), Heart rate (HR), Skin blood flow (SBF), Skin vascular resistance (SVR), Muscle blood flow (MBF), Muscle vascular resistance (MVR) as influenced by tilt and neck suction by -20 Torr. Flow and resistance values are in relative units calculated on the basis of 133-Xenon clearance.

RESULTS OF ECHOCARDIOGRAPHIC EXAMINATION DURING 7 DAYS FLIGHT ONBOARD SALIOUT VII, JUNE 1982.

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INTRODUCTION

The great physiological functions of human body are modified by microgravity exposure (1)(2). These disturbances appear later or sooner after the orbital insertion and either express themselves by clinical signs, such as space adaptation syndrom, or stay in an infraclinical stage, at least for short-term space flights. At the early beginning of the flight, the neurosensorial system undergoes the strongest disturbance (especially the vestibular system). At the same time, the disappearance of the hydrostatic pressure produces a new distribution of the blood mass within the body of the astronaut. Later, cardiovascular deconditioning , blood volume variations, hydromineral balance shift, calcium metabolism troubles, cosmic irradiations are of growing importance as the flight proceeds, in the absence of countermeasures.

Til now several studies have been reported on the cardiovascular system of the astronauts (3). However our knowledges on this subject will mainly be extended by using the modern, non invasive and more informative techniques inflight. Ultrasonic methods are presently most suitable for this purpose, because they allow to perform not only qualitative, but also quantitative evaluations of changes in haemodynamics and cardiac function. This was the reason of choosing Echography for examining the cardiovascular system in weightlessness during the joint Soviet-French spaceflight aboard Saliout VII, june 24th-july 2nd 1982. Inflight studies were performed on the French astronaut J-L. CHRETIEN with the participation of Soviet astronauts DJANIBEKOV and IVANTCHENKOF.

In weightlessness, the lack of hydrostatic pressure induces a shift of an important volume of blood towards the cephalad part of the body, and a change in distribution of extracellular fluid. These mechanisms lead, in one hand to a transient increase of the central blood volume, and in an other hand to significant variations of the venous pressure in almost all the body compartments. The new pressure gradients induce a progressive appearance of oedema in the upper part of the body (face, trunk,...) and may be also increase of blood volume into brain and lungs. The stimulation of central sensors can set Gauer's reflex on : ADH and aldosterone decrease, thirst reduction and increased water and electrolytes excretion (4).

Activity in orbit only needs a very light work from antigravity muscles. Postures changes do not stimulate vascular reflexes needed to control blood volume and haemodynamics in the inferior limbs in normal gravity. These two phenomena lead to the cardiac and vascular deconditioning.

OBJECTIVES

The principal aims of the Echography experiment were the followings :

- study of the regulation mechanisms of the overall cardiovascular function following change in body fluid distribution and appearance of a new pressure gradient in weightlessness ;
- evaluation of the consequences of these regulations on cardiac dimensions, contractility and ejection fraction, and also on cerebral and peripheral blood flow ;
- testing of the dynamic response of the cardiovascular system in microgravity when the pressure gradients are rapidly modified (cuffs test) ;
- at least comparison, on the same subject, of the results obtained inflight and on ground during simulation tests (5) (6) (7) (8).

Only results of cardiac studies will be presented in this paper.

MATERIAL AND METHODS

The ultrasound based device developed for the experiment has been built by Matra-Interelec company (Paris) starting from a prototype made in the Laboratory of Biophysics at the Faculty of Medicine of Tours. It includes 4 working modes which permitted to study blood flow in superficial vessels using Doppler method and duplex technique (B-mode + Doppler), and also to perform complete cardiac examination by time motion (T.M.) and bidimensional imaging (3.5 MHz).

The following cardiac structures have been studied by T.M. echocardiography :

- aortic root and left atrium at the level of aortic valves ;
- anterior and posterior mitral leaflets ;
- left ventricle below the mitral orifice, at the level of mitral chordae.

Bidimensional echocardiography was used to visualize dynamic views of short and long axis cross-sections of the left ventricle.

The operating astronaut is trained to use the probes on himself, and can control, thanks to a display monitor, all the information he wants to record. Ground base recordings were made 30 days and 3 days preflight. Inflight measurements were carried out the 2nd, 3rd, 4th and 6th days. The recovery period was studied the first and the third days. Then an ultimate recording was made 2 months after the flight, in association with an antiorthostatic test of 3 days, to be compared with microgravity results. All the data were stored inflight on magnetic tapes in order to be processed after the flight. 2D pictures and T.M. mode recordings can be operated in a conventional way. It should be noted that the quality of echocardiographic recordings was quite well and adequate for future processing and analysis. It was due to the quality of the equipment "Echography" and of the training of the astronauts at Gagarine Center.

From T.M. mode plottings and 2D pictures the main studies were carried out on the variations of the cardiac cavity volumes, the modifications of the ejection function of the left ventricle and the myocardial contractility (9)(10). The following parameters have been measured :

- aortic diameter AD (mm) ;
- maximum opening amplitude of aortic leaflets AA (mm) ;
- ejection time ET (s) ;
- corrected ejection time CET (s) ;
- left ventricle diastolic volume LVDV (ml) ;
- left ventricle systolic volume LVSV (ml) ;
- stroke volume SV (ml) ;
- heart rate HR (p./mn) ;
- cardiac output CO (l/mn) ;
- ejection fraction EF (%) ;
- mean velocity of fiber contraction (MVFC) (s^{-1}) ;
- left ventricular diameter shortening ΔF (%) ;
- mean systolic ejection rate MSER (ml/s) ;
- arterial pressure (mmHg).

The ventricular volumes were estimated by Teichholz formula :

$$\mathrm{LVDV} = \frac{7}{2.4 + \mathrm{LVDD}} \times \mathrm{LVDD}^3$$

$$\mathrm{LVSV} = \frac{7}{2.4 + \mathrm{LVSD}} \times \mathrm{LVSD}^3$$

LVDD = left ventricle diastolic diameter ;
LVSD = left ventricle systolic diameter.

The other parameters were calculated thanks to the following formula :

$$\mathrm{CET} = \frac{\mathrm{ET}}{\sqrt{\mathrm{RR}}}$$ (RR duration is measured from the E.C.G.)

$$\mathrm{EV} = \mathrm{LVDV} - \mathrm{LVSV}$$

$$\mathrm{CO} = \mathrm{EV} \times \mathrm{HR}$$

$$\mathrm{EF} = \frac{\mathrm{LVDV} - \mathrm{LVSV}}{\mathrm{LVDV}}$$

$$\Delta\mathrm{F} = \frac{\mathrm{LVDD} - \mathrm{LVSD}}{\mathrm{LVDD}}$$

$$\mathrm{MVCF} = \frac{\Delta\mathrm{R}}{\mathrm{ET}}$$

$$\mathrm{MSER} = \frac{\mathrm{EV}}{\mathrm{ET}}$$

The heart rate is averaged on 20 cardiac cycles. The measurements were carried out at rest and also during a pneumatic cuffs test 30 and 4 days before the flight, the 4th and 6th days in weightlessness and the 3rd day of the recovery period. This test consists in a positive counterpressure applied around the upper part of the thighs : 40 mmHg during 20 mn, 60 mmHg during 20 mn. Echocardiograms were recorded at the 10th mn of each pressure step.

All the results submitted here are averages from several measurements. Standard deviation was calculated followed by a Student test. Results with significant variations from basic values ($p < 0.05$) are plotted with a star on the curves.

RESULTS

During all stage of flight the physical condition of J-L. CHRETIEN was good and no complain or unpleasant sensation have been reported. During the analysis of echocardiograms no irreversible and clinically significant changes have been noted in geometry of cardiac cavities and aspect of cardiac structures (aortic and mitral leaflets, intraventricular septum, and posterior wall of the left ventricle.

The main results obtained before, during and after the flight are presented on figure 1. The heart rate keeps at an elevated value during all the flight and during the initial part of the recovery period. The first measurement inflight (2nd day) shows a decreased value of left ventricular systolic volume (LVSV) associated with high heart rate and can be explained as a consequence of neuro emotional stress. Increased values of the diastolic (LVDV) and systolic (LVSV) volumes the 3rd and 4th days should be observed, with return to basic values the 6th day. The stroke volume (SV) follows an evolution rather parallel to the ventricular dimensions. This leads to a more significant increase of the cardiac output (CO), which rises near 40 % the 4th day of the flight. The cardiac output keeps high during the early recovery period especially because heart rate remains higher than its basic value.

No impairing of the heart contractility is revealed during the flight, testified by the absence of pronounced changes of such indices as ejection fraction (EF), relative shortening of ventricular diameter (ΔF), ejection time (ET) and corrected ejection time (CET). However we can see that the velocity of fiber contraction (MVFC) and the mean systolic ejection rate (MSER) are increased, certainly as a consequence of increase of heart rate.

The whole observed phenomena are probably the consequence of the increased blood fullfilling of the cardiac cavities, caused by the new distribution of the blood towards the cephalad extremity. A similar evolution of the cardiac parameters is described by several authors during weightlessness simulation experiments by antiorthostatism, the increased fullfilling being a function of inclination of the subjects.

In order to clarify the consequences of blood pooling into the vessels of the lower part of the body, we used some specific functional test with cuffs placed around the tighs. This induces an artificial pooling of blood in the lower limbs. Figure 2 shows the variations of echocardiographic parameters during cuff test onground and inflight. To clarify, only results obtained on day 4 inflight are reported here (cuff test has been performed inflight on days 4 and 6). The pooling of blood into the inferior limbs normalizes central haemodynamic parameters by involving a decreased filling of the cardiac cavities and a quite pronounced (20 %) decrease of cardiac output which was associated with same decline of heart rate, and to a great extend with a decrease of stroke volume and LVDV. These effects on the haemodynamics have been more pronounced on the 4th day inflight. The observed changes and particularly the decrease of left

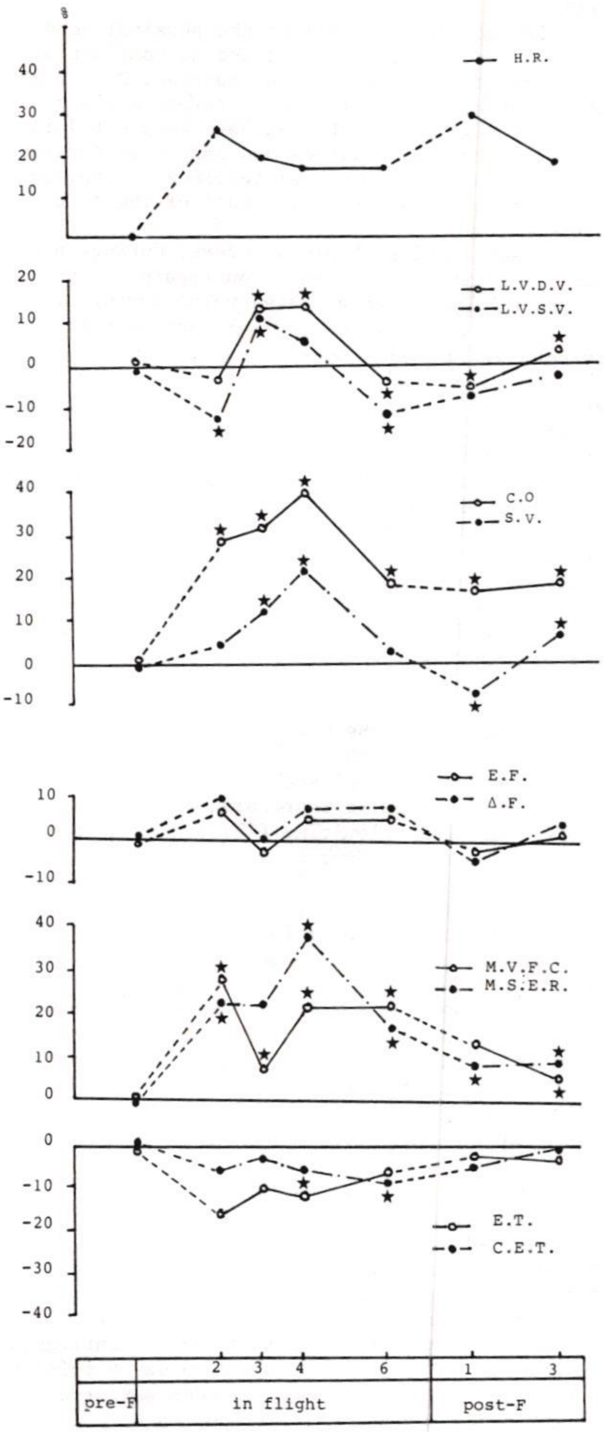

Figure 1 : Percentage of variation of cardiac parameters during and after the flight. Major changes are observed on heart rate (HR) cardiac output (CO) mean velocity of fiber contraction (MVFC) mean systolic ejection rate (MSER) and ejection time (ET).

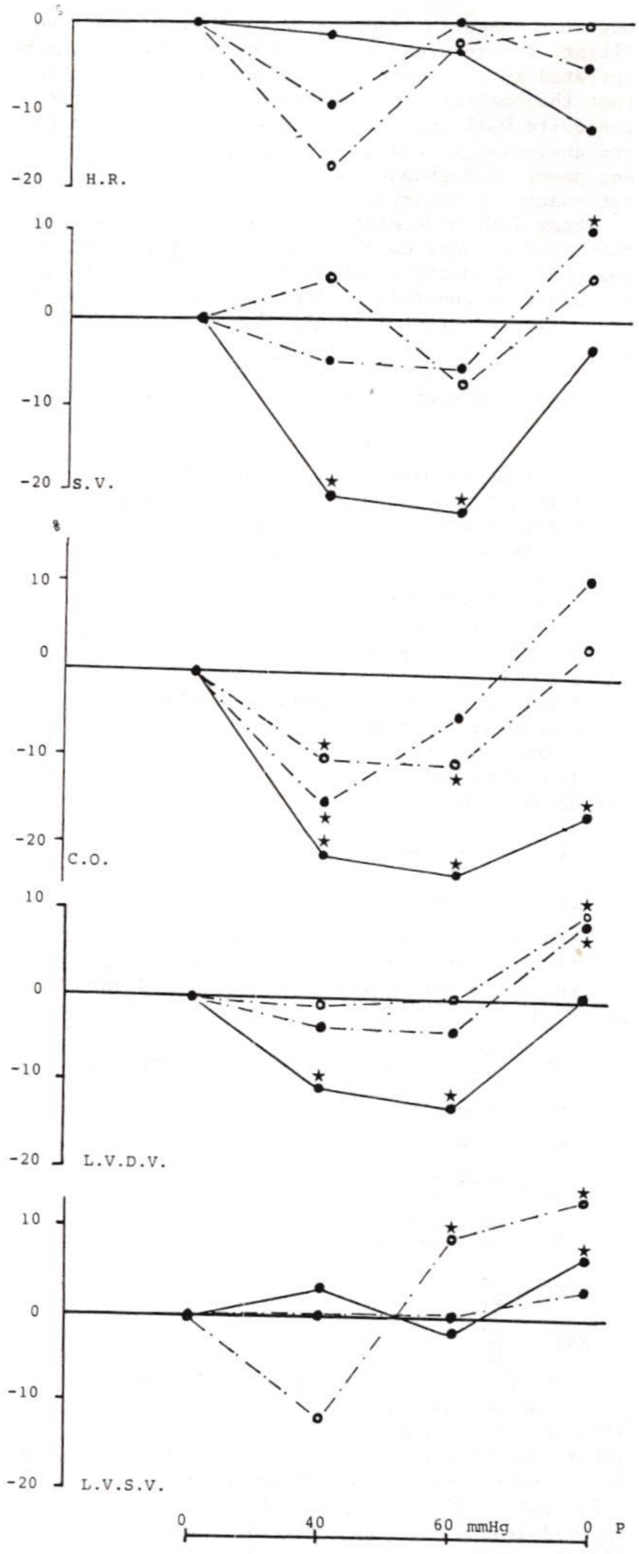

Figure 2 : Changes in cardiac parameters during cuff test (0.40 and 60 mmHg), before the flight (·—•—·), in flight day 4 (——•——), and after the flight (·—o—·).

ventricle diastolic parameters show that application of occlusion test permitted to reduce the degree of inflight changes in blood pooling, and may be to have the same efficiency as LBNP test (Lower Body Negative Pressure).

In continuing the experiment we consider that application of Echocardiography is very helpful because it allows to obtain new and informative data on the mechanisms of changes in cardiac performances and haemodynamics in spaceflight. The knowledge of these mechanisms is absolutely necessary for developing the effective, preventive and medically based prophylactic methods.

An antiorthostatic experiment was realized with the French astronaut the 60th day after the flight, at -6 degrees during 3 days. The response obtained is rather close to the one observed during the flight for the main cardiac parameters. This is testified by increase of stroke volume, LVDV and LVSV. However some differences have been observed : decrease of heart rate, and slight change in cardiac output, in absence of pronounced changes in echocardiographic indices and myocardial contractility. The absence of stress could partly explain these differences.

We must underline that all the results reported here correspond to the study of one subject, and that we should not draw definite conclusions for the mechanisms of the effects of weightlessness on cardiac haemodynamics, ejection function and myocardial contractility. To solve those problems, and to choose the optimal conditions of using the cuffs as a prophylactic mean, it is necessary to go on similar studies during spaceflights and ground simulation experiments.

CONCLUSIONS

The Echography experiment was performed during the seven days of the common Soviet-French flight in june 1982 aboard Saliout VII station. The astronauts were trained to use the apparatus developed by the Laboratory of Biophysics of the Faculty of Medicine of Tours, the C.N.E.S. and Matra firm. The ultrasound based device is able to visualize heart and vessels, to record movements of cardiac structures, and to provide the needed parameters for blood flow measurement in the superficial vessels. All the planned programm was carried out and the collected documents appear to be of good quality. Only results of cardiac exploration have been reported here. Additional publications will deal with data on vascular studies.

Recordings were made on J-L. CHRETIEN at the following dates : 30 and 3 days preflight, the 2nd, 3rd, 4th and 6th days inflight, the 1st, 3rd days after landing. An additional experiment with antiorthostatic test was performed 2 monts after landing. Among the main results obtained in cardiac studies, one can underline the increase of cardiac output, heart rate and stroke volume, the increase of left end systolic and end diastolic ventricle volumes and the stability of myocardial contractility. The use of pneumatics cuffs placed around the upper part of the thighs appears to be efficient in reducing the cardiac overload in weightlessness conditions. No irreversible pathological modification was observed in cardiac parameters and performances. The results of studies performed have confirmed one of the widely accepted hypothesis about influence of spaceflight factors on human cardiovascular system according to which in weightlessness human heart works in overloaded conditions (produced by volumic overload and may be pressure overload). Further studies are necessary to confirm these results.

Some important facts have been demonstrated during the Echography experiment :

- the ergonomy of the device revealed to be rather good as well as the training of the astronauts since all the programm was performed in time ;
- the increase of cardiac output without major modification of cerebral and femoral blood flows (shown by vascular studies (11)) leads to assume that renal and/or hepato-digestive circulation should increase during some phases of microgravity exposure. These phenomena must be studied in the next flights ;
- the pulmonary flow characteristics are also important to study because they directly influence the central venous pressure and consequently the size of cardiac cavities.

These remarks show the need for complementary studies, and also the interest of repeating the experiments, since it is uneasy to generalize results obtained only from one individual person.

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CHANGES IN INFORMATION PROCESSING ABILITY /IPA/, EEG, EOG USING PASSIVE ORTHOSTATIC AND ANTIORTHOSTATIC TEST

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ABSTRACT

In orthostatic position we have noticed tachycardia, bradypnea, pulse pressure decrease, lengthening of PEP and shortening of LVET, cardiac output and stroke volume values decrease while in antiorthostatic position we have seen bradycardia, tachypnea, pulse pressure increase, shortening of PEP and lengthening of LVET, stroke volume and cardiac output increase. Bit speed indicates increase both in orthostatic and antiorthostatic positions in accordance with the excitement of central nervous system. Comparison with rest both in orthostatic and antiorthostatic positions cerebral bioelectric activity increase significantly. Within it, the bigger energy level shifts toward fast frequencies. In antiorthostatic posture, nystagmoid eye movements can be noticed - characterized by fast and slow components.

INTRODUCTION

In our earlier investigations /1, 2/., corresponding to other authors we pointed out, that orthostatic and antiorthostatic loading induce substantial changes in circulation.

From the point of view of functional damage, antiorthostatic loading represents a more serious adverse factor as it is evidenced by our electroencephalographic and electrocardiographic studies /1/.

METHODS

Our investigations were performed in practically healthy pilots. On MEDICOR tilting table in horizontal position during 10 minutes, in orthostatic position during 20 minutes then in antiorthostatic position during 6 minutes, changes in pulse rate, respiratory rate, systolic and diastolic blood pressure and changes in PO_2 of capillary were measured by transcutaneous oxymetry on the skin of forehead, chest and leg. Measurements were performed by Hellige device complex.

We have recorded electrocardiograms in 12 leads, carotis mechanogram, horizontal and vertical electrooculograms, electroencephalograms by bipolar leads hemispherally and interhemispherally and their energy spectrum have been determined by Fourier analyse. We have determined pulse wave velocity and stroke volume, using a method developed by Bremser-Ranke.

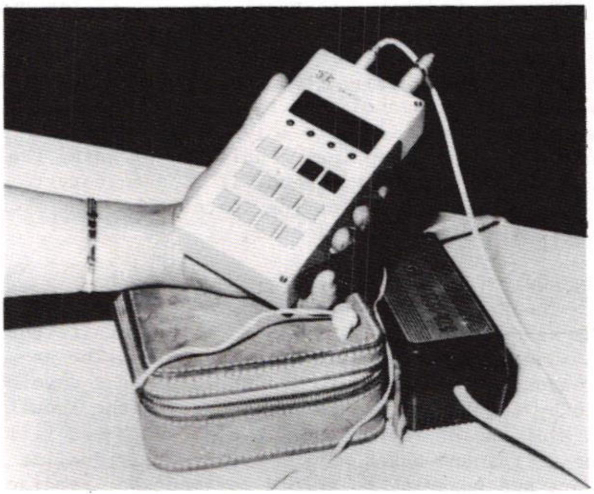

Figure 1. The device "Balaton".

Preceding examination then following orthostatic and antiorthostatic loading, we have determined changes in information processing ability /IPA/: sensory-motor reaction time, four-choice reaction time, four-choice selection time, processed information quantity, bit speed under double loading /in time force and during sound disturbance/ and in undisturbed circumstance. Measurements were performed by the device "Balaton" /MEDICOR, Hungary/ that have already been used during series of Intercosmos spaceflights. Method of this device and measurement has been developed by us /3, 4/. To follow changes in emotional tension, we measured changes in pulse rate and galvanic skin resistance. Results have been elaborated by PDP-11 and APPLE-II computer.

RESULTS

The 21 practically healthy pilots - considering the usual examination criterium - proved that they are persons with good tolerance.

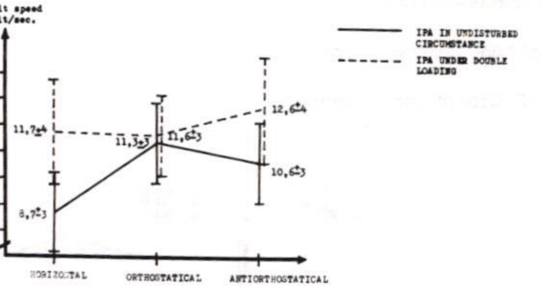

Figure 2. Change of information processing ability /IPA/ in different postures.

In Figure 2. can be seen changes in bit speed - from indices of information processing ability - in different postures in undisturbed circumstance and under double loading. Bit speed indicates increase both in orthostatic and antiorthostatic positions in accordance with the excitement of central nervous system. However psychophysiological reserves remain, as bit speed can be more increased under double loading in both tested postures.

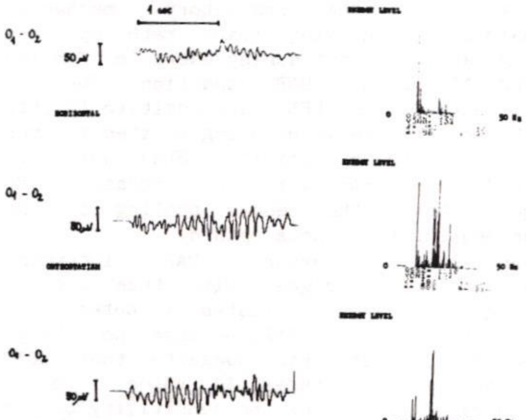

Figure 3. Changes in EEG spectrum in different postures.

Comparison with rest both in orthostatic and antiorthostatic positions cerebral bioelectric activity increase significantly /indicating increase of excitement of central nervous system. Within it, the bigger energy level shifts toward fast frequencies.

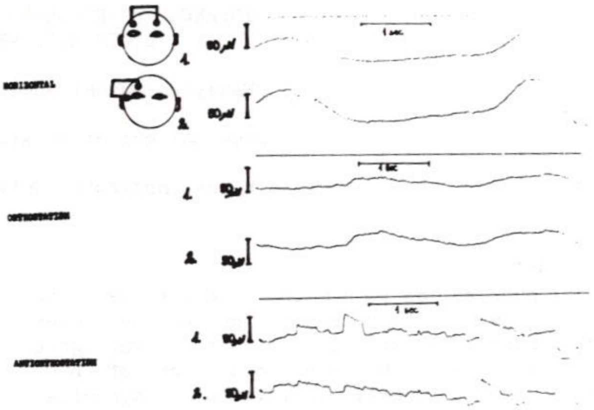

Figure 4. EOG changes in antiorthostasis.

On the basis of our data we can state that haemodynamic changes - in addition to others - play an important role in pathomechanism of vestibular disorders, caused by weightlessness. Haemodynamic changes does not influence unfavourably information processing ability, namely the current mental work capacity, moreover they do not decrease neither psychophysiological reserves. At the same time it means excitement of central nervous system can be noticed, as the perfect compensation of haemodynamical changes, induced by postural changes.

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CARDIOVASCULAR RESPONSES TO BICYCLE EXERCISE DURING LOWER BODY NEGATIVE PRESSURE

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INTRODUCTION

The purpose was to combine bicycle exercise and gravitational stresses as induced by lower body negative pressure in order to investigate the mechanisms behind the regulation of both central and peripheral cardiovascular dynamics. During short periods of lower body negative pressure (LBNP) sitting on a bicycle ergometer placed in a LBNP box the subjects exercised at different work rates with varied LBNP. Heart rate, cardiac output, blood pressure and muscle blood flow in the legs were measure during rest and exercise.

METHODS

Test subjects were 6 young healthy male subjects who gave their informed consent. They had all participated in previous experiments in our laboratory and were well aware of the implications of the experiment.

Cardiac output was measured by a rebreathing method using a mixture of acetylene (1.9%), oxygen (45%), and argon (9%) in nitrogen as described previously (Bonde-Petersen et al. 1981). The gasses were measured simultaneously on a mass spectrometer and the signals processes on-line on a PDP 11/34 computer.

Heart rate was monitored by electrocardiography (ECG) using an oscilloscope (DIASCOPE DS 521, Simonsen & Weel) which simultaneously displayed the ECG.

Blood pressure was measured by the conventional arm cuff method.

Leg muscle blood flow was measured by the local 133-Xenon clearance (Kety 1949, Lassen et al 1964) after injection of 50-100 uCi dissolved in 0.1-0.2 ml of isotonic saline into either right or left m. vastus lateralis and m. gastrocnemius. The gamma radiation was counted by two miniature cadmium telluride detectors weighing each 49 g (MEMOLOG, Novo Diagnostic System). The detectors were taped to the skin overlying the site of injection (Bojsen et al. 1982, Bojsen et al. 1983). Two experiments could be performed per experimental day, one for the right and one for the left-sided muscles. After the experiments the MEMOLOG portable memory (CMOS RAM) was read into the laboratory computer PDP 11/34 and the clearance rates were calculated off-line.

EXPERIMENTAL PROCEDURE

The subject rested for at least 30 min in the horizontal position while elctrodes etc. were mounted. He was then placed in the LBNP chamber which was a vertical plastic cylindre with a diameter of 650 mm and 1300 mm high sitting on a mechanically braked bicycle ergometer (Bonde-Petersen 1983) modified for remote control via the PDP 11/34. The entries for work power was via a screen with keyboard.

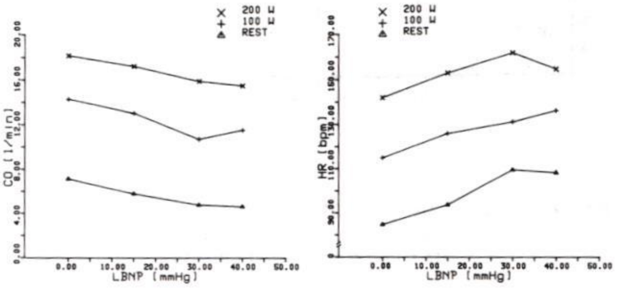

Fig. 1. CO and HR as a function of LBNP.

LBNP was applied at 0 mmHg (control) and -15, -30, or -40 mmHg respectively in combination with bicycle exercise at rest (control) or 100 and 200 watts.

RESULTS AND DISCUSSION

Cardiac output decreased and heart rate increased rectilinearly in parallel both during rest and exercise as a function of the decrement in LBNP (Fig. 1). This parallel displacement indicates that the regulatory mechanism responsible for driving heart rate up and reducing cardiac output during exercise funtion independently of the LBNP condition. The two stimuli, exercise and LBNP, are additive in this respect the increase in HR being related to the sympathetic nervous activity (SNA) which is increased during LBNP, while the decrease in CO will be related to the venous pooling in the legs decreasing the venous return.

Mean arterial pressure (MAP) increment during exercise was bigger with than without LBNP (Fig. 2). This indicates a potentiated effect of exercise and LBNP, because no change was seen during rest. This suggests that LBNP above a certain low threshold value - below minus 15 mmHg - increases the sensitivity of the arterial baroreceptors to the exercise stimuli. Above minus 15 mmHg no further effect of LBNP was recognised.

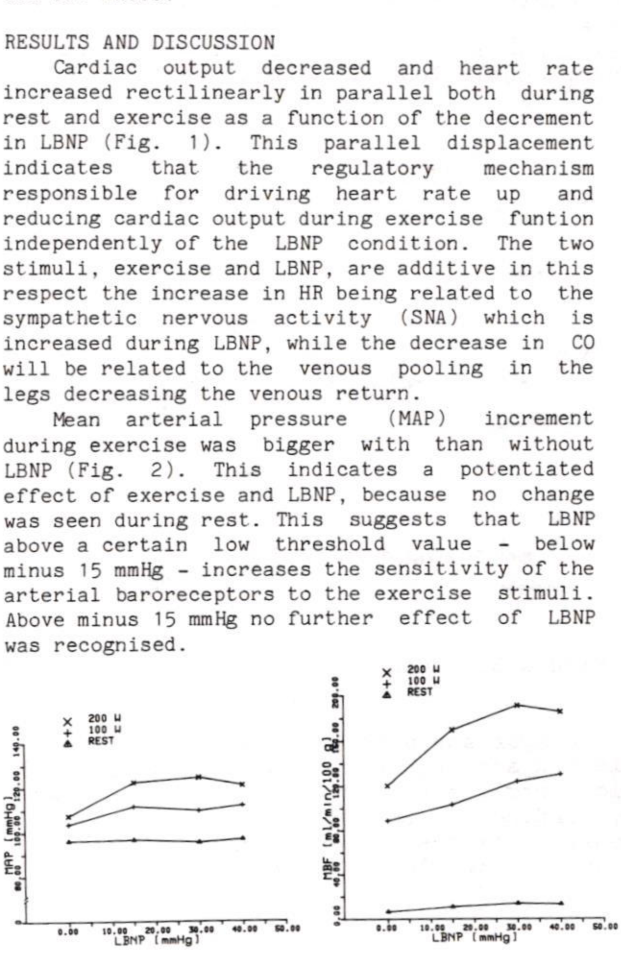

Fig. 2. MAP and MBF as a function of LBNP.

Muscle blood flow increased both during rest and exercise due to LBNP in the face of a decreasing cardiac output (Fig. 2). This indicates that the regulation of central and peripheral cardiovascular mechanisms under LBNP are independent. In addition to this a mechanical factor would play a role at least during rest the transmural pressure distending the arterioles thus inducing a fall in local vascular resistance.

We found the expected decrease in total peripheral resistance during exercise in the control situation (LBNP=0). However during LBNP in both rest and exercise, total peripheral resistance increased especially during rest (fig. 3). However, only during rest there is an effect of LBNP upon muscle vascular resistance which decreased. This was somewhat surprising because previously it has been shown (Henriksen 1977) that a distension of veins will increase the arteriolar resistance at least locally in the skin and this was suspected also to happen in the muscle. However, during exercise the local muscle vascular resistance (LMR) did not change much.

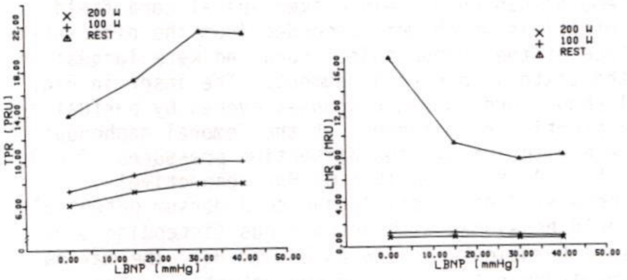

Fig. 3. TPR and LMR as a function of LBNP.

Fig. 4 shows as a summing up of the results for comparison. The effect of LBNP upon the delta HR values were similar both during rest and exercise except for at -40 mmHg LBNP during 200 watts exercise. This might prove that a similar regulation mechanism is active both during rest and exercise. From the right hand panel of Fig. 4 it is clear that combined stimuli of exercise and LBNP can evoke bigger changes but it seemed to have a certain saturation point at LBNP below -15 mmHg.

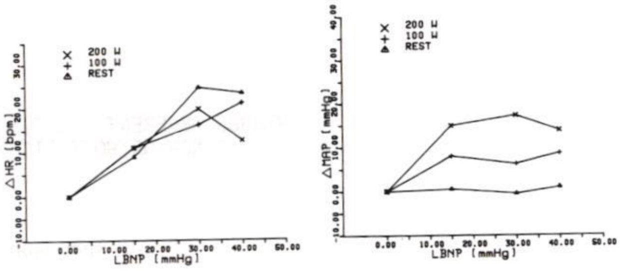

Fig. 4. Increments in HR and MAP over LBNP.

CONCLUSION

Heart rate and blood pressure are regulated through different reflex mechanisms during exercise in an increased gravitational field as simulated by LBNP. The muscle vascular resistance during exercise is exclusively metabolically regulated. During rest the vascular resistance in muscle will decrease during gravitational stress due to non-regulated mechanical distension of the vessels.

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VENOUS AFFERENT ELICITED SKELETAL MUSCLE PUMPING: A NEW ORTHOSTATIC VENOPRESSOR MECHANISM

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INTRODUCTION

The venous system is profoundly susceptible to gravitational accelerations; simple changes in posture initiate decreases in venous return that are a dramatic challenge to circulation. Many mechanisms have been described to contribute to orthostatic tolerance.

Previously reported circulatory control systems have not included sensory input from the peripheral venous systems; nor do these control mechanisms include a neural reflex control of the intramuscular venous capacitance. The peripheral venous systems are significantly involved in orthostatic hypotension because these are initial sites of orthostatic blood shifts and blood pooling.

Sensory innervation of the peripheral blood vessels have been described in numerous anatomical reports beginning in 1863. Recently our laboratory reported the projection of low threshold limb venous afferents to the spinal cord (1) and to the cerebral motor cortex (2). Subsequently, the interaction of these afferents with segmental spinal reflexes (3), and the responses of the lumbar spinal cord to limb vein distentions were reported (4).

The purpose of this report is:

1) to describe some of the functional transduction properties of sensory fibers which accompany and innervate the peripheral venous system.

2) to describe some of the connections of these afferents in the central nervous system to uncover potential functional roles of these fibers.

METHODS

The experiments were carried out in decerebrate-spinal cats. The surgical procedures were carried out in cats anesthetized with nitrous-oxide/halothan inhalation, anesthesia. The femoral vein was exposed at the medial thigh. The femoral-saphenous venous afferents were activated for study by electrical stimulation and also by two methods to stretch the wall of a segment of the femoral saphenous vein. Electrical stimulations were done by passing current pulses 2-3 mm longitudinally along the wall of the vein. One method of wall stretch was done by distention via perfusion of a cannulated segment of vein. The intravenous distending pressure was measured directly with a blood pressure transducer. The second means of wall stretch was done using an electromechanical transducer to produce controlled mechanical stretches of the wall of a segment of the femoral saphenous vein.

Evoked potentials were recorded with a silver ball electrode gently touching the exposed pial surface of the spinal cord.

RESULTS

A. Sensitivity of Venous Afferents to Vein Wall Stretch

Stretch of the vein wall of a segment of the femoral-saphenous vein evoked spinal cord field potentials which were recorded from the pial surface of the lumbar spinal cord and were largest in the sixth lumbar cord segment. The inset in Fig. 1 shows cord dorsum responses evoked by perfusion-distention of a segment of the femoral saphenous vein with intravenous distention pressures of 0.3, 2.5, 4.4, 8.0, and 15.5 mm Hg, respectively. These studies revealed that cord dorsum potentials could be evoked with intravenous distending pressure transients as low as 2-3 mm Hg. The maximal amplitude spinal cord evoked potentials were elicited by distending pressures of 18-20 mm Hg; half maximum responses were observed at 6-8 mm Hg distending pressure. The relative amplitudes of cord dorsum potential responses elicited by perfusion pressure transients from 1 through 72 mm Hg are shown graphically in Fig. 1.

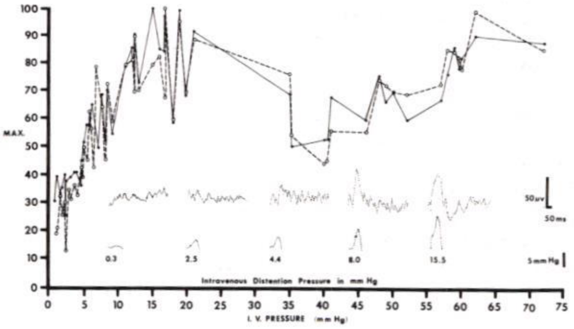

FIGURE 1. RESPONSE AMPLITUDE VS. VENOUS PRESSURE, Graph; Insets show responses and time course at 5 different pressures, (4).

Studies using controlled mechanical stretches of the wall of a segment of the femoral-saphenous vein revealed that stretches as small as 5 microns per mm of vein segment length could evoke cord dorsum potentials.

B. Venous Afferent Elicited Skeletal Muscle Pumping

Electrical stimulation of femoral-saphenous venous afferents elicits facilitation of hindlimb skeletal muscle motoneurons. This has been revealed through several different measures of motoneuron excitability. Shown in Fig. 2 is a single unit recording from a lateral gastrocnemius motoneuron. The lower trace shows the neuron's response to backfiring the muscle nerve with a train of three shocks at 500 Hz. The identity of the motoneuron is confirmed by the observation that the response frequency and latency are compatible with antidromic invasion rather than by synaptic excitation of the motoneuron. The motoneuron excitatory potential with superimposed discharge shown in the upper trace was elicited by venous afferent stimulation.

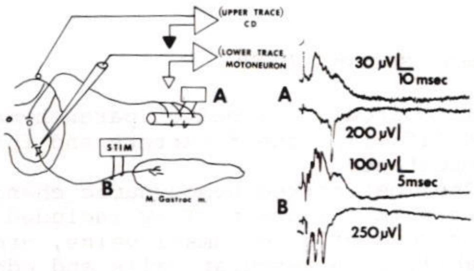

FIGURE 2. MOTONEURON RESPONSE TO VENOUS AFFERENT, upper trace, lower trace, response to stimulation of l. gastrocnemius n.

Simultaneous recordings of EMG activity in hindlimb muscles revealed that venous afferents elicit activation of both flexor and extensor muscles of the thigh and lower leg. This pattern is different from the reciprocal innervation pattern known for cutaneous and muscle afferents.

Recordings of the intramuscular pressure during electrical stimulation of the femoral venous afferents revealed phasic increases of several mm Hg pressure with abrupt onset and brief duration. Fig. 3, middle trace shows a record of the time course of an intramuscular pressure increase in the gracilis muscle elicited by electrical stimulation of the femoral venous afferents with a train of four shocks at twice threshold for the cord dorsum potential.

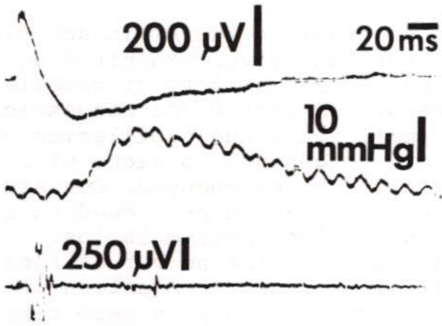

FIGURE 3. VENOUS AFFERENTS ELICITED INCREASE IN INTRAMUSCULAR PRESSURE, middle trace. Upper trace cord dorsum potential; Lower trace, EMG record from gracilis muscle, for reference.

In addition, we have observed that mechanical stretch of the wall of a segment of the femoral saphenous vein elicited motoneuron population excitatory potentials recorded from ventral root fibers of the seventh lumbar cord segment.

C. VENOUS AFFERENT VENOPRESSOR MODEL.

The preceeding data taken together indicate that venous afferents are sensitive to vein wall stretch and in addition have spinal reflex connections to skeletal muscle motoneurons.

We propose that these connections provide a means for limb venous afferent modulation of skeletal muscle tone.

Further, we propose that this modulation of skeletal muscle tone provides a rapid means of controlling the capacity of the intramuscular venous reservoir, through the long established effects of muscle tone counterpressure on muscle venous capacitance (5,6).

Since the femoral-saphenous vein is distended in the initial stage of orthostatic blood shift, we propose that the stretch sensitive venous afferents together with their reflex connections to motoneurons, combine to produce a substrate for an orthostatic muscle tonus-venopressor mechanism.

This mechanism may therefore be worth noting not only in the adaptation of the cardiovascular system to 1-G, but also in the development of cardiovascular deconditioning associated with 0-G missions (7).

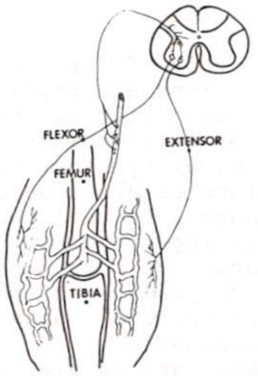

FIGURE 4. PROPOSED MODEL OF VENOUS AFFERENT ELICITED SKELETAL MUSCLE TONUS VENOPRESSOR MECHANISM.

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ANTIORTHOSTATIC HYPOKINESIA IN MONKEYS (EXPERIMENTAL MORPHOLOGICAL STUDY)

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The autopsy and histological examinations of organs and tissues of rhesus-monkeys have demonstrated that during head-down tilt they develop: a) blood redistribution accompanied by increased masses of brain, lungs, heart, liver, and kidneys; b) hypokinesia-induced changes; c) symptoms of an acute stress-reaction, and d) morphological manifestations of adaptive reactions aimed at stabilizing hemodynamics and fluid-electrolyte balance. These findings are in agreement with clinical and physiological data obtained in humans during head-down tilt studies.

At present a large body of data on man's clinical manifestations and physiological reactions to head-down tilt has been accummulated. However, it still remains unclear whether the clinical symptoms are only functional variations or are associated with structural changes. In this context, it is very interesting to perform a morphological investigation of nonhuman primates since their hemodynamics and phylogenesis are similar to those of humans thus allowing data extrapolation.

Head-down tilt tests were carried out on 12 rhesus-monkeys, eight of which were at -6° for 7 and 19 days and four served as controls. The autopsy has demonstrated three types of changes during head-down tilt: blood redistribution, hypokinetic syndrome, and stress-reaction. Blood pooling in the upper body was readily diagnozed on the basis of distinct blood congestion in veins and soft tissues, meninges and cerebral matter, as well as the parenchymatous organs - lungs, heart, liver, kidneys whose mass increased significantly. By day 19 the mass of the brain increased by 13% and that of the lungs, liver and kidneys by 20-30%. The mass of the left ventricle and the septum grew by 24% and that of the right ventricle by 6%. The soft tissues of the lower body and particularly of the limbs were ischemic and dry. Thymus involution, reduced spleen mass, adrenal hypertrophy and fat loss are considered as stress manifestations whereas decreased mass of lower limb muscles, especially antigravitational, are regarded as manifestations of hypokinesia per se.

The microscopic examination has demonstrated that hemodynamic disorders in the microcirculatory bed of parenchymatous organs differ in their pattern and functional importance.

The most marked hemodynamic changes were seen in the brain. They included persistent congestion of small veins, greater permeability of vascular walls and edematous perivascular spaces. The X-ray examination of temporal bones showed a more distinct pattern of finger-shaped depressions. In man this is usually a pathological sign of increased intracranial pressure. Cerebral microciruclatory disorders developed together with initial symptoms of hypoxic lesions of neurons and glial cells that are normally considered as reversible. For instance, Betz cells were of greater size and modified shape, they showed a swollen cytoplasm and partial tigrolysis. The only structure where more pronounced dystrophic changes in the form of single cell ghosts were seen was the dendate nucleus of the cerebellum. Eye examinations also revealed persistent congestion but dystrophic changes were observed mainly in the capillary endothelium. Electron microscopy examinations of endothelial cells demonstrated edema, increased number of pinocytotic vesicles, and myelin bodies in place of dead mitochondria. Nevertheless, there were no dystrophic changes in the retina of most animals.

In contrast to the brain and eyes, the lungs, heart and liver exhibited no signs of increased permeability of vascular walls in spite of distinct blood congestion. Although small veins and capillaries of the lungs were congested, no signs of edema were seen in the parenchyma. Consequently, lung hypervolemia can be viewed as a manifestation of blood accummulation.

In the heart the number of functioning capillaries and their congestion in experimental and control animals were similar while Thebesian veins of experimental monkeys were greatly dilated and congested, especially in the left ventricle. In view of the role Thebesian veins play in cardiopulmonary circulation (1) it can be assumed that during head-down tilt a major portion of the venous blood of the heart goes along the Thebesian veins to the left heart instead of the right heart as it is in the norm. The morphometric study revealed no signs of hypertrophic cardiomyocytes in both ventricles. In the liver blood congestion in the portal veins was not accompanied by greater permeability of capillaries or parenchymatous lesions.

Thus, the morphological investigations have shown that during head-down tilt blood redistribution is responsible for a greater mass of parenchymatous organs. The examinations have also made it possible to evaluate circulatory disorders in different vascular regions and to identify their consequences.

Changes of the second group were associated with hypokinesia per se. Variations typical of the early stage of hypokinesia were demonstrated microscopically in the hematopoietic system (inhibition of erythropoiesis) and in the musculo-skeletal system. The tibia showed greater vascularization of the compact substance, larger osteons and haversian's canals, disordered osteons, and enlarged areas with sinus resorption. Unlike rats, early symptoms of osteoporosis in monkeys were seen not only in the trabecular but also in the compact substance, whereas inhibition of bone growth which is very distinct in rats was less marked in monkeys. Similar changes in bones of monkeys exposed to 14-day hypokinesia have been reported elsewhere (2).

Finally, the structural study of the neuroendocrine system indicated that blood redistribution and hypokinesia may cause not only stress but also adaptive reactions involved in the development of a new hydration level and fluid-electrolyte balance.

For instance, during the first 7 days of head-down tilt morphological manifestations of inhibited ADH-vasopressin excretion were seen in the hypothalamus-pituitary neurosecretory system. This was evidenced by large accumulation of the neurosecretory substance in all compartments of the system, alongside with angiospasms. The reactions aimed at stabilizing fluid-electrolyte metabolism also included activation (by day 19) of the aldosteron-producing glomerular area of the adrenal cortex. Increased aldosteron was also observed in men during head-down tilt(7). The changes in calcitonin-secreting C-cells and parathyroid glands are essentially objective parameters of their involvement in hypokinesia-associated changes in Ca metabolism and bone structure. In the thyroid gland the count of C-cells producing calcitonin increased during the first 7 days and decreased by day 19 due to enhanced hormonal secretion to blood. The activation of parathyroid glands became distinct by day 19 which is in agreement with the data on an increased concentration of PTH in blood of bed-rested men (9).

Thus, the investigation of morphological reactions in rhesus-monkeys helped to identify the structural basis of many functional changes seen in man during head-down tilt and to demonstrate that head-down tilt of monkeys is an adequate animal model for studying mechanisms of reactions that develop during head-down tilt and at an acute stage of adaptation to weightlessness.

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CARDIOVASCULAR RESPONSES TO SPACEFLIGHT

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INTRODUCTION

The Cardiopulmonary system undergoes substantial adaptive changes during spaceflight. The effects of weightlessness are particularly important because many of the adaptive capabilities of the cardiopulmonary system have evolved with specific mechanisms to counter the continuous pull of Earth's gravity. Some of the changes in this system, such as cephalic fluid shifts, are the direct result of weightlessness; others appear to be concommitant adjustments and only indirectly related to the zero-gravity environment.

Despite the voluminous biomedical data obtained over the last 25 years of manned spaceflight, the time course and exact etiology of the cardiovascular adjustments to weightlessness are still poorly understood. Histochemical studies conducted on animals inflight are still too few in number and the information is too sparse to allow for a comprehensive understanding of the problem. The state of knowledge is further confounded by the medicalsafety and ethical considerations for the extensive use of countermeasures in manned missions. It is imperative that in the future flights, through carefully planned and executed human and animal experiments, a more precise knowledge is acquired for the development of rational countermeasures to facilitate adaptation to weightlessness and safe return to the Earth's environment.

BACKGROUND

Documented changes exhibited by the cardiopulmonary system in spaceflight stem from several observations:

Fluid Shifts

The most significant alteration that occurs in the cardiopulmonary system is the cephalic shift estimated at 1.5 to 2 liters from the lower extremities. Evidence for this shift of fluids has been obtained from:

1. Photographic Evidence - Photographs taken inflight show signs of periorbital puffiness, facial edema, and thickening of the eyelids (Thornton, Hoffler, & Rummel, 1977). The jugular veins and the veins in the temple, scalp and forehead appear full and distended. Although the inflight uplifting in the facial tissue may have been partly due to the absence of gravity, the fluid shifts probably also play a significant role, particularly through venous engorgement. The edema and venous engorgement do not subside even after some months in space. The crews' subjective feelings of nasal stuffiness and head "fullness," support the hypothesis of substantial fluid shifts.

2. Changes in Calf Girth and Leg Volume - Space travelers have typically demonstrated inflight decrements in calf girth of up to 30 percent. It appears that the major shift of fluids does occur rapidly, upon insertion into orbit. The rate of fluid shifts also appears to follow an exponential course, attaining a maximum within 24 hours and reaching a plateau or a new steady state within three to five days (Hoffler, Bergman, & Nicogossian, 1977). Calf volume measurements taken during long-term Soviet missions aboard Salyut 6 showed a similar pattern of decrease, with values reaching a plateau on the twelfth inflight day. Over 140-, 175-, and 185-day missions, fluctuations in leg volume followed a wavelike course of loss and recovery (Gazenko, Genin, & Yegorov, 1981a; Simonov and Kasian 1983; Nicogossian and Parker 1982).

The changes in limb volume and initial weight losses, particularly the rapid postflight recovery, indicate the occurrence of substantial cephalic fluid shifts inflight. On the basis of inferences drawn from the results of ground-based simulation studies (Burkovskaya et al., 1980; Katkov, Chestukhin et al., 1981; Nicogossian et al., 1979; Sandler, 1977) and inflight data (Gazenko, Genin, & Yegorov, 1981b), it appears that these fluid shifts result in a number of physiological readjustments. The immediate response is an initial increase in central blood volume, resulting in stimulation of cardiopulmonary mechanoreceptors. The activation of these receptors leads to an inhibition of the medullary vasomotor center, decreased sympathetic nervous system tone, and indirect effects on renal function via both neural and hormonal pathways. Thus, the complex events triggered by the fluid shifts initiate compensatory hypovolemia and the cardiopulmonary system becomes stabilized at a new pressure/volume relationship.

Inflight Measurements

The mean resting values of heart rate and systolic blood pressure tend to increase, while the diastolic pressures decrease as compared to preflight values. The observed arrhythmias and changes in electrical activity point to a sympathetic-parasympathetic neural imbalance, which could also be triggered by the fluid, electrolyte and endocrine changes. The consistent losses of body weight point toward a negative fluid balance in response to diuresis and/or diminished fluid intake (Nicogossian and Parker, 1982; Simonov and Kasian, 1983). Recent echocardiographic studies conducted onboard the Salyut-7 indicate initial increases and subsequent decreases in left atrial and left ventricular diastolic dimensions, which can be indicative of significant hemodynamic changes in the cardiopulmonary system generated as a result of small left and right atrial pressures. Additional findings consist of increased blood flow and compliance in the lower extremities (Thornton and Hoffler, 1977); decreases in cardiac stroke volume and shortened isovolumetric contractions time (Yegorov et al., 1981); decreased vital capacity (Sawin et al, 1976); unchanged work capacity inflight (Michel et al, 1977; Rummel et al, 1978; Gazenko et al, 1981a, 1981b); and possibly, increased cerebral perfusion. However, so far no gross indications of increased in cerebro-spinal fluid pressures have been documented.

Postflight Findings

Decreased orthostatic tolerance and weight has invariably been observed among space travelers postflight (Nicogossian and Parker 1982, Simonov and Kasian 1983, Gazenko et al 1976, 1981a, 1981b). Decreased circulating blood volume, fluid, electrolyte and endocrine changes, altered cardiac size (Nicogossian and et al, 1976), and cardiovascular dynamics as measured by echocardiographic changes have been all observed postflight. The recovery period has been quite variable and depending on the duration of spaceflight has taken as little as four days and as long as four weeks. Decreases in skeletal (especially antigravity) muscle mass has been also noted, which has led to the predictions of possible myocardial muscle mass decreases. More recently, small but reversible decreases in left ventricular muscle mass have been reported, utilizing echocardiography (Nicogossian and Parker 1982). In light of the restoration of the myocardial muscle mass by the seventh day postflight, it seems that direct myocardial tissue degeneration is unlikely and that the observed changes could be due to either intercellular hypovolemia.

Since 1980, NASA has placed its research thrusts on the mechanisms of cardiovascular deconditioning and development and testing of effective countermeasures. The prime technical objectives are to:

1. Study hemodynamic changes, especially inflight, utilizing both invasive and noninvasive procedures. These studies will allow a better definition of cardiac pressure/volume relationships, and the role played by the venous and arterial systems in regulating cardiac and pulmonary function in 0-g.

2. Study of the endocrine and neurohumoral aspects of cardiovascular physiology to ellicit the presence or absence of an active diuresis, the regulatory effects of altered electrolyte balance on myocardial function, and the effect of fluid shifts on baroreceptors and reflex control function.

3. Study of myocardial changes and temporal aspects of specific etiologic factors such as ionized calcium fluxes and myocardial isoenzymes, and sodium/potassium pump function.

4. Development and study of countermeasures for deconditioning, such as exercise, lower body negative pressure devices, salt and water loading and specific medications.

SUMMARY AND CONCLUSIONS

Experience gained so far indicates that the cardiopulmonary system acclimates satisfactorily to the space environment, and that human beings can maintain functional capacity for prolonged periods in space. However, this adaptation is not appropriate to one-gravity environment, and some cardiopulmonary responses may require up to three weeks postflight to return to preflight baselines.

Although the observations thus far suggest optimism with respect to the ability of the cardiopulmonary system to acclimate to the space environment, many basic questions remain unanswered. These questions which impact both the immediate and long-term aspects of spaceflight, need to be resolved before adequate prophylactic measures for extended space missions are developed.

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CARDIOVASCULAR RESPONSES TO HEAD-DOWN TILT IN YOUNG AND MIDDLE-AGED MEN

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INTRODUCTION

Head-down tilt was introduced by Kakurin and others (4) as a method for the study of cardiovascular adaptation to weightlessness. Subsequent studies, including crossectional comparisons of adaptations induced by actual space flight and by head-down tilt at moderate angles, have provided further support for head-down tilt as a simulation method. Qualitative and quantitative similarities with respect to body fluid shifts and post-intervention cardiovascular dysfunction have been established (1,7). The extent to which the exact time course of the response to weightlessness can be reproduced is still uncertain. The cardiovascular responses to microgravity and to head-down tilt are clearly more rapid than the responses to horizontal bed-rest (2). Comparison of echocardiographic measurements in space, obtained during the Soviet-French collaborative study (Pourcelot, personal communication) on Salyut-7 and during our head-down tilt studies suggest that cardiovascular changes may occur more rapidly during tilt than in space but more data are needed.

The vast majority of cardiovascular simulation studies have been performed in young healthy men (2,6), but current missions are flown by crews including female and male astronauts in their forties and fifties. Recent bed-rest (3,6,8) and tilt (Leach, CS et al., personal communication) studies including women and middle-age men have demonstrated that most features of the cardiovascular dysfunction induced by bed-rest develop independently of age and sex. However, there is no information on the effect of age on the response to head-down tilt. We have therefore compared the effects of a 24-hour period of head-down tilt at -5° in middle-aged and young men. Our hypothesis was that age-related differences exist since many aspects of cardiovascular function show significant age trends (5). In particular, we considered it likely that the blunting of the arterial baroreflexes with increasing age and the decreasing myocardial compliance (or increasing stiffness) would affect the responses.

RESULTS

We studied 5 men, age 41 to 48 years, and compared the results to data from our previous studies (which also include details on protocol and methods) in 10 young (22-30 years) men (1,7). The basic characteristics of the two groups are shown in Table 1. Height was similar but the older men were significantly heavier ($p<0.001$). Maximal oxygen uptake ($\dot{V}O_2$ max) was lower in the middle-aged group ($p<0.001$) but fitness levels were similar and slightly below average if normal age trends are taken into account.

The time course and magnitude of the central fluid shift were evaluated by measurements of leg volume. A decrease of 5% within 2 hrs was recorded in both groups. The decrease in total blood volume (measured by the RISA and ^{51}Cr techniques) after 24-hour tilt was slightly larger among the older men (0.51 compared to 0.35 liters) but the difference was not significant. Plasma renin activity was reduced in both groups, from a control level of 1.2±.2 to .5±.2 at 6 hours in the young and from .65±.2 to .17±.02 in the middle-aged men. Rates of urine production ($ml \cdot min^{-1}$) were increased during the initial 8 hours of tilt compared with the subsequent 16 hours, 2.19±.30 vs 1.15±.11 in the older group and 1.98±.29 vs 1.36±.12 in the young group. Thus, age had no effect on the time course or magnitude of the shifts in body fluids. However, the cardiovascular responses were significantly different.

Table 1
BASIC CHARACTERISTICS OF THE SUBJECTS

	MIDDLE-AGED	YOUNG
N	5	10
AGE, years	45±1	26±1
HEIGHT, cm	179±3	182±2
WEIGHT, kg	88±6	72±3
$\dot{V}O_2$ MAX, $ml \cdot kg^{-1} \cdot min^{-1}$	30±3	36±2
TOTAL BLOOD VOLUME, liters	6.28±0.34	5.03±0.62
MEAN ARTERIAL PRESSURE, mmHg	85±2	79±3

Mean values ± S.E. Differences with respect to age, weight and $\dot{V}O_2$ max were significantly different ($p<0.001$).

Central venous pressure (CVP) increased in both groups, but the increase was larger (4 vs 2.5 cm H_2O) and more sustained (6 hrs vs 1 hr) in the older group (Fig. 1). An increase (+5 mm in the young and +2 mm in the middle-age men) in left ventricular end-diastolic diameter (M-mode echocardiography) occurred in both groups, but the older men had a larger diameter in the control state (45±3 compared to 41±2 mm). There were no changes in contractile state as determined from echocardiographic estimates of left ventricular ejection fraction and mean velocity of circumferential fiber shortening. Both groups kept indirect arterial pressure constant throughout the experiment. Stroke volume (derived from measurements of cardiac output by the C_2H_2 technique and heart rate) increased initially in both groups but returned to baseline levels within 6 hours (Table 2), consistent with a transient increase in ventricular filling pressures and a Frank-Starling effect. The young men had compensatory bradycardia with a decrease from 75±4 to 64±2 beats/min during the initial 1½ hours, whereas the middle-age men had no significant heart rate changes.

Table 2
STROKE VOLUME (ML) DURING HEAD-DOWN TILT

	CONTROL	HEAD-DOWN TILT			
	SUPINE	30 min	90 min	6 hrs	20 hrs
MIDDLE-AGED MEN	104±4	111±7	107±7	97±8	98±7
YOUNG MEN	102±7	108±7	114±8	92±8	92±8

Mean values ± S.E.

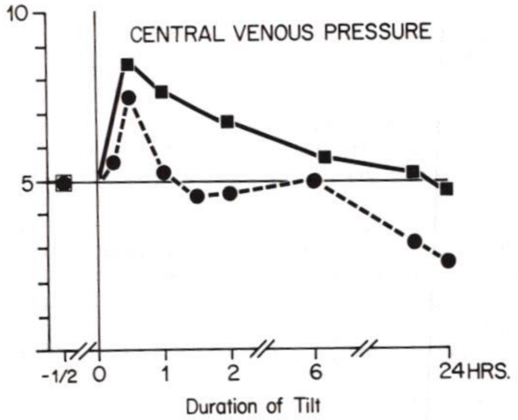

Figure 1. Central venous pressure (cm H_2O) before (supine rest) and during head-down tilt. Mean values from 5 middle-aged men (square symbols, solid line) and 10 young men (filled circles, dashed line).

As a consequence, cardiac output remained stable in the young men but increased significantly in middle-aged men from 6.9 to 7.9 $liters \cdot min^{-1}$. Post-tilt studies showed significantly increased heart rates during lower body negative pressure (LBNP) and submaximal exercise (Table 3) in both groups without significant age effects.

However, the exercise response tended to be altered less in the middle-age men as reflected by somewhat smaller decreases in maximal oxygen uptake (-10% or -3.0 $ml \cdot kg^{-1} \cdot min^{-1}$ compared to -22% or -7.9 $ml \cdot kg^{-1} \cdot min^{-1}$) and smaller increases in heart rate at submaximal levels (Table 3). The reverse was true for the LBNP response and the only incidence of pre-syncope or syncope during the combined studies occurred in one of the older men.

Table 3
HEART RATES (BEATS/MIN) BEFORE AND AFTER TILT

		PRE-TILT	POST-TILT	Δ
REST, supine	M	63±4	66±6	+3
	Y	58±3	73±4	+15
LBNP, -40 mmHg	M	68±5	90±8	+22
	Y	74±5	91±6	+17
SUB-MAX. EXERCISE	M	116±9	135±8	+19
	Y	118±5	144±5	+26

Mean values ± S.E. M-Middle-aged, Y-Young subjects. Submaximal exercise was performed in the upright position on a mechanical bicycle ergometer at a workload of 100 W.

In summary, the over-all pattern of adaptation to a 24-hour period of head-down tilt was similar in young and middle-aged men with a central fluid shift, a transient increase in ventricular filling, a diuresis and a blood volume decrease with a return within 24 hours to supine baseline levels for most cardiovascular measurements. The principal differences included a larger more prolonged increase in CVP in the older group, but changes in stroke volume were of equal magnitude. These findings are consistent with a decreased ventricular compliance in the older men. Arterial pressure was maintained constant in both groups but by different mechanisms, suggesting an age-related difference in baroceptor function. There was a reflex-induced bradycardia in the young men, but the middle-aged men responded with vasodilatation to the transient increase in ventricular filling and stroke volume. These findings indicate that at rest arterial pressure is more tightly regulated than cardiac output. Post-tilt, actual and functional hypovolemia was apparent in both groups. Further studies are required to evaluate a trend suggesting a greater effect on exercise performance in young and on LBNP response in middle-age men.

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INFLUENCE OF OPTOKINETIC STIMULATION AND IMMERSION ON EYE-HEAD COORDINATION IN MAN

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INTRODUCTION

The visual and proprioceptive afferent systems are known to be closely associated functionally and structurally with vestibular one. The recent neurophysiological studies point out to a direct involvement of the visual and proprioceptive signals in organization of the responses of vestibular nuclei (3, 1, 10). The populations of neurons responding adequately not only to the vestibular but to the visual and proprioceptive signals have been found in all the vestibular projection fields in all species from fishes to monkeys (4, 5). It is obvious that alteration of the gravitational loads, changing the parameters of the vestibular and proprioceptive receptor activity would disturb a precise and harmonic afferent interaction. But in what way? In an attempt to answer this question the characteristics of eye-head coordination in man have been studied in situations when the parameters of interacting inputs activity were altered by optokinetic stimulation and immersion.

The coordinated eye-head movements that follow the unexpected presentation of a visual target in the peripheral visual field have been used as an experimental model. In accordance with the data reported by 2, 6 an accuracy and timing of this reaction in primates and man is ensured by the precise coordination of three composing the reaction movements, namely, a saccade towards a target, a rotation of the head in the same direction and a compensatory counterrotation of the eyes providing the stabilization of the fovea on a target while the head is still moving. This coordination is controlled by a feedback mechanisms in which the crucial role play the vestibular afferent signals.

METHODS

The subjects performed a motor task of directing their gaze on a 1 deg luminoous target spot presented randomly in a horizontal perimeter 30 cm from the subject. Six target spots were spaced at 20 deg intervals from 60 deg to the left of the midsagittal plane to 60 deg to the right.

Horizontal eye and head movements were monitored, their amplitudes, velocities and time parameters were analyzed. In some experiments the galvanic vestibular threshold was also determined.

The characteristics of eye-head coordination have been studied in standard situations and under conditions of optokinetic stimulators. Dark and light strips of 3 cm wide each were projected to the same screen with the aid of optokinetic drum; the strips moved with the speed of 48-50 deg/s. To ascertain a role of proprioceptive signals the parameters of reaction were studied also before and after 7-day immersion hypokinesia.

RESULTS AND DISCUSSION

In control, the subjects performed the task quickly and precisely. The spatial and temporal pattern of the reaction was standard and stable. (Fig. 1). A saccade with a latent period of 230-290 ms was the first to appear. Then at a delay of 20-60 ms a head started to turn to the same direction. On completion of the saccade, when an eye reached the target but the head continued to move, a third component of the reaction - compensatory eye counterrotation - has appeared.

The amplitudes of saccades and head turns were always smaller than preset angular distance rising with an increase of a distance to the target and the amplitudes of head turns somewhat higher than saccade ones (Fig. 2). The maximum speeds of the saccades and head turns were also increased with an increase of the distance to a target. The maximum velocity of a compensatory counterrotation of the eyes in control was in a line with that of the head turn, the gain of vestibulo-oculomotor reflex determined as a relation of the head and eye counterrotation velocities was always close to 1.0 (Fig. 2). An accuracy of the eye-head coordination in control situation was always high: the number of errors did not exceed 5%, their magnitude ranged within 2-3 deg. As a whole, time of target catching in case of the targets of 20-60 deg did not exceed 320-460 ms (Fig. 2).

The OKS, not disturbing the spatial pattern of the reaction as a whole, changed substantially the parameters of its components and their interaction (Fig. 3). In this case, the performance of a task was characterized by facilitating of all

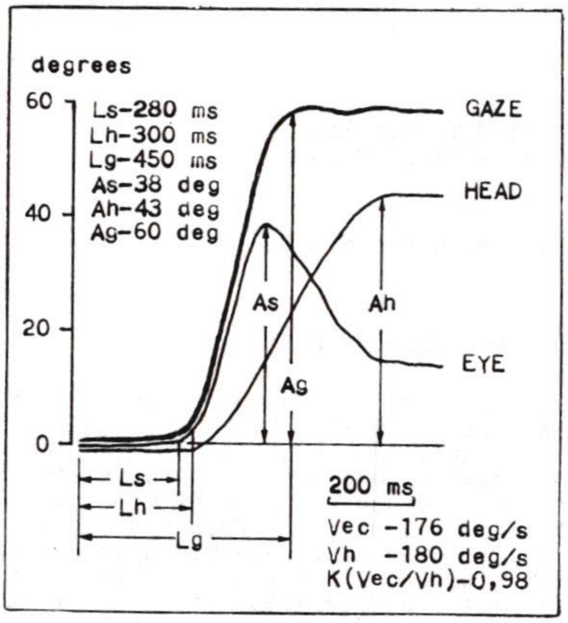

Fig. 1. Average diagram and parameters of the eye-head coordination. Target spot at 60 degrees; Ls- latency of saccade (ms); Lh - latency of head turn (ms); Lg - latency of gaze (ms); As - amplitude of head (deg); Ag - amplitude of gaze (deg); Vec - velocity of eye counterrotation; Vh - velocity of head movement.

the oculomotor components that was manifested by an increase of amplitudes of saccades (by 4-6 deg), their velocities (by 10-15%) and durations (by 5-10 ms) as well as by a significant rise of the velocity of compensatory counterrotation of the eyes (by 30-45% for the targets in a 40° and 60° range). This latter was linked to an increase of the gain of vestibulo-oculomotor reflex (up to 1.16-1.24) since the velocity of head turns in this case did not change significantly. An accuracy of eye-head coordination during OKS was obviously diminished: a substantial increase of an amplitude and amount of positional errors was noted - by the 5th minute of stimulation their amount was as great as 45-50%; simultaneously, the time of task performing increased by 150-180 ms (Fig.3).

An exposure to immersion hypokinesia for 7 days also decreased the reaction precision. In this case, the changes in the parameters of the reaction were somewhat different: the characteristics of the saccades changed insignificantly; the parameters of the second component of the reaction - head movements - were altered to a greater extent: their latencies increased by 20-30 ms, amplitudes - by 4-9 deg, velocities - by 30-50 deg/sec. However, an increase of the eye counterrotation velocity was still greater, so the gain of vestibulo-oculomotor reflex approximated 1.13-1.16 (Fig. 3). The number of positional errors after immersion reached 50%, the time of task performace increased by 40-50 ms. When OKS was used after immersion trend and magnitude of the reaction parameters changes were similar to those in the control data. However, OKS effects in this case were summed with the immersion ones and therefore were manifested significantly more clearly. (Fig. 3). An increase of saccade amplitude and a decrease of head turns elicited by OKS after immersion was more prominent. More significantly than in control increased after immersion the magnitude of vestibulo-oculomotor reflex, gain reaching 1.25-1.29. The number of errors in case of OKS after immersion was close to 100% (75-80%); time of the task performance raised more than 300 ms.

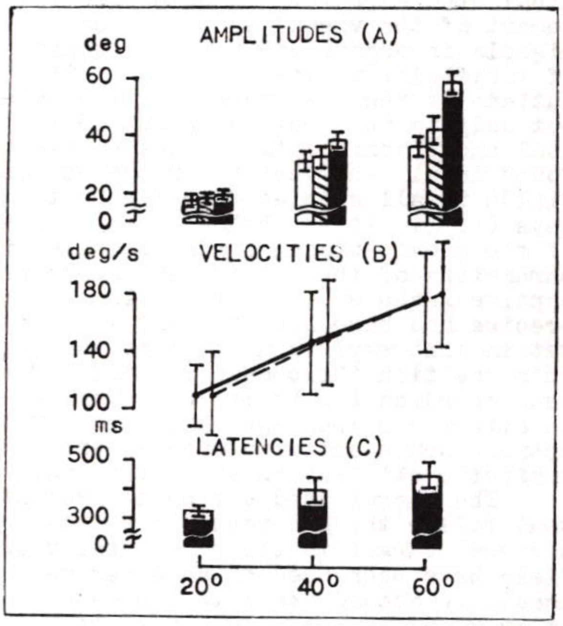

Fig. 2. Parameters of eye and head movements performed to different target locations. On the abscissa is shown the location of the target spots (deg). Open, striped and black bars show the data of eye, head and gaze movements correspondingly. Solid and dotted lines present the velocities of head turn and eye counterrotation respectively.

The results of the studies performed in a complete agreement with the ideas of (8, 9) and others demonstrate an important role of the visual and proprioceptive afferent signals in organisation of vestibular reactions and allow to clarify the mode of these effects. As follows from the experimental results, the influences of optokinetic stimulation and immersion on

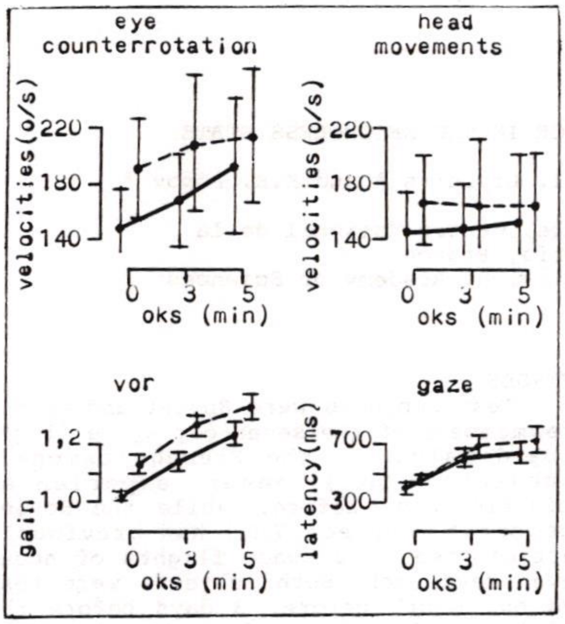

Fig. 3. Parameters of eye-head coordination in control (0) and after 3 and 5 minutes of OKS (3,5) before (solid line) and after (broken line) 7-day water immersion.

parameters of eye-head coordination were somehow similar: both effects were followed by facilitating the oculomotor sequenses of the reaction and increasing the vestibulo-oculomotor reflex gain. The latter was indicative of an increase of the vestibular excitability. The alterations of the galvanic vestibular thresholds recorded at the same time suggested that this increase of the excitability affected not only semicircular canals system but otolithic apparatus as well: in all situations mentioned, a marked decrease of the galvanic thresholds was recorded during OKS after immersion by 0.3-0.4 mA. Since the immersion hypokinesia is associated with a significant fall of the proprioceptive activity and in this case a facilitation of the vestibular responses is noted (7), it is concluded that the proprioception produces an inhibitory effect on the vestibular excitability. As opposed to it, the visual afferentation facilitates the vestibular activity. Of interest and importance is the fact of algebraical summation of two effects. Pointing to the independent mechanism of realization of influences of two afferent systems that play an important role in the control of vestibular activity, this fact predicts and explains an increase of "invasiveness" of optokinetic stimulation and a decrease of speed and precision of the eye-head coordination in weightlessness.

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A STUDY OF MECHANISMS OF POSTURE MAINTENANCE IN THE WEIGHTLESS STATE

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ABSTRACT

Adaptation of postural adjustments involved during voluntary elevation of the arm or of the whole body was studied during a seven-day space flight aboard Salyut-7. Results show an important forward inclination of the body at the beginning of the flight. Together with this inclination a redistribution of the electromyographic activities between ankle flexor and extensor muscles was observed. The findings suggests two types of adaptation of the central program of posture regulation to weightlessness.

INTRODUCTION

Maintening a stable posture during and after the movement of a boby part such as the elevation of the arm needs the estimation of postural constraints and the detection of the displacements of gravity center. Vestibular, proprioceptive, tactile and visual inputs play an important role in the correction of the postural equilibrium (1,2,3,4,5). These different sensorial modalities allow the establishment of central motor programs well adapted to the terrestrial gravitational field. In the weightless state, the control of the position signal of body segments with respect to the gravitational field is no more established. Furthermore, the gravitational mechanical constraints disappear. The sensiblity of numerous receptors involved in the postural control is also changed (2).

The purpose of this study was to evaluate the adaptation of motor control to the weightless state. This experiment will attempt to answer the following questions : Are terrestrial postural programs modified by the weightless environment ? Are they closely related to a terrestrial body scheme ?

METHODS

Test subjects were Soviet and French crewmembers of the seven-day space flight aboard Salyut-7. The French cosmonaut (subject J.L.C.) never experienced weightlessness before, while the Soviet cosmonaut (subject V.D.) had previously participated to 2 space flights of about seven days each. Both subjects were tested one month before, 3 days before the flight, and 3 days after the landing. Subject JLC was tested on the second, the third and the seventh day inflight. Subject VD was only tested on the fifth day inflight.

The postural reactions associated with the following movements were taken under study : 1) voluntary raising of the right hand, 2) voluntary tiptoeing, 3) unvoluntary body movement caused by the forward displacement of the support platform. In the first and the second condition the platform was fixed. For each experimental conditions ten identical trials were performed. Subjects were instructed to be perpendicular to the platform before, during and after each movement. Concerning the elevation on the tip toes subjects were asked to maintain this new positon during few seconds (rise-and-hold) or to return rapidly to the initial posture (rise-not-hold).

Figure 1 shows the experimental setup especially built for this experiment. Electromyograms of four right leg muscles (Biceps femoris, Quadriceps, Soleus, Tibialis anterior), hand movement acceleration, ankle joint angle and the position of the unfixed part of the support platform were recorded. Instantaneous position of various body segments in the saggital plane was recorded by means of a camera (32 images/s) which filmed small circular targets placed on the subject body.

Figure 1 : Diagram of apparatus.

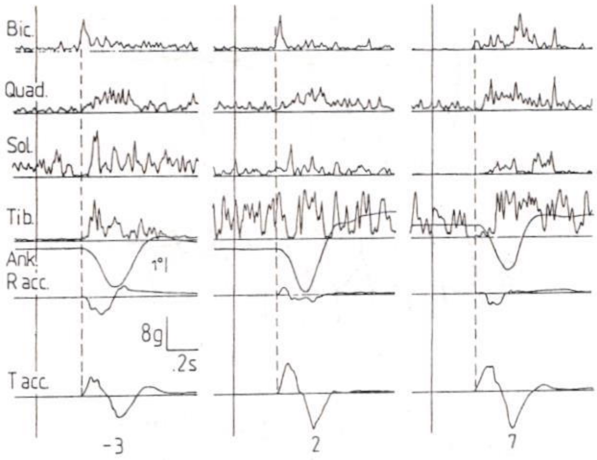

Figure 2 : EMG activities of Biceps femoris (Bic), Quadriceps (Quad), Soleus (Sol), Tibialis anterior (Tib), ankle displacement (Ank) and arm accelerations (R acc, T acc) recorded with subject JLC before(-3) and during the spaceflight (days 2 and 7).

RESULTS

1) Voluntary raising of the arm.

Postural adjustments induced by the voluntary raising of the arm in subject JLC three days pre-flight, on the second and on the seventh day inflight are shown on figure 2. These representative trials of movements performed for each day of experiment show that tonic activity of the ankle extensor muscle (Soleus) and of the ankle flexor muscle (Tibialis anterior) was modified in the weightless state. On the second day inflight the level of tonic activity of the ankle extensor is considerably diminished, while tonic activity of the flexor is enhanced, by comparison with pre-flight data. At the end of the space flight, tonic activity of both muscles decreased. Consistent with the findings of Belen'kii et al. (1967)(1), recordings obtained 3 days before the flight show an initial burst of activity of Biceps femoris before the initiation of the arm movement. In the same time, Soleus exhibited a readily desactivation which lasts after the onset of the arm movement. Consecutively to the arm movement the backward sway of the ankle is associated with the increasing activity of the four leg muscles. These anticipatory and compensatory activities are closely associated with maintenance of dynamic stabilization of the body in erect position. During the weightless state, before voluntary arm movement, the initial vigourous Biceps femoris activity was always present on the second day inflight, but was extremely reduced on the seventh day inflight. The most striking observation is related to the presence of a short disactivation period in Tibialis all along the flight. On the second day this desactivation appeared during a short period of time just after the initiation of the arm movement. On the seventh day it took place before the arm movement. Comparatively to the pre-flight tests, the amplitude of the backward sway of the ankle following the raising movement of the hand was similar (fig. 2). During the post-flight tests the same pattern of EMG activity as in pre-flight tests was observed. The profile and the amplitude of the ankle movement were also identical.

Based on cinematographic data, the initial postures and trajectories of body segments were reconstructed. Such recontruction is depicted on figure 3 when subject JLC performed an arm movement before, and during the flight. The most interesting observation is that the initial posture of the suject was

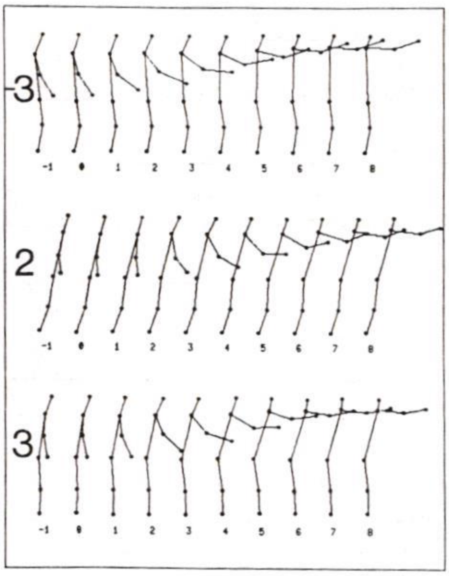

Figure 3 : Reconstruction of the posture of subject JLC during voluntary arm elevation before (-3) and during the spaceflight (days 2 and 3).

greatly forward inclined on the second day inflight. On the third day inflight, the initial posture of the subject is closely similar to the terrestrial posture. During the arm elevation, it could be noted similar displacements of the whole kinematic chain before and during the flight.

2) Voluntary tiptoeing.

It has been studied by Lipshits et al.(1981)(6) the temporal characteristics of the anticipatory activity of the postural muscles preceding the rapid elevation on the toes from the standing position. First, they observed an inhibition of the spontaneous activity of the Soleus following by a burst of activity in Tibialis which continued as far as the subject remained on the toes (fig. 4, rise-and-hold). However, if the subject returned immediately to the initial position, the anticipatory activity of the Tibialis was absent (fig. 4, rise-not-hold). The authors have proposed that the functional role of the anticipatory activity of the Tibialis is to cause displacements of the center of gravity into a new statically stable position. The same experiment performed in weightlessness shows similar results as depicted in fig. 4 for subject JLC tested 3 days before, on the third day inflight and 3 days after the flight. Indeed, the sequence of the motor patterns during voluntary tiptoeing was clearly preserved.

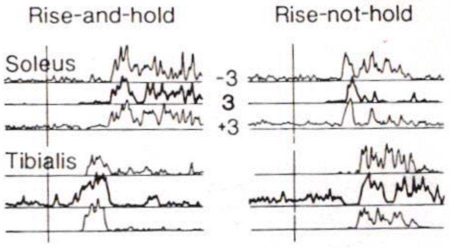

Figure 4 : EMG activities of Soleus and Tibialis during voluntary tiptoeing in subject JLC before (-3), during (day 3) and after the spaceflight (+3).

3) Unvoluntary body movement.

The brisk forward displacement of the platform on which subject stood erect, inducing a stretch reflex of the ankle flexor muscle (Tibialis anterior), was an interessant way to study the functional muscle stretch reaction of subject placed in weightlessness. In this situation, the displacement of the platform induces a burst of activity in

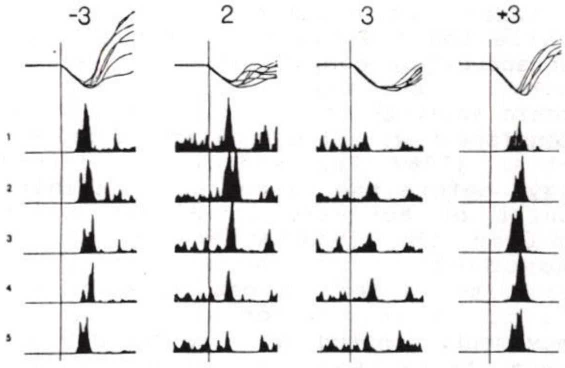

Figure 5 : EMG activity of Tibialis anterior during unvoluntary forward body movement in subject JLC before (-3), during (days 2 and 3) and after the spaceflight (+3).

Tibialis occuring 100 to 120 ms after the backward sway of the ankle joint. This phasic activity was well reproductible on earth as shown by figure 5 with subject JLC, 3 days before the flight. In weightlessness, we observed similar responses at the beginning of the session, and a gradual decrease of the amplitude of the burst after three trials. Finally the amplitude of the response was stabilized to about 40% of its initial value till the third day of the flight. Such result could be related to the less amount of inhibitory influence of the otolitic system upon postural flexor muscles.

DISCUSSION

These results show that subject placed in weightlessness could maintained an erect posture when they performed various movements. In contrast to the ground-based situation, the erect posture can be maintained primarily due to the predominant contraction of leg anterior surface muscles. This could be explained by the fact that the rest position of the ankle joint is a plantarflexion. So, the forward inclination of the subject (dorsiflexion) needs an important tonic activity in the ankle flexor muscle. This interpretation is in good agreement with our results showing a more important tonic activity of Tibialis anterior at the beginning of the flight when subject was largely forward inclined.

The findings are indicative of two types of adaptation of the central program of posture regulation to weightlessness : a fast adaptation stressed by a redistribution of motor commands between ankle flexors and extensors, and a slow adaptation characterized by the disappearance of anticipatory activation of Biceps femoris and the quantitative changes in EMG postural components seen on mission day 7.

The forward inclination and the respective position of body segments would be the sign of a persistent terrestrial attitude. This result together with the fact that the motor patterns induced by arm and body movements were similar to those observed on earth support the hypothesis that the control system underlying the stability of the posture is highly conservative. To adapt the motor strategies to a new environment such as weightlessness the control system would be based on a terrestrial body scheme (7).

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SLEEP-WAKE RESPONSES OF SQUIRREL MONKEYS EXPOSED TO HYPERDYNAMIC ENVIRONMENTS

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This study examines the sleep responses of primates to acute $3\ G_z$ environments. To investigate this question, loosely-restrained squirrel monkeys were exposed to 70 minutes of $3\ G_z$ during the day. The animals' behavioral state was polygraphically monitored (EEG, EMG, EOG) along with video and deep body temperature. During the control period, animals exhibited slow wave sleep (SWS) napping behavior. SWS occurred during approximately 20% of the control period. Body temperature was maintained at 38.7°C. At $3\ G_z$, SWS was inhibited for 5 minutes, after which SWS occurred at levels 50% lower than in the control period. During the post-centrifugation period, SWS was elevated above the control (50%) and hyperdynamic (100%) levels. Body temperature was depressed 1.5°C when the animals were at $3\ G_z$. Thus, hyperdynamic environments are capable of modifying primate sleep behavior, at least as a result of acute exposure. Further, the increased arousal in the hyperdynamic environment is correlated with a lower body temperature. This negative correlation differs from the normal positive correlation of arousal and body temperature.

Animals demonstrate a variety of physiological and behavioral changes as a result of acute exposure to hyperdynamic environments. Such changes in rats and monkeys include a significant depression of body temperature in excess of 1°C when these animals are exposed to $2\ G_z$ fields (1,2,3). However, in the earth gravity environment, body temperature decreases of this magnitude are highly correlated with changes in the arousal state of the animal (4). Specifically, a fall in body temperature is associated with sleep. Further, at $2\ G_z$ we have previously reported apparent changes in the sleep-wake behavior of animals monitored by direct video observation (2). The purpose of this study was to polygraphically define the behavioral state of squirrel monkeys acutely exposed to $3\ G_z$ fields for 70 minutes.

For sleep recording, the electroencephalogram (EEG), the electromyogram (EMG) and electrooculogram (EOG) were recorded continuously in six chronically prepared animals. Core body temperature was recorded with a thermistor inserted 6 cm past the anus and taped to the base of the tail. In some animals, the tail skin temperature was also monitored by securing a thermistor to the surface of the tail. At the end of the experiment the polygraphic data was scored at 30 sec intervals in the normal fashion. Body and tail temperatures were digitized in 1 min intervals. Plots and statistical averages were computer generated.

The experimental protocols were performed in the mid-afternoon. The protocol consisted of a 70 min pre-centrifugation period, followed by a 70 min hyperdynamic environment and a 70 min post-centrifugation period. The data were collected from 6 experimental subjects weighing 900 to 1100 grams. Each animal was exposed to the field once. The hyperdynamic environment was produced via centrifugation on an 18 foot diameter centrifuge.

During the control period the animals exhibited two separate behavioral states. Awake animals showed a low amplitude desynchronized EEG pattern concomitant with a high amplitude EMG showing bursts of activity (which were correlated with video monitored activity), and an EOG consisting of large amplitude eye movements. The nap-like behavior during the pre-centrifugation phase consisted solely of Slow Wave Sleep (SWS). The EEG was synchronized and of large amplitude. The EMG showed a reduction in amplitude with a loss of bursts of activity. Finally the EOG showed a tendency for slow rolling activity with the absence of large amplitude transient eye movements. This sleep pattern was similar to the stage I and stage II slow wave sleep patterns seen in humans. At no time during these day-time experiments was Rapid Eye Movement sleep observed in these animals.

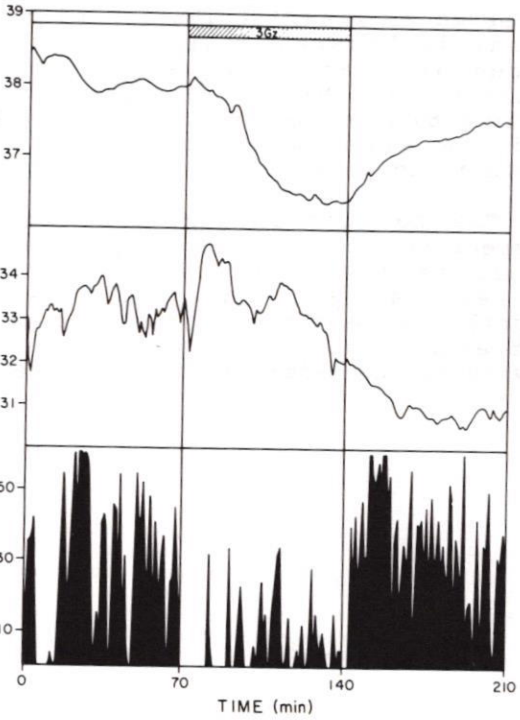

Figure 1. The responses of an animal to a $3\ G_z$ hyperdynamic environment. Plotted are the colonic (T_{co}) and tail (T_{ta}) temperatures ($^{\circ}$C) and the percent Slow Wave Sleep per minute. The time of the $3\ G_z$ field is indicated by the hatched area at the top of the figure.

Figure 1 is a plot of the responses of a single animal to this experimental protocol. The top panel shows the colonic temperature (T_{co}) response of this animal. The middle panel shows the tail skin temperature (T_{ta}) and the bottom panel shows the percent Slow Wave Sleep observed during each minute interval. As can be seen, during the pre-centrifugation phase the two temperatures were regulated at relatively stable levels while the animal showed an average of approximately 30% SWS. During the centrifugation phase, the body temperature fell over 1°C while the tail temperature showed a transient increase in excess of 1°C. SWS was totally inhibited during the first 10 minutes of centrifugation and only appeared transiently throughout the rest of the centrifugation phase. During the post-centrifugation phase the body temperature began to rise towards the control level while the skin temperature was depressed below control levels. SWS was elevated above the control level. Thus, during centrifugation there was an increase in heat loss which was concomitant with a fall in body temperature and an increased arousal of the animal. The mean fall in body temperature of all six animals was 1.5°C. During the post-centrifugation phase there was a recovery of body temperature with a reduction in heat loss and an increase in the amount of SWS present over control levels.

Figure 2 shows the average sleep response of all 6 animals exposed to the 3 G_z hyperdynamic fields and is plotted as percent SWS per 5 minute interval (mean + S.E.). During the control phase of the experiment, the animals demonstrated 21.2 ± 2.3% SWS during the 70 minute control period. With the onset of centrifugation, at 3 G_z the animals showed a significant reduction ($p < .05$) in the amount of SWS during centrifugation, with the percent SWS decreasing to 12.3 ± 1.6%. Further, there was an initial inhibition of sleep at the onset of centrifugation and a slow increase in the amount of sleep activity to a maximum level late in the centrifugation period. During the post-centrifugation phase there was an immediate increase in the amount of slow wave sleep to an average of 32.0 ± 1.0% for 5 minutes. This level was significantly elevated over the control level ($p < .01$).

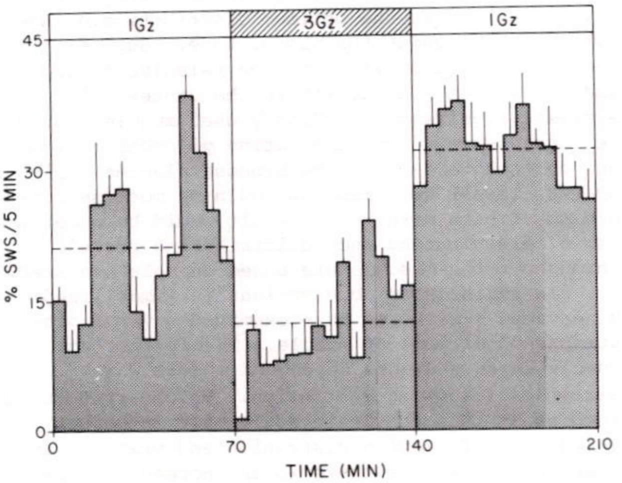

Figure 2. The mean sleep response of all 6 animals exposed to 3 G_z acceleration field. The data are averaged and plotted as percent SWS per 5 minutes (+ S.E.). The dotted lines in each 70 min segment represent the mean for that period. The time of the 3 G_z field is at the top of the figure.

Two findings are evident from these data. First, the sleep response of these primates is related to the intensity of the hyperdynamic environment, with an increase in arousal in this environment. Second, this arousal response is correlated with a lower body temperature. During the post-centrifugation phase a corollary response is observed. That is, there is an elevation in body temperature occurring simultaneously with increased SWS in these animals.

Over the last 25 years man has become increasingly concerned with the response of humans to both hyperdynamic and hypodynamic environments as a result of spaceflight and other practical concerns. Changes in fluid column pressures, dynamic loading of bones and muscles, growth, hormonal and neurological changes, all have led to studies examining practical aspects of changes in this gravitational vector. However, gravity is also a significant component of our environment which has been unchanging throughout our evolutionary development. As a result, changes in this parameter often produce unique and conflicting physiological responses. Thus, gravity becomes a useful and powerful tool in studying physiological responses.

The well-documented fall in body temperature occurs as a result of a significant increase in heat loss and a small decrease in heat production. This is a paradoxical response since body temperature, including hypothalamic temperature, is falling as a result of these peripheral changes in heat content. Normally, such a fall in temperature would produce a drive to decrease heat loss and increase heat production to elevate the body temperature back to normal. This response may result from an impairment of the thermoregulatory system since these homeotherms cannot maintain body temperature when thermally stressed in the hyperdynamic environment (1).

This study also produced opposing physiological responses. The increased arousal of the animals should lead to a hyperthermic if not normothermic condition. Since there is a dissociation between the arousal state and the fall in body temperature one could speculate that the coupling between these two physiological systems is not absolute. Further work is required to elucidate the mechanisms behind these responses. The hyperdynamic environment will be a useful tool in this regard.

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GLUCOCORTICOID SENSITIVITY, DISUSE, AND THE REGULATION OF MUSCLE MASS

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Glucocorticoids have a net catabolic effect on skeletal muscle. If the circulating concentration of these hormones is increased, the body musculature is placed in a negative nitrogen balance and loss of muscle mass occurs. However, if the receptor mechanism is governed by the law of mass action then the same process could occur by increasing the concentration of glucocorticoid receptor sites in a muscle. In this case a muscle would atrophy because it is hypersensitive to normal circulating concentrations of the hormone. Data has been developed in this laboratory suggesting that atrophy of a muscle such as occurs following immobilization may be caused by the affected muscles becoming hypersensitive to normal concentrations of glucocorticoids. Recently a new non-invasive immobilization procedure for use on small rodents has been developed. The procedure involves encasing the hind leg in light-weight, high-strength plastic. Studies employing this procedure are in progress.

WHY DOES MUSCLE NEED TO WORK?

A unique characteristic of skeletal muscle is the high degree of plasticity expressed in its protein mass. This plasticity, like that of the lipid mass of fat cells, is related to its fundamental role in general body energy metabolism. In mammals, forty to fifty percent of total body protein is maintained in the musculature. This protein, through the glucose-alanine cycle, is available for gluconeogenesis in the liver. Systemic demand on the protein mass of the musculature varies as a function of factors such as age, nutrition, and stress. Conversely, individual motor units have their own unique requirements for maintaining or expanding their protein mass which is determined by such factors as fiber-type and use. A reasonable postulate is that mechanisms must exist to distribute general systemic demands on the protein mass of the entire musculature according to the unique demands of individual motor units.

Hormones mediate the general metabolic relationship between the body and its musculature. Insulin mediates the deposition of nitrogen and carbon in the musculature whereas glucocorticoids mediate their withdrawal. Insulin is present in circulation primarily in the post-prandial condition. On the other hand, glucocorticoids are continuously present and respond to systemic conditions within the context of a complex diurnal pattern. Factors such as age, nutrition and stress impact the characteristics of this pattern.

Mechanical activity is central to expressing the unique demands of individual motor units. Used muscles show a net increase in protein mass while unused muscles show a net loss.

By what mechanism can the general demands of the system be coordinated with the unique requirements of individual motor units to provide optimum nitrogen distribution in the body? Several years ago, we observed that both immobilized (1) and denervated (2) muscles have an increased concentration of cytosolic glucocorticoid receptor sites. In the case of denervation, the increase was observed to occur very soon following nerve section and prior to any loss in muscle mass. It has been known for many years that increasing the circulating concentration of glucocorticoids causes a severe atrophy of the body musculature. If one assumes that mass action regulates the glucocorticoid receptor system, then a simple mechanism for the atrophy of an individual muscle (such as occurs with disuse) would be a Cushings-like atrophy mediated by an increased receptor concentration rather than by an increased hormone concentration. In addition, since the glucocorticoid influence on skeletal muscle is a net withdrawal of nitrogen, a mechanism of use-regulated glucocorticoid sensitivity would lead to the optimum distribution of body nitrogen throughout the musculature. Such a mechanism would provide for the relative demands of individual motor units within the context of systemic requirements. Highly used muscles would be less sensitive to circulating glucocorticoids and contribute less to the glucose-alanine cycle whereas lesser used muscles would be more sensitive and contribute more. The result would be a mechanism of distributing and redistributing protein throughout the musculature based on relative need.

In addition to observations on immobilized and denervated muscle, we have examined several other atrophy conditions of muscle. The levator-ani muscle is an androgen dependent muscle which atrophies following castration. We observed in our studies of this muscle in adult male rats that immediately following castration and prior to any loss in muscle mass there is an increase in the cytosolic concentration of glucocorticoid receptor sites. We observed similarly consistent results in our studies on dystrophic muscle. A common feature of virtually all dystrophies whether they be an animal model or one of the human conditions is the chronic loss of muscle mass. In our studies of both the chicken (3) and the mouse models we have observed that the down-regulation in the concentration of glucocorticoid receptor sites which occurs with maturation in the musculature of normal animals is substantially impeded. Thus the dystrophic animals maintain in their musculature abnormally high concentrations of glucocorticoid receptor sites. This observation is consistent with our initial hypothesis in that in the dystrophies there is a chronic loss of protein from the musculature which exceeds the demand of the system for substrates for gluconeogenesis and is accentuated by stress. In this respect there are certain parallels between the dystrophies and the situation existing in muscle disuse such as occurs under low gravity. In a weightless environment there is a chronic withdrawal of protein mass from the body musculature which exceeds systemic demand. This withdrawal is disproportionately focused on the more tonic (postural) muscles. That result is of particular interest since postural muscles are usually the least sensitive to the Cushings-like atrophy caused by increased concentrations of circulating glucocorticoids.

In situations of relatively stable muscle mass (eg. normal adult), more tonic red fiber muscles have both higher rates of protein turnover and higher cytosolic concentration of glucocorticoid receptor sites. Essentially, more tonic muscles are balancing a higher rate of protein degradation with a higher rate of protein synthesis. We have examined the relationship between protein synthesis protein degradation and glucocorticoid receptor concentration in a large number of conditions of muscle. In all cases, the rate of protein degradation and the cytosolic receptor concentration co-vary, but the rate of synthesis is independent.

Since high rates of protein degradation do not necessarily indicate atrophy, the question focuses on determining what factors regulate the balance between synthesis and degradation. To analyse the mechanism by which synthesis and degradation rates are balanced, we have developed a new non-invasive method of hind limb immobilization for rats. This method allows immobilization for various durations and later remobilization. It involves the use of precut patterns for forming a cast of a plastic-like material (Scotchcast; 3M Co.). The material is lightweight, less than 10% of the weight of a similar plaster cast, and is completely resistant to gnawing by the animals.

Although we have developed several cast forms which direct stretch to different muscles of the leg, the most extensive work has been completed on a form which affects approximately a right-angle immobilization of the ankle (Figure 1). This procedure has been carried out on 300-400 rats. As indicated in Table 1 the effect on the slow fiber soleus and fast fiber extensor digitorum longus is very similar to that which is observed as a result of weightlessness. The procedure which has been developed appears to provide a good model system for the study of disuse atrophy and recovery from disuse.

In summary, we have developed a hypothesis based on extensive analysis of several conditions of muscle atrophy: Glucocorticoid sensitivity of a muscle is a regulated characteristic and mechanical work is a major force in this regulation. Therefore, the weightless environment substantially removes this force especially from tonic muscles and may produce a condition of glucocorticoid hypersensitivity throughout the body musculature.

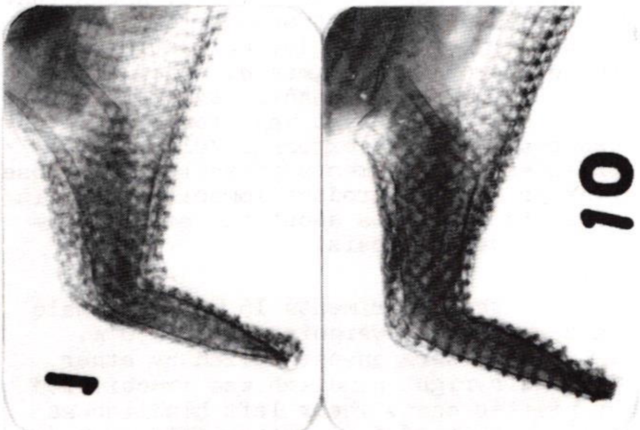

Figure 1. X-rays of casted legs.

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TABLE 1 MUSCLE WEIGHTS (% CONTRALATERAL CONTROL) AS A FUNCTION OF DAYS CASTED (TYPE A CASTS)

A. WET WEIGHTS

Days Casted	n	Extensor Digitorum Longus	Soleus
1	5	^{a}108.25 ± 8.09	^{a}97.61 ± 2.55
2	10	^{a}98.45 ± 8.78	^{a}89.88 ±11.44
3	10	^{a}99.54 ± 8.59	^{d}76.98 ± 8.52
6	8	^{d}86.17 ± 6.90	^{d}59.79 ±14.56
9	15	^{d}84.79 ± 6.69	^{d}50.80 ± 8.83

B. DRY WEIGHTS

Days Casted	n	Extensor Digitorum Longus	Soleus
1	5	^{a}104.18 ± 7.82	^{a}98.65 ± 10.10
2	10	^{b}93.11 ± 9.20	^{b}87.17 ± 17.17
3	10	^{a}90.30 ± 6.02	^{d}72.21 ± 6.74
6	8	^{c}78.97 ± 7.98	^{d}62.02 ± 14.23
9	15	^{d}83.46 ± 8.83	^{d}51.98 ± 12.12

a Not significantly different from control
b Different from control $P <.02$
c Different from control $P <.01$
d Different from control $P <.001$

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THE EFFECT OF IMMOBILIZATION ON THE RAT'S BONE

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INTRODUCTION

It has long been known from clinical observations that a state of inactivity /as staying long in bed or bone fractures/ leads to the development of osteoporosis. However, it has become recently known that weightlessness of astronauts leads to osteoporosis, too /1/. Immobilization proved to be a proper experimental model of osteoporosis induced by inactivity. This can be produced in different ways,for example, by denervation or by external /plaster cast/ or internal /ligaments/ fixation. We chose plaster cast to produce immobilization in order to gain data about the pathomechanism of osteoporosis.

METHODS

In the experiments 15 Wistar female rats were used, weighing about 250 g. 12 of them were anaesthetized by ether and their right hindlimb was immobilized by plaster cast. Their left hindlimb as well as that of 3 intact animals were used as controls. Rat were fed a standard diet. The animals were then killed by bleeding 8 weeks after plastering and their femurs, namely their length, periosteal and endosteal diameter in x-ray pictures were determined and on their basis a so called bone index was calculated. The density of bones studied by Zeiss densitometer by scanning according to the method of Gyarmati /2/. - 5-7 μm thick sections of tibia were stained by the method of Goldner, or haemotoxylin-chromotrope method. The calcification was demonstrated by Kossa reaction.

RESULTS AND DISCUSSION

We summarize the results of morphometrical /Table I./ and densitometrical measurements /Table II./. The reduction of mineralized bone substance was quantitatively proved. In the middle third of the bone, both the periosteal and endosteal diameters are significantly less though the reduction in the latter is less expressed. The thickness of compact bone shows a loss of 30% of its mass. Measurements at the level of distal metaphysis indicate a significant reduction of the number of trabeculae and along with that the mineral content was reduced, too.

Compared to the control the proximal epiphyseal cartilage of the tibia of immobilized animals, moderately broadened primarily by the thickness of maturing, hypertrophic zone. Vascularization and the number of cartilaginious cores decreased significantly in the metaphysis of plastered extremities, and osteoblasts were hardly detectable /Fig. 1.a/. All these phenomena indicate retardation in bone formation. Osteoclasts can be seen in the cortical bone localized subperiosteally in the Howship's lacunae /Fig. 1.b/. Capsules of osteocytes are broadened and cells become rich in process, this indicates resorbing activity of bone cells on the ground substance, that is osteocytic osteolysis /Fig. 2/. As a consequence there is a formation of fibrotic tissue.

Several questions can be raised having to do with the osteoporosis development under immobilization. Klein et al. /3/

TABLE I. Morphometrical analysis of femur

Group		L	D	d	D-d	$\frac{D-d}{D}$	D^2-d^2	$\frac{D^2-d^2}{DL}$	$\frac{D^2-d^2}{D^2}$
Immobilized	$\bar{x}$	38.09	3.46	2.49	1.07	30.2	6.55	0.046	51.4
n = 5	±SD	0.40	0.20	0.16	0.06	1.2	0.68	0.004	1.98
Control	$\bar{x}$	38.64	4.20	2.83	1.37	32.6	9.57	0.058	54.4
n = 5	±SD	0.50	0.09	0.12	0.12	2.8	0.85	0.005	3.83

L = length; D = periosteal diameter; d = endosteal diameter

TABLE II. Densitometrical analysis of femur

Group		Cortical bone		Spongious bone	
		μ 1/mm	Thickness of compact bone mm	Number of trabeculae	μ^+
Immobilized n = 5	$\bar{x}$	0.54	2.9	13.8	0.037
	±SD	0.035	0.2	2.0	0.006
Control n = 5	$\bar{x}$	0.59	2.7	22.8	0.055
	±SD	0.039	0.2	1.4	0.003

μ = relative density of unit compacta layer; u^+ = relative density of single trabecula

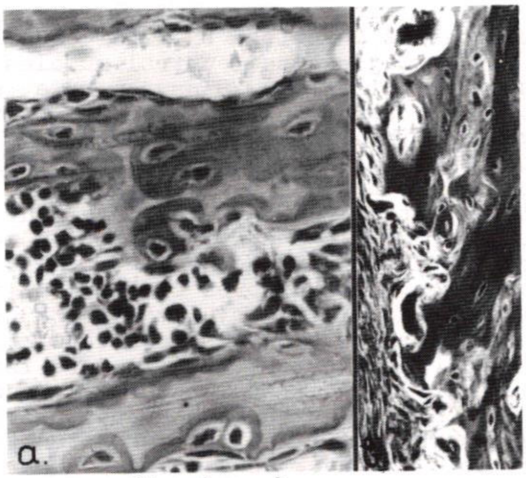

Fig. 1.a, b

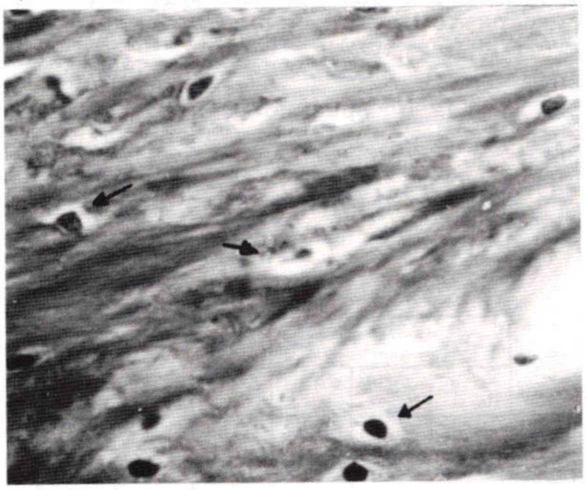

Fig. 2.

arrived at the conclusion that an increase of resorption results primarily in the reduction of bone mass. This can be reinforced also on the basis of our results, with accomplishment that osteoblasts can be hardly seen in the histological sections, which indicate retardation of bone formation as well. Several experiments emphasize the significance of the parathyroids. After the removal of parathyroid glands in dogs /4/ no osteoporosis developed after immobilization, which observation is supported by the experiment carried out on rats by Conaway et al. /5/. The question of the fall of mechanical stimuli has also been raised. These effects are important in sustainment and rebuilding of the bones /6, 7/. Reduction of stimuli leads to the decrease of rebuilding of the bones as well as to an increase of its resorption. As a consequence, the bone mass reduces. The decrease of the mechanical stimuli may cause impairment of the blood supply which leads to local changes in metabolism, and as a result, hormonal sensitivity of bone cells alters.

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EFFECT OF PHYSOSTIGMINE ON IMMOBILIZED RAT TONIC AND TETANIC SKELETAL MUSCLES

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The reactions of light meromyosin (LMM) (with specific antibodies and cholinesterase inhibitors) result a transformational change of myosin molecula, enhance the actin-myosin interaction and increase the degree and speed of muscular contraction, on the other hand, they inhibit relaxation (1). Such effect was shown by physostigmine which, as a cholinesterase inhibitor enters, in our assumption, into reaction with LMM. Simultaneously with our findings Horigome and Yamashita observed (2) that the modification by N-ethylmaleimide (NEM) of the SH groups localized in LMM results in an effect similar to that of physostigmine.

In our previous space biological experiments (3, 4) it was established that under the influence of weightlessness it is primarily the soleus muscle that suffers loss in weight, its myosin is transformed and takes up properties characterestic of the fast muscle. We pointed out a similar phenomenon (5) in the case of rats kept in plaster cast immobilization. We wished to clear up the molecular mechanism of this phenomenon in model experiments with glycerinated fibre preparations and by superprecipitation. We studied what the physostigmine sensitivity of the two types of muscles was like when no treatment was applied, and how it changed following immobilization.

METHODS

Female albino rats (Epimys norvegicus var albino, CFY strain) weighing 230-250 g were used throughout. The right hind limbs of the animals were fixed in a mildly flexed position according to Booth and Kelso (6) under full anaestesion with pentobarbitone sodium (50 mg/100 g body weight).

For our experiments, the extensor digitorum longus (EDL) muscles were used to represent the fast (tetanic) muscles and the m.soleus to represent the slow (tonic) muscles. The contralateral muscles were used as controls.

The total water content in muscle was obtained by drying 80-100 mg muscle to constant weight at 106° C.

The glycerinated muscle fibres were preparated as described by Szöőr et al. (7). The newly excised control muscles and those immobilized for 28 days were attached to rods corresponding to their original length and the myofibrillar membrane was exposed to osmotic shock. The preparations made in this way are available for use as early as after 48 hr. They maintained their contractile properties for 4-6 weeks stored in 50% glycerine at -20° C. The contraction of the fibres 0.25-0.4 mm in diameter was elicited by 5 mM ATP-Ca^{2+}. The isometric tension was recorded through a signal-converter. Myofibrils were isolated according to the method of Takács et al. (8). Changes in turbidity due to 1.15 mM ATP were determined spectrophotometrically in 1 cm cuvettes at 660 nm under continuous stirring following the method of Ebashi (9).

Myofibrillar Ca^{2+}-ATPase were determined according to the procedure described by Perry and Grey (10).

RESULTS

The degree of speed of contraction was significantly enhanced by physostigmine in both tapes of muscle.

	Maximal tension $g \times cm^{-2}$			Speed of contraction $g \times cm^{-2} \times min^{-1}$		
	control (n = 9)	5×10^{-4} M Phys. (n = 11)	10^{-3} M Phys. (n = 10)	control (n = 9)	5×10^{-4} M Phys. (n = 11)	10^{-3} M Phys. (n = 10)
m. soleus	5,81 ± 0,69	6,53 ± 0,66	7,28 ± 0,79	2,03 ± 0,54	2,88 ± 0,59	3,70 ± 1,08
	→	+ 12 %	+ 25%	→	+ 41%	+ 82%
m. EDL	3,01 ± 0,47	3,54 ± 0,26	3,96 ± 0,54	1,26 ± 0,23	2,11 ± 0,44	2,53 ± 0,36
	→	+ 17%	+ 31%	→	+ 67%	+ 101%

Fig. 1: Effect of physostigmine on glycerol treated m. soleus and m. EDL

Following 24 days of immobilization the ATP-induced tension to 50% in soleus muscle and in the case of EDL to 30%. An even greater decrease was observed in the contractile velocity of the muscles. In immobilized muscles the contraction enhancing and velocity-increasing effect of physostigmine was not observed.

	Maximal tension $g \times cm^{-1}$		Speed of contraction $g \times cm^{-1} \times min^{-1}$	
	immobilization (n=10)	10^{-3} M Phys. (n=9)	immobilization (n=10)	10^{-3} M Phys. (n=9)
m. soleus	0,32 ± 0,15	0,28 ± 0,13	0,07 ± 0,03	0,06 ± 0,03
	→	-14 %	→	-18 %
m. EDL	0,99 ± 0,31	0,71 ± 0,34	0,31 ± 0,15	0,51 ± 0,04
	→	-28 %	→	-35 %

Fig...: Effect of physostigmine on glycerol treated immobilized m.soleus and m.EDL

Superprecipitation experiments showed that as a result of immobilization the degree and velocity of superprecipitation decreased.

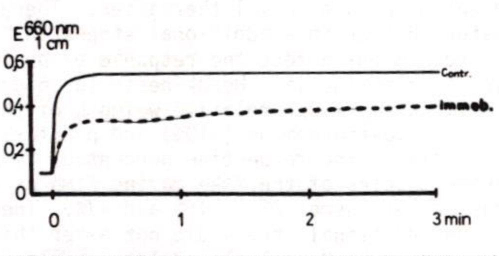

Fig. 3.: Effect of immobilization on the superprecipitation of myofibrillar proteins

According to our earlier experiments physostigmine accelerates superprecipitation following clearing at high K^+ concentration.

The superprecipitation of the control actomyosin develops only 58 min after clearing under the influence of 1 mM ATP, whereas 10^{-4} M physostigmine induces superprecipitation much earlier, i.e. in the 43rd min.

The degree and velocity of superprecipitation considerably decreases during immobilization, and physostigmine effect observed in the control muscles is also markedly decreased. One of the causes can be related to changes in ATPase activity.

Following immobilization there is considerable decrease in the activity of both muscle ATPases.

Muscle degeneration due to immobilizational atrophy results in the degradation and restructuring of the myosin molecule, this leads to the weakening of actin-myosin interaction. The decrease in the physostigmine sensitivity of the damaged myosin molecule also shows that normal contraction cannot be restored even by such factor as e.g. physostigmine treatment, which evoked enhancing and accelerating effect in the control experiments.

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PREVENTION OF METABOLIC ALTERATIONS CAUSED BY SUSPENSION HYPOKINESIA IN LEG MUSCLES OF RATS

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ABSTRACT

Rats were subjected to tail-cast suspension hypokinesia for 6 days with one leg immobilized in dorsal flexion by casting. Control animals were also tail-casted. The soleus, gastrocnemius and plantaris muscles of uncasted hypokinetic legs were smaller than control muscles. Dorsal flexion prevented atrophy of these muscles and caused the soleus to hypertrophy. The anterior muscles were unaffected by hypokinesia. The smaller size of the soleus of the uncasted leg relative to the dorsal flexed and weight bearing limbs correlated with slower protein synthesis and faster proteolysis. We also measured the capacity of this muscle to synthesize glutamine (gln), which carries nitrogenous waste from muscle. Although tissue homogenates showed higher activities of gln synthetase, the rate of de novo synthesis was not altered in intact muscle but the tissue ratio of gln/glutamate was decreased. Glutamate and ATP were not limiting for gln synthesis but availability of ammonia may be a limiting factor for this process in hypokinesia.

INTRODUCTION

We have been studying the metabolic response of skeletal muscle to decreased load bearing of the hindlimb and have reported previously (1) that the size of the soleus muscle is reduced considerably after 6 days, but no effect on the size of the extensor digitorum longus is evident at that time. One of the primary concerns of such a study is how we may prevent or at least offset partially the atrophy and attendant metabolic changes in leg muscles of hypokinetic rats. Several studies (2,3) have shown that passive stretch of muscle can lead to hypertrophy and decreased protein turnover. Therefore, we tested whether such treatment might be beneficial in the hypokinetic animals.

METHODS

Albino rats (130-170 g) bred by the Division of Animal Resources at the University of Arizona were suspended by a cast around the base of their tails consisting of orthopedic gauze and medical elastomer. By use of a clip, the rats were attached to a pulley which allowed them complete access to food and water, and the freedom to groom themselves. In this study we used three groups of rats. The weight-bearing control animals were tail casted but allowed to walk on all four limbs. A second group was hypokinetic with no load bearing of the hindlimb. The third group was also hypokinetic but with one leg free moving and the contralateral leg casted in a dorsal flexed position to stretch the posterior muscles and shorten the anterior muscles. Muscle incubations (4), protein turnover (5), glutamine assays (6) and measurement of glutamine synthetase (7) were carried out as described previously. Data were collected for 5-15 animals per group.

RESULTS AND DISCUSSION

Both the control and uncasted hypokinetic rats gained weight at a steady and similar rate over the six days of the experiment. In contrast, the additional stress of immobilizing one limb resulted in no weight gain prior to day 2 and a slower weight gain than normal thereafter. Therefore, we tested whether this additional stress in the leg casted rats might affect the response of posterior muscles to hypokinesia. Hypokinesia for 6 days led to significantly lower relative weights of the soleus (-26%), gastrocnemius (-10%) and plantaris (-9%) muscles. The respective percentage losses for these muscles of the free moving limb of the leg casted rats were -29%, -15% and -7%. Therefore, the additional stress did not alter this response. In the dorsal flexed leg, muscle stretch prevented the effect of reduced load bearing on the gastrocnemius and plantaris muscles and led to a 47% increase in the mass of the soleus. For anterior muscles, hypokinesia for only 6 days did not affect the relative mass of the extensor digitorum longus (EDL) or tibialis muscles. However, immobilization in a shortened position caused a small (4-8%) loss of muscle mass.

To account for the changes in muscle mass, we measured protein synthesis and degradation in the soleus and EDL muscles. Protein synthesis was 36% lower and protein degradation was 36% higher in the atrophied soleus. It seems apparent that the lower mass of this muscle in hypokinesia may be due to both slower rates of synthesis and faster rates of degradation of proteins. The finding is similar for the free moving limb of the dorsal flexed animals. However, in the stretched soleus muscle, the rates of these turnover processes were not different from normal values. In the EDL, hypokinesia had no effect on degradation but did decrease synthesis slightly. Doral flexion further lowered this process probably due to muscle shortening.

The enhanced turnover of protein in atrophying muscle requires the increased export of nitrogen derived from the degradation of certain amino acids by muscle. Both alanine (ala) and glutamine (gln) serve as important vehicles for removing excess nitrogen from muscle. Therefore, we measured the metabolism of these amino acids. The non-protein amino acid release includes amino acid in the medium derived from tissue pool losses and de novo synthesis. In the soleus muscle the total release of non-protein ala and its de novo synthesis were unaffected by hypokinesia. However, muscle stretch decreased these processes by 38-47% in the soleus. In contrast to this result for ala, the release of non-protein gln was 35% slower in the hypokinetic soleus muscle. Furthermore, the incubated tissue level of gln was 43% lower. However, the tissue levels of glutamate were unaffected by hypokinesia so that the ratio of gln to glutamate fell significantly. Stretching the soleus muscle of the hypokinetic animals prevented the changes in release of nonprotein gln, the tissue content of gln and the muscle ratio of gln to glutamate.

These data suggested that the capacity of hypokinetic soleus to synthesize gln might be lower. However, assays of glutamine synthetase activity in muscle homogenates showed a 2-fold greater activity than in the control muscles. Since enzyme activity was higher in hypokinesia, we considered whether ammonia may have limited the release of gln by this muscle. Addition of ammonium chloride had a similar effect on de novo synthesis of gln and the basal rates were similar for the two muscles. This result is surprising since the greater activity of glutamine synthetase in hypokinesia should have produced a larger effect of ammonia addition.

Since glutamate levels of muscle are not altered in hypokinesia, we determined whether ATP might have limited the rate of gln synthesis. Hypokinetic incubated muscles, however, had a 53% greater content of ATP than control incubated muscles. The tissue content of ATP in the stretched muscle was the same as in control muscles and the EDL showed no changes in hypokinesia. Clearly availability of glutamate and ATP are not limiting factors in the synthesis of gln by hypokinetic soleus.

Future investigations will consider further the possibility that the normal production of ammonia in these muscles may be lower. This idea is suggested by the higher levels of adenine nucleotides which normally provide ammonia to muscle by their deamination. Additional studies will also examine whether muscle stretch may prevent changes of muscle function in hypokinesia.

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PLASMA LEVELS OF NOREPINEPHRINE, EPINEPHRINE AND DOPAMINE DURING A 4-DAY HEAD-DOWN TILT WITH AND WITHOUT EXERCISE

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ABSTRACT -

The changes in sympatho-adrenal activity during a -6° head-down tilt were assessed in eight healthy men by measuring plasma norepinephrine (NE), epinephrine (E) and dopamine (DA) by a radioenzymatic method. Plasma catecholamine (CA) levels were unaltered after short-term (from 30 min to 10 h) or long-term (from 1 to 4 days) head-down tilt, and the association of regular exercise (50% V02 max during 2 hrs/d) with the tilt did not affect the results. These findings suggest that sympatho-adrenal activity is not significantly modified by head-down tilt at -6°.

INTRODUCTION -

Water immersion (WI) and bedrest in horizontal or head-down position have been proposed as experimental models to simulate the modifications observed during space flight (7, 11). Head-down tilt (HDT), in particular seems to be the most reliable ground based simulation to reproduce the hemodynamic alterations. This technique induces blood volume changes which are very close to those observed in weightlessness, namely a redistribution of fluids from the lower to the upper part of the body. This fluid shift results in a number of hormonal alterations intended to reduce central hypervolemia (11, 15). Although the involvement of the sympatho-adrenal system in hormonal changes after blood volume expansion in the thorax has been suggested (4, 13), no experimental proof is available in bedrest investigations, except the study of Chobanian (1)

In the present study we investigated in one group of subjects the effects of HDT on plasma CA levels. In a second group we tested the effects of regular exercise, used as a countermeasure in association with the HDT.

MATERIAL and METHODS -

8 healthy male volunteers men (24.1 ± 0.4 yr.old) participated in the study. The subjects were divided randomly into two equal groups.

Group I : Head-down tilt without exercise

On the first day (Do), the subjects remained supine and blood samples (0.5 ml) were taken at 9 am and 7 pm from one antecubital vein using an indwelling catheter.

On the second day (D1), the subjects were placed at 9 am in head-down position at -6° and blood samples were drawn at 9 (control) 9.30, 10, 12 am, 3 pm, 7 pm (fig. 1). Subjects remained in the head-down position until the 5th day at 9 am). Blood samples were taken twice a day at 9 am and 7 pm until the end of experiment (fig. 2).

Group II : Head-down titl with exercise

These subjects were submitted to the same experimental design, but in addition they exercised in the supine position on a bicyle ergometer for two 1-hr periods daily at 50% of their maximal oxygen uptake (10-11 am; 3-4 pm), except on day Do (no exercise) and day D1 (one exercise : 3-4 pm).

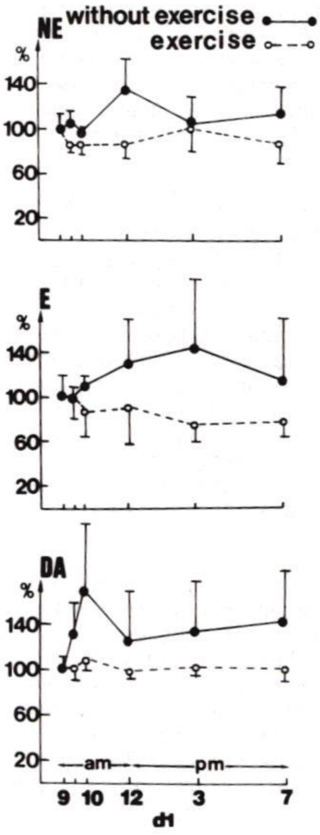

figure 1

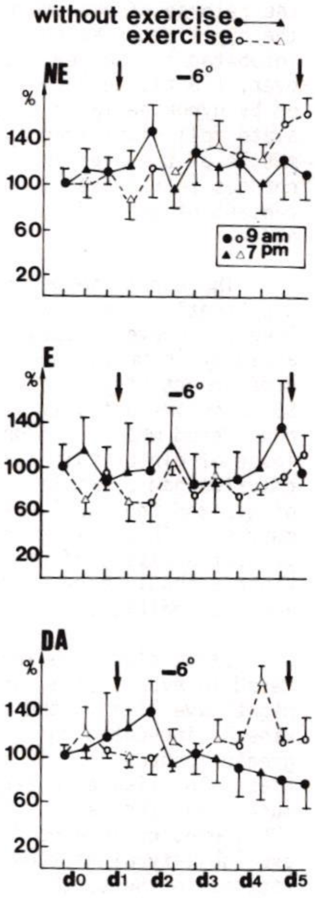

Figure 2

All subjects had a normal hospital diet (Na+ = 120 mmol/d; K+ = 60 mmol/d).

Plasma CA levels were determined according to a radioenzymatic method (2) and the results were analyzed by using a two-way analysis of variance.

RESULTS -

Data were expressed as a percentage of control levels as measured during horizontal bedrest preceding the head-down tilt.

As shown in fig. 1 and 2, the plasma CA levels were unaltered during HDT. Head-down bedrest associated with exercise resulted in a slight but non-significant increase in plasma NE levels at the end of tilt (D5) but failed to alter E and DA levels.

DISCUSSION -

HDT elicits a shift of body fluids from the lower to the upper part of the body, resulting immediately in a transient increase in central venous pressure (4, 11). This hemodynamic alteration may be detected for only a few hours because adaptive mechanisms lead to a decrease in plasma volume and a relative hypovolemia.

Endocrine alterations induced by hemodynamic changes associated with HDT thus include an early slight decrease in plasma renin activity, aldosterone and antidiuretic hormone, followed by increases in their release (5, 11, 15).

Hypothetically, from these findings, the following changes in sympathetic nerve activity during head-down tilt might be expected to occur successively :

1) an early decreased neural activity due to stimulation of cardiopulmonary receptors, as suggested by previous studies on sympathetic renal nerves in experimental animals (14),

2) an increase in sympathetic nerve activity possibly resulting from a reduction in intravascular volume following adaptation to HDT. Such an increase in sympathetic nerve tone would mediate the above cited hormonal changes.

The present study demonstrated that HDT failed to change plasma CA levels and this suggested that the activity of the sympatho-adrenal system was not significantly altered. Evaluating the sympathoadrenal activity in other experimental models simulating the hemodynamic effects of zero gravity has yielded conflicting results. Some authors failed to observe any significant variation of plasma CA during prolonged horizontal bed rest (1), or WI (3). Conversely, in similar situations, urinary NE concentrations were found decreased by JUCHMES (6) and SANDLER (13). More recently, KRISHNA (8) observed that a 4 hour head-out WI led to a significant decrease of plasma NE and to an increase of plasma DA. These opposit results are difficult to interpret and to compare, due to marked differences in study designs, particularly choice of experimental model and mode of evaluation of sympatho-adrenal activity.

Dynamic exercise was used as a countermeasure to offset the hemodynamic effects of HDT. Muscular work did not affect the results, except a slight but non-significant increase in plasma NE levels at the end of tilting.

In conclusion, this study demonstrates that neither short-term nor prolonged head-down tilt altered sympatho-adrenal activity, There is apparently therefore no evident relationship between peripheral sympathetic nerve function, as assessed by plasma CA measurements, and stimulation of cardiopulmonary receptors or neuro-endocrine changes induced by HDT.

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REVERSIBLE EFFECTS OF AN ALTERED GRAVITY FIELD ON MYOFIBRILLAR PROTEINS OF SKELETAL MUSCLES OF VARIOUS PHENOTYPES

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INTRODUCTION

The heterochronous reversibility of different physiological parametres of various types of skeletal muscles after gravitational overloading or weightlessness; perhaps; is based on the unequal velocity of renovation of contractile and modulatory myofibrillar proteins (I). Consequently for the understanding of the mechanism these phenomena is very important to clearing the degree of the including in the processes of the adaptive change working (actomyosin) and regulatory (modulatory proteins) apparates of muscle contraction. The purpose of this study was to determine the degree and reversibility of changes in contractile and regulatory proteins of two muscle types - slow and fast, after the action of Hypergravity Field.

METHODS

The white rats Wistar were exposed to acceleration 5Gz, daily 40 min during 2 weeks and were investigated after I and 30 days of the cessation of centrifugation. The acceleration vector was head--teil of animals. Native tropomyosin--trponin complex (Tm-Tn) was isolated from muscle by Graser a. Gergely (2) native actomyosin (NAM) - by KCI extraction and Ca-desensitazed actomyosin (DAM) was prepared by the remove of Tm-Tn complex. Aminoacid contents was determined by the analyzator of AAA-88I type and the calculation of protein compounds contents in actomyoson and Tm-Tn complex was examined on the SDS-gel by the electrophoretic method (3). The kinetic parametres of the superprecipitation of NAM and DAM were determined by the use of the optical equipment with the magnetic stirring camera.

RESULTS

In our experiments the two weeks periodic acceleration 4 or 5 Gz cases a significantly increasing of Ca-binding component (Tn-C) content in the Tm-Tn and decreases the content of ATP-ase inhibitor component (Tn-I) in slow muscles (Soleus m., medial head of triceps m.) In the fast muscles (brachialis m., extensor digitorum longus m.) the decrease of Tn-C content in Tm-Tn complex was observed. The content of troponin-T also changes. After 30 days readaptive period take place the restoration of the content various protein components but not completely in different types of muscle. (Fig.I). In the slow and fast muscles the content of tropomyosin reincreased, Tn-T-decreases in fast and reincreased in slow muscles.

It is interesting for the explain of the increasing in such experimental conditions the Mg - ATP-ase basal activity of actomyosin the decreqsing of the content of inhibitory troponin component -Tn-I(4).

The changes in the protein composition of Tm-Tn complex induced by acceleration is accompanied by the change of aminoacid composition. The adaptive changes in aminoacid composition of the Tm-Tn complex were more expressed in the postural muscles, valine and glutamine decreased and leucine and phenylalanine increased. In the fast muscles the ratios of valine-methionine and phenylalanine-histidine were higher. During a month readaptation period the relative content of individual amino acids recovered but not completely. In the case of leucin isoleucin, serin and arginin the subsequently changes are observed. These results indicate that the adaptive shifts in aminoacid composition are stipulated not only by changes in the protein components.

The kinetic parametres of superprecipitation of actomyosin in slow and fast muscles changes after the action of gravitational overloading in the opposite direction: the velocity of reaction decreased in the fast and increased in the slow muscles. More significantly changes is observed in the reaction of native actomyosin, which may be caused by the increasing of Tn-C and another hand by the decreasing TW-I. In this case Tn-C accelerated the reaction of superprecipitation in the presence of low Mg concentration. The change of the velocity of superprecipitation of DAM in our experiments suggest the appearance any adaptive changes in the molecular structure of contractile proteins; possible owing to increase the light chains content of myosin. Such assumption confirmed by results electrophorétic investigations of myofibrillar proteins (5) in the conditions of cosmic flight.

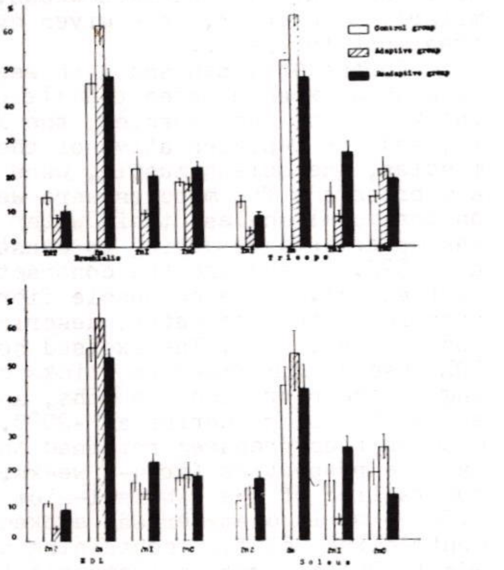

Figure 1. The content of troponine - tropomyosine components in various types of skeletal muscle.

It is interesting that after the removal of Tm-Tn complex native actomyosin the difference in the superprecipitation rate between the experimental and control animals was found only in postural muscles. After the month readaptation period the rate of actomyosin superprecipitation reaction became normal in the fast muscles and remained slightly elevated in the postural muscles. (Table 1).

The experimental results suggest that reversible adaptive changes appear not only in modulatory, but also in contractile proteins in myofibrils. There is many different patterns for the adaptive change in these two protein substrates in slow and fast muscles; During the month readaptation period the complete restoration of protein structures and function is not observed. For the understanding of the mechanism of different reaction slow and fast muscle is very important the fact that in fast muscle the changes in regulatory Tm-Tn complex are less marked.

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Table 1. The action of gravitational overloading velocity on the actomyosin superprecipitation

Muscles	Proteins	Velocity of Superprecipitation Control group	Adapt. group	Readapt. group
EDL	NAM	24.5 + 1.1	17.2 + 2.5	22.0 + 1.7
	DAM	27.0 ± 0.7	25.2 ± 1.6	26.3 ± 1.0
Brachialis	NAM	20.0 ± 1.3	18.0 ± 1.2	22.0 ± 1.8
	DAM	25.5 ± 1.0	24.5 ± 1.1	25.0 ± 0.2
Triceps	NAM	5.5 ± 0.6	16.6 ± 1.8	14.3 ± 1.3
	DAM	8.0 ± 0.4	13.3 ± 0.9	12.0 ± 1.1
Soleus	NAM	4.5 ± 0.3	10.0 ± 1.3	7.1 ± 0.8
	DAM	6.0 ± 0.2	8.7 ± 0.5	7.3 ± 0.3

The effect of hypokinesis and hypoxia on the contractile properties of muscles with different functions in rats

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As shown by experience in spaceflight during the last two decades, as a result of weightlessness, in the sense organs, in the circulatory, bone and muscular systems there occur such functional physiological changes that influence the physical working ability and physical state of astronauts /1, 2, 3/. The prolonged absence of gravitation leads to decreased functioning of the muscles. Increasingly affected by inactivity are the socalled antigravitational muscles, the ones that play a greater role in assuring motion and posture /4, 5/.

To simulate weightlessness under terrestrial conditions a number of experimental models have been elaborated /6, 7, 8/. The best method of all these has proved to be immobilization. In our previous experiments detailed examinations have been made on the effect of plaster-cast immobilization on the skeletal muscles of rabbits and rats /9, 10/. It has been established that atrophy develops fastest and to the highest degree in the slow /tonic/ muscles, its development in fast /tetanic/ muscles is much slower and attains a much lower degree /11/. With model experiments we participated in the Cosmos-1129 biosatellite project, and it proved that under weightlessness muscular atrophy develops similarly as in the case of plaster-cast immobilization /12, 13/.

The changes due to disuse have been investigated in hypokinesis combined with hypoxia, too. Plaster-cast immobilization involves disuse of muscles in certain extremities, whereas in the present experiments the animals were immobilized by being put in the stocks, thus their whole musculature was confined to relative rest.

METHODS

The experiments were performed with male rats of CFY strain with body weight 200-270 g. The animals were divided into four groups with 14 rats in each. The animals of the control group were allowed to move freely. The rats restricted in movement were kept in a special cage for 4, 8, 12 weeks, their feeding and watering was provided for inside the cage /14/. The members of the third group moved freely, however, for 24 hr prior to evaluation they were exposed to hypobaric hypoxia corresponding to an altitude of 7000 m above sea level. The fourth group was subjected to a combined treatment: the animals were kept in continuous hypokinesis till the 4th, 8th and 12th weeks, and similarly to group 3, were given hypoxic treatment for 24 hr.

In the 4th, 8th and 12th weeks the rats were exsanguinated then the representative of the fast muscles, the EDL muscle, and the representative of the slow muscles, the soleus muscle, were excised and prepared. The muscles were weighed on torsional scales, their water content was determined by drying to constant weight at 105°C. To examine the contractile properties, glycerinated muscle fibres were prepared after the method described by Szöőr et al. /15/. The excised soleus and EDL muscles were fixed to sticks corresponding to their original lengths, and were stored in 50% glycerine at -20°C. The muscles thus prepared retained their contractile properties for 4-6 weeks. The contraction of the fibres 1-2 cm long and 0.3-0.5 mm in diameter was evoked with a contracting solution containing 5 nM ATP-Ca^{++}. The changes in isometric tension were registered, through a signal converter, on a potentiometric recorder.

The significance of the differences between the experimental and control series was evaluated by the Student t-test.

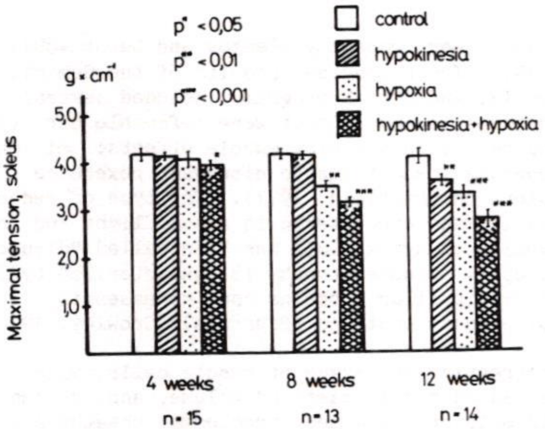
RESULTS

The body weight of the control animals rose from 200 g to 270 g in 12 weeks, whereas the increase was smaller in the rats kept under hypokinetic conditions, i.e. from 200 g to 233 g. The body weight of animals treated in the combined way was identical with those kept under hypokinesis, and no change in body weight was observed in the hypoxic group.

The weight of soleus and EDL muscles from animals kept under hypokinesis showed significant decrease as compared to the controls in the 8th and 12th weeks. The soleus muscle was more sensitive to the effect of hypokinesis than the fast EDL. In the hypokinetic group hypoxic treatment for 24 hr did not appreciably influence the weight of the muscles. In the hypoxic group no change in muscular weight was observed as compared to the controls.

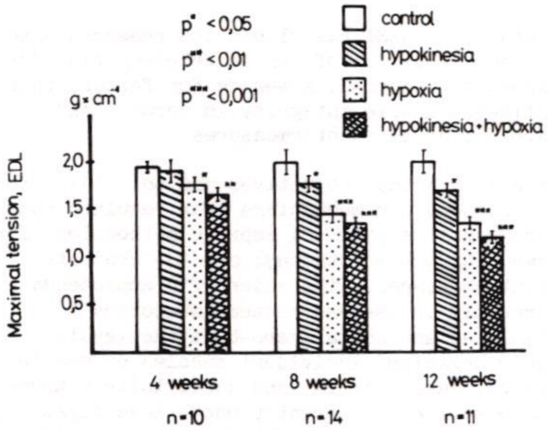
Examinations were made in each group to determine the dry weight of the muscles, however, no significant difference in water content was found. In the further step of the experiments the contractile properties of the glycerinated muscle fibres from animals of the control-, hypokinetic-, hypoxic- and combined-treatment groups were compared. The extent of contraction were referred to 1 cm fibrillar circumference.

In Fig. 1. the changes in the strength conditions of the soleus muscle are presented.



As a result of combined treatment a significant decrease in tension was observed as early as the 4th week, which was even more expressed in the 8th and 12th weeks. The significant decrease in tension took place only in the 8th and 12th weeks in the hypoxic groups, whereas significant decrease in strength was observed only in the 12th week in the hypokinetic groups.

In Fig. 2. the changes in the maximum tension of the fibrosis shown for the EDL muscle.



In the case of the EDL muscle much more significant changes were found than in the soleus muscle. Both the hypoxic and the combined treatment resulted in significant changes as early as the 4th week, which became highly significant in the 8th and 12th weeks. In the hypokinetic group significant decrease was found in the 8th and 12th weeks.

The rate of contraction was not changed appreciably significant by any of the various treatments.

In summary it can be established that immobilization decreases the body-weight growth tendency in the animals, which is, on the other hand, not influenced by hypobaric hypoxic treatment. Hypoxia results in the atrophy of the soleus muscle in a shorter time and of the EDL muscle in a longer time. This effect is manifested in the loss of weight and decrease in the contractile properties.

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MUSCLE AND THE PHYSIOLOGY OF LOCOMOTION

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INTRODUCTION

Among the problems associated with manned space flight is that of skeletal muscle atrophy. Degradation of skeletal muscles, especially of those used to counteract gravity on Earth, is commonly experienced in space flights. Its overt features include reduced volume of the limbs, particularly the lower extremities, and postflight reduction of muscle strength and exercise capacity.

Knowledge of the exact casual factors, pathogenesis, and practical methods of prevention of amelioration of the muscle atrophy of space flight is not sufficient to permit the rational development of dependable countermeasures that may be conveniently employed with a minimum of interference with the mission demands of space CREWS.

BACKGROUND

When the functional loading of the musculoskeletal system disappears, as during exposure to weightlessness, one of the body's responses is atrophy of skeletal muscles (Smith, 1978). Several authors reported postflight decreases in human muscle volume and mass, particularly in the lower extremities (Pestov and Geratewohl, 1975), and data from the Skylab series of space flights showed reduction in muscle strength of the limbs (Thornton and Rummel, 1977) and confirmed losses of mass of the leg muscles (Whittle, 1979).

Increased protein catabolism including breakdown of skeletal muscle has been a consistent finding in space flight. A reduction of the volume of the lower extremities and a persistent rise in urinary nitrogen, amino acids, and 3-methylhistidine excretion are some of the associated findings. Whether these changes are caused by zero-G or other factors in the space flight environment has not been fully determined, but weightlessness is generally thought to be the main etiologic factor (Bricker, 1979).

Numerous observations of American and Soviet space travelers, animals flown in space, and subjects of bed rest studies have generally demonstrated skeletal muscle atrophy. Associated effects include decreased muscle strength and tone and reduced work capacity (Pestov and Geratewohl, 1975). The biomedical results of the Gemini, Apollo, and Skylab programs included several features in common that were referable directly or indirectly to skeletal muscle effects: minimal to moderate loss of muscle nitrogen, pexercise tolerance (Dietlein, 1977). The type of reduced muscle mass encountered in space flight and in studies of hypokinesia has been called "disuse" atrophy. Disuse atrophy is characterized by loss of myofibrillar proteins and increases in collagenous proteins (fibrosis) (Crowley, 1976).

Progressive shrinkage of muscle cells, with decreased muscle mass and volume, and, ultimately, fibrosis of collapsed sarcolemmal sheaths are prominent histologic features of disuse atrophy (Robbins, 1967). The biologic mechanisms leading to this type of muscular atrophy are not well defined, nor is the question of possible prevention resolved. Most available evidence suggests that, despite attempts at intervention such as diet, hormones, and exercise, skeletal muscle atrophy continues throughout orbital flight featuring progressive loss of muscle and negative nitrogen balance (Whedon, 1978; Whedon et al., 1977).

The muscle atrophy experienced to date, in missions lasting from a few weeks to as long as six months, has apparently been reversible.

Since 1978, NASA has placed its research emphasis on the mechanisms of muscle deterioration during exposure to zero-G, a search for factors that influence muscle integrity in zero-G, and development of countermeasures.

NASA's technical objectives are to: (1) define the factors and mechanisms that regulate muscle mass and strength; (2) improve methodology for measuring muscle atrophy; and (3) evaluate countermeasures. The scientific approaches currently in use and contemplatedonses to simulated and actual zero-G at the levels of the whole organism, individual muscles or muscle groups, muscle cells, and subcellular components and molecules. Currently under investigation, or planned, are studies of tissue growth factors and their receptors, endogenous and exogenous anabolic stimulation of protein synthesis, a possible neurotrophic factor, and factors that may inhibit protein synthesis. Identification of intracellular proteases and definition of their influence on degradation of the contractile and structural proteins of muscle are also in progress or planned.

Although precise scientific documentation of its efficacy has not been possible for many cogent reasons, the available data and most expert opinion support the use of vigorous physical exercise during flight as a principal countermeasure against muscular deconditioning (Gazenko et al., 1981; Nicogossian and Parker, 1982). By 1975, the recommended inflight regimens prescribed three exercise periods daily for a total of 2.5 hours (Dodge, 1976). According to Thornton (1981), use of a combination of exercises and devices such as a passive treadmill, with the astronaut's body held by elastic cords so as to exert the equivalent of 1-G foot pressure, is the best way currently available to prevent or reduce some of the untoward effects associated with space flight, including muscle atrophy.

NASA's research to resolve the problem of muscle atrophy also include tests of various exercise regimens and devices programmed for some of the Space Shuttle flights, with the objective of improving techniques and equipment and determining optimum types, amounts, and schedules of exercises. Among the inflight experiments to be carried out in Spacelab 4 are those that will investigate biochemical, metabolic, and morphologic effects of weightlessness on skeletal muscle. In addition, NASA's Space Biology Program, which is separate from the mission-oriented Biomedical Research Program, includes studies of the effects of hypogravity on muscle biology. The Space Biology Program produces data that may well be applied to solution of problems of muscle atrophy.

SUMMARY

It is apparent that an adequate understanding of the patho-physiology and mechanisms involved in skeletal muscle atrophy associated with space flight is nonexistent. Consequently, impeded.

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THE NATURE AND CHARACTERISTICS OF A GRAVITATIONAL ATAXIA

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Disturbances of accuracy control of voluntary movements appear to be the constant consequences of the space flight and hypokinesia experiments: an increased time of motor tasks performance, a decreased precision of muscular efforts control, an increase of number of errors and diversity of the end positions of movements noted in the short-term space flights (1,5,7,8) and even more profound coordination disorders, showed up by an acute disturbances of upright posture, alterations of a posture sinergies and by the deep changes in a locomotor acts structure, observed after long exposures (2,3,4,6) suggest that the decrease of gravitational load serves as a trigger initiating the development of the significant shifts in activity of the motor control system. The mechanisms of these shifts that are logically evident are not understood yet. A decisive role in the genesis of the coordination disturbances in weightlessness is traditionally attributed to the muscular atrophy. However, the state of the movements control mechanisms influenced significantly by changes in the state of other parts of the motor system, namely, proprioceptors, spinal synergies, mechanisms of central integrations, which appear to be also affected greatly by hypogravitation, may be of a primary importance (8). For understanding the nature of coordination disturbances it seemed to be useful to provide a comparative study of the actual and simulated weightlessness effects in different parts of the motor system and motor control mechanisms as well.

Precise kinematic and electromyographic characteristics of voluntary movements differed by their organization - programmal and tracking; by speed - fast and slow; by controlled parameters - efforts, positions, integrated amplitude of EMG and by muscle groups performing the task - eye muscles, leg muscles, have been studied before, during and after exposures to simulated weightlessness (antiorthostatic bedrest, immersion) and after a short-term (7 days) and long-term (75-211 days) space flights as well.

The results confirmed the fact that the ataxic disorders are a natural consequences of gravitational unloading: the pronounced disturbances of accuracy control characteristics of all the movements under study have been observed in all the situations, that were mentioned. Thus, the magnitudes and numbers of errors, as well as variability of performance after 7-day immersion have increased significantly in the isometric contractions of leg muscles when subjects performed a task to control muscular efforts. This increase was more prominent in movements of dorsal flexion than in plantar one. The same results have been obtained when the errors and variability of the "isotonic" movements performance were analyzed after immersion. Alterations in movements structure characteristic to ataxia have been noted additionally: the task movements lost their fast and smooth patterns acquiring instead the pattern of slow movements, that gradually approximated the foot to a target.

A sharp decrease of motor control system abilities has been revealed when subjects were performing a special test of muscular efforts gradation during which they executed a series of the gradually increasing efforts over the minimum-to-maximum range with the minimal differences in values of the adjacent movements. In this case the number of gradations decreased significantly; the minimal efforts as well as an average increment of efforts in adjacent movements increased greatly. Even more pronounced were changes produced by immersion and weightlessness in characteristics of the complex-organized motor acts such as the eye-head coordination, corrective postural responses, etc.

The speed and dynamics of development of the coordination disorders bore a close resemblance to those of the development of the muscular tone shifts, recorded in the same experimental situations. In fact, the changes in the accuracy characteristics of the movement during immersion as well as a decrease of the muscle tone have clearly been observed beginning from the 2nd day of an exposure (Fig. 1); under hypokinetic conditions the both types of disorders developed slower, reaching the maximum by 15-30 days. The tonic alterations and the decrease of the motor control abilities were pronounced especially in the antigravitational musculature and movements executed by those muscle groups. This fact allowed to suggest that the atonia complicating the control processes may play an important role in the genesis of the ataxic disorders following gravitational unloading. A special series of experiments has confirmed a validity of this suggestion for the disorders, recorded in upright posture maintaining. A quantitative study into posture disorders manifestations induced by 7-day immersion in three experimental situations differed by the number of degrees of freedom (posture of a "thinker" with only one degree of freedom in an ankle joint, vertical standing on a platform and standing on a six support points) demonstrated clear correlation between the depth of disorders and the number of controlled links. Under conditions of artificial fixation of all the links except one in the posture of a "thinker" the posture disorders were minimal, and conversely, on increasing the number of degrees of freedom (in case of a six point support) the disturbances were the most prominent.

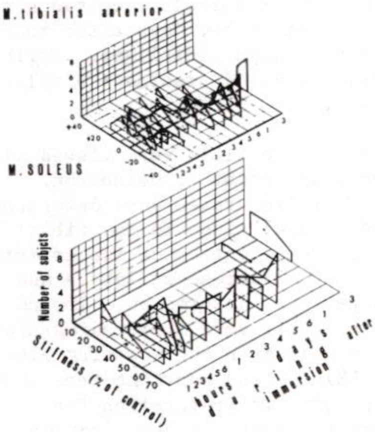

Fig. 1. Alterations of muscle stiffness of leg muscles at rest during 7-day water immersion. The figure shows the clear decline of soleus muscle stiffness during first 3-6 hours of immersion and absence of these changes in stiffness of m. tibialis anterior.

However, an increase in level of coactivation of antagonists, revealed in the same conditions, an occurrence of the analogous changes in characteristics of eye-head coordination and in reactions of maintaining an electromyographic activity level cannot be associated directly with muscular tone alterations. These disorders are more likely to be linked to alterations in regulatory systems abilities caused by shifts in activity of afferent inputs. In particular, the marked increase in the gain of VOR and in the level of the antagonists coactivation could be a natural consequences of an increasing excitability of central structures deprived, with the decrease of proprioceptive input activity, of normal level of inhibitory influences. Actually, an increase of excitability of spinal mechanisms and vestibular apparatus under hypokinetic conditions was confirmed by the studies of the parameters of T- and H-reflexes of soleus muscle and the threshold of the galvanic vestibular response.

Thus, an experimental analysis performed, determining the quantitative spectrum of accuracy control disorders in movements of different type, allowed to qualify them as atactic disorders and made it possible to define the main factors in the genesis of this type of ataxia which, judged from the analysis results, seem to be the changes of afferent inputs activity and linked to them a drop of tone of extensor muscles and a rise of excitability of central integrative mechanisms.

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ARRESTED BONE FORMATION DURING SPACE FLIGHT RESULTS IN A HYPOMINERALIZED SKELETAL DEFECT

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ABSTRACT

Rats flown in space for 19 days displayed a decrease in periosteal bone formation rate from 16.1 to 48.7% in tibio-fibular cross-sections. The least effect of space flight occurred at the posterior eminence of the tibia where the normal formation rate was the highest owing to intrinsic muscle forces. The arrest line separating flight and postflight bone was found to contain considerably fewer hydroxyapatite crystallites than surrounding bone. This hypomineralized defect appeared to be the result of an abnormal organic matrix. The arrest line was never found along the posterior aspect of the tibia.

INTRODUCTION

Previous studies have revealed a 44-47% decrease in periosteal bone formation rate in the tibia of rats flown in space for 18.5-19.5 days (1,2). This decrease in bone formation rate was found to be rapidly reversed upon return to Earth. Normal and elevated formation rates were recorded for the three-week period begun 3 to 5 days after touchdown (1,2). These previous investigations employed areal measurements to determine periosteal formation rates. Values of the appositional rate of bone formation at selected anatomical sites around the tibial cross-section were not computed.

Arrest lines, which occur when bone formation ceases and is later reinstated, were often found separating flight and postflight bone. The arrest line in the flight animals was originally delineated by light microscopy as a narrow, smooth distinct feature which did not stain for acid phosphatase activity (1). More recent studies have found the arrest line to be less mineralized than surrounding bone matrix (3). However, ultrastructural features of the arrest line have not yet been delineated.

The objectives of this investigation were a) to determine the effect of space flight on the appositional bone formation rates at selected anatomical sites around the periosteum of tibial cross-sections, and b) to determine the location and ultrastructure of the arrest line.

METHODS

Undecalcified transverse sections were obtained from the tibio-fibular junction of rats flown aboard the Cosmos 782 and 936 biosatellites. Details of the experimental conditions on these biosatellites have already been published (1,4). Briefly, animals received tetracycline injection 1 or 3 days prior to the 19-day space flight. One group of rats was sacrificed soon after landing. Other animals received a second tetracycline injection 3-4 days after touchdown and were killed 25 days postflight. Earth based control animals received treatment similar to the flight rats.

Sawed sections of the tibia were viewed with incident beam fluorescent illumination. Appositional formation rates were determined along selected radial lines in the tibial cross-section. The location of the arrest line in relation to the fluorescent labels and anatomical aspect was determined. Ground sections of the tibia were microradiographed and subsequently examined in the scanning electron microscope (SEM). These sections were then embedded in plastic in preparation for ultramicrotomy and transmission electron microscopy (TEM). Selected sections were decalcified prior to embedment for TEM.

RESULTS

The control periosteal formation rates varied from 4.7 μm/da along the anterior aspect of the tibia to 11.4 μm/da at the posterior eminence (Fig. 1). The effect of space flight was to decrease the formation rate by 16.1 to 48.7%, the least effect seen where the normal formation rate was the highest.

An arrest line was found paralleling the periosteal surface, superimposed on or located just within the second tetracycline label. The arrest line was discontinuous, never appearing in the region of the posterior eminence. It was found in microradiographs to be a radiolucent line. SEM of ground sections revealed the arrest line as a groove-like feature. The depth of the groove was less than 10% of the section thickness. In the TEM the arrest line was found to be a zone about 3 μm wide. The bone in the arrest zone contained considerably fewer hydroxyapatite crystallites than the adjacent flight and postflight bone matrix. However, the pericanalicular bone appeared to be mineralized normally. The arrest zone appeared to contain fibrous material, presumably collagen generally aligned parallel to the defect. A convoluted feature was also always seen in the arrest zone.

DISCUSSION

Previous studies have already shown that the effect of space flight on bone formation differs in different bones. No effect was found in the mandible (5); the decrease in formation rate in the humerus was about 1/2 that found in the tibia (2). These previous results indicated that the effect of space flight was greater in weight bearing bones. Our present results demonstrate that variations in the effect of space flight on formation rate can be found within the same cross-section of a bone. The decrease in formation rate was the least in the region of the posterior eminence where the normal formation rate was the highest owing to the contribution of intrinsic muscle forces. The relative influence of gravity-related forces on bone formation was greatest along the anterior aspect of the tibia. The fact that bending forces in the tibia would be expected to result in higher stresses along the periosteal than endosteal surface might explain why little effect of space flight was found along the endosteum (1).

The arrest line was never found at the posterior eminence where the decrease in formation rate was only about 16%. The location of this defect in areas were the reduction in averaged formation rate was highest confirms that it is, indeed, a feature marking the cessation of bone formation.

Ultrastructural study of the arrest line indicates that it is a hypomineralized defect resulting from abnormal precipitation of hydroxyapatite crystallites. The fact that the pericanalicular bone in the arrest zone is normally mineralized suggests that the defective bone is the result of abnormal matrix that does not properly mineralize. As a result of being hypomineralized, the arrest zone is more susceptible to abrasion as seen in SEM of ground sections and to fracture as observed during handling of the specimens (Table I).

In conclusion, cessation of periosteal bone formation during space flight does not occur where intrinsic muscle forces continue to act on the tibia. The skeletal defect resulting from arrested bone formation during space flight is a mineralization defect which appears to be related to an abnormal organic matrix.

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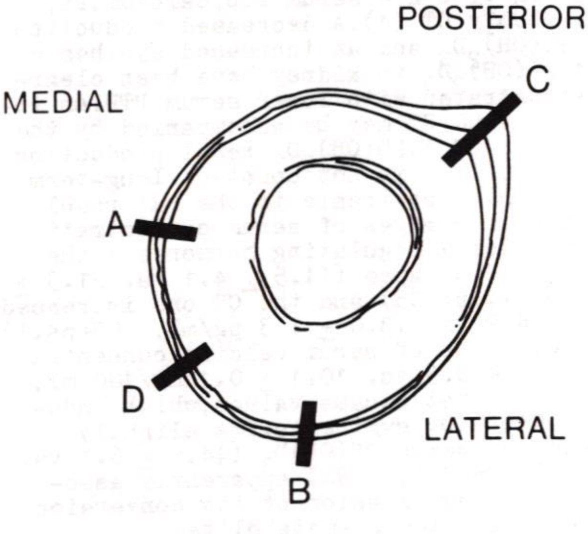
Table I

CHARACTERISTICS OF THE ARREST LINE

1. 3 µm wide
2. Hypomineralized
3. Abnormal organic matrix
4. Less resistant to abrasion than normal bone
5. Plane of mechanical weakness



Site	Control Formation Rate, µm/da.	% Change from Control Rate in Space
A	5.6	-46
B	5.1	-48
C	11.1	-16
D	4.7	-49

Figure 1. Schematic showing a cross-section of the rat tibia. The control formation rates and % changes from the control rate resulting from space flight are given at selected sites in the cross-section.

SPECIFIC REGULATION OF CALCIUM-PHOSPHORUS METABOLISM DURING HYPOKINESIA AND WEIGHTLESSNESS BY VITAMIN D_3 ACTIVE METABOLITES

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Calcium metabolism, growth and remodelling of bone tissue are regulating by hormonal system including parathyroid hormone (PTH), vitamin D and calcitonin (CT). Vitamin D functions exlusively after its biotransformations to hormonaly active form(s) : 1,25-dihydroxyvitamin D (1,25$(OH)_2D_3$) and possibly 24,25-dihydroxyvitamin D (24,25$(OH)_2D_3$) (1,2).

It was previously shown that in hypokinetic rats the production of 1,25$(OH)_2D_3$ suppressed and the one of 24,25$(OH)_2D_3$ enhanced in kidney as well as the accumulation of the last metabolite diminished in intestinal mucosa and bones (3).

The major regulators of vitamin D metabolism in vivo are serum PTH, calcium, Pi, and probably CT (4). A decreased production of 1,25$(OH)_2D_3$ and an increased synthesis of 24,25$(OH)_2D_3$ in kidney have been clearly demonstrated with lower serum PTH. An increased serum CT may be accompanied by the diminution of 1,25$(OH)_2D_3$ renal production, but this effect is not constant. Long-term (one month) hypokinesia in the rat resulted in such changes of serum concentrations of calcium-regulating hormones : the PTH level decreased (11.5 ± 4.1 vs. 21.3 ± 2.4 mIU/ml, $p<.05$) and the CT one increased (21.4 ± 4.2 vs. 13.0 ± 1.3 pg/ml, $.05<p<.1$). The diminution of serum calcium concentration (9.0 ± 0.3 vs. 10.1 ± 0.1 mg/100 ml, $p<.01$) have not became values, which induced 1,25$(OH)_2D_3$ synthesis. The slightly increase of serum 25$(OH)D_3$ (44.9 ± 6.1 vs. 32.9 ± 3.7 ng/ml, p NS) apparently associated with suppression of its conversion to dihydroxylated D_3-metabolites.

Prolonged hypokinesia caused a decrease of calcium absorption in the small intestine in vitro, especialy in young rats (Table 1, Figure 1). This effect may be a result of declined circulating level of 1,25$(OH)_2D_3$. It must be emphasized that active calcium transport largely decreased when intensive growth had been ended. Obviously the diminished need of this mineral with delay of bone growth in hypokinesia or in connection with ageing is conditioned by hormonal changes (a decrease of concentrations of 1,25$(OH)_2D_3$, PTH and an increase of CT level in serum). It is possible that such changes in endocrine system responsible for the maintenance of calcium homeostasis are connected with the inhibition of bone remodelling and turnover under these conditions.

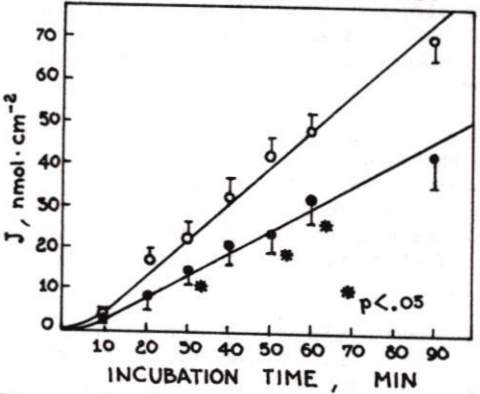

Figure 1. Calcium absorption by uneverted sacs of the small intestine of adult rats after one month of hypokinetic exposure.

It was previously demonstrated that the suppression of rat bone growth during severe long-term hypokinesia was attended by a decline in bone density, a decrease of the calcium, phosphorus and hydroxyproline content per bone volume unit, a diminution of the Ca/P and a slightly increase of Ca/hydroxyproline ratio in bone tissue (5). The prophylactic administration of 24,25$(OH)_2D_3$, 1,25$(OH)_2D_3$ and especialy of their combination to hypokinetic rats produced optimized effects on the bone chemical composition (3-5). This influence of active D_3-metabolites may be associated with an enhance of bone mineralization. In this context, it seemed interesting to investigate the effects of 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$ on histomorphometric parameters of rat bones in prolonged hypokinetic exposure.

The study demonstrated a significant inhibition of growth of long bones in lenth and width, smaller thickness of the diaphyseal cortical plate, smaller thickness of the growth cartilage plate, and osteoporosis of the trabecular bone spongiosa. The administration of 24,25$(OH)_2D_3$ or the composition of 1,25$(OH)_2D_3$ and

Table 1. Intestinal calcium transport and serum calcium levels in hypokinetic rats.

Group of animals	Serum Ca, mg/dl	Calcium transport			
		$^{45}Ca_{in}/^{45}Ca_{out}$	J_{ms}	J_{sm}	Accumulation into the tissue
			nmol·cm^{-2}·hr^{-1}		
Young rats					
Control	10.2 ± 0.2	5.42 ± 0.61	30.6 ± 4.1	5.52 ± 0.90	24.1 ± 3.3
Hypokinesia	9.4 ± 0.2*	3.48 ± 0.47*	18.7 ± 2.0*	5.48 ± 0.34	13.2 ± 2.8*
Adult rats					
Control	9.8 ± 0.1	2.03 ± 0.34	8.26 ± 0.90	4.17 ± 0.44	3.86 ± 0.82
Hypokinesia	8.5 ± 0.3*	1.75 ± 0.59	6.76 ± 0.59	3.88 ± 0.70	3.04 ± 1.10

Active Ca transport by everted sacs of the small intestine in vitro was measured as described elsewhere (5); J_{ms} and J_{sm} = the Ca flux from mucosa to serosa and from serosa to mucosa respectively. The initial mass of young and adult rats was 98.5 ± 6.3 and 259 ± 6.2 g respectively. The duration of hypokinesia was one month. Values are mean ± SE. * $p < .05$ from control value.

$24,25(OH)_2D_3$ to the hypokinetic rats resulted a significant restore in the rate of linear growth as well as in the volume and weight gain of long bones (Table 2). At the same time, one failed to demonstrate the positive effect of these D_3-metabolites upon the width of growth cartilage plate whereas the volume of primary and secondary spongiosa reached the control level in case of $24,25(OH)_2D_3$ administration and enlarged when $1,25(OH)_2D_3$ was used (Table 3). Normalization of volume of the trabecular bone spongiosa in hypokinetic rats given $24,25(OH)_2D_3$ was the result of a width and sizes increase of bone trabeculaes, but not their number. Apparently it associated with the stimulation by this metabolite first of all bone mineralization. This ossified effect of $24,25(OH)_2D_3$ prevented osteoporotic changes of the trabecular bone however its structure became more rough. $1,25(OH)_2D_3$ stimulated the ossification too, but its resorptive action obviously was dominated.

The interaction of systemic and local factors in disorders of calcium metabolism and bone state in hypokinesia is unclear. Our data allow the conclusion that an alteration of calcium absorption in the small intestine and a delay of bone growth and mineralization in hypokinetic rats may be associated with a change in the production of vitamin D_3 dihydroxyleted metabolites and/or a modification in the sensitivity of target tissues to these. The certian differences of effects of hypokinesia and weightlessness on rat bone tissue does not allow to extrapolate with confidence obtained results to the situation of real spaceflight though our previously data indicates a slightly decrease of calcium absorption in vitro by the small intestine of suspended rats.

Table 3. Relative volume of primary and secondary spongiosa in the proximal end of the tibia.

Group of animals	Volume of spongiosa in per cents
Control	9.9 ± 0.5
Hypokinesia	7.1 ± 0.4*
Hypokinesia + $1,25(OH)_2D_3$ (0.15 µg)	8.8 ± 0.9
Hypokinesia + $24,25(OH)_2D_3$ (1.25 µg)	10.0 ± 0.6

Table 2. Evalution of effects of vitamin D_3 metabolites on the parameters of bone growth.

Vitamin D_3 metabolite	Dose, μg	Growth in lenth	Growth in width	Volume gain	Mass gain
$1,25(OH)_2D_3$	0.03	-	-	±	±
$1,25(OH)_2D_3$	0.15	-	+	±	-
$24,25(OH)_2D_3$	0.25	+	+	+	+
$24,25(OH)_2D_3$	1.25	++	++	+	++
$1,25(OH)_2D_3$ + $24,25(OH)_2D_3$	0.03 + 0.25	++	++	++	++
$1,25(OH)_2D_3$ + $24,25(OH)_2D_3$	0.03 + 1.25	+	±	+	+

(++) = maximum positive effect, (+) = positive effect, (±) = possible positive effect, (-) = no effect.

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THE EFFECTS OF IMMOBILIZATION ON CORTICAL BONE IN MONKEYS (M. NEMESTRINA)

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INTRODUCTION

In space or simulated conditions of weightlessness, man reveals a significant loss of calcium. Weightlessness induced bone loss has been of considerable scientific and operational interest during the last decade. During this period, the immobilized primate has been applied in our laboratory to develop a suitable MODEL for a multidisciplinary evaluation of disuse osteoporotic processes. During extended periods under zero-G conditions, the static stress distribution in the bone and its metabolic requirements are altered. In outer space, the effective terrestrial acceleration is reduced and except for transient dynamic loads due to physical exertion, the body is suspended in equilibrium. This marked reduction in mechanical stress, combined with physical inactivity, leads to a significant loss of calcium as evidenced by local or uniform atrophy of trabecular bone. Ultimately, osteoporosis may develop. Earthbound periods of immobilization or long-term bedrest mimic this condition by producing a local disorder of bone known as disuse osteoporosis. Osteoporosis appears to be due mainly to an increase in the rate of resorption without a compensatory increase in bone formation. The pathologic picture of disuse osteoporosis is no different from that of other osteoporoses such as nutritional, endocrine, and senile osteoporosis, in which both cortical and trabecular bone is affected. Still, the underlying mechanisms of this alteration are not known and the consequences of adaptive bone loss have not been fully evaluated. Our multidisciplinary studies were designed to evaluate structural and compositional changes of tibial cortical bone during short- and long-term restraint of monkeys, and during different periods of recovery after restraint. A principal objective of our studies has been to develop useful adjuncts to human research through studies of the osteoporotic process, and factors which relate to the evaluation of potential risk factors, and factors dealing with the reversibility of this process. Defining various stages and temporal events in the development of disuse osteoporosis as well as validation of noninvasive measures of bone quality can have important applications in future research with humans.

MATERIAL AND METHODS

Short-term restraint (up to 4 weeks) studies were performed with rhesus monkeys (M. mulatta); both short-term and long-term restraint (4 weeks to 7 months) studies were done with pigtail monkeys (M. nemestrina). A hypogravic-hypodynamic evironment was simulated by restraining the animals in a semirecumbent position according to the techniques of Howard et al. (1). This procedure reduces normally occurring stresses in the lower legs. In general, the animals complete the studies without abrasions or contusions. There was little body weight loss. Since in all these studies there was no evidence of gastric ulceration or gross pathology in the heart, liver, lungs, kidneys, and adrenals, it is very obvious that this restraint system is atraumatic and therefore suitable for physiological investigations. Noninvasive techniques such as X-rays, photon absorptiometry, computed tomography, and bone bending stiffness measurements were applied to evaluate the effects of short- and long-term restraint on structure and mechanical properties of bone. In addition, histopathology and electron probe analysis of the distribution and composition of bone minerals was studied.

RESULTS

Immobilization of adult monkeys results in characteristic losses of tibial cortical bone. Radiographically, disuse osteoporosis is associated with resorption cavities at the endosteal surface, with an apparent splitting of the inner cortex, well-defined intracortical striations, subperiosteal loss, and surface erosion extending to the juxta-articular area (2). The intracortical striations seen in radiographs resemble the resorption cavities which are characteristic of senile osteoporosis, hyperparathyroidism, and renal osteodystrophy. The losses during 6 months of restraint tended to occur predominantly in the anterior portion of the proximal tibiae. Tomography demonstrated endosteal widening and, qualitatively, a reduction of mineralized tissue (2). Norland bone mineral analysis revealed that the proximal tibiae show the greatest losses of bone mineral which range between 23% and 31% after 6 months of restraint (3). In recovering monkeys even after 15 months, bone mineral content did not necessarily return to normal levels. In addition to X-rays, tomography, and bone mineral analysis, the <u>in vivo</u> bending rigidity was studied (3). Bending rigidity in the anterior-posterior plane was measured by an impedance probe technique. Forced vibrations of the bones were induced with an electromechanical shaker, and force and velocity at the driving point were determined. After 6 months of restraint, the largest changes of bone stiffness ranged from 36% to 40%. Approximately 8 1/2 months were required for restoration of normal bending properties, even though mineral content in the cross section was not restored.

Histological changes of cortical bone occur very rapidly. The demonstration of numerous and extensive resorption cavities in areas of haversian bone, particularly in the anterior proximal tibiae confirm the radiographic and tomographic data. Compact bone in this particular area consists principally of closely packed secondary and tertiary generations of haversian systems delineated by an outer circumferential layer of lamellar bone. Within one month of restraint, resorption cavities can be seen at the endosteal surface and within the cortical layer of bone. The most unique changes occur in single osteons which lose their typical lamellar pattern and reveal an accumulation of amorphous material at the boundary of the deteriorating osteon (3). This material has a strong affinity to metachromatic dyes. Electron probe analysis of this stage of a deteriorating osteon, reveals a loss of 25% to 30% of calcium and phosphorus (4). After 10 weeks of restraint the external lamellar bone is thin and irregular. There are large resorption cavities subperiosteally and throughout the cortex. Some resorption cavities are lined by mononuclear cells, others contain mostly components of bone marrow and hematopoietic tissue. Additionally, numerous fusiform cells are present. Some larger resorption cavities still contain portions of unresorbed bone. Occasionally, multinucleated osteoclasts are detectable within the resorption cavities. After 6 months of recovery there is appositional growth in the cortex, recognizable by the presence of several arrest lines, sometimes deep in the cortical tissue. Newly formed lamellar bone appears to be replaced by haversian systems, so that after 15 months of recovery the cortex consists mostly of first generations of haversian systems. After 40 months of recovery from restraint, cortical bone in the proximal tibia appears normal with numerous secondary and tertiary osteons.

Microradiographs of tibial cross sections exhibit the structural components of bone. The light and dark osteons reflect the radiographic or mineral density of the material. In normal tissue there are only few dark, younger osteons, reflecting a low turnover rate of osteons. During restraint, there is an absence of dark osteons. During 6 months of recovery, all areas of former resorption cavities are filled with new, less radiographic dense bone. Such areas appear as huge single osteons. Image enhancement by back scattered electron image confirms the different density in individual osteons (5).

Osteons from control, restrained, and recovered animals were ranked subjectively in regard to their radiographic density, and analyzed with the electron probe. The largest differences in the bulk mineral (Ca, P, F) content between the lightest and darkest osteons is 18% to 20%. In immobilized animals, additionally, the average bulk mineral content in all osteons is decreased about 4% to 6%. No preferential loss of specific elements could be detected. During recovery, bone shows again normal levels of mineralization (6).

DISCUSSION AND CONCLUSION

The present studies are a multidisciplinary approach to evaluate the pattern of development of osteopenia in the immobilized monkey model.

Cortical bone is potentially high reactive, and during immobilization, bone loss occurs rapidly and reflects the imbalance between resorption rate and bone formation rate. Early signs of bone deterioration within 1 month are only detectable in histological preparations. Large portions of osteons undergo structural and compositional changes which are thought to be the initial stages of resorption and subsequent cavity formation. In addition to bone loss through resorption cavity formation, the remaining hard tissue undergoes demineralization. Pavlova et al. (7) also reported decreases in tibial mineral saturation in young dogs immobilized by confinement.

The responses to a hypodynamic environment could have serious consequences. The data show that there can be considerable decreases in bone bending stiffness (3).

Earlier studies of disuse osteoporosis with documented bone loss in man (8) concluded that there is no restitution of hard tissue after remobilization; but our investigations show that cell proliferation and cellular activity increase sufficiently to restore normal properties of compact bone. However, the recovery process is slow. Three patterns of recovery seen include a) massive new bone growth with refilling of the resorption cavities, b) re-mineralization of older bone, and c) lamellar bone apposition, peri-osteally. Responses of the primate model suggest primary focal regulation of bone. Initial stages of resorption cavity formation occur in whole osteons, and the reversibility of bone loss after disuse osteoporosis is associated with refilling of each cavity with new bone. At each site of sequential remodelling, the volume of new bone formed is comparable to the volume previously resorbed.

In conclusion, the immobilized monkey model can provide answers specifically in regard to the reversibility of osteoporosis.

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MOTION SICKNESS SUSCEPTIBILITY RELATED TO ACTH, ADH AND TSH

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INTRODUCTION

Variations in individual susceptibility to motion sickness have prompted the search for physiological and biochemical parameters which might be of value in predicting an individual's susceptibility to motion sickness or contributing to our understanding of neurochemical mechanisms underlying the malady. A year ago we reported our research on the influence of stressful motion on the circulating levels of human growth hormone (hGH), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), cortisol, norepinephrine and epinephrine. Significant increases in the levels of ACTH, cortisol, epinephrine, and norepinephrine were noted. Considering the possibility that certain hormonal responses to stressful motion might serve an adaptive role enabling man to function in a stressful motion environment, we have now measured basal levels of hormones in our experimental subjects before presentation of a stressful motion stimulus. Subjects classified as less susceptible to motion sickness were compared with those of higher susceptibility to test the hypothesis that endogenous levels of certain hormones might be indicative of an individual's susceptibility to stressful motion.

METHODS

Five males and three females, aged 20 through 44 years, participated in the study. All subjects were in normal health and had normal vestibular function. Each of the subjects was at least moderately susceptible to motion sickness as measured by three different provocative motion tests. These tests included a fixed RPM coriolis sickness susceptibility index (9), an off-vertical-rotation test (4), and a sudden-stop test with eyes open (3). The motion sickness stressor used to measure an individual's susceptibility to stressful motion was generated by requiring the blindfolded subject to execute standardized head movements while seated in a rotating chair. A staircase velocity profile was used with 40 head movements being performed at each successive 2 RPM step. Each subject completed two trials on this stressor. They began the staircase profile at 1 RPM. The ceiling on the velocity profile was 35 RPM. The subject was continually monitored for signs and symptoms of motion sickness and the test trial was terminated when the Malaise III level of motion sickness was reached (2). The symptoms present at the end of the test trial and the number of head movements were recorded. Each subject then returned to the laboratory each week for the next ten weeks for a blood draw. The blood samples were used to measure blood levels of ACTH (8), cortisol (1), vasopressin (10), TSH (6), angiotensin (5), aldosterone (7), and hGH (11). After each blood draw the subjects were tested on the rotating chair as part of a separate experiment (in press). Thus, each subject anticipated the same degree of stressful motion on each visit to the laboratory.

RESULTS AND DISCUSSION

The levels of ACTH and vasopressin measured before exposure to stressful motion were two-fold higher in the less-susceptible subgroup (Table 1). Cortisol showed no significant difference but tended to move in a direction opposite to that of ACTH. This behavior is not unusual because cortisol normally inhibits the release of ACTH. TSH was elevated in the less-susceptible subgroup but the difference was not statistically significant. No significant differences were noted in the levels of angiotensin or aldosterone (data not shown). It is remarkable that the differences between the susceptibility subgroups were greater for a given hormone than for any of the changes induced by exposure to stressful motion. This observation suggests the need for further consideration of the hypothesis that endogenous levels of certain hormones may be indicative of an individual's susceptibility to stressful motion.

Because of the episodic and circadian influences on the release of the hormones we measured, it is necessary to discuss our experimental design in further detail. Our design entailed the determination of each subject's motion sickness susceptibility using the staircase velocity profile motion stressor (n=2), followed by an analysis of multiple blood samples (n=10) obtained at weekly intervals. Based on our many years of determining an individual's motion sickness susceptibility and on the basis of the results of the three additional provocative motion tests employed, we feel confident these tests adequately characterize an individual's susceptibility and that an individual's susceptibility to motion sickness is a fairly consistent behavioral measure. However, we measured high variability in the levels of each hormone for each subject. For instance, it was possible to find individual ACTH values ranging across a five-fold range (average = 2-3 fold). Because of this variability we felt

Table I Motion Sickness Susceptibility and Hormone Levels

Subject[1]	Susceptibility	Basal Hormone Levels			
	Head Movements[2]	ACTH[3]	Vasopressin[3]	TSH[4]	Cortisol[5]
JP	105	47 + 11	2.8 + 0.5	3.2 + 0.6	9.0 + 2.0
AN	110	42 + 19	4.6 + 1.4	3.9 + 1.0	10.6 + 3.3
JS	155	36 + 14	3.0 + 1.4	3.1 + 0.7	16.3 + 5.9
LB	180	37 + 13	2.7 + 1.3	3.7 + 1.0	10.9 + 3.4
Average	138 + 18	40.5 + 2.5	3.3 + 0.4	3.5 + 0.2	11.7 + 1.6
BB	198	80 + 25	9.4 + 7.9	3.8 + 1.0	7.5 + 4.0
PE	212	71 + 20	3.9 + 1.4	4.8 + 1.6	8.8 + 1.8
TL	275	78 + 20	7.6 + 3.7	4.8 + 0.6	7.0 + 1.4
RL	425	62 + 17	3.8 + 1.4	2.9 + 0.6	12.8 + 3.7
Average	278 + 52	72.8 + 4.1**	6.2 + 1.4*	4.1 + 0.5	9.0 + 1.3

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[1] JP, PE, and TL are female. Individual entries represent means + 1 SD (n = 10). Averages represent means + 1 SEM. * $p < 0.05$, ** $p < 0.01$ by comparison with the high-susceptibility subgroup (unpaired t-test). [2] n = 2, [3] pg/ml, [4] IU/ml, [5] g/dl.

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it was necessary to obtain repeated measures of blood hormone content so that we might be able to characterize an individual subject's average daytime hormone level.

Further research is needed to clarify whether these hormonal differences are related by cause or effect to the individual's motion sickness susceptibility or simply correlated with that susceptibility. Experiments are currently underway at the Johnson Space Center with drugs which profoundly influence the pituitary-adrenal-cortical axis (dexamethazone and metyrapone) to determine the precise role of ACTH in motion sickness.

The possibility remains that individuals possessing higher resistance to motion sickness might also display significantly or entirely different neurochemical profiles as compared to susceptibles. ACTH, vasopressin, TSH, and other as yet unrecognized factors may all be important neuromodulators in this regard. Because of the close biological and biochemical relationships between ACTH, vasopressin, enkephalin, beta-endorphin, melanocyte stimulating hormone and beta-lipoprotein, it is also possible that other neuromodulators may play a role in determining a subject's susceptibility and might be coreleased with the hormones we have measured.

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DISTRIBUTION OF FLUIDS IN THE BODY OF THE CENTRIFUGED RAT

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Chronic exposure to forces of multiple g's by centrifugation should alter the fluid equilibria established at terrestrial gravity as has been found to be the case in the weightless rat (3). Many of these alterations would be simple reversible responses to changes in the existing physical forces. But exposure to an elevated g-level throughout the period of rapid growth might result in some persisting changes. Here we have looked for such persisting changes in the rat by comparing a group centrifuged as adults with groups centrifuged for prolonged intervals starting shortly after weaning.

METHODS - Female Sprague-Dawley rats were kept at 22 ± 2°C with 12 hours of light and 12 of darkness per day and with food (Purina Laboratory Chow) and water available ad libitum. The experimental groups were centrifuged chronically at 4.2 times terrestrial gravity with controls at terrestrial gravity.

In the Growth Study the rats were 35 days old and ∼70 gm live mass at the start of centrifugation. Random samples (usually 8 animals) from each group were sacrificed for body composition studies at 0, 28, 63, 105, 179 and 308 days after the start of centrifugation. In the Adult Study the rats were ∼4 months of age and ∼325 gm live mass at the start of centrifugation. At the end of 60 days of chronic centrifugation centrifuged and control groups were sacrificed for body composition studies. Other aspects of the growth study (5) and the adult study (6) have been published earlier.

After decapitation and bleeding out, each rat was sheared closely with animal clippers and dissected into the following components: heart, liver, kidneys (Growth Study only), lungs with trachea (Growth Study only), gastrointestinal tract corrected for content (gut), brain and spinal cord (CNS), skin, total skeletal muscle and total bone. On each component water was determined by freeze-drying and fat content by Soxhlet extraction with petroleum ether (BP 30-60 C). Water and fat of the total body were obtained by summation. Further details are provided in (4).

The bleeding out technique expelled 61.2 ± 1.4% (mean of 8 rats ± SE) of the blood volume calculated as 7% of total body mass less fur and gut content (1). This minimized errors due to unknown volumes of blood within other components. Also, the interval of approximately 1 to 3 hours between termination of centrifugation and sacrifice insured that reversible changes with short time constants would be reversed before body composition analysis occurred. Thus it is unlikely that we were looking at short term changes in either blood volume or in other fluid compartments within the components which we examined.

RESULTS AND DISCUSSION - The results are summarized in Table 1. Kidneys, lungs, CNS and bone showed no changes and have been omitted. The table contains 2 columns of data on absolute masses (grams of water and solids) and 2 on relative masses (water fraction of component and fraction of total water in component). Wet mass changes are determined by the changes occurring in water and solids. The relative masses in general reflect changes in the absolute masses but the logic connecting the two is frequently obscure, probably because of statistical variations.

The absolute masses of total fat-free body ↓, gut → and muscle ↓ responded similarly to centrifugation in both Growth and Adult Studies. Heart and liver both showed decreases in the Adult Study but were constant in the Growth Study. Skin wet mass and skin mass of water were greater than control levels in the Growth Study.

The data on water fraction of individual components in Table 1 show increases in 4 instances, i.e., liver (two), gut and muscle which are attributable to disproportionate changes in water and solids during centrifugation. With respect to the relative distribution of total body water, since the total fat-free body mass decreased during centrifugation, components which either remained constant or increased in mass eventually contained a larger fraction of the mass of total water. Thus, the fraction of total water in gut and skin went up as a result of centrifugation and that in muscle went down.

Since the data in Table 1 were obtained by comparing smaller centrifuged animals with larger control animals, the observed differences could be attributed to either the change in body size or to centrifugation per se. To segregate the roles of these two causative agents we have applied to the Growth Study the "allometric equation" $Y = aX^b$ which is regarded as a simple and satisfactory model of individual component growth relative to total body growth (2). The double logarithmic plot employed in Fig. 1 linearizes the relationship, the slope of each line yielding the respective value of the exponential constant (b) for each component. The curve for total body water in Fig. 1 is representative of most body components in that the differences between centrifuged and control groups shown in Table 1 disappear and there is a single population which is affected only by log FFBM. Possibly centrifugation during growth directly stimulates a reduction in growth of total body mass and then most individual components, acting secondarily, participate proportionally in that process. At any rate, in this type of plot control rats and centrifuged rats fall close to a single line with respect to most individual components.

By contrast skin values for fat-free wet mass, water and solids show two populations in the figure, the centrifuged groups being ~10 to 18% higher than controls at all levels of log FFBM. Also a comparison of slopes by the method of Student's t shows that with increasing FFBM skin solids increased more rapidly than skin water in control groups ($P<.001$) as well as centrifuged groups ($P<.01$).

Our strategy of comparing adult rats centrifuged for only a few weeks with those centrifuged throughout the period of rapid growth yielded differences of interest but none which were truly persistent. Mass changes in the heart and liver in the Adult Study (Table 1) probably represent transient responses which disappeared in the longer Growth Study. By contrast the more prolonged centrifugation of the Growth Study increased both water content and solid content of skin, independently of any changes in total body mass (Fig. 1). Thus skin showed simultaneous responses to centrifugation and to body size, the first characterized by a rise in the position of the curve on the ordinate and the second by a slope constant (b) which in each case was close to the value for comparable controls. The skin values were unique in that they were the only increases in mass associated with centrifugation and the only responses to centrifugation per se.

None of the changes in response to centrifugation were truly persistent. Retirement from the centrifuge to terrestrial gravity reversed both the changes in fat-free wet masses, as previously shown (6), and in water and solids of skin, as shown in Fig. 1.

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Table 1. Fluid-solid composition of body and 5 components

	Growth Study				Adult Study			
			H_2O Fraction				H_2O Fraction	
Compon	H_2O (gm)	Solids (gm)	in Compon (%)	of Total (%)	H_2O (gm)	Solids (gm)	in Compon (%)	of Total (%)
Total body	↓	↓	→		↓	↓	→	
Heart	→	→	→		↓	→	→	
Liver	→	→	↑	→	↓	↓	↑	→
Gut	→	→	→	↑	→	→	↑	↑
Skin	↑	→	→	↑	→	→	→	↑
Muscle	↓	↓	↑	↓	↓	↓	→	→

All components are fat-free. Criterion for rejecting null hypothesis is $P<.01$. ↑, ↓ and → indicate: experimentals higher, lower than controls and no statistically significant difference.

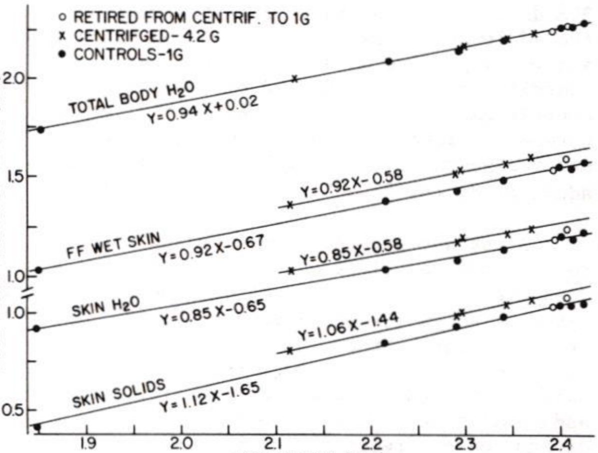

Fig. 1. Allometric plot of composition versus fat-free body mass (FFBM). The lines were fitted by the method of least squares to the individual data but for clarity only group means are plotted. The group retired for 116 days has returned to the control line while the group retired for only 57 days is intermediate between centrifuged and controls.

GENERAL PRINCIPLES AND METHODS OF ANIMAL EXPERIMENTS FLOWN ON COSMOS BIOSATELLITES

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Biological experiments in space flights form an important line of research in space biology and physiology. They can be performed onboard manned and unmanned space vehicles. Of particular importance among the latter are biosatellites, i.e. earth artificial satellites that carry various biological objects (1).

In the last decade the Soviet Union performed biological studies onboard five biosatellites of the Cosmos series. The purpose of these studies was: a) to investigate the effect of weightlessness on the structure and function of various organisms (2,3); b) to explore the possibility of using artificial gravity to optimize the functional state of living organisms in space flight (4); c) to study the modifying effect of weightlessness on the radiosensitivity of mammals.

The preparation and conduct of experiments onboard Cosmos biosatellites involved the following areas: a) ground-based development of flight studies (Table 1); b) preflight preparation (Table 2); c) flight experiments (Table 3); and d) postflight studies and data processing (Table 4). Ground-based development of flight studies is very labor and time consuming. The results of the ground-based studies carried out essentially determine the methods of studies in space flight.

When developing rat experiments onboard Cosmos biosatellites, the following requirements were specified:

- restriction of motor activity of rats in flight studies should be minimal and should not affect significantly the physiological systems;
- life-support system should meet the biological requirements (food and water supply, gas exchange, energy exchange, temperature profile, etc.) of the animal species.

Using the above requirements, it was decided to fly unrestrained rats housed in cages for one animal in earlier flights and for a group of animals in later flights.

When planning rat experiments with artificial gravity, the following requirements were specified:

- the centrifuge arm should be as large as possible, i.e. as allowed by the interior size of the biosatellite and its equipment;
- the environmental parameters for the centrifuged animals should be identical to those for the weightless animals (temperature, humidity, ventilation, food and water supply);
- the rate of centrifugation should be selected in such a way as to generate 1.0 g in the animal area.

In view of the fact that physiological, morphological and biochemical parameters of 1 g rats have been adequately studied, it was hoped that artificial gravity (1.0 g) would provide good controls and would become a simple method to answer the question whether artificial gravity can be employed to maintain normal functions of the vital systems of the animal body in space flight.

In order to investigate the combined effect of weightlessness and ionizing radiation on animals, a special experimental protocol was developed. On flight day 10 the rats were irradiated with two doses of gamma-radiation. The Cesium-137 source had the following characteristics: activity 325 mC, energy 0.661 MeV and half-life time 26.6 years. The maximum dose of whole-body irradiation (800 ± 85 rad) was selected such as to initiate radiation sickness. The early stage of radiation sickness and postradiation recovery developed during the remaining 10 days of the weightless flight.

The specific feature of the Cosmos experiments was their complete automation. Due to this, the quality and reliability of every component of the life support system and scientific equipment should be very high. In view of this, the launch of every biosatellite was preceded by bioengineering tests in the mockup.

The goals of these tests were:

- examination of the equipment performance under the conditions similar to real flight;
- physiological and hygienic evaluation of animal life-support systems;
- investigation of the composition and time course of the changes of the mockup atmosphere;
- assessment of the effects of the environment and other parameters of animal handling on the animal's physiology para-

TABLE 1. Stage of Ground-Based Development of Flight Studies

- Development of flight programs and selection of adequate experimental protocols
- Development of methods of study
- Selection of animal models
- Development of methods of animal training
- Substantiation of bioengineering requirements for animal life-support systems
- Development of principles of life-support systems and biotelemetric monitoring
- Development of animal diets
- Biological evaluation of mockup and flight units of life-support system and scientific equipment

TABLE 2. Stage of Preflight Preparation

- Veterinary and physiological examination of animals, their selection and training for flight and control studies
- Transportation of animals to the launch-site and their placement onboard the biosatellite
- Final testing of life-support system and scientific equipment
- Prelaunch monitoring of capsule environment

TABLE 3. Stage of Flight Experiments

- Acquisition and processing of telemetric information
- Monitoring of the flight experiment
- Synchronous experiment in the biosatellite mockup
- Vivarium control experiment

TABLE 4. Stage of Postflight Studies and Data Processing

- Biomedical investigations at the recovery site in a field laboratory
- Transportation of animals and biomaterial to Moscow
- Laboratory investigations
- Treatment, analysis and discussion of results
- Preparation of reports

meters;

- final refinement of investigations for flight study.

The test results were used either to qualify the experimental equipment for space flight or to identify the failures to be eliminated before final approval.

Almost simultaneously with the flight study synchronous control experiments were carried out on the ground. Their purpose was to simulate as close as possible the effect of space flight factors, except for weightlessness, on the biological material. This was necessary to compare the flight and synchronous results and to differentiate weightlessness-induced effects from those produced by other space flight factors, in particular maintenance and handling. Synchronous studies as well as bioengineering tests were conducted in ground-based mockups.

The program of physiological, morphological and biochemical investigations in rats studied onboard Cosmos biosatellites was developed in such a way as to yield the main bulk of information in postflight studies. In this context, in order to obtain data undistorted by readaptation to 1 g, the post-recovery examination of animals, including their sacrifice, organ removal and conservation, were performed at the recovery site in the field laboratory. Each laboratory included a landrover, tents for scientific investigations, containers with equipment, heating and ventilation system, power supply, etc. After these primary examinations all the biomaterial was brought to Moscow for further analysis at R+1. The general principles and methods used in the rat studies onboard Cosmos biosatellites are to be used, after revision and refinement, in the primate study.

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DEMOGRAPHIC CONSIDERATIONS IN GRAVITATIONAL BIOLOGY

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INTRODUCTION

Understanding the consequences of different gravitational fields on individual organisms is, at least by loose definition, the field of gravitational physiology. The term acute in this field usually encompasses short exposure, often of a few minutes or hours, while the term chronic is normally reserved for longer exposure, usually over a large fraction of the individual's lifetime.

A necessary but sometimes limiting constraint of all scientific disciplines is the use of time scales to delimit their scope. Chronic in one field may denote a time period which is thought of as acute in another. That is to say, as science moves from one level of biological organization to another, time frames change as do the units of biological interest.

The purpose of this paper is to explore a different time frame and biological unit in gravitation biology. Instead of being concerned with the specific effects of different gravitational fields on the individual, I will address the consequences of potential effects in terms of a population of individuals. My assumption here is that different organisms respond in different ways over a range of acceleration fields and that these responses ultimately affect population survival and reproduction. These represent the characteristic variables in the field of demography--the study of populations.

The paper is organized into 3 parts. First, I present a brief overview of the field of demography --assumptions and importance. Second, I establish a conceptual framework for working in this area in gravitational biology and present the results of a simulation study using a set of life table parameters for the flour beetle, Tribolium castaneum. Third, I briefly discuss the major points of the analysis and the implications of addressing demographic questions in gravitational biology.

BACKGROUND

The foundation of quantitative demography is the stable population model set forth by Lotka (4). The assumptions of this model are: 1) the population is closed (no migration); 2) each sex is dealt with independently of the other (female sex normally addressed); and 3) birth and death rates are constant. The model demonstrates that a population obeying these rules will eventually evolve to a stable state where both its growth rate (also known as the intrinsic rate of increase, r) and the proportion in each age class (stable age distribution) become constant. An example of this is given in (1). The stable model is important for basically two reasons. First, it enables one to interrelate and compare qualitatively different units (survival, fertility and development) through the intrinsic rate of increase. Second, more complicated aspects of the population renewal process can be better understood with reference to this case.

Another concept in demography which has direct relevance to gravitational biology is that of demographic equivalence. This concept, as introduced by Coale (2) states, "A difference in mortality is considered equal to a difference in fertility if the two cause an equal change in the intrinsic rate of increase of a population." The reason this concept is important to gravitational biology is that there are differences both in degree and in kind in how an organism responds to hyper- vs hypo-gravitational fields (5). For example, it is conceivable that an organism exists which, if their populations were exposed to a 2g field, would experience heavy mortality. But those which attain maturity may reproduce normally. On the other hand if a population of this same organism was exposed to a .5g field, their reproduction may suffer but their mortality may remain unaffected. The point here is that the intrinsic rate of increase of the populations may be identical in both cases but for quite different reasons. Population growth rate is the unifying principle over the gravitational spectrum in this context.

ANALYSIS AND DISCUSSION

The conceptual relationship between acceleration field and the intrinsic rate of increase of organisms exposed to these fields is given in Fig. 1. Several aspects of this graph merit comment. First, I assume that the intrinsic rate of increase for all organisms, unselected for non-earth gravitational tolerance, is at a maximum at 1g. This is little evidence or reason to think that this is not the case.

Second, the intrinsic rate of increase can be used as a quantitative measure of fitness or adaptation for organisms subjected to different g-fields. It is a commonly used measure of fitness in population genetics and evolutionary biology because it is a collective measure of an organism's life history traits. A trait which is pre-adaptive

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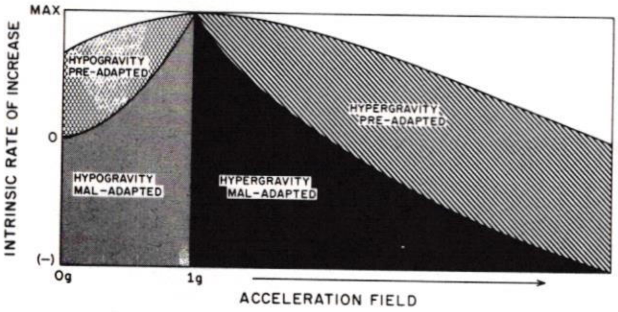

Fig. 1. Diagrammatic relationship between acceleration field and population growth rate.

in a hypo-gravitational environment may be mal-adaptive in a hyper-gravitational environment. And vice-versa.

Third, a conceptual framework such as this may help workers in gravitational biology focus more sharply on the basic questions of what constitutes a pre-adaptive or a mal-adaptive trait in organisms rather than attempting to randomly screen many species over a wide variety of conditions. For example, it has been suggested that a terrestrial organism best adapted for movement in a weightless environment would be shaped as a sort of aerodynamic fish. Of course, no such animal exists but by abstracting the question one can make the generalization that wings are probably mal-adaptive to weightless environments. I am certain that with more thought and experimentation similar generalizations could be made on other life history traits which affect survival and reproduction in different g-fields.

To examine how different gravitational fields might affect the age distribution of populations I conducted the following computer simulation study consisting of two parts. First, I obtained the life table values for the flour beetle, T. castaneum, from a previously published study (3) and computed the intrinsic rate of increase and the fraction in each stage (egg, larvae, pupae, adult) if the population was growing at this fixed rate. Second, I separately reduced 1-of-5 factors from this original life table (egg, larval, pupal and adult survival; adult fecundity) until the intrinsic rate of increase was zero for each case. This level represents a stationary population. I then computed the percent of the total population in each stage. The results of this study are given in Table 1.

TABLE 1. Results of changing different life history parameters in T. castaneum populations on their stable stage distribution.

Life History Trait Reduced	r	% in Stable Distribution			
		Egg	Larvae	Pupae	Adults
No reduction	.10	26.1	67.9	2.9	3.1
Egg survival	.00	4.4	28.9	5.8	60.9
Larval survival	.00	51.7	37.4	.2	10.7
Pupal survival	.00	12.7	85.2	.8	1.3
Adult survival	.00	10.1	71.3	15.1	3.5
Fecundity	.00	3.2	22.5	4.9	69.4

Several generalizations can be made based on these results. First, reducing survival in a particular pre-adult stage tends to decrease the percent of the population in the subsequent stage to a much greater degree than the percent in the stage whose survival was reduced. Furthermore, which stage gains the largest percent of the total when pre-adult survival is manipulated is seldom straightforward.

Second, increasing adult mortality actually increases the proportion of adults in the population relative to the unconstrained case. This was not expected. However, the explanation for this phenomenon is as follows. Although the direct effect of increasing adult mortality is to make the population more youthful, the indirect effect is to reduce population growth rate thus "age" the population. In terms of population age structure, the "aging" effect of slower growth rate overrides the direct effect of increased adult mortality.

Third, reducing growth rate by decreasing fecundity has a simple and direct effect on population age structure. Most of the population shifts to the older stages (pupal and adult).

MAJOR POINTS OF ANALYSIS

Three major points emerge from this examination. First, the effects of different gravitational fields have intuitive effects on population growth rate but, due to the intrinsic properties of population renewal process, may have counter-intuitive effects on population age structure. Second, gravity will affect all physiological systems of an organism simultaneously but (by definition) in qualitatively different ways. Qualitative differences cannot by meaningfully compared outside of a demographic context. Third, population analysis in gravitational biology represents the intermediate step between the physiological and the evolutionary. Individuals are selected for but populations evolve.

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SCALING OF METABOLIC RATE ON BODY MASS IN SMALL MAMMALS AT 2.0 *g*

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We have recently advanced the hypothesis that the classic Kleiber mammalian allometric relationship between metabolic rate (MR) and total body mass (TBM),

$$\mathrm{MR, kcal \cdot hr^{-1}} = 2.82(\mathrm{TBM, kg})^{0.756}, \quad r = 0.998,$$

should be altered by changes in gravitational loading (9,10). We postulate that augmentation of gravitational loading, as in chronic centrifugation, should produce an increase in both the positioning constant and the exponent, while abatement of gravitational loading, as in spaceflight, should result in a decrease in both.

It has been shown by other investigators that the oxygen consumption rate (2,5), daily maintenance feed requirement (12), and thyroxine blood levels (4) are increased after a number of days of chronic centrifugation. The increase in these physiological parameters clearly indicates that augmentation of normal gravitational loading results in an increase in metabolic rate.

It should be pointed out, however, that when animals are first subjected to chronic centrifugation a transitory anorexia (1,4,6,13) and depressed body temperature (3,4,7) occur which last 2-10 days depending upon age and species of the animal as well as the onset rate and level of acceleration imposed. Thus, comparisons of long-term effects of increased gravitational loading on steady-state metabolic energy requirements are probably best made at least 2 weeks after reaching the augmented loading level by stages. Although the transitory effects are reversed within several days, the animals are left with an appreciable body mass decrement which persists for months as long as the augmented loading is imposed (4,11,13).

PROCEDURES

We have previously described a 4-species animal model for study of metabolic scale effects which provides a body mass range of 0.1 to 4.0 kg, comprising 6 each metabolically mature hamsters, rats, guinea pigs and rabbits. Measurement of individual metabolic rates under standard conditions yields the Kleiber allometric relationship with appropriate accuracy (9). We have now used this model in a preliminary test of our hypothesis that doubling gravitational loading by chronic centrifugation at 2.0 *g* should produce an increase in the values of the positioning constant and exponent of the Kleiber equation.

An open-circuit metabolic apparatus was designed such that 6 individual metabolic chambers were mounted with one degree of freedom at a radius of 2.7 m on a large centrifuge. The apparatus permits continuous measurement of oxygen consumption rate ($\dot{V}O_2$) of animals occupying the chambers while the centrifuge is running. Individual cages similar to standard vivarium cages, with provision for feed and water, were also mounted at the same radius to house the animals between $\dot{V}O_2$ measurements.

Male animals 8 months of age were procured from commercial breeders and, after a week of stabilization in the vivarium, $\dot{V}O_2$ was measured in the stationary centrifuge metabolic chambers for 6 hr. The mean of the 2 lowest hourly values was taken to represent metabolic rate at 1.0 *g*. The animals were transferred to the centrifuge housing cages, and rotation was begun at 1.5 *g* for at least a week before going to 2.0 *g*. Once a week the centrifuge was stopped briefly to weigh the animals, transfer them to the metabolic chambers and resuming rotation for 6 hr to measure $\dot{V}O_2$. The centrifuge was stopped a second time to return the animals to the housing cages, and rotation was resumed.

RESULTS

Results of the experiment with 10 rabbits are shown in Fig. 1. Metabolic rate was significantly increased at 2.0 *g*, and stabilized after 2 weeks. Total body mass exhibited a significant decrement of about 10% within the first week at 1.5 *g*, which persisted for the following 8 weeks of the experiment. Metabolic intensity was significantly increased by about 36% over the 1.0 *g* value after 2 weeks at 2.0 *g*. It should be noted that the standard errors of the means for both total body mass and metabolic intensity were very nearly equal to the height of the symbols in Fig. 1.

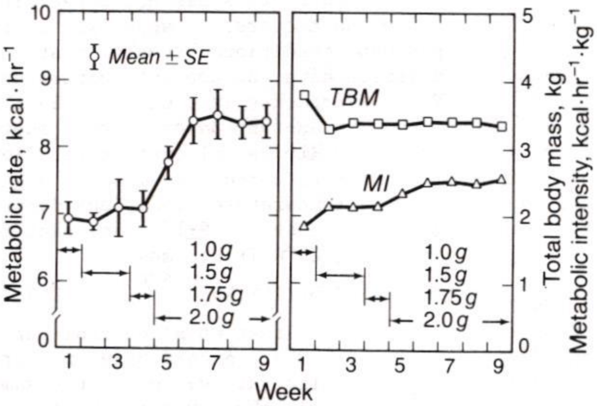

Fig. 1. Effect of chronic 2.0 *g* on metabolic rate, total body mass and metabolic intensity in rabbits.

Similar results were obtained with all 4 species, and a comparison of the mean values of metabolic intensity at 1.0 *g* with those measured after 2-4 weeks at 2.0 *g* is shown in Fig. 2. The hamsters, rats and rabbits exhibited highly significant increases ($P < .001$) in metabolic intensity at 2.0 *g*, while the guinea pigs showed only a marginally significant increase ($P < .10$) because of considerable variability in the measurements. The guinea pigs also displayed a greater decrement in total body mass compared with the decrement observed in the other 3 species. Statistical analysis of the percentage changes in metabolic intensity revealed a significant correlation of the increase in metabolic intensity with body size, indicating that the larger the animal the greater the increase in metabolic intensity at 2.0 *g* compared with 1.0 *g*.

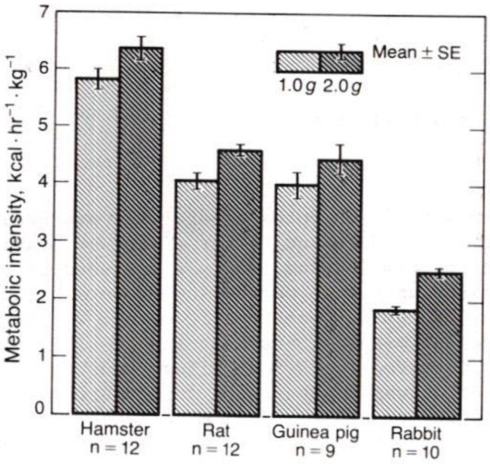

Fig. 2. Effect of chronic 2.0 *g* on metabolic intensity in 4 species.

DISCUSSION

Allometric analysis of the metabolic rate data as a function of total body mass was carried out for all 43 animals with the following result

$$MR_{1.0g}, \mathrm{kcal \cdot hr^{-1}} = 3.16(\mathrm{TBM,kg})^{0.679}, \quad r = 0.983$$

$$MR_{2.0g}, \mathrm{kcal \cdot hr^{-1}} = 3.66(\mathrm{TBM,kg})^{0.732}, \quad r = 0.991$$

Analysis of covariance (14) revealed that the positioning constants are significantly different at $P < 0.001$ and the exponents at $P = 0.038$. Thus, the results confirm our hypothesis that both parameters should be greater at 2.0 *g* than at 1.0 *g*.

While the findings were gratifying in one sense, they were disturbing in that the absolute values of the 1.0 *g* allometric parameters did not agree with the classic Kleiber parameters. Reappraisal of the centrifuge experiment conditions revealed that through a misunderstanding the ambient temperatures at which the $\dot{V}O_2$ measurements were made was 25±1°C for all 4 species. In another paper at this meeting (8) we showed that the Kleiber allometric relationship between metabolic rate and body mass is significantly perturbed as temperature conditions depart from the thermoneutral zone of 29±1°C, and that for our 4-species model at 24°C it becomes

$$MR_{24°C}, \mathrm{kcal \cdot hr^{-1}} = 3.51(\mathrm{TBM,kg})^{0.676}, \quad r = 0.987,$$

a result quite similar to that obtained from our centrifuge animals at 1.0 *g*. Accordingly, we corrected our 2.0 *g* metabolic rate values by the same factors required to adjust the 1.0 *g* metabolic rate values to the normal Kleiber thermoneutral values, and performed another allometric analysis to yield

$$MR_{2.0g}, \mathrm{kcal \cdot hr^{-1}} = 3.30(\mathrm{TBM,kg})^{0.813}, \quad r = 0.993.$$

Analysis of covariance showed that the positioning constant at 2.0 *g* is increased by 17% from that at 1.0 *g* at the $P < .001$ level, and the exponent is increased by 8% at the $P = 0.008$ level.

These findings lead us to conclude that augmented gravitational loading does indeed shift the allometric relationship between metabolic rate and body size by an increase in both allometric parameters, and that therefore gravitational loading is an important contributor to mammalian metabolic energy requirements. Thus, we continue to predict that abatement of gravitational loading in spaceflight will result in a lowering of both allometric parameters in the Kleiber equation.

ACKNOWLEDGEMENT

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THE EFFECT OF HYPERGRAVITATION ON NUCLEIC ACID METABOLISM IN THE RAT LIVER

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INTRODUCTION

Liver is the subject of many investigation with the purpose of studying the effect of various physiological and patologicalfactors.It is an organ with many functions, which plays a leading role in metabolic processes in mammalian organism. Development and functioning of liver begins early in embryogenesis. Processes developping in nucleic acids are the basis of such important sides of cell activity as proliferation, tissue and cell differentiation. Thus, there are reasons to suppose, that disorders in nucleic acid metabolism under the effect of various extremal factors, including hypergravitation, can play an impotent role in the mechanisms of the transfer of genetic information.

The purpose of the present study was to investigate the effect of hypergravitation 2g on RNA-synthetic system of rat liver and the content of nucleic acids in this tissue.

METHODS

Female Wistar rats about 300 g body weight, were used.Experemental animals (IO rats) during gestation days I4 through 2I were centrifuged at 2 g. Synchronous control animals (5 rats) were held during the experiment in one room with the experemental rats. 5-IO hours later after the experiment was completed the rats were decapitated and liver of adult animals and their 2I-day fetuses was taken for investigation.

Rat liver nuclei were isolated with hypertonic sucrose by Widnell and Tata (I).The purity of nuclei was controled under microscope. Part of the nuclei was used to determine RNA synthesis (transcription), the rest of the nuclei were used to get solubilisated enzyme RNA polymerase (2). RNA synthesis in nuclei and enzyme activity were measured by incorporation of rarioactive precursor ^{3}H-UMF in acid-insoluble product of RNA-polymerase reaction. The content of nucleic acids in tissue was assayed by the method of Blobel and Potter (3) and expressed in mg/g wet weight of tissue.

RESULTS

Isolated nuclei is a convenient model to investigate the RNA-synthetic system of mammalian cells. Nuclei,isolated from the tissue of rat liver, for a certain time retain the ability of synthesising RNA identical to the one synthesised in vivo.

Results of the investigation of RNA synthesis in rat liver nuclei are shown in Table I.

TABLE I. The effect of hypergravitation on RNA polymerase activity and transcription in rat liver nuclei (CPM/ g of DNA).

Age	Group	RNA polymerase activity	Transcription
Adult	Controle	3I.8±3.I	204±II
	Experiment	29.4±2.5	202±I2
2I-day fetuses	Control	33.9±I.4	I77±20
	Experiment	32.2±I.I	I87±9

It demonstrates that transcriptional activity of 2I-day fetuses was very close in experimental and control animals. No changes were also noticed in RNA synthesis in nuclei isolated from the liver of adult rats in conditions of changed gravitation.

RNA polymerase plays an important role in regulation the synthesis of different types of RNA from DNA template. The activity of this enzyme can change considerably depending on different physiological and pathological conditions. Table I shows RNA polymerase activity in adult rats and their fetuses. It demonstrates that a twofold increase of a gravity field (compered to the Earth level) did not change the activity of solubilisated enzyme in adult rats and their 2I-day fetuses.

The sdudy of transcription and RNA-polymerase activity in rat liver of both adult rats and 2I-day fetuses showed that there were no changes in these systems. Therefore, these systems were sufficiently resistant to hypergravity effect.

It is well known that the DNA concentration in indivisible cells is a constant. The level of DNA may become higher as the result of its increased synthesis, which usually happens before mitose. Table 2 shows the concentration of DNA and RNA in liver tissue.

TABLE 2. Concentration of nucleic acid in rat liver tissue (mg/g of tissue).

Age	Group	RNA	DNA	RNA/DNA
Adult	Control	I6.I±0.7	3.5±0.4	4.6
	Experiment	I6.6±0.4	3.7±0.2	4.5
2I-day fetuses	Control	I4.I±0.5	5.8±0.4	2.8
	Experiment	I4.8±0.5	6.0±0.4	2.5

The data demonstrate that a twofold increase of the gravitation (compared to the Earth level) did not affect the content of nucleic acids in the liver of adult rats and 2I day fetuses. Thus, RNA content (mg/g of tissue) was very close in experimental and control adult rats. RNA content in 2I-day exerimental and control fetuses did not differ either. DNA content in the liver of experimental and control adult rats under the effect of hypergravitation was much the same. It shoud be noted that the RNA/DNA ratio in experimental and control 2I-day fetuses decreased (almost twofold), which indicates the changes of nuclear-cytoplasmic ratios in a cell. This increase of index in fetuses as compared to adult animals is caused by lower concentration of RNA and higher concentrarion of DNA in liver cells during prenatal period. These results are in good agreement with the data of other authors, who noted a lower RNA/DNA ratio in tissues during fetal period (4). Published data indicate that the number of cells, synthesising DNA, considerably increased in liver of 2I-day fetuses(5).

Thus, we investigated nucleic acids metabolism in a very important period of cell activity- in the period of active synthesis of DNA before mitose.

The present study show that hypergravity 2g did not affect the content of DNA and RNA in rat liver tissue of adult and 2I-day fetuses. RNA-polymerase system in rat liver was also resistant to the effect of expore to hypergravitation. The reult indicate that no changes were noticed in RNA synthesis and RNA-polymerase activity of adult rats and 2I-day fetuses. The gene expression may be regulated by the enzyme RNA polymerase which transcribe the information directly from the genome. Its activity in vivo is known to change under various condition. The fact that no changes were noticed in the activity of this enzyme in our experiment allows to conclude that exposure to 2g cannot disturb molecular mechanisms of the gene expression in the mother-fetus system.

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IS THERE AN ORIENTATION OF THE NUCLEI IN MICROPLASMODIA OF PHYSARUM POLYCEPHALUM?

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INTRODUCTION

Gravity is acting on cells in two ways which are only partially known and investigated:

a) The most evident gravity effects are indirect or secondary ones. They are caused in the following way: Gravity interacts with any mass of an organism. This results in weights of the different masses. The weight of each mass of the same kind like blood, bones and muscles is transformed into vectorial forces and hydrostatic pressures. These forces and pressures - and not gravity itself - are influencing the single cell from outside. Sometimes cells are more or less free of such mechanical stresses; e.g. cells of the connecting tissue if they are positioned at the real upper side of an organism with respect to the direction of gravity or all cells of a submersed organism. Tertiary effects have to be defined if secondary effects release a humoral or nervous signal chain before the final reaction takes place.

b) Direct or primary gravity effects theoretically are always present for each cell. In this case gravity is interacting with masses located inside the cell.

These influences (a and b) lead to mixed signals the origin of which (a or b) cannot be discriminated by testing organ reactions alone or single cells within their tissue.

Masses for direct or primary perception may be nuclei, nucleoli, condensed chromosomes and mitochondria. The low density and small size of these cell particles are making it difficult to decide on their possible function as primary acceleration receptors (1, 2, 3). On our fast rotating clinostats (4, 5) we found some indications of direct gravity effects; e.g. Sobick (6) found a shortening of mitosis in the slime mold Physarum polycephalum.

Physarum is a giant cell forming a flat network with diameters of up to one meter. It resembles a network of flowing hot magma with a closed front zone and a typical network behind that front. For the flowing magma the pattern is partly determined by gravity. In the case of Physarum, however, we do not know how differentiation takes place and if gravity is playing a role at all. Does Physarum, or migrating cells during the embryogenesis of higher organisms, use gravity as a reference signal for orientation? If this proves right, a reference pattern inside the cell must exist (7). In special cases we can observe polar or orientated structures (e.g. plant amyloplasts) possibly belonging to this hypothetic reference system. Todd (8) observed the orientation of the mitotic spindles in mammalian tissue culture cells. He did not find any regularities.

During our work with Physarum we observed that nucleoli show a typical polar arrangement at the periphery of the slime mold's nuclei up to four hours before mitosis starts. This arrangement should theoretically lead to a cork- tumbler reaction of the nuclei (9). In fact, the nuclei are revealing an orientation at that stage, but at first sight it is independent of gravity.

This paper demonstrates the orientation behavior of the Physarum nuclei and describes the first efforts made to analyse the phenomenon.

METHODS

Microplasmodia, a special growing type of the slime mold Physarum polycephalum, were grown in shuttle cultures (10). We used a special microchamber in which the microplasmodia were slightly pressed (6). After an adaptation time they differentiate into flat active forms of up to 200 µm, which can be observed in vivo by light microscopy. In good preparations the peripheral nucleoli within the nuclei can be observed for several hours (Figure).

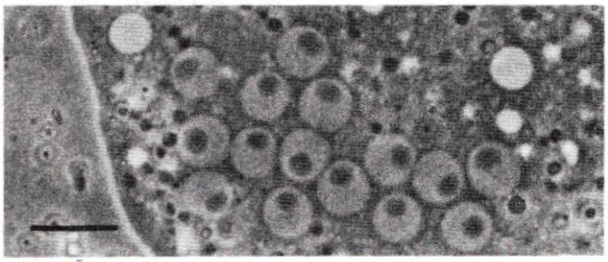

Figure Flattened microplasmodium within the microchamber shows nuclei with peripheral nucleoli. Bar represents 10 µm.

- For decoupling the nuclei from a hypothetic cytoskeleton and for inducing a possible cork-tumbler reaction we used different concentrations of benzamide (up to 100 mM) in phosphate buffer (15 mM) (11).
- Vertical, horizontal and clinostat light microscopes together with video cameras and video timelapse registration were available. The clinostat (horizontal) microscope could provided for inverse illumination capability.

RESULTS

The results were obtained in three types of light microscopes differing in their orientation towards gravity: normal vertical (condition 1) microscope, horizontal (condition 2) and fast clinostat (85 rpm; condition 3) microscope. Under these three conditions we observed two orientation reactions of the nuclei of Physarum polycephalum:

a) About two hours after mitosis, the nucleoli move from their central position close to the nuclear envelope. Up to prophase, the nucleoli are turned away from the incident light, i.e. they are directed towards the observer. During the first hours of this orientation the nuclei are showing two kinds of movements:
    - Tumbling movements were brought about by turns around their center. The period length of the tumbling is 20-40 seconds. The angle of tumbling is 70-90 degrees. During about one hour one of 20 nuclei will make a 360 degree turn. Few minutes or few hours before mitosis the tumbling movements stop (used condition 1, 2, 3).

- Nuclei frequently show linear movements in all directions during their life time but seldom during mitosis (used condition 1, 2, 3).

The orientation of the eccentric nucleoli into one direction is indicated by:

- The impression gained during the observation of series of real time or timelapsed pictures.
- The repeated observation of different focal planes of the nuclei in the microscope reveals the position of the nucleoli. We are sure that an optical illusion is not involved.

b) A second type of an orientation reaction is frequently shown by the daughter nuclei some minutes after mitosis. In this phase the nucleoli are reconstructed from irregularly distributed dense granular material. This makes it possible to observe turning movements of the nuclei with axes parallel to the light beam; the impression is similar to that of moving toothed wheels in a clock work (used condition 1 2, 3).

We realized three groups of polar influences acting parallel to the optical axis of the microscope and tried to find out which of these influences might have caused the observed orientation:

a) The microplasmodia preparations are slightly pressed between the two plane-parallel walls of the microchamber orientated perpendicular to the microscope's light beam. This possibly forces an orientation on the nuclei. However, we prevented such orientated arrangement by using thicker preparations where the nuclei were free to move parallel to the light beam (used condition 1, 2, 3).

b) For the viability of the microplasmodia it is necessary that one wall of the microchamber consists of a nutrient agar plate. This includes a polarity of the surfaces of the two plates (agar/ glass). Changing these chemical and surface polarities has no effect on the orientation reaction (used condition 1, 2).

c) One can think of light as a further polar stimulus. We tested two conditions to prove an orientated light reaction of the nuclei:

- Omnidirectional homogeneity of the stimuli (including darkness).
- Inversion of the direction of the incident light.

The first condition is fulfilled if there is no illumination. One should expect that the orientation of the nuclei becomes a random one but this was not the case. Surprisingly we got the same result with nuclei revealing linear shifting but no tumbling movement (used condition 1, 2).

In trying to fulfil the second condition we involuntarily offered the first one again. When the light beam was inversed by way of a special mirror system and different wave lengths (450-630 nm) were used, we found no influence on the original orientation of the nuclei (used condition 1, 2). This may be caused by a diffusing light reflection of the relative thick agar plate (2.5 mm) which is now on the other side of the object relative to the incident light. So the microplasmodia are illuminated by the direct and reflected light. This again represents condition number one. Preparations without a thick agar plate were not successful. We suspect fibrilar structures (cytoskeleton) to fix the nuclei so avoiding the expected cork-tumbler reaction. We tried to decouple the nuclei using the depolymerizing substance benzamide. We could not find a clear reaction against benzamide even in high concentrations.

DISCUSSION

It is well known that the orientated growth of plants depends on light and gravity. The polar structure of the Physarum nuclei and their orientation indicate that the same mechanisms are involved. Solving this problem might enable us to get some knowledge of a general gravisensitivity of cells. At present we cannot prove the observed orientation to be a light - or a gravireaction. Despite that it is difficult to think of other stimuli. If light is involved, the cell, after a first stimulus, would fix the orientation of its nuclei due to an inherent reference pattern which dominates the regulation of the orientation at least until the next mitosis. Simultaneously such a hypothetic regulatory system may be forced to compensate the influence of gravity. This can be concluded from orientation reactions of the nuclei on the three types of microscopes used.

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THE EFFECTS OF WEIGHTLESSNESS AND INCREASED GRAVITY ON HEMOPOIETIC STEM CELLS OF RATS AND MICE

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ABSTRACT. Changes in the number of hemopoietic stem cells (CFU-s) were studied in rats during the recovery and selected post-recovery days after an 18-day flight on the biosatellite Cosmos 1129, and in mice after long-term hypergravitation (+ 1.0 g). Both changes in gravity result in a decrease in the CFU-s number with subsequent gradual restoration of the CFU-s pool.

INTRODUCTION

Both hypo- and hypergravity have been known to have effects on a wide variety of biological and medical functions, from the cellular to the population levels. The experiments on the biosatellite Cosmos 936 as well as observations made in terrestrial laboratories with the use of hypergravitation indicated that many disturbances caused by changes in g decrease upon return back to 1.0 g (1).

A depression of the red blood cell formation in the bone marrow of mice observed on post-recovery days after the Apollo 11 mission (2), as well as the same depression observed in rats after their stay on board the biosatellites Cosmos 605 and Cosmos 782 (3,4) indicated that factors associated with space flight may produce changes in hemopoiesis. Since the required number of peripheral elements is provided by normal proliferation and differentiation of hemopoietic stem cells, the size of the pool of hemopoietic stem cells of the bone marrow was analyzed after a flight of the rats on the biosatellite Cosmos 1129, and after a long-term stay of the mice under hypergravitation.

METHODS

Weightlessness was achieved by the stay of rats of Wistar strain 18 days in space on board the biosatellite Cosmos 1129 (5). The following groups of animals were used: (1), an experimental group - a flight group; (2), a synchronous model control group - kept under the same conditions as the experimental group, but on Earth; (3), vivarial control groups - placed in the conditions of a terrestrial vivarium. For the hemopoietic stem cell examination the spleen-colony assay method (6) was used, which was adapted (7) for the application in rats. The assay systems used are described in detail in a previous paper (5). For a histological examination, serial sections 70-100/um thick were prepared from the longitudinal surface of the spleens and the bones in which the colonies formed from the transplanted hemopoietic tissue were counted and expressed per spleen or femur.

The effect of increased gravity on hemopoietic stem cells was studied in female mice (CBA x C57Bl)F1, aged 10 weeks at the beginning of the experiment. The increased gravity of 1.0 g relative to the Earth gravity field was obtained by centrifugation 5 hours per day for 5 days. An exogenous spleen colony assay (6) was used in the wholebody ^{60}Co-gamma irradiated isogenic recipients.

RESULTS

After transplantation of the bone marrow of rats on the recovery day following an 18-day stay in space, the mean value of colony count (CFU-s) was found to be lower, both in the spleen and the bone marrow (Table 1), than that after an identical amount of bone marrow from rats of the control group ($P < 0.01$). The decrease in CFU-s number was on average 4- to 5-fold for Cosmos 1129 as compared with the synchronous model group. The size of the CFU-s pool in the femoral bone marrow, was assessed on day 12 after transplantation of a similar amount (1.5×10^6) of bone marrow nucleated cells from the Cosmos 1129 experiment group. The bone marrow taken on the recovery day gave a seven times lower amount of CFU-s, and that of the 6th post-flight day a four times lower number of CFU-s, than was the level of CFU-s of the synchronous model group. The drop in the bone marrow CFU-s number observed in the Cosmos 1129 experiment can be caused by a damage of the stem cells, or by their migration from the bone marrow, or by a decrease in the seeding efficiency of the transplanted bone marrow, but the small number of experimental animals made it not possible to undertake a more detailed analysis of the changes observed.

Long-lasting hypergravitation (+ 1.0 g, 5 hrs, 5 days) resulted in a drop of the number of CFU-s in the spleen as well as the bone marrow of mice (Table 2). Changes in the CFU-s count in the spleen started

Table 1 Average number of microcolonies of the haemopoietic tissue (± S.E.M.) grown in the spleen and in the femoral bone marrow of rat recipients of bone marrow hemopoietic stem cells from rats on the recovery day after the stay on the biosatellite Cosmos 1129

Group	Average number of colonies after 1.5 x 10^{6} n.c. injected			
	n	In spleen	n	In femur
Experimental flight	8	5.8 ± 0.83^{o}	6	16.3 ± 3.9^{o}
Synchronous model control group	8	25.8 ± 1.4^{x}	6	68.3 ± 9.7^{x}
Vivarial control group	8	16.6 ± 1.6	12	43.2 ± 5.4
Endogenous background	15	2.8 ± 0.6	8	3.1 ± 0.43

n = number of femurs or spleens examined, o versus x, $P < 0.01$
n.c. = nucleated cells

Table 2 Some characteristics of femoral bone marrow, spleen and thymus of mice following 1.0 g (5 hrs for 5 days) hypergravitation

Group	Mean number of CFU-s ± S.E.M. (x 10^{6})			Cellularity ± S.E.M. (x 10^{6})		
	n	Bone marrow	Spleen	Bone marrow	Spleen	Thymus
Control	10	145 ± 12^{o}	115 ± 8^{o}	16.1 ± 0.6	137 ± 28	118 ± 24
Days after hypergravitation						
1	10	103 ± 10	44 ± 6^{x}	13.6 ± 0.5	96 ± 3.2	84 ± 16
3	10	74 ± 6	62 ± 4	13.6 ± 0.4	95 ± 2.8	67 ± 12
6	10	85 ± 9	62 ± 6	14.7 ± 0.2	107 ± 3.6	104 ± 32
11	10	100 ± 14	46 ± 4	14.5 ± 0.4	105 ± 1.3	105 ± 22

n = number of animals, o versus x, $P < 0.01$

earlier and lasted longer than those in the bone marrow.

The maximum drop in the CFU-s number reached 62 % in the spleen and 49 % in the bone marrow, as compared with the control group level. Up to day 3 after hypergravitation, spleen cellularity decreased to 41 % and thymus cellularity to 43 % of the control. An involution of lymph organs produced by the effect of hypergravitation may be a result of stress reactions. A decrease in the spleen cell number and thymus cellularity was observed also in rats after their 22-day stay on board the biosatellite Cosmos 605 (1). Our results may therefore indicate that a gravity change within the range of ± 1.0 g relative to the Earth gravity field (zero g in space flight, and hypergravity during centrifugation) has the signs of a stress reaction. The latter could be the cause of the observed decrease in the number of the stem cells of the hemopoietic tissue compartments. This possibility is supported by a marked decrease in the CFU-s number in the bone marrow of mice after hydrocortisone administration (8). However, further experiments are needed to elucidate this consideration.

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HEMATOPOIESIS IN ANTIORTHOSTATIC, HYPOKINESIC RATS

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Rats exposed to antiorthostatic, hypokinesia showed the following effects which are comparable to those seen in man during or after space flight; weight loss, reduced food and water consumption, transient increases in peripheral hematocrit and RBC count, decreasing MCV and reduced reticulocyte count. In addition, the hemoglobin P50 was shifted to the right. A significant shortening of RBC t1/2 was only seen after suspension. Changes in leukocyte and platelet numbers in suspended rats were also comparable to those in man during space flight but leukocyte PHA sensitivity in rats showed no consistent alteration. The results demonstrate that this model reproduces many of the hematological effects of space flight and has potential as a tool in understanding the hematopoietic response to zero gravity.

INTRODUCTION

Rodents exposed to antiorthostatic, hypokinesia have proven useful in understanding the muscle (1) and bone (2) manifestations associated with changes in the direction of the gravity vector on the body. Here we provide descriptive information concerning the effect of antiorthostatic, hypokinesia on the hematopoietic system of rats. The data suggest antiorthostatic, hypokinesia produces marked peripheral hematological effects although it is not yet clear whether these are a direct consequence of the altered gravity vector, i.e., antiorthosasis, of hypokinesia, of both, or are somewhat indirect and secondary to, for example, the reduced food and water consumption.

MATERIALS AND METHODS

Male, Sprague-Dawley, rats were used throughout in groups of at least 5 animals. Rats were suspended either from the tail or in a rigid body harness at a head-down angle of approximately 20 degrees. Body weight, and food and water consumption were monitored daily.

Blood samples were routinely obtained from the lateral tail veins and were processed using standard hematological techniques for complete blood counts (CBC), reticulocytes, and for the determination of the oxyhemoglobin dissociation curve from which the P50 and Hill coefficient (n) were derived. Radioiron (^{59}Fe) was used to assess red blood cell (RBC) production, ^{125}I-human serum albumin to determine plasma volume (PV), and ^{51}Cr-labeled RBC to measure red blood cell mass (RCM) (3). The disappearance of similarly labeled cells was followed over 3-4 week periods from which the RBC t1/2 was estimated. Peripheral blood leukocyte reactivity was assessed in PHA-stimulated cultures.

Giemsa-stained histological preparations of bone marrow and spleen were scored for differential cell counts using conventional criteria.

RESULTS

Changes in the rate of weight gain during suspension were similar for rats weighing either 140-160 gm or 300-350 gm at commencement of the studies. Within the first day of suspension, the rats lost approximately 6% of their body weight and failed to regain their normal rate of growth through 7 days. Post-suspension, the control RATE of weight gain was rapidly re-attained but the suspension-induced DEFICIT in weight was not entirely recovered for at least 21 days. The changes in weight were associated with decreases in food and water consumption during suspension which were largely made up post-suspension.

Alterations in the hematological responses to suspension were independent of animal size and mechanism of suspension. Significant and consistent increases in RBC count (Figure 1) and hematocrit were seen within the first 1-2 days of suspension. By the 7th day these parameters were of similar values to the controls. MCV (Figure 1) showed a steady decline throughout suspension and even into the first 1-2 days of "recovery". However, hemoglobin concentration (Figure 1) did not change significantly during suspension because MCH decreased from 24.9±1.1 to 21.8±0.6 pg. Reticulocytes were moderately suppressed during suspension and the P50 was "shifted to the right" from 39.0±0.5 mm Hg to 41.0±0.1 mm Hg. Post-suspension, the hematocrit, RBC count, and hemoglobin concentration were significantly below pre-suspension values for variable periods of time and a reticulocytosis was observed on the 3-4 "recovery" day. Preliminary data indicate a reduction in blood volume, plasma volume, and RCM during suspension. The disappearance rate of ^{51}Cr-labeled RBC was reduced during suspension. Post-suspension, the RBC were cleared more rapidly than normal whether the cells remained in the previously-suspended rats or were transferred to normal animals.

The number of peripheral blood lymphocytes was variably suppressed during suspension and no consistent effects were observed on their in vitro sensitivity to PHA. Granulocytes and platelets showed no change in concentration dur-

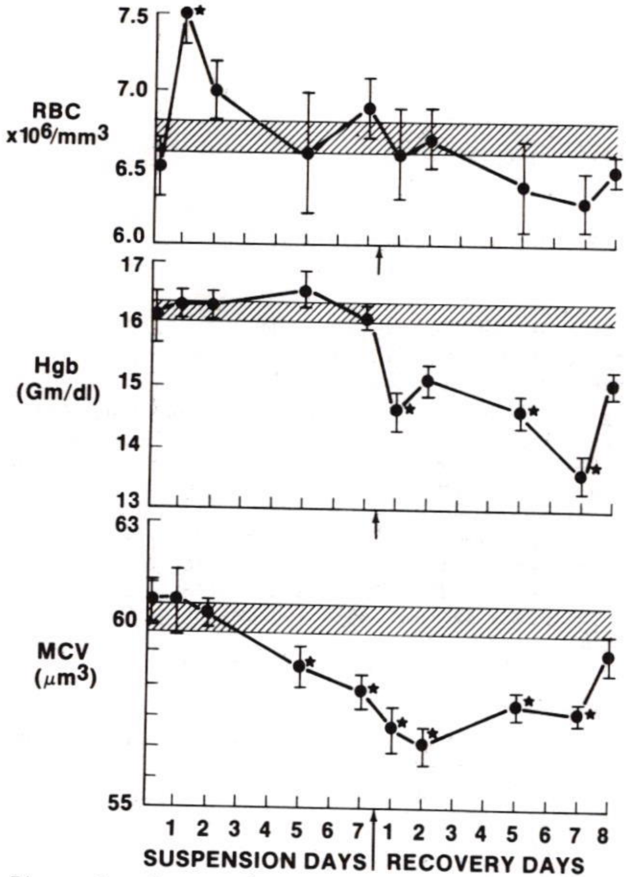

Figure 1. Changes in some erythroid characteristics of rats during suspension. Mean ± SEM. $*P<0.05$ from control values represented by shaded areas.

ing suspension. Post-suspension, a moderate granulocytosis and lymphocytosis were noted. Bone marrow cellularity and spleen weight were both reduced during suspension. A decrease in bone marrow erythroid elements and an increase in large lymphocytes in the spleen were seen.

DISCUSSION:

Significant hematological data during and/or after space flight are only available for rats (4) and man (5). The data now presented offer aninteresting insight into what may be expected when comparable data are available from both rodents and man after actual space flights and, at the same time, can be compared with the current information. Thus, suspended rats have the following characteristics in common with man in space; a reduction in body weight, reduced food consumption, transient hemoconcentration (increased RBC count and hematocrit), a low MCV and reticulocytopenia. These changes, together with the now reported P50 shifts, are causes or expected consequences of suppressed erythropoiesis which would be expected to result in a RCM decrease which our preliminary data are suggesting. One of the most interesting and unexpected findings from the anti-orthostatic, hypokinesic rats was the alteration in RBC survival. While post-space flight data from man suggest no change in RBC survival (5), an increased random destruction has been reported in rats (4). Evidence for enhanced RBC destruction with the rodent model was only observed after suspension and this may contribute to the post-suspension decrease in RBC count, hematocrit, and hemoglobin concentration. Similar to man after space flight, post-suspension rats show a significant leukocytosis but, unlike man, this is not associated with any consistent change in the in vitro lymphocyte reactivity to PHA. Platelet counts appear to remain unchanged in both anti-orthostatic, hypokinesic rats as well as in man following space flight.

The present data suggest that changes in the gravity vector, even in an animal such as the rat with only a small hemodynamic gradient, do produce significant hematological effects. It remains to be determined whether these changes are specific sequalae of altered gravitational forces, revealing some previously-unrecognized regulatory mechanisms, or are secondary to the hemoconcentration-producing cephaled fluid shift, or are non-specific effects of either the reduced food and water consumption, or of the relative inactivity. All of these factors have been implicated in the etiology of the so-called "Anemia of Space Flight" in man (5).

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THE EFFECTS OF HYPERGRAVITY ON THE RATE OF ANTIBODY FORMATION IN THE RAT

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ABSTRACT

This experiment was designed to measure the immune response in acutely stressed and chronically centrifuged hyper-G-adapted male rats. Rats were exposed to 2.1G and 3.1G. Acutely stressed animals were injected with sheep red blood cells (SRBC) on the day of initial exposure to hyper-G, and were chronically centrifuged for 10 to 15 days after immunization. Hyper-G adapted rats were chronically centrifuged for 28 days prior to antigen injection, and for 21 days after injection. Booster injections were given and serum samples taken at intervals from 3 to 9 days after the initial and booster injections. Antigen dose, injected ip, ranged between 1.35×10^6 SRBC and 1.38×10^9 SRBC/100 g. body weight. Pair-fed and *ad libitum* fed noncentrifuged controls were used. No significant differences in anti-SRBC antibody titers were found between centrifuged and control animals, although there were some changes in WBC counts and a significant increase in adrenal gland size in acutely stressed animals.

MATERIALS AND METHODS

All experiments were carried out using a 12 ft. radius centrifuge, with a fixed inner and outer position on each of the 10 radial arms. The centrifuge was rotated at 26.1 rpm, producing a G-intensity of 2.1G at the inner position, and of 3.1G at the outer position. The cages were mounted on pivotal yokes, so that they were free to tilt when the centrifuge was run, subjecting rats to G-forces perpendicular to the floors of the cages.

Altogether, seventy-two weight-matched, 63- to 65-day-old Sprague-Dawley male rats (Bantin-Kingman, Milpitas, CA) were used. Blood was collected from the tail, and standard laboratory methods were used to measure the parameters shown in the tables.

EXPERIMENTAL STUDIES

The first, third and fourth experiments were designed to study the immune response in rats injected with antigen during the acute stress response to centrifugation, when corticosteroid levels were elevated (1). Pair-fed controls and 48-hour-fasted controls were used so that any changes observed in immune function could be attributed to hyper-G exposure, and not to differences in food consumption.

The second experiment examined the effects in hyper-G-adapted rats which were chronically centrifuged for four weeks prior to antigen injection. The food consumption of these hyper-G-adapted rats approximated that of their controls fed *ad libitum*.

All animals were injected with 0.005 ml SRBC suspension/g. body weight. SRBC suspension concentrations (v/v%, in saline) varied as noted below.

Experiment 1. Acute-Stress-Exposed Rats-10% SRBC

Rats were divided into three groups of six rats each as follows: a) maintained at 1.0G and fed *ad lib*, b) maintained at 1.0G and fasted for the first 48 hours of the experiment, then fed *ad lib*, c) centrifuged at 3.1G and fed *ad lib*. Food and water consumption for all groups were recorded. All animals were injected ip on day 0 of the experiment with a 10% suspension of SRBC containing 2.76×10^9 SRBC/ml. Parameters were measured on the days shown in Table I. There were no significant differences in antibody titers or hematocrits on days 5, 7 or 9, but WBC counts were lower in the group exposed to 3.1G on day 9. (Mann-Whitney U-Statistic, two-tailed, $p=0.032$).

Experiment 2. Hyper-G-Adapted Rats-0.5% SRBC

Rats were divided into three groups of six rats each as follows: a) maintained at 1.0G for 28 days, b) centrifuged at 2.1G for 28 days, and c) centrifuged at 3.1G for 28 days. All rats were fed *ad lib* and were injected on day 0 of the experiment with a 0.5% SRBC suspension, which contained 1.05×10^8 SRBC/ml. They were bled on days 3, 5, 7, and 9, and were given a booster injection containing 6.0×10^7 SRBC/ml on day 13. They were bled on days 16 and 20. As shown in Table II, there were no changes in any of the parameters measured on the days indicated.

Experiment 3. Acute-Stress-Exposed Rats-0.5% SRBC

Experiment 3 was similar to experiment 1 except for the following changes:

1.) The three groups used consisted of a 1.0G control, a 2.1G group and a 3.1G group.

2.) the 1.0G and 2.1G groups were pair-fed to the 3.1G group.

3.) The antigen dose was smaller. The animals were injected with a 0.5% SRBC suspension containing 1.19×10^8 SRBC/ml on day 0. They were given a booster injection containing 1.65×10^8 SRBC/ml on day 7.

Table III shows there were no differences in antibody titers between the three groups, although WBC counts were increased in the 2.1G and 3.1G exposed rats on days 7 and 14 (t-test: day 7, $p<0.005$; day 14, 1.0G vs. 3.1G, $p < 0.05$, 1.0G vs. 3.1G, $p<0.01$.).

Experiment 4. Acute-Stress-Exposed Rats-0.016% SRBC

This experiment was identical to experiment 3 with the following changes:

1.) A 0.016% SRBC suspension was used; the initial injection contained 3.1×10^6 SRBC/ml, and the booster injection contained 2.7×10^6 SRBC/ml.

2.) No WBC counts were done.

In Table III, anti-SRBC titers are shown. On days 7 and 14, antibody titers of rats exposed to 2.1G were lower than those of the rats maintained at 1.0G and centrifuged at 3.1G. On day 14 those differences were significant. (Mann-Whitney U-Statistic, two-tailed: day 7, $p=0.026$; day 14, 1.0G vs. 2.1G, $p=0.008$, 2.1G vs. 3.1G, $p<0.016$.)

Table I. Acute effects of hypergravity on antibody titers, WBC counts, and hematocrits

	Days post-immun.	1.0G Control	1.0G Nutritional control	3.1G
Ab Titers[a]	5	7.0±0.7(6)[c]	7.8±1.0(6)	7.4±1.0(5)
	7	9.2±0.3(6)	8.5±0.3(6)	8.8±0.5(5)
	9	8.5±0.3(6)	8.5±0.3(6)	8.5±0.2(5)
WBC[b]	9	1.37±0.12(5)	1.39±0.14(5)	1.06±0.09(5)
Hct	9	52±0.7(5)	53±5.7(5)	50±1.6(5)

In this and the following tables:

[a] Titers are expressed as powers of 2 (X ± SE)

[b] $10^4/mm^3$

[c] Number of rats is in parentheses

Table II. Antibody titers, WBC counts and hematocrits in hyper-G-adapted rats

	Days post-immun.	1.0G Control	2.1G	3.1G
Ab Titers[a]	7	7.7±0.6(6)[c]	7.2±0.8(6)	8.2±0.2(5)
	9	7.7±1.0(6)	8.3±0.3(6)	8.4±0.2(5)
	16	8.8±0.4(6)	8.0±0.4(6)	9.2±0.4(5)
WBC[b]	9	1.24±0.08(6)	1.03±0.11(6)	1.01±0.15(6)
	16	1.17±0.19(6)	0.90±0.08(6)	0.99±0.15(6)
Hct	7	48±1.3(6)	45±2.0(6)	44±1.8(6)

[a,b,c] See footnotes to Table I.

Table III. Antibody titers and WBC counts in acutely stressed rats

	Days post-immun.	1.0G Control	2.1G	3.1G
EXP. 3				
Ab Titers[a]	5	4.5±0.4(6)[c]	4.3±2.1(4)	4.2±0.7(6)
	7	6.3±0.5(6)	6.0±0.7(4)	5.2±0.6(6)
	14	7.2±0.3(6)	7.5±0.7(4)	6.8±0.2(6)
WBC[b]	7	0.91±0.10(6)	1.07±0.06(6)	1.73±0.16(6)
	14	0.77±0.06(6)	1.08±0.11(6)	1.31±2.09(6)
EXP. 4				
Ab Titers[a]	5	0.6±0.6(5)	0.0±0.0(6)	0.3±0.2(6)
	7	3.0±0.7(6)	0.7±0.4(6)	3.3±0.7(6)
	14	6.5±0.3(6)	4.8±0.3(6)	5.8±0.3(6)

[a,b,c] See footnotes to Table I.

DISCUSSION

Hypergravity induces many physiological alterations, including increased levels of corticosteroids, which are immunosuppressive (2). Many other kinds of stress can impair the immune system (3). No differences in hematocrits were seen in any of these experiments. However, in experiments 1, 3, and 4, measuring the acute effects of hyper-G, WBC counts were decreased in the 3.1G-exposed animals in experiment 1, and were increased in 2.1G- and 3.1G-exposed animals in experiment 3. Variations in WBC counts due to centrifugation have been observed (4). In the hyper-G-adapted animals (expt. 2), there were no alterations in WBC counts.

Antibody titers were unchanged, except in expt. 4, where a decrease in titers of 2.1G-exposed animals was seen. This effect was only observed with the lowest antigen dose used.

This study demonstrates the remarkable homeostatic capacity of the immune system under G forces which produce changes in other physiological systems.

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THE EFFECT OF HYPERGRAVITY ON THE PRENATAL DEVELOPMENT OF MAMMALS

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The effect of hypergravity on the prenatal development of mammals was investigated in several studies. Volkova et al. (6) exposed female mice, nonadapted to hypergravity, to 2 g 11-12 h after mating. This exposure was followed by gestation interruption, embryo's death and blastomere lysis. Gurjian (1) and Oyama et al. (3) investigated the effect of centrifugation on mammals who, prior to fertilization, were exposed to hypergravity, i.e. were adapted to centrifugation. In this situation there were no developmental anomalies or they were less marked than in the studies. Ishay et al. (2) exposed rats to 1.04-1.47 g beginning with gestation days 2-4 or 10-13. Some of the animals exposed to 1.43 g and over showed gestation interruption and others - a smaller number of pups in the litter.

The purpose of this study was to investigate the effect of 2 g on the prenatal development of mammals during the last trimester of the gestation period associated with an intensive fetal growth. No data of this type is available in the literature.

Five-month female rats of the Wistar strain were centrifuged at 2 g during gestation days 14 through 21 (the rate of rotation was 33.3 rpm with a pause once a day from 9.00 to 9.30 a.m.). The reproductive function of the centrifuged rats was compared with that of the synchronous controls exposed to the factors that are concomitant with centrifugation (noise, air ventilation, etc.) and that of vivarium controls. The animals were examined 5 to 10 h after they were removed from the centrifuge on gestation day 21.

During the exposure to 2 g the centrifuged rats lost on an average 3 grams. The weight loss of the synchronous controls was similar. The vivarium controls gained approximately 60 grams during the same period. The centrifuged and synchronous rats showed thymus involution as compared to pregnant and non-pregnant vivarium controls ($p < 0.001$).

The gestation was attended by anemia. The hemoglobin concentration in blood and the count of erythropoietic cells in bone marrow of the centrifuged rats were significantly lower than in the synchronous or vivarium controls ($p < 0.01$).

The lymphocytes/neutrophils ratio in blood in the course of gestation decreased due to a reduction of the lymphocyte count and an increase of the neutrophil count, being identical in the centrifuged rats, synchronous and vivarium controls. The lymphocyte count in bone marrow of the centrifuged rats was significantly lower than in the two control groups ($p < 0.01$).

The centrifuged and synchronous rats exhibited no difference between such parameters as embryonic mortality, abnormal fetuses and abnormal placentas (Table I). As compared to the vivarium controls, these parameters slightly declined, apparently, due to the stress-reaction of the maternal body that was seen both in the centrifuged and synchronous rats. This stress-reaction included growth delay, thymus involution and other changes mentioned above. The number of alive fetuses in the centrifuged rats and controls was nearly identical (Table I) with their morphological signs being consistent with their calendar age.

The fetal weight of the centrifuged rats was significantly lower than in the synchronous controls (3.71 g versus 4.24 g, $p < 0.01$); the placental weight of the centrifuged rats was lower than in the vivarium controls but did not differ from the synchronous controls (Table I).

The exposure of the mother-fetus system to hypergravity was accompanied by a delayed skeletal development which included: significant reduction of the ossified areas in the folds of limb bones, shortening of the tail, enlargement of the fontanel as compared to the fetuses of the synchronous and vivarium controls (Table II).

Thus, the centrifugation of female rats at 2 g during the last trimester of their gestation period did not prevent the development of the normal number of alive fetuses, in spite of the stress-reaction of the maternal body which was accompanied by a marked stimulation of catabolic processes and growth delay. The difference in the fetal condition of the centrifuged and control rats was small.

Since previous experiments have demonstrated that hypergravity and weightlessness cause a number of similar (nonspecific) changes which include delayed growth

TABLE I. Parameters of the Reproductive Function of Female Rats Exposed to 2 g during Gestation Days 14-21

GROUP	N	PRENATAL DEATH (%)	ALIVE FETUSES	WEIGHT (g)		MALFORMATIONS (%)	
				FETUS	PLACENTA	FETUS	PLACENTA
2 g	10	24.2±4.2 $p_v < 0.05$	10.1±0.6	3.71±0.12 $p_s < 0.01$	0.45±0.01 $p_v < 0.01$	8	7
SYNCHRONOUS CONTROL	5	35.0±10.8	9.6±1.6	4.24±0.09	0.48±0.015	8	6.3
VIVARIUM CONTROL	15	12.6±2.6	11.4±0.8	4.0±0.1	0.55±0.03	0	1.6

TABLE II. Development of Fetal Skeleton in Rats Centrifuged at 2 g

GROUP	N	LENGTH OF OSSIFIED AREAS (mm)					UNFUSED OSSIFIED AREAS OF STERNUM	FONTANEL WIDTH (mm)
		FEMUR	TIBIA	ILIUM	ULNA	SCAPULA		
2 g	17	2.4±0.05 $p_{v,s} < 0.01$	3.0±0.05 $p_v < 0.002$	2.0±0.03 $p_v < 0.01$ $p_s < 0.02$	3.5±0.04 $p_v < 0.001$ $p_s < 0.02$	3.0±0.04 $p_v < 0.02$	0.5±0.1 $p_v < 0.01$ $p_s < 0.001$	2.9±0.05 $p_{v,s} < 0.002$
SYNCHRONOUS CONTROL	10	2.6±0.05	3.2±0.1	2.1±0.01	3.8±0.1	3.1±0.05 $p_v < 0.02$	0	2.5±0.1
VIVARIUM CONTROL	10	2.6±0.04	3.4±0.1	2.2±0.05	4.0±0.1	3.4±0.1	0.1±0.1	2.5=0.1

and thymus involution(4), our findings suggest, in our opinion, that mammals flown at 0 g during the last trimester of their gestation period may also produce normal fetuses. From the data in the literature and our earlier investigations (5, 7) it follows, however, that gestation days 14 through 21 show greater resistance to adverse environmental effects as compared to other developmental stages. Therefore, it may be expected that exposure to 2 g at earlier gestation stages may give rise to more marked changes in the reproductive function.

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THE EFFECT OF HYPERGRAVITY ON FLUID-ELECTROLYTE METABOLISM IN RAT FETUSES

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Modified gravity produces significant changes in fluid-electrolyte balance (2). This conclusion has been drawn from examinations of adult mammals, whereas the information about gravitational effects on fluid-electrolyte metabolism of developing embryos is practically unavailable. The purpose of this investigation was to study fluid-electrolyte metabolism in pregnant rats and developing embryos during chronic centrifugation.

MATERIALS AND METHODS

Samples were obtained from white rats exposed to 2 g at the last trimester of gestation. Experimental conditions are described in the paper by Serova et al. published in this Supplement. Ten animals, exposed to 2 g, 5 synchronous controls and 5 vivarium controls were examined.

Samples of the myocardium, liver, different areas of kidney and tibia of pregnant rats, placenta, whole fetus, and fetal kidney were put into quartz vials, weighed in a VLAO-100 balance, and dried in a thermostat at 105° until their weight became constant. Then the samples were cooled in an exsiccator to bring them to room temperature and weighed again. After that concentrated HNO_3 was added and the samples were kept in a dry-air bath at 80° to provide complete dissolution of organic substances. Electrolytes were dissolved in 0.01 n HCl; then sodium and potassium were measured in a Zeiss-III flame photometer, in air-propane flame; magnesium, calcium, copper and iron were measured in a Hitachi (model 508) absorption spectrophotometer, in air-acetylene flame.

RESULTS

The vivarium rats gained 60 g during gestation days 14 through 21. The centrifuged rats lost about 3 g during this period and the synchronous rats gained only 2 g. The weight of fetuses from the centrifuged females was 12.1% less than that of synchronous controls. The placenta weight of the centrifuged rats was also lower (by 12.7%) than that of the synchronous controls. The kidney weight of the fetuses of the three groups was identical. However, water content as calculated per dry matter of placentas, whole fetuses and fetal kidneys of the centrifuged rats did not differ from the controls (Fig. 1).

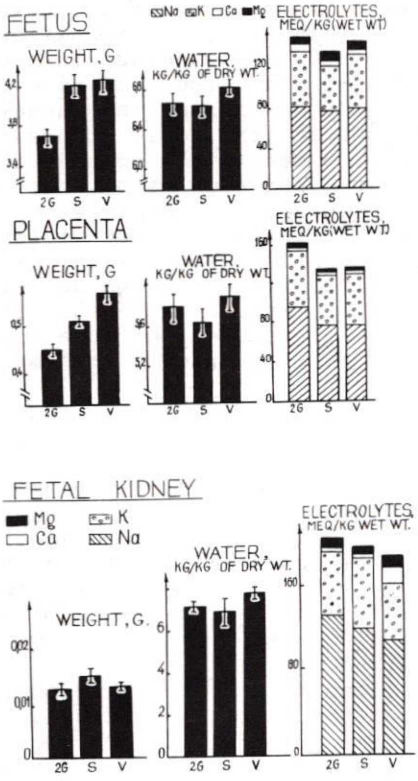

Fig. 1. Water and electrolyte content of the fetus, fetal kidney and placenta of female rats, exposed to 2 g on gestation days 14-21 (2 g - centrifuged rats, S - synchronous control, V - vivarium control)

It can therefore be concluded that water balance of developing mammals exposed to chronic centrifugation remains unaltered. Comparison of the data from the three groups suggests that the effect of stress-factors causes a nearly identical decrease of body weight gain in the centrifuged and synchronous adult females. Conversely, a decrease of fetal weight occurs only in response to centrifugation. The lack of differences in the water content of developing fetuses, their kidneys and placentas of animals from the three groups indicates that the system of fluid-electrolyte homeostasis of a developing mammalian body is well protected from environmental pathogenic effects.

The fetal development strongly depends on the amniotic fluid. Since it is impossible to determine its volume directly, its mass was calculated as the difference between the uterus mass and the mass of fetuses and placentas divided by the number of fetuses. The amniotic fluid mass was reduced in both centrifuged and synchronous rats. Therefore, the decrease in the amniotic fluid volume can be attributed to the effect of nonspecific stress-factors.

The electrolyte composition of developing fetuses was examined in great detail. It is known that the effective cell function largely depends on the capacity to maintain constant the ionic composition and osmotic concentration of body fluids. When calculated per body water, the content of sodium, potassium and magnesium in the centrifuged fetuses does not differ from that in both control groups (Fig. 1). The calcium content of the fetuses as calculated per wet and dry matter increases. These findings are of interest because adult humans exposed to microgravity show marked calcium losses (1).

Analysis of the total content of various elements in whole fetuses gives evidence that, in spite of the decrease of body mass, the content of potassium, calcium, magnesium, copper, and iron in the centrifuged animals does not decline. As a result, the centrifuged fetuses as compared to the synchronous controls show a higher content of potassium, calcium, and iron, when calculated per wet and, particularly, per dry matter.

These findings make it possible to discuss the factors responsible for the reduction of fetal mass during centrifugation. It should be indicated that the decrease involved not only the mass of the fetuses but also that of dry matter. Hence, the content of organic substances lowered. In the centrifuged fetuses the potassium content calculated per dry matter was higher than in the synchronous fetuses. It is known that potassium is primarily an intracellular ion. It can thus be postulated that in centrifuged fetuses the formation of new cells develops at a normal rate but the mass of organic substances of extracellular origin decreases. The lack of differences in the copper and ion content of the fetuses of the three groups suggests that the synthesis of the compounds containing these trace elements remains unchanged. In particular erythropoiesis is not involved (erythron iron makes 60-70% of total iron of the fetal body). The centrifuged placentas show higher amounts of sodium, calcium, and unaltered quantities of potassium, magnesium and the trace elements. The content of the electrolytes and trace elements of fetal kidneys was similar in the three groups (Fig. 1).

The centrifugation brought about no significant changes in the content of water, electrolytes and trace elements in the myocardium, liver and tibia of the pregnant rats. In kidneys, especially in their papillar area, the content of water, iron, and copper decreased and that of sodium and potassium increased. These changes were seen in both centrifuged and synchronous rats and, therefore, can be attributed to the effect of nonspecific stress-factors that accompany centrifugation.

Our findings allow the conclusion that exposure to hypergravity may modify the fetal mass, with water and electrolyte balance remaining unchanged.

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BIOLOGICAL EFFECTS OF WEIGHTLESSNESS AT CELLULAR AND SUBCELLULAR LEVELS

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ABSTRACT

The structure and function of procaryotes and plant eucaryotes at different levels of evolutionary development are discussed in relation to space flight effects. It has been shown that the scope of changes of the bacterial ultrastructure induced by weightlessness depends to a certain extent on the initial conditions of culture growth. Biological effects of weightlessness at the subcellular level of eucaryotes increase with flight time; they also enhance in more complex plant forms; this may be due to intercellular interactions in the tissue system. It is assumed the cell differentiation in weightlessness develops within the framework of normal genetic programs. Biochemical and physiological processes vary significantly due to the lack of rigorous pre-determination.

The pattern of rearrangement in the structure and function of the plant cell in weightlessness is discussed with respect to the problem of adaptation to zero-g.

INTRODUCTION

Studies in cell reproduction, differentiation and vital activity, i. e. processes underlying the growth and development of organisms are of essential importance for solution of fundamental and applied problems of space biology (1, 2). The performed methodical experimental techniques made it possible to use the ultrastructural cell organization and its changes as one of reliable indicators for estimating the extent of spaceflight factors effect on cell functioning. The bacterium Proteus vulgaris (procaryote), unicellular green alga Chlorella, protonema of the moss Funaria hygrometrica, meristem and root cap of pea and arabidopsis plants (eucaryotes) used in space biological experiments with developing organisms were taken as objects for studying organisms of various levels.

RESULTS

Experiments with the procaryotes have shown that the degree of structural manifestation of the space flight effect depends to a certain extent on the initially prescribed conditions of the culture growth. Under optimum growth conditions the cells of Proteus vulgaris grown in orbital flight differed from the control ones in a number of ultrastructural organization indices. So, distribution of cytoplasm containing ribosomes over the whole cell volume is more uniform, the membrane gyrosity is more pronounced, occurrence and volume of fine-granular inclusions are increased, availability of compact-arranged DNA fibrils forming various configurations. Addition of a modifying agent (2, 3, 5-triphenyl tetrazolium bromide) to the medium and anaerobic growth conditions permitted revealing the differences which were not displayed under optimum aerobic conditions. Rather essential differences are established in localization of electron-dense almost insoluble formazan deposits resulted from a specific reduction of tetrazolium salts. This was laid as a basis for a supposition on changes in the membrane permeability pattern occurring when cultivating a facultative aerob under anaerobic conditions which is intensified by factors of spaceflight. New differences in the Proteus ultrastructural organization - formation of cell wall evaginations more expressed in the experiment and formation of intricate plasmalemma invaginations, which is not typical of G-negative bacteria, observed when triptose is available in nutrient medium and under facultative anaerobiosis are undoubdetly evidence for essential changes in cell metabolism pattern under spaceflight conditions. It should be mentioned that during orbital flight under such initially modified conditions the growth of Proteus culture, as distinct from optimum growth conditions (growth acceleration in the experiment) and unfavourable conditions (growth inhibition in the experiment), did not differ significantly from the such in the control variant. In this connection an assumption is advanced on an adaptation role of complicated membrane structures formed by plasmalemma in cells of the experimental variant.

Experiments with developing organisms

have also shown that biological effects of spaceflight conditions become stronger with prolongation of the time of the experiment. In a 10-day experiment no essential differences were found in growth indices and ultrastructural organization between experimental and control cultures of Chlorella (strain LARG-1), which testifies to a normal, on the whole, functioning of cells during the experiments. Application of a morphometric analysis made it possible to reveal a tendency to a decrease in the volumes of plastids and their structural components -starch grains and thylakoids. Under a more prolonged space flight - 28 days, alongside with on accelerated biomass gain, in the experimental variants there also occurred a more rapid ageing of the culture. This was clearly pronounced in the ultrastructural organization and in relative volumes of cytoplasmic organelles and reserve substances.

It is shown that the weightlessness effect becomes stronger with complication in plant organisms, which might be due to intercellular interactions in tissue system of multicellular forms as compared to unicellular ones. So, in particular, under weightlessness the root meristem cells are characterized by the presence of mitochondria with on electron-dense matrix and well-developed, relatively regularly arranged cristas. This is not typical of meristematic cells. The well known correlation between ultrastructural and functional peculiarities of mitochondria gives reason to make a conclusion on a significant intensification in the functional activity of meristematic cell mitochondria under weightlessness. An unusual alteration of a peripheral cistern of dictyosome secretory pole is an ultrastructural peculiarity of meristematic cells under weightlessness of no less interest. Taking into account the role of the Golgi apparatus in synthesis and transport of a greater part of polysuccarides incorporated into the cell wall composition,it is possible to assume that the visible alterations in the cistern of the dictyosome secretory pole reflect to some extent disturbancy in these processes. It is not impossible that structural rearrangements of dictyosomes of the Golgi apparatus as well, particularly in processes of assemblage, growth and specialization of membranes, which undoubtedly will disturb dynamic equilibrium between metabolism phases in meristematic cells.

An increased volume of condensed chromatin in nuclei of the root cap central statenchyma cells might show a decrease in the nuclei functional activity.

In central statenchyma cells of in the root cap in pea and arabidopsis plants growing under spaceflight conditions there is no clear localization of amyloplasts-statoliths: they are located in various parts of a cell. The amyloplasts stroma becomes of lower electron density as compared to the control variant, the volume and number of starch grains are decreased (in 7-day pea plants). In plastids of root cap central statenchyma cells in 18-day arabidopsis and pea plants starch either is absent or represented by single round or oval grains located in the organelle central part in a slice. The central statenchyma cells of experimental plants are characterized by a progressive vacuolization with an increasing flight period. The absence of a gravitational stimulus causes to a failure in due gravireception system functionability. The normal spatial arrangement of plant organs under weightlessness is due to phototropism and chemotropism compensation for a geotropic reaction impossible under there conditions. This phenomenon offers new interesting opportunities for studying tropisms in plants under zero gravity becouse the action of only a single, one or another factor can be studied in an absolutely pure form.

Rearrangements of cell organization found at a structural level, including increased frequency of electron-dense globules occurrence in cell membrane components identified as Ca^{++}-binding centres, indicate to changes in cell metabolism and confirm data available in literature based on biochemical and physiological methods. The character of changes in the structural-and-functional organization of a plant cell under weightlessness without anomalies in processes of mitosis, cytokinesis and differentiation of vegetative organ tissues, gives reasons to assume that rearrangements of cell metabolism under zero gravity occur within the limits of existing genetic programmes.

During a definite time of zero gravity effect the cell functioning provides on the whole the implementation of cytodifferentiation and morphogenesis programmes genetically determined on the Earth. Changes in biochemical and physiological processes are highly significant because of the absence of initially rigid determination. In the light of the data obtained, the immediate task of further studies seems to be revealing of cell metabolism links which are most sensitive to the effect of weightlessness.

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RESPONSE OF AMPHIBIAN EGG CYTOPLASM TO NOVAL GRAVITY ORIENTATION AND CENTRIFUGATION

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Descriptive and experimental evidence for the compartmentalization of the cytoplasm of Xenopus laevis eggs into yolk platelet density compartments is presented and discussed.

Gravity orientation of amphibian eggs has been used by various investigators as a probe for studies on amphibian pattern formation. Recent observations from our laboratory revealed that inverted Xenopus eggs display substantial pattern reversal and can develop normally. Since the determinants of early pattern formation reside in the egg cytoplasm, gravity driven perturbation provides an experimental tool for studying the manner in which the cytoplasmic program which regulates early postfertilization embryogenesis is organized (Neff et al., 1983). In this report we presend descriptive and experimental evidence which indicates that the cytoplasm of Xenopus laevis eggs is compartmentalized into a set of distinct and morphologically identifiable yolk platelet density compartments. We describe and discuss the response of these compartments in unfertilized, fertilized and artifically activated inverted eggs to normal gravity (1 x g) and hypergravity (10-183 x g).

The mature freshly spawned (unfertilized) Xenopus egg exhibits a distinct animal/vegetal polarity of external pigment. After fertilization the egg rotates within the perivitelline space created by the release of cortical granules. The darkly pigmented animal hemisphere opposes gravity and the lightly pigmented vegtal hemisphere faces gravity. As a starting point for this investigation normally oriented unfertilized eggs were fixed and embedded in plastic and sectioned midsagittally at 0.5, 1.0, and 4.0 um (Neff et al., 1983). Figure 1a indicates that the egg cytoplasm can be divided into a set of morphologically distinct yolk platelet zones or compartments that show clear animal/vegetal asymmetry as well as radial symmetry about the animal/vegetal axis. It should be understood that other cytoplasmic components such as lipid bodies, glycogen granules, macromolecules, etc. may also show unique asymmetric distributions.

The small yolk mass (SYM) occupies most of the animal hemisphere and contains predominantly small yolk platelets (<4u, diameter). The yolk platelets of the centrally located-clear cytoplasm (CC) are less densely packed. The intermediate yolk mass (IYM) consists mostly of intermediate sized yolk platelets (4-8 um diameter). The vegetal yolk mass (VYM) occupies most of the vegetal hemisphere and contains mostly large yolk platelets (>8 um diameter). The subcortical vitelline layer (SVL) was discovered experimentally in inverted eggs and is 30 to 60 um thick. It is located in the vegetal hemisphere between the cortex and the LYM. The SVL contains the germ plasm and a variety of small, intermediate, and large yolk platelets.

After fertilization there is a sequence of asymmetrical surface contractions and internal cytoplasmic displacements (Fig. 1a) that coincide with the establishment of the future dorsal side of the egg and subsequent primary embryonic axis formation. Those events are usually oriented opposite the sperm entrance point (Ubbels et al., 1983).

Yolk platelets isolated on a continuous isopycnic density gradient are not uniformly dense. The large yolk platelets tend to be more dense than the small ones. (Radice et al., 1981). Midblastula stage blastomeres isolated on a continuous density gradient are also not the same density. The larger blastomeres contain the larger yolk platelets and are more dense than the smaller blastomeres (Neff and Malacinski, 1982). Preliminary cytological examination of Xenopus eggs which were maintained in an inverted orientation from fertilization onwards show substantial but not complete gravity driven compartment rearrangements (Fig. 1c; Neff et al., 1983). These results led us to hypothesize that the cytoplasm of Xenopus eggs is organized into a set of discrete compartments. Those compartments possess unique morphologies and physical properties (such as density and viscosity) and perhaps contain unique regulatory macromolecules.

We examined whether compartments behave as discrete entities in 1 x g inverted eggs. Inverted eggs were fixed sequentially after fertilization (T= 0.07, 0.25, 0.50, 0.75, 1.0, first cleavage, and 3.0, third cleavage), embedded in plastic, and midsagittally sectioned at 0.5 um. Figure 1c indicates that the intermediate and large yolk compartments move toward gravity as coherent masses with very little mixing between the compartments along the periphery of the egg. The central cytoplasm and small yolk mass shift against gravity. At T=0.75 the compartment shift is substantial, but not complete. The large yolk mass occupies the animal hemisphere (which faces gravity). The small yolk mass occupies the vegetal hemisphere. The SVL is intact in the original vegetal hemisphere. This general pattern is maintained through the third cleavage and subsequent embryogenesis. The compartment shifts are asymmetrical. Most inverted eggs are not exactly 180° inverted. Rather, they are slightly tilted with respect to the animal/vegetal axis. The shift toward gravity occurs on the "downtilt" side of the egg regardless of the sperm entrance site. Preliminary evidence from our laboratory has shown that the direction of the large yolk mass shift, rather than the sperm entrance site, determines the future dorsal/ventral polarity of the inverted egg. On the other hand, eggs which are inverted exactly 180° show symmetrical compartment shifts and subsequent abnormal early pattern formation. We conclude that compartments behave as discrete entities and they appear to have unique physical properties. Some compartments move toward gravity (LYM, IYM), and others against it (CC, SYM). The SVL is apparently not affected by gravity orientation.

The cytoplasm of the unfertilized egg does not rearrange in response to inversion (Fig. 1b; Radice et al., 1981). We examined whether compartment release is a result of sperm penetration or a general property of egg activation. Unfertilized, inverted eggs were artifically activated by electrical shock (79 V, 1-2 seconds) in 100% Steinberg's solution (pH 7.4). The compartment shift was identical to unfertilized, inverted-control eggs. It appears, therefore, that compartments are preloaded into unfertilized eggs during oogenesis and are released from one another by egg activation.

Next, we determined the response of the compartments of unfertilized and fertilized egg to increased g forces. Unfertilized, and fertilized eggs were chemically dejellied (1.75% cysteine) and embedded in 9% gelatin (175 Bloom)--10% Steinberg's solution (pH 7.4), inverted, and centrifuged for 5 minutes at various gravitational forces (10-183 x g). Normal orientation, inverted, centrifuged, and non-centrifuged eggs were fixed and embedded in plastic or paraffin and sectioned mid-sagittally (4 um and 8 um).

Figure 3a shows an unfertilized, inverted egg centrifuged at 67 x g for 5 minutes. No substantial compartment shift or packing of yolk platelets occurred. Either longer centrifugation or higher g forces did, however, result in some yolk platelet packing.

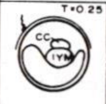
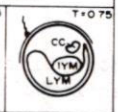
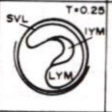
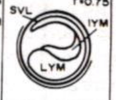
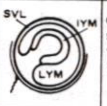
Figure 3b shows an inverted fertilized egg at T=0.33 centrifuged at 18 x g for 5 minutes. The compartments shifted as discrete masses with little mixing. The SVL maintained its integrity. Both the degree and type of compartment shift were similar to inverted eggs maintained at 1 x g.

Figure 3c shows an inverted, fertilized egg (T=0.33) which was centrifuged at 67 x g for 5 minutes. The compartments dispersed and the yolk platelets packed. The SVL was also disrupted. The integrity of the compartments can clearly be overcome by substantial g forces.

These results can be unified into a "density compartment" model for egg dorsalization. It has the following features: Eggs are preloaded with compartments during oogenesis without respect to gravity orientation. Activation by sperm or other stimuli releases the preloaded compartments to respond to internal forces (eg. sperm aster growth) as well as external forces (i.e. gravity). Those forces break the radial symmetry of the egg. The symmetry breaking establishes the cytoplasmic organization and compartment interactions that may be necessary for subsequent early pattern formation.

This model unifies the phenomena of animal/vegetal and dorsal/ventral polarity into a single concept. The model predicts that subcompartments may exist and postulates that compartment interactions might establish new and unique molecular interactions which are important for pattern specification. The breaking up of the compartments by centrifugation may account for the developmental abnormalities such as twinning. A straight forward interpretation of this model predicts that <u>Xenopus laevis</u> eggs will have difficulity establishing a primary embryonic axis in the microgravity environment of outer space.

This research was funded in part by NASA grant NAGW-60.

Fig	Orientation	Experiment T=0.0	Results	
1a		fertilized, normal orientation at 1g	T=0.25 	T=0.75 
b		unfertilized, inverted at 1g		
c		fertilized, inverted at 1g	T=0.25 	T=0.75 
2		electrically artificially activated, inverted at 1g	T=0.25 	T=0.75 
3a		unfertilized, inverted, centrifuged, 10-67g for 5 minutes		no major compartment shift or packing of yolk platelets
b		fertilized, inverted, centrifuged, low speed ≤18g for 5 minutes		compartment shift similar to Figure 1c above
c		fertilized, inverted, centrifuged, high speed ≥34g for 5 minutes		breaking up of compartments and SVL, packing of yolk platelets

Diagramatic representation of experiments and results of density compartment investigation. Diagrams depict mid-sagittal cytological sections. Abbreviations are described in text.

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THE EFFECT OF A 90-DAY HYPODYNAMY ON THE NEUROHUMORAL SYSTEM, EGG LAYING AND METABOLISM OF PROTEINS IN JAPANESE QUAIL

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ABSTRACT

Egg laying Japanese quails were exposed to the hypodynamy for 0,5,30,60 and 90 days. The concentration of catecholamines in hypothalamus and plasma and plasmatic level of corticosterone, thyroxine, triiodothyronine and estradiol were analyzed. The egg production, body weight and concentration of proteins, DNA, RNA in m.pectoralis and m.fibularis completed the picture of changes. The long-term hypodynamy evokes markedly changes in the neuroendocrine system binding preferently at the beginning and the last days of hypodynamy. In experimental groups exposed to 30 and 60 days of hypodynamy, the indicators of neurohumoral activity give the symptoms of a temporary adaptability.

INTRODUCTION

Though the specific situations of space flight such as weightlessness or strongly limited space create nonphysiological situations for living organisms |5|, the results obtained from series of experiments in rats confirm that several weeks spent in the orbit do not result in intensive changes of neurohumoral functions |8,16|.

For testing of some basic physiological functions in Japanese quail under the conditions resembling space flight a specialy adapted model of unforced restriction was used as a simulation of weightlessness under the conditions of Earth surface. Our previous experiments with a short and long-term unforced restriction showed a rapid activation of neuroendocrine system |6|.

MATERIALS AND METHODS

Adult females of Japanese quail |Coturnix coturnix japonica| of an average weight of 135 g at the extensive egg-laying period were used. The animals were housed at 23 °C ± 2 °C under a 14:10 h light:dark cycle and provided with starter for turkeys and water ad libitum. The quails were divided into 5 groups |8 animals per group|. The control birds were kept in individual cages. The quails of other groups were restricted for 5,30,60 and 90 days. The hypodynamic quails were hung in the tight jackets preventing them from moving and touching the bottom of the cage with the legs. Food and water intake remained unrestricted. The quails were taken in the experiment in such way that all the groups could be decapitated on the same day. Blood was collected in heparinized tubes. From the isolated brain the hypothalamus was dissected on a cooled plate. From the skeletal muscles samples were taken from musculus pectoralis and from musculus fibularis. A sample from liver was taken as well.

The concentration of catecholamines in hypothalamus and plasma was estimated by a radioenzymatic method |7|, the level of thyroxine |3|, triiodothyronine |15| and estradiol |Sorin kits-Italy|, in plasma was measured by specific radioimmunoassay, corticosterone |10| by a protein binding assay. Dry matter of the skeletal muscles and liver was obtained by lyophilization. Protein concentration in muscles and liver was estimated according to Lowry et al. |9|. DNA and RNA in the same tissue was determined with a modification of spectrophotometric method described by Pecháň |13|. The data were evaluated by the analysis of variance, the values are expressed as means ± S.E. The differencies between means were calculated by Duncan's test. Experimental data on the egg laying were analysed using a mathematical model |4|.

RESULTS

The catecholamine content in hypothalamus considerably changed during the hypodynamy |Fig. 1|. On the 5th day noradrenaline plus adrenaline were significantly depressed. On the 30th and 60th day their concentration approached to the level of the control group while on the 90th day a significant decrease of that was found. The concentration of dopamine was also markedly decreased on the 5th day of restriction and remained at the same level during the whole 90-day experiment.

Total catecholamines in plasma significantly increased on the 5th day

TABLE 1. Body weight, dry matter and DNA content, protein and DNA, RNA and DNA ratios in thigh muscle

Days of hypodynamy	0	5	30	60	90
Body weight	135,00 ± 4,09	111,00 ±** 4,94	119,60 ±** 4,01	116,71 ±** 5,37	117,00 ±** 7,03
Dry matter |mg.g^{-1} wet tissue|	279,09 ± 4,51	278,88 ± 3,88	276,95 ± 6,25	275,24 ± 6,22	273,12 ± 4,48
P-DNA |ug.g^{-1} wet tissue|	21,68 ± 1,50	30,30 ± 2,60	28,01 ± 3,50	25,48 ± 1,57	24,42 ± 2,22
$\frac{\text{Protein}}{\text{P-DNA}} \cdot 10^{-2}$	117,90 ± 8,86	69,69 ±** 5,98	85,47 ±* 19,24	82,44 ±* 9,02	92,77 ± 16,10
$\frac{\text{P-RNA}}{\text{P-DNA}}$	0,78 ± 0,07	1,21 ±** 0,08	0,97 ±* 0,03	0,93 ±* 0,05	0,94 ±* 0,01

P-DNA, P-RNA - phosphorus DNA, phosphorus RNA. Each value represents the mean ± S.E. of 7-8 quails.
* Significantly different from the value of control, $P < 0,05$.
** Significantly different from the value of control, $P < 0,01$.

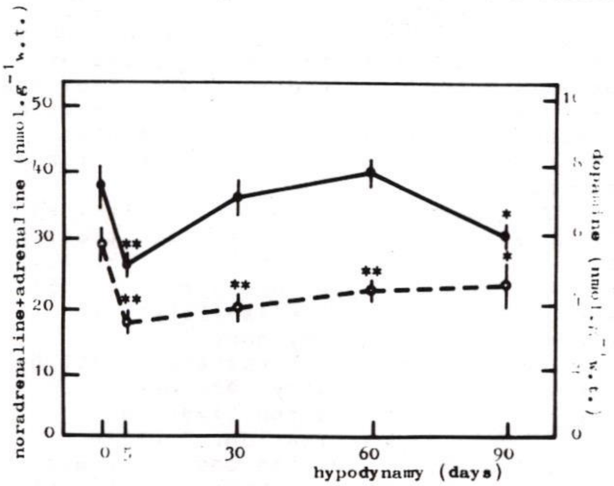

Figure 1. The effect of hypodynamy on noradrenaline + adrenaline and dopamine content in hypothalamus. Each value represents mean ± S.E. of 7-8 quails; ●—● noradrenaline + adrenaline; o--o dopamine; * $|p<0,05|$, ** $|p<0,01|$ - significant difference vs.control.

|Fig. 2| while their level on the 30th and 60th day did not differ significantly from controls. On the 90th day a significant rise was again noticed.

The corticosterone concentration in plasma showed highest values on the 5th day |Fig. 3| and was maintained up to the 30th day of the hypodynamy |Fig. 3|. It was on the level of the control group on the 60th day and after another 30 days its concentration significantly increased.

The reaction of the thyroid gland to long-time hypodynamy was observed via the thyroxine and triiodothyronine level |Fig. 4|. While the concentration of the former hormone did not change throughout the experiment, the latter one remained significantly increased from the 30th day up to the 90th day.

The activity of gonads was studied via the plasmatic estradiol level and the intensity of egg laying |Fig. 5|.

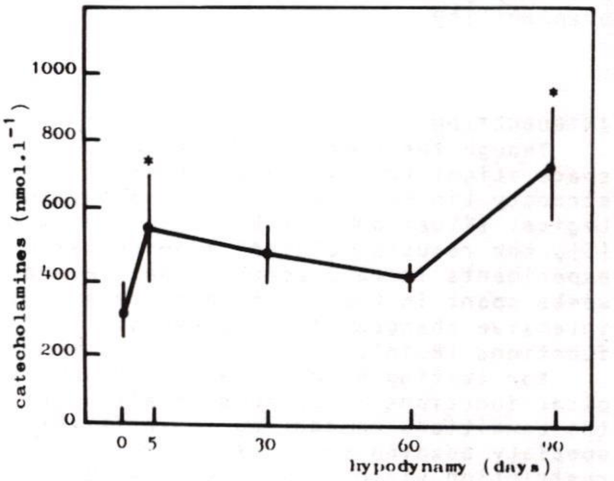

Figure 2. The effect of hypodynamy on catecholamine concentration in plasma. Each value represents mean ± S.E. of 7-8 quails; * $|p<0,05|$ - significant difference vs.control.

Estradiol in plasma decreased most markedly on the 5th day of the experiment and remained significantly decreased up to the 90th day. The egg laying was also inhibited. The lowest egg laying was recorded between the 3rd and 9th day, the highest one between the 58th and 64th day, representing 25 % resp. 82 % of the initial level of egg laying. The

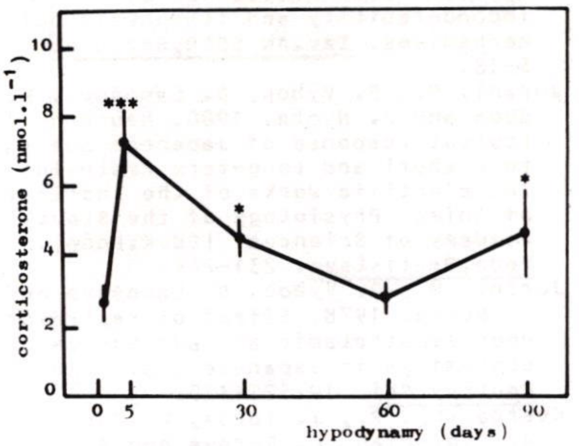

Figure 3. The effect of hypodynamy on corticosterone concentration in plasma. Each value represents mean ± S.E. of 7-8 quails; * |$p<0{,}05$|, *** |$p<0{,}001$| - significant difference vs. control.

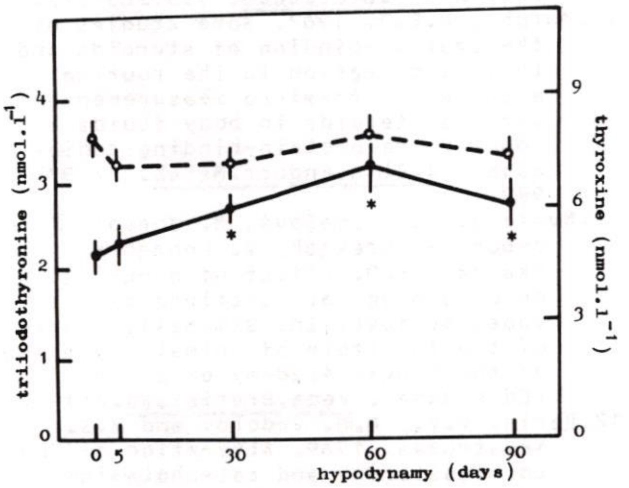

Figure 4. The effect of hypodynamy on the triiodothyronine and thyroxine concentration in plasma. Each value represents mean ± S.E.; ●—● triiodothyronine; o--o thyroxine; * |$p<0{,}05$| - significant difference vs. control.

aproximation by means of mathematical model, explaining 83 % of the total variation in recorded data, resulted in evaluation of the time of the maximum decrease of egg laying T_{min}= 7.2 days and the time of reaching the maximum in egg-laying during hypodynamy T_{max}= 63 days

Body weight of Japanese quail significantly decreased already on the 5th day of hypodynamy and remained at this level throughout the whole experiment |Tab. 1|. In skeletal muscles |m.pectoralis and m.fibularis| the dry matter content and DNA was not changed. We recorded significant increase in the RNA/DNA ratio of m.fibularis begining on the 5th day and lasting to the 90th day of the hypodynamy. On the contrary decrease in the protein/DNA ratio up to the 60th day was observed in this muscle. The parameters mentioned above were not significantly changed in breast muscles. Significant differencies were observed neither in the dry matter of the tissue nor in the DNA content, RNA/DNA and protein/DNA ratio in the liver.

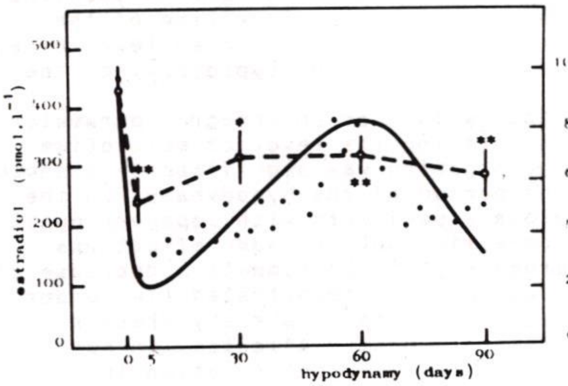

Figure 5. The effect of hypodynamy on estradiol concentration in plasma and egg laying. Each o value represents mean ± S.E. of 7-8 quails; each o value represents the mean of 3 days egg laying; o--o estradiol; ●—● egg laying; * |$p<0{,}05$|, ** |$p<0{,}01$| - significant difference vs.control.

DISCUSSION

The response of the organism to stress is associated with a decrease of hypothalamic catecholamines. The work of Parin et al. |12| confirms that 30-day hypodynamy lowers the hypothalamic catecholamine content in rabbits. Our previous experiments with acute hypodynamy in Japanese quail refer to the decline of noradrenaline plus adrenaline in the first minutes of hypodynamy, whereas dopamine started to raise after four hours |7|. The content of catecholamines in the hypothalamus was raised during the second week |6|.

The plasma catecholamine level considerably increased as shown in previous experiments with a short-term or long-term hypodynamy in Japanese quail |6,7| or in domestic fowl |11|.Such response being very similar to that described under similar conditions in rats |2|. Corticosterone, another adrenal hormone characterizing the level of stress, significantly increased during hypodynamy except of the 60th day of hypodynamy. This completes the picture of adrenal cortex function from our previous works |6,11|. The increased level of corticosterone due to a long-term hypodynamy is known also in rats |2|.

The changes of triiodothyronine level refers to the higher measure of peripheral deiodation of the thyroxine caused by long-term hypodynamy. In the previous experiments with short-term hypodynamy in which only thyroxine level was determined its decline was demonstrated |6|.

A significant decline of estradiol level associated with a decreased egg laying |6| after a 3-week hypodynamy of Japanese quail was recorded. An identical picture of a permanent decline of the estradiol level and a lowered level of egg laying is provided by hypodynamy on the 90th day.

Body weight as an integral parameter characterizing the level of metabolism of the organism was significantly changed in the period of the hypodynamy. In the previous experiments with Japanese quail and domestic fowl the same effect was observed |6,11|. In mammals a decrease of body weight was demonstrated |14| under the same conditions, whereby skeletal muscles were mostly affected. In our experiment a different reaction in protein level between m.pectoralis and m.fibularis was observed. While in the former case the protein content did not change, in the latter one its decline occured, thus indicating its increased break down. Presumably muscle distrophy reported in some histological works |1| occurs due to a decreased strain of thigh muscles during hypodynamy. On the other hand, increased RNA values during hypodynamy express a more intensive protein synthesis. Due to hypodynamy the total metabolism of muscle proteins is apparently enhanced with the predominance of katabolic processes.

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RESISTANCE OF MATURE ARABIDOPSIS PLANTS TO MECHANICAL DEFORMATION IN RELATION TO G-FORCE DURING DEVELOPMENT

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ABSTRACT *Arabidopsis* plants were grown in centrifuge tubes under well standardized culture conditions. Each plant was subjected to centrifugation (roots out) for 10 min at one of a series of centripetal forces between 7 and 390*g*. No deformation was observed in plants centrifuged at less than 35*g*. An "average" degree of deformation was attained at about 60*g*. All plants exposed to more than 95*g* were maximally deformed but none was broken nor otherwise damaged irreversibly even at 390*g*. In every case new shoot growth continued normally after the centrifugation.

A plant population grown on horizontal clinostats (0.5 rpm) under culture conditions exactly the same as for the upright plants responded to centrifugation stress in a way that was not substantially different from the response pattern of the plants cultured upright at 1*g*.

INTRODUCTION There are indications that some structures of higher plants develop in response to normal gravitational stress but whether plant growth is sensitively adapted to the magnitude of the *g* force has not attracted much attention on the part of experimental plant morphologists. If such adaptations occur they may also be physiological, especially in herbaceous plants whose stature depends chiefly on maintenance of cell and tissue turgor. Conceivably such plants *might* be capable of regulating cell turgor in response to prolonged growth at a gravitational stress higher or lower than normal.

We performed exploratory experiments to evaluate the resistance of mature *Arabidopsis thaliana* plants to increased mechanical stress due to loading by a controlled centripetal force and to determine whether that resistance was different when the test plants were grown in simulated weightlessness on horizantal clinostats.

METHODS Aseptic cultures were established in 50 ml centrifuge tubes. In each tube one seed was placed on the surface of 10 ml of nutrient agar. The tube was covered with a layer of 0.5 mil Saran Wrap secured by a rubber band. Growth occurred under constant conditions of temperature (24° ± 1°C) and fluorescent illumination (150 f.c. Wide Spectrum GroLux coming from above the seedlings). When plants were 21 days old each tube was subjected to a 10 min. period of centrifugation at a controlled *g* level. The *g* force was imposed in the normal axial direction (centrifugation was "roots out"). The speed of the DC centrifuge was adjusted by a variac and monitored with a stroboscope. To avoid overshooting the desired *g* value while bringing the centrifuge up to speed, the stroboscope was set at a predetermined value and the centrifuge speed was controlled by the variac to achieve as quickly as possible and thereafter to maintain the desired rpm. Generally it required from 40 to 60 seconds for the centrifuge to come up to speed so that for at least the last 9 min. of the period of centrifugation each plant was exposed to exactly the prescribed *g* stress. The condition of each plant was noted before and after centrifugation.

PLANT RESPONSE It was possible to recognize six categories of mechanical effects due to centrifugation. In order of increasing severity of plant collapse these were:

I. No detectable effects.
II. Incipient collapse; shoot no longer erect but not seriously deformed.
III. Partly collapsed; stem leans against wall of tube or tip may be bent over.
IV. Collapsed; considerable portion of stem pressed against agar surface but some parts still raised above the surface; some rosette or bract leaves extended; no breakage.
V. Complete collapse; all of flower stem, branches, and most or all leaves pressed firmly against agar surface; no breakage.
VI. Complete collapse; evident breakage.

Obviously the differences between categories cannot be assumed to be quantitatively equal even though the progressive array of response categories does represent some measure of plant resistance to axially directed mechanical stress. The several categories are roughly in order of severity of plant deformation and the array can be considered as a crude scale of plant resistance to collapse.

RESULTS In order to grasp the response pattern or trend of the data we may safely ignore the undoubted nonlinearity of the plant response scale and array the individual plant scores in relation to the centripetal *g* force each plant experienced. Figure 1 shows the array of responses thus "plotted". The curve was fitted by eye to the central trend of the data obtained from three separate experiments. In categories I through V the plants were not killed. In a few cases of incipient collapse (II) the bent portion of the plant stem achieved partial recovery although this was unusual. In *all* cases *new* shoot growth subsequent to centrifugation was erect and healthy even though the entire shoot system may have been completely collapsed and the older parts remained so. In no case was the plant killed by centrifugation even up to the highest *g* values imposed (390 *g*). Breakage (VI) was never observed. Deformation as the result of applied mechanical stress was detected only above 35*g* and was constant in all cases above 95*g*. A rough estimate of the centripetal force needed to produce "average" deformation was about 60*g* for 10 min.

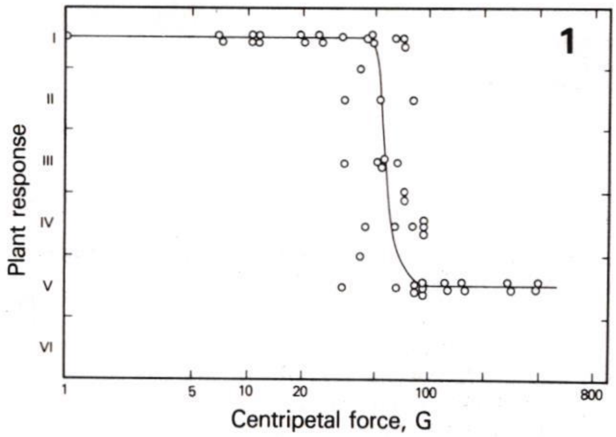

Figure 1. Categories of response (ordinate) to 10 min exposure to centripetal force (abscissa) by mature, green, herbaceous plants (*Arabidopsis thaliana*) that had been cultured under standardized conditions in normal upright (1*g*) orientation.

We note that some readers may instinctively compare the plant's average stress resistance to 36,000*g*·sec to the much lower values that have been reported for man and even much smaller mammals. It seems evident that the higher plants are somewhat more overbuilt than are the higher animals.

EFFECT OF G-STRESS DURING PLANT CULTURE Since Fig. 1 characterizes the response pattern of a population of plants grown to maturity in normal gravity, we wanted to compare that result with the response pattern of plants cultivated on horizontal clinostats (simulated microgravity). We used the same culture conditions in all respects except that plant growth occurred during continuous rotation on horizontal clinostats (0.5 rpm). Figure 2 shows the array of results. Again the data points were fitted by eye with a solid line marking a central trend. To facilitate comparison, the line from Fig. 1 is reproduced as a broken line in Fig. 2. In view of evident variation within each response pattern we would not want to claim that differences were significant.

DISCUSSION If the *g* force that prevailed during culture of our test plants affected their ability to withstand unfamiliar, high, *g* stress, that conditioning influence was not enough to make a confidently identifiable difference in our relatively crude measurement of their resistance. We note, however, that the cells of higher plants, unlike those of animals, characteristically deposit around themselves very strong walls that permit the occurrence of positive (and negative) pressures of impressive magnitude--in the stems of herbaceous plants, commonly as high as 0.5 to 1 x 10^6 Pa (5 to 10 bars) and at times as low as -1 or -2 x 10^6 Pa (-10 or -20 bars). Since plant cells and therefore stem tissues are built to withstand such an enormous range of pressures, perhaps we should not find it surprising that stem stiffness is not much affected by the presence or absence of a small additional compression force attributable to only a few grams of mass loading.

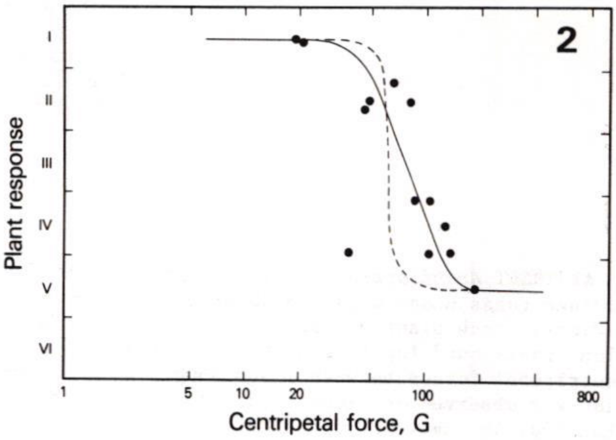

Figure 2. Categories of response (ordinate) to 10 min exposure to centripetal force (abscissa) by mature, green, herbaceous plants (*Arabidopsis thaliana*) that had been cultured under the same conditions as those that contributed data to Fig. 1 except that the plants had been grown in ***simulated*** hypogravity on horizontal clinostats.

ACKNOWLEDGEMENTS The technical assistance of R.P. Kuneiwicz and P. O'Dowd was especially helpful. Direct financial assistance was provided through a contract between the NASA Ames Research Center and the University of Pennsylvania (NAS 2-22432) and by a NASA Headquarters grant in support of research (NGR 39-010-104).

CLINOSTAT EFFECTS ON SHOOT AND ROOT OF ARABIDOPSIS

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ABSTRACT - In the clinostat environment, Arabidopsis thaliana (L.) Heynh. plants grown in cultures having limited gas exchange with the external atmosphere developed "above" the agar media a large mass of roots which eventually covered the stem. Cultural conditions were 0.5 rpm, 350 ft.c. from cool white fluorescent lamps, and 25°C. Limited gas exchange culture tubes were covered with Saran Wrap or mylar film while free gas exchange cultures were plugged with cotton or polyurethane foam. Average shoot-root dry weight ratio of clinostatted, limited gas exchange plants was 3.39 ($p<0.001$) as compared to 10.1 for upright stationary and 10.3 for vertically rotated controls. Average dry weight (48.3 mg) of all clinostatted shoots was greater than those reported by other investigators (15.1 mg). Finally, shoots of our clinostatted plants were almost 2x heavier ($P<0.01$) than our controls (28.7 mg, 26.7 mg), a result contrary to findings of previous investigators where the clinostatted plants are the lightest (clinostat 15.1 mg, controls 16.5 mg and 17.2 mg).

INTRODUCTION - With the near advent of long duration space missions having large crews, information is required on how to grow multiple generations of plants for food and oxygen in a micro-gravity environment. Studies have been done on growing plants from seed to seed both in the weightless environment of space (Parfenov and Abromova 1981; Yudin 1981), and under simulated weightlessness on Earth using clinostats (Kerminskiy and Tarkhanovskiy 1980; Merkis, et al. 1976). The difficulty of growing plants as indicated by the poor growth or failure to form seed in space and on clinostats, has revealed a need for precise cultural information on growing plants from seed to seed under controlled in vitro conditions using simulated or true weightlessness.

Besides precise information on growing specific plant species, the growth responses to various cultural methods and imposed environmental conditions need to be documented in detail. Such responses must be well understood in order to delineate and identify plant responses that are primarily due to gravitational rather than to cultural conditions. In so doing, a basis for more precise studies on plant responses to gravity can be developed.

In vitro methods for growing plants from seed to the flowering and fruiting stage under normal gravity have been investigated (Hoshizaki 1982). Seed to seed growth of Arabidopsis thaliana (L.) Heynh., the mouse ear cress, and Cardamine oligosperma, Nutt., bitter cress, was accomplished only in those cultures having a free gas exchange with the ambient atmosphere (free). Whenever tightly sealed limited gas exchange (limited) conditions were used the plants failed to produce seeds.

In vitro studies were expanded to examine the gravitational effect by using clinostats. During these experiments, an abnormal root growth was noted on plants growing in those limited cultures being subjected to clinostat treatment. Part of the roots were growing away from the agar media along the wall of the container and appeared to be growing faster than roots in the media. Such growth was not seen in free cultures.

This change in the growth response of the clinostatted root forms, in part, the basis of this report. To a better understand these findings, an explanation of the cultural and experimental conditions that produced this change in root growth is now given.

MATERIALS AND METHODS - The experimental apparatus was comprised of a clinostat with its plant holders and fluorescent lamps for the light source, both enclosed in a rectangular container. Three identical clinostat systems were used in each experiment. The system used as the horizontal clinostat treatment was oriented with its clinostat axis parallel to the horizontal plane. Two controls were placed with their clinostat axes vertical. One served as the "stationary upright" control. The plants were not rotated. The second system served as the vertical rotation control and the plants were rotated. The clinostat conditions were 0.5 rpm, 350 ft.c. from cool white fluorescent lamps at plant level, and 25°C air temperature. Temperature was maintained by blowing in 50 percent relative humidity air from the temperature- and humidity-controlled room.

Seeds of Arabidopsis thaliana (L.) Heynh., Columbia strain were obtained from Dr. E. Meyerowitz, California Institute of Technology, and are traceable to Dr. G.P. Redei, University of Missouri. Seeds were surface sterilized with 5 percent sodium hypochlorite solution, and aseptically planted into autoclaved 25 x 200 mm culture tubes containing 20 ml of nutrient agar media supplemented with sucrose and glutamic acid (Brown, et al. 1976). The agar concentration was increased from 0.8 to 1.2 percent to preclude agar media separation from the tube wall. For cultures having limited gas exchange with the ambient atmosphere, a single layer of Saran Wrap (Dow Chemical) or mylar (DuPont) film was stretched over the mouth of the tube and held in place with two rubber bands tightly stretched over the film 1 cm and 3 cm below the rim. The relative humidity (RH) in limited cultures was near 100 percent as surmised from the continuous presence of condensate over much of the interior wall of the culture tube. For cultures having free gas exchange with the ambient atmosphere, cotton or polyurethane foam plugs were used. The RH was presumed to range from 50 to 100 percent since the minimum RH of the room was 50

TABLE 1. SHOOT AND ROOT DRY WEIGHT AND SHOOT-ROOT RATIO AT HARVEST OF ARABIDOPSIS THALIANA GROWN IN VITRO CLINOSTATS

CLINOSTAT TREATMENT	PLANT GROWTH MEASURED	CULTURAL CONDITIONS					
		FREE GAS EXCHANGE			LIMITED GAS EXCHANGE		
		n	mean	±S.E.	n	mean	±S.E.
UPRIGHT STATIONARY	SHOOT/ROOT RATIO	7	8.88	±1.11	9	10.12	±0.79
	SHOOT DRY WT. mg	8	36.40	±6.00	9	21.60	±8.32
	ROOT DRY WT. mg	7	5.91	±1.39	9	2.33	±2.11
VERTICAL ROTATION	SHOOT/ROOT RATIO	9	7.48	±0.98	10	10.29	±0.75
	SHOOT DRY WT. mg	10	23.85	±5.36	10	29.80	±7.89
	ROOT DRY WT. mg	9	3.19	±1.22	10	2.78	±2.00
HORIZONTAL ROTATION	SHOOT/ROOT RATIO	8	5.75	±1.04	10	3.39	±0.75^1
	SHOOT DRY WT. mg	8	49.36	±6.00	10	47.47	±7.89^2
	ROOT DRY WT. mg	8	9.20	±1.30	10	19.17	±2.00^1

THE SHOOT/ROOT RATIOS ARE OBTAINED BY DIVIDING THE DRY WEIGHT OF THE SHOOT OF AN INDIVIDUAL PLANT BY THE DRY WEIGHT OF ITS ROOTS. 1 $P<0.001$, 2 $P<0.05$.

percent. The walls of the free cultures had minimal condensate which was limited to areas adjacent to tube holder surfaces and agar surface.

Plants were harvested two months after planting, and the shoot and root dry weights recorded. Shoot weights included stems, leaves, flowers, seed pods, and seeds when present. Non-orthogonal analysis of variance was used.

RESULTS AND DISCUSSION - Shoots of plants subjected to clinostat treatment were significantly heavier than those of plants grown in a stationary upright position or rotated about a vertical axis (Table 1). Weights of our clinostatted shoots were much heavier than those reported by Merkis, et al. (1976) and may be indicative of strain differences in the plants used or differences in the cultural methods and conditions. Their findings of a lower mean dry weight (15.1 mg) than our dry weights (49.4, 47.5 mg) suggest a need to examine thoroughly cultural methods as they affect plant growth response (Berry, et al. 1981). Analysis of the free and limited shoot weights revealed that the clinostat effect resided mostly in those shoots grown in limited cultures (Table 1). Similar analysis of roots indicated no significant growth response to clinostatting under free conditions. However, clinostatted roots in limited cultures were heavier than controls. This significant weight increase of roots (clinostat 19.17 mg, controls 2.33 and 2.78 mg) influenced the shoot-root ratio and completely masked the two-fold weight increase of the shoots.

In the limited gas exchange environment, shoot and root growth increased significantly when plants were clinostatted. One may speculate that the presence of condensate on the wall of the culture tube provided a milieu for the roots to grow into regions not available to roots growing upright or in cultures having little or no condensate on the wall. Sepaskhah and Boersma (1979) have shown that roots grow more in solutions having higher osmotic potential. If the condensate on the walls of our cultures was due to transpiration and not to guttation, the high osmotic potential would be ideal for root growth. In some cases, the root masses were large enough to entangle and obscure the stems (see Figure 1). Such a response was not seen in cultures where the walls were dry. The type of root growth response seen in the limited cultures may be a detriment in culturing and testing plants in weightlessness under conditions where transpired and excess media moisture may condense on container walls.

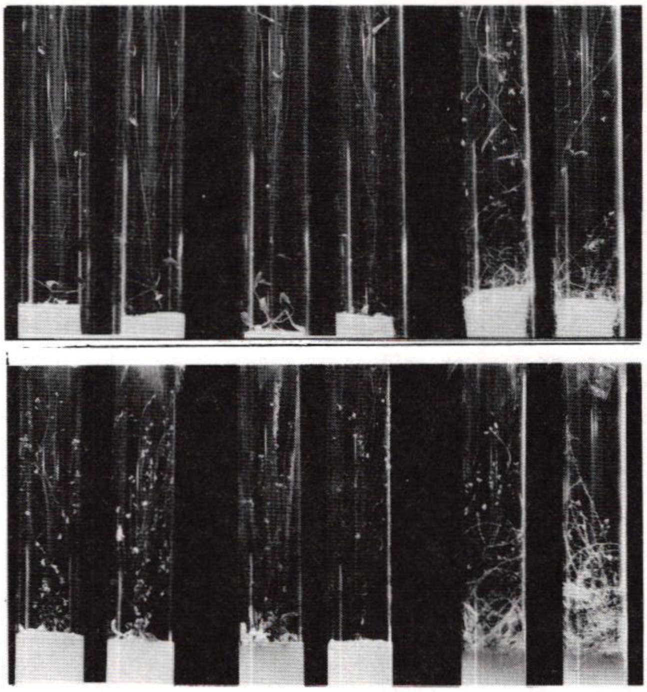

Figure 1. Root growth response of Arabidopsis. Top - free gas exchange. Bottom - limited gas exchange. From left to right - upright stationary, vertical rotation, and horizontal rotation. See text for more information.

The basis of the increase in mean shoot dry weight in the clinostatted plants is not clear at the present time. Further investigations are being made to differentiate gravitational responses from responses to cultural conditions. (Supported by NASA Contract NAS7-918.)

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CHRONIC HYPOKINESIS AND 3 PERIODS OF THE STRESS REACTIVITY IN RATS

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In view of the effect of a permanent hypokinesis on bodyfunctions it is necessary to consider the duration of the influence of stressors.

19 parameters from the endocrine, brain-metabolic, behavioural and visceral functions were tested after 1, 3 and 6 weeks hypokinesis and 12 hours free motility as an intermittent scheme).

The results indicated to 3 stages:

1. Inhibition
2. Immobilization of adaptive processes
3. Premorbide stage

The catecholamines (Fig. 1) show a periodical course. After 1 weeks hypokinesis a rise, after 3 weeks a very distinct rise and after 6 weeks a drop in the hormonal level appears.

According to that changes periodical processes are to seen also in the brain metabolism and in the level of performances in conditioned avoidance-learning (Fig. 2).

Opposite to the results after 1 week, where the protein-synthesis as well as the avoidance learning is restricted, an optimal level in the centralnervous parameters and in the metabolic activity of brain is to observe after 3 weeks stress.

The negative emotional stress-state caused by prolonged hypokinesis stress (6 weeks) with an important restricted avoidance learning capacity is not to compensate by the only a few arised metabolic processes of brain.

To compare all parameters recorded the datas were plotted in such a way, that the highest value was related to a maximum and the lowest one to the minimum.

The important rise of the brain metabolism after 3 weeks hypokinesis induces to a mobilization of adaptive processes, whát after 6 weeks stress is to proofe no more (Fig. 3).

Simultaneous to the metabolic changes disturbances are observed in the avoidance-learning and the sleep-behaviour (Fig. 4).

The phasic course is to proofe as a trend also in the endocrine functions, exceptionally in acetylcholine (Fig 5). Additionally are to note functional dissoziations between

1. Catecholamines and Ach. Whereas the catecholamines are arised, the Ach-level is not changed after 3 weeks.
2. Between the stimulated adrenergic and cholinergic system it is noticable the more evident rise of the NA-level.
3. Between corticosterone and ACTH after 6 weeks hypokinesis.

The dissoziation of functions, which normaly react in the same way, induces to the presence of pathogenic processes.

The systolic bloodpressure and the blood sugar level show a continuous rise during the time of the influence of the hypokinesis.

The 3 stages, mentioned at the beginning of these report could be based by the following mechanisms:

1. A protective inhibition against exogenic influences as defined by PAWLOW
2. An arised adaptation as a resistance-stage as defined by SEYLE
3. A dissoziation of functions with permanent hyper-or hyporeactive reactions

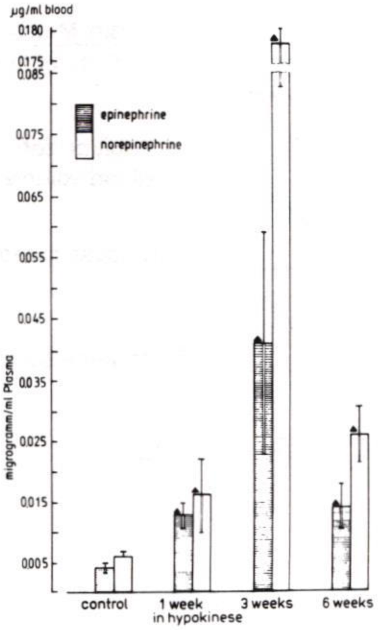

Figure 1. Periodical course of catecholamines during hypokinesis

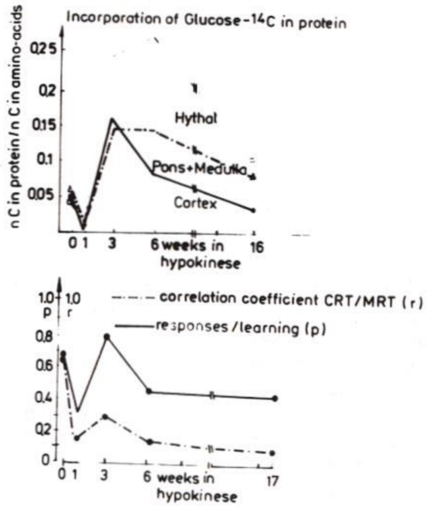

Figure 2. Brain metabolism and avoidance-learning

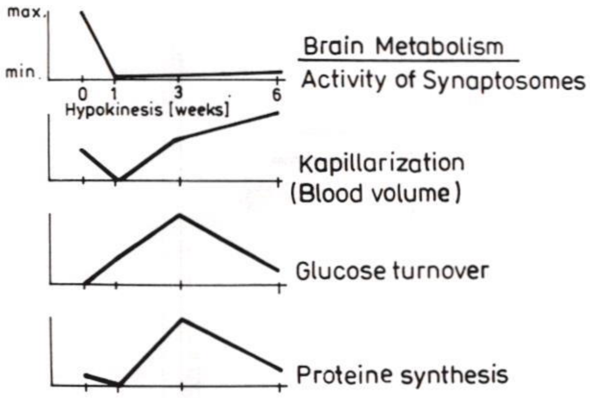

Figure 3. Brain metabolism

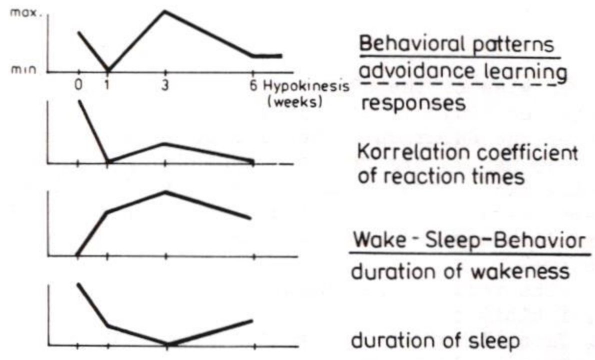

Figure 4. Avoidance-learning and sleep-behaviour

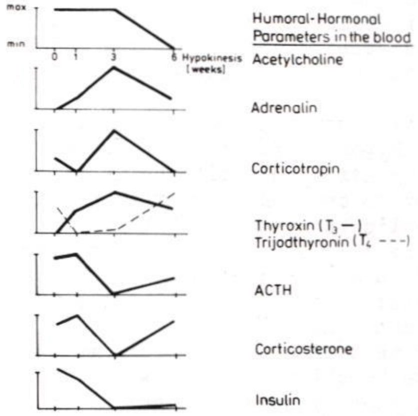

Figure 5. Endocrine functions

THE INFLUENCE OF HYPOKINESIS ON THE ASYMMETRIC DISTRIBUTION OF NORADRENALINE IN THE NEOCORTEX, HIPPOCAMPUS AND SUBRENALS OF RATS

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The functional asymmetry of brain hemispheres in animals and in the man is relevant to adaptive processes. Studies in pilots and cosmonauts have shown, that the asymmetry and specially the handednes plays a important role.

Oke et al. /1/ described an asymmetric distribution for NA in the CNS (thalamus) of rats. Further it's know from the literature, that there are stress induced changes in the functional asymmetry of brain.

These study was aimed to investigate the influence of hypokinesis on the asymmetric distribution of catecholamines in the neocortex, hippocampus and subrenals.

METHOD

3 groups, consisting of rats in each case, were subjected to 1, 3 or 6 weeks hypokinesis respectively. The content of NA was determined by a method described by Chang. The samples were taken from the different brain tissues during the activity maximum of rats (beween 5 and 6 p. m.) and during the winter time.

RESULTS

In the control group we got the following results in the different areas, tested:

	left	right
neocortex	554±86 ug/g	462±56 ug/g
hippocampus	641±147 ug/g	1064±216 ug/g
subrenals	1056±207 ug/g	732±138 ug/g

The data were significant on the $p<0,01$ level.

It's to seen that the distribution is contrallateral between the neocortex and hippocampus and analogous between the neocortex and subrenals.

After 1 week immobilization the asymmetric distribution of NA in the neocortex and hippocampus disappears:

	left	right
neocortex	532±81 ug/g	540±84 ug/g
hippocampus	593±123 ug/g	555±335 ug/g

The following datas of the organ weight indicate, that the differences in the NA-levels are not to reduce to the different mass of these structures:

	subrenal	hippocampus	neocortex
right	28±6 g	50±10 g	223±31 g
left	29±5 g	47±11 g	217±28 g

The datas are significant on the $p<0,05$ level.

The levelling of the asymmetric NA-content in the subrenals after 3 weeks hypokinesis is to seen in the following datas:

subrenals	left	1211±278 ug/g
	right	1117±287 ug/g

But to seen is also an elevation of the NA level after 3 weeks comparing to the first week.

The levelling of the NA-content in the both hemispheres is to observe also after 3 and 6 weeks hypokinesis respectively.

It's remarkable that there is a positive relationship between the duration of the hypokinesis influence and the NA-concentration specially in the hippocampus.

hippocampus	1. week	6. week
left	593±123 ug/g	910±145 ug/g
right	555±335 ug/g	953±60 ug/g

DISCUSSION

In the study reported could be proofed, that the functional pathway neocortex - hippocampus - subrenals, which is important to emotional reactions and stress is characterized by an asymmetric distribution of NA. That fact induced, that the asymmetry of brain may be proofed in the peripher organs too.

Obviously is the asymmetric distribution of NA to find under normal conditions. Hypokinesis as stressor (as we could show) induces a levelling of the functional lateralization.

Therefore it's supposed that the asymmetric functions have a relevance to the adaptive processes.

The delay of the levelling in the left-right differences of the subrenals could indicate that the phylogenetic younger structures are more sensitive to hypokinesis stress than the elder ones or that the adaptive processes are used earlier by the organism respectively.

The study, I reported there, combines, probably for the first time, the problems of asymmetric functions with the problems of adaptive processes of of stress. It's of course quite clear, that in further studies these relationship must be investigated deeper.

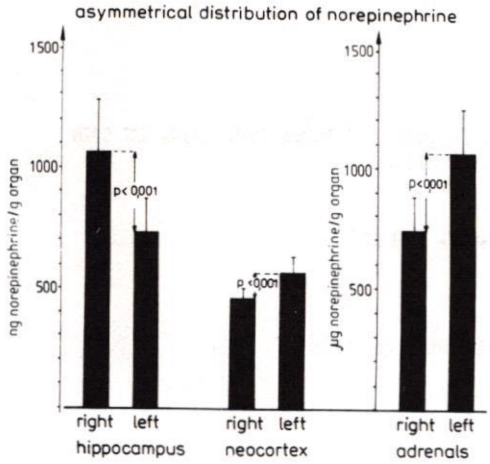

Figure 1. Concentration of noradrenaline in the tissue of the left and right hemispheres of the neocortex, hippocampus and subrenals in rats under control conditions

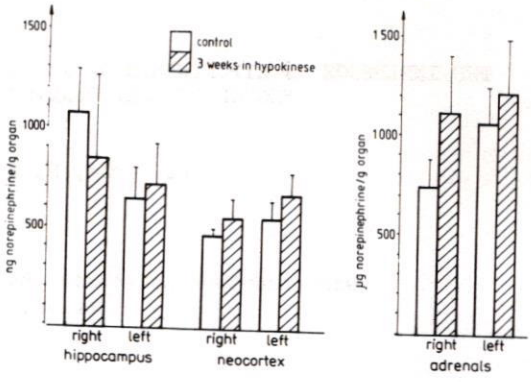

Figure 3. Concentration of noradrenaline in the tissue of the left and right neocortex, hippocampus and subrenals in rats under control conditions and in the week 3 of hypokinesis influence

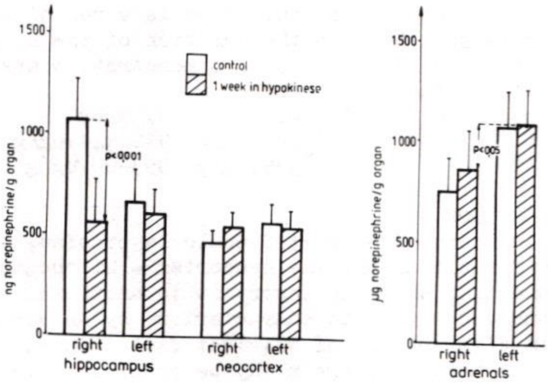

Figure 2. Concentration of NA in the tissue of the left and right hemispheres of the neocortex, hippocampus and subrenals in rats under control condition and in the week 1 of hypokinesis influence

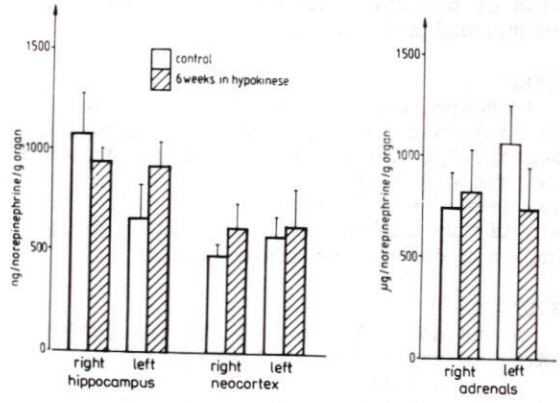

Figure 4. Concentration of NA in the tissue of the left and right hemispheres of the neocortex, hippocampus and subrenals in rats under normal condition and in the week 6 of hypokinesis influence

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SLEEP CHANGES IN RATS INDUCED BY PROLONGED HYPOKINESIS AND TREATED BY SUBSTANCE P

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INTRODUCTION

Many studies during the last years have indicate to different hypokinesis induced changes in body functions. The results are important also with regard to the conditions of weight_lessness during cosmic flights. But in these studies was not considered the sleep /1/. On the other side it is evident, that the sleep is relevant to the adaptive and protective mechanism of the organism /2/.

Our study was aimed, to investigate the influence of a prolonged, intermittend immobilization on the qualitative and quantitative charateristics of sleep and to test the anti-stress effect of SP /3, 4/ by using a hypokinesis-model.

In previous studies we could demonstrate, that changes in the different parameters are depending on the duration of the hypokinesis influence. Thereby the plasma adrenaline level shows the following picture:

norm: 0,004 µg/ml ± 0,0008 µg/ml

hypokinesis:		
1. week	3. week	6. week
0,013± 0,002 µg/ml	0,041 ± 0,018 µg/ml	0,014 ± 0,003 µg/ml

As the changes in sleep were simultaneous to the catecholamines highly affected after 3 weeks immobilization, we choose that kind and exposition of hypokinesis for our studies in sleep-changes.

METHOD

The tests were carried out in 40 male wistar rats (200-250g), divided in3 groups: control-group, hypokinesis and hypokinesis+SP. The EEG and EMG was recorded polygraphically over 2 hours during the circadiane activity minimum (8.00-12.00 p.m.) and on 4-5 consecutive days. During the recording the rats were not fixed, so that they could move around the experimental box.

Substance P (Arg-Pro-Lys-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) and SP-analogon SP-A (Lys-Phe-Ile-Gly-Leu-Met-NH_2) was injected i.p. in a dosage of 125 µg/kg and 250 µg/kg 1 hour before the EEG recording was startet. The sleep structure from the native EEG and EMG recording was criticized by the minute-values of the visual analysis.

RESULTS

On an avarage the controls show a well-balanced sleep-structure (Fig. 1) with the following portions of the different vigilance-stages:

I	active wakefulness	15,8±9,7%
II	passive wakefulness	10,4±0,4%
III	superficial sleep	23,5±1,1%
IV	deep slow sleep	42,0±1,3%
V	paradoxical sleep	8,3±0,3%

After 3 weeks hypokinesis the sleep-structure was changed in an important extent by reducing, the sleep-stages, above of all the deep slow sleep in favour of the wakefulness (fig.2) I-28,3±2,96%, II-23,3±4,2%, III-21,7%±1,76%, IV-23,3±4,1%, V-3,4±0,62%.

The cyclogramm of these stressed animals is signed by a frequent awaken (Fig. 1). In general in comparing the different days (Fig. 3) and the latency of the different stages (Fig. 4) these animals show a motoric hyperactivity as well as a labil sleep behaviour and problems to fall a sleep.

That facts indicate a disturbed adaptional ability to the experimental situation during the sleep-recording.

In testing the SP-level in blood by means of the radioimmunoassay-method was observed a drop in SP. But as these rats were treated by SP-and SP-A-injections, it appeared the physiological normal sleep structure (Fig. 1, 2, 3, 4) I-15,0±1,6%, II-8,3±0,4%, III-18,3±2,5%, IV-50,0±4,3%, V-8,4±1,2%.

By checking after going of the peptides, it was demonstrated, that the described effect has been maintained up to 96 hours. Rebound of the paradoxical sleep was not to seen.

DISCUSSION

The studies reported here have shown, that prolonged hypokinesis acts in serious qualitative and quantitative disturbances as well of sleep as of the ability toadapt. These symptoms disappeared to a large extent by SP- and SP-A applications. That means, it was possible, to normalize the disturbed sleep in its structure and dynamic by SP. But on a normal sleep-profile these peptids have not any effect. Therefore in regard of these peptids it could be demonstrated an inductive effect for the delta- and paradoxical sleep.

The described effect of SP is considered as an non-specific effect, as the vegetative functions as well as the hypokinesis induced changes in the adrenaline-level could be normalized additionally /4, 6/. The following mechanisms are supposed to be the basis for the SP-effect:

1. Substitutional effect
2. Inhibitory influence of SP an the biosynthesis of catecholamines and on catecholaminergic neurons /6, 7/
3. Stimulating SP effect directly on dopamin and indirectly on the GABA and serotonin release.

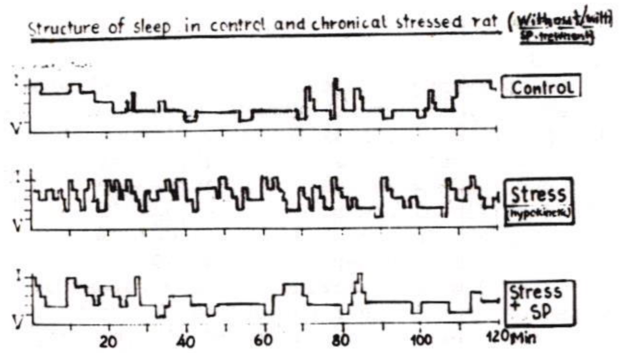

Figure 1.

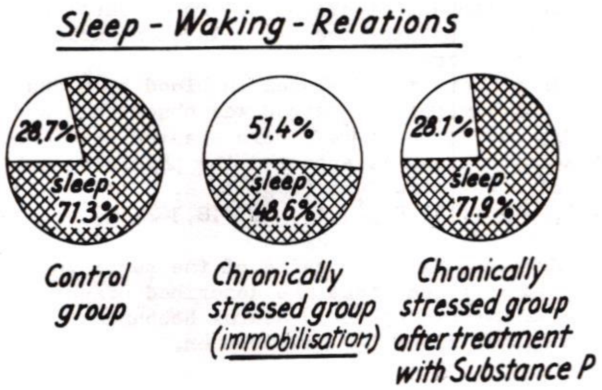

Figure 2.

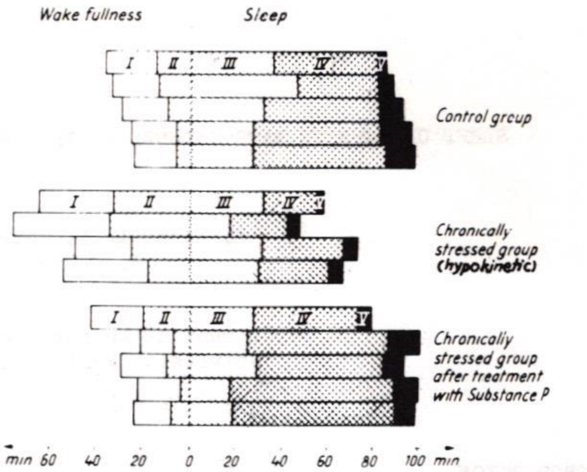

Figure 3.

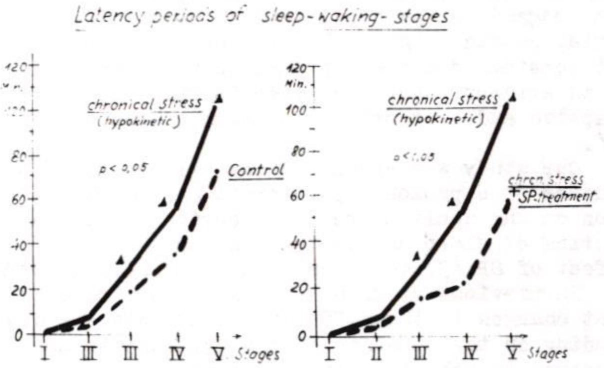

Figure 4.

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AUTOMATIC SLEEP STAGE ANALYZER TO DETERMINE THE PHYSIOLOGICAL SLEEP PROFILE IN MAN, MONKEY AND IN RATS

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Sleep studies, as it is known, are connected with a large quantity of dates.

Therefore the clinical medicine demands for an automatic sleep analysis. Beyond it physiological laboratories and groups engaged in animal experiments call for a multivariable use of such analyzer.

In these study is presented with reference to the visual EEG, EMG and EOG analysis an automatic sleep analyzer, worked out, constructed and tested in our laboratory and useful for studies in rats, monkey and in man.

Subjecting the special electrophysiological courses of each species (man, monkey, rat) the analyzer detects in the EEG-frequency bands the avaraged activity including its intensity and duration. Regarding to the muscle-tonus (man, monkey, rat), the rapid eye movement (man, monkey) and the spindle activity (man) is identified the actual sleep stage in intervalls of 30 sec.

The results of the frequency analysis and the sleep cyclogramm is plotted immediately. Further the analyzer is coupled with a microcomputer.

In comparing the visual with the automatic analysis in rats, as it was done by an extendend amount of dates, we got a correspondence of these two analytic ways of about 80%.

Generally in the sleep-stage analysis 2 task are to distinguish.

1. Simple single analysis supporting the visual analysis

2. Automatic detection of sleep stages

Variables signals, complex pattern and important inter- and intraindividual differences are to consider in the automatic analysis.

Because of that an automatic analysis can be used only with a simultaneous control by the experimenter. Therefore the algorithm of the automatic analysis should be similar to that of the visual analysis.

So an adaptable hybrid-system was developed consisting of:

1. The sleep analyzer, analysing the informations (EEG, EMG, EOG) and detecting the actual sleep stage

2. A computer-programm doing the required corrections and the statistic calculation.

The results are plotted in each period of the analysis, so that the experimenter may influence active on the system.

Construction and method of working of the sleep analyzer are represented in the following figures.

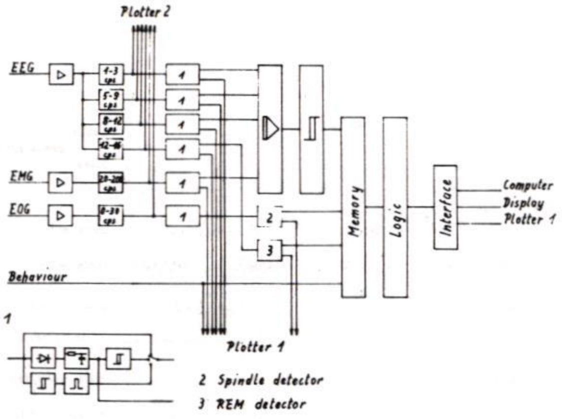
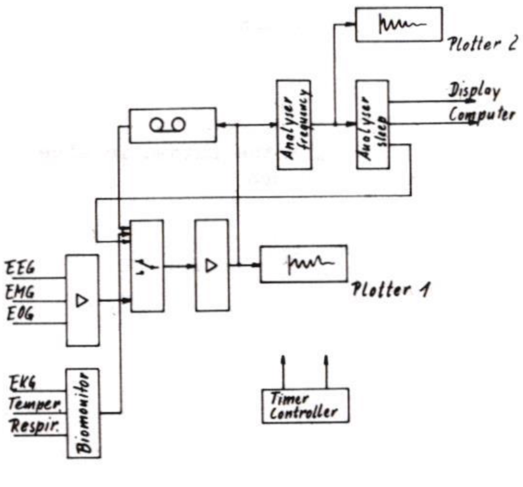


Figure 1. Technical structure of the sleep analyzer



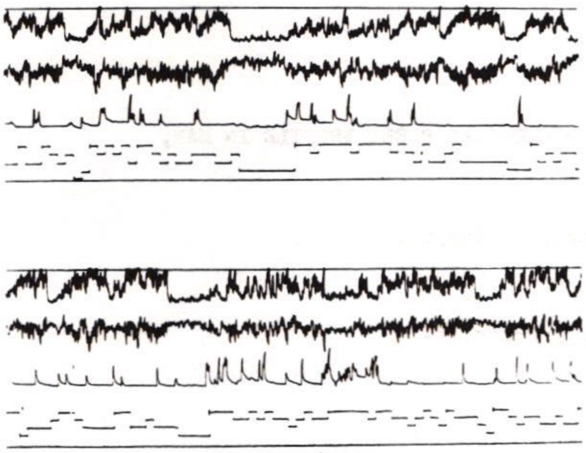
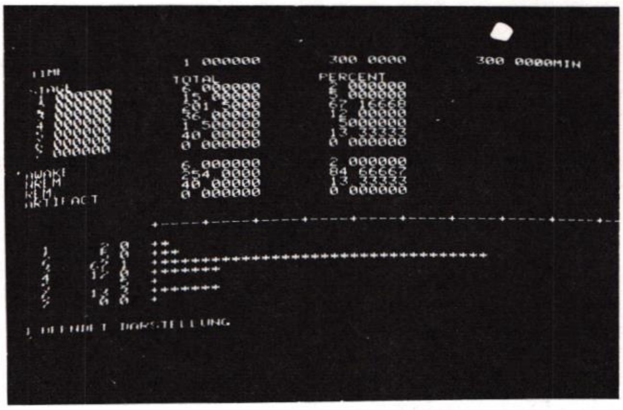


Figure 2. Examples of sleep analysis in 2 rats
From above to below for each rat
- delta-activity
- theta-activity
- muscle-activity
- sleep-stages



```
                1 000000        300 0000        300 0000MIN
TIME
                TOTAL           PERCENT
                6 000000        2 000000
                15 00000        5 000000
                201 5000        67 16668
                36 00000        12 00000
                1 500000        500000
                40 00000        13 33333
                0 000000        0 000000

AWAKE           6 000000        2 000000
NREM            254 0000        84 66667
REM             40 00000        13 33333
ARTIFACT        0 000000        0 000000

BEENDET DARSTELLUNG
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Figure 4. Avaraged distribution of sleep stages in the course of 5 hours

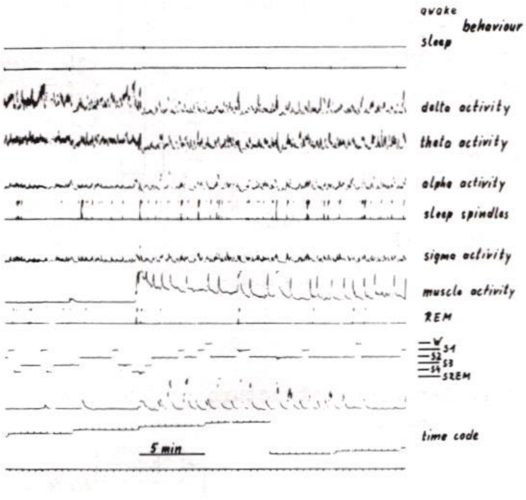

Figure 3. Examples for the automatic sleep analysis in man

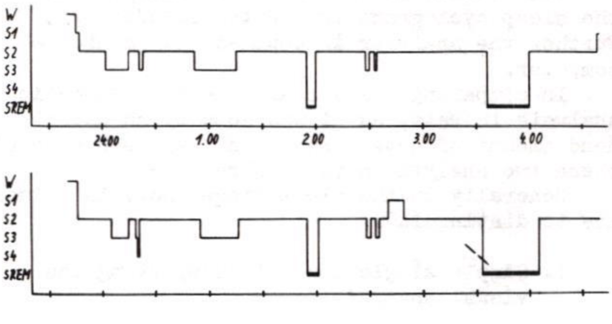

Figure 5. Comparsion of the visual (above) and automatic (below) analysis

THE EFFECT OF EMOTIONAL STRESS PRIOR TO THE ONSET OF CENTRIFUGATION ON ACCELERATION TOLERANCE IN PILOTS

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ABSTRACT

Centrifuge examination undertaken in the pre-start period /PSP/ on 80 student pilots indicated that an optimal reaction of the circulatory system appears to be an increase in systolic and diastolic blood pressure /$P_{S,a}$ and $P_{D,a}$/,heart rate /HR/ and blood flow velocity /V_m/ within the limits of mean reactions,with an additional increase in this parameters just before starting moment. Strongly expressed emotion in the PSP influences upon the +GzT decrease.

INTRODUCTION

In many studies an increase in physiolological activity which occurs in pilots during different stages of flight,as well as in particulary stressing tests,have been pointed out /1,2,3/. Authors have variously attributed this phenomenon to the effects of workload and to psychological or emotional stress. Similar states can be observed in the PSP of the human centrifuge examination.

METHODS

The examinations were carried in a group of 80 student pilots aged 21-22 who underwent,for the first time in their lives,great +Gz accelerations in the centrifuge. The centrifuge technique was based on using linearly increasing acceleration of 0,1 G/s until loss of vision field was observed in the subjects. In this study only the analyses of HR value, $P_{S,a}$,$P_{D,a}$, and V_m, were undertaken. The physiological data were registered two minutes prior to the start of the centrifuge.The control values were estimated in the day preceding the centrifuge tests. The examination results achieved under control conditions and during PSP were compared with the acceleration values affecting the subjects at the blood flow arrest moment in the temporal artery.This moment was accepted as the objective criterion for the +GzT.The analysis of blood circulation parameters was carried in each case in division into 3 groups. The division was based on statistic distribution of the results,so that the first group included the subjects who had the lowest values of the parameterin question,and in the third group - the highest.Mean +GzT applied for inter-group comparisions, was calculated for each group.

RESULTS

HR increase in PSP was confirmed in all subjects,but the correlation value between HR and +GzT was negative /$-0,581$ $p<0,01$/. The analysis in groups /Fig.1/should suggest that it is the range of HR increase since the moment of starting the centrifuge,that plays an important role.It can be also concluded from analysis of $P_{S,a}$ that, when compared with control values,this parameter increase varries, being respectively: 34% in group I,19,8% in group II, and 14,8% in group III. +GzT was the lowest in group I,which results in concluding that it is the control value of $P_{S,a}$,and not its increase in PSP which clearly influences achieving a higher +GzT.$P_{D,a}$ showed significant differences in groups I and III.In group I,a $P_{D,a}$

decrease immediately preceded starting the centrifuge,and the +GzT was the lowest, whereas in group III both $P_{D,a}$ and +GzT were the highest.It indicates that higher values of $P_{D,a}$ positively influence +GzT. It can be concluded from Fig.1 result analysis,that a marked V_m increase on the head level at the moment of starting the centrifuge,observed in subjects in groups I and II,positively influences on +GzT. If V_m remains on this increased level during the initial period of centrifuging as well,+GzT reaches the highest values. In contrast to these groups,the subjects in group III occur to have lower blood flow velocity values at the starting moment,which may influence upon the decrease of +GzT.

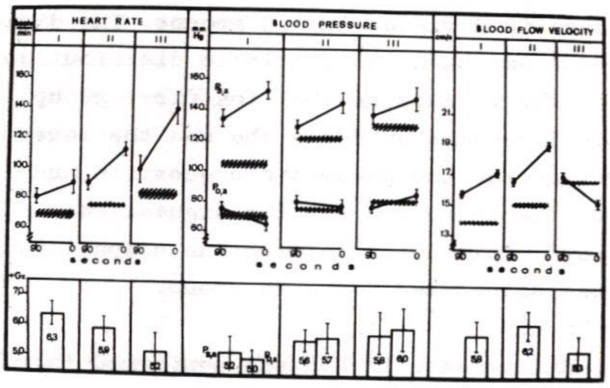

Fig.1. Circulatory parameters during control and PSP compared with GOR +GzT in division into 3 groups

Interindividual differences were observed in circulatory system parameters, which supposedly resulted from both prestart emotions and compensatory reactions of the organism.It has also been noted that in subjects with high HR,a high amplitude of $P_{S,a}-P_{D,a}$ was often found with evident decrease in $P_{D,a}$ and in V_m.Those subjects were able to endure low acceleration values only.Such circulatory parameter reactions suggested that an evident decrease in cardiac stroke volume,resulting from shortening of the time required for refilling the ventricles,occurs in those subjects.It is possible that in those cases circulatory reactions were influenced by a decrease in peripheral vascular resistance resulting from high level of adrenaline in blood.It should be marked that subjects with such reactions reach maximal compensatory possibilities limits, even though acceleration values are still small;when this stimulus is continuously increased,those reactions are broken too early.These subjects have been classified as excessively emotional.Analysis of both HR and V_m in PSP,and then at the initial phase of centrifuging,results in a conclusion that those parameter indexes have significant influence on +GzT.Subjects who reached the highest +GzT,had the lowest HR under 2-3 G and their V_m,in spite of being gradually decreased at this period /when compared with the other subjects/, was also continued on a higher level than the control values.It should be pointed out that despite the decreased HR at this phase of centrifuging,those subjects showed the highest increase in both parameter values in PSP.

Conclusion: one of the more effective compensatory reactions of the circulatory system is the increase in both parameter values,especially when prior to the starting moment.However,this should be growing in parallel to the acceleration increase, until this value reaches the level at which a decrease in V_m on the head level begins.

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OSMO- AND VOLUMOREGULATION IN RATS WITH HEREDITARILY CHANGED HORMONAL BALANCE

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ABSTRACT. Water and ion excretion after intragastric injection of 5 ml/100 g b.w. of water, complete Ringer or 1% NaCl solution was compared in Wistar rats(WR), homozygous Brattleboro rats, which lack the antidiuretic hormone (DI) and also in spontaneously hypertensive rats (SHR) which are characterised by a higher antidiuretic hormone (ADH) secretion. In response to the alteration in the body fluid volume and osmolality,the rats of all starins with varying hormonal balance were found to be able to excrete hypo- and isoosmotic loads successfully enough. However, they maintained the volume and osmotic homeostasis by different mechanisms. Depending on the pretest body hydration and the hormonal level, both regulating systems exhibit a prevalent effect respectively and thus determine different effectiveness of the correction and the structure of renal reaction to the load.

INTRODUCTION. As follows from space flight observations, the period of adaptation to weightlessness and readaptation to the Earth gravity is attended by significant alterations in the kidney function. The similar results have been obtained when studying the immersion effect on fluid-electrolyte metabolism in test subjects (1,2). The correlation between volume-, osmo-, and ionoregulation and, respectively, the kidney reaction to the water and salt loads are able to change depending on the initial hormonal level. Thus, it would be interesting to study the kidney reaction to hypo- and isotonic loads in rats with hereditaryly changed hormonal status.

METHODS. Experiments were performed on Wistar rats with a normal hormonal balance (WR), homozygous Brattleboro rats (DI), which are lacking in antidiuretic hormone (ADH) and have the increased activity of the renin-angiotensin system (RAS), and also on the spontaneously hypertensive rats (SHR) characterised by a high ADH secretion and abnormal activity of RAS. Before the experiments, animals were on a normal diet, water was available. During the experiments,rats were loaded per os, via catheter,with the water or salt solutions (complete Ringer or 1% NaCl), 5 ml/100 g b.w. and decapitated 4 hr after the loading. To evaluate the renal function under the load,the following criteria were used: the rate of urinary flow (V),the glomerular filtration rate (GFR), the osmotically free water reabsorption ($T^c_{H_2O}$), the total and fractional sodium excretion (E_{Na}, $F{\cdot}E_{Na}\%$),at the peak of diuretic or natriuretic reaction.

RESULTS. The load with Ringer solution (Fig.1) induced, in all animals, a characteristic volumeregulating response - gain of V,which was due to GFR increase and reduction in the reabsorption of sodium and water bound with it. The $FE_{Na}(\%)$ rose in all animals, and the $T^c_{H_2O}$ changed insignificantly. The most prominent increase in E_{Na} was revealed in SHR.An exaggerated natriuresis in this strain had been also found in similar experiments previously(3).

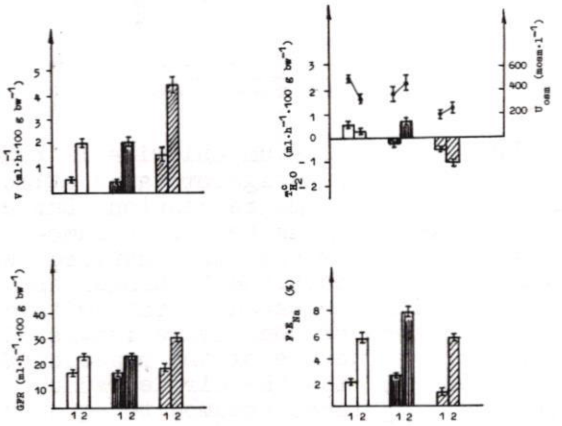

Figure 1. Effect of Ringer solution load on the renal water and sodium excretion in Wistar, SHR, and Brattle boro rats. 1 - basic level; 2 - peak of the reaction.
□ - Wistar rats, ▥ - SHR, ▨ - Homozygous (DI) Brattleboro rats.

The diuresis in DI after loading was higher than in WR or SHR.The enhanced diuretic response was mainly due to the rise of GER and was not followed by change in urine osmolality. The intensity of natriuretic reaction and the mechanism of its development differed no significantly from that of WR.

The water load (Fig.2) induced changes both in volume and osmolality of the circulating fluid. WR and SHR showed the diuresis peak by the end of an hour. The E_{Na} at the diuresis maximum did not differ from that of resting level in all strains studied. Thus, the development of the diuresis after water load was, primarily, due to the osmoregulating reaction. The most enhanced V, GFR and most profound reduction of $T^{c}_{H_2O}$ at the reaction peak were observed in SHR. However, this peak was not prolonged and SHR excreted less water volume than WR. DI rats responded to the volume and water loads in different ways. After the loading no significant increase in diuresis was observed, the decrease in osmotically free water reabsorption was minimum in comparison with other rats. The rats lacked ADH did not practically respond to the hypotonic water load, however, permanently high diuresis permitted them to excrete it.

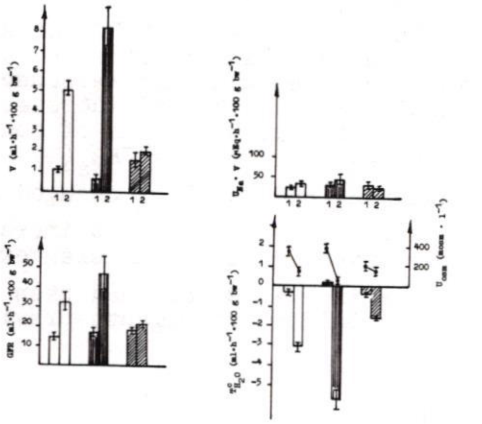

Figure 2. Effect of water load on the renal water and sodium excretion in Wistar, SHR, and Brattleboro rats. 1 - basic level; 2 - peak of the reaction.
□ - Wistar rats, ▥ - SHR, ▨ - Homozygous (DI) Brattleboro rats.

The isotonic sodium chloride is considered by many investigators as a stimulus involving the volume regulation. But complex interrelations between volume- and ionoregulating systems are noticed when comparing the reactions to Ringer and 1% NaCl solutions ingested by rats with different degrees of body hydration. As Fig.3 shows, the structure of the reaction (% of value changes with the circle radius being a resting level assumed to equal 100%) to the Ringer solution differed from that one of 1% NaCl. The former stimulated the volumoregulstory response and in neither case the increase in osmotically free water reabsorption was observed. The increase in FE_{Na}% was significantly less than after sodium chloride loading. The structure of the reaction to the 1% NaCl load depended on the level of body hydration. When the isotonic NaCl solution was given to hydrated animals there was no volumoregulating reaction with "throwing off" the isoosmotic sodium solution, but a clear cut rise of the urine osmolality (to 700-800 mOsm/l) as a result of increase in $T^{c}_{H_2O}$ was revealed. In other words, the effect of the ion balance was registered, id. the excretion of sodium and simultaneous increase in free water reabsorption. And when the U_{osm} was more than 600 mOsm/l, there was a regular volume regulation without significant changes in $T^{c}_{H_2O}$.

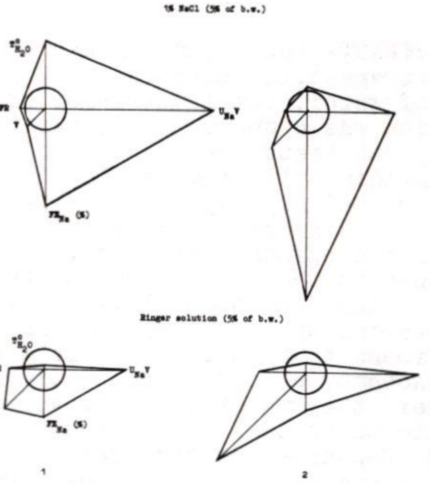

Figure 3. Changes in diuresis (V), glomerular filtration rate (GFR), reabsorption of osmotically free water ($T^{c}_{H_2O}$), total sodium excretion ($U_{Na}V$), and fractional sodium excretion (FE_{Na} %), in % from basic level of each index, after the load with 1% NaCl and complete Ringer solution; 1 - basic urine osmolality less than 400 mosm/l, 2 - urine osmolality more than 600 mosm/l.

Thus, in response to body fluid volume and osmolality changes, the rats of all strains studied were found to be able to excrete hypo- and isoosmotic loads successfully enough. However, they maintained the volume and osmotic homeostasis by different mechanisms. Depending on the pretest body hydration and the hormonal level, both regulating systems exhibit a prevalent effect respectively and thus determine different effectiveness of the correction and the structure of the renal reaction to the load.

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THE ROLE OF TISSUE RECEPTORS AND SPECIFIC STRUCTURES OF MEDULLA OBLONGATA IN THE FLUID DISTRIBUTION

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Adequate information about the work performed by the organ and its blood supply can be obtained from measuring pH in the effluent fluid, i.e. in venular blood and in small lymph vessels. These areas contain numerous poorly specialized endings of non-myelinated nerve fibers termed "tissue receptors" (5,6). These endings can be excited by various mechanical and chemical stimuli of sufficient intensity, including pH variations that occur in the arterial blood under normal physiological conditions (4). Increase in metabolites and acidification of effluent fluids enhance slow impulsation along the C-afferents originating in the organ. This could be expected to lead to a reflex recovery of the lost harmony (1). Disorders in the normal hydrostatic status that occur in weightlessness or during postural effects also result in disturbed agreement between the work performed and the blood supply. This gives rise to a number of autonomic shifts that constitute motion sickness and cannot be easily tolerated. The present paper discusses the compensation of such shifts by the excitation of tissue receptors.

In our experiments on dogs we simulated an increase of tissue somatic impulsation using electrostimulation of afferent fibers of somatic nerves. The electrostimulation parameters provided a highly efficient involvement of C-afferent fibers that are directly connected with preanglionar neurons of the sympathetic compartment of the autonomic nervous system. The study of central circulation responses did not show significant differences in the haemodynamic effects of electrostimulation of the tibial nerve and the brachial plexus. At the same time the redistribution of cardiac output depended on the topography of the source of afferent impulsation. For instance, electrostimulation of the tibial nerve produced cardiac output redistribution into the cranial compartments while electrostimulation of the brachial plexus - to the caudal compartments. Enhanced somatic afferent impulsation during tilt tests (at 70° with the head up or down) substantially increased circulation tolerance. As it was expected, electrostimulation of the tibial nerve during antiorthostasis proved most efficient. The effect of enhancement of somatic afferent impulsation included improvement of qualitative and quantitative characteristics of stabilization of the major circulation parameters (Fig. 1), correction of the distribution of cardiac output between the anterior and posterior body compartments with the headward blood flow being more stable and close to the initial level, and increase of outflow of venous blood from the dependent compartments (3).

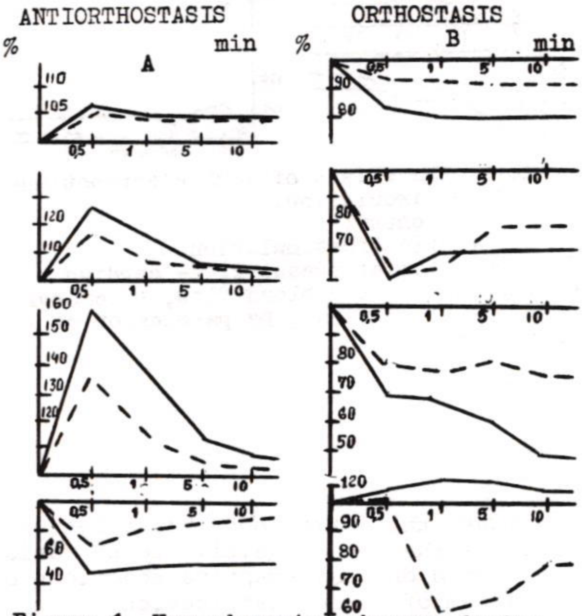


Figure 1. Haemodynamic changes during electrostimulation of cranial(A) and caudal(B) somatic afferent fibres.

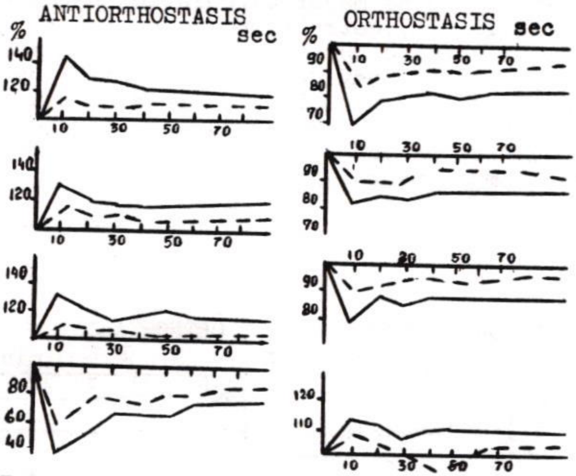
———— control

\- - - - electrostimulation

1- mean arterial pressure, 2- cardiac output, 3- carotid blood flow, 4-femoral arterial blood flow. (By permission of Kholin S.F.).

The ubiquitous tissue receptors initiate both vascular reactions and reflex reactions aimed at modifying the circulating blood volume (2).

Many authors have recently described chemoreceptor structures of the ventral surface of brain stem (VSBS) which are sensitive to pH of the blood and liquor. Our investigations have demonstrated that electrostimulation of VSBS structures produces not only respiratory reactions and changes in the circulatory systemic parameters but also redistribution of cardiac output in the cranial direction. Excitation of the VSBS structures during ortho- and antiorthostatic exposures enhance circulation compensatory reactions (Fig. 2).



Fgure 2. The effect of VSBS electrostimulation on circulation.
—— control
- - - - electrostimulation
1- mean arterial pressure, 2- cardiac output, 3- carotid blood flow, 4- femoral arterial blood flow. (By permission of Enes A.E.).

Thus, the animal body has a system of receptors that are sensitive to pH variations which ensures adaptive reactions of the system of fluid distribution.

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INTERSEROSAL FORCES: THE PRESSURE ENVIRONMENT OF THE CENTRAL CIRCULATIONS AND NATURE'S INTERNAL "G SUITS" (Part II - The Thoracic Containers, Analysis Via The DSR)

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The stresses of positive G_z acceleration on the cardiopulmonary system are countered in part by several physiological and anatomical mechanisms. In Part I of this report (see E. H. Wood and E. A. Hoffman, The Physiologist, this issue), we have discussed several of the body's naturally occurring "G Suits" such as the pericardial sac and the cranium. Protection of the lung and its circulation against the adverse effects of increased G exposure has proven to be the Achilles' heel (3,7,8,24,27). Particularly significant gravitational effects on lungs are pulmonary arteriovenous shunting, acute mediastinal emphysema, and atelectasis (27).

This paper will focus upon the gravitational effects on the thoracic contents (including the mediastinal structures) and the possible importance of regional lung geometry and its alteration by the gravitational vector.

It has been proposed and demonstrated (6,9,26, 27) that there is a vertically-oriented gradient in lung expansion with the non-dependent alveoli being more expanded than those in the dependent lung region. The concept of a gradient of lung expansion was used to explain non-uniform distribution of inspired gas (4,17) on the basis of the "S" shaped pressure-volume curve of lung parenchyma and the gradient in regional lung expansion (25). Although this explanation has proven to be useful, it is well-recognized that it does not elucidate primary mechanisms. Glazier and West (6) suggested that such non-uniform lung expansion could be explained on the basis of weight of the lung hung below a given horizontal cross-sectional area of lung parechyma. Mead (16) proposed that one source of variation in regional lung surface expansile forces might be due to a mismatch between the lung and chest wall shapes. Agostoni (1, pp 108-116) provides references regarding work related to these views as well as work addressing the question of the relative importance of the mediastinal contents in influencing regional lung geometry.

Recently we have asked the question: "What is the effect of gravity on the lung under otherwise identical conditions of respiratory effort?". Using a computer-based biplane video x-ray technique (8,9,14,22), changes in distance between radiopaque lung parenchymal markers have been used to study regional lung expansion in intact anesthetized dogs. Three weeks prior to study, apical and basal parenchymal markers were placed percutaneously in the lungs of five dogs (9). Intermarker distances (IMD) measured at end expiration (FRC) were less at the apex (average 31% ± 0.85 SEM) and unchanged or slightly greater at the base (average 13% ± 5) when head-down as compared to the head-up positions during spontaneous breathing. Next, spontaneous respiration was abolished with IV succinylcholine and IMD's were measured immediately after successive reversals between head-up and head-down positions with the airway closed, after subsequently opening the airway, and again after a period of positive pressure breathing (PPB). As shown in Figure 1, changes in apical and basal IMD values in response to change in body position (with no intervening breath and lung air content held constant-shaded regions of Fig. 1) were similar to those observed in the spontaneously-breathing animal. This is consistent with the primary causative role of gravity under these circumstances. Airway pressure changed from 0.0 to +16.0 cm H_2O during head-up to head-down tilt and from 0.0 to -9.2 cm H_2O (one dog) during head-down to head-up tilt. This is most probably due to the weight of the abdominal contents and perhaps of the mediastinal structures.

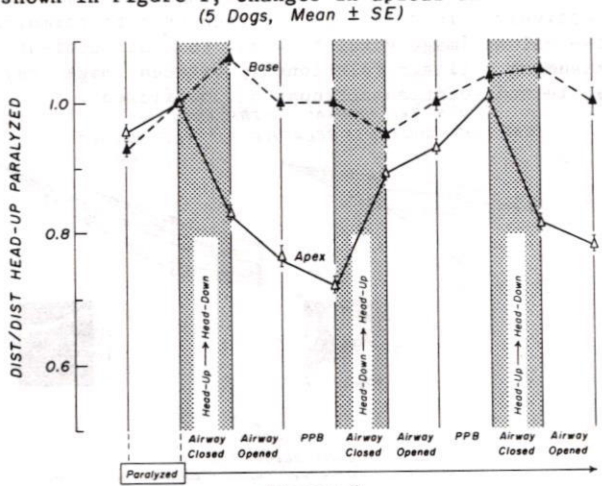


Figure 1

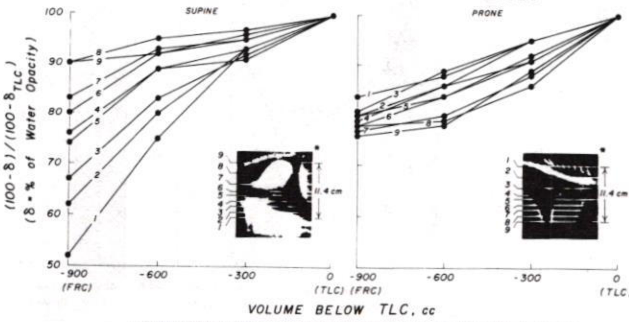
Although gravity is implicated in the changes in regional lung expansion, defining the various mechanisms by which gravity functions requires the ability to assess the three-dimensional cardiopulmonary geometry and regional lung function simultaneously.

It is our belief that studies regarding gravitationally-mediated effects on lung function remain inconclusive largely because of measurement ambiguities. For instance, nitrogen washout curves yield simple, readily "flyable" (26) estimates of regional inhomogeneities. However, mechanisms for the development of the curves are only vaguely understood (5). Similarly, Xe^{133} techniques provide a rough sampling of regional lung function, and measurements are generally made in isolation from other important phyiologic and anatomic parameters. Pleural pressure measurements and their interpretation have proven to be highly controversial (9,10).

To study the gravitationally mediated alterations in lung geometry and function in the 1 G environment, we are employing the Dynamic Spatial

Reconstructor (DSR, 12,18,19). The high temporal resolution volumetric scanning capabilities of the DSR offer the opportunity to gain previously unobtainable knowledge of the amounts of regional distribution of pulmonary gas and blood volumes as well as regional and global geometric information. Images of cross-sectional slices obtained via DSR scanning and subsequent computer reconstruction are composed of a two-dimensional array of numbers, each number indicating the x-ray attenuation coefficient (density) of an individual three-dimensional cubic voxel. The volume image is generated from a stack of thin sections and stored as a 3-D array of numbers. Up to 240 contiguous 0.9 mm thick transverse images of the body are obtainable in 1/100 second repeated 60 times per second. We are able to sample the voxel values within the region of the mainstem bronchus and the cardiac chambers as measurements of air and "water", respectively. These values are then used to normalize the volume image voxel by voxel to % air content assuming a linear relationship between image gray scale and roentgen attenuation coefficient.

VENTRAL - DORSAL DISTRIBUTION OF
LUNG AIR CONTENT RELATIVE TO TLC VALUES



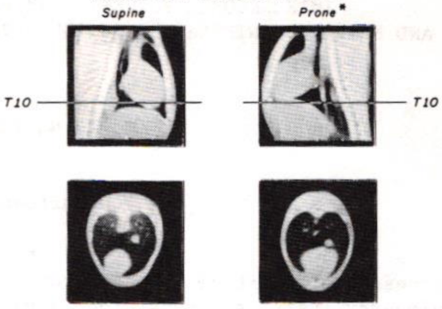
* Supine & Prone Sagittal Sections Showing Orientation of Coronal Slices Used for Sampling

Figure 2

Figure 2 shows data from a typical anesthetized dog scanned at three static inflation steps prone and supine. the horizontal brightened lines superimposed on the image of a thin sagittal slice indicate the levels at which coronal sections of the lung field were sampled. Regional air content (100 - % water opacity) of the lung is normalized to regional air content at total lung capacity (TLC) and plotted against lung inflation steps. In the supine position, decreasing slopes of the curves with increasing vertical height of the lung field sampled demonstrate greater regional volume change in the dependent versus non-dependent lung regions. In the prone dog, these regional differences are significantly reduced or abolished (11). Note the lack of consistent order of the curves suggesting minimal influence of vertical location within the prone lung. These relationships are quite consistent with data obtained using lung parenchymal markers (14).

Figure 3 demonstrates the dramatic change in shape of both chest wall (transverse sections at T10 level - lower panels) and diaphragm (thin midsagittal sections - upper panels) which occurs when the dogs are in the supine versus prone positions.

CHEST WALL CONFIGURATION OF
SUPINE AND PRONE DOG
(14.5 kg, Pentobarbital Anesthesia,
1.0 sec DSR Scan, 1.5 mm Slice)



* Spinal Suspension

Figure 3

CHANGE IN LATERAL RIB CAGE DIMENSIONS
WITH CHANGE IN BODY POSITION

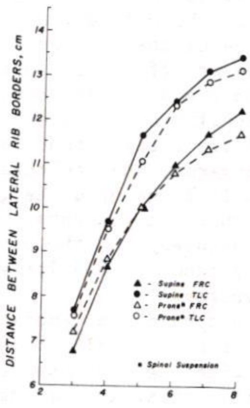


Figure 4

To investigate the contribution of change in rib configuration to this change in shape of the chest wall, we measured the distance between lateral rib borders from mid coronal sections of the same DSR scans prone and supine at FRC and TLC (Figure 4). Concomitant changes in distance between dorsal and ventral rib insertions using the intervertebral discs and mid-sternebra as landmarks are demonstrated in Figure 5. While the lateral rib dimensions at FRC are not significantly altered with a change from the supine to prone position, the ventral-dorsal dimenions increase considerably. In fact, the prone ventral-dorsal dimension at FRC exceeds the supine TLC dimensions of each rib (Figure 5). Studies of dogs at autopsy suggest that this change in rib shape may occur primarily at the costochondral junction between the costal cartilage and the body of the rib. This junction appears to serve as a pivot point giving the ribs a second degree of freedom, the first being the so-called bucket handle motion whereby the rib pivots about its ventral-dorsal insertions. How these changes in rib cage and diaphragm configuration might translate into variations in regional lung expansion has not been fully investigated. Tonelli and colleagues, using projection images

CHANGE IN DORSAL-VENTRAL RIB CAGE DIMENSIONS
WITH CHANGE IN BODY POSITION

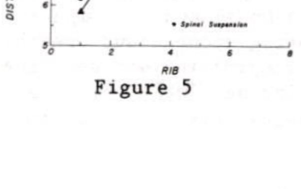
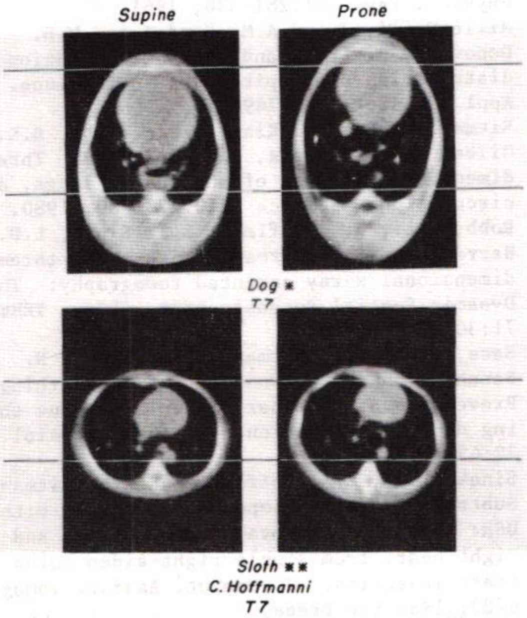


Figure 5

with inherent superposition problems, suggest that lobar slippage minimizes the effects of altered configuration of the diaphragm on regional ventilation (23).

Hoffman et al. (11) have shown that the ventral-dorsal gradient in lung expansion is present in supine, absent in prone, and is present in the non-dependent lung of the dog in the right and left lateral decubitus body positions. These observations, along with observations of the relative positions of the heart within the thorax (unpublished data), suggest that configuration of the chest wall and diaphragm may not be a major cause of this non-uniform lung expansion, but rather, cardiac orientation within the thoracic cavity could be of primary importance.

Data from a scan of a two-toed sloth (Coloepus Hoffmanni - loaned to us by Dennis A. Meritt of the Chicago Lincoln Park Zoo) also showed a ventral-dorsal gradient in regional lung expansion supine which disappeared in the prone body position (unpublished data). As demonstrated in Figure 6, the large shape change of the compliant chest wall of the dog prone vs. supine (top panels) is absent in the sloth (lower panels) which has approximately 21 ribs and a relatively non-compliant chest wall.



* Pentobarbital Anesthesia - 1.5 mm Thick Transverse Slices (Suspended from Spine, Prone)

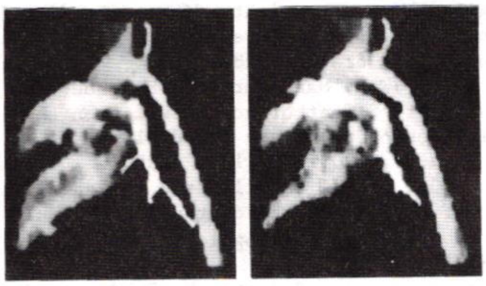
** Ketamine Anesthesia - 1.0 mm Thick Transverse Slices

Figure 6

Although there is some overall lung volume change in the sloth supine vs. prone, the shift in the diaphragm is in a piston-like movement with essentially no change in shape (unpublished). The common finding between dog and sloth is the shift in the heart from a dorsal location to a more ventral location when turning both dog and sloth from the supine to the prone body posture. We hypothesize that the ventral position of the heart in the prone body posture serves to push the ventral lung to a lateral position and in doing so imposes a geometry which provides for greater uniformity of regional expansion. In the supine position, the ventral lung regions fill in the space left by the heart and thus the ventral lung regions become relatively more expanded. Bar-Yishay and colleagues (2), using a finite element technique, have recently demonstrated the additional effect which the weight of the heart might have on regional lung expansion.

Related to the effect of the heart on the lung is the question regarding the ability of the heart to function within the constraints of the lungs and pericardium, i.e., its natural "G suit" environment. Given the changes in regional and global lung compliance which occur due to change in body position, change in gravitational force environments, and normal respiration; how does the heart protect itself against the altered afterload which it might encounter by "dragging" the variably compliant lung with it during contraction? Again we are employing the use of the DSR to study this question. The 3-D shaded surface displays (12) shown in Figures 7 and 8 demonstrate our ability to detect the three-dimensional shape and dimensions of the cardiac chambers with associated great vessels and the surrounding myocardium. Chamber shape and dimensions are visualized by opacification via a vena caval injection of radiopaque contrast agent (Renovist) (21). Accuracy of these volumes detected via DSR scanning has been shown to be within 5% (13,15). From such an imaging technique we have found, in a series of preliminary experiments on dogs, the total cardiac volume (including heart muscle, myocardial blood volume, and cardiac chamber volume) does not change between end diastole and end systole. This may account for the maintenance of normal cardiac output when the air in the lungs is replaced by liquid fluorocarbon in conjunction with total body water immersion in a rigid container (20). It appears as if the base of the heart functions as a piston in that it moves towards the apex as the ventricular chambers empty and the atrial chambers increase volume by a nearly equal amount. Thus, the heart is well designed to function within its distended natural G suit - the pericardium.

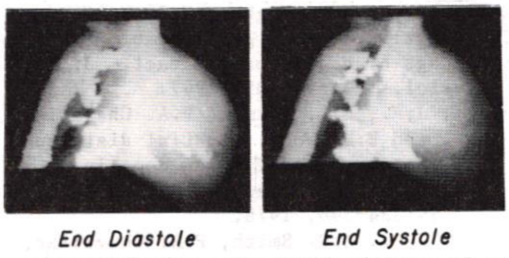
SURFACE DISPLAY: CARDIAC CHAMBERS & MAJOR VESSELS



End Diastole End Systole

Figure 7

SURFACE DISPLAY: IN VIVO HEART & MAJOR VESSELS



End Diastole End Systole

Figure 8

In conclusion, the total picture of cardiopulmonary function as it is affected by the gravitational force environment requires a degree of simultaneity of measurements previously unobtainable. To complete this expose on the "new image of cardiopulmonary function", we have compared the area under cardiogreen indicator dilution curves with the area under dilution curves obtained by x-ray volumetric imaging, of the passage of a radiopaque contrast agent (Renovist) in the main pulmonary artery and its major branches. An 8 Kg dog was anesthetized and three levels of cardiac output were studied. Relative changes from control of the area under the dilution curves obtained by the two methods differed by less than 10% (Figure 9). These data offer encouragement regarding our future ability to measure regional flowing blood volumes throughout the lung which, when coupled to measurements of regional variations in air content, will allow for the unveiling of important ventilation/perfusion relationhips.

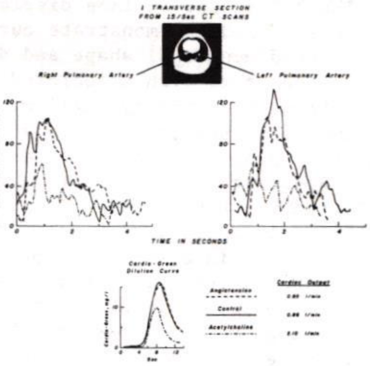


Figure 9

The DSR promises to open up a new approach to gravitational physiology.

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THERMOREGULATION IN COLD- AND NONCOLD-ACCLIMATED RATS COLD EXPOSED IN HYPERGRAVIC FIELDS

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INTRODUCTION

Cold-acclimated rats challenged by cold-exposure activate effectors for heat production in a manner that differs quantitatively from noncold-acclimated rats. This difference allowed the design and execution of experiments that have helped elucidate the mechanisms underlying the impaired temperature regulation observed in rats exposed to hypergravic fields. The intent of this paper is to discuss such experiments. The opening section places these experiments in context with studies on effects of gravitational fields on temperature regulation as a whole. Subsequent sections focus on the altered neural control of specific thermogenic mechanisms.

Oyama et. al. (9) were the first to demonstrate that when rats are exposed to a constant hypergravic field their core temperatures fall. This observation, confirmed in numerous subsequent studies (1,3,4,6,7,11), is noteworthy because it indicates that even though ambient temperature is maintained at 22-23 °C, a relatively small increase in gravitational field (e.g., from 1 G to 2.5 G) can evoke a large drop (about 2.5 °C) in core temperature, T_c (9). In contrast, when challenged at 1 G with seemingly more intense stressors (e.g., exposure to ambient temperatures of 2 °C), rats are able to appropriately activate thermogenic effectors and maintain T_c. Thus, the thermoregulatory system is functionally impaired by hypergravic fields. The fall in T_c occurring in rats at hypergravity has also been seen in dogs (8) and monkeys (2). In all of these mammals there is a downward drift in core temperature, and stabilization of T_c occurs at lower levels even though ambient temperature is maintained at 22-23 °C.

By lowering or raising ambient temperature, the ability of centrifuged animals to regulate T_c by activation of effector mechanisms can be more fully investigated. Several studies have employed a one hour period of cold exposure (about 5 °C) to test the dynamic responses of rats in constant hypergravic fields (3,4,6,11). Over the range of 1.5 to 4 G there was increasing impairment in temperature regulation as shown by larger differences in T_c between the end and beginning of the one hour period of cold exposure (4). In fact, the decrease in core temperature, ΔT_c, that results from a one hour exposure at 7 °C can be described by the equation $\Delta T_c = 2.3G - 0.79$ for rats restrained in acrylonitrile holders during exposure to 1.5 to 4.0 G (4). These studies involving responses to cold ambient temperatures have laid the basis for further experiments where the altered mechanisms underlying this impairment have been investigated (6).

Such experiments have focused on the neural controllers that activate effector mechanisms involved in heat conservation (vasoconstriction) and heat production (shivering and nonshivering thermogenesis, the latter being the conversion of chemical energy to heat by mechanisms other than shivering). The argument has been advanced that different neural controllers are affected by the gravitational field for different periods of time and that normally coordinated activities of the neural controllers are disrupted by hypergravity (6). The intent of the present study was to evaluate further this possibility by examining the ability of cold-acclimated and noncold-acclimated rats to cope with cold exposure in a hypergravic field -- the effector mechanisms in each being quantitatively very different. That is, in cold-acclimated rats that are cold exposed, most (80% or more) of their cold-induced heat production is generated via nonshivering thermogenesis, with little being generated by shivering. In contrast, cold-exposed, noncold-acclimated rats generate most of their heat by shivering thermogenesis rather than nonshivering pathways (5). Comparing the responses of the two acclimated groups allows functional assessment of the central neural controllers for the two types of heat production.

METHODS

Twenty-four Long-Evans, hooded, male rats were maintained on a 12 hour light-dark cycle and provided with water and food (Simonsen's white diet) ad libitum. One group, CA was cold acclimated by exposure to 5 °C for at least 6 weeks prior to exposure to hypergravic fields. The noncold-acclimated group, RT, was housed at a room temperature of 23 °C for 6 weeks prior to use in the experiments. RT and CA rats were similar in weight, 441 ± 21 g and 444 ± 20 g, respectively. The RT rats used in the experiments were younger (128 ± 17 days) than were the CA rats (168 ± 12 days).

Core, tail and ambient temperatures were measured using Veco 32A12 thermistors; oxygen consumption was monitored in a closed-system. As oxygen was consumed it was replaced from a Krogh-type spirometor (CO_2 was absorbed by soda lime placed beneath a wire screen on the floor of the respiratory chamber.) Oxygen consumption was continually recorded using a Varian chart recorder and temperatures were monitored with Houston Omnigraphic recorders.

Rats were exposed to hypergravic fields (3 G) on a 2.1 m radius centrifuge. The centrifuge chamber was mounted so that it could rotate during centrifugation with the resultant of the centrifugal and gravitational fields always perpendicular to the chamber floor. The direction of the field was thus unchanged (although the amplitude was varied from 1 to 4 G) and was orthodynamic with respect to the animal [$-G_x$ cf. (1)].

Data were analyzed using a t-test for comparing 2 means.

RESULTS

In one series of experiments, oxygen consumption was measured in unrestrained CA and RT rats under four different conditions: (a) T_a = 23 °C at 1 G; (b) T_a = 8 °C at 1 G; (c) T_a = 23 °C at 3 G; and (d) T_a = 8 °C at 3 G. In a single trial a rat was exposed to several of these conditions. That is, from time t = 0 to 1 hr., T_a = 23 °C at 1 G (condition a); then from t = 1 to 2 hrs., T_a = 8 °C at 1 G (condition b). The rat was then exposed from t = 2 to 3 hrs. to T_a = 8 °C at 3 G (condition d), from t = 3 to 4 hrs. to T_a = 8 °C at 1 G, and finally at t = 4 to 5 hrs. back to T_a = 23 °C at 1 G. The rates of oxygen consumption were measured over the whole 5 hour period. The values listed in Table 1 were obtained for the last 30 minutes of each of the first 3 one hour intervals of the trail (i.e., t = 0.5 to 1 hr., t = 1.5 to 2 hrs., and t = 2.5 to 3 hrs.) In both CA and RT rats, the rates of oxygen consumption between t = 1.5 and 2.0 hrs. were similar to those at t = 3.5 to 4 hrs., and the rates of oxygen consumption between 0.5 and 1 hr, were the same as those between 4.5 and 5.0 hrs. These latter results indicate that the metabolic responses of the rats were not irreversibly altered during the course of a given experiment.

In another series of experiments unrestrained rats were exposed to the following paradigm: from time t = 0 to t = 1 hr., T_a = 23 °C at 1 G (condition a); for t = 1 to 2 hrs., T_a = 23 °C at 3 G (condition c).

As summarized in Table 1, rates of oxygen consumption in both RT and CA rats at 1 G were comparable at 23 °C and also at 8 °C, each approximately doubling during cold exposure (T_a = 8 °C). However, in 3 G fields at T_a = 8 °C, the rates of oxygen consumption of CA rats were significantly greater than were those of the RT rats.

In a second type of experiment using restrained rats, T_c was measured to assess the thermoregulatory ability of rats in hypergravic fields. At 23 °C, 1 G, T_c fell in both CA and RT rats during the initial period of restraint, stabilizing at 36.7 ± 0.4 for RT and 37.1 ± 0.3 °C for CA rats by the end of 1 hour. When the rats were then exposed to 3 G at 23 °C, both CA and RT animals showed an additional fall in T_c, and the magnitude of this fall was similar in the two groups. However, when the rats were subsequently exposed to 10 °C at 3 G, there occurred a smaller fall of T_c in the CA vs. the RT rats (Table 1).

TABLE I. Effect of cold acclimation on the rate of oxygen consumption and on the core temperature of rats exposed to cold and hypergravity[a]

Unrestrained rats	Noncold acclimated rats	Cold acclimated rats
Oxygen consumption[b]		
at 1G, 23 °C	18.7 ± 1.8 (5)	21.2 ± 0.5 (5)
at 3G, 23 °C	18.0 ± 1.0 (5)	22.8 ± 1.7 (5)*
at 1G, 8 °C	44.2 ± 2.6 (5)	40.3 ± 3.1 (5)
at 3G, 8 °C	28.8 ± 1.1 (5)	39.0 ± 1.2 (5)*
Restrained rats		
Core temperature changes[c]		
at 1G, 23 °C[d]	−0.6 ± 0.1 (5)	−0.3 ± 0.1 (5)
at 3G, 23 °C[e]	−2.2 ± 0.1 (5)	−2.2 ± 0.5 (5)
at 3G, 10 °C[f]	−1.9 ± 0.6 (5)	+0.1 ± 0.5 (5)

[a] Values are X ± SEM for the number of rats in ( ). Rates of oxygen consumption represent those measured during the last 30 min. of the 1 hr. period (see text). Values that differ significantly ($p < .05$) between CA and RT rats are designated with an asterisk (*).

[b] Oxygen consumption values have units of mls $O_2 \cdot min^{-1} \cdot kg^{-1}$.

[c] Core temperature values have units of °C.

[d] Difference in T_c from .5 to 1 hour at 1 G and 23 °C.

[e] Difference in T_c for the initial hour at 3 G, 23 °C.

[f] Difference in T_c over a second hour at 3 G with an added period of cold-exposure.

DISCUSSION

At 3 G, restrained CA rats challenged with a 1 hour period of cold exposure more closely regulated their core temperature around normal levels than did similarly exposed RT rats (the fall in T_c occurring during cold exposure at 3 G was less in the CA vs. the RT rats). These findings are consistent with those of previous experiments at 2 G with rats acclimated to 5 °C prior to cold exposure at hypergravity. In these 2 G experiments, the change in T_c after one hour of cold exposure was only -0.2 °C for the CA rats while for the RT animals it was -0.8 °C (1).

The observation that CA rats more effectively maintained warm core temperatures when cold exposed at hypergravity is consistent with the finding that these cold-exposed, CA animals increase their heat production to a significantly greater degree than do the RT rats. That is, the unrestrained CA rats almost doubled their rate of oxygen consumption when cold exposed (8°C) at 3 G (compared to their responses at 23 °C at 1 G). In contrast,the rate of oxygen consumption in RT rats exposed to 8 °C at 3 G was only 60% above that at 23 °C, 1G. The reduced response of the RT rats has been attributed to (a) the fact that cold-induced heat production in RT rats primarily reflects shivering while in CA rats there is a shift to nonshivering thermogenic pathways (5) and (b) shivering in the rats tends to be depressed at hypergravity as a result of increased demands on the skeletal musculature for postural bracing (6).

The results of experiments with cold exposed CA and RT rats at 3 G are summarized in Figs. 1,2 and 3. Figure 1 shows receptors for subcutaneous, T_{su} and deep body temperature, T_b (i.e., receptors in the hypothalamus and spinal cord) relaying signals to parallel controllers in the central nervous system (10). Signals from the controllers then activate thermoregulatory effectors. In CA and RT rats at 23 °C, shivering, NST and heat conservation effectors are not activated to offset the fall of T_c at the onset of centrifugation (Figs. 2,3). By the end of 1 hour at 3 G, 23°C, the low rate of oxygen consumption and fall of tail temperature indicate that heat conservation effectors have recovered but thermogenic effectors have not.

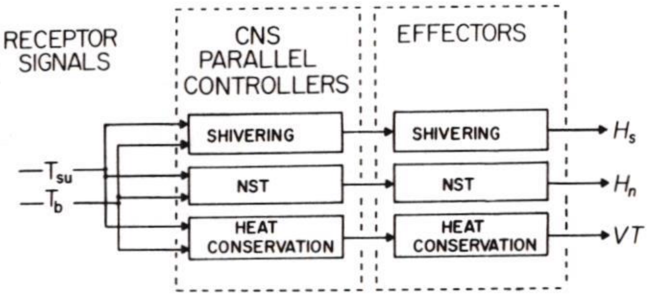

Figure 1. Parallel neural controllers and effectors. Signals from subcutaneous receptors are denoted by T_{su}, signals from other receptors (e.g., those in the hypothalamus and spinal cord) denoted by T_b. Heat production from shivering and nonshivering thermogenesis is represented by H_s and H_n, respectively. Heat is conserved by increasing vasomotor tone, VT.

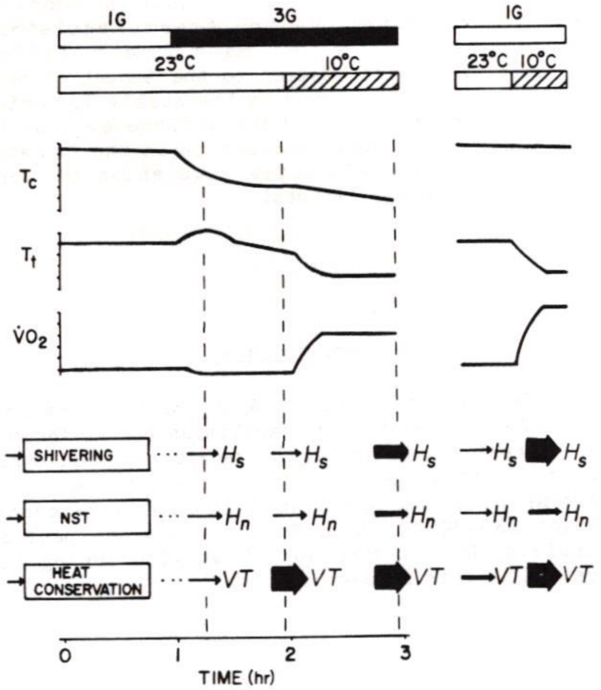

Figure 2. Effector activity, core temperature, T_c, tail temperature, T_t, and oxygen consumption, $\dot{V}O_2$, during periods of 1 G and 3 G exposure at 23 °C and 10 °C in noncold-acclimated rats. The size of the arrows represents the magnitude of effector activity.

Note: Curves in this figure (and in Fig. 3 below) are idealized (not plots of actual data).

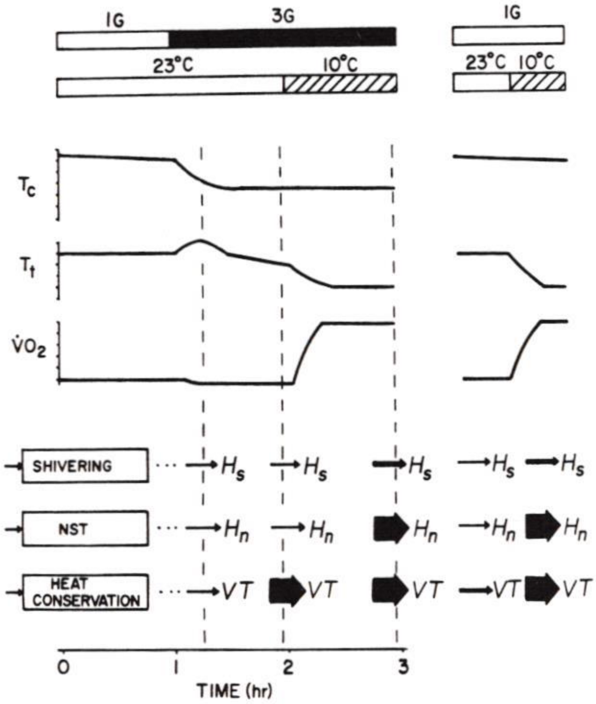

Figure 3. Effector activity, T_c, T_t, and VO_2 in cold-acclimated rats.

During exposure to the combined stressors of cold and hypergravity, the thermoregulatory responses of the CA and RT rats differ quantitatively with respect to the amount of heat generated. As indicated by the stable T_c during 3 G exposure at 10 °C, this difference (due to greater rates of heat production in the CA rats) results in more effective regulation of core temperature in the CA rats.

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Biological Scaling from Cells to Environment: A Prelude to Gravitional Explanations

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The influence of gravity on animals depends on body mass (M). Small insects are oblivious to gravity, but are dominated instead by surface forces. Mammalian tolerance of gravity is inversely related to M. Gravity has been implicated as the cause of the familiar $M^{3/4}$ scaling of basal metabolism in at least three ways: 1) a compromise between demands of thermoregulation and gravity, 2) the preservation of elastic similarity to resist buckling due to gravity, and 3) as an energetic cost of gravitational tolerance.

First I review the allometry of basal metabolism, thermoregulation, maximum aerobic activity, and estimated total daily metabolism in nature. Then I examine the gravitational hypotheses that seek to explain $M^{3/4}$ scaling. Finally, the allometry of lifetime energetics and life histories are surveyed. I conclude that it is not yet possible to decide which explanation is correct, and that resolution of this problem may require a broad interdisciplinary approach.

INTRODUCTION

Kleiber's mouse-to-steer curve (17) establishing the $M^{3/4}$ scaling of metabolic rates was only an empirical correlation, but abundant confirmation begs for causal explanation. Gravity has been invoked as a factor in metabolic scaling in at least three ways. However, conformity to $M^{3/4}$ rates and $M^{1/4}$ times occurs throughout the biology of homeotherms--in reproduction, growth, and ecology, indicating a need for allometric survey of the full spectrum from cell to population biology before accepting causal explanations from within a single discipline. Limits to applicability of M scaling must also be recognized. Metabolic scaling changes when comparisons are from conditions other than basal thermoneutrality.

METABOLIC SCALING

Basal Metabolism ($\dot{E}_{min}$). Kleiber's rule (17) relating E_{min} and (body mass, M)$^{3/4}$ has been verified repeatedly even in unicellular animals (12a). The physiological support (minute volume, cardiac output, renal clearance, has compatible $M^{3/4}$ scaling (2,5,6). Recently Kleiber's rule has been questioned by Heusner (13,14,15) who argues that mammalian $\dot{E}_{min}$ data should be regressed, not as an interspecific log E_{min} vs log M that yields the familiar 3/4 slope, but as a series of intraspecific regressions with parallel slopes of $\sim 2/3$. The allometric coefficients a (in $\dot{E}_{min}=aM^b$) were positively correlated with body size (13,14) but it was suggested later (15) that a "may represent a physiological intensive property", intensive properties being size-independent. The data came from rodents (mice, rats) at the small end of the size range, carnivores (cats, dogs) in the midrange, and artiodactyls (sheep, cattle) at the large end, but it appears that body size rather than phylogeny dominates a. In fact, that trend is necessary to generate the steeper slope in the interspecific regressions that lump all eutherian mammals.

Thus, we can assess the influence of body size over the full range attained in a class as: 1) a single, simple regression that has one slope (3/4 for E_{min}) and one coefficient, or 2) separate intraspecific regressions that may have parallell slopes and an array of coefficients. Either procedure is valid for showing the effects of size (10). Heusner's average intraspecific regression exponent, 0.67, may be explained as a surface area function, but revived "surface law" seems inadequate without explaining the positive allometry of the coefficients a. The logarithmic form of the allometric equation is:

$$\log \dot{E}_{min} = \log a = b \log M \qquad (1)$$

where $\dot{E}_{min}$ is in watts, a is the intercept with the vertical axis, b is the mass-dependent slope, and M is mass in kg. Heusner's values for a in 7 species are a function of M:

$$\log a = 0.52 + 0.115 \log M \qquad (2)$$

Substituting from eqn. (2) and Heusner's mean b (0.67) in eqn. (1):

$$\log \dot{E}_{min}+0.52+0.12 \log M+0.67 \log M \qquad (3)$$

Heusner has, in effect, broken down the mass-dependence of the Kleiber equation into two components. Consolidating the log M terms gives a combined slope of 0.79, essentially Kleiber's 3/4.!

Thermoregulation. The lower critical temperature (T_{lc}) is the lowest environmental temperature (T_e) at which E_{min} is sufficient to maintain a normal body temperature (T). Below T_{lc}) E must be increased in linear proportion to the differential T_b-T_e:

$$\dot{E} = h\ (T_b-T_e) \qquad (4)$$

where h (watts/°C) scales as $M^{1/2}$. Substituting scalings into eqn. (4) at $T_e=T_{lc}$, note the $M^{1/4}$ dependence of the differential (T_b-T_{lc}):

$$\dot{E}_{min}=h(T_b-T_{lc}) \propto M \quad \propto M^{1/2}\ (T_b-T_{lc}) \qquad (5)$$

Below T_{lc}, $\acute{E}$ is not proportional to M . Thus M scaling in thermoneutrality combines the $M^{1/2}$ of the heat transfer coefficients and the $M^{1/4}$ of thermoneutrality.

Maximum Aerobic Activity. Studies of running locomotion (26) and subsequent analysis (11) reveal another aspect of metabolic scaling. The total E during running is the sum of two costs (\$), transport \$ that is linearly proportional to speed (u) plus a "zero-speed" or postural \$:

$$\text{watts}=\dot{E}_{zero\ u} + (\$_{transport}) \times (u) \qquad (6)$$

The allometric terms for the whole animal zero u and $\$_{transport}$ are 6.03 $M^{0.70}$ and 10.7 $M^{0.68}$, respectively(26). The scaling of total $\dot{E}$ becomes, at the trot-gallop transition and at maximum aerobiosis(11), respectively:

$$\dot{E} = 6.03M^{0.70}+10.7M^{0.68}x1.53M^{0.24}=24.7M^{0.86} \quad (7)$$

$$\dot{E} = 6.03M^{0.70}+10.7M^{0.68}x3.06M^{0.15}=39.0M^{0.79} \quad (8)$$

Although b (0.79 to 0.86) is somewhat larger than basal 0.75, the factorial scope, for increase above the zero-u level is much more significant for larger mammals ($M^{0.68+0.24}=M^{0.92}$; $M^{0.68+0.15}=M^{0.83}$).

Field Metabolic Rates. In nature, the total energy requirements or average metabolic rate per day would combine basal maintenance, thermoregulatory, and activity costs. Estimates from time and energy budgets as well as from measurements of isotopic water turnover both indicate that metabolic rates have a lower scaling exponent of 0.60 to 0.67 (12,27). Thus there are several exponents to consider in evaluating any influence of gravity.

GRAVITATIONAL EXPLANATIONS OF $M^{3/4}$SCALING

At least three explanations based on gravity have been given for Kleiber's scaling: compromise, elastic similarity, and gravitational tolerance.

Compromise. Numerical simplicity makes an attractive hypothesis, attributed to Kleiber by Pace and Smith(21). Gravitational loading is proportioned to M while heat loss scales as $M^{1/2}$. Thus $M^{3/4}$ scaling of $\dot{E}_{min}$ may be a compromise between the two. Mathematically, the universal $M^{3/4}$ compromise would give heat loss three times the influence of gravity.

$$3M^{1/2}M \propto M^{3/4} \quad (9)$$

Considering the great range in World climates, would natural selection have been so inflexible between metabolic demands of the tropics and the arctic?

Elastic Similarity. Two animals of different sizes but similar in their elastic limits or resistance to buckling will have different proportions of diameters relative to limb and truck lengths. McMahon proceeded from Rashevsky's analysis of relative sagging due to self-loading to contribute the first direct prediction of $M^{3/4}$ scaling for $\dot{E}$ (19,20). However, in mammals other than the Artiodactyla, scaling of characteristic lengths does not conform to elastic similarity. Alexander (1) has detailed the objections and alternatives to elastic similarity that indicate its inadequacies for tying metabolic scaling to gravity.

Gravitational Tolerance. Small mice can tolerate greater gravitational forces than rats, and rats can tolerate more g's than dogs. This g-tolerance scales as $M^{-0.14}$ (8). The metabolic incremental cost for overcoming the pull of gravity would then be expected to be proportional to the inverse, $M^{0.14}$. Economos (8) derived gravitational cost term, with an exponent of 0.89 (=0.75 + 0.14). When added to a surface law term ($M^{0.67}$). This fits the mouse-to-elephant metabolic plot with a slightly smaller error than the familiar $M^{0.75}$ plot. Perhaps the $M^{0.12}$ scaling of the coefficient a, that combined in equation (3) to yield the $M^{0.79}$ scaling, is not significantly different from Economos exponent of 0.14; gravity might have its influence there. If so, why does it appear as Heusner's step-function between species, rather than as a continuous consequence of size increase within a species?

PHYSIOLOGICAL TIME AND LIFETIME METABOLISM.

Over one-half of the body mass is apparently proportioned the same in both large and small mammals; muscle, blood, heart, lungs, and spleen. Thus the capacity to do work and to supply oxygen to the tissues is scale as $M^{1.0}$. Physiological times such as cycle times of the heart and respiratory system as well as metabolic turnover times scale as $M^{1/4}$, (18). Capacity ($M^{1.0}$) divided by time ($M^{1/4}$) gives a volumetric rate function scaled as $M^{3/4}$.

The maximum observed longevity (t_{max}, assumed to represent the physiological maximum) of mammals is scaled almost like the shorter physioligical cycles (yr= $11.6M^{0.20}$;23). The $\dot{E}_{min}$ for lifetime per kg body mass would be the product:

$$\dot{E}_{min}x\ t_{max}x\ M^{-1} =(3.8M^{0.76})(3.15\cdot 10^{7}s/yr)/M^{-1}= 1.39\cdot 10^{6}\ kJ/kg\cdot lifetime)M^{-0.04} \quad (10)$$

The lifetime cumulative mechanical fatigue caused by gravity would be the product of mass and lifespan, or force·time, scaling as $M^{1.2}$, a scaling that exceeds that for skeletal mass.

LIFE EXPECTANCY AND METABOLISM IN NATURE

The scaling exponent for list expectancy (t_{exp}, da.) is greater than that for t_{max} (3), just as the scaling for metabolism in nature (E_{wild}, J/da) is less than $M^{3/4}$ (12). Thus large mammals and birds tend to live a time, but at $\dot{E}$ reciprocally less intense than those of smaller animals. In the case of the mammals, this is:

$$t_{exp}\ x\ \dot{E} \div M^{1.0} = (1854M^{0.35})\ (802M^{0.66})/M^{1.0}= 1.49\cdot 10^{6}\ kJ/(kg\cdot lifetime) \quad (11)$$

This is essentially independent of size ($M^{0.01}$) and of the action of gravity effected disproportional detriment to the lives of larger mammals, why would their life expectancy tend further towards the physiological maximum?

LIFE HISTORY SCALING AND METABOLISM

One might expect the energetic demands of reproduction, growth, and population density and turnover to be proportional increments of E_{min} or daily E. The allometric regressions now available seem to fit this expectation.

Reproduction. The number of births per year per member of the population is 1.26 $M^{-0.33}$(28). This scaling effectively cancels that for life expectancy ($M^{+0.35}$) giving a size-independent number of about 12 births per lifespan, perhaps an ecological counterpart of the size-independent physiological design constants identified by Stahl (25).

Growth. Growth times show, in general, the $M^{1/4}$ scaling of physiological times, and mean growth rates are scaled like $\dot{E}_{min}$ $M^{\sim 3/4}$ (review: 5).

Population Turnover. The time for replacement of a standing crop (ratio of biomass of population to annual productivity) scales in mammals as $M^{0.29}$ to $M^{0.33}$ (review: 4).

Population Density. The density of herbivorous mammals scales inversely to $\dot{E}_{min}$ so the product of metabolism per individual and number of individuals per hectare of habitat is size-independent, approximately(7). Thus collectively, the small species and the large ones harvest similarly at the population level.

CONCLUSION

The ultimate significance of physiological measurements is in the lives of animals in nature where the mechanisms and proportions evoloved. Our correlations have not established a causal role for gravity in quantitative scaling. Until such a role is established, we cannot limit our thinking to the comfortable confines of one discipline.

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HAEMODYNAMICS UNDER CHANGED GRAVITY

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ABSTRACT

Microgravity was simulated by "dry" immersion of 1, 3 or 7 days. Before and after immersion hemodynamic parameters were measured during ergometry tests. The major goal of this study was to investigate cardiovascular changes during exercise performed after a short-term exposure to simulated weightlessness. Another purpose was to identify a relationship between renal fluid losses during immersion and cardiovascular changes after the exposure.

METHODS

This study was carried out on 22 healthy volunteers, aged 25-32. Weightlessness was simulated by water immersion (so-called method of dry immersion /1/). In the 1st experimental run (8 test subjects) the exposure was 1 day, in the 2nd (8 subjects) it was 3 days, and in the 3rd (6 subjects)- 7 days. The test subjects were given a standard diet. They performed an exercise test of 100 Wt in the supine position on a bicycle ergometer.

The following parameters were measured at rest and during ergometry at 8th and 15th min: oxygen consumption ($\dot{V}O_2$), CO_2 production (the exhaled air was collected in a Douglas bag); cardiac output ($\dot{Q}$) which was determined by the CO_2 rebreathing method (according to /2/), stroke volume ($\dot{Q}$s) and rate-pressure product (heart rate x systolic pressure) that were derived by calculation.

During the control and immersion stages the daily fluid balance was determined as the difference between fluid consumption and excretion.

RESULTS AND DISCUSSION

Prior to immersion the cardiorespiratory parameters of all the test subjects were adequate to the exercise load and duration.

1st experimental run.After 24 h immersion $\dot{Q}$ increased by 19.5% while heart rate and stroke volume grew by 6.7% and 7.2%, respectively (the data at rest). These cardiovascular changes described by many authors /3, 4/ seem to reflect blood congestion in the upper body.

In the middle of the exercise test post-immersion $\dot{Q}$ decreased by 10.5% and at 15th min did not differ from the control value. At 8th min $\dot{Q}$s decreased by 15% and by the end of the ergometry test only by 5.8%. Heart rate was 11.5% higher than the pre-immersion value at the end of the exercise test with an insignificant difference earlier in the test and during recovery. $\dot{V}O_2$ differed from the control value in the middle of the test but slightly and at the end of the test decreased by 7.5%.

The rate-pressure product increased by 18% at the end of the ergometry test. This was largely associated with a higher heart rate because systolic pressure remained essentially unchanged after immersion.

2nd experimental run.After 3-day immersion the minute respiratory breathing, $\dot{V}O_2$, heart rate did not change and $\dot{Q}$ diminished due to a stroke volume decrease; these are the data at rest.

During ergometry $\dot{Q}$ was identical to the control value and $\dot{Q}$s was 8% lower at every test stage. $\dot{V}O_2$ declined by 10.6% and 8.8% at 8th and 15th min, respectively. Heart rate increased slightly at 8th min and 10.6% at 15th min. After immersion heart rate recovered but slowly. The rate-pressure product was by 15.6% higher than the pre-immersion value only at 15th min.

3rd experimental run. After 7-day immersion heart rate at rest was 9.4% higher than in the control period. This heart rate increase may explain an increase by 11.2% of $\dot{Q}$ since $\dot{Q}$s remained unchanged.

During the ergometry test after immersion the following changes were seen.

$\dot{Q}$ did not differ significantly from the control values. $\dot{Q}$s decreased by 10.8% at 15th min. $\dot{V}o_2$ increased by 8.1% at 8th min and by 10% at 15th min. Heart rate was about 11% higher than the control level beginning with the first minutes of the test. After immersion heart rate slowly returned to the norm. The rate-pressure product increased by 10.6% mostly at 8th min.

During immersion fluid losses exceeded fluid consumption by 823, 1169, and 1350 ml in the 1st, 2nd, and 3rd experimental runs, respectively.

Variations in $\dot{Q}$ were of different type: after 1- and 7-day immersion the parameter increased by 19.5% and 11.2% and after 3-day immersion decreased by 5.4%.

This dynamics of $\dot{Q}$ may reflect the phasic pattern of cardiovascular adaptation to immersion.

During post-immersion exercise tests (as compared to pre-immersion data) $\dot{Q}$ did not change significantly. The only exception was the 1st experimental run in which a 10.5% decrease was found. However, in spite of this $\dot{Q}$ invariability, $\dot{V}O_2$ decreased after 1- and 3-day immersion and increased after 7-day immersion. It is probable that immersion brings about a distortion in the close correlation between $\dot{Q}$ and VO_2 that exists in normal conditions.

The value of $\dot{Q}s$ tended to decline as immersion continued. For instance, after 1-day immersion the decrease was by 5.8%, after 3-day immersion by 8%, and after 7-day immersion by 10.8%.

Existing literature indicates different factors that may be responsible for the $\dot{Q}s$ decrease during exercise tests performed after exposure to immersion, hypokinesia or real weightlessness. These factors include: decreased tolerance to the upright posture /5/, deteriorated contractile function of the myocardium /6/, reduced circulating blood volume /3, 7/.

In our investigations carried out on supine subjects the concept of the major role of orthostatic disorders can hardly be applied.

Recent investigations by A.M. Babin (1983) who used echocardiography demonstrated that short-term (up to 7 days) immersion produced no changes in heart contractility.

The changes in $\dot{Q}s$ detected in our study are likely to reflect variations in the hydration status of the body; they may be associated with renal fluid losses that increase with immersion duration. In parallel with our measurements, K.I. Gogolev evaluated plasma losses during the 2nd experimental run: they varied from 11 to 22%. These changes are suggestive of circulatory adaptation aimed at reducing blood congestion in the upper body which is typical of immersion reactions.

Our study showed that the rate-pressure product during the exercise test increased after the immersion of different duration. However, the 18% increment at 15th min of the exercise test following 1-day immersion was the greatest.

Thus, our investigations give evidence that an early stage of simulated weightlessness is accompanied by changes in cardiac output. This is associated with its increase after 1- and 7-day immersion and a slight decrease after 3-day immersion. This variation of $\dot{Q}$ points to a phasic pattern of processes in the human body at an acute stage of adaptation to simulated weightlessness.

Man's tolerance to a workload of 100 Wt performed in the supine position on a bicycle ergometer declines after exposure to immersion-hypokinesia of 1, 3 and 7 days. This is indicated by a higher, as compared to the pre-immersion level, increment of heart rate, increase of the rate-pressure product, decrease of stroke volume. The work capacity decreases to a greater extent after 7-day immersion.

The decline in the reserve capabilities of the cardiovascular system during exercise tests after water immersion shows individual variability. This is to be taken into consideration when applying means and methods that may affect man's physical performance.

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NASA Space Biology Program

The advent of the space age provided the first access to the "gravity-free" state and an opportunity to manipulate gravity from its norm of one down to zero. Therefore NASA has assumed the responsibility to investigate the biological significance of gravity and thereby expand biological knowledge.

Objectives

The objectives of NASA's Space Biology research program are *1*) to investigate the biological significance of gravity; *2*) to use gravity to solve relevant biological questions; and *3*) to enhance our capability to use and explore space.

Goals

The goals of the Program are *1*) to enhance our knowledge of normal physiological adaptive mechamisms in both plants and animals and thereby provide new insight into both normal and pathological mechanisms; *2*) to provide for the multiple generation survival of plants and animals in space through an understanding—and ultimately control—of the affects of gravity on development, adaptation, and evolution; and *3*) to enhance plant productivity through an understanding and control of gravitational and related environmental stimuli and the manipulation of response mechanisms.

The achievement of such goals depends on answers to basic scientific questions that include the following.

1) Does gravity influence fertilization and early development and can fertilization and early development proceed normally in a near 0–G environment? If gravity does affect fertilization and early development, what are the sensitive physiological systems and how are they affected? If early development is affected by gravity, is it a result of an affect on the parent or the direct affect on the embryo itself?

2) What is the role of gravity in the formation of structural elements, such as lignin, cellulose, chitin, and bone calcium, at the molecular as well as at the more complex organizational levels?

3) What role does gravity play in calcium-mediated physiological mechanisms and in calcium metabolism?

4) What is the gravity-sensing mechanism? How does it perceive information? How is the information transmitted to evoke a response?

5) How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology, and behavior of organisms? *Or* how do gravitational and other environmental stimuli interact in their control and direction of living forms? Can the action of gravity be replaced by different stimuli?

Strategy

The strategy so far has been to manipulate gravity on earth and develop weightless simulation models to develop and test gravitational hypotheses; to identify gravity-sensitive biological systems and interacting environmental response mechanisms; to address valid gravitational biological questions on earth when possible; and to plan and design future space experiments. As space-flight opportunities, either manned or unmanned, become more prevalent, increasing emphasis will be placed on flight experiments. Similarly, as longer flight missions become available, emphasis will be directed toward biological questions that require longer periods of microgravity for adequate experimentation.

Program Content

The program has been divided into the following three broad areas: *1*) the role of gravity in reproduction, development, maturation, and evolution; *2*) gravity receptor mechanisms (these include the identification of the organ or site of gravity reception and the biological systems and mechanisms that transmit the information to a responsive site); and *3*) the physiological effects of gravity (this includes the biological mechanisms by which living systems respond and adapt to altered gravity, particularly that of the space environment, as well as the interactive affects of gravity and other stimuli and stresses on the physiology, morphology, and behavior or organisms).

This NASA program in space biology is carried out intramurally by the NASA Research Centers and by a system of extramural grants. Qualified scientists interested in learning more about the program and the development of research proposals should contact:

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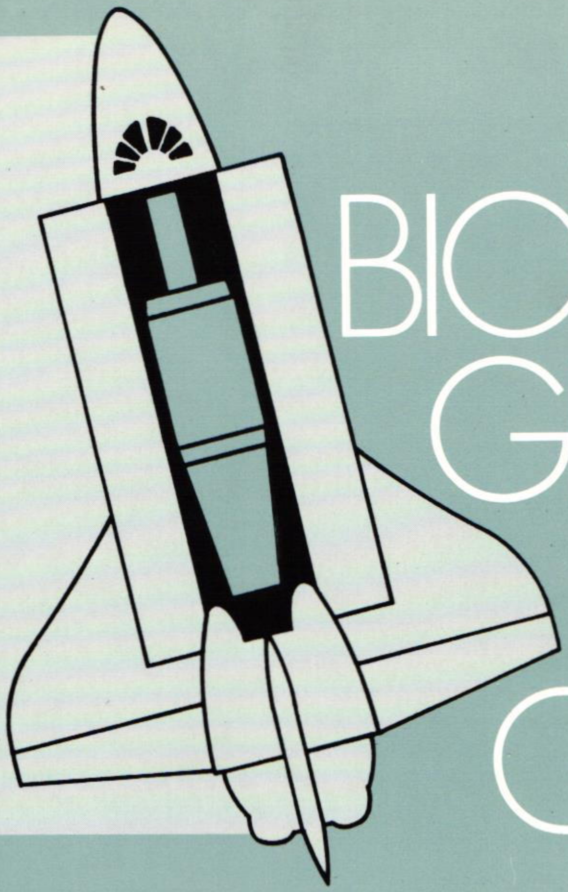

SPACE BIOLOGY GRANT PROGRAM