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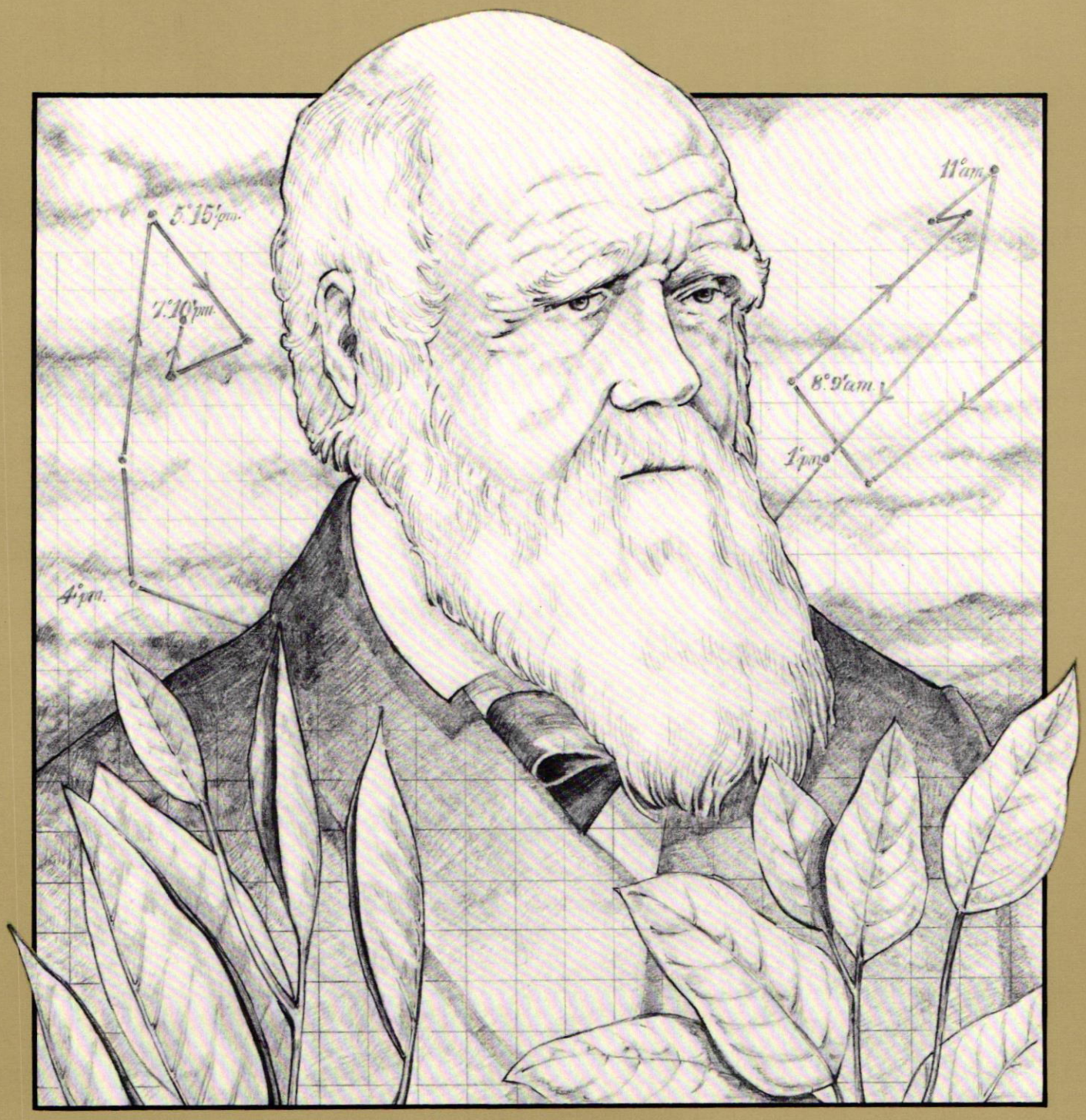

Proceedings of the Sixth Annual Meeting of the IUPS Commission on Gravitational Physiology

18–21 September 1984
Lausanne, Switzerland

International Union of Physiological Sciences
Commission on Gravitational Physiology

PRELIMINARY ANNOUNCEMENT

IUPS Commission on Gravitational Physiology – Seventh Annual Meeting 13–18 October 1985 Niagara Falls, New York, USA

The Seventh Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences is being planned for Niagara Falls/State University of New York, Buffalo, NY, USA, 13–18 October 1985. The meeting will be held in conjunction with the Fall Meeting of the American Physiological Society.

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 symposia by invited speakers on several topics in gravitational physiology.

It is planned to publish the Proceedings of the Seventh 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.

Dedication

Charles Robert Darwin (1809–1882) to whom this volume is dedicated is and probably always will be best known for his *Origin of Species.* Nevertheless, after publication of the *Origin* in 1859, Darwin continued to publish on biological topics. The *Origin* has even been called an "heuristic prologue" to Darwin's chiefly botanical studies published in the last two decades of his life (1). He wrote six books after the *Origin,* two of which dealt with causes and purposes of plant movements. *The Movements and Habits of Climbing Plants* (1875) was an extension of a journal article published earlier, and what has been called his "most influential botanical work" (2), *The Power of Movement in Plants* (1880), with his son Francis as coauthor, is for gravitational physiology a 19th century classic (3).

Had Darwin not published the *Origin* when he did, he would not have enjoyed the scientific credit nor borne the brunt of criticism for the secular, evolutionary, "new science"—impossible to reconcile with special creation. Instead those honors would have gone to Alfred R. Wallace, his fellow naturalist. Nevertheless, Darwin's original experiments and observations in the sensory physiology of plants ensured for him an important place in the early development of gravitational physiology, and he would be well remembered for those seminal contributions alone.

Darwin's formal education, first at Edinburgh University, where for two years he found medical lectures, as he put it, "intolerably dull" and then three years at Cambridge to study for the clergy where his " time was sadly wasted," did not prepare him uniquely for tackling physiological problems. In those years, however, he indulged a taste for natural science by taking field trips, collecting minerals and beetles, learning first hand about countryside geology, and, as he specifically noted, making good use of the library.

More significant than formal course work were Darwin's opportunity to study on his own certain aspects of botany, to develop his natural talent as a keen observer, and most important of all to serve as the (unpaid) naturalist on the historic around-the-world voyage of H.M.S. Beagle.

Among the attitudes that we now believe importantly shaped Darwin's way of thinking and pursuing his scientific goals were his insistence that observation be coupled with speculation (or, as we would say today, experimentation cojoined with testable theory) and his frankly teleological concept of biological designs, which served him well in the years that he was marshaling evidence for the *Origin of Species.* These views help us to understand the personal background for his contributions to what we now call gravitational physiology.

Darwin lived about a century too early for him to have achieved solutions (in our modern sense) to basic problems of gravitational physiology; his achievement was to discover some of those problems. By his own rueful admission Darwin had no talent for mathematics. He was neither inclined nor able to construct mathematical models to account for biophysical mechanisms that interested him. In the 1860s and '70s what we now call biochemistry practically had not truly begun; its subject area was not much advanced beyond herbal medicine. The nature of enzymes and their mode of action were beginning to be understood only decades after Darwin's death. Nevertheless, his botanical publications provided original, thoughtful, and seminal observations on two kinds of plant growth movements—tropistic responses to photic and gravitational stimuli and presumably nastic oscillations, which Darwin recognized were responsive to gravity but, as he thought, not dependent on it. In both of these examples he identified new areas for physiological research that are important for us today.

Darwin recorded relatively slow growth movements of plants without the benefit of our modern, time lapse, cinematography. He attached a finely drawn out filament of glass to the tip of a growing organ and observed it through a fixed glass plate. By sighting along the direction of the filament a dot was made on the glass with a sharp pointed stick dipped in thick Indian-ink; afterward the series of dots made at different times would be joined by straight lines. Thus a record was obtained of the course of movement, which often was patently oscillatory. Darwin coined his own term for these movements, *circumnutation,* which was the principal subject of his *Power of Movement of Plants.* As he wrote, "Circumnutation is of paramount importance in the life of every plant; for it is through its modification that many highly beneficial or necessary movements have been acquired" (3).

Darwin's genius identified new areas for physiological research. He was the first to observe— and he explicitly recorded it as original—that the plant's gravity sensors were highly localized in the tips of growing roots and shoots but that the effect of their stimulation was to alter growth rates of tissues some millimeters or even centimeters away. As he summarized it, ". . . we now know that it is the tip alone which is acted on, and that this part transmits some influence to adjoining parts, causing the latter to bend" (3).

There followed a series of experiments by physiologists in a number of European laboratories who tried to understand the kinetics of transmission of that "influence." Ultimately it was associated with a naturally occurring substance, which, by definition, was a hormone. The substance, at first called "Auxin," later was identified chemically as a relatively simple organic compound, indole-3-acetic acid.

The progress of understanding at each step of that succession of investigations was in a way unusual, for it all seems as near as biological science ever approaches to a simple, logical sequence of experiments. From the beginning (Darwin's 1880 monograph) to the separation of the first known plant growth regulating hormone in the 1920s and its chemical identification in the 1930s, each experiment was designed as a follow-on from a previous result; there were almost no accidental or serendipitous discoveries along the way. Thus one may confidently trace the growth of this research field all the way back to its Darwinian origin.

Today we know of dozens of naturally occurring organic compounds, commonly grouped in five distinct classes, that have growth-regulating power, although we still are not able to explain to our full satisfaction how any of them regulates plant growth.

In a very practical sense today's multimillion dollar chemical growth-regulator industry was established on the foundation of Darwin's astute observations of plant growth responses to gravitational and other stimuli. This relationship should not be overlooked by those among us who share responsibilities for allocating public or private resources for research. Darwin was financially independent, and he surely felt no obligation to justify his work on any but intellectual grounds, but had it been otherwise it seems most unlikely that, during his lifetime, he could have anticipated any financial rewards stemming from his researches.

After more than a century Darwin's classic monograph on circumnutation (3) still is read and often cited by gravitational physiologists, and since it is nearly devoid of pedantic technical terminology, it stands as a quite readable reference and inspiration for our students with some curiosity about the history of our science.

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

The "Sixth Annual Meeting of the IUPS Commission on Gravitational Physiology" was held at the Institute of Plant Biology and Physiology of the University of Lausanne, Switzerland, September 18–21, 1984. More than 100 registrants from 14 countries attended the presentation of 70 research papers on topics in gravitational physiology.

The meeting opened with four invited symposium papers covering recent spaceflight results and future plans for space life sciences research in the USSR, USA, Western Europe, and Japan, followed by two papers from Japan and the USSR on the neural regulation of cardiovascular function in rabbits and dogs, respectively, during changes in body orientation. In the afternoon of the first day, four invited symposium papers from the USA on plant bioregenerative systems were presented.

The second and third days of the meeting, plus a night session on the last day, were devoted to 46 voluntary papers on gravitational research results from plant and animal forms ranging from *Arabidopsis* to man. The topics included cardiovascular function, cell division, circadian rhythmicity, circumnutation, embryonic development, endocrine function, energy metabolism, fluid and electrolyte balance, gravitropism, muscle function, population dynamics, respiratory function, skeletal function, temperature regulation, and vestibular function.

On the final day of the meeting, the morning session comprised four invited symposium papers from Western Europe and the USA on cardiovascular and vestibular effects of spaceflight in man, together with two papers from the USSR on motor and vestibular function in the Cosmos 1514 monkeys and on results from the pregnant rats flown in Cosmos 1514. In the afternoon eight invited symposium papers from Japan, Western Europe, and the USA were given on the topic of gravity and plant reactions.

The sessions were lively and were characterized by much discussion, and the consensus was that the meeting was highly successful.

The follow pages are presented as the Proceedings of the meeting by arrangement with the American Physiological Society. In the interest of timeliness and economy they are published without editorial review. Financial support for the preparation of the Proceedings has been provided by the US 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.

The seventh annual meeting of the IUPS Commission is scheduled to be held in conjunction with the Fall Meeting of the American Physiological Society in Niagara Falls, New York in October 1985, and the eighth annual meeting is to be held in Tokyo, Japan in early November 1986. All are welcome, and detailed announcements are forthcoming.

H. Bjurstedt, Sweden, *Chairman*
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K. Kirsch, GFR
I. Kozlovskaya, USSR
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H. Saiki, Japan
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OPENING REMARKS

H. Bjurstedt
Chairman, IUPS Commission on Gravitational Physiology

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It is an honor and a great pleasure for me to welcome you to this Annual Meeting of the International Commission of IUPS on Gravitational Physiology, the sixth of its kind. The fact that our Annual Meetings continue to attract biologists in great numbers and from many countries means that the importance of gravitational physiology is winning world-wide recognition, and that the Annual Meetings of our Commission are regarded as a useful forum for discussing ideas, methods and instrumentation for continued experimental research in gravitational physiology.

The printed program of this Meeting outlines the activities for the 4 days which are before us. Apart from five sessions of free communications in various areas of gravitational physiology, four sessions are organized in the form of symposia of invited papers. Some of the highlights of these symposia are the results of plans drawn up during our 1983 Meeting in Moscow. Thus, one of the symposia will deal with the role of gravity in bioregenerative systems, a problem of fundamental scientific interest and of special significance in connection with long-term life support in future space stations. Another symposium is devoted to various aspects of gravireactions in plants. Our Meeting being hosted by Professor Pilet's Institute, it is only natural that the field of gravitational plant physiology is well represented in the scientific program.

Of great interest to the Commission are the research opportunities offered by the Shuttle/Spacelab system. The Spacelab 1 mission, a joint venture of the European Space Agency and the US National Aeronautics and Space Administration took place in November and December 1983. This project conducted a number of path-breaking experiments in zero-gravity biological and metals research. This first flight can claim a number of discoveries and scientific firsts. The Commission is pleased to note that many results from the biological experiments will be presented and discussed at this Meeting. At our recent Meeting in Moscow the coming flight of the Kosmos 1514 Primate Biosatellite was awaited with keen anticipation. It is a great pleasure for us to note that our first symposium sessions will highlight results from this flight and also additional results from other USSR life science experiments in space. The Commission is pleased to acknowledge that the number of submitted papers for the four symposia and five open sessions totals 80, which means that the volume of the scientific contributions to our Annual Meetings continues to increase.

As has been the case for our previous five Annual Meetings, The Proceedings of this Meeting will be published in The Physiologist by arrangement with the American Physiological Society. Financial support for this purpose has been kindly provided by the US National Aeronautics and Space Administration. This organization as well as the European Space Agency have generously put funds at disposal to support and facilitate also in other ways the organization of the scientific program. We like to believe that the wealth of information contained in the Proceedings of our previous and present Annual Meetings will serve a most important function in providing background data and impetus for future research projects. In the past we have been able to publish our Proceedings within only a few months of the event, and we hope to maintain this tradition.

It is appropriate on this occation to acknowledge the excellent work expended by Professor Pilet and his staff in making it possible for the Commission to hold its Sixth Meeting in the beautiful city of Lausanne, and to enjoy the new and splendid facitlities in the Biology Building on the University Campus at Dorigny. The Commission is grateful for his thoughtfulness in solving many problems involved in the organization of the scientific program, and for his efforts associated with other preparations and arrangements for our Meeting, which will greatly contribute to facilitating the scientific exchange and otherwise making our Meeting a success. We congratulate Professor Pilet to his new Institute, and we are sure that he and his colleagues in other branches of biology will profit greatly from being able to work under the same roof. Let me conclude these remarks by introducing our gracious host Professor Pilet.

PROBLEMS OF GRAVITATIONAL PHYSIOLOGY AND THEIR SOLUTION IN COSMOS FLIGHTS

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Abstract

In orbital space flights the effect of microgravity, artificial gravity, and combined microgravity and ionizing radiation on the structure and function of various physiological systems as well as processes of growth and development were investigated. Exposure to microgravity didn't cause pathological changes but led to nonspecific and specific variations in tissues, organs and physiological systems. The major stages of animal and plant growth and development proceeded in a normal way. Microgravity did not modify the radiation effect on mammals. Artificial gravity produced a normalizing effect on the animal and plant status.

Introduction

Gravity is one of the most stable and essentially invariable factor of the biological evolution on the Earth. The Invariability of the gravitational field explains the fact why regular investigations in gravitational physiology started only in early 50ies. At that time it became possible to generate short-term weightlessness using ballistic and suborbital rockets. Later, experiments on gravitational physiology were carried out onboard aircraft (in parabolic flights), space vehicles, and on laboratory centrifuges and clinostats.

An important contribution to the investigations of gravitational physiology was made by biosatellites of the Cosmos series. The Cosmos studies constitute part of the research program of space biology and medicine. Their purpose is to study the physiological role of gravity and to clarify the mechanisms of adaptation of living systems to weightlessness which is undoubtedly of great importance for astronautics as well as for general biology and physiology.

General Biology Experiments

Numerous experiments on the biosatellites have convincingly demonstrated that gravity produces no direct effect on unicellular organisms or cultured plant and animal cells (3,7,8). It has been also shown that gravity does not play a significant role in the major developmental stages of plants, insects, fish and birds (7,2,5). The rat embryology experiment on Cosmos-1514 has given evidence that the weightless state does not impede the processes of organogenesis in mammals.

It would, however, be an oversimplified approach if we assume that gravity is an absolutely indifferent factor for intracellular processes, growth and development of various organisms. Firstly, gravity produces an indirect effect on the unicellular organisms via their environment. Secondly, in the case of cells integrated into an organism gravity can exert both an indirect and a direct effect, primarily, on the specialized gravity-sensitive cells and the cells of the tissues that in the course of their evolutionary development have adapted to constant mechanical loads (weight-bearing tissues, cardiovascular system). Thirdly, although it is obvious that weightlessness is not an obstacle for the primary stages of ontogenesis, many problems of growth and development of various species under the conditions of altered gravity remain obscure. These problems include specific features of morphogenesis, stability of ontogenesis, evolutionary adaptation, etc.

The biosatellite studies have contributed not only to the clarification of above problems of gravitational physiology, but also to the elucidation of the effects of weightlessness on the structure and function of the mammalian body. These researches have made it possible to better understand the mechanism of adaptive reactions to the weightless state and thus to generate a more solid scientific foundation for space biology and medicine.

Rat Experiments

Having in view the necessity of studying the effect of weightlessness on various tissues and organs of mammals, we used white rats of the Wistar strain in the flights of our five biosatellites. The reason is that the Wistar rats are very well studied laboratory animals. Besides, our idea was to fly such a number of rats on each biosatellite that would be adequate to get statistically significant samples.

The rat experiments were carried out in specially designed cages that allowed the animals to remain unrestrained. At first the rats were kept in individual small-size cages, i.e., a rat per cage, then in a large cage for a group of ten rats.

The rat experiments demonstrated that during 18-22 days of flight the animals were in good condition: they consumed the flight diet in adequate quantities, their motor activity and body temperature varied within normal limits, and, in addition, they developed a new 6-hour rhythm in response to the food supply 4 times a day (1,5).

Postflight the health state of the rats was also satisfactory. The most distinct changes were seen during the first five days (delayed weight gain, diminished motor activity and physical endurance, modified reactivity of the vestibular system, etc.).

Morphological and biochemical investigations carried out on postflight days 1-2 did not reveal any pathological changes in tissues and organs that can be attributed to the effects of weightlessness. Such changes as hypoplasia of the lymphoid tissues of the thymus, spleen and lympth nodes, as well as the decreased concentration of the somatotrophic hormone in plasma gave evidence that adaptation to weightlessness obeys the patterns of general biology which are typical of prolonged effects of unusual environmental effects, i.e., it is accompanied by a complex of nonspecific changes of the type of a moderately expressed chronic stress. Morphological and biochemical signs of an acute stress were also detected but they were induced by the transition from zero-g to 1 g (1,5,6).

Metabolic studies as well as provocative tests with water and potassium loads of rats revealed weightlessness-induced fluid-electrolyte changes that were very similar to those in man. The changes seen in fluid-electrolyte balance are listed in Table 1.

Table 1

Fluid-Electrolyte metabolism and renal function

- increase in water content of the viscera
- decrease in water content of the skin and the musculo-skeletal system
- decrease in total water content of the body (6.7 %)
- decrease in the extracellular fluid
- retention of Ca, Na, K and water postflight
- decrease in the K pool of the body
- increase in the kidney weight (28%)
- blood congestion in the medullary substance of the kidneys
- decrease in the K concentration in the wet substance of the kidneys.

Morphological examination have shown that one of the possible mechanisms responsible for the change in fluid-electrolyte metabolism is a rearrangement of the functional state of kidneys in weightlessness. This is evidenced, in particular, by a decreased concentration of potassium in the wet substance of various segments of the kidney, blood congestion, an increased number of the functioning capillaries in the medulla of the kidney, and an increased content of water of the cortex and medulla of the kidney.

Examinations of the musculo-skeletal system of the rats showed a correlation between the atrophy of skeletal muscles in weightlessness and the degree of their involvement in the antigravitational function on the Earth. Most distinct atrophic changes were detected in the muscles of the calf, specifically, in the soleus muscle. These changes included a loss of muscle mass, a reduction of the cross-section area of, mainly, slow myofibers, a partial transformation of slow muscles into the fast ones, and a decline of the strength properties of muscles. The basic factor responsible for these changes in weightlessness was the elimination of static load on a large group of muscles that maintain posture and form tonic movements. A certain contribution to the development of the above weightlessness-induced changes in the structure and function was also made by a rearrangement of the neurotrophic regulation and a poorer blood supply of the muscles.

As was the case with the muscle system, the greatest changes in bones were seen in the weight-bearing skeleton segments, i.e., in limb bones and vertebrae. The changes detected in bones are listed in Table 2. These changes were generated not only by an elimination of the static load on the skeleton in the weightless state but also by a rearrangement of the hormonal regulation of the bodily function, viz., a decrease in the plasma concentration of the somatotrophic hormone, a decrease in the production of calcitonin and an increase in the production of parathormone.

Table 2

Limb Bones

- reduction of the rate of periosteal bone-formation,
- decrease of the appositional growth,
- delay in the maturation of osteoids,
- osteoporosis of spongy compartment,
- loosening of the metaphyseal spongiosa,
- reduction in the rate of bone growth in length,
- decrease of the ash content,
- decrease of the calcium content,
- decrease of the mechanical strength.

Artificial gravity of 1 g generated by an onboard centrifuge (Cosmos-936) prevented the development of structural and functional changes in the hemopoietic and endocrine systems, musculo-skeletal apparatus, myocardium and fluid-electrolyte metabolism. This suggests that artificial gravity can in principle be used to maintain the functional state of the human body within normal limits in long-duration space flights.

Inflight exposure of animals to gamma-irradiation in doses 200±25 rad and 800±85 rad from a Cesium-137 source did not change significantly the adverse effect of radiation on the mammalian body in the weightless state. The coefficient of the modifying effect of weightlessness on the radiation sensitivity of most physiological systems was around 1.0 and increased to 1.2 only with respect to some blood morphology parameters.

The rats that for 18-22 days were exposed to weightlessness exhibited essentially complete normalization of most structural-functional parameters characterizing various physiological systems by the 25-27th day of the recovery period. The only exception was the animals that were irradiated at a dose of 800±85 rad: their recovery period proved longer.

Thus, the rat experiments onboard biosatellites of the Cosmos series provided an adequate topography of the changes occurring in various parts of the functional systems that are involved in specific and nonspecific reactions of adaptation of the animal body to space flight factors, primarily to weightlessness. It

should be indicated that the major body of scientific data in the rat studies was obtained during postflight examinations.

Primate Studies

The necessity of enlarging the scope of physiological examinations of animals directly in space flight dictated the studies on primates. The first in the USSR space flight of two nonhuman primates, rhesus-monkeys (Macaca mulatta), was performed in December 1983; it was a five-day flight of the biosatellite Cosmos-1514.

The major goal of this study was to investigate the pattern of adaptation of the vestibular apparatus and the cardiovascular system to weightlessness at an early stage. Other experiments, the study of the motor function, higher nervous activity, biorhythms, etc., were of secondary importance (4).

The vestibular and motor examinations were carried out on the primate Abrek, aged 3 yrs 7 mo, weighing 3.5 kg. The cardiovascular studies were performed on the primate Bion, aged 5 yrs, weighing 4.64 kg. The physiological parameters of both primates recorded in flight are listed in Table 3.

Table 3

Physiological Parameters of Primates Recorded Inflight

Parameter	Abrek	Bion
EEG of the sensorimotor area of the cortex	+	
Neurogram of the vestibular nuclei	+	
EOG	+	
Mechanogram of head movements	+	
EMG of femoral muscles	+	
Total motor activity	+	+
Core body temperature	+	+
Skin temperature	+	+
ECG	+	+
Rheoplethysmogram	+	+
Linear flow velocity in the common carotid artery	+	+
Pressure in the common carotid artery	+	+

The health status of the animals in flight was monitored with respect to such parameters as heart rate, body temperature, food consumption, juice consumption, time spent to perform test-programs, as well as TV-data and performance of life-support systems.

Onboard the biosatellite each primate was in moulded chair supplied with a restraint system and was housed in a BIOS-Primate capsule. The capsule was equipped with an air heater, light source, food and juice nozzles, waste collector, light indicator on a panel, sticks of arm and leg actographs. etc. In flight both primates could see each other.

During the first two days both primates were drowsy and inert, in particular Bion; they made no sharp movements with the head or the body; their faces, especially the lips and the neck, were enlarged. By flight day 3 the behaviour of Abrek became normal; he began to turn his head and showed interest in the surrounding objects; the edematous enlargement decreased significantly. The behaviour of Bion also improved but became normal only by the end of flight day 4.

During the 5-day flight Abrek consumed 396.0 g of the paste-like diet and 616 ml of the juice which was about 2.5 times less than he normally consumed on the ground within the same time period. Bion consumed 319.0 g of the diet and 200 ml of the juice which was 4 times less that he typically consumed on the ground within the same time interval.

During two preflight days and five days of flight body weight losses of Abrek and Bion were 7.4% and 8.6%, respectively. This was associated with body dehydration: at R+1 or 2 the circulating blood and plasma volume decreased by 27% on the average; the extracellular and interstitial fluid diminished by 33%, and venous hematokrit increased by 9% (Korolkov, Lobachik).

Preflight Abrek was trained to perform complex instrumental motor reactions used to measure the function of the semicircular canals (Program No. 1), motor system (Program No. 2), and higher nervous activity (Programs Nos. 1, 2).

Analysis of Program No. 1 data (head turns and arm manipulations) showed that throughout the flight Abrek worked without errors and was given juice as a reward. The time of the program completion was within normal limits, i.e., no more than 25 min. As to the gaze fixation reaction, Kozlovskaya et al. found a decrease in the movement amplitude on flight days 1-2 and a subsequent increase on flight days 3-4. The velocity of head movements towards the target remained diminished till the end of the flight. Simultaneously, beginning with flight day 1 the velocity ratio of the eye counterrolling and head turns increased drastically. All these changes in the gaze fixation reaction indicated that in the weightless state the excitation of the semicircular canals increased substantially. Other data concerning the vestibular function of the primates in weightlessness are being processed.

Throughout the flight Abrek performed Program No. 2 that included stereotyped leg movements. There were however sessions when Abrek did not respond to the signals and did not perform the test. The number of correct tests, i.e., the tests that were rewarded with juice, also decreased.

On the basis of analysis of the myograms and kinematic parameters of leg movements, Kozlovskaya et al. identified three types of motor changes. The first type developed almost immediately after insertion into orbit and was due to a muscle tone decline as a result of which the magnitude of muscle effort decreased drastically. The second type developed on flight day 2 and was caused by the compensation of muscle effort deficiency due to the involvement of a large number of contracting myofibers. The third type chan-

ges developed on flight days 4-5 and can be considered atactic, i.e., associated with a disorder in the stable function of central mechanisms of regulation due to changes in proprioceptive afferentation.

It should be noted that after the 5-day orbital flight the circumference of upper and lower extremities of the primates diminished. The decrease of the circumference of the extremities involved in Program No. 1 (the left arm pushing the stick of an actograph) and Program No. 2 (the left leg pushing the stick of an actograph) was less than in the nonworking extremities (Korolkov).

The cardiovascular studies revealed no significant changes in the ECG amplitude-interval parameters of both primates or pathological changes in the heart in flight (Melnichenko, Badakva). Heart rate variations in flight were distinctly periodic: at night and early in the morning heart rate decreased. The cardiac output variations of Bion were induced by changes in heart rate and stroke volume that were similar in sign (Badakva). Total peripheral resistance in flight was lower than in preflight tests (Fig. 1).

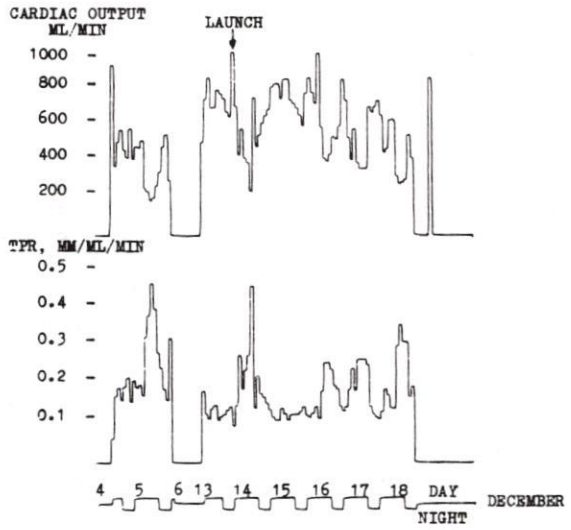

Fig. 1. Cardiac output and total peripheral resistance (TPR) of Bion in weightlessness.

The data on the changes in flow velocity and pressure are being processed and analyzed.

Body temperature of both primates varied within 36-38° C with a maximum in the daytime and a minimum at night. The average inflight body temperature of animals was 0.5° C lower than in ground experiments (Klimovitsky, Alpatov). (Fig. 2)

The primate examination at the recovery site showed that both animals were in good shape, they were active and adequately reacted to the people around them. They displayed no traumatic lesions. Arterial pressure in Abrek and Bion was 155/50 and 105/50 mm Hg, respectively.

Postflight Abrek was exposed to a complete range of physiological examinations. Bion died 69 hrs after touchdown as a result of volvulus of part of the ileum. The biomaterial was taken under histological examinations.

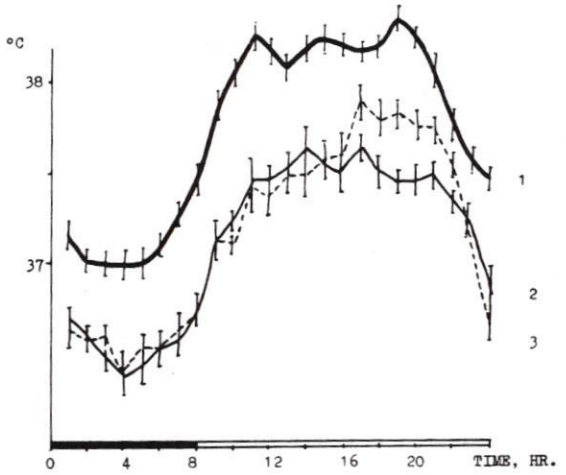

Fig. 2. Body temperature of primates in weightlessness:
1 - Ground experiments
2 - Abrek
3 - Bion

Postmortem histological examinations carried out by Savina et al. demonstrated that 5-day exposure to weightlessness did not produce any atrophic changes in leg muscles, inhibition of erythropoiesis, increased red blood cell decomposition, distinct thymus involution or significant delipoidization of the adrenals.

Having in view the data from the primate studies on Cosmos-1514, it can be concluded that rhesus-monkeys are an adequate experimental model for a study of physiological mechanisms of adaptation to weightlessness.

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SPACELAB AND THE U.S. BIOMEDICAL SPACE RESEARCH PROGRAM

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It can be argued that the U.S. biomedical research program in space which began in the early 1960s has finally come of age in the 1980s with the introduction of Spacelab. At the outset, I should concede that my orientation is that of an academic researcher who sees the space environment as an opportunity to elucidate some of the fundamental physiological problems in man and other living creatures. Understandably, NASA's approach over the last 20 years has concentrated on operational requirements, that is activities designed to promote the safety, well-being, and productivity of man in space. Naturally this remains an important objective but with the introduction of Spacelab the research horizons can be widened and it should be possible to attract outstanding scientists from the academic community to take advantage of this new and unique physiological environment.

In spite of the operational flavor of most of the work in the U.S. biomedical program to date, a number of features of physiological interest have emerged. While the earliest measurements in the Mercury program, for example, were limited to simple physiological monitoring such as ECG, the measurements increased in sophistication through the Gemini, Apollo, Skylab and Apollo-Soyuz series. In addition, a number of important observations have been made on unmanned missions. As a result of measurements to date in the biomedical field, several areas of concern have been identified. These include the cardiovascular system with the evidence of in-flight deconditioning and subsequent orthostatic intolerance, vestibular system with the high incidence of space sickness which is particularly important in short missions such as those characteristic of the space shuttle, bone and mineral metabolism with the evidence that decalcification continues unabated for long periods in weightlessness, impairment of muscle function as indicated by loss of strength and histological changes, blood alterations including a reduction in blood volume and red cell mass, and renal and endocrine changes related to alterations in the distribution and volume of body fluids.

With the introduction of Spacelab, there are now opportunities for much more sophisticated experiments which can go beyond the immediate operational needs of promoting the well-being and productivity of man in space. A feature of Spacelab is the tremendous versatility of configurations including the pressurized core and experimental modules, and the unpressurized pallets. It should be emphasized that Spacelab was designed for a variety of scientific disciplines of which life sciences is only one. However, the pressurized modules which provide an orbiting shirt sleeves environment with extensive racks of laboratory equipment and comfortable experimental conditions provide a breakthrough for biomedical experiments.

Just as important as the introduction of new hardware is the extensive utilization of astronaut scientists including mission specialists and payload specialists. These are trained to be intimately familiar with the experimental payload and function essentially as co-investigators who can deal with unexpected contingencies, equipment repair, and the other surprises that always occur in laboratory work. The close links between these orbiting scientists and the principal investigators on the ground via the Payload Operations Control Center and the Science Monitoring Area provide a tremendous potential for working through difficult experiments which never go exactly as planned. Indeed, one of the most notable features of Spacelab 1 was the extent to which the mission and payload specialists were able to undertake equipment repair even when this had not been planned for.

Spacelab 1 was launched on November 28, 1983 and returned on December 8. Although in some respects this was a proving flight, it turned out to be remarkably productive. In all, about 70 investigations were carried out using 38 experimental facilities, and the investigators came from 11 European countries, Japan, Canada and the United States. About half the payload was selected by the European Space Agency, ESA, and half by NASA. This joint use of the first Spacelab reflected the fabrication of Spacelab in Europe. The total payload weight was about 3,000 kg.

The scientific disciplines on Spacelab 1 included astronomy and solar physics, space plasma physics, atmospheric physics and earth observation, materials sciences, and life sciences. The European life science experiments will be described in a report by Dr. Heinz Oser in this symposium, and many of the U.S. life science projects will be covered by individual papers so I will not go into details. Suffice to say that the U.S. program included studies on the effects of prolonged weightlessness on the humoral response in man, influence of space flight on erythrokinetics in man, nutation of Helianthus (sunflower) in a microgravity environment, circadian rhythm during spaceflight in Neurospora, radiation environment mapping, vestibular responses to zero gravity, and vestibulo-spinal reflex mechanisms. Preliminary reports of all these experiments together with those devoted to the physical sciences were published in Science 225: 163-234, 1984.

One of the most striking findings from the Spacelab 1 life sciences experiments was caloric nystagmus during weightlessness. Because it has often been argued that this type of nystagmus is due to convective movements (which are abolished by weightlessness) this result came as a surprise and the implications are not yet clear. Another interesting observation was the low venous pressure measured in an arm vein on mission days 0, 2 and 7. This was unexpected because of the evidence for the cephalad movement of body fluid in early weightlessness but it may be that the first measurement which was made 22 hours into the mission was too late to catch the increase in pressure. Another possibility is that arm venous pressure does not accurately reflect central venous pressure though it is difficult to see how the former could be lower. Other interesting findings included poorer mass discrimination by crew when asked to judge the mass of 3 cm diameter balls of mass 50 to 64 grams, reduced activation of lymphocytes exposed to the mitogen concanavalin A, circumnutation in sunflowers at zero g, and altered circadian rhythm in Neurospora.

Spacelab 2 will be solely devoted to the physical sciences. Spacelab 3 which will actually fly before Spacelab 2 is again a mixed discipline mission with some life science experiments. An important emphasis will be on testing sophisticated measurement systems and the projects will include studies of the research animal holding facility (RAHF), an extensive biotelemetry system, the dynamic environment measurement system, and a urine monitoring system. Some of these elaborate facilities will be used extensively on Spacelab 4. Another experiment on Spacelab 3 is an investigation of operant conditioning (biofeedback) in an attempt to reduce the incidence of space sickness.

The most ambitious Spacelab mission from the physiological point of view in the near future is Spacelab 4 which is dedicated to life sciences. It will carry a whole series of experiments under the headings of vestibular physiology, cardiovascular/pulmonary physiology, renal/endocrine physiology, blood physiology, bone physiology, muscle physiology, and gravitational biology. Table 1 lists the experiments which are planned, and these presumably reflect the perception of NASA and its advisors on the most important areas in biomedical space research at the present time. Present indications are that this mission will be divided into two flights, SLS-1 and SLS-2, the first to be launched in January 1986. If this missions fulfills its scientific expectations, it should certainly be a turning point in the U.S. space life sciences program.

Just as important as the introduction of the new hardware and the increased participation of scientist astronauts, is the increasing involvement of the academic community in life science space research. Until now, much of the research has necessarily been carried out by NASA laboratories with limited involvement of the universities, and many of the findings have been published in technical reports which are not easy to find. By contrast, we now see a move to the open literature with the quality control that is characteristic of top class peer review journals. This brings space physiology into line with other areas of environmental physiology.

We can also hope that the increasing involvement of the academic community will result in increased pressure for rigorous peer review of projects for flights where only a fraction of submitted proposals can be accommodated. The United States has developed a peer review system in the National Institutes of Health which is the envy of many other countries, but it has yet to be fully adopted by NASA where the perceived importance of the projects to the Agency, that is the operational needs, have often dominated the selection. But changes are occurring rapidly and we can hope that the space environment will soon take its place along with other areas of environmental physiology as a means of elucidating basic physiological processes in man and other living creatures.

Table 1-Experiments Planned for Spacelab 4

Vestibular

- Human vestibular function and adaptation to microgravity.
- Morphologic changes in rat otoliths.

Cardiovascular/Pulmonary

- Cardiovascular changes in weightlessness measured by echocardiography, blood pressure measurement and other studies.
- In-flight study of human cardiovascular deconditioning measured by a rebreathing technique.
- Carotid sinus baroreceptor reflexes in man studied using a pressurized neck cuff.
- Effect of weightlessness on human pulmonary function using a battery of single breath and multibreath and rebreathing tests.
- Microvascular changes in rats studied via a chronic skin flap technique.
- Cardiovascular adaptations to weightlessness in rats by means of indwelling catheters and flow meters.

Renal/Endocrine

- Analysis of blood and urine samples in astronauts to obtain information on fluid, electrolyte, renal and circulatory status.
- Fluid and electrolyte changes in squirrel monkeys by means of urine collection and implanted arterial/venous catheters.

Blood

- Radioisotope studies of human red cells to study reduction in red cell mass.
- Pre and post-flight hematologic measurements in rats.
- Erythropoiesis in rats.
- Effects of stress and weightlessness on lymphocyte activation and function.

Bone

- Double isotope technique to measure calcium metabolism in man.
- Calcium metabolism during weightlessness in rats.

Muscle

- Protein metabolism in man studied by labelled glycine.
- Functional capacity of rat skeletal muscle determined by pre- and post-flight exercise tests and tissue analysis.
- Morphological, biochemical and histochemical changes in rat muscle.
- Role of myosin isoenzymes in the alteration of muscles in rats.

Gravitational Biology

- Gravitropic bending of oat seedlings exposed to a transverse gravity field.
- "Twisting" response of wheat seedlings in plants simulated by blue light.
- Effects of weightlessness on the symmetry of developing embryos studied using amphibian eggs fertilized in orbit.
- Effects of space flight on circadian rhythms and body temperature regulation of squirrel monkeys.

ON THE RESEARCH ACTIVITIES IN THE FIELDS OF GRAVITATIONAL PHYSIOLOGY AND THE SPACE LIFE SCIENCES PROGRAM IN JAPAN

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Gravity, as the oldest and steady environmental factor, has covered all the processes of development, growth, activity, behavior and evolution of terrestrial life. Since the 1950's, 1st decade of space age, provocated and supported by the finding on space life science (SLS) field, the sciences on biodynamics was recognized to be a more important field than before. In this review paper, the interaction between gravitational physiology (GP) and SLS activities will be discussed, introducing the topics in the active programs in Japan during the recent years. 1. ON THE GP TOPICS IN THE SPACE RESEARCH ACTIVITIES OF THE FOLLOWING ORGANIZATIONS OR THE PROJECTS. 1). Japan Society of Aerospace and Environmental Medicine. 2). Japan Rocket Society. 3). Japan Microgravity Utilization Society. 4). Space Development Committee, the Federations of Economic Organizations. 5). Space Shuttle Utilization Program (NASDA) and Japanese Rocket Utilization Program (SAC). 6). Space Station Program (SAC). 7). Symposium, Study Group etc.(ISAS). 2. ON THE ACTIVITIES OF THE PROPER GP SCIENCE, THAT IS NOT NECESSARILY RELATED DIRECTLY TO THE SPACE PROJECTS. 1). The activity of research team on gravitational physiology, supported by the Ministry of Education. 2). Others. In conclusion, the systematic knowledge of the GP seems to make essential base for many fields of physiology. For the abnormal gravity of extra-terrestrial environment,space exploration stimulate the physiological scientist to make them awake to the biological significance of the terrestrial gravity. Territory of the GP is far reaching, so that, we have fortunated to have the plenty of colleagues. The international cooperation in wider fields is expected to be successful.

INTRODUCTION

Origins of scientific knowledges,on the feilds of gravitation,is considerably old, which is able to trace back to Archimedes, Leonardo Da Vinci, Galileo, Newton et al, and they involve the sprouts of many physiological findings. As the main topics of gravitational physiology, that of medical physiology on the various types of acceleration, passive motion by surface vihecles, airplanes or parachute etc. were enumerated, but as the another important targets for scientific approach, gravitational factors in the biological body, such as body weight, size and shape, or load factors or stimulating factors, such as exercise, muscle work,nutural buoyancy in water or lifting in air, and physical training procedure were also drawn the attention. Since the 1950's, 1st decade of space age, physiologists paid more attentions to the basic significances of gravitational problems in the physiological sciences than before. Because biogravitational problem was proved asa main biological factor in outer space life. Low gravity or microgravity, 0 gravity negative gravity were given another new significances over in geo- or normo-gravity. Far from it, it successfully provocates the general physiologist to awake a new field for their research work. This is in turn one of the important benefit of space life sciences for the physiological sciences. Supported by the findings on space life sciences fields, achieved in the real outer space projects and in the various simulating procedures of them, the sciences on BIODYNAMICS were recognized to be a more important fields than before. In this review paper, the interaction between gravitational physiology and space life sciences activities will be discussed, introducing the up to date topics in the active program in Japan during present decade.

1. ON THE TOPICS IN THE ACTIVITIES OF THE FOLLWOING ORGANIZATIONS OR THE PROJECTS. 1) Japan Society of Aerospace and Environmental Medicine. Japan Aviation Medicine and Psychology Society,that was found in an old table on the Systems of Aerospace Medical Research in Japan in 1961,[5] developed to Japan Society of Aerospace and Environmental Medicine now. They have had many committees for the promotions of space biology since 1963. They are chronologically, Space Medicine and Psychology Promotion Cottee, Biosatellite Cottee, Bioastrnautics Cottee, and Space Rescue Cottee. The last 2 committees'activities hold a close relations with the cottees in the national space program, that were started in action since around of 1975 under such working bodies as Science and Technology Agency (STS) or National Space Development Agency of Japan (NASDA). So that, such committees in the society were dispersed. They are serving for the activities of such national cottees. Last July, Aviation Medical Center was estabilished as a pilot's check facility, independently from the Company. Stuffs of this society contributed on this achievement. This society, as only one academic society in this field in Japan, is also contributing to the promotion of the gravitational and accelerational research activity in the physiological sciences or medicine through the scientific or enlightening meetings.

In 1965, 23rd IUPS meeting was held in Tokyo, this society held the meeting with environmental physiologists and space physiologists in the participants of the congress. The good communications of many gravitational physiologists were attained combined with the satellite symposium on environmental physiology. Main scientists in the gravitational physiology, Profs. Parin, Margaria, Gauer, Karlson, who were the main staffs at the establishment of this commission (IUPS gravitational commission) and other younger scientists, who are now in the center of the activity of this fields, were found at the meeting. 2) Japan Rocket Society. This society were estabilished following the starting of rocket development in Japan, and a member of the international Astronautical Federation (that was estabilished 1955). The International Symposium Space Technology and Sciences-Tokyo, that were held by this society in every other year, have been a precious international activity in Japan, including the biomedical session from the 3rd meeting, for the 1st time. At this meeting in 1961 , Prof. von Beckh served as

a chairman, and the session developed successfully. Last May - June, we had the 14th ISTS-Tokyo meeting and Dr. Bucchanan (NASA) et al, main staffs of USA space medical scientist attended the meeting, Dr. Bucchanan is expected as invited speaker at the coming meeting of JSASEM in Tokyo. Such efforts to keep the relationship with international organizations are one of the characteristics of these societies. 3). Japan Microgravity Utilization Society (1983 established). Spring of last year, it was established , and from this year, membership registration is started. This is one of the society, that is supported by national scientists and physiologists, who are active in the field of government level space activity. It seems to be more applicational than ELGRA. Good cooperations between government and non-government facilities will be expectable. 4). Space Activity Promotion Council, The Federation of Economic Organization, (developed in 1968 from special cottee for peaceful utilization of space that was estabilshed 1961). In this mighty group, with a back up of the industrial world, especially, recently, following the promotion of international activity, their activity on the introduction and study of international information source are increasing. 5) Space Shuttle Utilization Program (NASDA) and Japanese Rocket Utilization Program (SAC). Figure 1 shows the space-related organizations in Japan, where is found the parts of gravitational physiology concern. In the Space Shuttle Utilization Program (NASDA), life sciences project is going to be made following the material processing program. On a tentative plan of the 1st material processing test, Japan life science payload, consisiting on one double rack, will be launched in JFY 1985, desirable. The results of the 1st selection of life sciences experiments shows number of items, after announcement of opportunity released from every source. And for the life sciences, 17 items were selected from 27 colleted items. Totally 62 items were selected from 103. In the conclusion of final selection (the 2nd selection) by Space Shuttle Utilization Committee of NASDA, the items were reduced 12 items through the results of terrestrial pre-experimetns (Table 2). Table 1 is that in 1971. In parallel with these payload development, Japan plans to have own payload specialists.[2] Such announcemnet opportunity procedure came down from December 1, 1983 to Jannuary 31, 1984. 554 applicants were registered through the 1st paper test, at April 4, 1984. The evaluation of the 2nd test, that is general medical test and psychological test, is just going to finish at the end of this month. 15-16 subjects are expected to be selected in this time. In future, test on some special function will be perfomerd as the 3rd examination. The test items for this examination will be the next[3] items. 1) Treadmill and ergometer test. 2) LBNP test. 3) Spin loading test. 4) Straight lineal acceleration test. 5) Continuously recorded ECG test for 24 hours. These examinations are continuously scheduled to be completed finally before the end of March, 1985 with the 7-8 subjects as the selected candidate. September of 1985 they are going to remove to America (NASA) and will be given training & will be tested for the final examination. Finally 3-4 candidates will be selected as payload specialists. Anyway, those schedule were postponed about 1.5 years mainly by the economical reason.

In the Japanese Rocket Utilization Program (SAC), the experiment is performed as complement and study during or after space shuttle project. As a way of approach to FMT. The rocket #8 was launched in summer of 1979 in Japan at Tanegashima Space Center of NASDA and recovery system was performed successfully and technique seems to be most important for Life Science Program, too. After such microgravity flight, series of flight using T-500A is planing, and after space lab test, we have an intention for the time being, to perform 2nd and 3rd projects. For the completion of the program, the co-operation between the Japanese Life Science Committees and NASDA et al, seems to be most important for the sound and effective development of the science. 6). Space Station Program (SAC, Special Panel). This was composed at 1982, fall. This special pannel,[1] was started to prepare for the cooperation with NASA, together with Canada and European Space Agencies. Last month, SAC of JAPAN prepared the contract note for NASA as the 1st step on this problem. They include the projects theme on many fields, one of them is the life science projects. As shown in Table 3, they consist of 4 theme, include the contents acquired from 38 proposed theme. The activity of this pannel is now accelerated by the progress of Space Shuttle Utilization Program and the situation of international cooperation. 7). Symposium & Study Groups (SAC). For instance, last year, Japan Society on Biophysics made a symposium on Space Life Sciences at Gife University as a part of the Annual meeting of the society. During 2-3 years, a few meetings, were held by Japan Society for Aviation and Astronautics, also, ISAS having some study group on the life sciences for space station. They include some biogravitational problems.

2. ON THE ACTIVITIES OF THE PROPER GRAVITATIONAL PHYSIOLOGICAL SCIENCE, THAT IS NOT NECESSARILY RELATED DIRECTLY TO THE SPACE PROJECT. There are the activities of research team of gravitational physiology,[4] supported by Ministriy of Education, and recognized by National Committee for physiological Sciences, Science Council of Japan. This is the first authorized group activity named "Gravitational Physiology" in Japan. The targets of their research are not limited on microgravity, but include the problems of all quantities and types of gravity. The activities of this group was partly reported in the International Union of Physiological Sciences, Commission on Gravitational Physiology and that of International Union of Physiological Sciences, Commission on Gravitational Physiology was refered for the considerations of the research work of Japanese group. Originally, the National Committee for physiological sciences, Science Council of Japan has been affiriating to IUPS, and also has been incorporated with the physiological scientific societies in Japan, Therefore, since 1973 International Commission on Gravitational Physiology was composed in IUPS, this situation aroused the interests of Japan Physiological Society to gravitational Physiology. A research team on gravitational physiology, supported by the academic potentiality of the scientists of such above-mentioned societies, was composed in 1977. This team made efforts to have joint meeting with related societies, such auto-nomic nervous system physiology group, or physical fitness group, etc. We have nice cooperation with the scientists in such fields. Table 4 shows the main theme and group scientists of the study group of the graviyational physiology.

SUMMARY

The structure and function of the body, genetic problem, environmental conditions such as aquatic, land and other behavioral characteristics of life on earth have adapted to survive under a terrestirial gravity. The systematic knowledge of the gravitational physiology seems to make es-

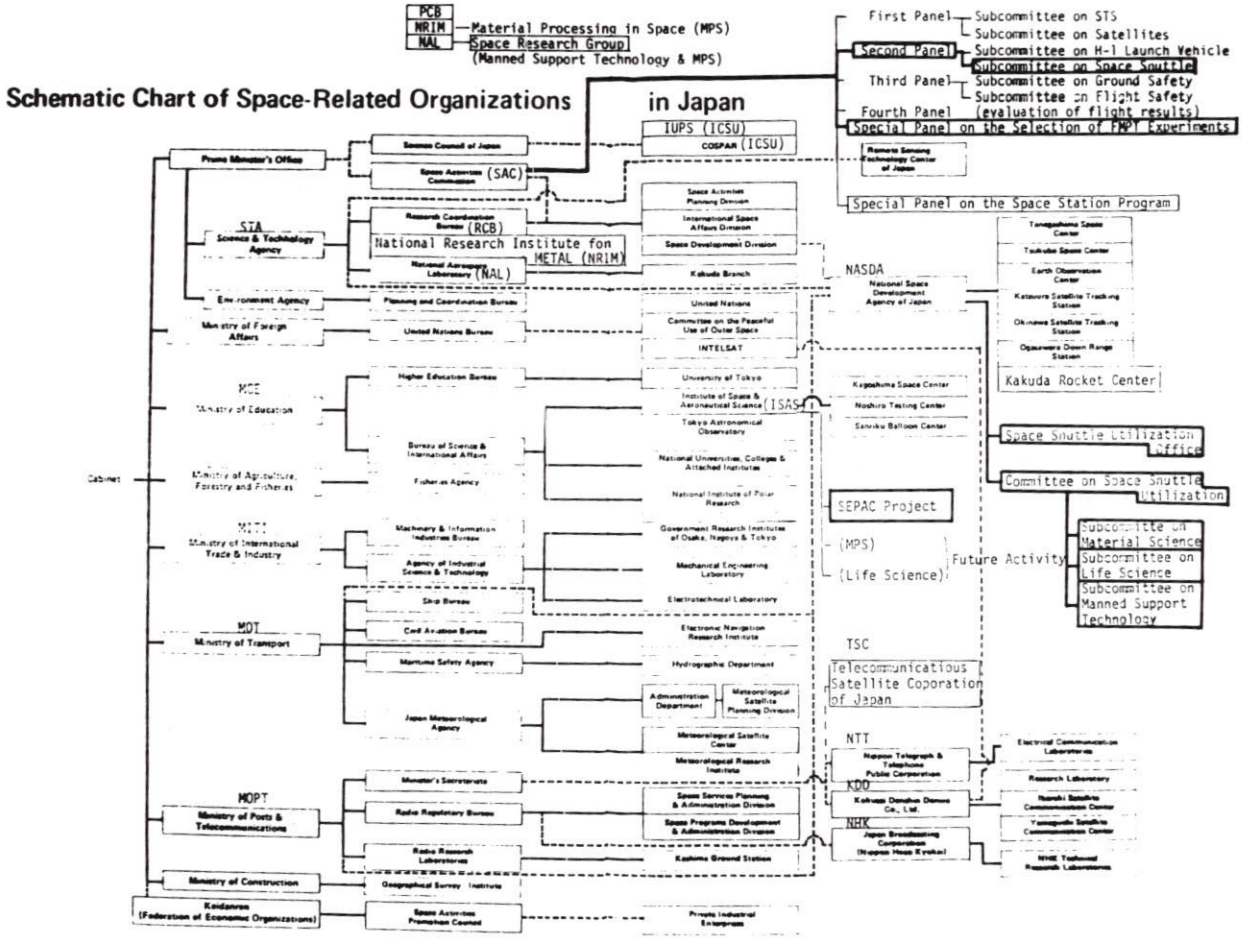

Fig. 1 Schematic Chart of Space-Related Organizations in Japan

Table 1 Bio-medical 11 Theme for Space Researches, Proposed by Working Group of Post-Apollo Program, Space Activity Committee (SAC) JAPAN. (1971)

LS- 1. Behavior control under weightlessness.

LS- 2. Circadian rhythm of human functions under space environment.

LS- 3. Charactaristics of muscle work.

LS- 4. Cardio vascular neurological studies on the adaptation to space environment.

LS- 5. Basal and work metabolism under weightlessness.

LS- 6. Physiological functions under abnormal gravitational environment.

LS- 7. Effects of 0-extreme low gravity to the development and growth of animals.

LS- 8. Interaction of biological body and magnetic fields.

LS- 9. Biological effects of solar ultraviolet radiation on micro organism.

LS-10. Biological effects of 0-gravity and radiation in space to myxomycete.

LS-11. Biological effects of 0-gravity and radiation in space on the cell divisions and growth of plants.

Table 2 Bio-medical 12 Theme, for Space Schuttle Utilization Researches, Selected by Space Schuttle Utilization Cottee (NASDA) JAPAN. (July, 1984)

- Estabilishment of the conditions for the successful separation of bio-substances by electrophoresis under 0-gravity.
- Separation of each cells by free flow electrophoresis.
- Development of enzyme crystalization through the application of 0-gravity.
- Effects of 0-gravity on the ultra microscopic microstructure and the physiological functions of the cells on mamaria.
- Effects of 0-gravity to the genesis and growth of bone and cartilage.
- Biogenetic effects of space radiation and HZE.
- Study on the effects of space radiation to biological body, and development of the counter measure.
- Studies on the adaptation of astronauts and payload specialist during space flight – endocrinological reactions and metabolic changes – .
- Studies on the visual-vestibular control of behavior and locomotion during process of adaptation to 0-gravity.
- Studies on the stability of visual function under space environment.
- Studies on the characteristic of sensori-motor functions under 0-gravity – study on the characteristic of hand work – .

sential base for many fields of physiology. For the abnormal gravity of extra-terrestrial environment, space exploration stimulates and provocates the physiological scientists to make them awake to the biological significance of the terrestrial gravity. Therefore, territory of the gravitational physiology is far reaching, so that, we have fortunated to have the plenty of colleagues. The international cooperation between different organization must be emphasized as most important factor for the progress of these sciences.

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Table 3. Space Station Theme in the Field of Mission on Life Sciences (Proposed by SAC, Sept., 1984)

Space Station Theme in the Field of Mission on Life Sciences
(proposed by SAC, Sept. 1984)

Mission	Performed in 1 ATA Pressurized Part of Space Station	
Life Sciences (4 theme)	Theme	Included Proposals (Sept. 1984)
	Biology	7
	Space Medicine	17
	Eco Life Support Systems	11
	Biotechnology	3

Table 4. Research Items of Research Team on Gravitational Physiology

a.

Research items of research team on gravitational physiology, supported by Ministry of Education, Japan.

I. Environmental adaptation on the field of gravitational physiology

II. Gravitational physiological study on the adaptation of physical fitness

K. Asahina
T. Fukuhara
T. Fukunaga
M. Masuda
G. Mitarai
K. Miyakawa
T. Nagasaka
Y. Ohmura
K. Oohara
H. Saiki (Chairman)
S. Watanabe
K. Yamamoto

b.

I. Environmental adaptation on the field of gravitational physiology

1. Training and heat production mechanism under various gravitational conditions.
   1-1. Effects of gravitational conditions on the physical training of human subjects.
   1-2. Effect of acceleration on the thermal regulation of biological body.
2. Metabolic adaptation during abnormal gravitation exposure.
   2-1. Metabolic adaptation during hypogravics exposure.
   2-2. Heat balance under hypodynamics or rats, and its adaptability.
3. Posture control under abnormal gravity conditions.
   3-1. Posture control of human subjects under hypogravis.
   3-2. Vestibular reflex to changes of gravitational conditions.
4. Neurophysiological mechanism of appetite under abnormal gravitational conditions.
5. Micro-circulation under gravitational stress.
6. Activity of circulatory center under various gravitational conditions.

c.

II. Gravitational physiological study on the adaptation of physical fitness.

1. Approach from the gravitational physiology.
   1-1. Gravity and working capacity - I.
   — On the blood flow in extremities —
   1-2. Gravity and working capacity - II.
   — Effects of training on the circulatory responses for the inclination of body axis —
   1-3. Gravity and working capacity - III
   — Hypodynamics tolerability and physical fitness levels —
   1-4. Reflex and characteristic of voluntary activity during parasympathicotonic conditions.
2. Approach from the thermal regulation physiology.
   2-1. Thermal regulation of rat during hyper G exposure.
   2-1-1. Sexual cycles depending differences of thermal regulatory function during hyper G exposure
   2-1-2. Comparison between hyper G exposure and dosage of capsaicin on the thermal regulatory functions.
   2-2. Heat production and loss under various gravitational exposure.
3. Approach from the respiration and circulation physiology.
   3-1. Central regulation of respiration and circulation under abnormal gravity and adaptability of physical fitness.
   3-2. Gravitational physiological study on the circulatory adaptation.
4. Approach from the posture maintenance reflex physiology.
   4-1. Study on the gravitational factors on the posture regulation of upright position.
   — Regulation during simulated hypodynamics induced by water immersion —
   4-2. Study on the posture balance on the seesaw platform.
   — Posture control on the seesaw platform. Analysis of side wards elements. —
5. Approach from the nutritional physiology.
   5-1. Hypothalamic regulatory mechanism of food intake and autonomic reaction under abnormal gravity.

The European Life Sciences Experiments on the first Spacelab Mission - a Summary Report.

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Abstract:

The first mission of the European built manned space laboratory, Spacelab, had its first mission accomplished on board the U.S. Shuttle Columbia STS-9 flight, Nov.-Dec. 1983.

During the 10 day mission, 9 European and 7 U.S./NASA experiments in the field of life sciences were carried out. A summary account is given below.

1. Introduction

The Spacelab-1 mission (SL-1), a joint venture of the European Space Agency (ESA) and the National Aeronautics and Space Administration (NASA) took place during the period of 28 Nov.- 8 December 1983.

The mission payload was multidisciplinary, and Life Sciences was one amongst 6 different disciplines. Although the primary goal of SL-1 was to test the Spacelab system under orbital conditions (Verification Flight Test) a great number of scientific investigations could be carried out which produced substantial results.

The preliminary results of the Life Sciences experiments have been presented by the investigators already at several symposia and meetings. Most of them have also been published in SCIENCE, July 1984 edition, to which specific reference is made for more detailed information (1).

Although the experiments had been already selected for flight in late 1976, a delay of almost three years after the originally planned launch date had to be taken into account. During the mission, all Life Sciences experiments used appr. 50% of the crew time, underlining the importance of manned space flight for this kind of investigations. In orbit operations were for the first time strongly supported by an intensive crew/ground operations staff/investigators interface, allowing for early and real time data analysis on ground, and - where necessary - subsequent changes to the experiment protocol. This interface had certainly a major share in the overall mission success. The unexpected addition of a tenth mission day contributed to significant data retrieval to the benefit of all disciplines.

Life Sciences experiments which involved the crew as test subjects, started appr. 6 months prior to the mission with baseline data collection, and continued for nearly one week after landing to allow for meaningful interpretation of adaptational changes or direct effects of microgravity. All other Life Sciences experiments ran in parallel ground controls for assessment of and comparison with in flight obtained data; the inflight specimens could be retrieved within hours after landing, for subsequent analysis in the laboratory.

The author attempts to summarise in a short, concise fashion the experiment description and quotes early, partially preliminary results.

2. The European Life Sciences Experiments on SL-1

It would go beyond this publication to describe in full detail all the experiments. Therefore, only a short description is given of the purpose and the major objectives, the methods applied and some remarks about the data evaluation and those results which are believed to be of immediate interest to the reader.

2.1 Three-Dimensional Ballistocardiography in Weightlessness - 1ES028; principal investigator Prof. A. Scano, University of Rome

The experiment was aimed at tri-axial recordings of a ballistocardiogram (BCG) under unique conditions: a test subject free-floating in weightlessness. The tracings obtained were to be compared with tracings recorded on the same subject on the ground, primarily to obtain a 'calibration - BCG under ideal conditions'.

The human body reacts to each heartbeat with little movements caused by the acceleration forces of the blood which is ejected from the heart into the major vessels. BCG is a method which records these periodic motions as a series of waves. BCG's are considered as useful indicators of cardiovascular performance and allowing for biomechanic interpretations.

The test subject wore a dorsal plate fitted with mini-accelerometers and one ECG-lead, all of which were connected to a miniature tape recorder. Recordings were obtained during rest, crouched body positions and after physical exercises.

Preliminary analysis of the pre-, in- and postflight data collected, showed that only the $-G_z$ waves were significantly increased during flight, whereas the G_y-waves produced useful data but were less significantly increased. The G_x-waves could not be interpreted. The BCG displayed age-related and axis-dependent differences.

2.2 Measurement of Central Venous Pressure and Determination of Hormones in Blood Serum during Weightlessness - 1ES026/32; principal investigator: Prof. K. Kirsch, University of Berlin

Due to fluid redistribution from the lower limbs to the upper part of the body (which occurs in the absence of hydrostatic forces, i.e. during weightlessness) it was speculated that the pressure in the low-pressure system might be elevated, and

as a consequence a negative water balance should ensue.

The main goal of this investigation was, therefore, to obtain quantitative data on changes in the distribution of body fluids and associated mechanisms with respect to the balance of water and minerals.

The method employed consisted of a conventional strain gauge, connected with a needle which was put into the anticubital vein. The signals obtained were observed in an oscilloscope, and stored on a tape recorder. After measuring the venous pressure, blood was drawn for subsequent hematocrit and various hormone level determinations.

Inflight data were compared with pre- and postflight data collected shortly before launch and after landing. Preflight data indicated a gradual increase in central and peripheral venous pressure, a decrease in hematocrit, and in general a slight weight gain. The inflight data pointed, however, to a marked decrease in the central venous pressure in all subjects and a slightly elevated hematocrit. The values obtained immediately after landing showed a comparatively high venous pressure, with a decrease in hematocrit despite the negative water balance. Appr. 12 hours after re-adaptation to the 1-G environment the venous pressure reached the lowest value during the whole study. Only within a week after landing all parameters gradually returned to preflight values. The investigators concluded that the fluid shift is a highly dynamic process which takes place within 3 to 6 hours.

2.3 Personal Miniature Electrophysiological Tape Recorder - 1ES030; principal investigator: Dr. H. GREEN, CRC, Harrow

The main goal was to collect physiological data on more representative individuals of the 'general population' than highly trained astronauts. In addition, the equipment used was to demonstrate the suitability of standard commercial equipment for research in space.

A standard OXFORD Medilog Recorder, battery-powered, was worne by the payload specialist, and connected to electrodes recording EOG, ECG, and during the sleep an EEG. Although the hardware experienced some difficulties during the experiment operation (e.g. tape jamming), useful data could be collected on ECG's (some 60 hours continuous recording) and on EEG's, notably on the REM-sleep-phases. Preflight baseline data were compared with inflight data, taking two variables into account: 12 hours time shift and weightlessness. Inflight REM sleep increased at the beginning up to 50%, whereas the normal value was between 20-25% of total sleep time. No pathological functions, however, were recorded during the entire study.

2.4 Effects of Rectinlinear Accelerations, Optokinetic and Caloric Stimulations in Space - 1ES201; principal investigator: Prof. R.D. von Baumgarten, University of Mainz

This experiment comprised a number of subsets of different scientific objectives. In terms of crew operations/time it was the most demanding Life Sciences experiment on SL-1. The study aimed at illucidating the function and response of the complex vestibular system with its associated reflex mechanisms in dependence of gravity induced cues. Exposure to weightlessness was thought to be the appropriate stimulus to obtain meaningful results. The inflight tests were complemented by extensive pre- and postflight baseline data collection.

Measurements included the threshold determination for the perception and direction of linear oscillations; vestibulo-ocular reflexes elicited by angular and linear movements, oculo-motor and posture responses to optokinetic stimulations, and responses to caloric stimulation.

The test subject was secured in a body restraint system (BRS) and fitted with a 'helmet' structure holding the associated test equipment: EOG amplifiers; CCD-camera with infrared illumination in front of one eye, for recording eye movements in the x-y-axis and real time downlink to the ground; a TV-monitor in front of the other eye to provide optokinetic stimuli as well as a target cross; caloric stimulation of both ears by means of heated or cooled air insufflation.

Although the analysis of all test results is not yet completed, some significant findings shall be pointed out here:

- threshold measurements for linear oscillatory motion at 0,3 Hz in the x, y, and z body axes were made such that both detection and direction of motion were recorded. In general, there was an elevation of threshold levels as compared to preflight values. This may indicate that the adaptation from 1G to OG reduces the gain of the sensory system processing signals from vestibular and somesthetic gravi-receptors.

- vestibulo-ocular reflexes (VOR) were studied both with angular and linear acceleration stimuli: the results obtained (and to be confirmed by future experiments) suggest that the adaptation to microgravity might depend (amongst others) on increased dependence, on visual as opposed to vestibular mechanisms, or, that under 1G conditions the otoliths make a significant contribution to the gain of the VOR.

- caloric nystagmus, currently based on the theory that thermal convection of the fluid within the labyrinth is responsible for this reflex mechanism, was to be tested under O-G conditions: on both subjects the presence of caloric nystagmus could be demonstrated inflight which suggests that also other mechanisms than thermal convection must be responsible. For the authors it appears therefore possible that caloric nystagmus on ground is partially or fully caused by the same (yet to be determined) mechanism.

2.5 Mass discrimination during weightlessness - 1ES025, principal investigator: Dr. H. ROSS, University of Sterling

This experiment aimed at the comparison between the perception of mass under O-G conditions and the weight on Earth, concerning the nature of information that man uses to judge weight and mass.

The test apparatus included 24 small weighted balls and a set of record cards. The balls, all of the same shape and size, varied in mass from 50 - 64 g, in 2 g steps. Comparisons (using the same hand) of always two different pairs were marked for the "heavier" one on the record sheet.

Thresholds for mass discrimination under O-G were found to be higher by a factor of about 1.8, as compared with weight discrimination on the ground. This suggests that humans are not as sensitive to inertial mass cues as they are to weight, and that adaptation can only partially compensate for loss of gravity.

2.6 Effects of Weightlessness on Lynphocyte Proliferation - 1ES031; principal investigator: Dr. A. COGOLI, ETH Zürich.

The major objectives of this experiment were to test whether cells in general are sensitive to gravity, and whether functional changes occur, in particular on cells responsible for the immune response while depriving them from G vector cues.

Cultures of human lymphocytes were exposed during O-G to Concanavalin A, a mitogen capable to transform resting T-lymphocytes into activated (dividing) cells; the rate/magnitude of activation was determined by incorporation of tritiated thymidine into DNA. The study aimed also at conclusions on the cell membrane properties.

As the main finding it could be demonstrated that the inflight lymphocytes showed a marked depression of the activation as compared with those of the ground control cultures. In comparison also with previous studies under simulated O-G (clinostate) and hypergravity (centrifuge) there is a support for the hypothesis that microgravity depresses whereas high gravity enhances cell proliferation rates. The effects of gravity are particularly strong in cells undergoing differentiation. Although this experiment was done in vitro, and no extrapolation to changes in vivo can be made, and although the very mechanisms involved in gravitational effects on cells are still unknown, the investigators conclude on the basis of their results the remarkable proof that cells are sensitive to gravity.

2.7 Advanced BIOSTACK experiment - 1ES027; principal investigator: Prof. H. BÜCKER, IAM, DFVLR Cologne

This study aimed at both the determination of the radiobiological importance of cosmic radiation particles of high charge and high energy, as well as evaluating the physical components occured during the SL-1 mission, including dosimetric data for the different shielding environment.

For this purpose, 4 different sets of 'BIOSTACKS' have been mounted into the Spacelab rack, on the Spacelab floor and on the pallet respectively. Monolayers of biological test organisms were sandwiched between thin foils of different types of nuclear track detector material.

Based also on previous spaceflight experiments, it is concluded that the average radiation dose-equivalent (expressed in millisieverts, m Sv, per year), even when hundredfold increased, is not the 'prohibitive' limit for living organisms, including man: It is rather the radiobiological quality of numerically minor components of the cosmic radiation.

The evaluation of the physical and biological components indicate that in general the biological specimens survived this exposure in good condition, and the dosimetric data show a distinct dependence on the shielding environment.

2.8 Microorganisms and Biomolecules in the hard space environment - 1ES029; principal investigator: Dr. G. HORNECK, IAM, DFVLR Cologne.

The major objective was to determine the response of a resistant microbial system to 'space conditions' and to selected components of this hard environment. 316 dry samples of bacillus subtilis spores were exposed to the vacuum and/or to the full ultraviolet spectrum (170 nm) or selected ranges of peak wavelengths of 220, 240, 260 or 280 nm.

Growth disturbances, membrane damages, and structural changes in enzymes and proteines are possible consequences of prolonged exposure to space conditions, for which precise answers were sought.

An exposure tray with four compartments was mounted on the pallet. Two compartments were vented to the outside, the two others hermetically sealed at 1 bar. An optical filtering system allowed for exposure to various wavelengths of solar UV-radiation.

Exposure to the vacuum of space for 10 days reduced the viability counts (colony formers) to about 50 percent, and increased the mutation frequency about tenfold. Similar vacuum-treated samples on the ground did not affect or only slightly inactivate the spores. The response to solar UV-irradiation showed a higher sensitivity in vacuo than at 1 bar. The UV-sensitivity was measured in terms of UV-fluences that reduced survival to 10 percent. Also, DNA cross-linking was induced by solar UV-irradiation, both in vacuo and at 1 bar; the amount of free DNA decreased with increasing UV-fluence. The interpretation of apparent differences in the photobiological and photochemical data between flight and ground simulation experiments, however, will require more statistical analyses.

3. Conclusions

The SL-1 mission was for Life Sciences very important since it provided the science community of a wider range for the first time in Europe with a set of remarkable, and sometimes even unpredictible data. Overall, the mission can be termed as a success.

The importance must be also seen in the light of a learning process, particularly in how to achieve a mature space experiment; the author believes that the time has passed to call this science community 'newcomers' to space. Future experiments and missions already in the planning, indicate that a second generation, more mature in its approach, has opened the round for a broad spectrum of Life Sciences investigations in space, justifying the expenses associated with this research, and always bearing in mind the aim for a better understanding of living matter on the earth.

EXPERIMENTS		GENERAL FIELD	SUB-DISCIPLINE	SPACE RELATED ENVIRONMENT
IES 028	3-D - BALLISTOCARDIOGRAPHY	Human Physiology	Cardiovascular System and Body Fluid Control	ZERO G
IES 026	MEASUREMENT OF CENTRAL VENOUS PRESSURE			
IES 032	SERUM HORMONE LEVEL DETERMINATION			
IES 030	ELECTROPHYSIOLOGICAL TAPE RECORDER		Neurosensory System, Reflex Mechanisms	
IES 201	VESTIBULAR EXPERIMENTS		Psychosensory Function	
IES 025	MASS DISCRIMINATION			
IES 031	LYMPHOCYTE PROLIFERATION		Cell Growth and Development	
IES 027	ADVANCED BIOSTACK	Radiation Biology	Microbial Systems and Biomolecules	- COSMIC RADIATION (HZE PARTICLES) - SOLAR IRRADIATION - SPACE VACUUM
IES 029	EFFECTS OF RADIATION ON BIOLOGICAL SYSTEMS			

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EFFECT OF GRAVITY ON NEURAL OUTFLOW FROM THE CENTRAL RESPIRATORY AND VASOMOTOR CONTROL MECHANISMS IN THE RABBIT

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The alterations in the neural outflow from the central respiratory and vasomotor control mechanisms induced by passive postural changes were quantitatively analyzed by means of power spectrum and pulse-weight correlation. Experiments were performed on vagotomized rabbits with intact carotid sinus nerves, anesthetized, paralyzed and maintained by artificial ventilation. Spontaneous efferent discharges of the renal sympathetic and phrenic nerve activities were recorded simultaneously. Both head-up (30-45°) and head-down (30°) procedures caused significant increases in the end-tidal CO_2 concentration and in the total power of renal sympathetic and phrenic nerve activities and prolongation of the period of the phrenic respiratory discharge. Cardiac rhythm in the renal sympathetic nerve activity was intensified by head-down tilting. Respiratory discharge in the renal sympathetic nerve activity became more regular and shifted from the inspiratory-expiratory phase-spanning phase toward the inspiratory phase during the head-up position.

The influence of the force of gravity on the cardiovascular dynamics has been studied by many investigators. They noticed the important role of the baroceptor reflex mechanisms to compensate and maintain the cardiovascular homeostasis against some changes in the gravity (7, 9, 10, 12, 14, 16). In most of these studies, the conventional parameters such as arterial and venous blood pressure, heart rate and respiratory mechanogram were measured. Efferent phrenic and sympathetic nerve activities are useful for further studies on the central regulatory mechanisms compensating the effect of gravity as more direct indicators of the neural outflow from the central respiratory and cardiovascular control mechanisms (13). Changes in these nerve activities produced by postural changes, however, were scarcely described (15) because of the recording-artefacts accompanied with the tilting procedures. Most of successful nerve recordings during postural changes were analyzed by means of integration or pulse count. These analytical methods revealed the time course of the changes of the overall activity, but did not provide the informations connected with the rhythmic components in the nerve activity. It is well known that peripheral sympathetic nerve activity is modulated in the cardiac and respiratory rhythms (1, 5). The phrenic nerve activity consists of respiratory rhythm and high frequency oscillation of 60-100 Hz reported by Dittler (8). In recent years, power spectrum (4) and correlation analyses were applied for the purpose of the quantitative evaluation of rhythmic components in the nerve activity (3, 6). In the present study, using power spectrum and correlation, we have undertaken detailed quantitative analyses of the effects of postural changes on the followings; cardiac and respiratory rhythms in the renal sympathetic nerve activity, respiratory rhythm and high frequency oscillation in the phrenic nerve activity, and the phase relation between respiratory volleys in both the nerve activities (2, 11).

Methods

Experiments were performed on rabbits weighing between 2.2 and 3.6 kg. The animals were immobilized with gallamine triethiodide, anesthetized with ether and maintained by artificial ventilation with monitoring of end-tidal CO_2 and O_2 levels. Tidal volume of artificial ventilation was regulated in order to maintain the end-tidal CO_2 level at around 4%. Vagus, depressor and cervical sympathetic nerves were cut in the neck bilaterally, while the carotid sinus nerves were remained intact. Arterial blood pressure in femoral artery and ECG were recorded by conventional techniques. Electrical recording-artefacts accompanied with the experimental postural changes were possibly minimized using implanted bipolar platinum electrodes insulated with silicon rubber. Spontaneous efferent discharges were simultaneously recorded from the central cut end of the right phrenic and renal sympathetic nerves. The animals were placed in the supine position on a tilting table and were tilted for 5 to 10 min to the head-up position (at 30° or 45°) or the head-down position (at 30°). Electrical activity of both the nerves was stored on magnetic tapes for subsequent data processing using a data recorder (TEAC, R-81) together with arterial blood pressure, ECG and CO_2 concentration in expired air. For a quantitative evaluation of alteration in the nerve activities produced by postural changes, power spectral and pulse-weight correlation analyses were performed by means of a computer (NEC San-ei, 7T-08).

Results

Humoral and cardiovascular effects of postural changes (Table I)

Tilting to the head-up position at 30° caused an increase in CO_2 concentration and a decrease in O_2 concentration in expired air. Systolic and diastolic blood pressure fell gradually accompanying a decrease in the pulse pressure during the head-up position. A slight increase in the heart rate was caused by head-up tilting. Tilting to the head-down position at 30° caused a more pronounced increase in the end-tidal CO_2 level than head-up tilting. The mean arterial blood pressure slightly elevated by head-down tilting. No appreciable change in the pulse pressure and heart rate was observed during the head-down position.

Changes in the neurogram of the renal and phrenic nerve activities

Typical recordings of the renal sympathetic and phrenic nerve activities were shown in Fig. 1. In the phrenic nerve activity, regular volley discharges exhibited the central respiratory rhythm different from the cycle of artificial ventilation. In the renal sympathetic nerve activity, slow rhythmic burst discharge synchronized with phrenic respiratory volley, though it was more irregular than that in the phrenic nerve activity. Both head-up and head-down tilting procedures induced a prolongation of the phrenic respiratory period and an increase in amplitude of the phrenic and renal nerve activities. The respiratory volley in the renal sympathetic nerve activity became more regular during the head-up tilting-period.

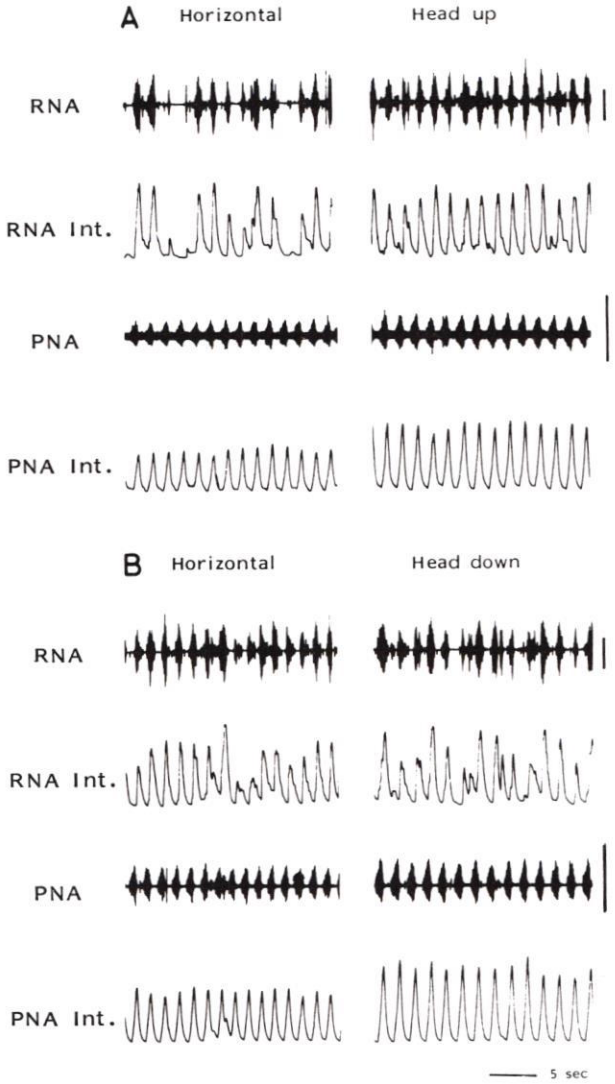

Figure 1. Effects of postural changes on the renal sympathetic and phrenic nerve activities. A: Head up, 3 min after head-up at 30°. B: Head down, 3 min after head-down at 30°. RNA, renal sympathetic nerve activity; RNA Int, leaky-integrated neurogram of renal sympathetic nerve activity; PNA, phrenic nerve activity; PNA Int, leaky-integrated neurogram of renal sympathetic nerve activity. Horizontal calibration is 5 sec. Vertical calibrations for nerve activities are 100 uV.

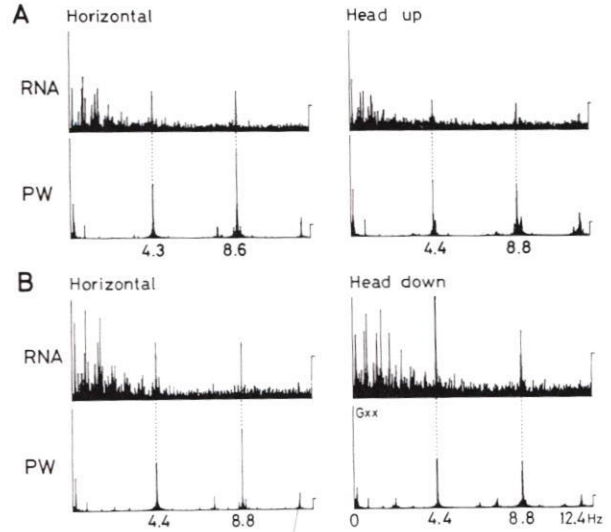

Figure 2. Alteration in cardiac-related activity produced by tilting to the head-up position. A: Horizontal position (left) and 3 min after head-up at 30° (right). B: Horizontal (left) and 3 min after head-down (right) position (30°). RNA: Power spectra of renal sympathetic nerve activity. PW: Power spectra of arterial pulse wave. Ordinate represents power. Abscissa is frequency. Resolution is 0.0244 Hz. Corresponding peaks are indicated by dotted lines.

Power spectral analysis of renal sympathetic nerve activity

Figure 2 shows the power spectra of the renal sympathetic nerve activity and arterial pulse-waves filtered with a low pass of 20 Hz. In the power spectrum of the renal sympathetic nerve activity, many peaks were observed. One of these peaks had a frequency corresponded to the rate of phrenic respiratory volley, and two peaks at 4.3 -4.4 and 8.6-8.8 Hz coincided with the two peaks in the power spectra of arterial pulse waves respectively. These two peaks in the power spectra of the renal sympathetic nerve activity were termed cardiac-related peaks. The total power of the renal sympathetic nerve activity was increased by both tilting procedures. The power value of the band-width of the cardiac-related peaks was markedly increased by head-down tilting but was unchanged or slightly decreased by head-up tilting (Fig. 2 and Table I). The cardiac-related peaks shifted slightly toward the higher frequency side in the power spectrum during the head-up tilting-period.

Power spectrum of phrenic nerve activity

In the power spectrum of the phrenic nerve activity, the total power of 0-1,000 Hz increased during head-up and head-down tilting-period (Table I). The peak with a frequency corresponding to the rate of respiratory volley was shifted toward the lower frequency side by both the tilting procedures. Figure 3 shows the power spectra of phrenic nerve activity filtered with a band pass of 50-1,000 Hz. A sharp peak was found near 100 Hz in the power spectrum. This peak indicates the existence of the high frequency oscillation in the phrenic nerve activity. This peak rose during the period either head-up or head-down tilting-priod (Fig. 3).

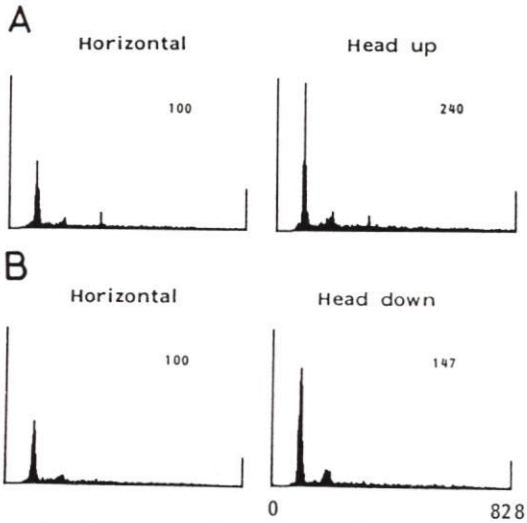

Figure 3. Power spectra of phrenic nerve activity filtered with a band pass of 50-1,000 Hz. A: 3 min after tilting to the head-up position (30°). B: 3 min after tilting to the head-down position (30°). Figure in the upper right of each power spectrum indicates the total power refferring to the control value as 100. Resolution is 3.26 Hz.

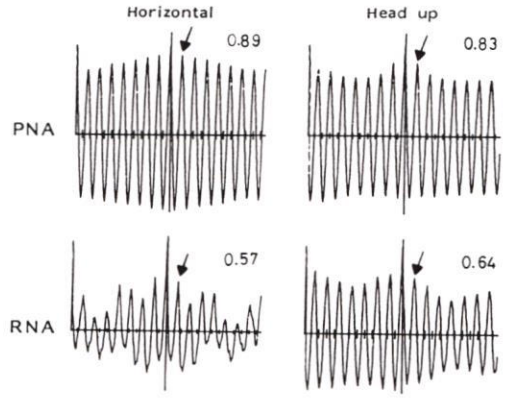

Figure 4. Autocorrelograms of the phrenic and renal sympathetic nerve activities. 3 min after head-up at 30° (right). PNA: Autocorrelogram of phrenic nerve activity. RNA: Autocorrelogram of renal sympathetic nerve activity. Ordinate of the correlogram is autocorrelation function. Abscissa is lag time (900 msec/division). Address bin is 90 msec. Arrow indicates the first peak at which the autocorrelation coefficient was calculated. Figure in the upper right of each correlogram represents the value of autocorrelation coefficient.

Regularity of the respiratory rhythm in the renal sympathetic and phrenic nerve activities

The regularity and period of the respiratory rhythm in the phrenic and renal nerve activities were quantitatiyely evaluated in the autocorrelogram of nerve activity. In the autocorrelogram of the phrenic nerve activity, the interval between the ordinate (lag time: 0) and the first pericentral peak in the first quadrant indicates the period of the rhythmic activity. The regularity of the respiratory rhythm was quantified as an autocorrelation coefficient of the first peak in the autocorrelogram. The period of the phrenic respiratory rhythm was prolonged by head-up and head-down tiltings (Table I). The autocorrelation coefficient of phrenic nerve activity was unchanged by both the tilting procedures. The autocorrelation coefficient of renal sympathetic nerve activity increased during the head-up tilting-period, but unchanged during the head-down tilting-period (Fig. 4 and Table I).

Phase relation between respiratory discharges in the renal sympathetic and phrenic nerve activities

Cross-correlation analysis between phrenic and renal nerve activities revealed the phase relation between respiratory discharges in the two nerve activities. Figure 5 shows the cross-correlogram between phrenic and renal nerve activities. In the cross-correlogram, the interval between the ordinate and the first pericentral peak in the first quadrant means the lag time from the maximum point of the pulse density in the phrenic nerve activity to that in the renal sympathetic nerve activity. The ratio of the lag time (d) to the respiratory period (P) in the cross-correlogram was termed phase index (=d/P, Fig. 5). The phase index was decreased by the head-up tilting accompanying an increase in the

Table I. Effects of head-up and head-down tiltings.

	N	Horizontal Mean ± SD	Head-up 30-45° Mean ± SD	Horizontal Mean ± SD	Head-down 30° Mean ± SD
Arterial blood pressure[a] (Torr)					
Systolic	15	124 ± 23	107 ± 34*	128 ± 17	132 ± 14
Diastolic	15	82 ± 21	72 ± 27*	84 ± 16	89 ± 14*
Mean	15	96 ± 22	83 ± 29*	98 ± 16	105 ± 16*
Pulse pressure	15	41 ± 8	35 ± 9*	43 ± 8	42 ± 5
Heart rate (/min)	15	246 ± 39	251 ± 36*	245 ± 35	245 ± 41
End-tidal CO_2 (%)	15	4.1 ± 0.9	4.3 ± 1.0*	4.0 ± 0.8	4.5 ± 0.9*
O_2	15	16.1 ± 1.4	15.7 ± 1.5*	16.4 ± 1.2	15.4 ± 1.5*
Phrenic nerve activity					
Respiratory period (sec)	15	1.39 ± 0.23	1.49 ± 0.25*	1.36 ± 0.23	1.53 ± 0.23*
Total power (%)	15	100	137 ± 43*	100	147 ± 50*
Autocorrelation coefficient[b]	15	0.80 ± 0.05	0.78 ± 0.14	0.80 ± 0.06	0.82 ± 0.07
Renal sympathetic nerve activity					
Total power (%)	8	100	139 ± 20*	100	118 ± 13*
Cardiac-related peak power[c] (%)	8	8 ± 6	6 ± 4	7 ± 5	16 ± 8*
Autocorrelation coefficient[b]	8	0.30 ± 0.17	0.50 ± 0.23*	0.30 ± 0.20	0.30 ± 0.20
Cross-correlation coefficient	8	0.42 ± 0.18	0.52 ± 0.26*	0.42 ± 0.18	0.41 ± 0.17
Phase index	8	0.35 ± 0.05	0.29 ± 0.06*	0.33 ± 0.07	0.28 ± 0.07

a): Femoral arterial blood pressure was measured placing the manometer in the same niveau of the heart. b): Autocorrelation coefficient was obtained by normalizing the correlation function at the first peak with a value at zero lag in the nonnormalized autocorrelogram. c): Value of cardiac-related peak power was represented as percentage of each total power. *, $p<0.05$.

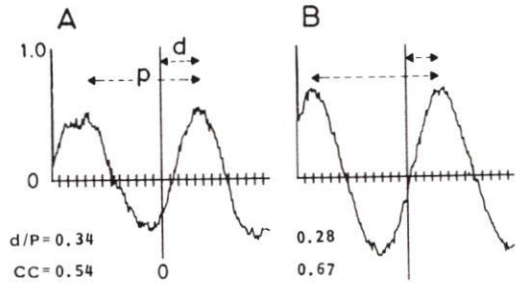

Figure 5. Pulse-weight cross-correlogram between the phrenic and renal sympathetic nerve activities. A: Horizontal position before the postural change. B: 3 min after tilting to the head-up position at 30°. These cross-correlograms were obtained by computer-summing (10 times). Ordinate is cross-correlation function. Abscissa is lag time (100 msec/division). Address bin is 10 msec. d, the lag time from the ordinate to the first pericentral peak in the first quadrant. P, the interval between the two peaks. d/P, phase index. CC, cross-correlation coefficient at the first peak.

cross-correlation coefficient. A decrease in the phase index means that the phase of respiratory discharge in the renal sympathetic nerve activity shifted from inspiratory-expiratory phase-spanning toward the the phrenic inspiratory phase (Fig. 5 and Table I). The change in the phase index produced by head-down tilting was not statistically significant.

Discussion

A remarkable increase in the end-tidal CO_2 concentration and a prolongation of the volley period of the phrenic nerve activity during the head-up and head-down tilting periods suggest that a fraction of the increase in the nerve activity was mediated by the overall central chemosensitivity to CO_2 including the central chemosensitive areas. Power spectral analysis of the renal sympathetic nerve activity revealed that the change in the power value of cardiac and respiratory components in the nerve activity was differential in response to the short-term postural changes for 5-10 minutes. Though both the head-up and head-down tilting procedures increased the total power of the renal sympathetic nerve activity, the power of cardiac-related peak was increased only by head-down tilting. Thus, the neural outflow from the central vasomotor control mechanisms may consist of the functionally heterogeneous fractions. An increase in the autocorrelation coefficient of renal nerve activity and a pronounced shift in the phase relation between respiratory discharges in the phrenic and renal sympathetic nerve activities were caused by tilting to the head-up position, but not by tilting to the head-down position. This finding suggests that the functional coupling process between the central respiratory and cardiovascular control mechanisms in the brainstem is dynamic and especially sensitive to the head-up position. In the next step of the present study the mechanisms and physiological significances of thse alterations in the nerve activity would be analyzed in relation to the relevant main factors: neural inputs from the equilibrium apparatus; arterial and other baroreceptors; proprioceptors; cerebellum as well as the related areas in the central nervous system; and changes in humoral factors such as P_{CO_2}, P_{O_2} and endogenous vasoactive substances.

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ALGAL BIOREGENERATIVE SYSTEMS FOR SPACE LIFE SUPPORT

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Abstract

Regenerative life support systems should 1) recycle water, 2) exchange carbon dioxide and oxygen, 3) produce food, and 4) contain and recover wastes. Eighty-five percent by weight of man's requirements would be satisfied by accomplishing the first two tasks. Algal photosynthetic gas exchange systems have been evaluated for exchange of carbon dioxide and oxygen and production of biomass. Maximum theoretical overall electrical efficiency using artificial lights is 4% or about 6.3 g dry algae $Kw\text{-}hr^{-1}$. Attainable efficiences are about 2%. The functional dependence of yield per unit volume on surface-to-volume ratio requires 1) a large illuminated surface, 2) minimum culture depth, and 3) interrelation of irradiance (power input) with area and volume and compromise between these parameters for any intended application. Hence a two-man capacity (1200 liters oxygen per day or 1200 g dry algae per day) algal gas exchange system should require from 15-20 m^2 illuminated surface and about 32 Kw of power.

Attainment of our national goals in space will eventually necessitate development of regenerative space life support systems. Eighty-five percent by weight of man's requirements for life support would be met by recycling water and oxygen (Table I). Several physical, chemical, and biological processes have been proposed and evaluated for CO_2 removal, O_2 generation, water recycle, and waste control. The biological approach has as its model the complex life-support system that has evolved on earth and offers the potential advantage of food production. According to Myers (1) the biological approach to space life support originated in 1951, when Heinz Specht (2) suggested that man's respiratory requirements might be balanced by photosynthesis of green plants, i.e., substitution of man for the mouse in the Priestley experiment. This paper reviews the requirements for use of algal systems for bioregeneration and presents data on the design and evaluation of a two-man capacity algal photosynthetic gas exchange system.

TABLE I

Human turnover in grams per day

Input		Output	
Moderately active*			
H_2O	2,200	H_2O	2,540
O_2	860	CO_2	980
Food (dry)	520	Solid waste (dry)	60
Relatively inactive†			
H_2O	2,268	H_2O	785
O_2	544	CO_2	644
Food (dry)	544	Solid waste (dry)	‡

*Data from Clamann (30).
†Data from Welch (168).
‡Not measured.

(from Miller and Ward, SAM-TR-66-11, 1966)

Algal systems were the focal point of extensive research and development efforts funded by the Air Force and the National Aeronautics and Space Administration during a 10-year period from about 1955 to 1965. However, these efforts lacked the advantage of a mission-oriented goal, such as a planetary base, and were essentially abandoned by 1967.

Much of the early work on algal systems was not based on known principles of algal physiology. As a consequence, what appeared to be highly contradictory data accumulated in the literature which served to fuel debates on the power, volume, surface area, and weight requirements for the respiratory support of man. Extant literature on algal gas exchange systems was reviewed in 1966 by Miller and Ward (3) in an attempt to reconcile the existing database and provide a theoretical basis for algal gas exchanger design. Analysis of data, either published or calculated, for 29 different algal systems served to illustrate the interrelationships between algal growth and the requirements for power, surface area, and volume (Table II). Electrical efficiencies were generally less than half of the 4% theoretically achievable. Maximum productivity and greatest efficiency were obtained in light-limited optically dense cultures. In optically dense cultures, light intensity decays with optical thickness and gradations in light with culture thickness give rise to gradations in cellular photosynthetic rates. Hence, in dense cultures, the overall rate is a composite of the cellular rates for each differential increment of culture depth, and may be determined by integration. The Bush equation demonstrates, in a qualitative sense, the overall effect of increasing irradiance on dense culture performance:

$$R = \frac{E_m I_s}{J}\left(\ln \frac{I_o}{I_s} + 1\right)$$

where I_o is the incident irradiance, I_s the saturating irradiance, E_m the maximum efficiency of light utilization, J the heat of combustion of algae, and R the overall growth rate per unit area.

TABLE II

Comparison of algal photosynthetic gas exchanger design parameters, yields, efficiencies, and one-man support requirements

Reference	Algal species*	Temp. (°C.)	Culture chamber design	Total vol.† (liters)	Mode Oper.‡	Illumination system—lamps			Incident illuminance§ (ft.-c.)	S.A.* (m.²)	S.A./vol. ratio†† (cm.²/liter)	I.P./S.A. ratio‡‡ (kw./m.²)	Yield§§		Efficiency‖‖		One-man support¶¶		
						No.	Type‖	Position#					(gm./m.²-day)	(gm./liter-day)	(gm./kw.-hr.)	(%)	S.A. (m.²)	Vol. (liters)	I.P. (kw.)
Bovee et al.	C.p.	39	Panel	380	SC	369	30w-FL	E	1,000	22.3	590	0.41	17	1.2	1.5	0.90	35	500	17
Bowman and Thomae	C.p.	38	Annulus	4	SC	5	40w-FL	I & E	350	—	—	—	—	0.7	0.6	0.38	—	857	42
Casey et al.	C.p.	39	Rect. tank	4,560	SC	124	215w-FL	I	—	33.4	70	0.80	30	0.2	1.6	1.02	20	3,000	17
Cook	C.p.	25	Cylinder	10	C	3	100w-FL	E	—	0.44	440	0.68	11	0.5	0.7	0.45	55	1,200	36
Davis	C.p.	25	Annulus	0.1	SC	6	300w-IL	E	7,000	0.015	1,510	119	86	13	0.03	0.02	7	46	—
Eley and Myers	C.e.	25	Annulus	0.36	C	9	15/20w-FL	I & E	—	0.13	3,640	1.3	16.5	6.0	0.5	0.32	36	100	50
Gafford and Fulton	C.p.	40	Dome	9	SC	—	Sunlight	E	Var.	0.5	—	—	12	1.5	—	—	14	400	—
Gafford and Fulton	C.p.	40	Panel	14	SC	—	Sunlight	E	Var.	0.5	500	—	50	1.76	—	—	12	341	—
Gaucher et al.	C.p.	37	Annulus	4	SC	1	1500w-QL	I	10,000	0.13	330	11.4	202	6.7	0.74	0.47	3.0	90	34
Gaucher et al.	C.p.	39	Annulus	11	C	3	1500w-QL	I	35,000	0.17	150	15.4	157	2.4	0.30	0.19	3.8	250	83
Hannan et al.	C.p.	38	Rect. tank	22.8	C	3	1500w-QL	I	15,000	0.23	100	19.6	120	1.2	0.25	0.16	5.0	500	100
Hannan et al.	C.p.	38	Cylinder	6.2	C	6	1500w-QL	I	23,000	0.25	390	28.3	350	14.1	0.52	0.33	1.7	43	48
Hemerick	C.p.	39	Lenticular	12	B	1	1500w-QL	I	11,000	0.56	460	2.7	100	4.5	1.5	0.96	6.0	133	17
Hemerick and Benoit	C.p.	39	Annulus	12	C	1	1500w-QL	I	35,000	0.10	140	13.6	180	1.4	0.79	0.50	3.3	428	32
Krall and Kok	A.n. and Sc.	32-38	Rect. tank	182	SC	276	42w-FL	I	2,000	14.6	800	0.55	34	2.7	2.6	1.66	18	222	9.6
Leone	C.p.	38	Annulus	5	C	1	1500w-QL	I	6,000	0.14	280	8.6	115	3.2	0.56	0.36	5.2	188	45
Matthern and Koch	C.p.	38	Cylinder	2.8	C	4	1500w-QL	I	—	0.09	325	46	410	13.0	0.37	0.24	1.5	46	68
Meleshko	C.sp.	40	—	65	B	—	IL	E	9,300	—	—	—	—	246	—	—	—	3.5	—
Myers and Graham	C.e.	25	Cylinder	1.01	C	—	IL	E	—	0.0020	20	—	71.0	0.14	—	—	85	—	—
Myers and Graham***	C.e.	25	Cyl. w/cone	0.86	C	—	IL	E	—	.0017	17	—	155	0.31	—	—	39	—	—
										.0178	207							
Newland and Price	C.sp.	29-34	Annulus	3.0	C	2	20w-FL	I	900	0.23	1,800	0.17	8.4	0.6	2.0	1.28	71	1,000	12.5
Shuler	C.p.	39	Annulus	1.8	C	1	1500w-QL	I	9,000	0.06	380	14.7	240	9.0	0.68	0.43	2.5	67	37
Tew et al.	C.p.	38	Annulus	1.2	C	1	150w-FL	I	—	—	—	—	—	3.0	1.0	0.64	—	200	24
Thacker and Babcock	C.p.	25	Glass tube	68	B-SC	120	40w-FL	E	2,000	5.8	1,530	0.82	14.4	1.4	1.2	0.77	42	429	21
Wallman et al.	C.p.	38	Rect. tank	600	C	36	215w-FL	I	2,500	10.7	220	0.72	65	—	3.5	2.24	9.2	—	7
Ward et al.	C.sp.	38	Panel	8	C	8	110w-FL	E	3,500	1.0	2,000	0.88	50	6.0	2.4	1.53	12	100	10.4
Zuraw et al.	C.p.	39	Rect. tank	57	C	5	1500w-QL	I	—	0.32	60	24.6	165	1.1	0.28	0.18	3.6	545	89
Zuraw et al.	C.p.	39	Ann.-dome tank	66	C	5	1500w-QL	I	—	0.95	145	7.9	100	1.2	0.54	0.35	6.0	500	46
Zuraw and Adamson	C.p.	39	Ann.-dome tank	53	C	10	1500w-QL	I	15,000	0.95	180	15.8	121	2.2	0.32	0.22	5.0	273	78

No distinction is made in this table between original (cited) and calculated data.

*Abbreviations are:
C.p. — *Chlorella pyrenoidosa.*
C.e. — *Chlorella ellipsoidea.*
A.n. — *Anacystis nidulans.*
Sc. — *Scenedesmus.*
C.sp. — *Chlorella species* (strain unspecified).

†Total volume includes both culture vessel and external recirculation volume when applicable.

‡Mode of operation:
B: Batch.
SC: Semicontinuous (periodic harvest).
C: Continuous (continuous harvest).

‖Type of illumination source including nominal wattage:
FL: Fluorescent lamp.
QL: Quartzline lamp.
IL: Incandescent lamp.

#Position of lamp with respect to culture:
E: External.
I: Internal.

§Incident illuminance upon culture vessel. All data are cited figures.

**Area of culture exposed to the incident irradiance.

††Surface area per unit illuminated culture volume.

‡‡Illumination power input per unit surface area.

§§Yields are grams dry algae per unit illuminated surface area and per unit total culture volume.

‖‖Efficiency is overall conversion of electrical energy into algal cells. Percent efficiency is based on 5.5 kcal./gm. dry algae.

¶¶Based on a one-man oxygen requirement of 600 liters per day (equivalent to the production of 600 gm. dry algae per day).

S.A. — Illuminated surface area.
Vol. — Total culture volume.
I.P. — Input (electrical) power for illumination.

***This paper and the one above demonstrate the use of a diffusing cone for attenuation of irradiance. Surface areas are base (input) over lateral (output) of the cone. Surface-to-volume ratios are based on input and output light areas. Yield is based on input area.

(from Miller and Ward, SAM-TR-66-11, 1966)

Figures 1-3 present experimental and calculated data for the 29 algal systems reviewed by Miller and Ward; theoretical curves were generated with the Bush equation. The "fit" of the experimental data is surprisingly good. The trade-offs between power, surface to volume ratio, and yield per unit area as a function of power input were used as the basis for design of a man-rated algal photosynthetic gas exchanger (4).

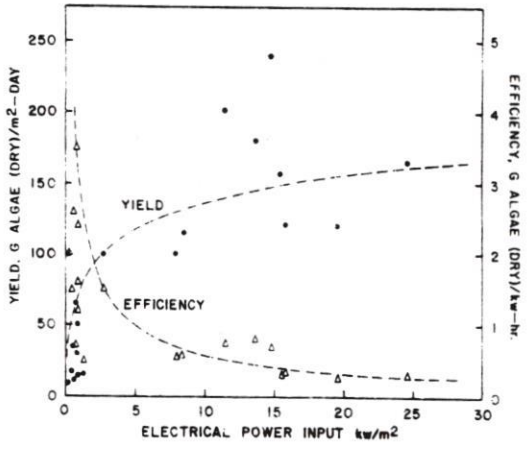

Figure 1. Yield per unit area and efficiency, g algae/kw-hr., as a function of electrical power input for illumination, kw. Data are for photosynthetic exchangers listed in Table II. ●-denotes yields; Δ-denotes efficiencies. Curves calculated from Bush equation for a hypothetical lamp 20% efficient in converting electrical to visible light energy. Parameters in the Bush equation were assigned these values: E = 0.20; I_S = 3.5 mw/sq cm; J = 5.5 kcal/g algae (from Miller and Ward, SAM-TR-66-11, 1966)

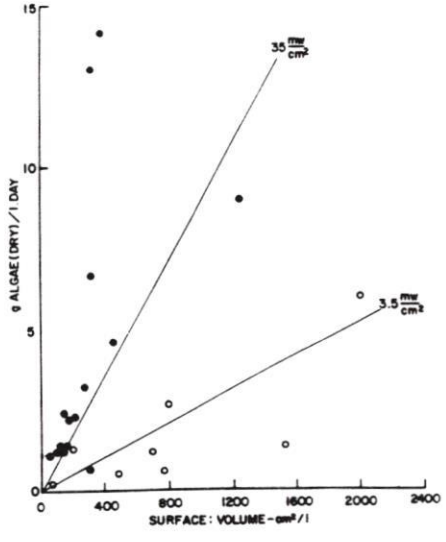

Figure 2. Yield per unit volume as a function of surface to volume ratio. Data are photosynthetic exchangers in Table II. ○-denotes fluorescent lamp sources; ●-denotes quartzline sources. Curves were calculated from the Bush equation with values assigned in Figure 1. Parameter is incident irradiance (from Miller and Ward, SAM-TR-66-11, 1966).

The essential features of the two-man algal gas exchanger developed by the USAF School of Aerospace Medicine (built under contract by Beckman Instruments, Inc., Fullerton, Calif.) are shown schematically in Figure 4. The process flow is composed of two subsystems, liquid and gas. Liquid algal culture flows continuously through a closed-loop circuit containing separate process units for culture illumination (panels), gas contact (sintered stainless steel spargers), and gas separation (centrifugal action). The system is housed in two consoles (Figure 5), one for liquid-gas contact and separation (left) and the other for culture illumination and temperature control (right). Algal culture is exposed to fluorescent light bilaterally in as many as 14 thin, transparent plastic panels composed of 16

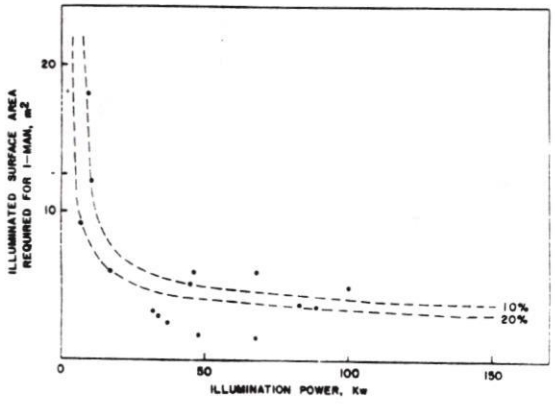

Figure 3. Illuminated surface area required for one-man support as a function of illumination power. Data are for photosynthetic exchangers in Table II. Curves were calculated from Bush equation with assigned values given in Figure 1. Parameter is hypothetical efficiency for electrical to visible light energy conversion (from Miller and Ward, SAM-TR-66-11, 1966).

polycarbonate tubes, 117 cm long, 7.5 cm wide, and 0.5 cm deep. Each panel has 2.28 m^2 of illuminated surface (both sides) and contains about 6.8 liters of culture. Fluorescent lamp banks are sandwiched between algae panels to provide an irradiance of about 8 mw/cm^2 to each side of adjacent culture panels. The power required for illumination, calculated from nominal lamp wattage, is 2.64 Kw per bank. A total culture volume about 135 liters is

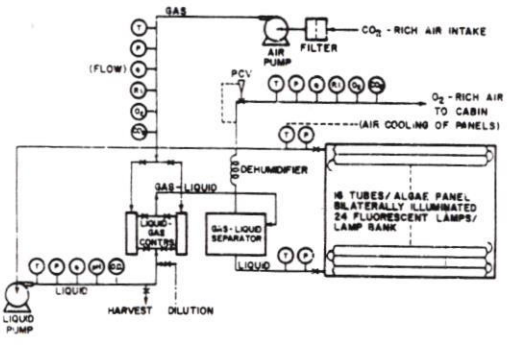

Figure 4. Process flow schematic of two-man capacity algal photosynthetic exchanger. Solid lines indicate flow elements. Dashed lines indicate control elements (from Miller, et al., SAM-TR-69-64, 1969).

circulated continuously through the system at a rate of 160 liters/minute. Performance of the two-man rated algal gas exchange system was evaluated during the period from March 1967 to June 1968 (4). Growth and gas exchange data were developed for three species of algae: *Synechococcus lividus*, a thermophilic unicellular

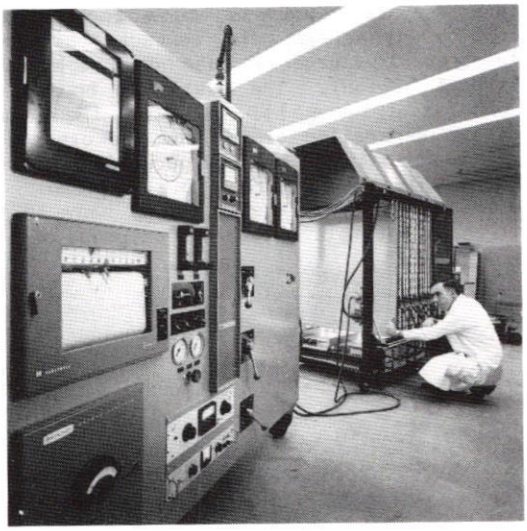

Figure 5. Face view of two-man capacity algal photosynthetic exchanger. Process console at left; illumination console at right.

blue-green alga and two thermotolerant green unicellular forms, *Chlorella sorokiniana* and *Oosystis polymorpha*. Several tests of variable duration in days were made with each alga. Table III presents data for the highest production rate achieved with each alga. The

TABLE III

Growth of algae in two-man algal gas exchanger

Parameter	*S. lividus*	*C. sorokiniana*	*O. polymorpha*
Duration, days	2	7	15
No. algae panels	8	10	11
No. light banks	9	11	11
Temperature, °C	50	39	38
Production rates			
g/day	322*	900	1148
g/m^{2}day	17.7	39.5	48.0

*About 1 liter of O_2 is produced per g dry wt of algae produced; the oxygen requirement of one man is about 600 liters per day (after Miller, R. L. *et al.*, SAM-TR-69-64, 1969).

relatively short test periods resulted from the experimental protocol rather than problems with algal growth. The long-term reliability of steady-state algal cultures was adequately demonstrated even though algal production rates did not meet design specifications. Culture tube fouling due to sticking of algae was common with *C.sorokiniana; O. polymorpha* demonstrated the highest production rates with no visible fouling after weeks of operation. Extensive operation of the system revealed a design flaw in the culture flow-liquid-gas contacting system which resulted in severe CO_2 limitation of algal growth. When the input concentration of CO_2 was increased, the production of *O. polymorpha* approached the design specification of 1200 g dry algae per day. Modification of the hydraulic system to insure CO_2 saturation should have allowed the system to sustain an areal production rate of 60 to 70 g/m^2 per day using either *C. sorokinianaor O. polymorpha*. However, the system was not modified or tested further.

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ALGAL CULTURE STUDIES RELATED TO A CLOSED ECOLOGICAL LIFE SUPPORT SYSTEM

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Long-term cultures of Scenedesmus obliquus were maintained in an annular air-lift column operated as a turbidostat. We observed a linear relationship between the dry weight of the cultured cells, their cell number, and their chlorophyll content over a broad range of cell density at constant illumination. Thus, the cells did not appear to be adapting to differences in growth rate or light intensity during these experiments. Productivity vs dry wt rose linearly until the cell density reached a level at which light became limiting; at this point ~ 89% of the photosynthetically active radiation (PAR) was being absorbed. The maximum dilution rate of the system corresponded to a doubling time of 13.8 hr, about half the maximum growth rate generally observed at this temperature. Productivity at the maximum was ~ 80% of the maximum theoretical productivity. The rather low incident intensities (~ 10% of full sunlight) were a main contributing factor to the high light utilization efficiencies obtained in this system, since the cells were never driven into light saturation.

In many respects, algae would be ideal plant components for a biologically-based closed life support system, since they are eminently suited to the closely coupled functions of food production and atmosphere regeneration.* In this communication, we report some findings on the (steady-state) continuous culture of Scenedesmus obliquus, a physiologically well-characterized green alga with good growth characteristics.

METHODS

Description of Culture Apparatus

Long-term cultures were maintained in annular air-lift columns operated as turbidostats. These continuous culture systems are based on the earlier work of several other groups, notably Myers and Clark (J. Gen. Physiol. 28, 103-112, 1944); also, Kuhl and Lorenzen (in Methods in Cell Physiology, Vol. I, D. Prescott, ed., pp. 159-187, Academic Press, NY, 1964); and Ammann and Lynch, (Appl. Microbiol. 13, 546-551, 1965). A primary goal of the design and construction of our culture systems was to provide a means to control and monitor important physiological parameters, such as light flux, light absorption, temperature, and growth rate. Another important consideration was our goal of constructing a system that was harvestable on demand. This latter requirement precluded the use of a chemostat system; instead, we use the turbidostat system described below.

Figure 1 is a diagram of the culture apparatus constructed and used in our laboratory. The culture system is made of transparent glass and consists of three concentric, cylindrical chambers.

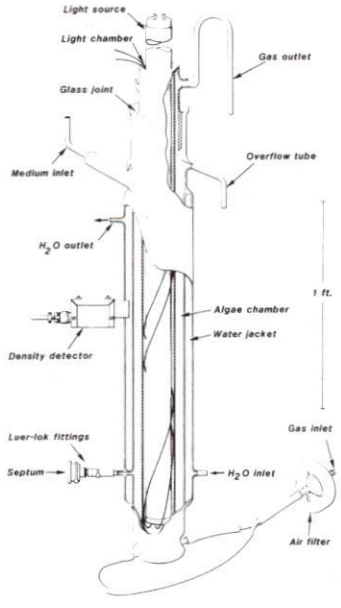

Figure 1.

The innermost chamber houses the illuminating source (a standard 40-watt, cool white, high-output, fluorescent bulb). The middle chamber, with a volume of 890 ml and a width of 1.0 cm, contains the algal culture. Temperature control is provided by a refrigerated bath (Neslab RTE-4; ± 0.01°C temperature control from -30°C to +100°C) which circulates water through the outermost chamber.

Cell density is maintained by monitoring the light transmission through the culture using a photoconductive cell (Clairex CL604L). The output of this photocell is amplified, integrated (to remove the ac component from the light), and digitized. This value is then compared to a preset digital reference. When the processed photocell output exceeds that of the reference for 4 s, a peristaltic pump is turned on for a preselected period of time allowing for the addition of medium to the culture. A corresponding volume of suspension is displaced from the algal chamber via the overflow tube. After a latent time of 40 s to

* This idea was clearly recognized by an earlier generation of scientists (see, e.g., Bioregenerative Systems, NASA SP-165, 1968). A similar program is being carried out in the USSR (Gitel'son, I., et al., Problems of Space Biology, Vol. 28, Experimental Ecological Systems Including Man, NASA Technical Translation F-16993, 1975).

allow for mixing of any newly added medium, the monitoring cycle is reinitiated. Turbidity is monitored and controlled by a microcomputer (SYM-1, Synertek Corporation) and ancillary electronics built in house. With this microcomputer system, eight culture systems can be controlled simultaneously.

Either air or CO_2-enriched air is admitted through the fritted bottom of the culture module at a flow rate of about 800 cm^3/min; this flow rate is sufficient to provide relatively rapid mixing and prevent cell settling.

Measurements of Cell Characteristics

Chlorophyll was determined by adding an aliquot of algal culture to a 1:1 mixture of Triton X-100 and 5% KOH in MeOH, heating at 63°C for 3 minutes, and centrifuging. Optical density was measured at 645 nm. Packed cell volume (PCV) was determined by centrifuging (clinical centrifuge, high speed) 1 ml of algal culture in a modified hemotocrit vessel. Cell density was determined using a Coulter Counter Model TAII with PCAII accessory. Dry weight determinations involved filtering a 10-ml aliquot of algal culture through glass fiber filter (approximate retention 2.6 µm), rinsing thoroughly with distilled water, and drying at 110°C. (The filter paper was dried overnight at 110°C prior to weighing; dried samples were cooled to room temperature in a dessicator before weighing.) The growth rate of the culture was determined by measuring the volume that overflowed during a defined time period (usually 16-24 hr). The productivity (mg/hr) of an algal culture is defined as the product of the dry weight (mg/ml) of the culture and the overflow rate (ml/hr).

Light Measurements

Light intensity was measured with an ISCO Model SR spectroradiometer between 400-700 nm in increments of 25 nm; this data was integrated to yield light intensity in units of $\mu W/cm^2$. Measurements were made at 8 points around the culture apparatus, and the values averaged to correct for any lack of symmetry. Light efficiency was calculated using the absorbed light intensity (cal/hr) and the biomass productivity, converted from mg/hr to cal/hr, assuming a conversion of 5.5 cal/mg. (See, e.g., R.L. Miller and C.H. Ward, in Atmosphere in Space Cabins and Closed Environments, pp. 186-222, K. Kammermeyer, ed., Appleton-Century-Crofts, New York, 1966.)

Carbon and Nitrogen Analyses

A 10-ml volume of algal culture was centrifuged at 10,000 RPM for 10 min, and an aliquot of the supernatant acidified with 0.05 ml concentrated H_2SO_4/ml supernatant. Glycolic acid was determined by heating (100°C for 20 min) the acidified supernatant in a 4-fold greater volume of 0.01% 2,7-dihydroxynaphthalenediol in concentrated H_2SO_4 (Calkins, Anal. Chem. 15:762, 1943). Absorbance was measured at 530 nm with a Cary 15 spectrophotometer. Total excreted carbon was determined by measuring the chemical oxygen demand of the supernatant (Oceanographic International Corporation's Standard Ampule C.O.D. method). The nitrate concentration of the supernatant and growth media were determined on a Dionex Model 16 ion chromatograph. Nitrogen analysis of the freeze-dried algal pellet was done by an outside laboratory (Galbraith Laboratories, Knoxville, TN).

RESULTS AND DISCUSSION

Characteristics of Cultural Cells

Figure 2 illustrates the linear relationship between the dry weight of the cultured cells (mg/ml), their cell number (cells/ml), and their chlorophyll content (µg chl/ml). These data indicate that the relationships between cell mass, cell population, and chlorophyll/cell are constant over the range of culture conditions tested. Thus, the cells do not appear to be changing or adapting to differences in growth rate or light intensity

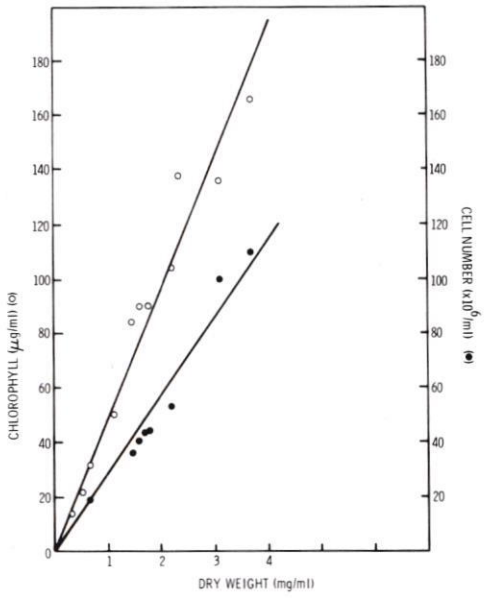

Figure 2.

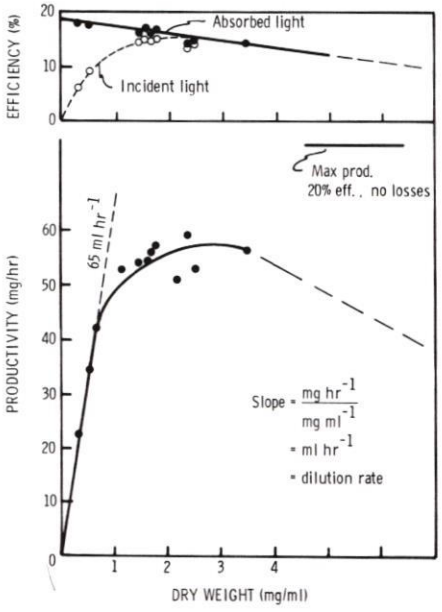

Figure 3.

during these experiments (see, e.g., Myers, Proceedings of the IBP/PP Technical Meeting, Trebon, 1970). The slopes of the two lines (computed by standard statistical techniques) are 2.8×10^7 cells/mg dry wt and 48.3 µg chl/mg dry wt, respectively. This corresponds to 1.73×10^{-6} µg chl and 3.6×10^{-5} µg dry cell mass per cell.

Productivity and Light Efficiency

Figure 3 illustrates the relationships between culture productivity (lower panel) and light utilization efficiency (upper panel) vs dry weight observed in a series of experiments in which Scenedesmus was maintained in the continuous culture system shown in Figure 1. The productivity vs dry weight curve rises linearly until the cell density reaches a level at which light becomes limiting (~ 1.4 mg ml^{-1} or ~ 48 µg chl ml^{-1}). At this point, ~ 89% of the photosynthetically active radiation (PAR) is being absorbed. In the initial linear portion of the curve, productivity is limited by cell growth at the given light intensity. The slope of this initial portion reflects the maximum dilution rate of the system (dimensions of ml hr^{-1}). In the present instance, this rate is 65 ml hr^{-1}, which corresponds to a doubling time of 13.8 hr. This fairly low growth rate (about half the maximum growth rate generally observed at this temperature) reflects the rather low intensity of the light source (~ 10% of full sunlight at the inner wall of the algal culture chamber). These low incident intensities are a main contributing factor to the high light-utilization efficiencies obtained in this system, since the cells are never driven into light saturation (see Appendix).

The slope of the line drawn through zero and any point on the productivity curve corresponds to the dilution rate, and therefore the doubling time and growth rate, at this point. The productivity curve rises to a value of ~ 58 mg hr^{-1} at ~ 3 mg ml^{-1}, which is ~ 80% of the maximum theoretical productivity (~ 20% on an energy basis; see, e.g., Radmer, R. and B. Kok, in Encyclopedia of Plant Physiology, Vol. 5, New Series, pp. 125-135, A. Trebst and M. Avron, eds., Springer-Verlag, Berlin, 1977). One would predict that the productivity would gradually decrease at very high cell densities, since increasing amounts of biomass (with finite and significant maintenance energy) would be supported by a constant amount of absorbed incident light (~ 97% absorption at 2.5 mg ml^{-1}). However, it is not practical to obtain such data in the present system.

The upper panel of the figure shows the efficiencies* of absorbed and incident light as a function of cell density (dry weight). The efficiency with respect to absorbed light (solid line) appears to be a steadily-decreasing (linear ?) function of cell mass, reflecting 1) lack of light saturation due to the low incident intensity (see above), and 2) the significant maintenance energy required by the (increasing) biomass. If we assume the reality of the linear relationship, the maximum efficiency of absorbed light in this system is 19%. The slope of the line, 1.28% $(mg/ml)^{-1}$, reflects losses due to maintenance energy, which is probably linear over the rather narrow conditions tested. Since 100% efficiency corresponds to 0.0064 W hr mg^{-1}, the maintenance energy is 8.19×10^{-5} W hr mg^{-1}, and one would predict that the culture would reach light compensation at a dry wt of 14.3 mg ml^{-1}.

The efficiency with respect to incident light (dotted line) reflects the balance between light absorption and utilization (the initial ascending phase) and losses due to the cellular metabolism (descending phase). In the present system, the maximum light utilization efficiency occurs at 1.7 to 2.0 mg ml^{-1} dry wt. The descending phase becomes identical to the "absorbed light" curve at high cell densities.

Nitrogen Utilization Efficiency

Krauss et. al. [Proc. 21st Plenary Meeting, Committee on Space Research (COSPAR), 1978] reported that a significant fraction of the NO_3^- nitrogen provided to Chlorella cultured in a "recyclostat" was lost, probably as N_2O. The release of this gas into the atmosphere of a closed system could cause grave problems for the air regeneration system, as well as contribute to a lack of closure of the nitrogen cycle.

Because of these earlier results, one of our goals was to determine the nitrogen balance of Scenedesmus cultures, and specifically, whether compounds such as N_2O were excreted into the medium. Our initial approach was to determine the nitrogen levels of the nutrient medium, cell-free efflux, and harvested algae.

The results of these experiments (Table I) show surprisingly good agreement between added and recovered N (average 100.5% recovery) and suggest that the nitrogen entering the culture (as NO_3^-) was either incorporated in the algae or appeared as NO_3^- in the efflux supernatant. We have had no indication to date that the nitrogen is lost by the system. Although these results do not prove that there is no nitrogen loss, they do suggest that any loss must be small (e.g., < 1%), at least for NO_3^-.

TABLE I

Sample	Nitrogen (mg/l)			Recovery
	Medium	Supernatant	Algae	(%)
1	339	216	127	101.2
2	293	120	171	99.3
3	290	123	171	101.4
4	290	122	168	100.0

Because our data obtained with Scenedesmus point to a very low production of nitrogenous by-products, we have not attempted to determine N_2O directly. (Current data indicate that the N_2O

* These light efficiency measurements contain the following primary sources of error: 1) a small volume at the bottom of the culture (~ 10% of the total volume) that is not significantly illuminated; 2) the overflow cell density is only 89% of the reactor density; 3) the light measurements represent the average of a somewhat asymmetrical cylindrical light field; and 4) room light was not excluded (< 3% of total incident light). All these errors are relatively small, and the more significant ones tend to cancel.

concentration in the gas stream would be too low to monitor directly.) We have observed only traces of NO_2^- (< 1 ppm) in the effluent supernatant.

Long-Term Culture

We maintained continuous Scenedesmus cultures of this type for about 4 months before voluntary shut down. The cultures were monitored daily for packed cell volume (PCV), chlorophyll, dry weight, reproduction rate, and pH, and intermittently for glycolate, total N, and microbial contamination. Representative data from these long-term experiments is shown in Figure 4. Note that the culture system displays good long-term stability.

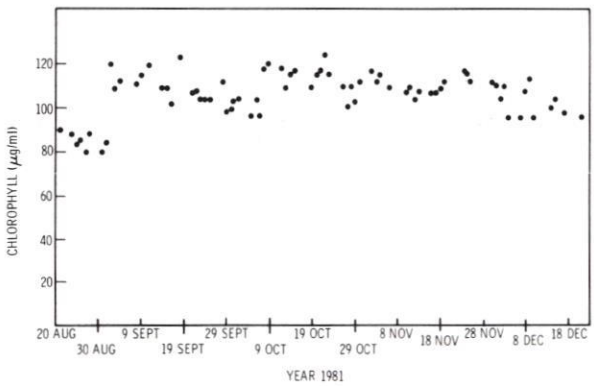

Figure 4.

We addressed the interrelated topics of algal by-product excretion and microbial contamination by periodically assaying the culture supernatant. To date, we have detected no significant glycolate (< 1 ppm of this primary algal excretory product), or excreted carbon (< 25 ppm) while our cultures were in the steady state. Parallel microbial assays in some cases indicated a low (0.1 - 0.01%) non-algal biomass that did not change appreciably (with time) with respect to amount or species composition. These findings suggest that microbial contamination should not be a significant problem in such cultures because 1) the algae seem to excrete little or no organic compounds; and 2) microbial populations, even when present, do not take over the culture.

APPENDIX

Relationship of Light Intensity to Turnover of the Photosynthetic Apparatus

One of the primary limitations of most photosynthetic organisms is that they do not perform well in strong light (e.g., full sunlight). The photosynthetic apparatus operates somewhat like a lens; approximately 200 "light-harvesting" chlorophyll molecules transfer light energy to a reaction center, with a corresponding increase in the effective light intensity per center. Consequently, efficiency can be very high in weak light, but drops off rapidly at intensities approaching that of bright sunlight, due to the rather slow (ms) turnover of the dark reactions (see, e.g., R. Radmer and B. Kok, in Encyclopedia of Plant Physiology, Vol. 5, New Series, pp. 125-135, A. Trebst and M. Avron, eds., Springer-Verlag, Berlin, 1977).

Figure 5 is an idealized cross sectional diagram of our algal culture system. The light flux values (in units of photosynthetically-active quanta $cm^{-2}s^{-1}$) were measured in the absence of algae. Note that they closely follow a 1/r relationship, suggesting that the total flux is conserved (except for losses due to reflection and absorption by the glass walls) during its passage through the concentric cylinders.

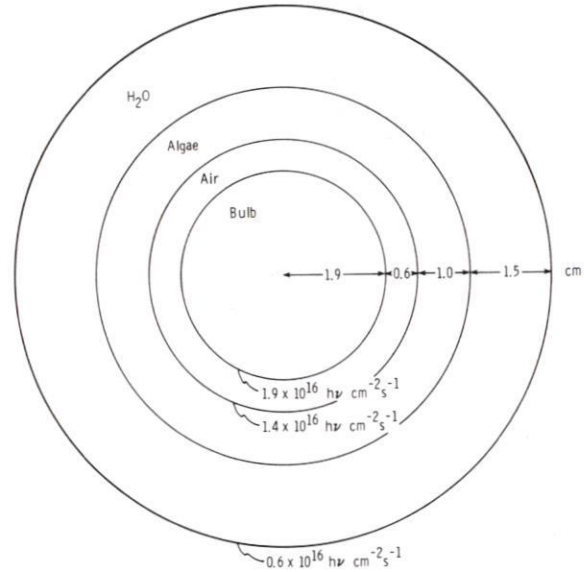

Figure 5.

A rough estimate of the light flux per reaction center can be made as follows. Our data indicate that Scenedesmus has a specific absorption coefficient of ~ 0.6 l cm^{-1} g^{-1} over the spectral range of 400-700 nm (specific data not shown), and a chlorophyll content of 4.8% (Figure 2; see also J. Myers, in Encyclopedia of Chemical Technology, pp. 33-51, R. Kirk and D. Othmer, eds., Interscience, NY, 1957). Note that 0.6 l cm^{-1} g^{-1} corresponds to (0.6) (1000 cm^3) cm^{-1} g^{-1} or 600 cm^2 g^{-1}. Thus, the equivalent specific absorption is 1.2×10^4 cm^2 (g chlorophyll)$^{-1}$, which is about 10% of the extinction coefficient observed at the absorption maxima. Since one g chlorophyll ≈ 6×10^{20} molecules, the molecular cross section (= extinction coefficient) is 0.2×10^{-16} cm^2 (chlorophyll molecule)$^{-1}$.

The maximum quantum flux that the algae are subjected to is 1.4×10^{16} hν cm^{-2} s^{-1} or 0.28 hν molecule^{-1} s^{-1}. Since each reaction center is connected to ~ 200 chlorophyll molecules (see above), the maximum quantum flux will result in the transfer of ~ 60 hν s^{-1} to each reaction center.

This value is well below the generally accepted maximum reaction center turnover rate of ~ 100 s^{-1} (Radmer and Kok, op.cit.). Thus the photosynthetic dark reactions are able to keep pace with the light flux, the system does not become light saturated, and high light efficiencies can be obtained.

ACKNOWLEDGEMENTS

This work was carried out under a contract from the National Aeronautics and Space Administration (Contract NAS2-10969). We thank A. Venables for technical assistance during the early stages of this project.

The test apparatus included 24 small weighted balls and a set of record cards. The balls, all of the same shape and size, varied in mass from 50 - 64 g, in 2 g steps. Comparisons (using the same hand) of always two different pairs were marked for the "heavier" one on the record sheet.

Thresholds for mass discrimination under O-G were found to be higher by a factor of about 1.8, as compared with weight discrimination on the ground. This suggests that humans are not as sensitive to inertial mass cues as they are to weight, and that adaptation can only partially compensate for loss of gravity.

2.6 Effects of Weightlessness on Lynphocyte Proliferation - 1ES031; principal investigator: Dr. A. COGOLI, ETH Zürich.

The major objectives of this experiment were to test whether cells in general are sensitive to gravity, and whether functional changes occur, in particular on cells responsible for the immune response while depriving them from G vector cues.

Cultures of human lymphocytes were exposed during O-G to Concanavalin A, a mitogen capable to transform resting T-lymphocytes into activated (dividing) cells; the rate/magnitude of activation was determined by incorporation of tritiated thymidine into DNA. The study aimed also at conclusions on the cell membrane properties.

As the main finding it could be demonstrated that the inflight lymphocytes showed a marked depression of the activation as compared with those of the ground control cultures. In comparison also with previous studies under simulated O-G (clinostate) and hypergravity (centrifuge) there is a support for the hypothesis that microgravity depresses whereas high gravity enhances cell proliferation rates. The effects of gravity are particularly strong in cells undergoing differentiation. Although this experiment was done in vitro, and no extrapolation to changes in vivo can be made, and although the very mechanisms involved in gravitational effects on cells are still unknown, the investigators conclude on the basis of their results the remarkable proof that cells are sensitive to gravity.

2.7 Advanced BIOSTACK experiment - 1ES027; principal investigator: Prof. H. BUCKER, IAM, DFVLR Cologne

This study aimed at both the determination of the radiobiological importance of cosmic radiation particles of high charge and high energy, as well as evaluating the physical components occured during the SL-1 mission, including dosimetric data for the different shielding environment.

For this purpose, 4 different sets of 'BIOSTACKS' have been mounted into the Spacelab rack, on the Spacelab floor and on the pallet respectively. Monolayers of biological test organisms were sandwiched between thin foils of different types of nuclear track detector material.

Based also on previous spaceflight experiments, it is concluded that the average radiation dose-equivalent (expressed in millisieverts, m Sv, per year), even when hundredfold increased, is not the 'prohibitive' limit for living organisms, including man: It is rather the radiobiological quality of numerically minor components of the cosmic radiation.

The evaluation of the physical and biological components indicate that in general the biological specimens survived this exposure in good condition, and the dosimetric data show a distinct dependence on the shielding environment.

2.8 Microorganisms and Biomolecules in the hard space environment - 1ES029; principal investigator: Dr. G. HORNECK, IAM, DFVLR Cologne.

The major objective was to determine the response of a resistant microbial system to 'space conditions' and to selected components of this hard environment. 316 dry samples of bacillus subtilis spores were exposed to the vacuum and/or to the full ultraviolet spectrum (170 nm) or selected ranges of peak wavelengths of 220, 240, 260 or 280 nm.

Growth disturbances, membrane damages, and structural changes in enzymes and proteines are possible consequences of prolonged exposure to space conditions, for which precise answers were sought.

An exposure tray with four compartments was mounted on the pallet. Two compartments were vented to the outside, the two others hermetically sealed at 1 bar. An optical filtering system allowed for exposure to various wavelengths of solar UV-radiation.

Exposure to the vacuum of space for 10 days reduced the viability counts (colony formers) to about 50 percent, and increased the mutation frequency about tenfold. Similar vacuum-treated samples on the ground did not affect or only slightly inactivate the spores. The response to solar UV-irradiation showed a higher sensitivity in vacuo than at 1 bar. The UV-sensitivity was measured in terms of UV-fluences that reduced survival to 10 percent. Also, DNA cross-linking was induced by solar UV-irradiation, both in vacuo and at 1 bar; the amount of free DNA decreased with increasing UV-fluence. The interpretation of apparent differences in the photobiological and photochemical data between flight and ground simulation experiments, however, will require more statistical analyses.

3. Conclusions

The SL-1 mission was for Life Sciences very important since it provided the science community of a wider range for the first time in Europe with a set of remarkable, and sometimes even unpredictible data. Overall, the mission can be termed as a success.

The importance must be also seen in the light of a learning process, particularly in how to achieve a mature space experiment; the author believes that the time has passed to call this science community 'newcomers' to space. Future experiments and missions already in the planning, indicate that a second generation, more mature in its approach, has opened the round for a broad spectrum of Life Sciences investigations in space, justifying the expenses associated with this research, and always bearing in mind the aim for a better understanding of living matter on the earth.

EXPERIMENTS		GENERAL FIELD	SUB-DISCIPLINE	SPACE RELATED ENVIRONMENT
IES 028	3-D - BALLISTOCARDIOGRAPHY	Human Physiology	Cardiovascular System and Body Fluid Control	ZERO G
IES 026	MEASUREMENT OF CENTRAL VENOUS PRESSURE			
IES 032	SERUM HORMONE LEVEL DETERMINATION			
IES 030	ELECTROPHYSIOLOGICAL TAPE RECORDER		Neurosensory System, Reflex Mechanisms	
IES 201	VESTIBULAR EXPERIMENTS		Psychosensory Function	
IES 025	MASS DISCRIMINATION			
IES 031	LYMPHOCYTE PROLIFERATION		Cell Growth and Development	
IES 027	ADVANCED BIOSTACK	Radiation Biology	Microbial Systems and Biomolecules	- COSMIC RADIATION (HZE PARTICLES) - SOLAR IRRADIATION - SPACE VACUUM
IES 029	EFFECTS OF RADIATION ON BIOLOGICAL SYSTEMS			

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PHOTOSYNTHETIC PRODUCTIVITY AND VIBRATION/ACCELERATIONAL STRESS CONSIDERATIONS FOR HIGHER PLANTS IN BIOREGENERATIVE SYSTEMS

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Photosynthetic higher plants have been identified as key constituents of bioregenerative life support systems to be deployed in space. Although alternative, physiochemical methods might be used for air revitalization and waste recycling, chemosynthetic food production has not yet been perfected, and production of edible biomass by means of the photosynthetic process is the main driver for using higher plants in a regenerative life support system. Of course, higher plants also can scavenge respiratory CO_2 and evolve fresh O_2 in light, do quite a good job of purifying sewage water by means of transpiration, and may be able to recycle some inorganic wastes as plant nutrients (Fig. 1) (1). If the food production loop is ever truly closed within a space-deployed regenerative life support system, one food production scenario will be an all-vegetarian diet. There are various scenarios of vegetarianism, ranging from generous to modest to severe. The strictest scenario involves at least one representative from 3 categories of food crop: a protein crop, a carbohydrate/calorie crop, and a mineral/vitamin/fiber crop. The food production group of the NASA Closed Ecology Life Support Systems (CELSS) program is conducting research to optimize production of candidate species from each of the major categories of food crop. Studies are underway with soybean, wheat and potato, and leaf lettuce. Our laboratory emphasizes the controlled environment production of leaf lettuce. Our objective is to obtain the greatest possible production of edible lettuce biomass in the smallest possible growing space in the shortest possible time. There are multiple reasons why we have chosen to work with this food crop: Nutritionally, it is no better than many other salad crops, but it would contribute essential vitamins and minerals to a balanced vegetarian diet, especially if it were the only representative of that category. In its favor, leaf lettuce growth rate is very responsive to environmental manipulation; it has a relatively short production cycle; and unlike crops where reproductive development is involved in a production cycle, lettuce sustains air revitalization and biomass accumulation capacity throughout production. Furthermore, it maintains a high proportion of edible biomass, which is important because only digestible matter represents a useful net gain within a bioregenerative life support system. Leaf lettuce also is very tolerant of NH_4^+ in nutrient solutions, which could be valuable in recycling wastes containing reduced nitrogen. The crop also is suitable for various forms of soilless culture, including hydroponics, aeroponics, and nutrient film technique; it has a diminutive stem, which should lessen tropism problems in a microgravity environment; there is a substantial data base of information to build upon in optimizing this crop; and astronauts and cosmonauts should find it psychologically satisfying to grow in a confining and austere space habitat. In short, leaf lettuce is a good crop to grow in CELSS.

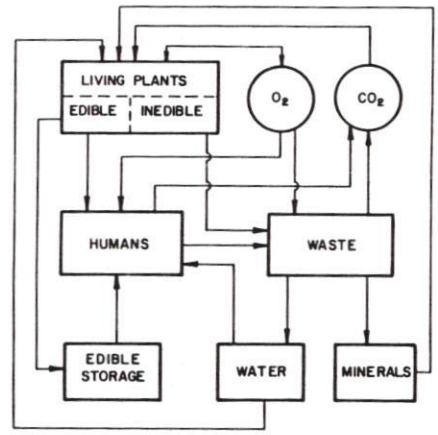

Figure 1. A regenerative life support system based on higher plants for food production and air revitalization.

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Our experimental approach involves manipulation of environmental parameters (2,3) and application of growth-regulating chemicals in order to enhance lettuce growth. One approach is to enrich atmospheric CO_2. To accomplish this, we use transparent chambers in which up to 36 lettuce seedlings can be grown initially, and through which atmosphere containing known amounts of CO_2 flows at a controlled rate. Plants growing in these chambers are harvested at intervals for growth analysis. In one study, CO_2 enriched to 1000 $\mu l\ l^{-1}$ together with 442 $\mu mol\ s^{-1}m^{-2}$ of photosynthetically-active radiation (PAR) for 20 hr day^{-1} resulted in 63% greater yield of 'Waldmann's Green' lettuce relative to those plants grown at a similar radiation at 350 $\mu l\ l^{-1}$ CO_2. Lettuce was harvested 19 days after seeding, which was 13 days after the onset of treatment.

We also have compared the yield of 'Waldmann's Green' leaf lettuce grown at enhanced light levels from a bank of parabolic reflector flood lamps filtered through water. Plants in both chambers were grown at 350 $\mu l\ l^{-1}$ CO_2, but one chamber was maintained at 920 $\mu mol\ s^{-1}m^{-2}$ of PAR, and another at 450 μmol. High photosynthetic photon flux density, or PPFD, increased yield 43% relative to lettuce grown at low PPFD. In addition, high PPFD caused a significant 4% increase in relative growth rate or RGR after 19 days.

Since yield of 'Waldmann's Green' was enhanced either by increasing PPFD or CO_2 alone, we examined the effect of the combination of these two parameters on lettuce growth. Plants were grown at 1000 $\mu l\ l^{-1}$ CO_2, with either 428 μmol of PAR or 905 μmol of PAR. A substantial 19% boost in RGR resulted in a 68% increase in dry weight of the high vs. the low-PPFD treatment. Clearly, there is a synergism between high PPFD/high CO_2 in stimulating growth of 'Waldmann's Green' lettuce,

and we observed the same pattern when plants were grown at 1500 µl l^{-1} CO_2 with high vs. low PPFD. By day 19, plant dry weight was greater for plants grown at 895 vs. 425 µmol of PAR. In addition, harvest index, the proportion of edible biomass, was not altered by high or low PPFD at high CO_2.

To further enhance lettuce yield, we are investigating effects of triacontanol, a 30-carbon primary alcohol reputed to have growth-stimulating effects with several plant species. A colloidal-dispersion of triacontanol at 10^{-7}M was sprayed to runoff on lettuce plants in one chamber 4 and 8 days after seeding. Both chambers were illuminated with 900 µmol $s^{-1}m^{-2}$ of PAR and maintained at 1500 µl $l^{-1}CO_2$. Leaf fresh and dry weight, as well as root and plant dry weight were stimulated by as much as 18% with application of triacontanol. The RGR of triacontanol-treated seedlings in this experiment was 555 mg of new dry weight assimilated each day for every gram of dry weight already present.

The combined effect of high PPFD, high CO_2, and triacontanol applied to 'Waldmann's Green' lettuce plants growing hydroponically also amounted to an average yield rate of 54 g dry weight of leaf lettuce per m^2 of growing area per day, during the exponential phase of growth. This contrasts with a figure of 4 g m^{-2} day^{-1} for open-field agriculture and 16 g m^{-2} day^{-1} for previous controlled-environment studies with lettuce. We feel that further progress can be made in optimizing production of this crop, particularly by shortening the lag phase of seedling development.

It is obvious from the lettuce studies that the ability to achieve high photosynthetic productivity is a desirable option for plant production in a bioregenerative life support system. However, another NASA-sponsored project in our laboratory involving effects of mechanical stress on plants is a cause for concern regarding the ability of plants to be productive in a microgravity space environment. Periodic gyratory shaking reduces stem elongation, but also dry weight gain and therefore photosynthetic productivity. We have been conducting mechanical stress research in a controlled environment with soybean, another CELSS candidate species which can provide protein for a vegetarian diet. Soybean plants shaken 3 times daily at 240 rpm on a gyratory platform were shaken for 5 minutes each time for 15 consecutive days. There were statistically significant reductions in dry weight gain, leaf area expansion, and stem elongation, but no significant differences in leaf specific weight or leaf specific water content. Growth dynamics analysis of shaken and undisturbed soybean plants during exponential growth further corroborated the interpretation that seismic stress inhibits photosynthetic productivity. The 4% inhibition of RGR found was due entirely to a decline in net assimilation rate, a measure of photosynthetic efficiency, which is measured as increments of dry weight gain per unit leaf area per unit time. The mechanical stress effect on RGR would have been even stronger if leaf area ratio had not partially compensated by increasing in response to stress.

Photosynthetic rates of whole plants then were monitored before, during, and following the morning, afternoon, and evening shake treatments.

Measurements taken on day 15 of treatment indicated no significant difference in CO_2 assimilation between control or shaken plants even for the afternoon and evening treatments, when seismic stress had been received only 5 hours earlier. However, photosynthesis began to drop within seconds after a shake began, and 5 minutes after the shake ended photosynthesis averaged 15% less than that of controls. Significant decreases lasted from 40-80 minutes following treatment, with morning treatments having the greatest effect and lasting the longest.

To determine what might be causing these transitory decreases in photosynthetic rate, transpiration and photosynthetic rates were measured for both upper and lower leaf surfaces of individual, attached soybean leaves. Twenty-five minutes after the afternoon shake, significant inhibition of both photosynthesis and transpiration had occurred, but only on lower leaf surfaces. Leaf resistance analysis also reflected these changes: there were significant increases in diffusive resistance through stomates for both water vapor and CO_2 only on lower leaf surfaces. Mesophyll resistance to CO_2 uptake was not significantly affected by shaking. Thus, transitory stomatal aperture reduction of the lower leaf surface appears to account for the reduction in photosynthetic productivity of soybean caused by periodic seismic stress.

The seismic disturbances that attend spacecraft acceleration, maneuver, machine operations, and even astronaut activity are a cause for concern, not only for their effects on plant growth and tropisms, but because they have gravity equivalents. Pulses of vibration in space may mimic gravity. Or will they retard tropistic curvature? Many questions remain regarding whether seismic disturbance will be a serious confounding factor for plant research in a microgravity environment.

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ACHIEVING MAXIMUM PLANT YIELD IN A WEIGHTLESS, BIOREGENERATIVE SYSTEM FOR A SPACE CRAFT

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Limitations to maximum plant yield are photosynthesis, respiration, and harvest index (edible/total biomass). Our best results with wheat equal 97.5 g total biomass m^{-2} day^{-1}. Theoretical maximums for our continuous 900 µmol photons m^{-2} s^{-1} = 175 g carbohydrate, so our life-cycle efficiency is about 56%. Mineral nutrition has posed problems, but these are now nearly solved. CO_2 levels are about 80 µmol m^{-3} (1700 ppm; ambient = 330 ppm). We have grown wheat plants successfully under low-pressure sodium lamps. The main factor promising increased yields is canopy development. About half the life cycle is required to develop a canopy that uses light efficiently. At that point, we achieve 89% of maximum theoretical growth, suggesting that most parameters are nearly optimal. The next important frontier concerns application of these techniques to the microgravity environment of a space craft. There are engineering problems connected with circulation of nutrient solutions, for example. Plant responses to microgravity could decrease or increase yields. Leaves become epinastic, grass nodes elongate, and roots grow out of their medium. We are proposing space experiments to study these problems.

Recent studies (Keller and Olson, 1984) indicate that higher plants (and perhaps algae) might play a key role in an economical life-support system for an orbiting space craft or for one on a long-duration mission from the earth into deep space. Such a bioregenerative life-support system has been called a Controlled Environment Life-Support System (CELSS). The U. S. National Aeronautics and Space Administration (NASA) is currently supporting several projects intended to provide baseline information for the design and construction of a CELSS.

A group of us at Utah State University are working on one of these projects entitled Studies on Maximum Yield of Wheat for the Controlled Environments of Space Craft. I am principal investigator, and Bruce G. Bugbee is co-principal investigator. Bugbee has spent full time working on this project for the past three years and is the real moving force behind the work reported in this paper. Other members of the team include Rulon S. Albrechtsen, John G. Carman, William F. Campbell, and Wade G. Dewey. In addition to our CELSS project, I am principal investigator of a project supported by the Space Biology Program of the Life Sciences Division of NASA. This project is entitled Gravitropism in Leafy Dicot Stems. The fundamental problems studied in our gravitropism project will hopefully find application in our CELSS project.

The goal of the plant part of a CELSS is to achieve maximum yields of edible biomass for standard inputs of physical mass (equipment, water, nurients), volume, energy, time, and human control and effort expended. Some of these problems (equipment, volume, and human inputs) are essentially engineering ones, to be solved after the biological problems have been mastered. The key biological problem to be investigated in ground-based studies is to achieve maximum yields per unit energy input, in minimum growing space, and in minimum time. Accordingly, yields are expressed as grams of edible biomass per watt per square meter per day: g W^{-1} m^{-2} day^{-1}. In our studies and in this report, however, we use units of moles of photons per square meter per second so that our results can be compared with other photosynthesis research. Yet watts are more appropriate units for a bioregenerative system in which energy input is critical.

Thanks to basic research in photosynthesis, maximum theoretical yields can be calculated. These calculations set the theoretical limits that could be achieved in a CELSS and hence establish the ultimate goals. The limitations are concerned primarily with photosynthetic efficiency (basic mechanisms of photosynthesis and radiation interception by the leaf canopy), the ratio of photosynthesis to plant respiration, and the harvest index (edible biomass/total biomass).

Consider the present state of the art based on our project with wheat. (There are also projects being run by other investigators on potatoes, lettuce, soybeans, algae, etc.) Our plants are grown in growth chambers in which light energy is provided by flourescent tubes combined with high-pressure sodium lamps. At the moment we achieve 900 µmol photons m^{-2} s^{-1}. These energies are provided 24 hours per day resulting in a total daily photon flux of 78 mol m^{-2} d^{-1}. (Natural sunlight reaches levels of 2000 µmols per m^{-2} s^{-1} at noon; about 60 mol m^{-2} d^{-1}). Under these conditions we have achieved a complete life cycle from planting to harvest in 62 days (90 to 100 days are typically required in the field). Biomass production has been as high as 97.5 g m^{-2} day^{-1}. In 62 days, we have obtained a total biomass of edible grain of 1488 g m^{-2} (dry weight). Hence our wheat yield is 24 g m^{-2} day^{-1} and our harvest index equals 0.246 (24.6%). Our total yields are excellent, as discussed below, but our harvest index could be almost twice our measured value according to the best agronomic experience.

Theoretical maximum yields can be calculated as follows: our light energy in the range of the spectrum utilized in photosynthesis is 900 µmol m^{-2} s^{-1} multiplied by 0.9 absorbance and divided by 12 photons per molecule of CO_2 fixed, which equals 67.5 µmols CO_2 m^{-2} s^{-1}. This value multiplied by 30 µg carbohydrate (µmol CO_2)$^{-1}$ equals 2025 µg carbohydrate m^{-2} s^{-1}. Since there are 86400 seconds per day, the theoretical maximum yield equals 175 g carbohydrate m^{-2} day^{-1}. Our efficiency (100 x 97.5/175) so far is thus 56%.

How can we close the gap between 56% efficiency and 100% efficiency? There are many parameters to manipulate. Consider a few.

1. TEMPERATURE. So far we have not really studied temperature. We use values (28°C) reported in the literature to be optimal for wheat with CO_2 enrichment. We might vary temperature between "day" and "night," but this would probably not help under our continuous 24-h irradiation. Higher temperatures could shorten the life cycle but would probably decrease yield per day. We don't expect further studies of temperature to increase efficiencies much, but there could be surprises.

2. HUMIDITIES. There are two possible adverse effects if humidity is too high. First, because transpiration is reduced under such conditions, leaf cooling is less, and leaf temperatures may increase above optimal levels when irradiation is as high as it is in our chambers. Second, because transpiration is reduced, mineral uptake may be reduced. Evidence from recent experiments in our laboratory suggests that this is an important effect when CO_2 levels are elevated, which causes partial stomatal closure. It is easier for us to maintain optimal nutrient conditions within plant tissue when humidities are lowered. This does not appear to be the case if plants were not growing so rapidly under the ideal conditions that are provided.

3. NUTRIENTS. We grow our plants with their roots in aerated, circulated, nutrient solutions. We find that mineral nutrient concentrations in these solutions can be very critical, and responses to nutrients can change as other parameters change--as just noted for humidity. We have expended much time developing adequate nutrient solutions and techniques to provide them, but we have not yet solved all the problems. Our youngest plants sometimes show deficiency symptoms that disappear as the plants mature (i.e., when they reach about 2 weeks of age). Iron, manganese, phosphate, and other nurients can be problems, especially as the pH increases rapidly as nutrients are absorbed. We have been able to control pH within fairly narrow limits by providing a balance of ammonium and nitrate ions and by using an automated system to add acid when needed. Ammonium ions are exchanged for hydrogen ions produced in the plant roots, decreasing pH, and nitrate ions are exchanged for bicarbonate ions from the roots.

4. CARBON DIOXIDE CONCENTRATION. CO_2 is typically limiting at ambient levels (320 ppm = 15 $\mu mol\ m^{-3}$). Yields are greatly increased when CO_2 levels are raised around the plant leaves. We elevate to 80 $\mu mol\ m^{-3}$ (1700 ppm). Stomates tend to close completely when CO_2 levels are elevated too high, but so far we have not been able to reach CO_2 levels that are high enough to produce this effect. (We must modify our measuring equipment to do this.) It would be possible to manipulate other gases, and lowering oxygen levels would also increase rates of photosynthesis, probably without stomatal closure. So far we have not invested the time and money required for such a study.

5. THE RADIATION ENVIRONMENT. Light is the energy source for photosynthesis and is thus critical to plant yield. There are several aspects of the light environment that must be studied.

A. Light Level (Irradiance). Increasing irradiance would not help in the above example to raise efficiency; indeed, it might lower the efficiency of photosynthesis if the process had already reached light saturation. If saturation had not been achieved, however, an increase in light level might raise absolute yields expressed as $g\ m^{-2}\ s^{-1}$. With today's technology it is difficult and expensive to obtain light levels much above the 900 $\mu mol\ m^{-2}\ s^{-1}$ that we use, although it can be done. We would like to provide irradiances high enough to satisfy ourselves that saturation had been achieved and that further increases would only lead to decreased efficiency on an energy basis. Yet at the moment there seem to be more fruitful approaches to try.

B. Light Quality (Spectrum). The balance of wavelengths can be modifed in an almost infinite variety of ways, so there is much room for experimentation. We have taken one of these approaches with rather interesting results. We have grown healthy wheat plants that produce normal grain under low-pressure sodium lamps. The energy from these lamps is nearly all confined to one line in the spectrum at 589 nm. The lamps are efficient at producing light energy, and so they are worth considering for future use in a CELSS. Furthermore, we find that seconday metabolites (specifically lignin) are reduced under these lamps. Activities of two key enzymes (PAL and TAL) in the synthesis of secondary metabolites are greatly reduced in tissues grown under these lamps. This could mean that primary metabolites (starch, protein, fat) could be higher in the plants, although we have not yet shown this to be the case.

In general, light quality has many important photomorphogenetic effects. For example, light quality might influence the partitioning of assimilates in such a way that harvest index is increased.

C. Light Cycling. Daylength (photoperiod) has profound effects on many plant responses including flowering, seed filling, tillering (formation of axillary stems in grass plants), and dormancy. Hence photoperiod can and does influence the duration of the life cycle as well as the harvest index. A few plants including wheat have high yields and short life cycles under continuous high light levels. Thus our choice of plant may be especially fortunate in this regard. Nevertheless, future experimentation might show that yields can be increased per watt of input energy by subjecting the plants to various light-dark or bright-dim cycles. Many other species respond favorably to such treatments.

D. Canopy Development. Our results so far show that this is probably the most important parameter. The above calculation assumes that 90% of the incoming radiation is absorbed. Early in the life cycle, this is clearly not the case, since there is much space between plants. The leaf-area index (LAI) expresses the number of layers of leaf tissue through which a given ray of light must pass (on average) before it strikes the ground. For maximum absorbance of incoming radiation, a high LAI is essential. Wheat reaches an LAI of 6 to 8 in the field and has reached 14 in our controlled conditions. In the early part of the life cycle, however, LAI is inadequate, and it takes about half of the life cycle to close the canopy so that most of the radiant energy is absorbed. At that point, light at the bottom of the canopy is only about 10 to 20 $\mu mol\ m^{-2}\ s^{-1}$. Absorption is extremely high.

We have modified one of our growth chambers so that we can measure photosynthesis of an entire canopy. To do so, we had to close virtually all of the leaks in the chamber; we had to install large cooling coils so that they could function at close to ambient temperature, preventing condensation of

free water that would absorb CO_2; we had to seal the roots out of the chamber volume so that their respiration would not influence measurements; we accurately measure the volume of incoming air and its CO_2 concentration; and we measure the concentration of CO_2 in the outgoing air. When all of this is done, we can calculate the amount of CO_2 being absorbed by the canopy, and we find that it is as high as 60 µmol CO_2 m^{-2} s^{-1}. This compares with 67.5 µmols CO_2 m^{-2} s^{-1} in our above calculation of the theoretical maximum photosynthetic rate based on absorbed energy. Thus the efficiency (100 x 60/67.5) equals 89%. This value, approaching the maximum possible, suggests that we cannot improve efficiency very much by manipulating parameters as suggested above, although attempts to do so are probably still worth the effort. The important manipulations have apparently already been made.

In terms of obtaining maximum yields of grain for astronauts, however, two extemely challenging problems remain: first, it is essential to close the canopy as quickly as possible. Second, the harvest index must be increased. There may be engineering ways to close the canopy (plants close together when young, spreading as they mature?), although simple solutions are not readily apparent. We have every possibility of increasing the harvest index based upon experience in the field. Here is where manipulations of parameters might be highly profitable.

Another important approach is to select cultivars that have a high genetic potential for rapid canopy closure and high harvest index. We have tested about 250 cultivars, and we find great differences in their growth, yield of grain, and harvest index under our conditions. Harvest index is usually highest in dwarf (30-cm) cultivars, but so far their overall yields are relatively low. Particularly promising are uniculm cultivars that produce only one or a few tillers per plant. Such a cultivar could be planted with plants very close to each other so that the canopy closes early in the life cycle. The two cereal breeders on our project (Albrechtson and Dewey) have already incorporated some desirable agronomic features into a uniculm cultivar. In essence, they are designing a special wheat plant for a CELSS.

Still another approach is to reproduce highly productive hybrids (hybrid vigor), which normally do not breed true, by tissue cloning. Single cells might be used. Carmen and Campbell are just now initiating research on this expanded part of our project.

6. What is the Role of Gravity in all This? Microgravity could strongly influence many of the above ideas and conclusions. There are two kinds of problems: first, how plant responses might be affected by microgravity; and second, the engineering problems unique to weightlessness. As to engineering problems, for example, under microgravity conditions there is no convection to help move fluids (air and nutrient solutions). These must be moved by creating pressure gradients and allowing bulk flow. (We must pump and blow.) Furthermore, there is no gravitational acceleration to hold liquids in their containers, to it is essential to close all systems that contain fluids. It is comforting, however, that space engineers now have had much experience with these problems, which are certainly not unique to space farming.

What are some of the plant responses to microgravity and their possible effects on yield? The most obvious plant response observed on plants grown under simulated microgravity conditions (i.e., on clinostats) or in our limited experience in space experiments is a downward bending of leaves called epinasty. Stems may also twist in various directions, and sometimes roots grow out of the medium. In our project on gravitropism, we have tested the idea that leaf epinasty observed on a clinostat might be caused by mechanical stresses rather than simulated weightlessness. We are unable to produce epinasty by mechanical stresses, but we can produce epinasty even when mechanical stresses are greatly reduced, providing weightlessness is simulated (by carefully inverting plants at 20-minute intervals). These results support the idea that plant responses observed on clinostats will also be observed under microgravity conditions. So far experiments in space have borne this out. (Unfortunately, the rather extensive Russian work with higher plants in the space environment has not been well reported in the literature available to us.) Consider some possible effects of microgravity on plant responses that might influence yield.

At the cellular level, preliminary results indicate that lignin synthesis might be reduced (Cowles et al., 1982) and that root cells might exhibit important abnormalities (Slocum and Galston, 1982).

We expect that vegetative growth will be influenced in various, sometimes subtle ways that might be of only moderate interest to space biology but that could significantly reduce (or perhaps even increase; Hoskisaki, 1983) yields of edible plant material. Germination, for example, might be affected, and we have every reason to expect that orientation of stems, leaves, and roots will be upset by microgravity--unless the orientation can be reestablished by unidirectional light. Mature nodes of grasses elongate when they are rotated on a clinostat, and this could happen to our wheat plants in microgravity. Possible effects on yield remain completely unknown. The formation of axillary shoots in grasses (tillering) is strongly influenced by plant-growth-regulator levels in the tissues, (Johnston and Jeffcoat, 1977), and the observed leaf epinasty suggests that such plant growth substances are upset by microgravity. Thus tillering will quite likely be influenced by microgravity; it is certainly known to be highly sensitive to nutrient levels, light, temperature, photoperiod, and perhaps other parameters. The partitioning of assimilates to leaves, roots, stems, and especially maturing fruits and seeds will certainly be influenced by the arcitecture of the plant as it develops under weightlessness and might well be influenced by changed levels of growth regulators.

Reproductive growth could also be influenced in many subtle ways that might ultimately influence yield. These include flower initiation (known to be highly sensitive to many parameters including hormone balance), pollen development, embryosac formation, pollination, fertilization, seed, filling, and, finally, maturation of seeds and fruits. The viability of the resulting seeds might also be influenced by microgravity (although there is no real evidence to suggest this). Most of these plant responses could be studied using the presently available plant growth unit (PGU). This unit has already been used in a few space-shuttle experiments and could be used to study germination, orientation, elongation of mature grass nodes, pollen and embryo sac formation, pollination, fertilization, seed development, and viability of

resulting seeds. Tillering, partitioning of assimilates, photosynthesis and respiration rates, and flower initiation, will probably require more sophisticated facilities even for initial studies. Ultimately, all of these factors should be studied with highly sophisticated growth chambers (having larger volume, high light levels, and other features controlled) to see how they all combine to influence edible yield.

If anywhere near enough food could be produced in a CELSS to support the astronauts in a space craft, all the oxygen used by these astronauts would be regenerated, and the carbon dioxide produced would be converted to carbohydrate or other plant material. Actually, because not all the plant material produced in plant growth can be eaten and digested, the wastes (over 50% under the best of conditions) will have to be oxidized (burning or wet oxidation) to keep oxygen levels from becoming too high and carbon dioxide levels from dropping so low that they might cause "altitude" sickness in astronauts.

To summarize, future space research must emphasize:

1. The engineering techniques of growing plants under weightlessness.

A. Optimizing environment to maximize yields.

B. Doing this with small, light-weight, efficient equipment that requires low energy inputs and is not too expensive. The greatest possible energy savings could be achieved if sunlight could be used directly for plant photosynthesis, since it is extremely expensive and inefficient to convert sunlight to electrical energy and then use this to produce light for plant growth.

2. Plant responses to microgravity conditions.

A. Developmental responses, as outlined above.

B. Biochemical responses including those influenced by plant growth substances.

C. Yield, which is a practical summation of the other responses.

What if plant yield is substantially reduced under microgravity conditions in spite of good engineering control of all parameters? Will it be necessary to spin the space farm to provide an artificial gravity? This is a possibility that should be kept in mind during all the studies mentioned in this paper. If centrifugal accelerations are necessary to get proper yields, this fact will strongly influence the design of future space vehicles that utilize a CELSS.

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EFFECTS OF NON-WEIGHT BEARING ON FRACTURE HEALING

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The relationship of weight bearing (WB) to bone metabolism presents a unique problem for fracture management. A number of transitory as well as prolonged conditions (bed rest, space flight, paralysis and disuse) result in osteopenia. In transitory situations a return to weight bearing usually corrects the osteopenia. The need to investigate the effects of non-weight bearing (NWB) on bone metabolism and morphology led Morey to develop an inverted suspension cage (ISC) for rats. This suspension cage was modified by Sweeney and used in this study to compare the effects of WB and NWB on fracture (fx) healing in the rat. Methods: The ISC allows the rat to be suspended by the tail in a preparation similar to human tranction. Animals were divided into four treatment groups: fx-WB, fx-NWB, nfx-WB, nfx-NWB. Fractures were induced by osteotomies in the right fibula of each fx-ed rat. The animals were placed in regular or ISC cages. Tetracyclines were administered on selected days. Results of this experiment paralleled those of our previous experiment, despite the use of the fibula rather than the femur as the fractured bone. Weight: NWB animals showed no weight loss during the 36 days; however, fx-NWB animals showed significant weight loss at 9 days but recovered this loss by 18 days. Histology: In the fx-NWB animals there was minimal periosteal involvement, poor callus formation, osteopenia and delayed chondro- and osteogenesis. The Tetracyclines amply demonstrated the inhibition of chondro- and osteogenesis in NWB animals. Conclusion: Weight bearing is an essential component of fx healing in rats. WB influences callus formation, periosteal bone formation and chondro- and osteogenesis. On the other hand, NWB delays and alters the process of fx healing in rats. In the absence of significant weight loss, suspension stress was not considered a major factor in delayed fx healing in rats.

The relationship of weight bearing (WB) to bone metabolism presents a unique problem for fracture management. A number of transitory as well as prolonged conditions (bed rest, space flight (2), paralysis, and disuse (1)) result in osteopenia. In transitory situations a return to weight bearing usually corrects the osteopenia. The need to investigate the effects of non-weight bearing (NWB) on bone metabolism and morphology led Morey (3) to develop an inverted suspension cage (ISC) for rats. This suspension cage was modified by Sweeney and used in this study to compare the effects of WB and NWB on fracture healing in the rat.

Materials and Methods:

Treatment groups.

The 72 experimental animals were equally divided into WB and NWB groups. In turn the 36 rats in each of these groups were divided into fx (18) and nfx (18) groups. Six animals in each group were sacrificed at 9, 18, and 36 days. Each group received fluorescence (tetracyclines) tags on the schedule shown below:

Groups	Days Tetracycline Injected
9d	1 and 8
19d	1, 6, 15 and 17
36d	1, 6, 31 and 35

The tetracyclines were given IP (75mg/kg). No unusual distress was noted on or following injection.

Fibular Osteotomy

Rats were anesthetized with sodium pentobarbitol, 0.1 ml per 100 g body weight. After sleep was induced they were shaved over the lateral aspect of the right lower leg and the skin preped with Betadine solution. Rats were brought into the operating room and draped sterily. A 1 cm incision was made on the lateral aspect of the lower leg above the fibula. Facia was incised and muscle plains were bluntly dissected down to the fibula. An osteotomy was performed with a synthes mini compressed air oscillating saw with a 0.5 x 6 mm blade. Cooling was effected with normal saline. Facia and skin were closed with 6-0 nylon interrupted sutures. Rats were observed until fully awake and then returned to their respective cages WB or suspended NWB.

Suspension (ISC)

Adult Long Evans rats, 9-11 months of age with an average weight of 519±63 g were used. Rats were restrained in a hospital bath towel and a tail suspension device similar to human skin traction applied to the proximal two-thirds of the tails in the following manner. Rats tails were: washed with a warm, mild soap solution; rinsed thoroughly with warm water; cleaned with 70% ETOH and dried with a warm air blower; use gauze for each tail cleaning step; sprayed with tincture of Benzoin and again dried with a warm air blower. A 1 x 15 cm strip of orthopedic traction tape was applied to the lateral side of the proximal two-thirds of the tail. A spreader block was placed between the two sides of the traction tape to separate them and afford an attachment of the x-y device. The traction tape and tail were wrapped with 2.5 cm bias cut stockinette and secured with a 1 x 3 cm piece of tape (Fig. 1).

The ISC allows the rat to be suspended by the tail in a preparation similar to human traction. The rat is suspended from a low-friction x-y device permitting unrestricted movement within the cage. This type of suspension evoked minimal stress as evidenced by eating and drinking habits. In addition, the animals did not appear to lose weight.

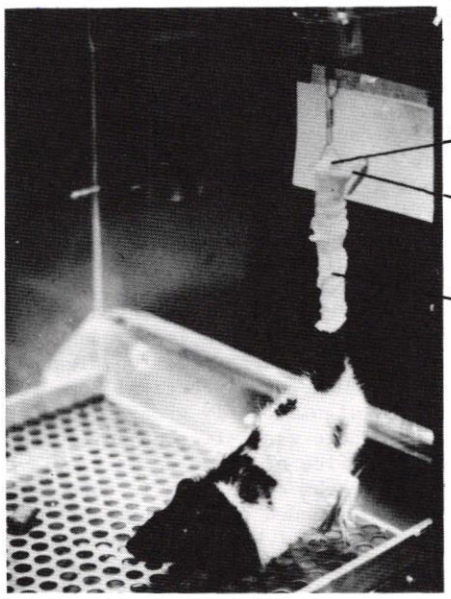

FIGURE 1

At the termination of each experimental group the animals were euthanized and the following bones were removed from each rat: the fx-ed and non-fx-ed fibulae, femora, ulnae, radii and a portion of the spinal columns. The entire fx-ed bone and its pair were fixed in neutral formalin for processing in methyacrylate. A portion of all other bones was fixed in neutral formalin. The non-decalcified plastic sections were stained with Goldner's or left

unstained for examination by polarized light microscopy and UV microscopy to visualize fluorochrome labels.

Results

Histology: The most marked differences in fracture healing in NWB rats were: increased osteopenia, increased cortical vasculature. The callus of NWB rats was larger but lacked the organization usually seen in fracture healing.

Polarized light: The major difference involved the woven bone appearance of the remodelling callus of NWB rats when compared to WB rats. This difference was particularly striking in the newly formed bony trabeculae. Distinct parallel arrays of collagen fibers were present in WB rats whereas in NWB rats the collagen was present in arched arrays in woven bone. The cortex of NWB rats showed decreased collagen organization from the internal to the external circumferential lamellae. This was especially prominent in the fracture region (Figs. 2 and 3).

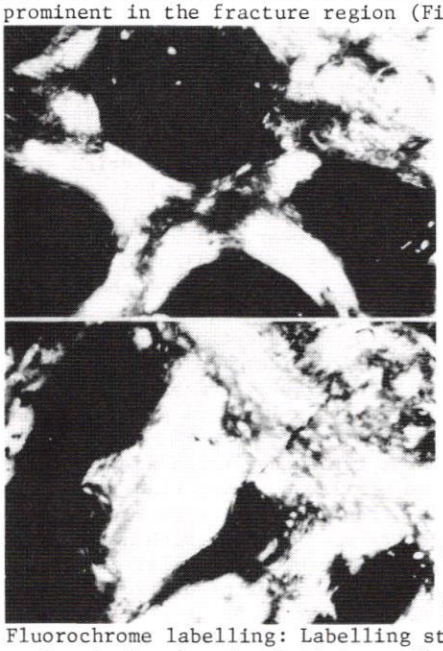

FIGURE 2

FIGURE 3

Fluorochrome labelling: Labelling studies in control animals showed a bridging callus with a marked increase in periosteal new bone formation. This was in contrast to the callus of suspended animals which was in disarray with little evidence of remodelling. In both control and suspended animals labelling was seen in association with infrequent osteons. Figure 4 illustrates fluorochrome labelling in the cortex of a fx-NWB rat. Two distinct fluorochrome labels can be seen, FLA and FLB. The space between them indicates the amount of cortex formed between injection and sacrifice of the animal. Figure 5 is of a control animal in the fx-WB group. Four distinct fluorochrome lines can be seen FLA, FLB, FLC, FLD. In comparing the two figures the difference in the amount of cortical bone formed in the WB rat is significantly greater than that formed in the NWB. There was a five day difference in the dates of sacrifice of these animals, 31 versus 36 days, however, the difference in bone formation was not solely depended on this time but rather reflects the impact of WB on osteogenesis. NWB rats showed a marked increase in the cortical vascular bed. The majority of vessels extended from the periosteal layer. However, a contribution by the intramedullary vessel could not be negated. Within the callus vascularity was comparable between the two groups. Fluorochromes were present in calluses of both groups but was more pronounced in NWB rats indicating on-going remodelling.

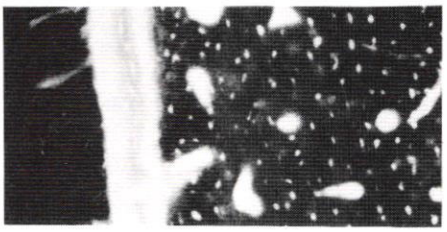

FIGURE 4

FIGURE 5

Conclusion: Fracture healing in NWB rats was different from healing in WB animals. In the WB groups healing was characterized by increased osteopenia, delayed callus remodelling and abnormal remodelling of the associated cortex. The process of callus formation was delayed 18-36d in the NWB animals. In WB rats a well developed callus was present with bridging accomplished at 18 days. In the NWB only a poorly formed callus was present at 9 days and bridging was not evident until 36 days. With the delay in callus there was also a delay in chondro- and osteogenesis. Cartilage islands were present in the WB at 9-18 days and in the NWB 36 days. We concluded that weight bearing was essential for appropriate callus formation, early chondro- and osteogenesis, and normal mineralization.

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TISSUE FLUID SHIFT, FORELIMB LOADING, AND TAIL TENSION IN TAIL-SUSPENDED RATS

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Abstract: The tail suspension model (head-down tilt) simulates hypogravity in terms of musculoskeletal loss in the rat. However, little is known of tissue fluid shifts and body weight distribution in this model. Tissue fluid pressures were measured by wick catheters in 12 Munich-Wistar rats before, during and after 48 hrs. of tail suspension (about 30° head-down tilt). Subcutaneous tissue fluid pressure in the neck increased from -2.2 ± 0.4 (normal horizontal position) to +4.0 ±1.5 cm H_2O ($p<0.05$) during tail suspension, indicating a cephalic fluid shift and significant edema during head-down tilt. In a separate study, 6 rats were suspended at 30-70°, and forelimb load and tail tension were measured by a balance and force transducer, respectively. Approximately 50% of body weight (BW) was loaded on forelimbs at a head-down tilt angle of 30° and forelimb load declined linearly to 10% BW at 70°. Furthermore, tail tension increased from 50% BW at 30° to 85% BW at 70°. These results indicate that less than normal loads are applied to forelimbs of rats suspended at angles >30° and that the tail bears an increasing proportion of the rat's body weight at head-down tilt angles >30°.

Introduction: Human studies suggest that blood and tissue fluids are shifted from the legs to the chest and head in response to weightlessness or simulated weightlessness. This fluid shift is documented by leg volume loss, facial edema and negative water balance during weightlessness (7, 11) and by similar responses, plus a pronounced diuresis during weightlessness simulation (1-3). Also, interstitial fluid pressure of leg tissues decreases significantly during water immersion (6) and 5° head-down tilt (5). During weightlessness, tissue dehydration in legs of humans is probably due to loss of a hydrostatic pressure gradient from heart to foot with consequent lowering of microvascular blood pressures in dependent tissues (3).

In animals, specifically rats, the tail suspension model (head-down tilt) is a well-documented maneuver to simulate weightlesssness in terms of loss of bone (8) and atrophy of antigravity muscles (10). However, little is known of fluid shifts and body-weight distribution in this animal model of zero-gravity simulation. Therefore, the objectives of this study were to : 1) measure shifts of interstitial fluid in this model and 2) determine distributions of body weight as function of angle of head-down tilt in the rat.

Methods: Eighteen Munich-Wistar rats (140-280 gm) were suspended by the tail using methods of E.R. Morey (8). In fluid shift studies of 12 rats, wick catheters (4) were inserted in subcutaneous tissue of the neck during general anesthesia (100 mg/kg inactin intraperitoneally). After recovery, interstitial fluid pressures were continuously monitored during 2 hours of normal ambulation, 48 hours of tail suspension (about 30° head-down tilt), and again after 24 hours of normal, horizontal position. Interstitial fluid pressures were measured by a pressure transducer, connected to a strip-chart recorder (Fig. 1).

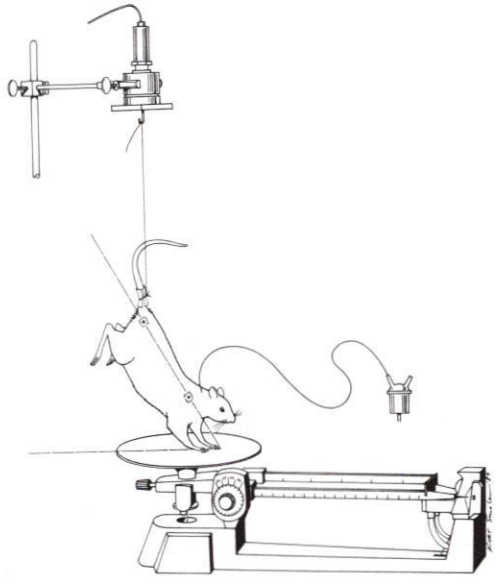

Figure 1. Measurement of neck subcutaneous pressure, load on forelimbs, and tail tension in the tail-suspension model of simulated weightlessness. Angle of head-down tilt is measured by a goniometer using a line through the dorsal aspects of the tail base and shoulder.

In separate experiments, 6 rats were suspended at 30°, 40°, 50°, 60° and 70° while the load on forelimbs and tail tension were measured by a balance and force transducer, respectively (see Fig. 1). The rats were blindfolded to minimize their activity and maximize accuracy of the body-weight measurements. Weights were expressed as a % of body weight for each rat.

Results: Subcutaneous tissue fluid pressure in the neck increased significantly from -2.2 to +4.0 cm H_2O ($p<0.05$) after 48 hours of head-down tilt at about 30° (Table 1). Subcutaneous pressure was still slightly elevated after 24 hours of readjustment to horizontal position.

Table 1. Fluid shift indicated by increased interstitial fluid pressure in neck of tail-suspended rats (mean pressure ± S.E. cm H_2O)

Normal, baseline	-2.2 ± 0.4
30° head-down tilt (48 hours)	+4.0 ± 1.5
24 hours post-tilt	-1.1 ± 1.2

Body-weight distributions were linearly related to tilt angle (Fig. 2). At 30°, a normal load of 50% body weight was placed on the forelimbs. Tail tension represented the other 50% of body weight at this angle. Forelimb load decreased linearly to 10% of body weight at 70° (correlation coefficient r = 0.995), whereas tail tension increased to 85% of body weight at 70° (r = 0.996).

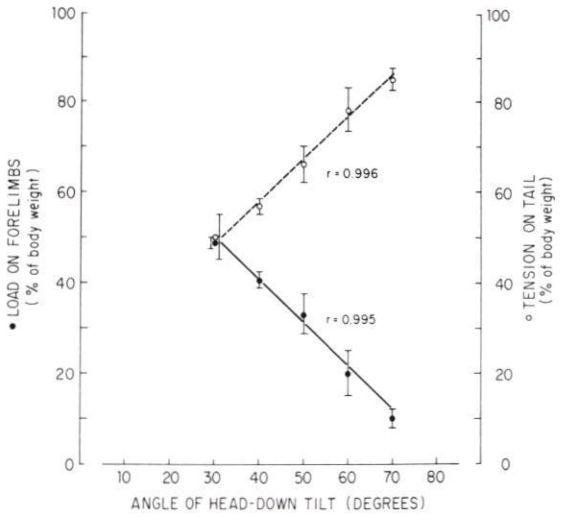

Figure 2: Load on forelimbs and tail tension (both expressed as % of body weight) as a function of head-down tilt angle in the rat.

Discussion: The tail-suspension model produces a cephalic fluid shift and significant edema in the rat neck during head-down tilt, and thus, provides a good animal model for simulating zero-gravity in terms of a fluid shift. This fluid shift may be an important factor in the loss of musculoskeletal tissues previously documented for this model.

Normal loads are applied to forelimbs of rats suspended at 30° with loads decreasing linearly at angles greater than 30°. In the tail-suspension model, the tail bears an increasing proportion of the rat's body weight at tilt angles over 30°. These tensions represent abnormal and high loads which are deserving of further study. Finally, our studies of body-weight distributions during head-down tilt in the rat suggest that rats exposed to harness suspension (9) may be abnormally loaded over their chest and abdomen.

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THYMIC INVOLUTION IN THE SUSPENDED RAT MODEL FOR WEIGHTLESSNESS: DECREASED GLUCOCORTICOID RECEPTOR CONCENTRATION

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Hindlimb muscle atrophy, thymic involution and adrenal hypertrophy in rats during spaceflight can be simulated using suspension models. Skeletal muscle and thymus are sensitive to glucocorticoids (GC) and previous studies have demonstrated that muscle atrophy in suspended rats is associated with increased GC receptor concentration. The objectives were to confirm thymic involution during suspension, and determine if involution correlated with increased GC receptor concentration. Seven days of antiorthostatic (AO) suspension of rats produced a significant ($P<0.001$) reduction in thymic wet weight not associated with an alteration of % water content. GC receptor concentration (pmol/mg protein) decreased 20% ($P<0.025$) in thymus glands from 7 day AO suspended rats. Suspension, therefore, is associated with involution of the thymus but this is not dependent upon AO positioning. Thymus GC receptor concentrations were depressed in 7 day suspended rats, in contrast with previous observations on skeletal muscle, suggesting that different mechanisms may underlie these responses.

INTRODUCTION

Differential muscle atrophy, thymic involution and adrenal hypertrophy have been observed in rats exposed to weightlessness in the COSMOS series of biosatellites (2). Caren et al (1) have previously indicated that thymic involution occurred in the suspended rat simulation of responses to weightlessness. The suspended rat model is also associated with hindlimb muscle atrophy and adrenal hypertrophy (4,5). The sensitivity of skeletal muscle (6) and the thymus gland (3) to adrenal glucocorticoids (GC) has been well documented. This sensitivity is mediated by receptor proteins exhibiting specific affinities for GC. The hormonal responsiveness of a tissue is defined both by the concentration of the free hormone and the tissue concentration of specific hormone receptors. We have previously reported (5) that hindlimb muscle disuse and unloading produced by suspension of rats is characterized by differential atrophy of selected hindlimb muscles (soleus gastrocnemius = plantaris extensor digitorum longus). This is associated with an increase in GC receptors which reflects the differential nature of the muscle atrophy (7). The initial objective of the present experiments was to confirm that thymic involution occurred in the whole body suspended rat, the time course of this response, its relationship to the orientation of the gravitational vector, and the potential for recovery responses. The second objective was to determine if thymic involution correlated with an increased tissue concentration of GC Greceptors, suggesting similarity in the potential mechanisms underlying the effects of suspension on skeletal muscle and the thymus.

METHODS

Age-matched male Sprague-Dawley rats weighing 180 - 200g were suspended in a denim/velcro harness in an antiorthostatic (AO, head-down tilt 20°) or orthostatic (O, horizontal) position as previously described (4) or maintained in metabolism cages for 7 days. One group of rats was removed from suspension after seven days and allowed to recover in metabolism cages for a further seven days. Rats were sacrificed by decapitation, thymus glands excised and wet and dry weights determined. Tissues were homogenized in buffer (10mM Tris, 1.5mM EDTA, 10mM monothioglycerol, 10mM molybate and 10% glycerol, pH 7.5), cytosol prepared by centrifugation at 100,000 x g for 30 minutes, and incubated in duplicate for 24 hours with increasing concentrations of ^{3}H-dexamethasone (0.2 - 50nM) in the absence (total binding) and presence (nonspecific binding) of a 100-fold excess of unlabeled dexamethasone at 2°C. Incubations were terminated by adsorption of free steroid with dextran-coated charcoal, centrifuged, and aliquots of the supernatant counted to determine hormone binding. Specific binding to receptor sites was determined as the difference between total and nonspecific binding. Maximum binding (Bmax) and apparent dissociation constants (Kd) were determined by Scatchard analysis. Statistical comparisons were made with the Student-t test. Levels of statistical significance are given in the text and tables.

RESULTS AND DISCUSSION

The absolute (mg) wet weight of the thymus gland decreased rapidly during the first two days of AO suspension (Table 1). This weight loss continued at a reduced rate through the remainder of the seven day suspension period and resulted in a 50% decrease in tissue weight during the first week of suspension. A similar response (52% decrease) was observed if the tissue weight was expressed relative to body weight, indicating a true involution. These results confirm the previous observations of Caren et al (1), who observed a 30% decrease in thymus wet weight between days 10 and 16 of suspension. Alterations in thymic weight were not the result of changes in per cent water content as this parameter remained relatively unchanged. Postsuspension recovery was associated with increased absolute and relative thymic weights, suggesting that the effects of suspension on the thymus were reversible. Quantitatively similar alterations in thymic weights were observed in rats suspended in an O position (data not shown), indicating that head-down tilting was not required for the thymic response.

Since suspension was associated with hypoplasia of the thymus gland, GC receptor content of thymus tissue was investigated to determine if involution could have resulted from alterations in tissue sensitivity to GC. Preliminary studies indicated that the receptor assay was linear with respect to protein con-

centration in the incubations. Since intact animals were utilized, it was also necessary to investigate the effects of endogenous GC on receptor measurements. Thymus cytosol prepared from adrenalectomized rats was preincubated with or without various concentrations of corticosterone, the primary GC in the rat, prior to receptor determinations to determine the extent of exchange with ^{3}H-dexamethasone. Corticosterone at physiological concentrations did not significantly effect determination of Bmax or Kd using the techniques described above. GC receptor concentrations in thymus glands obtained from both seven day AO and O suspended rats were decreased significantly compared with age-matched metabolism cage controls (Table 2). This significant reduction in receptor concentration was not associated with an alteration in the apparent dissociation constant of the receptor, suggesting that the kinetics of the hormone receptor interaction were unaffected by suspension.

The response of thymus GC receptor levels to suspension-induced involution was distinctly different from that observed in atrophic hindlimb muscles from suspended animals (6). While muscle atrophy was associated with elevated GC receptor levels, involution of the thymus correlated with a decreased GC receptor content. This could suggest that different mechanisms underly the responses of these tissues to suspension. It is of interest, however, that GC receptor levels in the thymus were responsive to suspension. This might indicate that GC receptor alterations in skeletal muscle and thymus follow a different time course and that the results from the two studies are not strictly comparable.

Table 1. Wet weights and % water contents of thymus glands from control (C), antiothostatic (AO) suspended and recovery (R) rats.

Group	Day	Wet Weight (mg)	Wet Weight (mg/100g)	Water Content (%)
C	0	597±37* (8)	322±18 (8)	79.7±0.1 (8)
AO	1	502±33+ (8)	291±15 (8)	79.3±0.1 (8)
AO	2	385±24+ (9)	236±14+ (9)	79.6±0.1 (9)
AO	3	346±42+ (6)	212±20+ (6)	79.8±0.1 (6)
AO	7	301±21+ (9)	166±9+ (9)	77.7±2.2 (9)
C	7	625±24 (9)	270±21+ (9)	79.5±0.2 (9)
R	7	650±55 (5)	255±11+ (5)	79.5±0.2 (5)

*Mean ± SEM (number of observations)
+Significantly (P<0.05) different from day 0 C

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Table 2. Maximum specific ^{3}H-dexamethasone (Bmax) and apparent dissociation constant (Kd) of thymic cytosols prepared from age-matched control (C) and seven day suspended antiorthostatic (AO) and orthostatic (O) rats.

	Bmax (pmol/mg. protein)	Kd (nM)
C	0.87 ± 0.04* (7)	2.8 ± 0.3 (7)
AO	0.66 ± 0.07+ (5)	2.8 ± 0.4 (5)
O	0.68 ± 0.04+ (5)	3.7 ± 0.7 (5)

*Mean ± SEM (number of observations)
+Significantly (P<0.025) different from C

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CARDIOVASCULAR AND HORMONAL (ALDOSTERONE) RESPONSES IN A RAT MODEL WHICH MIMICS RESPONSES TO WEIGHTLESSNESS

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Cardiovascular responses and fluid/electrolyte shifts seen during spaceflight have been attributed to cephalad redistribution of vascular fluid. The antiorthostatic (AO) rat (suspended, head-down tilt of 15 - 20°) is used to model these responses. This study documents that elevated blood pressures in AO rats are sustained for periods of up to seven days, compared with presuspension values. Increased blood pressures in AO rats suggests a specific response to AO positioning, potentially relatable to a cephalad fluid shift. To assess a role for hormonal regulation of sodium excretion, serum aldosterone levels were measured. Circulating aldosterone concentrations were seen to increase approximately 100% during seven days of AO suspension concurrently with a pronounced natriuresis. These results suggest that aldosterone may not be inolved in the long term regulation of increased Na^+ excretion in AO animals. These studies continue to show the usefulness of models for the development of animal protocols for space flight.

INTRODUCTION

The overall objective of these studies is the continued testing of an animal model which can be used to assess changes in cardiovascular related fluid and electrolyte imbalances seen during weightlessness. In general, cardiovascular responses as well as fluid and electrolyte shifts seen during spaceflight have been attributed to a cephalad redistribution of vascular fluid. This hypothesis, however, remains to be confirmed in the rat. The suspended head-down tilted (antiorthostatic = AO) rat is used as a model to simulate these responses.

Initial studies (6) showed that antiorthostatic positioning of rats for one and three day periods resulted in significantly elevated blood pressures (mean arterial pressure, MAP, and systolic and diastolic pressures, SP and DP). These initial studies further revealed that at day three of suspension there appeared to be a reduction in the response to the additional stress of head-up tilt (70°). This was interpreted as an adjustment to AO positioning. Questions remained, however, as to whether AO suspended rats adapt to lengthened periods of suspension and the capacity of the cardiovascular system to recover following removal from suspension.

Previous reports from this laboratory have demonstrated that AO positioning of the rat resulted in pronounced diuresis, natriuresis and kaliuresis (2). Of the various endocrine factors which could be involved in these fluid and electrolyte disturbances, we have focused on the role of aldosterone. While others have emphasized the potential role of antidiuretic hormone, less attention has been given to an examination of the involvement of aldosterone. Aldosterone, synthesized in the zona glomerulosa of the adrenal cortex, acts upon the renal tubular epithelium to promote retention of sodium and a concomittant loss of potassium. Assuming that sodium is the primary ion regulated by this endocrine pathway, then the natriuresis associated with AO suspension could be accompanied by a depressed circulating level of aldosterone.

The specific objectives of the present work were: 1) to determine blood pressures in rats suspended in an AO position for up to seven days, 2) to examine the readjustment of cardiovascular parameters following removal from suspension, and 3) to assess serum aldosterone concentrations and urinary excretion of water, Na^+, and K^+ during AO suspension of rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180 - 200g were suspended for up to seven days in a denim/velcro harness in a head-down tilt position (15° - 20°) as described previously (2,5). Three to four days prior to suspension a polyethylene cannula (PE 50) was surgically placed in the left carotid artery and exteriorized behind the neck. Blood pressures were determined by connecting the saline-filled cannula to a Statham-Gould pressure transducer (model P23) with the diaphragm held at heart level. Pressures were monitored on a Grass (model 79) recording system. Blood pressures were measured daily between 12:00 and 15:00 hr in unanesthetized and undisturbed rats in the animal quarters. Presuspension recordings were made for each rat and, therefore, each animal served as its own control.

A separate series of rats of similar size were suspended for aldosterone studies and compared with age-matched animals maintained in metabolism cages with ad libitum access to food and water. All animals were sacrificed by rapid decapitation and trunk blood collected, allowed to clot in the cold, and the serum separated by centrifugation and stored at -20°C prior to analysis. Serum aldosterone was determined with a commercially available solid phase radioimmunoassay (Diagnostic Products, Los Angeles, CA). Urine was collected under oil on the day of sacrifice and the volume determined gravimetrically. Urinary Na^+ and K^+ were determined with a Nova 1 Na^+/K^+ analyzer.

RESULTS AND DISCUSSION

It is evident that MAP, SP, and DP show a consistent increase during the seven day period of AO suspension and there is a rapid fall to control levels within one day after removal from the suspension apparatus (Table 1). Pulse pressures remained relatively unchanged throughout the experiment. Heart rates (HR) increased from presuspension levels of 420 bpm to 460 bpm during suspension and showed a slight further increase during postsuspension recovery.

These data agree with earlier findings (6) in which we reported elevated blood pressures during the initial stages of suspension. These findings differ, however, from those reported by Popovic (7) which indicate a fall in MAP during the first two days of AO suspension followed by a return to control levels for the remainder of the seven day suspension period. Although the HR reported by Popovic (7) are slightly lower than those in this report, both studies suggest that HR remains relatively unchanged during AO suspension and increases slightly after removal from AO suspension.

One prominent question which bears further study is in the post suspension adjustments. Altered blood pressures show a rapid reversal to control levels. This is similar to the rapid postsuspension recovery seen in fluid and electrolyte parameters. While the evidence is circumstantial, it indicates a potential relationship between fluid/electrolyte and cardiovascular alterations and suggest the need for further research in this area.

Serum aldosterone concentrations increased during the seven days of AO suspension (Table 2). This was unexpected since urinary output of sodium was increased on days three and seven. Urinary K^+ was increased only on the seventh day of suspension. Serum levels of Na^+ and K^+ were unchanged during seven days of AO suspension (data not shown). Thus the hormonal response was opposite to that hypothesized and was unexpected in light of previous observations on urinary output of aldosterone in Skylab crewmen (3). However, previous studies of bedrest subjects has indicated that AO positioning in the human is associated with elevated plasma aldosterone (1, 4). Further investigation is required to seek an explanation for these divergent findings. It is evident, however, that in the suspended rat aldosterone does not appear to be involved in the long term regulation of the natriuretic effects of AO positioning.

In summary, blood pressure data confirm our previous observations. In addition, during seven days of AO suspension there are sustained increases in MAP, SP, and DP with no apparent adaptation, and following removal from suspension there is a rapid (within 24 hours) readjustment to control levels. Serum aldosterone concentrations increased during AO suspension in contrast with the original hypothesis that the natriuresis associated with AO positioning would be associated with decrements in serum aldosterone (Supported by NASA grant NSG 2325.)

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TABLE 1. Blood Pressures in mmHg: (mean arterial = MAP, diastolic = D, systolic = S, pulse = PP) and heart rate (HR = bpm) in head-down tilted rats (15-20°).

	0	1	3	7	R1
MAP	102±2 (15)	109±13 (6)	114±5 (5)	121±5 (8)	102±2 (4)
D	88±2 (15)	95±5 (6)	101±6 (5)	107±5 (8)	86±2 (4)
S	130±2 (15)	137±4 (6)	145±6 (5)	149±17 (8)	134±6 (4)
PP	41±2 (15)	42±2 (6)	43±6 (5)	46±2 (8)	42±4 (3)
HR	423±11 (14)	460±13 (6)	457±14 (5)	451±9 (8)	471±8 (4)

0 = Pre suspension
1, 3 and 7 = days of suspension
R1 = 1 day recovery

Data are: mean ± SEM and numbers of rats (n)

Table 2. Serum aldosterone (pg/ml) and urinary excretion of Na^+ and K^+ (mEq/100g/d) in metabolism cage control (MCC) and antiorthostatic (AO) suspended rats.

Group	Aldosterone	Na^+	K^+
Day 0 MCC	78.4±30.3 (12)	0.85±0.06 (9)	1.62±0.09 (9)
Day 1 AO	157.1±35.6 (12)	0.83±0.10 (8)	1.31±0.09 (11)
Day 3 AO	115.5±22.2 (5)	1.09±0.13 (6)	1.77±0.18 (6)
Day 7 AO	163.6±51.7 (9)	1.30±0.08 (8)	2.15±0.16 (8)

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ARTERIAL PRESSURES, FLUID ENERGY, POSTURE AND + Gz ACCELERATIONS

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During maneuvers in high performance aircraft, pilots are exposed to high sustained + Gz accelerations (HS + Gz) i.e. high longitudinal accelerations applied during a period of time which is significant compared to the response time of physiological reactions. The effect of such accelerations on blood circulation is that they enhance phenomena observed during the change from the supine to the standing position. The change in the distribution of potential pressure energies between the various terms of BERNOULLI's equation is a function of the magnitude of the load factor. The same applies for one of its consequences : the increased heart contractile power. The left ventricle must indeed, take over the gain in gravitational energy which appeared between the base of the heart and the base of the skull and consequently must consume more ∿P bonds per second. After repeated exposure to HS + Gz rabbits exhibit arterial hypertension associated with a persisting increase in the maximum rate of rise of the left ventricular pressure (dP/dt max). The decrease observed in myocardial glycogen is inversely proportional to the increase in dP/dt max.

High sustained + Gz accelerations are applied to the human body during fighter aircraft maneuvers. They are observed whenever the speed vector, tangent to the trajectory, changes. The trajectory is curved and the acceleration vector directed toward the curve depression is projected along the axis of the spine and main blood vessels. What is of concern is not only that new generation fighter aircraft may sustain a much higher load factor than previous aircraft, but that this load factor may apply much longer thus for a significant period of time as far as physiological responses are concerned. Our purpose was to study the consequences of repeated exposure to high sustained + Gz acceleration on the functional state of the cardiovascular system.

In man, lying on a table, the acceleration of gravity is perpendicular to the axis of the main vessels. Thus the mean arterial pressures are approximately 100 mmHg in the aorta (P1) and 95 mmHg in distal arteries (P2). In fact BERNOULLI's equation shows that in reality these pressures are respectively P1 + ρGz, P2 + ρGz, etc...(P is the transmural pressure, ρ is the blood density, G is the acceleration of gravity and z is the position of the point of measure relative to the reference level of potential energy). Evidently it has become common practise to omit the expression ρGz in results.

On a standing man who pivoted around an imaginary axis crossing his heart the gravity vector becomes parallel to the axis of vessels. What is happening between the base of the heart and the base of the skull ? The head moved further from the center of the Earth by a distance h'. On carotid arteries we observe a drop in transmural pressure exactly compensated by a gain in gravitational energy ρGh'. The change in position induced a drop in transmural pressure, therefore decreased parietal stretching, hence the involvement of baro-receptors "fortunately" located between the left ventricle and the base of the skull. This involvement suppresses the inhibition of the heart and sympathetic system created by the four inhibiting nerves. This sympathetic response includes a cardiac inotropic effect and a systemic vasoconstrictor effect acting on veins and arteries. The installation of this new circulatory equilibrium approximately corresponds to an increase in pressure (RMS) of about 20 mmHg in the carotid area. If, in ρGh, G is multiplied by 6 or 7 during a combat maneuver the phenomenon is increased by the same proportions.

We may then wonder whether repeated exposure to this aggression may not risk causing persisting alterations of the functional state of the cardiovascular system.

The experiment was conducted on three groups of rabbits : placed on a centrifuge, restrained, and control. The centrifuged animals were placed in the restraining system, with the axis of large blood vessels parallel to the horizontal arm of the centrifuge. The animal's head was oriented toward the centrifuge axis. During a four-minute test, the animals were exposed to three consecutive runs at 8-9 Gz reached with onset rates of 0.8-1 $G.s^{-1}$, and maintainedat a plateau for 20-40 s. Such exposure was repeated 3-4 times a week for 3 to 6 weeks.

Each animal was anaesthetized on the day following the last exposure (ethylurethane 1.25 g/kg, i.p.). Electrodes were placed on the limbs, to record the ECG. A femoral catheter was connected to a Hewlett Packard (HP 1280C) pressure transducer. A mikrotip 4 F MILLAR pressure transducer was inserted into the carotid artery and pushed into the left ventricle. The arterial femoral pressure (AP) and the left ventricular pressure (LVP) were measured. The first derivative (dP/dt max) of the LVP was calculated (HP 8814 A). The animals were then mechanically ventilated ; the chest was opened and the heart was frozen in situ using Wollenberger clamps. Myocardial ATP, ADP, AMP, creatine phosphate (CP) and glycogen (GLY) concentrations were determined. The restrained animals were submitted to the same protocol except that accelerations were plateaus of less than 0.4 Gz x 4 mn. The control animals were not restrained.

Results are summarized in the table which indicates, for each parameter, the mean value m (top line, underlined), the standard error of the mean (middle line) and the group population (bottom line in brackets). For each parameter the three means were compared by variance analysis.

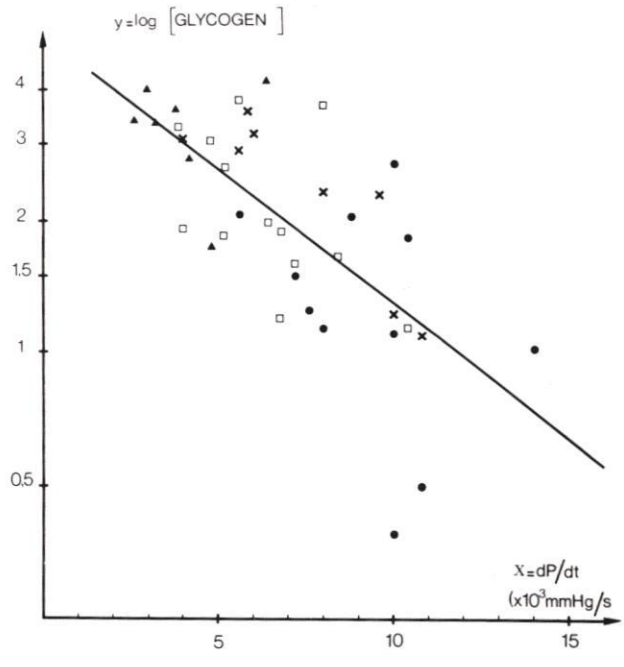
When the F-test for overall comparison is significant, means are compared two by two according to the method described by Scheffé (m*, $p < 0.05$; m**, $p < 0.025$; m***, $p < 0.01$ compared to the controls). Systolic and diastolic LVP and AP are given in mmHg, dP/dt in mmHg/s, heart rate (HR) in pulses/mn, ATP, ADP, AMP and CP in µmoles per 100 mg of fresh weight, and glycogen in µmoles of glycosyl units per 100 mg of fresh weight.

	Centrifuge	Restrained	Control
AP systol	133.6 * / 2.6 (12)	135.7 ** / 3.8 (9)	122.1 / 2.7 (15)
AP diastol	85.0 ** / 2.7 (12)	80.4 / 3.9 (9)	73.1 / 2.1 (15)
LVP systol	123.1 * / 6.7 (11)	121.2 * / 5.5 (10)	107.9 / 1.9 (16)
dP/dt max	9055*** / 720 (11)	7067 / 837 (9)	5980 / 342 (15)
HR	325 / 5 (12)	321 / 10 (11)	313 / 5 (16)
ATP	0.420 / 0.017 (9)	0.434 / 0.023 (8)	0.456 / 0.015 (12)
ADP	0.073 / 0.008 (9)	0.069 / 0.005 (6)	0.067 / 0.005 (10)
AMP	0.018 / 0.003 (7)	0.016 / 0.003 (4)	0.013 / 0.002 (9)
CP	0.671 / 0.065 (9)	0.638 / 0.086 (8)	0.786 / 0.051 (12)
GLY	1.45 * / 0.26 (9)	2.48 / 0.37 (7)	2.44 / 0.27 (11)

A significant increase in femoral arterial systolic values is observed in restrained and centrifuged animals. The increase in diastolic AP values is significant only for centrifuged animals. Systolic LVP values significantly increase in both groups of animals. The maximum rate of rise of ventricular pressure (dP/dt max) significantly increases in centrifuged animals, due to the steadily increasing power of heart contractions : not only pressures are higher in the left ventricle (14 % increase in systolic LVP) but also the maximum rate of rise of LVP is even higher (50 % increase since dP/dt varies from 6000 mmHg.s^{-1} in control animals to 9000 mmHg.s^{-1} in centrifuged animals).

The myocardial ATP concentrations decrease in centrifuged animals but the differences observed in the means are not significant, probably because of the relatively small size of our groups. The same is true for CP concentrations.

The myocardium of centrifuged animals is significantly depleted in glycogen compared with restrained and control animals. This observation is confirmed by the relationship (figure) between the amount of stored myocardial glycogen and dP/dt max. In the figure, squares □ represent control animals, crosses × represent restrained animals and dots • represent centrifuged animals ; in this latter group the maximum rate of rise of LVP is high and the glycogen reserves are low. Triangles ▲ represent rabbits which suffered a fracture of the lumbar spine during excessively high-G centrifuge tests. Their heart was excised within 24 hours, i.e. before spinal shock disappeared : dP/dt collapses and glycogen reserves are high. The correlation coefficient observed for the whole set of data is -0.68, i.e. the correlation is high ($p < 0.001$ with 37 degrees of freedom).



These observations made on anesthetized animals submitted to their last test at least 24 hours earlier show the stress imposed on the myocardium and the persistence of sympathetic response after repeated exposure to HS + Gz accelerations. The two types of stress (centrifuge + restraint ; restraint alone) determine the appearance of two types of arterial hypertension. Systolic AP and LVP significantly increase in restrained animals. The function of the cardiovascular system is more perturbed in centrifuged animals since the significant changes affect not only systolic AP and LVP but also diastolic AP and the rate of rise of LVP. Significant glycogenolysis is associated with these phenomena. It is known to be a major metabolic response of circulating catecholamines.

Remembering that these results were obtained on a small rodent, the question may be raised : a pilot repeatedly exposed to HS + Gz may tolerate each exposure without too much apparent trouble (1), but one can wonder at what cost for his myocardium, and with what hazards for his future cardiovascular functional state ?

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Human responses to repeated high G simulated aerial combat maneuvers.
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CARDIOVASCULAR CHANGES WITH HEAD-DOWN ANKLE SUSPENSION

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The purpose of the work here reported was to study the effects of head-down inversion on central and peripheral circulation. Ten healthy men, aged 21-30 years, participated in the study. The basic experimental design consisted of measurements taken in three phases: vertical head-up, horizontal supine and vertical head-down. Blood flow changes were assessed through measurements of electric impedance. Blood pressure changes were measured non-invasively. In relation to the standing position head-down suspension resulted in a non-significant lowering of the heart rate, a significant increase in mean blood pressure in the arms, an increase in stroke volume and cardiac output (which became the same as in the horizontal position) and a decrease in leg blood pressure. In assuming the head-down position there was a shift of fluids from the legs to the thorax suggested by impedance changes. The peripheral vascular resistance in the lower extremities markedly decreased, this resulting in an apparent increase in leg blood flow. The increase in blood pressure in the cephalad portion of the body is a potential risk for those practicing head-down suspension.

INTRODUCTION: Tilting procedures have been extensively used to study body responses to postural changes. Since postural changes imply changes in the relationship between the saggital axis of the body and the gravitational vector, tilting procedures have been found useful in the interpretation of the effects of weightlessness on humans during space flight. More recently, inversion procedures with head-down position have been proposed for the purpose of relieving pressure on vertebral articulations. This unloading of the vertebral column is supposed to result in a number of medical benefits. The scientific literature is not lacking papers reporting the physiological effects of head-down [1-4] tilting but most of these papers do not deal with total head-down suspension and many do not focus on the overall cardiovascular effects which constitute the principal objective of this report.

METHODS: Ten healthy men, aged 21 to 30 years, participated in the study (Table 1). These were normal individuals not engaged in any kind of physical training program. The basic experimental design consisted of three phases: vertical head-up, horizontal supine and vertical head-down. Each phase lasted 5 minutes. Changes of electric impedance in the chest and in the calf were recorded with the Minnesota Impedance Cardiograph[5]. Blood pressure measurements were taken non-invasively in the arm and in the ankle. A tracing of the electrocardiogram was recorded throughout the study. Blood samples were withdrawn from an arm vein to determine the hematocrit in the supine position before the subject was tilted head-down. The following measured and calculated variables were used in the data analysis: systolic (SBP), diastolic (DBP) and mean blood (MBP) pressure in the arms and legs, heart rate (HR), stroke volume (SV), cardiac output (C.O.), peripheral blood flow per 100 gm of tissue ($\Delta V/100$), mean chest and calf impedance (Zo) and the first time derivative of chest impedance. These variables were all recorded continuously except for blood pressure, which was recorded once every minute. The statistical results are based on the observation made between the fourth and fifth minute of every phase. Stroke volume was calculated from the chest impedance recording using the equation proposed by Kubicek[5]. The blood resistivity factor in that equation was corrected for the subject's venous hematocrit according to the equation proposed by Hill[6].

Table 1. SUBJECT CHARACTERISTICS, INDIVIDUAL DATA, MEANS AND STANDARD DEVIATIONS FOR AGE, WEIGHT, HEIGHT, HEMATOCRIT AND BLOOD ELECTRIC RESISTIVITY.

SUB-JECT	AGE (years)	WEIGHT (Kg)	HEIGHT (cm)	HCT (%)	ρB (Ωcm)
1	21	72	196	48.5	132.0
2	21	77	197	43.5	121.8
3	22	80	185	39.0	112.1
4	22	85	175	43.0	120.5
5	30	72	180	43.0	120.5
6	30	85	182	43.0	120.5
7	22	77	182	37.0	107.9
8	27	64	175	43.0	120.5
9	22	90	200	45.0	124.7
10	29	82	175	49.0	133.1
$\bar{x}\pm SD$	24.6 ± 3.9	78.4 ± 7.7	184.7 ± 9.6	43.4 ± 3.7	121.7 ± 7.7

RESULTS: Measurements of heart rate, arm blood pressure, stroke volume, cardiac output, thoracic impedance, segmental leg impedance and blood pressure and flow in the legs, were recorded every minute. The changes of those variables in going from the vertical head-up to vertical head-down position are summarized in table 2.

Table 2. CHANGES OBSERVED COMPARING VERTICAL HEAD-UP AND VERTICAL HEAD-DOWN POSITIONS. HR=heart rate. SBPa and MBPa=systolic and mean blood pressure in arm. SV=stroke volume. CO=cardiac output. Zo(c)=chest mean impedance. SBPl and MBPl=systolic and mean blood pressure in leg. $\Delta V/100$=leg blood flow. Zo(l)=mean impedance in leg.

	N	Vertical Head-up ($\bar{x}\pm$S.D.)	Vertical Head-down ($\bar{x}\pm$S.D.)	P-value
HR (beats/min)	10	69.7±5.6	64.4±7.3	< 0.200
SBPa(mmHg)	10	111.9±15.4	140.6±8.9	< 0.005
MBPa(mmHg)	10	88.0±9.8	115.6±9.2	< 0.001
SV(ml)	10	93.8±22.5	150.2±36.2	< 0.001
C.O.(l/min)	10	6.52±1.6	9.7±2.6	< 0.001
Zo(c) (Ω)	10	19.4±3.0	16.9±2.5	< 0.001
SBPl(mmHg)	10	217.7±23.5	5.8±6.8	< 0.001
MBPl(mmHg)	10	166.6±19.6	2.1±2.2	< 0.001
$\Delta V/100$ (ml/min/100ml)	10	3.3±2.4	8.1±3.3	< 0.001
Zo(l) (Ω)	10	46.7±15.7	60.4±17.5	< 0.005

DISCUSSION: In changing position from supine to vertical head-up, heart rate and mean blood pres-

sure in the arm increased. Stroke volume and cardiac output decreased. The mean blood pressure in the ankles increased and segmental impedance decreased suggesting a decrease in leg blood flow. Opposite changes in mean thoracic and leg impedance suggest that thoracic fluid volume decreased and segmental leg volume increased. In going from the head-up to the head-down position the heart rate increased but the change was statistically not significant. The mean pressure in the arm, stroke volume and cardiac output increased significantly, although the latter measurements were not significantly different from those observed in the supine position. There was an increase in segmental impedance suggesting an increase in segmental leg blood flow.

The interpretation of these observations is not free from difficulties. Papers reporting experiments with head-down suspension which contain cardiovascular observations are scarce. The literature reporting results with head-down tilt of various degrees is abundant but no paper has been found reporting 90° head-down experiments. Also, tilting and head-down suspension do not create the same physiological situation. The support or lack of it of the body by the limbs is an important factor in determining the cardiovascular response. The site where blood pressure is measured relative to the heart level determines a variable hydrostatic factor[7] which must be taken into consideration. In our study, the pressure in the arm during head-down suspension was taken with the arm pending along the head. The assumption was that pressures measured in this position of the arm would give an approximate assessment of the hydrostatic factor in the carotid system. If this assumption is correct, the hydrostatic factor in the carotid system is, according to our measurements, approximately 27 mm Hg. The position of the cuff was approximately 35 cm from the heart level. Considering that 1 cc of blood exerts a pressure equivalent to 0.735 mm Hg., 35 x 0.735 = 25.7 mm Hg, which is approximately the arm pressure increase observed in this study.

It has been repeatedly observed that in the normal individual the assumption of the standing position is accompanied by a drop in stroke volume and cardiac output as it has been the case in the present investigation. This is attributed to a drop in central pressure caused by a shift of body fluids of approximately 500 ml from the thorax to the legs. It is assumed that if the body is totally inverted the reverse will occur, i.e., there will be a shift of fluids to the thorax that will increase stroke volume and cardiac output, an assumption that some tilt experiments have supported. Our measurements of stroke volume and cardiac output in the supine position and those in the vertical head-down position were not statistically different. This contradictory finding is not an isolated one. In a previous study[3] atrial pressure measurements during -75° tilt showed that in the inverted position there was no apparent full imposition of the hydrostatic effects of blood in venous columns caudad to the diaphragm. Also there seems to be marked individual variability in the magnitude of this hydrostatic factor. This variability expresses itself in positive and negative responses in stroke volume and cardiac output. There were, however, in our study changes in thorax and leg mean impedance suggesting that fluid shifted from the legs to the thorax.

The increase in magnitude of segmental leg impedance during head-down suggests an increase in arterial blood flow. Changes in segmental leg impedance took place in the same direction as changes in mean leg impedance, contrary to what occurred in the chest. We do not know how to interpret these findings and are not sure that the blood flow changes suggested by leg impedance changes are real. It should be pointed out, however, that there were no signs of ischemia in the feet.

Previous studies have shown that head-down tilt of -75° is accompanied by an increase in venous pressure of 15 to 20 mm Hg at the base of the skull. There must be also an increase in capillary pressure. An increase in capillary pressure is probably the cause of vessel rupture which is manifested by the periorbital and neck petechiae which are observed in some individuals when suspended head-down[8]. In another study[9] systolic and diastolic pressures in the central retina artery have been found to be increased and the pressure in the intraocular fluid also.

The findings of this and previous studies provide evidence of cardiovascular changes during head-down suspension that are consistent with the interpretation of an undue stress of the cephalad circulation. Such stress puts in risk persons with hypertension, glaucoma, vascular fragility, cardiac insufficiency and spinal instability.

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THE LUNGS, "ACHILLES HEAL", OF AIR BREATHERS IN CHANGING GRAVITATIONAL-INERTIAL FORCE ENVIRONMENTS

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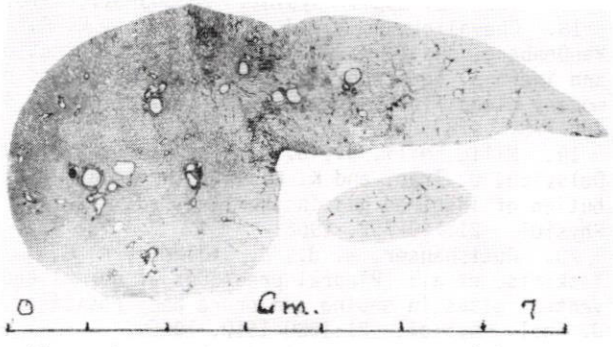
Most internal organ systems such as the heart, brain and systemic circulations are enveloped by interserosal force environments which manifest inherent vertical gradients that vary in direct proportion to changes in the magnitude and/or direction of the gravitational-inertial force environment to which the organism may be subjected (1). Consequently, these organ systems have a relatively high degree of resistance against functional or anatomical structural damage due to changes in the force environment (2).

However, due to the intra-pulmonary pressure imbalances which occur at high levels of acceleration consequent to the large differences between the essentially zero specific gravity of the intra-alveolar respiratory gases and the surrounding blood and tissue, "nature's stratagem" for protecting other organ systems against changes in the force environment is not effective relative to the lungs (1). The disturbances in pulmonary ventilation/perfusion relationships with changes in body position at 1 G (3), the uniform occurrence of arterial desaturation with increases in the magnitude of the force environment (4), and the disruption of lung parenchyma in chimpanzees exposed to 40 G in a water immersion capsule (5) documents the susceptability of the lungs to change in the force environment (6, 7). Fig. 1 shows a lung section taken from a dog sacrificed following several exposures to accelerations of 2, 4, and 6 G.z. Of note are the over-distended ventral alveoli in non-dependend regions in the thorax, and alveolar collapse in the dependent dorsal region. Over-distended apical alveoli (insert) were also observed.



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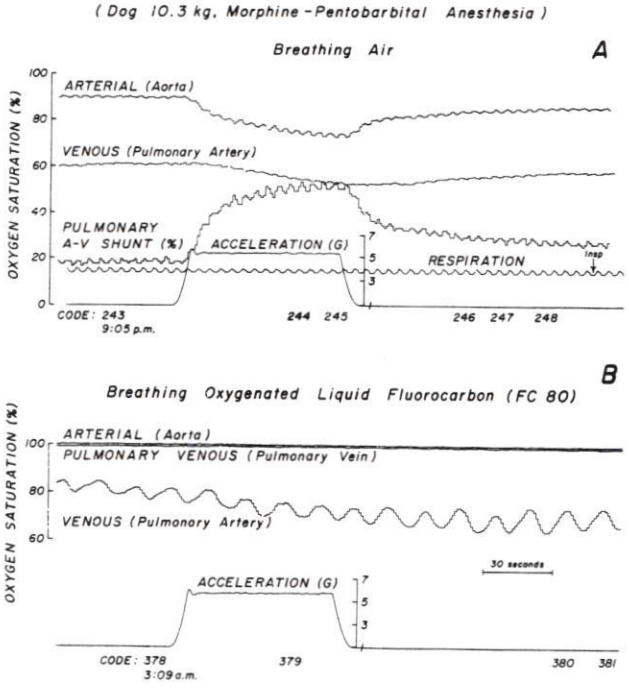
The prevention of arterial desaturation during increases in the force environment achieved by replacing the respired gas by a very high oxygen content liquid with a specific gravity similar to blood and tissue (Fig. 2) documents the physical basis of this disturbance (8)

This, along with the military value of enabling humans to withstand large changes in the force environment (9-11), as well as possible pathogenic significance in some disease states (12), has encouraged continued study of the affects of gravity and inertial forces on the magnitude and regional distribution of interpleural forces (13,14), and associated changes in the spatial distribution of lung parenchymal strains (15), ventilation (16), and perfusion (17, 18; Fig. 3).



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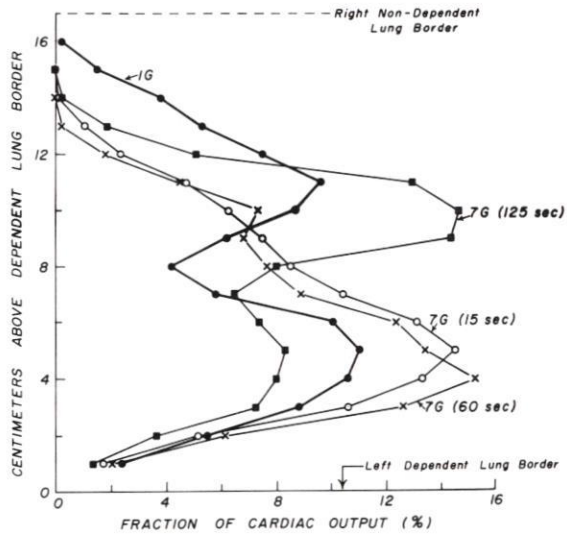
VARIATION IN VERTICAL DISTRIBUTION OF PULMONARY BLOOD FLOW WITH DURATION OF EXPOSURE TO AN INCREASED FORCE ENVIRONMENT
(Dog, Left Decubitus Position, IPPB with 99.6% Oxygen)



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Since ventilation of the essentially passive alveolar system is mediated by transmission across the potential pleural space of the cyclic forces generated by the diaphragmatic, thoracoabdominal respiratory musculature system, measurement of the time varying magnitude and spatial distribution of interpleural forces is of basic importance to respiratory and gravitational physiology (6, 19).

There has been general agreement as to the magnitude of interpleural pressures at dependent sites in the thorax (14), and also a concensus that the magnitude of the expansile forces acting on non-dependent regions of the lungs increases with vertical height in the thorax (6, 19). However, because of the divergent values obtained by different measurement methods, there is little agreement concerning the magnitude of this vertical

gradient in interpleural forces (20, 21).

The physiologic significance relative to pulmonary mechanics of the relatively large vertical gradients in pleural liquid pressures of about 0.7 cm H_2O/cm of vertical height have frequently been disregarded on the basis that the pressures in pleural liquid are not directly related to the so-called surface pressures which are presumably responsible for lung expansion (22). However, more recent studies using new independent methods suggest that the magnitude of the pleural expansile forces in non-dependent regions of the thorax are at least as large or larger (i.e. pressures as negative or more negative) than measurements of pleural liquid pressures suggest (23, 24) in the apical regions of head-up dogs. Possibly this discrepancy may be due to the existence of significant adhesive forces between the visceral and parietal pleura in these apical non-dependent lung regions, where the thickness of the pleural space may be decreased to the degree that significant molecular forces between the closely apposed surface may be present (23). If such forces increase in direct proportion to the level of the force environment as liquid pleural pressure measurements suggest (6, 13), it is not surprising that their magnitude may be sufficient to produce actual disruption of lung parenchyma as observed at high levels of acceleration (5, 6). In any event, quite certainly the vertical gradient in the interserosal force environment which surrounds the lungs is a causative factor of differences with vertical height in the thorax of lung strains, perfusion, and ventilation which occur at 1 G, presumably magnify during the launch and re-entry phases of space flight (Fig. 4), and perhaps cease at zero G (25).

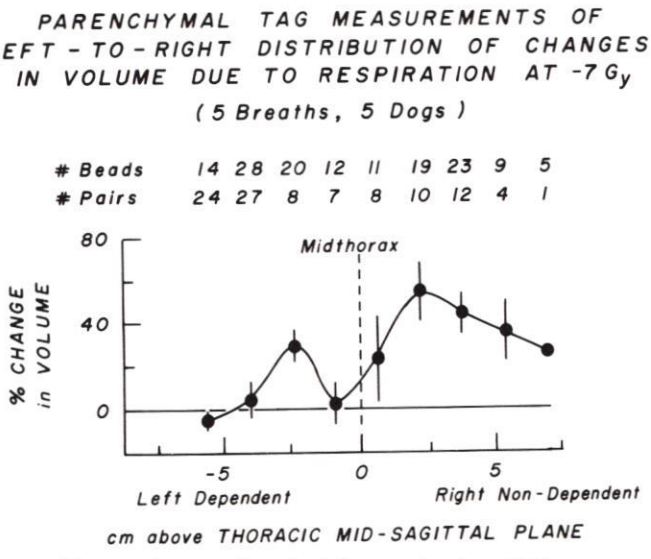

Figure 4 reproduced with permission (16)

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PLASMA RENIN ACTIVITY DURING 5-HOUR ANTIORTHOSTATIC HYPODYNAMIA

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This study was designed to investigate the renin secretion in the course of a head-down bed rest, which is considered a reliable model for ground-based weightlessness simulation. Serial measurements of plasma renin activity (PRA) were realized in 6 adult volunteers submitted to the 3 following postural tests. Day 1 : 7 hours sitting. Day 2 : 1 hour sitting; then 5 hours horizontal supine, finally 1 hour sitting. Day 3 : 1 hour sitting, then 5 hours head-down bed rest (-10°), finally 1 hour sitting. The order of the postural tests was randomized for each subject. The results show that a 5-hour head-down tilt results in a progressive decline of PRA. This decline occurs earlier, and is more pronounced than the one observed during horizontal bed rest. The kinetics of the renin response to a 5-hour head-down bed rest is similar to that observed during a head-out water immersion of the same duration.

INTRODUCTION

Horizontal or head-down bed rest is widely used as an experimental model for ground-based weightlessness simulation, particularly for the study of the cardiovascular and hormonal systems. As observed during actual exposure to zero gravity, bed rest induces a fluid shift from the lower to the upper part of the body. The resulting central hypervolemia is supposed to be more pronounced in the head-down than in the horizontal position.

A number of experiments performed in human as well as in animals, have demonstrated renin secretion to be specifically sensitive to changes in intrathoracic blood volume. Therefore this study was undertaken i) to investigate the short-term variations of renin secretion in the early stages of bed rest, and ii) to compare in this respect horizontal and head-down bed rest.

MATERIAL and METHODS

Subjects -

Six normal male volunteers (age 22-27 yr) participated in this study. Details of the protocol were explained to each individual and their informed consent was obtained. Three days before the begining of the study, and during the whole study period, they received the same controled diet containing approximatly 140 mM Na+ and 70 mM K+ per day.

Protocol

The experiment consisted of three postural tests realized on the same week, monday, wednesday and friday. The order of the postural tests was randomized for each subject. The first postural test consisted of seven hours spent in the seated position. The second test consisted of one hour sitting, then five hours in the horizontal supine position, and finally one hour sitting. The third test consisted of one hour sitting, then five hours 10 degrees head-down tilt, and finally one hour sitting. In each case, the study was started exactly at 8 h. Blood pressure and heart rate were monitored throughout the experiment. Serial blood samples were obtained from each subject :

- at 9 h. i.e. after one hour spent in the seated position. This served as the control value.
- then at 9.10, 9.20, 9.30, 10, 11, 13, 14 h.
- and finally at 15 h, i.e., for postural tests two and three, after one hour of recovery period during which the subjects returned to the seated position.

Measurements and Calculations

On each blood sample, the following parameters were measured :

- plasma sodium, potassium, creatinine and osmolality using conventional laboratory methods,
- hematocrit
- plasma renin activity (PRA) using our radioimmunoassay of angiotensin I generation (10)

The results were analyzed statistically using a two-way analysis of variance and a Dunnett's test (1).

RESULTS

During the course of the three postural tests there was no significant variations in heart rate, blood pressure, hematocrit, plasma sodium and potassium (fig. 1). The control PRA

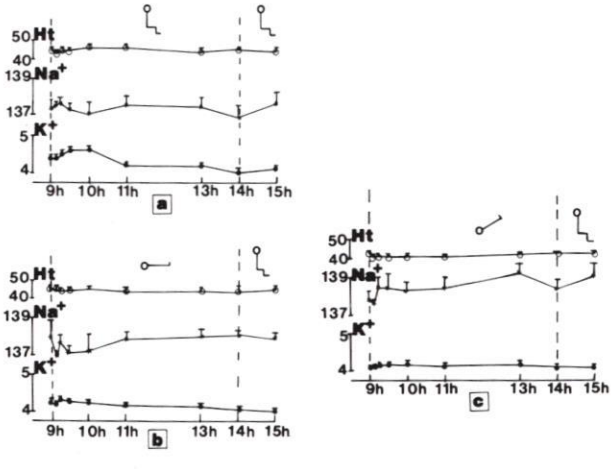

Fig. 1 - Time course of hematocrit (Ht), plasma sodium (Na+) and potassium(K+) throughout the three postural tests.

values, measured at 9h, were of the same magnitude : 152 ± 27.9 ng/l/min (postural test 1), 140± 25.6 ng/l/min (postural test 2), 177 ± 22.7 ng/l/min (postural test 3). As shown in figure 2, PRA remained unchanged throughout the seven hours spent in the seated position (postural test 1).

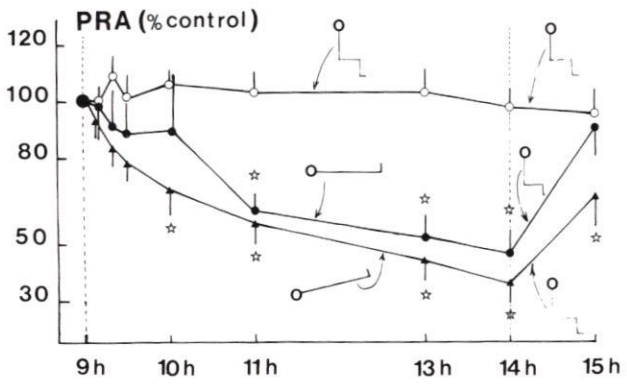

Fig. 2 - Time course of PRA changes throughout the three postural tests. The results are expressed as the percentage of the control value.
* = $p < 0.05$ vs control value

Conversely, assumption of the horizontal position (postural test 2) led to a decrease in PRA. This decrease became significant from the second hour, and three hours latter, PRA reached approximatly 47% of the control value. After resuming the seated position for one hour, PRA returned to its control value. Head-down tilt (postural test 3) also led to a decrease in PRA. By comparison with postural test 2, this decrease was more precocious (statistically significant from the first hour), and tended to be more important : at 14 h, i.e. after five hours of head-down tilting, PRA was only 35% of the control value. After the last hour in the seated position, PRA rose, but did not return to its control value.

DISCUSSION

The data from this study demonstrate that a 5-hour 10° head-down bed rest results in a progressive decline in PRA, which occurs earlier, and is more pronounced than the one observed during an horizontal bed rest of the same duration. Previous studies of PRA changes within the very first hours of head-down tilting have led to conflicting results : PRA has been reported to decrease, by Güell (3), London (8) and Leach (7), whereas unchanged values were observed by Volicer (11), Nixon (9) and Hargens (4). Several factors in the study designs may account for these discrepencies. First, the titl angles which were used varied from -5° to -10°. Secondly, these was differences in the definition of the reference PRA value, i.e. the one which served as the control for comparison to head-down tilt. But the major lack in previously published data is that of a time-control experiment where the subjects would be kept in a standardized reference posture for the same duration of time than the horizontal or head-down bed rest. Such a time-control experiment would allow detection of eventual fluctuations in PRA levels which would not be linked to postural changes, but rather to circadian rythms. For these reasons, particular attention was paid in our study to ensuring such appropriate controls, thus allowing a true comparison between observations realized in different postural conditions.

The origin of the renin lowering effect of the head-down tilting remains to be established. An activation of arterial high-pressure baroreflexes is unlikely, since we did not observe any significant variations of blood pressure. The participation of cardiopulmonary low-pressure baroreflexes is more likely (6). Whatever its origin, it must be pointed out that the reduction of renin secretion induced by head-down tilting is only transient : from the 10th-12th hour of tilt, a progressive increase of the secretion occurs, so that during prolonged observations (up to 14 days) high PRA values are regularly found (3, 5, 7, 11).

Finally, it is of interest to observe that the kinetics of the renin response to a 5-hour head-down bed rest is similar to that observed during head-out water immersion (2).

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ROLE OF CARDIAC VOLUME RECEPTORS IN THE CONTROL OF ADH RELEASE DURING ACUTE SIMULATED WEIGHTLESSNESS IN MAN

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ABSTRACT

Hemodynamic responses and antidiuretic hormone (ADH) were measured during body position changes designed to induce central blood volume shifts in ten cardiac and one heart-lung transplant recipients to assess the contribution of cardiac volume receptors in the control of ADH release during the initial acute phase of exposure to weightlessness. Each subject underwent 15 min of a sitting-control period (C) followed by 30 min of -6° headdown tilt (T) and 30 min of resumed sitting (S). Venous blood samples and cardiac dimensions were taken at 0 and 15 min of C; 5, 15, and 30 min of T; and, 5, 15, and 30 min of S. Blood samples were analyzed for hematocrit, plasma osmolality, plasma renin activity (PRA), and ADH. Heart rate (HR) and blood pressure (BP) were recorded every two min. Plasma osmolality was not altered by posture changes. Mean left ventricular end-diastolic volume increased ($P < .05$) from 90 ml in C to 106 ml in T and returned to 87 ml in S. Plasma ADH was reduced by 20% ($P<.05$) with T and returned to control levels with S. These responses were similar in six normal cardiac-innervated control subjects. These data may suggest that cardiac volume receptors are not the primary mechanism for the control of ADH release during acute central volume shifts in man.

INTRODUCTION

Exposure to simulated models of weightlessness such as water immersion and antiorthostatic (headdown) bedrest increases sodium and water excretion, and decreases plasma renin activity, aldosterone, and antidiuretic hormone (ADH) (1,6). According to the Henry-Gauer hypothesis (1), sudden shifts of fluids from the legs and abdomen into the chest are interpreted by atrial receptors as an increase in total blood volume. Such stimulation of atrial volume or baroreceptors would result in a decrease in plasma ADH release from the neurohypophysis and a consequent increase in sodium and water excretion. However, since most of the experimental evidence in support of this hypothesis has come from studies using the dog as the experimental model (2,5), the importance of the proposed Henry-Gauer atrial receptors in the control of ADH responses to acute central blood volume shifts in man has been challenged by recent observations that vagotomized non-human primates exhibit significant diuresis during water immersion (3) and volume expansion (7).

If atrial receptors contribute significantly to the control of body fluid and electrolyte regulation through ADH inhibition or stimulation, then individuals with little or no atrial afferent output, i.e., partial or complete denervated hearts, should exhibit little or no reduction in ADH when exposed to posture changes designed to simulate the acute central blood volume shifts that occur during the initial phases of entry into and re-entry from spaceflight. Therefore, the purpose of this study was to test this hypothesis by measuring hemodynamic responses and ADH during body posture changes in one heart-lung and ten cardiac transplant recipients and compare these physiological responses to those of normal-innervated-control subjects.

METHODS AND PROCEDURES

Three groups of subjects volunteered to participate in this study: 1) 10 cardiac transplant recipients with partial or complete denervation; 2) 1 heart-lung transplant recipient with nearly complete denervation; and, 3) 6 normal cardiac innervated subjects (controls). All transplant subjects were at least one-year post-surgery. Descriptive data are presented in Table 1.

TABLE 1. SUBJECT DESCRIPTIVE DATA

SUBJECT GROUPS	N	AGE yr	HEIGHT cm	WEIGHT kg
CARDIAC INNERVATED CONTROLS	6	44 ±4	175 ± 5	77.2 ±8.5
CARDIAC TRANSPLANTS	10	40 ±3	180 ± 2	73.2 ±2.5
HEART-LUNG TRANSPLANT	1	41	185	71.8

Values are mean ± SE.

The subjects underwent exposure to and return from antiorthostatic tilt designed to simulate the initial acute effects of weightlessness and re-entry to 1 g. The experimental protocol consisted of a 15-min resting-control period (C) in the seated position followed by 30-min exposure to -6° headdown tilt (T) followed by a 30-min recovery consisting of a return to the upright-seated position (S). The subjects were instructed to remain as motionless and relaxed as possible.

Just prior to the initial 15-min resting-control period, a 21-gauge needle with polyethylene catheter was inserted into the left arm antecubital vein and plasma volume (PV) was measured with a modified Evans blue dye (T-1824) dilution method. Blood samples were collected without stasis at 0 and 15 min of C, at 5, 15, and 30 min of T, and at 5, 15, and 30 min during S. Duplicate microhematocrit (Hct) determinations were made immediately after collection of each blood sample and the change in plasma volume from the initial seated-control position was calculated from the changes in Hct. Plasma antidiuretic hormone (ADH) concentration and plasma renin activity (PRA) were determined using standard radioimmunoassay techniques.

Heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressures were measured every 2 min before, during, and after T. A Hewlett-Packard ultrasonic echocardiography system using M-mode was used to determine an index of heart volume changes at 0 and 15 min of C; 5, 15, and 30 min of T; and, 5, 15, and 30 min of S.

Changes within groups were evaluated by using single-factor analysis of variance with repeated measures. Comparisons between groups were determined with the unpaired t-test. The null hypothesis was rejected when $P<.05$ and non-significant differences were denoted by NS.

RESULTS

Mean cardiovascular and hormonal responses during C, T, and S are presented in Table 2. For the control subjects, T increased heart volume ($P < .05$) and decreased heart rate ($P <.05$) from 63 ± 2 bpm in C to 56 ± 2 bpm during T and returned to 63 ± 3 bpm with S. Except for an initial elevation in SBP at 5 min of T ($P < .05$), SBP and DBP were not significantly altered during

body position changes. Compared to C values, plasma volume was significantly increased during T but returned to C levels following S. T induced a 20-30% reduction ($P < .05$) in plasma ADH levels which returned to C values upon resumption of the seated position during S. PRA decreased slightly ($P < .05$) during T and remained depressed during S.

The cardiac transplant subjects had higher ($P < .05$) heart rate and blood pressure levels during C with lower circulating plasma volume ($P < .05$) compared to the control group. However, the cardiovascular responses in the cardiac transplants were similar (NS) to those measured in the controls (Table 2). For the transplant subjects, mean HR decreased ($P < .05$) from 101 ± 4 bpm in C to 94 ± 4 bpm during T and returned to 101 ± 4 with S. SBP was elevated ($P < .05$) during tilt but DBP was not altered by body posture changes. Plasma volume increased by 6.3% ($P < .05$) during T and mean heart volume increased ($P < .05$) from 90 ml in C to 106 ml during T and returned to 87 ml in S. Plasma ADH was reduced by 20% ($P < .05$) during T and returned to C levels following resumption of the upright-seated position in S. The heart-lung transplant recipient demonstrated similar responses in cardiovascular adjustments and plasma ADH levels as those measured in the cardiac-transplant and control subjects (Table 2).

TABLE 2. CARDIOVASCULAR RESPONSES AND ANTIDIURETIC HORMONE (ADH) DURING SEATED CONTROL (C), -6° HEADDOWN TILT (T), AND RESUMPTION TO UPRIGHT SITTING (S).

	NORMAL INNERVATED			CARDIAC TRANSPLANT			HEART-LUNG TRANSPLANT		
	C	T	S	C	T	S	C	T	S
HR, bpm	63 ±2	58* ±3	65# ±4	101 ±4	96* ±4	101# ±4	100	99	100
SBP, mmHg	115 ±4	116 ±4	113 ±5	131 ±5	142* ±8	129# ±4	129	131	130
DBP, mmHg	82 ±5	82 ±4	80 ±5	99 ±3	106 ±6	100 ±2	91	91	92
PV, ml	3355 ±230	3521* ±238	3268# ±193	2933 ±155	3120* ±180	2947# ±161	3190	3475	3167
ADH, pg/ml	3.0 ±.6	2.3* ±.7	3.0# ±.8	2.5 ±.7	1.8* ±.5	2.8# ±.9	5.6	3.8	6.0

Values are mean ± SE.

* $P < .05$ vs corresponding control value
\# $P < .05$ vs corresponding tilt value

DISCUSSION

In the present study, -6° headdown tilt induced an acute headward shift of fluids sufficient to enlarge the heart volume in cardiac and heart-lung transplant as well as normally innervated control subjects as indicated by a significant increase in echocardiographically measured left ventricular end-diastolic volume. Subsequent hemodynamic changes in all subjects included an inward shift of extravascular fluids to the vascular space resulting in a transient increase in plasma volume and a reflex reduction in heart rate with essentially no change in arterial blood pressure. In addition, all transplant and innervated-control subjects demonstrated a decrease in plasma PRA and ADH. These hemodynamic and hormonal responses were reversed by the 30 min of resumed sitting following headdown tilt. Therefore our data demonstrate that cardiac and heart-lung transplant recipients have mechanisms by which ADH secretion can be controlled during acute central blood volume shifts.

Although the role of atrial volume receptors in the control of ADH secretion has been demonstrated in dogs (1,2), recent data from studies using the non-human primate have suggested that cardiac receptors may play little role in regulating blood volume. Cervical vagotomy (4) or complete, selective cardiac denervation (7) failed to attenuate the diuretic response to volume expansion or water immersion in the monkey. However, ADH was not measured in these animals. The present study is unique in that plasma ADH levels were measured during central blood volume shifts induced by body-posture changes in humans with cardiac denervation. Our data are consistent with previous observations in the vagotomized and cardiac-denervated monkey and suggest that the control of ADH secretion in man during the acute stages of simulated weightlessness exposure cannot be completely explained by the Henry-Gauer reflex since plasma ADH changes were similar in heart-lung and cardiac-transplant subjects during acute central blood volume shifts compared to cardiac-innervated controls. However, caution must be taken in the interpretation of similar ADH responses observed in heart transplant recipients and normal cardiac-innervated subjects since residual atrial tissue from the recipient heart containing afferent nerve endings is left during the surgical procedure and the functional capacity of this receptor area is unknown. However, the role of any residual-receptor area in the control of ADH in cardiac-transplant subjects appeared negligible since similar hemodynamic and ADH responses were observed in the heart-lung transplant recipient who represented nearly complete denervation.

In conclusion, the results of the present study demonstrated that the responses of heart rate, arterial blood pressure, plasma volume, plasma renin activity, and antidiuretic hormone to acute central blood volume shifts induced by posture changes designed to simulate the initial phases of entry into and return from weightlessness are similar in partial and complete cardiac-denervated subjects compared to their normal cardiac-innervated controls suggesting that cardiac and heart-lung transplant recipients have mechanisms by which blood volume can be regulated by altering plasma ADH levels. These data are consistent with the hypothesis that the control of ADH secretion during acute central volume changes associated with the initial stages of exposure to weightlessness in man cannot be explained by a role of cardiac-volume receptors alone.

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GARDIOVASCULAR RESPONSES TO +G OR -G STIMULATED DURING POST-EXERCISE IN MILD SUPINE PEDALLING IN -60 mm Hg LBNP

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After leg exercise, an impending syncop is caused by a gravity intolerance due to mild LBNP (1). The gravity intolerance is brought about by diminished cardiac filling and decreased systemic arterial pressure, which are caused by decreasing venous return (3).

In the present study, when mild supine pedalling was performed, cardiovascular responses to the post-exercise period in a -60 mm Hg LBNP were investigated. In specially, the decreased venous return with LBNP was stimulated by a 0 mm Hg LBNP or a 10 mm Hg LBPP in the first experiment, and also by a leg exercise in the second experiment. Changing venous return with muscle pump or LBPP must put back the decreased cardiovascular responses with LBNP into the normal levels.

Method

Subjects participated were seven healthy female students aged 20 to 22 years old. However, two of the subjects fainted during one of the experiment, so the present study was based on results taken from five subjects. Before practical experiments, maximal exercise testing on each subject was carried out on a cycle ergometer. The mean body weight for the subjects was 54.5 Kg and mean maximal oxygen uptake was 36.5 ml/Kg/min. The subjects lay horizontally on a table and the lower half of their bodies were placed in a lower body pressure box with an ergometer(Monark). The exercise load was a 500 kpm/min in all experiments. Through the experiments, oxygen uptake was continuously measured by an online system with a mass-spectrometer and spirometer. Heart rate (HR) was recorded by means of an ECG with a chest lead. Arterial pressure on the left upper-arm was measured with the conventional ausculation method and the mean arterial pressure (MAP) was calculated depending on the measurements. Left ventricle systolic time interval was determined by keeping a record of mechanocardiography which was obtained from simultaneous high speed recordings of an electrocardiographic lead, a carotid arterial pulse tracing and phonocardiogram. Cardiac output was measured by the means of acetylen gas rebreathing technique(2). Fore-arm blood flow(FBF) was measured by Whitnny's method with a rubber strain-gauge in the right forearm (4). Each of other parameters used was calculated depending on these measurments.

After a 15 minute rest period 20 minutes of exercise were performed under a -60 mm Hg LBNP in a supine position, and then a 20 minute period of recovery was observed for each of the conditions. That is, for the first five minutes of the recovery period, the subjects were exposed to -60mmHg LBNP. After five minutes of recovery the exposing condition changed to a 10 mm Hg LBPP or 0 mm Hg LBNP.
Two control experiments were carried out a 10 mm Hg LBPP or in a 0 mm Hg LBNP.

Results

In LBNP condition, HR which was significantly higher than that in the control decreased during post-exercise ($p<0.01$), and did not return to the resting level, staying at about 100 bpm. However, suddenly changing from LBNP to 0 mm Hg LBNP or LBPP caused the HR to dramatically drop to the control level or below the control.

Not only during exercise but also during recovery in LBNP oxygen uptake (VO_2) was a little higher than in 0 mm Hg but significantly lower than in LBPP ($p<0.01$).

The MAP in LBNP stayed at a significantly lower level($p<0.01$) during exercise and also dropped sharply during post-exercise as compared with that in 0 mm Hg LBNP or in LBPP. When changing from LBNP to LBPP during recovery period, MAP was significantly elevated from about 80mmHg to about 100 mm Hg ($p<0.001$) where it stayed constant (Fig.1).

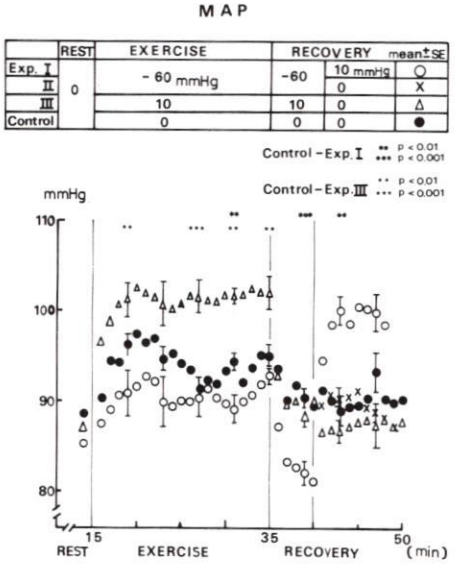

Fig. 1, Changing in mean arterial pressure every minute.

During recovery, although cardiac output(CO) and stroke volume(SV) were significantly decreased in LBNP as compared with those in 0 mm Hg LBNP or to LBPP ($p<0.01$), changing from LBNP to 0 mm Hg LBNP or to LBPP meant that CO and SV were highly elevated and total peripheral resistance(TPR) and systemic arterio-venous O_2 difference (A-V O_2 Diff) decreased (Fig.2).

When repeating exercise after post-exercise, pre ejection phase per left ventricle ejection time (PEP/LVET) stayed the same constant as that of the control level,

although the value did not get back into the control level during the five minute recovery period in LBNP. As shown in Fig. 3, in this case, even in LBNP CO and MAP increased,but were a little bit lower than those in the control condition.

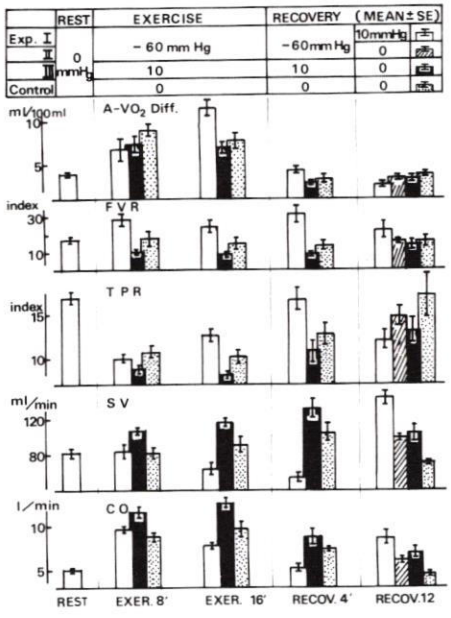

Fig.2, Responses of cardiac output (CO), stroke volume (SV), total peripheral resistance(TRP), forearm vascular resistance(FVR) and systemic arterio-venous O2 difference (A-V O_2) Diff) during exercise and recovery in each experimental condition.

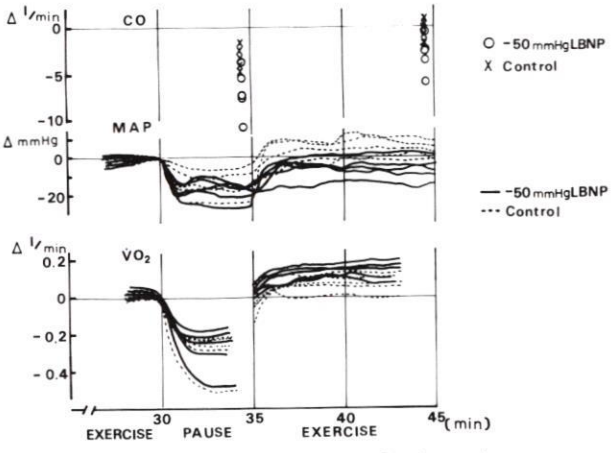

Fig. 3, Tracing of changing in oxygen uptake (VO_2), mean arterial pressure (MAP) and cardiac output (CO) during pause and re-exercise in each of subjects.

Consideration

Using the results obtained from the present experiments, gravity intolerance due to decreased systemic arterial pressure during post-exercise in LBNP could be returned to normal condition by an increase in venous return with LBPP, and this was indicated by an increase in SV. Consideration such as this have also been reached in re-exercise experiment after five minute recovery in LBNP. That is, even if gravity intolerance occurred during post-exercise in LBNP, when the exercise was repeated again, CO and MAP increased at a similar level to that of pre-exercise as heart contractility was getting back to a normal state in spite of the decrease in cardiac efficiency indicated by SV/LVET. Because PEP/LVET would suggest heart contractility and ejection fraction, the fact that heart contractility and/or ejection fraction in re-exercise in LBNP stayed the normal level would be due to an increase in CO with increasing venous return with strengthing muscle pump.

In conclusion, our results showed that gravity intolerance observed by cardiovascular responses at post-exercise in LBNP is returned to normal condition by means of the increase in cardiac output with muscle pump, for example, LBPP or exercise stimulation, which makes venous return increase with increasing muscle pump.

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BLOOD PRESSURE RELATED WITH MUSCLE ACTIVITY AND/OR CAROTID ARTERY COMPRESSURE DURING MILD SUPINE EXERCISE EXPOSED TO LBNP

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In resting man in lower body negative pressure condition (LBNP), progressive venous pooling with decreasing stroke volume and cardiac output eventually cause a fall in arterial pressure(3).On the otherhand, even if the sympathetic activation is clearly nonuniform, vasoconstriction mediated by the arterial baroceptors are probably related to a predominance of low-pressure effect at mild LBNP and further to compressing carotid sinus with progressive venoud pooling and decreasing arterial pressures and pulse pressure at mild LBNP (1).

In the present study, as exposed to progressive venous pooling in LBNP, a mild supine exercise was performed and cardiovascular responses to muscle activity and pushing carotid artery during the exercise were investigated.

Method

Subjects participated were five healthy female atudents aged 20 to 22 years old. Before practical experiments,maximal exercise testing on each subject was carried out on a cycle ergometer.The mean body weight for the subjects was 53 Kg and the mean maximal oxygen uptake was 38.7 ml/kg /min. The subjects lay horizontally on a table and the lower half of their bodies were placed in a lower body pressure box with an ergometer.The exercise load was a 300 kpm/min in the first experiment or a 500 kpm/min in the second experiment. Through all these experiments, oxygen uptake($\dot{V}O_2$) was continuously measured by an online system with a mass-spectrometer and spirometer. Heart rate(HR) was recorded by means of an ECG with a chest lead.Arterial pressure on the left upper-arm was measured with the conventional ausculation method and then mean arterial pressure (MAM) was calculated depending on the measurements. Left ventricle systolic time interval was determined by keeping a record of mechanocardiography which was obtained from stinultaneous high speed recording of an ECG lead, a right carotid arterial pulse tracing and phonocardiogram.Forearm blood flow (FBF) was measured by Whitney's method with a rubber strain-gauge in the right forearm. Cardiac output(CO) was measured by means of athetylene gas rebreathing technique(2). Mean electromyography (m-EMG) on the quart femoral part in the right leg was recorded in the first experiment.Each of other parameters used was calculated depending on these measurements.
In the first experiment, after a 10 minute rest in -30 or -50 mm Hg LBNP 15 minutes of exercise were performed in each LBNP of them. In the second experiment, the left carotid artery was pushed by a 15 mm Hg during the last five minutes of resting period and of exercising period, respectively.

Results

During rest period, LBNP caused progressive increase in HR, and decrease in MAP and FBF, but it did not effect on $\dot{V}O_2$. In this case, pre-ejection phase per ejection time(PEP/LVET) and CO decreased with degree of LBNP. During exercise, $\dot{V}O_2$,m-EMG, and HR increased with degree of LBNP, bur MAP, CO and FBF decreased.

MAP during exercise was significantly correlated to CO, stroke volume/LVET (SV/LVET), FBF, and arterio-venous O_2 difference (A-$\dot{V}O_2$ Diff), but did not to HR and $\dot{V}O_2$.

As shown in Fig.1, m-EMG during exercise was significantly correlated to every parameter such as MAP, total peripheral resistance (TPR), SV, $\dot{V}O_2$, end tidal CO_2 % ($FECO_2$) and A-$\dot{V}O_2$ Diff. Important observation was that although TPR increased with increasing m-EMG, MAP and SV decreased with that.

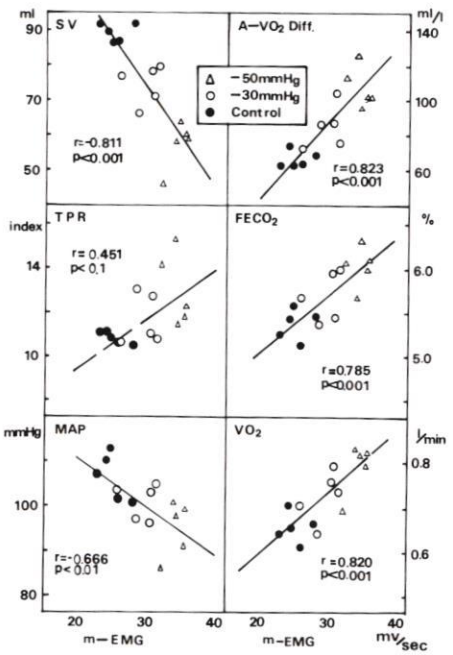

Fig.1, Relationships between m-EMG and MAP, $\dot{V}O_2$, TPR, $FECO_2$, SV, and A-$\dot{V}O_2$ Diff during exercise

Pushing the carotid artery in resting men in the second experiment,$\dot{V}O_2$ did not change, but $FECO_2$ and ventilation (VE) decreased slightly and A-$\dot{V}O_2$ Diff increased slightly. During exercise, these parameters were responsed almost the same as them in the rest, except VE which its changing fushion in LBNP with pushing carotid artery increased progresively in spite of the decrease in $FECO_2$.

In Fig.2, the averaged effect of pushing carotid artery on SV/LVET, CO, MAP, HR and forearm vascular resistance (FVR) during each period of rest and exercise is

shown. Not only in the control but also in LBNP a decreasing tendency during pushing carotis artery was observed on SV/LVET, CO and MAP, and the decreasing tendency was done on FVR. These tendencies were also similarly observed even during exercise, except FVR which decreased further by pushing.

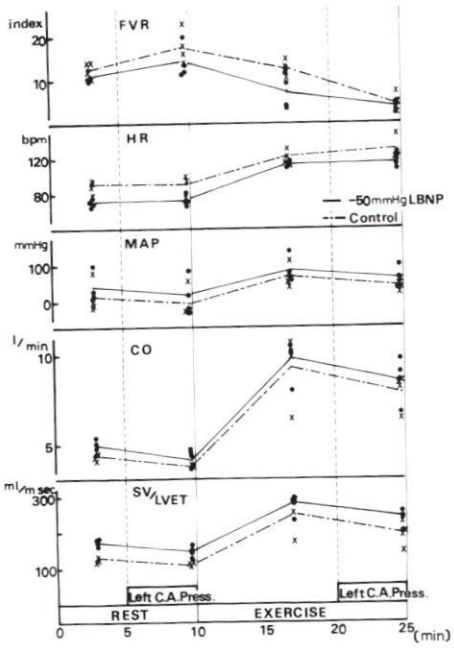

Fig. 2, Changing fushions of SV/LVET, CO, MAP, HR and FVR during rest and exercise with pushing carotid artery (mean of the subjects)

Cosideration

In the first experiment, the exercise load was quite mild (about 30 % VO_2 max). In this case, the degree of LBNP affected on VO_2 during exercise as shown in the increase in VO_2 with increasing degree of LBNP.Also, the affection is suggested on decreasing cardiac efficiency with decreasing MAP, because SV/LVET correlated with MAP is probably indicated as an index of cardiac efficiency. An important observation is on the increases in CO and FBF and the decrease in A-VO_2 Diff with increasing MAP which decreased with degree of LBNP in spite of the same mechanical exercise load, because these relationships indicate the progressive decrease in venous return with LBNP even during mild exercise and thus CO is still an executive controller of systemic blood pressure. That is, even if exercise is performed in LBNP, when the load is mild, despite the muscle pump progressive venous pooling occured brings the decrease in SV and CO and this eventually causes a fall in systemic arterial pressure.

On the otherhand, the increase in m-EMG with VO_2 suggests the increase in muscle mass. According to this, the relationship between m-EMG and MAP means that the increase in muscle mass at an exrcise load causes a fall of systemic arterial pressure regardless of the increase in TPR, $FECO_2$, or A-VO_2 Diff. The fact that SV was conversely correlated to m-EMG, at least, brings such as a conclusion that in the case of below 30 % VO_2 max exercise the muscle pump is too weak to make venous return increase in LBNP. In this case, it is doubtable if some afferent informations from exercising muscle are related to systemic arterial pressure control, as increasing muscle mass with degree of LBNP.

In the second experiment, pushing the carotid artery caused the decrease in MAP and FBF in resting men.This means that vasoconstriction mediated by the arterial baroceptors are related to a predominance of lowpressure effect with compressing carotid sinus due to pushing the carotid artery during rest in normal cndition and also in LBNP. During exercise at about 40 % VO_2 max, however, despite the decrease in MAP and CO the FVR was rather decreased by pushing carotid artery. Therefore, it is possible that an activity of low-pressure baroceptors in carotid simus is not sure in exercising men, and the decrease in systemic arterial pressure is due to a fall of CO, even if the carotid artery is pushed regardless of LBNP.

In conclusion, systemic arterial pressure control during exercise in LBNP is eventually related with progressive venous pooling and thus with decreasing CO. But the importance is such an unexplainable fact that at the same mechanical load MAP during mild exercise was gradually fallen with degree of LBNP regardless increasing muscle mass and thus increasing VO_2.

Summary

Exposing to progressive venous pooling in LBNP, cardiovascular responses during mild supine cycling were investigated in the relation to muscle activity or compressing carotid sinus. Even in LBNP, systemic artery pressure control is at least related with hemodynamic change according to the degree of venous pooling but not positively with muscle activity.

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NON-INVASIVE ASSESSMENT OF HEART CONTRACTILITY CHANGES DURING A 7 DAY 6° HDT 0-G SIMULATION

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Decreasing venous return to the heart by means of LBNP suction can be used to dynamically test heart function. STI are used to assess cardiac performance. Linking these two non-invasive methods we evaluated contractility changes on 6 male volunteers during a 7 day 6° HDT 0-g simulation. LVET and PEP values were plotted as a function of preload. The PEP curves obtained at the end of the HDT period were shifted upwards in comparison to the ones obtained under control conditions. LVET curves shifted in the opposite direction signalizing the same trend of contractility change. From our results, and taking into account changes of heart size and heart rate, we conclude that a depression of the contractile state of the heart must be discussed as a part of the mechanisms involved in the cardiovascular adaptation to 0-g simulation.

Introduction:

One of the aims of a 7 day 0-g simulation by 6° head-down tilt (HDT), was to test a method that should help to describe the changes of cardiac performance that occur in weightlessness or during its simulation. The data found in the literature dealing with this question are controversial.

The method had to be non-invasive, safe, of relative easy implementation, and should ideally make it possible to analyse heart function on a beat-to-beat basis, thus a lowing a dynamic testing, so as e.g. ventricular function curves do. Ventricular function curves are obtained varying preload. One way of reducing venous return to the heart is to apply negative pressure to the lower body with a lower body negative pressure (LBNP) device (2, 3, 5, 7) .

Methods:

As parameters of ventricular function we chose systolic time intervals (STI), a currently utilized non-invasive method for assessing cardiac performance (4, 6). We limited our interest to two STI: pre-ejection period (PEP) and left ventricular ejection time (LVET). STI were measured from the ECG and first derivative of the thoracic impedance. We utilized a portable device that permits continuous measurement of ECG, whole body electrical impedance, thoracic impedance and its first derivative. The data were collected three times during the simulation: Once pre-bedrest, i.e. in the control period, once at the end of the bedrest period, and finally five days after bedrest in the recovery phase.

Results:

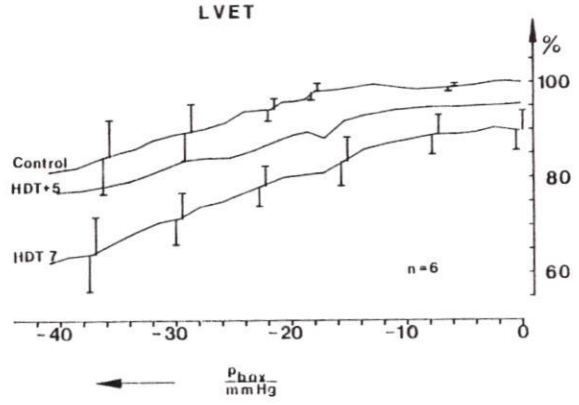

Figure 1. LVET response curves (mean + sd). Preload reduction by LBNP. 100% = resting values during control period.

Fig. 1 shows the mean values and the standard deviations of the curve obtained from 6 volunteers. The curve designated "HDT 7" was obtained at the end of the bedrest period and is shifted downwards to a significant lower level with respect to the control. The curve designated "HDT+5" was obtained on the 5th day of the recovery period and it is not possible to say to which collective it belongs, statistically. Its tendency is towards control. The scattering of the PEP values makes it difficult to interpret the data depicted in Fig. 2.

However, the same trend as the one described for LVET can be recognized in the clear shift of the "HDT 7" curve with respect to the control.

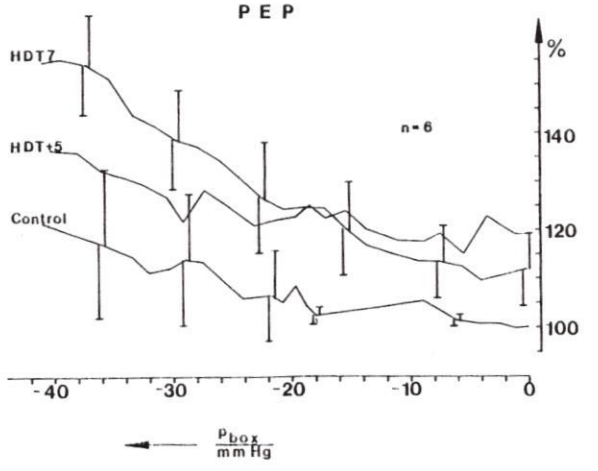

Figure 2. PEP response curves (mean + sd) Preload reduction by LBNP. 100% = resting values during control period.

Discussion:

We reported elsewhere (1) of ventricular function curves obtained from animal experiments, utilizing PEP and LVET as parameters. Fig. 3 shows the PEP and LVET curves obtained from one animal. Three different contractile states can be discriminated. Note the dependence of both parameters on left ventricular end-diastolic pressure (LVEDP) and the shift of the curves indicating changes of the contractile state.

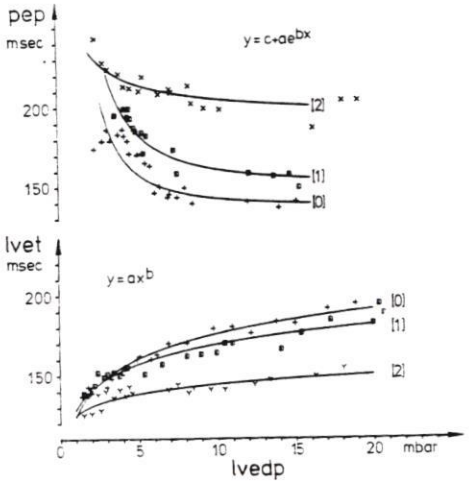

Figure 3. Ventricular function curves obtained from one animal. Three different contractile states are depicted.

The shape of the ventricular function curves represent the dependence of ventricular performance on preload and the shifts represent change of the contractile state. Curves [0] were obtained under control conditions. Curve [1] indicates depressed contractility: the PEP curve is shifted upwards and the LVET curve is shifted downwards. Curve [2] shows further depression of the contractile state.

Recordings of cardiac parameters measured during an LBNP manoeuvre performed in the range 0 to -40mmHG are shown in Fig. 4.

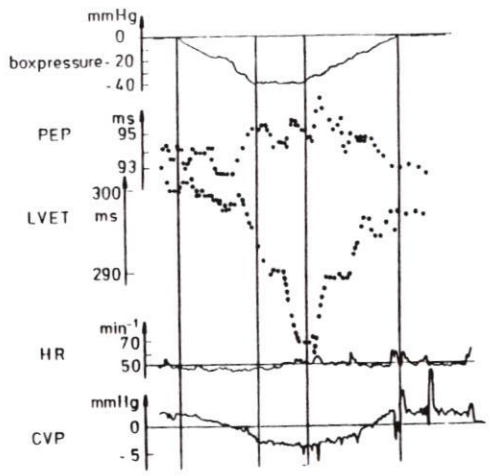

Figure 4. Recordings of cardiac parameters during an LBNP manoeuvre on a human test subject.

LVET and PEP respond in consequence of a diminishing central venous pressure, even in this case where heart rate remains constant. It is well known that these parameters are influenced by a number of factors: arterial pressure, heart rate, preload, and the inotropic state of the heart.

Resting heart rate was similar at the times of measurements; also there were no significant changes of arterial pressure during microgravity simulation. The variations of aortic pressure and heart rate that result from an LBNP manoeuvre in the range 0 to -40 mm mercury cannot account alone for the observed shifts of the curves. Therefore it is concluded that also heart function can be affected by microgravity. If this phenomenon is to be evaluated only as adaptation mechanism or if by continuous exposure to weightlessness without adequate countermeasures, impairement of heart function is to be expected remains an open question that requires further investigation.

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PHYSICAL FITNESS AND ITS EFFECT ON FACTORS AFFECTING ORTHOSTATIC TOLERANCE

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It has been proposed that high levels of physical fitness ($\dot{V}O_{2max}$ > 50 ml O_2/kg/min) results in a more compliant lower limb during lower body negative pressure (LBNP). Subsequently, we have reported no difference in leg compliance between high fit subjects ($\bar{x}$ $\dot{V}O_{2max}$ = 70.2 ml O_2/kg/min) and average fit subjects ($\bar{x}$ $\dot{V}O_{2max}$ = 41.3 ml O_2/kg/min). Independent assessment of leg compliance without LBNP was carried out on six fit (F) subjects (mean $\dot{V}O_{2max}$ = 62.5 ± 1.9 ml O_2/kg/min) and six unfit (UF) subjects (mean $\dot{V}O_{2max}$ = 35.4 ± 2.8 ml O_2/kg/min), P < 0.01. Leg compliance (V_C) was assessed in the supine position by measuring changes in leg volume between leg occlusion pressures of 0, 20, 40, 60, 80 and 100 torr. A summary of the results are listed below:

	Height (cm)	Weight (kg)	Body Surface Area (m^2)	Leg Compliance ($ml \times 10^{-2}$/torr)
F $\bar{x}$	177.9	71.4	1.89	7.86
UF $\bar{x}$	181.3	90.4	2.09	6.80
P Value	NS	<0.05	<0.05	NS

Further investigation of these same subjects using phenylephrine HCL as an alpha-agonist challenge resulted in the following data obtained during steady state hemodynamics at an infusion rate of 60 µg/min.

	HR (beats/min)	DBP (torr)	HR/DBP (ratio)	$\dot{Q}$ (l/min)	Calculated PVR (units)
F $\bar{x}$	41.4	96	0.45	6.07	18.71
UF $\bar{x}$	56.6	82	1.22	7.15	14.31
P Value	<0.01	<0.02	<0.05	NS	<0.05

In conclusion our investigations suggest, in contrast to previous reports, that the high pressure baroreceptor and its reflex response to hypotension was altered by endurance training.

Introduction

In their review Klein et al. (1) questioned the concept of endurance training as an appropriate means of preparing for environments which challenge the blood pressure control system during orthostatism. These challenges may be transiently severe (+7 to +9G_z) in nature (e.g. a high velocity turn in a fighter aircraft), or prolonged duration, yet less severe (+1 to +2G_z) in stress, as observed during shuttle re-entry. The primary reason presented to explain the loss of blood pressure control in an exercise fit population during orthostatic challenges similar to those identified above was based upon the finding of a casual yet strongly positive relationship between maximal aerobic capacity ($\dot{V}O_{2max}$) and lower limb compliance (2). A consequence of this increase in lower limb compliance during a challenge using lower body negative pressure (LBNP) was a decreased tolerance to LBNP because of an increased rate of change in leg volume (2). More recently, we found no difference in the rate of change in leg volumes or calculated lower limb compliance, during LBNP to -50 torr, when comparing six average fit ($\bar{x}$ $\dot{V}O_{2max}$ = 41.3 ± 2.9 ml O_2/kg/min) subjects with eight high fit ($\bar{x}$ $\dot{V}O_{2max}$ = 70.2 ± 2.6 ml O_2/kg/min) subjects (3). Hence, the effects of endurance training on the factors affecting lower limb compliance remain in question and form the basis of the present investigation.

Materials and Methods

Twelve healthy adult male volunteers ages ranging from 25 to 32 years were used as subjects in these studies. Special efforts were made to include individuals involved in intense athletic training. Each subject provided informed consent in writing. The study had the approval of the Human Research Review Committee of the Texas College of Osteopathic Medicine. Preliminary examinations including a resting 12-lead electrocardiogram (ECG), a health questionnaire evaluation, and a treadmill exercise stress test to the level of $\dot{V}O_{2max}$.

The Bruce maximal stress test protocol (4) was utilized for all subjects to determine $\dot{V}O_{2max}$. During the test, ventilation volumes were determined minute by minute using a dry gas meter (model CD_4; Instrumentation Associates) connected to the inspiratory side of an Otis-McKerrow breathing valve. A continuous measurement of the O_2 and CO_2 content in expired air was obtained by sampling from a 10-liter Plexiglas mixing chamber connected to the expiratory side of the breathing valve. Measurements of the gas concentrations were made using a respiratory gas analyzer-mass spectrometer (Perkin-Elmer model 1100AB) calibrated against known standard gases. All variables were recorded on a multi-channel pen recorder (Soltec model #3313) and properly corrected for time delays, temperature, and pressure to determine the necessary standardized (STPD and BTPS) outputs as averages for each minute of exercise.

Multiple ECG leads (standard and augmented limb leads and V_5) provided a continuous monitor trace on an oscilloscope, and a 10s ECG recording was obtained at 1-min intervals during the test and immediately before the end of the test. An auscultatory brachial artery blood pressure was determined at 3-min intervals during the test and at test termination. A plateau of O_2 uptake ($\dot{V}O_2$) during the final stages of the test, maximum heart rate (HR), and the appearance of the subject at the termination of the test were used as both qualitative and quantitative criteria for determining the maximum effort (5).

After determination of the individual's $\dot{V}O_{2max}$, the subject was assigned to one of two groups according to his level of $\dot{V}O_{2max}$. By design, the $\dot{V}O_{2max}$ data separated the subjects into two distinctly different groups. Individuals were designated fit (F) when their $\dot{V}O_{2max}$ was > 58 ml O_2/kg/min and unfit (UF) when their $\dot{V}O_{2max}$ was < 40 ml O_2/kg/min. Table 1 provides a descriptive summary for both groups of subjects.

TABLE I
Description of Subjects

		age (yrs)	ht (cm)	wt (kg)	BSA (m^2)	$\dot{V}O_2$max (ml/kg-min^{-1})	SBPrest (mmHg)	DBPrest (mmHg)	HRrest (beat/min)
F	$\bar{x}$	30.5	175.9	70.8	1.86	62.1	118.3	74.8	49.6
N=6	SE$\bar{x}$	1.8	2.8	3.1	0.05	1.9	2.9	2.8	3.4
UF	$\bar{x}$	27.8	180.0	94.5	2.12	33.2	115.6	73.9	67.8
N=6	SE$\bar{x}$	1.9	2.5	8.4	0.07	2.0	2.4	3.0	2.6
p		NS	NS	<0.02	<0.01	<0.01	NS	NS	<0.01

On a separate day, following the assessment of $\dot{V}O_{2max}$, and without prior activity providing a residual effect, the leg compliance (V_C) of each subject was assessed in the supine position, see Figure 1.

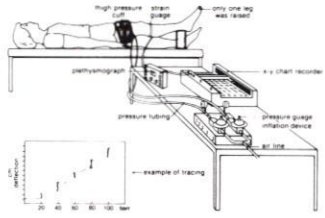

Figure 1
SCHEMATIC DRAWING OF PROCEDURES USED TO DETERMINE CHANGES IN LEG VOLUME UNDER THE STRAIN GAUGE

The leg compliance (V_C) was determined by measuring changes in leg volume (LgV) between leg occlusion pressures of 0, 20, 40, 60, 80 and 100 torr. Changes in LgV were derived from changes in calf circumference. Leg circumference (LgC) was measured by a dual-loop mercury-silastic Whitney strain guage plethysmograph. The loop was placed in a midcalf position with wooden spacers to ensure that the loops of the gauge were maintained 1 cm apart. The output of the gauge was amplified (Parkes Electronics model 271) and recorded on a strip-chart recorder (Physiograph model DMP-4B). A calibration curve for each gauge was obtained before and after each LBNP exposure. Initial total leg volumes (LgV) were calculated from measurements of circumference at 12 well-defined points using Simpson's rule (6). This volume did not include the volume of the foot. LgC changes, measured as percent of original circumference, were assumed to be representative of changes throughout the leg as a whole and converted to equivalent volume changes in LgV. Leg occlusion was provided by a thigh cuff automatically and progressively inflated to the required pressures, after stabilization of circumference was observed on the recording, see insert of Figure 1, the LgC measurement was made.

On a separate day following the assessment of V_C the subjects α-receptor sensitivity was determined using phenylephrine hydrochloride (PE) via a venous cannula at infusion rates of 6, 12, 20, 40 and 60 µg/min, see Figure 2.

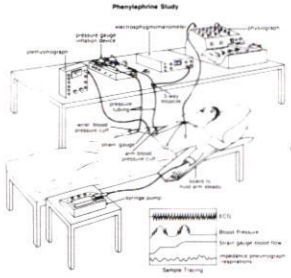

Figure 2
Schematic drawing of the system used to produce α-challenge by infusing phenylephrine hydrochloride at 0, 6, 12, 20, 40, and 60 µg/min infusion rates.

At rest and during the period of stable hemodynamics for each infusion rate, heart rate (HR) using 10 sec averages of R-R intervals, blood pressures (BP), cardiac output ($\dot{Q}$) using a CO_2 rebreathing technique (7) and forearm blood flow (BF) measured by strain guage plethysmography were determined.

Results

As noted in Table 1, the subjects used were 12 healthy adult male volunteers designated as F with an average $\dot{V}O_{2max}$ = 62.1 ml O_2/kg/min. and UF with an average $\dot{V}O_{2max}$ = 33.2 ml O_2/kg/min., p < 0.02. However, resting systolic, diastolic and hence mean blood pressures were not different (p > 0.05).

Figure 3 graphically describes the differences in initial calculated calf volumes under the 1 cm strain guage between the F ($\bar{x}$ = 107.5 ± 2.5 cm^3) and the UF ($\bar{x}$ = 125.5 ± 6.1 cm^3) p < 0.01, as well as identifying the difference in variances between the two groups.

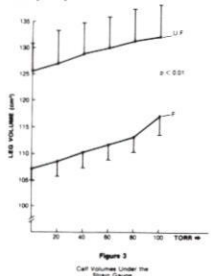

Figure 3
Calf Volumes Under the Strain Gauge

These differences were maintained over the complete range of occlusion pressures. Hence, changes in absolute volume per unit of occlusion pressure were not different between the two groups p > 0.05, see Figure 4.

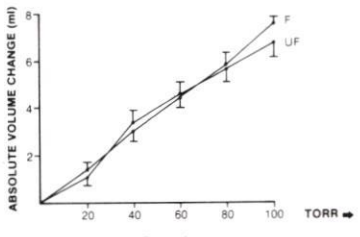

Figure 4

Changes in leg volume under the strain gauge

During PE infusion from 0 to 60 µg/min the absolute change in HR was the same in both groups. The difference observed in the resting HR, see Table 1, of 68 beats/min for the UF and 50 beats/min for the F was maintained at 60 µg/min where the HR of the UF = 62 beats/min and the F = 42 beats/min ($p < 0.01$), see Figure 5.

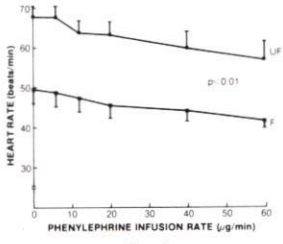

Figure 5
Changes in Heart Rate of Both Groups with Increasing Doses of Phenylephrine

The major difference that was observed was that the F subjects had a significantly higher diastolic blood pressure increase over the course of the infusion, see Figure 6.

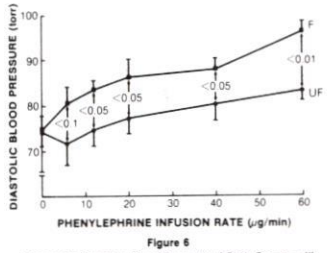

Figure 6
Changes in Diastolic Blood Pressure of Both Groups with Increasing Doses of Phenylephrine

The infusion resulted in a 21 torr increase for the F and only 9 torr increase for the UF (at 60 µg/min), $p < 0.01$. These differences in response were observed at the lowest infusion rates of 6 µg/min. The changes in systolic and calculated mean blood pressure were similarly different between groups.

Discussion

The data obtained in these investigations confirm our previous findings (3) of no difference in lower limb compliance between F and UF subjects. In this present investigation limb compliance (V_C) was assessed passively and would not have involved differences in afferent or efferent vasomotor inputs mediated by the baroreflex response to the LBNP induced hypotension. Once again one must question the finding of Luft et al. (2) with respect to their reported differences in V_C between F and UF subjects. Furthermore, one could suggest that the strongly positive relationship they observed between V_C and $V\dot{O}_{2max}$ was one of chance and that the relationship between LBNP tolerance and V_C or $V\dot{O}_{2max}$ was more casual rather than causal.

When PE infusions were used to produce a pure α-receptor challenge the F subjects responded markedly whereas the UF subjects appeared to have less sensitive α-receptors, see Figure 6. This increase in pressure was accomplished primarily by a greater vasoconstriction, see Figure 7, evidenced by a greater reduction in forearm blood flow and a greater increase in peripheral vascular resistance of the F compared to the UF subjects.

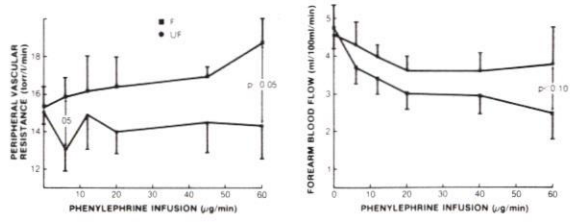

Figure 7
Changes in Forearm Blood Flow and Calculated Peripheral Vascular Resistance

These changes were mediated without alterations in metabolism or cardiac hemodynamics. It was felt that in the light of a constant cardiac output the difference in the alterations in resistance and flow were primarily mediated by differences in the α-receptor sensitivity.

Despite the F subjects having a greater increase in diastolic and systolic blood pressure the heart rate depression observed in both groups was the same. A calculated baroreflex index of sensitivity ($\Delta HR/\Delta DBP$) was used to clarify the differences between F and UF subjects. From 0 µg/min to 60 µg/min the $\Delta HR/\Delta DBP$ for the F = 0.45 and for the UF = 1.22, $P < 0.01$. In addition, this index was negatively correlated to $V\dot{O}_{2max}$, $r = -0.5$, $p < 0.1$ by the expression $\Delta HR/\Delta DBP = -0.04\ V\dot{O}_{2max} + 2.8$. These data suggest that the baroreflex response to hypertensive stress was modified by high $V\dot{O}_{2max}$ levels.

In conclusion, we have shown that factors affecting blood pressure control are altered by high $V\dot{O}_{2max}$ levels. In addition, similar to our previous report (3) we noted an attenuated baroreflex response in the high fit subjects. Thereby suggesting that the interaction of other reflexes impinging on the baroreflex (8,9) are in some way modified by exercise training which results in a high level of $V\dot{O}_{2max}$.

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EFFECT OF DIFFERENT RESTING POSITION ON URINARY KALLIKREIN ACTIVITY IN NORMAL MAN.

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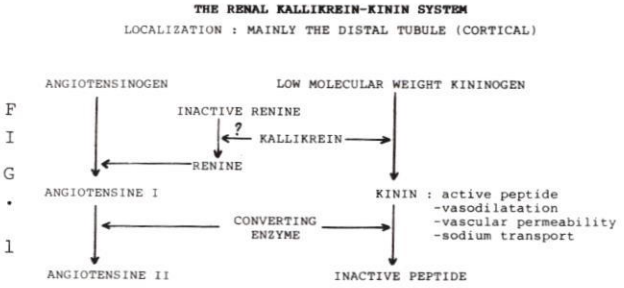
Renal kallikrein is an enzyme which could be involve in several putative functions:sodium and water balance,renal blood flow regulation... In this study we reporte the results of 3 normal subjects which have been submitted to three different protocols:day 1 seven hours in the sitting position,day 2 five hours in the supine position,day 3 head-down tilt (HDT)at -8° for five hours.In day 2 and 3 one hour control in the sitting position was observed before and after the experiment.For the three subjects the results show the same pattern of evolution. In day 1 no significant change in diuresis and in kallikrein activity was observed,in day 2 and 3 change in position either supine or HDT provide effective stimuli to increase kallikrein excretion.Since kallikrein activity remained steady only in the sitting position,this position could appear suitable for determination of baseline value.

INTRODUCTION

Its now well documented that micro-gravity induces several modifications in the cardiovascular and endocrine systems.There is a redistribution in body fluids specially blood,lymph and interstitial fluid and also a modification of the activities of endocrine systems such as the renin angiotensin aldosterone system.Fif.1 shows the interactions between the renal kallikrein kinin system (RKKS) and the renin angiotensin system (R.A.S.).The renal kallikrein is a serine protease acting on a liver glycoprotein,mainly the low molecular weight kininogen to generate kinin which is the active peptide of the system.The organisation of the RKKS is very parallel to the RAS with a major interconnexion via the converting enzyme acting on both side.

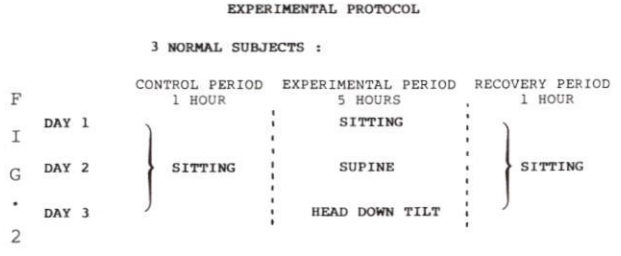
The study of the kallikrein system is of interest because of several hypothetical functions in cardiovascular and renal homeostasis (1).This system has been involved in sodium and water balance with conflicting results either natriuretic (2) or antinatriuretic (3).The RKKS has also been involved in renal blood flow regulation because of the potent vasodilataor effect of kinins and also in the activation of prorenine,at last in vitro.This study was designed to investigate the effect of changes in position on the variations of endocrine systems in order to establish a reference position for the determination of baseline values.



F I G . 1

MATERIAL AND METHODS

Three normal subjects of about 24 years old were submitted to 3 different protocols.Fig.2 shows the experimental protocol.On day 1 the subjects remained in the sitting position during seven hours.On day 2 the subjects remained in the supine position during five hours and on day 3 the subjects were submitted to five hours in head-down position at -8°.In day 2 and 3 one hour control period in the sitting position was observed before and after the experimental period.At the beginning of each period it was asked to the subjects to drink 100 ml ofwater.For urine samples it was asked to the subjects to void their bladder at the end of each hours.Urinary kallikrein activity was measured by the action of an urine aliquote incubated during 30' at 37°C with a crude preparation of kininogene,after the incubation period the generated kinins were measured by radioimmunoassay.Urinary kallikrein activity is express in µg of bradykinins generated per minute of incubation and per hour of total volume of urine (µgBK/'/h).



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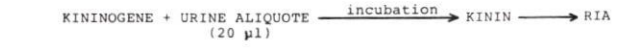
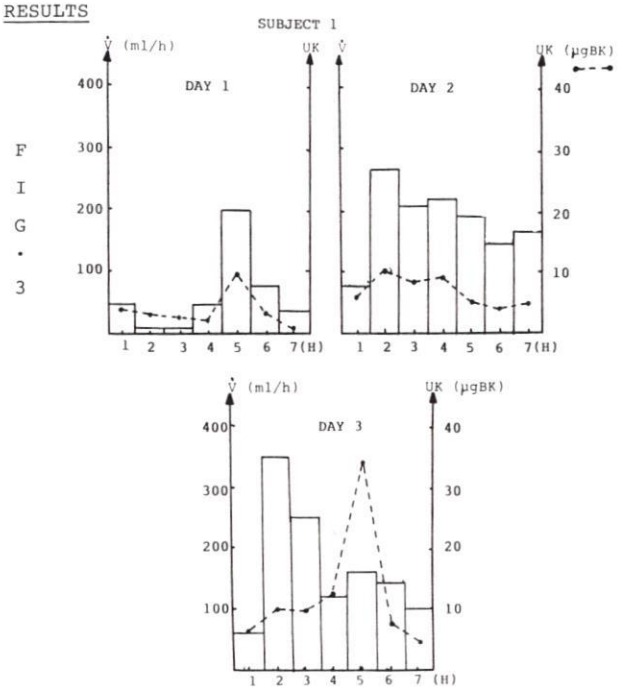


RESULTS



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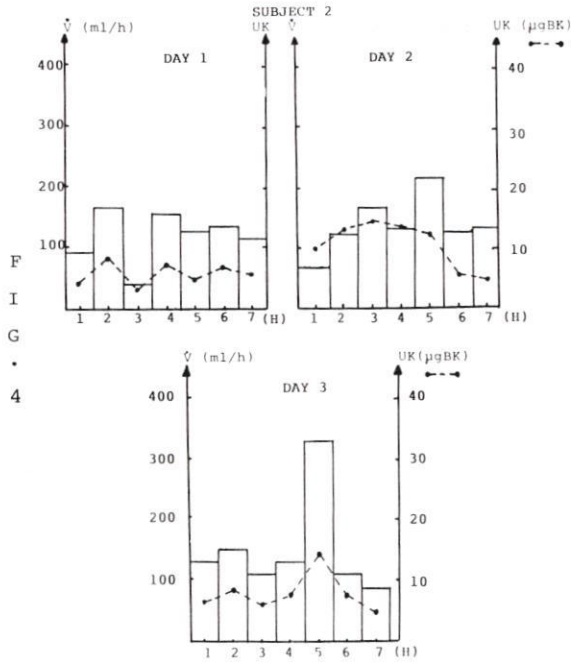

FIG. 4

The present data came from 3 normal subjects(six were run but only 3 subjects had provided urinary samples at each period).The individuals results for subjects 1,2 and 3 are plotted respectively on figure 3,4 and 5.In the sitting position (day 1)for the 3 subjects,urinary kallikrein activity (UKal A) and diuresis remained around means values which are for UKal A (4,33±2,47 µgBK/'/h and for diuresis (76,62±55,7 ml/h).On day 2,in supine position, UKal A did not rise beyond a first initial increase which is at least 50% higher than the first hour control in the sitting position.In the head-down position (day 3) UKal A had shown a progressive increase reaching for subjects 1 and 2 a maximal value 4 hours after the beginning of HDT, respectively 34,64 µgBK/'/h and 14,5 µgBK/'/h and 5 hours after for subject 3 (17,4 µgBK/'/h).It is interesting to remark that all the subjects,in the recovery period,returned to the control value observed at the first time period either on day 2 or day 3.

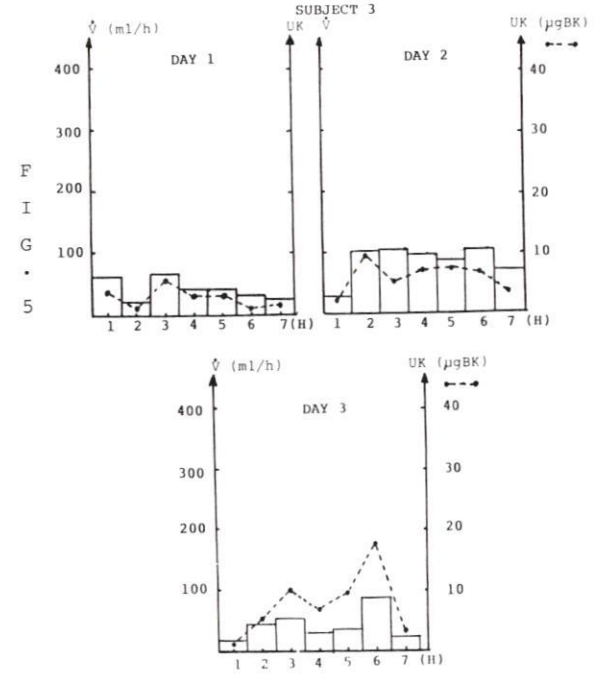

FIG. 5

DISCUSSION

There are very few studies reporting the evolution of the UKal A excretion during changes in position and specially during a head-down Tilt.Because of the small number of subjects it was impossible to run statistical analyse.However the interpretation of separate data subject by subject shows a tendency to increase UKal A excretion when the subjects are submitted to the translation from sitting to supine or to head-down position.Two main arguments reinforce this interpretation,during seven hours in the sitting position UKal A remained steady, furthermore after changes in position this activity returned to control value during the recovery period,this indicates that the methodology is reliable.The reasons and the role of this increase during supine and head-down position remain to be elucidate.However,some factors have been reported to influence renal kallikrein excretion,among which are:renal artery pressure volume expansion,Mineralocorticoïds (2),antidiuretic hormone (4)and angiotensin II (2).In several conditions, the renin angiotensin aldosterone system and kallikrein excretion are dissociated,i.e water immersion to the neck does not affect kallikrein (esterase) excretion in man,in spite of the fact that this manoevre depresses the renin-angiotensin aldosterone system (5,6).An other investigator A.MIMRAM (7) reports during a tilt test from supine to upright (+60°),a decrease in urinary kallikrein esterase activity with an augmented plasma renin activity.These decrease in UKal A is abolished by captopril a very potent inhibitor of the converting enzyme and thus of the Angiotensin II generation. These data are consistent with our results and the increase in UKal A excretion might reflect an increase of the intra-renal kallikrein system in response to an elevated local angiotensin II concentration in order to protect the renal circulation. In conclusion,in spite of the small number of subjects,UKal A excretion remains steady after seven hours in the sitting position;this position appears suitable for determination of baseline value.The change in position from sitting to supine or head-down position induces an increase in UKal A excrerion and this increase seems to be more pronounced during head-down tilt.A greater number of subjects are necessary to confirm these preliminaries results. Since the role of the renal kallikrein kinin system is not well known and because of the studies are reporting variations of this system in pathological conditions,head-down tilt appears as a usefull manoevre to stimulate and to study this system in physiological conditions.

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EFFECT OF MUSCULAR EXERCISE DURING 4 DAYS SIMULATED WEIGHTLESSNESS ON ORTHOSTATIC TOLERANCE.

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In this study,the effectiveness of muscular exercise to prevent orthostatic intolerance (OI),was investigated during a 4 days head-down tilt (HDT)at -6° in two groups of four young males,one control and one submitted twice a day at one hour of bicycle exercise.To evaluate orthostatic tolerance two tilt tests were performed,one before the HDT and one after the HDT. Plasma renin activity (PRA)plasma arginine vasopressin and plasma catecholamines were measured.Heart rate (HR)and blood pressure (BP) were recorded every minutes all through the test.In both groups,the two tilt tests have produced an increase in HR.After the HDT, the tilting at +85° has induced an increase in PRA in both groups,but no difference was observed between the two groups.During the second tilt test,OI was only demonstrated by the exercise groups.These results suggest that under our experimental conditions,physical exercise is ineffective to prevent OI.

INTRODUCTION

Its now well known that exposure to weightlessness even for short periods,induces significant changes in the cardiovascular and endocrine systems in man (1,2).Following return to earth gravity Astronauts show an alteration in orthostatic tolerance characterized by tachycardia,decrease in blood pressure, impairment of exercise capacity and as a general rule difficulties in readapting to normal activity (3,4).Exposure to microgravity induces a rapid shift of about 1500 to 2000ml of blood,lymph and interstitial fluid from the lower to the upper part of the body.This fluid shift leads to a cardiovascular deconditioning.For better understanding the cardiovascular modificationswhich occur in microgravity and also to develop effective and convenient counter measures,ground based simulation methods have been developed.Among these methods Head-down Tilt (HDT) is a very useful method because it reproduces the orthostatic intolerance (OI)observed in crew members after space flight.The purpose of the present investigation is to study the effectiveness of muscular exercise,during a 4 days head down tilt, to prevent OI.

MATERIALS AND METHODS

The experiment was explained to the volunteers, medical students',and their consent obtained.Eight young healthy male (24,1±0,4 years old) have been divided into two equal group.One group served as control (CG)the other(exercise group:EG)was submitted twice a day at one hour of bicycle exercise, always in the anti-orthostatic position,performed at 50% of their maximum respective oxygen,uptake, corresponding to an energy expense of about 1000 Kcal.The subjects were free from any drug and fed on a normal hospital diet the total caloric intake being about 2500 Kcal/day,Na^+:120mEq and K^+ 60mEq with free fluid intake,air temperature:23°C. Two days before starting the experiment an active orthostatic test was performed and the data obtained have served as control values.The orthostatic test was carried out on a tilt table,after 60 minutes in supine position the subjects were gently tilted at +85° for 15 minutes then,bring back to the horizontal position.Two blood samples were taken from an antecubital vein via a catheter implanted one hour before,in order to prevent puncture side effects,two minutes before tilting at +85° and at the tenth minutes in the upright position. Blood samples were immediately centrifuged at +4°C and the plasma frozen at -20°C until assays. On these plasma samplesthe following parameters were measured:plasma renin activity (PRA),plasma arginin vasopressin (PAV)and plasma catecholamines:epinephrine (E),Norepinephrine (NE)and Dopamine (DA). Heart rate (HR),systolic and diastolic blood pressure respectively (SBP)and (DBP)were recorded every minutes all through the test with a monitor. After a 24 hours'control period in horizontal position,the subjects were tilted at -6° for four days. The subjects were free to move,the only restriction was to keep their heads above bed level.On the fifth day,the subjects were bring back to the horizontal position and an active orthostatic test was performed in the same experimental conditions as before the HDT.

RESULTS

Tilt test before HDT (B.HDT).

For the hormonal parameters,in response to the tilting at +85°,no change in PRA,PAV and catecholamines have been observed in both groups.

For the systemic responses,individual HR and BP responses are plotted on Figures 1(control group) and 2 (exercise group).The tilting at +85° produces an increase in HR for all subjects,but BP is not affected by the change in position.

Tilt test after HDT (A.HDT)

No significant change have been observed in PAV and catecholamines.However this second tilt test (eg day 5) induced the same magnitude increase in PRA in both groups when tilted at +85°:C.G had shown an increase of about 55% E.G:about 80%.It is also of importance to point out that supine values before the tilt test are six fold higher in both groups when compared to the supine values, measured before the first tilt test (eg day-2).This results indicated the well documented effect of several days in head-down position.

For the systemic responses,HR and BP (Fig.1 and 2),it is interesting to remark that HR was always higher after the HDT than before for all the subjects.For the CG (subject 1,2,3)in spite of the small variations observed,blood pressure have been well regulated.For two subjects of the EG the tilt test have been terminated prematurely because of clinical evidence of faintness respectively at the 14th minutes (subject n°5)and the 10th minutes (subject n°6)in the upright position.For the two other subjects (subjects 7 and 8)we can also observe a transitory fall in BP at the end of the tilt but without symptoms of faintness.

DISCUSSION

The present investigation was designed to examine the effect of muscular exercise on the occasional prevention of OI after 4 days in head down position. In our experiment the intensity of exercise has been chosen in order to reproduce the activity of

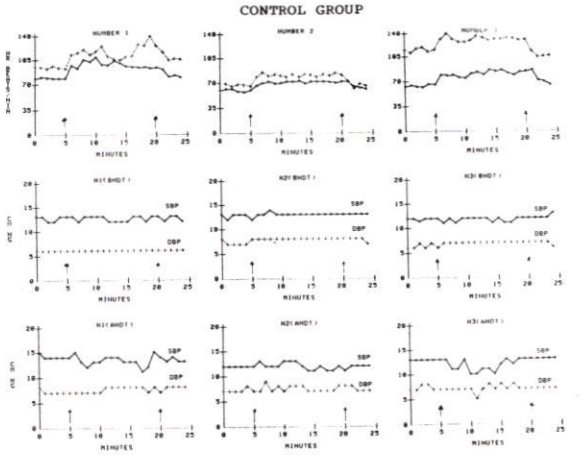

FIG. 1

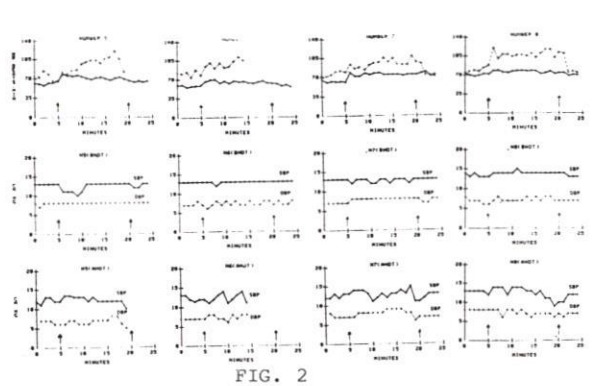

FIG. 2

Fig.1-2 : Individual responses of heart rate (HR), blood pressure (BP).

—HR before HDT --- HR after HDT.At the first arrow the subjects were tilted at +85° and at the second arrow bring back to the supine position.The two others graphs indicate SBP and DBP berofe HDT in the second line graphs and after HDT in the third line graphs.

a sedentary subject,i.e 2600 Kcal/day divided in the following way:1600 Kcal for the basal metabolism and 1000 Kcal for the physical exercise.The method employed to study the orthostatic tolerance has been the active orthostatic test because it reflects a normal physiological activity rather than passive Tilt,which is more effective but non physiological stimulus.

The increase in HR observed in all subjects during both tilt tests could be explain by the fact that the change in posture from supine to upright position leads to a rapid shift of blood in the legs.This new redistribution of blood volume is perceived by cardio-vascular stretch receptors which send this information to the central nervous system and in response,there is an arteriolar vasoconstriction and an increase in HR.The main purpose of this reflex mechanism is to maintain arterial blood pressure.There is also a neurohormonal response characterized by an increase in the secretion of PAV (Gauer-Henry reflex),an increase in PRA and in catecholamines.Our results show no significant variation of this 3 hormonal systems except for PRA after HDT;in fact individually (not shown)all the subjects have shown these classical responses but the non significant variations obtained are probably due to the well known fact that great variations exist from one subject to another within each group,the very important standart error or the mean are testfy to that conclusion.The increase in resting PRA after the HDT compared to the supine position of the first tilt test could be explain by the following fact;during the first day of HDT there is an inhibition of the renin angiotensin aldosterone system (RAAS)with an important natriuresis and,in response of this loss we assist to an important increase in the activity of the RAAS.Orthostatic intolerance found in subjects submitted to physical exercise can not be attributed to a decrease in sympathetic nervous activity.In actual fact plasma NE can be considered as an index of sympathetic nervous activity (5,6)and the results obtained in our experiment show that NE response to the second tilt test is not significantly different from those observed during the first tilt test;this would be in agreement with the results of CHOBANIAN et al(7).On the other hand it is possible that the intensity of the exercise to which the subjects were submitted during the 4 days'period was not sufficient.So,maybe a shorter time exercise with a stronger intensity would had been effective.We can also suggest that physical exercise has produced an increase in vascular bed in the legs.An other hypothesis suggested by STEGMANN et al.(8) could be that physical exercise might produce an increase in vagal tone;in this condition,the cardiovascular and neurohormonal responses become inefficient to maintain arterial pressure during the change from recumbent to upright position.Therefore,administration of a vagolytic drug half an hour before the end of an immersion period prevent the OI in subjects who have been submitted to physical exercise during this immersion (8).Other studies ought to establish if these association,exercise and vagolytic drug really prevent, in all cases,the OI observed after HDT at different negative angles.In conclusion,our study has shown that under our experimental conditions,physical exercise was ineffective to prevent OI and even have had an opposite effect.Besides,our study has also shown that the individual variations are not negligble and that they ought to be taken in mind for the interpretation of the results,particullary when the number of subjects is reduced,which is very frequent in the experiment of weightlessness simulations.

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PLASMA CATECHOLAMINES AND CENTRAL HEMODYNAMICS DURING SIMULATED ZERO GRAVITY (HEAD OUT WATER IMMERSION) IN HUMANS

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ABSTRACT

The variation in plasma epinephrine (PlE) and nor-epinerphrine (PlNE) with increased central venous pressure (CVP) was examined in 8 healthy males on two occasions after overnight food- and fluid restriction: During control the subjects sat upright outside the pool for 6 hours wearing a water perfused garment (water temperature = 34.6 centigrade). During water immersion to the neck (WI) they assumed the same position in a stirred water bath for 6 hours (water temperature = 35.0 centigrade). Central venous pressure, Systolic arterial pressure, and cardiac output increased during water immersion as compared to control while diastolic arterial pressure was unchanged. Heart rate (HR) was decreased by 10 bpm throughout immersion. When Plasma Epinephrine and Plasma Norepinephrine during WI were compared to control values significant decreases were found. The experiment indicates that the decrease in HR is controlled by sympathetic nervous activity which is diminished during WI.

INTRODUCTION

The purpose of the present study was to investigate if water immersion (WI) as a model of simulated weightlessness for 6 hours would influence the sympathetic nervous activity (SNA) in humans. This would explain, at least in part the depression of heart rate (HR) previously reported during these circumstances. We have previously reported a depression of the catecholamine levels during 6 hours WI in four subjects (3), but since no effect of WI on SNA could be measured by Epstein et al (4), while Krishna (5) reported a decrease in plasma norepinephrine during WI, it was felt that this discrepancy in the literature needed further elucidation.

MATERIAL AND METHODS

Eight healthy males age 19-28 y., weight 61.8-92.8 kg, and height 168-199 cm participated in the experiment. All had a negative history for hypertension, cardiovascular- or kidney diseases. Informed consent was obtained after the subjects had read a description of the experimental protocol. During WI to the neck (sternoclavicular notch) the subject was sitting in a swimmingpool kept at thermoneutral temperature (35.0 centigrade). During control experiments the subject was sitting beside the swimmingpool wearing a garment perfused with water taken from the pool. In this way the subjects had the same skin temperature as during water immersion.

Central venous pressure (CVP) was measured through an indwelling 60 cm long catheter. The pressure transducer was placed at level with the subjects right nipple.

Systolic (SAP) and diastolic (DAP) arterial pressures were measured with a mercury sphygmomanometer (Erkameter 300) and a stetoscope. The beginning of the fourth sound of Korothoff indicated DAP. Heart rate was measured by palpating the radial artery.

Cardiac output was measured with the acetylene rebreathing technique described previously (2).

For one hour pre- and poststudy the subject sat in the chair beside the pool both during the control study and WI. During control, the subject was sitting in the chair for 6 hours wearing the water perfused garment while during WI the subject sat in the swimmingpool also for 6 hours.

Blood samples were drawn 30 min before the experiment, at 4, 5, and 6 hours, and 1 hour after the experiment for analyses of epinephrine and norepinephrine. At the same times Cardiac output, HR and blood pressures was recorded

Data were evaluated statistically by paired t-tests. A significance level of 0.05 was accepted.

RESULTS

During control, CVP was 2.2±1.3 mmHg (mean±SE) and 3.3±1.2 mmHg at 20 min and 6 hours, respectively, while corresponding values for WI were 10.9±1.5 mmHg and 10.0±1.4 mmHg, respectively ($P<0.01$).

Systolic arterial pressure increased significantly by 7-10 mmHg ($P<0.05$) after 2 hours of WI, while diastolic arterial pressure was unchanged.

Heart rate (HR) was decreased by 10 bpm throughout immersion.

Plasma epinephrine (PlE) increased significantly ($P<0.05$) during the 8 hours sitting control, but plasma norepinephrine (PlNE) remained unchanged. When PlE and PlNE during WI were compared to control values significant decreases were found (PlE decreased from 0.363±0.0056 ng/ml to 0.0175±0.0025 ng/ml, while PlNE decreased from 0.2862±0.0141 ng/ml to 0.1288±0.0249 ng/ml at 6 hours of control, respectively WI conditions).

There was a positive significant correlation between PlE and PlNE on one side and HR on the other side during both control and WI experiments.

Mean arterial pressure and CVP correlated negatively to PlNE.

Cardiac output increased during water immersion by around 1 l/min compared to control values which varied between 5 and 6 l/min. After WI there was a drop in cardiac output down to 4.8 l/min.

DISCUSSION

The present experiment indicates that the HR is controlled by sympathetic nervous activity also during WI because a positive statistical significant correlation was found between HR and plasma catecholamines. The exact mechanism behind this finding is not clear because it is not known to which extent peripheral and central nervous factors participate in this regulation. One possibility is that the high pressure baroreceptors which stimulated to an increased negative feed back by the increased stroke volume during WI. The increased cardiac output also found in connection with the increased central venous pressure fits with the Starling relation for the heart. The physiological counter regulatory mechanisms elicited from the dynamic part of the high pressure baroreceptors in aorta and the carotid sinuses via the cardiovascular center directed towards the peripheral

Table 1 Central venous pressure (CVP), Heart rate (HR), Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), Cardiac output (CO), Plasma epinephrine (PlE) and Plasma norepinephrine (PlNE) during the control and the water immersion (WI) experiments

Time	CVP	HR	SAP	DAP	CO	PlE	PlNE
	Torr	bpm	Torr	Torr	l/min	ng/ml	ng/ml
-30 min							
control	2.175	79	96.6	59.3	5.794	0.025	0.303
WI	2.871	77.3	94.5	58.4	5.556	0.029	0.336
4 hours							
control	1.950	80.5	96.6	58.8	5.988	0.034	0.286
WI	9.038	67.9	103.8	59.5	6.635	0.018	0.127
5 hours							
control	4.088	79.6	94.4	56.8	5.860	0.035	0.298
WI	9.675	66.6	102.1	59.8	7.054	0.015	0.140
6 hours							
control	2.650	80.3	94.5	56.4	5.582	0.036	0.286
WI	9.188	67.1	102.3	58.0	6.638	0.018	0.129
1 hour recov.							
control	4.557	83.3	91.9	57.0	6.306	0.048	0.298
WI	1.500	80.5	92.0	58.9	4.762	0.047	0.294

cardiovascular system relaxing the arterioles and decreasing the total peripheral vascular resistance (1) are evidently not completely successful, since arterial pressure remained elevated.

The present investigation support the notion that WI deloads the SNA in the same way as does bedrest with a decrease in catecholamines as one of the effects. Thus these pressure hormones are added to the list of hormones described in litterature (e.g. arginine vasopressin, renin, angiotensin, aldosterone) which are also reported to decrease.

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CENTRAL VENOUS PRESSURE AND PLASMA ARGININE VASOPRESSIN DURING WATER IMMERSION COMBINED WITH INFUSION AND HEMMORRHAGE IN MAN

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ABSTRACT

After overnight food and fluid restriction 8 normal males were examined twice: 1) During water immersion to the neck (WI) for 6 hours combined with intravenous infusion of 2000 ml of isotonic saline from the 2nd to the 4th immersion hour (WI+2000), and 2) during WI for 6 hours combined with hemorrhage of 500 ml of blood from the 2nd immersion hour over 30 min (WI-500). WI+2000 increased central venous pressure (CVP), systolic arterial pressure (SAP), diuresis, natriuresis, kaliuresis, and osmolar clearence compared to WI-500, while hematocrit, hemoglobin concentration and urine osmolality decreased. Heart rate, plasma osmolality, plasma sodium, plasma potassium and free water clearence did not change when comparing the two studies. Plasma arginine vasopressin (pAVP) was significantly higher after 6 hours in WI-500 compared to after 6 hours in WI+ 2000. In conclusion, there was a significant correlation between CVP and pAVP during the two studies indicating a relationship, while during a poststudy fall in arterial pressure after WI-500, decrease in SAP and MAP correlated significantly with increase in pAVP.

INTRODUCTION

Gauer and Henry in 1976 (4) stated that central volume expansion through stretch of atrial receptors suppresses AVP secretion leading to an increased diuresis. In humans, the water immersion model has been found usefull as a means of inducing central blood volume expansion (2,4). Through extensive studies, Epstein (2) demonstrated profound increases in diuresis, natriuresis, kaliuresis and suppression of plasma renin and plasma aldosterone. Epstein et al. (3) also demonstrated a suppression of plasma AVP (pAVP) during immersion. In none of these studies central venous pressure (CVP) was measured.

During WI for 6 hours we found a suppression of pAVP in humans simultaneously with an increase in CVP when compared to a corresponding seated control period (Norsk et al. submitted, 1984). Therefore, to examine whether the increase in CVP is responsible for AVP suppression during WI, we performed the following experiment which in one study combined WI with isotonic saline infusion and in another combined WI with hemorrhage in the same subjects in order to create a change in CVP during the same experimental conditions.

MATERIAL AND METHODS

Eight healthy males were studied twice: 1) During WI for 6 hours combined with infusion of 2000 ml of isotonic saline from the 2nd immersion hour to the 4th (WI+2000), and 2) during WI for 6 hours combined with hemorrhage of 500 ml of blood from the 2nd immersion hour over 30 min (WI-500). During WI the subject was sitting in a specially constructed chair with arms on leans above the water in a swimmingpool kept at 35.1(0.2) degrees cel.(mean(SD)).

Central venous pressure (CVP) was measured through an indwelling 60 cm long catheter (Intracath) introduced through a cubital vein until the tip was placed in an intrathoracic vein.

Systolic (SAP) and diastolic (DAP) arterial pressure were measured with a sphygmomanometer and a stetoscope.

Heart rate (HR) was measured by palpation of the radial artery.

The concentration of AVP in plasma (pAVP) was measured by radio immuno assay (1). The pAVP values during and after infusion were corrected for increase in plasma volume (dPV in %) in order to reflect AVP secretion.

Plasma osmolality (pOSM) was measured with an osmometer by freezing point depression.

Hematocrit (Hct) was measured on a centrifuge and hemoglobin concentration (Hgb) in a spectrophotometer with a cyanid method.

Percent changes in plasma volume (dPV) were calculated from concomitant changes in Hct and Hgb.

Plasma concentrations of sodium (pNA) and potassium (pK) were measured in a flame photometer.

Urine was sampled at hourly intervals and diuresis (V), osmolality, sodium excretion (UNa), potassium excretion (UK) and free water clearence (C) measured and calculated.

RESULTS

WI increased CVP, which in the two studies attained the same level before infusion or hemorrhage. CVP was significantly increased by WI+2000 when comparing with WI-500 by approximately 4 mm Hg.

pAVP was decreased during WI. Comparing WI+2000 to WI-500, pAVP was significantly lower at 6 h (1.6(0.2) (mean(SE)) vs. 2.0(0.2) pg/ml). During 0.5 h of recovery from WI, pAVP increased to 3.0(0.6) pg/ml after WI-500 and to 1.7(0.2) pg/ml after WI+2000 ($p<0.05$ when comparing the two studies.

SAP increased during WI+2000 when compared to WI-500, but only significantly at the 5th hour of immersion. DAP and mean arterial pressure (MAP) did not change significantly when comparing the two studies except after 0.5 h of recovery from WI, where MAP was significantly lower during WI-500 than during WI+2000.

WI decreased HR in both studies, but neither WI+2000 nor WI-500 further changed it.

There was a weak negative but significant correlation between CVP and pAVP when plotting paired observations in the 8 subjects from the two studies ($r=-0.39$, $y=-0.05X + 2.2$, $p<0.001$, $n=183$, Fig. 1). When plotting the change in pAVP against the change in SAP during the recovery period after WI in the two studies, there was a significant negative correlation ($r=-0.76$, $y=-0.07X - 0.43$, $p<0.001$, $n=16$). During the same period of recovery the change in MAP also correlated well with the change in pAVP, but slightly weaker ($r=-0.67$, $y=0.06X +0.11$, $p<0.01$, $n=16$). SAP, DAP and MAP did not correlate significantly with pAVP when plotting all paired observations from the two studies.

WI+2000 increased V, UNa, and UK significantly when compared to WI-500. Free water clearence did not change significantly. There was no change in pNa, pK and pOSM. Hct and Hgb decreased during WI+2000 and dPV increased to 5.3(1.1) % at the 2nd immersion hour and to 18.0(1.6)% at the 4th hour due to infusion.

DISCUSSION

Our data confirm earlier observations (Norsk et al. submitted) and the results of Epstein et al. (3) that WI suppresses pAVP. Further more we have demonstrated that comparing further volume expansion (WI+2000) with volume contraction (WI-500) during identical conditions (WI), pAVP decreased, even though this parameter was corrected for hemodilution during infusion.

Johnson et al. (5) found a significant correlation between change in left atrial transmural pressure vs. change in plasma ADH. Such a correlation has not been established in humans. In our experiment we have found a weaker but significant correlation between CVP and pAVP (Fig. 1).

In animal experiments, hemorrhage is a wellknown stimulus for ADH or AVP rerlease (6). Share and Rocha e Silva et al. (7,8) concluded from dog experiments, that change in blood volume during hemorrhage correlated well with pADH during a period with unchanged arterial pressure. When on the other hand arterial pressure decreased due to a substantial hemorrhage, there was a better correlation between MAP and pADH. We can confirm this in humans concerning pAVP.

CONCLUSIONS

From this experiment the following is concluded:

1) There appears to be a weak negative but significant correlation between CVP and pAVP in humans, suggesting a relationship.

2) During a significant decrease in arterial pressure, there is a significant negative correlation between the change in SAP and MAP with the change in pAVP.

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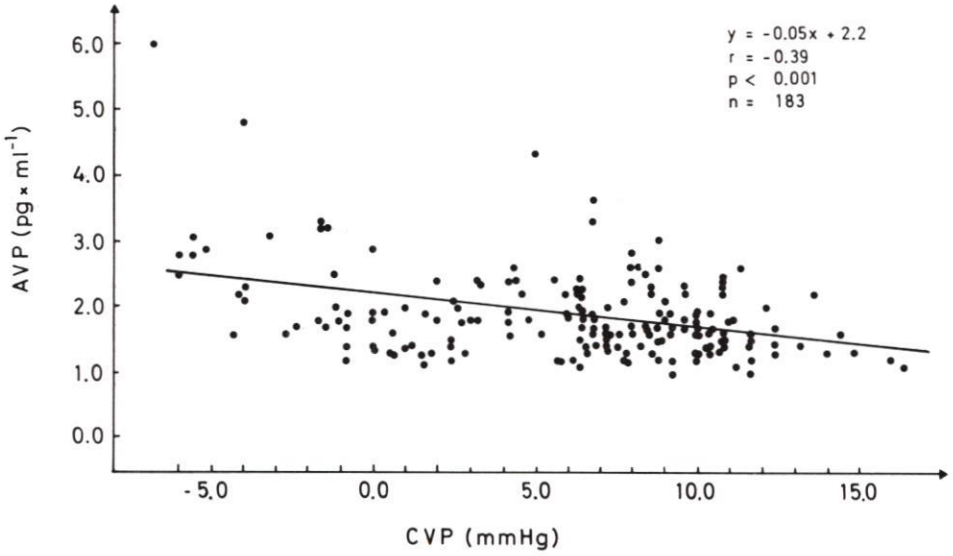

FIG 1. Plasma concentration of arginine vasopressin (AVP) in pg/ml versus central venous pressure (CVP) in mm Hg. Paired observations from 8 subjects before, during and after water immersion combined with infusion or hemorrhage. The equation of line of regression is AVP = -0.05·CVP + 2.2 . Significant slope ($p < 0.001$, $r = -0.39$ and $n = 183$.

PLASMA NOREPINEPHRINE RESPONSES TO STIMULATION OF COLD RECEPTORS AND VOLUME RECEPTORS

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Abstract

If plasma concentrations of norepinephrine ($[NE]_p$) are lowered by expansion of the intrathoracic blood volume and raised by cooling of the body, then intrathoracic volume receptors may limit the rise of plasma norepinephrine concentration ($[NE]_p$) during cold-water immersion. Six healthy men were exposed to a control condition (27.6°C air), thermoneutral water (35°C), cold air (14.8°C), and cold water (29.8°C). Immersion in thermoneutral water reduced $[NE]_p$ from 0.34 ng/ml to 0.20 ng/ml. Mean $[NE]_p$ increased to peak values of 1.5 ng/ml in cold air ($P < 0.05$) and 1.1 ng/ml in cold water ($P < 0.05$), with no significant differences being observed between the $[NE]_p$'s in cold air and cold water. Despite an apparent expansion of intrathoracic blood volume, immersion in cold water did not significantly reduce $[NE]_p$ below values measured in cold air. Therefore, volume receptors did not limit the increase of $[NE]_p$ in response to cold stress during cold-water immersion.

Introduction

Immersion in chilly water may, in addition to activating cold receptors, stimulate volume receptors by expanding the intrathoracic blood volume. It has been postulated that sympathetic vasomotor nerve activity is depressed by volume receptors and stimulated by cold receptors (2). If plasma norephinephrine concentrations ($[NE]_p$) vary in relationship to vasomotor nerve activity, then intrathoracic blood volume expansion may oppose the effect of cold stress on $[NE]_p$ during cold-water immersion. Consequently, cold-induced elevations of $[NE]_p$ should be significantly lower in cold water than in cold air.

Methods

Six men experienced equivalent exposures to hydrostatic pressure when immersed to the neck in either cold water (29.8°C, unstirred) or thermoneutral water (35°C, unstirred). Exposure to cold air (14.8°C, 37% r.h., air velocity < 4 m/min) was predicted to cause the same rate of body heat loss as in the cold-water exposure. A fourth exposure, to thermoneutral air (27.6°C, 33% r.h., air velocity < 4 m/min), provided for control measurements of norepinephrine. Consumption of alcoholic beverages, caffeinated beverages, cheese, vanilla, and bananas, was prohibited 24 hours before the exposure. One antecubital vein was cannulated before a subject began sitting for 2 hours in the baseline environment (27.6°C air). Baseline measurements of $[NE]_p$, oxygen uptake ($\dot{V}O_2$), heat loss, and vital capacity were made before the subject continued sitting an additional 6 hours in each of the exposure environments. $[NE]_p$ was measured by radioenzyme assay.

Results

The control environment maintained $[NE]_p$ at constant values of 0.30-0.36 ng/ml throughout the exposure period. Two minutes of exposure did not alter the baseline measurements of $[NE]_p$ in any environment (Fig. 1).

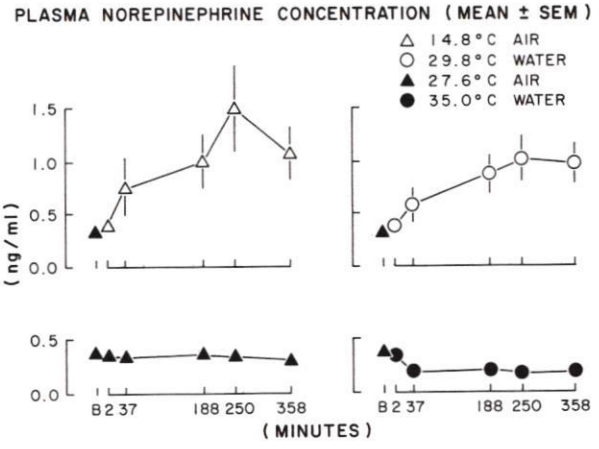

Figure 1. "B" denotes baseline measurements. Two-way ANOVA revealed that $[NE]_p$ was changed by the interaction of environment with time ($F_{15,75} = 8.1$).

Immersion in thermoneutral water reduced $[NE]_p$ from 0.34 ng/ml to 0.20 ng/ml. Every subject experienced reductions of $[NE]_p$ between the 2nd and 37th minutes of immersion in thermoneutral water, but the magnitude of reduction was not statistically significant. Both cold environments significantly raised the $[NE]_p$ above control measurements by the 188th minute of exposure, eventually resulting in peak values of 1.5 ng/ml in cold air and 1.1 ng/ml in cold water. There were no significant differences between the $[NE]_p$'s in cold air and cold water. Linear regression analyses indicated that mean $[NE]_p$ correlated with mean rectal temperature ($r = -0.94$) and mean $\dot{V}O_2$ ($r = 0.67$).

1. $[NE]_p = -0.75\ T_{re} + 27.95$
2. $[NE]_p = 4.70\ \dot{V}O_2 - 0.96$

Water immersion significantly reduced control vital capacity by 0.42 liters in thermoneutral water and 0.67 liters in cold water, with no significant differences between the vital capacity changes in cold water and thermoneutral water. The effect of cold air on vital capacity was not significant. Fluctuating intensities of chills and shivering were experienced by the subjects during their exposures to 14.8°C air and 29.8°C water, but not in the thermoneutral environments. Control $\dot{V}O_2$, 0.24-0.28 liters/min, was not significantly different from measurements in thermoneutral water. Cold temperature significantly raised the $\dot{V}O_2$, causing peak rates to occur at the end of the exposures to cold air ($\dot{V}O_2$ = 0.38 liters/min) and cold water ($\dot{V}O_2$ = 0.43 liters/min). Metabolic rates in cold air and cold water were not significantly different from each other. Mean rates of heat loss were 45 W/m^2 (control), 54 W/m^2 (thermoneutral water), 71 W/m^2 (cold water), and 66 W/m^2 (cold air) during the final 3 hours of exposure.

Discussion

We observed comparable increments of $[NE]_p$ at equal rates of heat loss in cold air and cold water. Other stimuli of norepinephrine release, such as exercise and abrupt changes of posture, were excluded from the experiment. The elevations of $[NE]_p$ in cold water were consistent with the theory that stimulation of superficial and deep cold receptors caused overflow of norepinephrine from vasomotor nerve synapses into the circulation. Keatinge et al. (3) observed that brief, intense stimulation of cutaneous cold receptors raised the $[NE]_p$ by 0.32 ng/ml. The more extensive cooling of our subjects raised $[NE]_p$ by 1.16 ng/ml above control values, indicating that deep cold receptors recruited plasma norepinephrine's response to cold stress.

Krishna et al. (4) observed significant reductions of $[NE]_p$ after 3 hours of water immersion; however, this was not confirmed by Epstein et al. (1). Our findings were consistent with a reduction of norepinphrine release into the circulation during the first half hour of immersion, since it seemed unlikely that thermoneutral water immersion would reduce the $[NE]_p$ in all subjects as a matter of chance. Either the thermal or hydrostatic properties of thermoneutral water may have lowered the $[NE]_p$'s by amounts which were relatively insignificant when compared to the effects of cold stress. The fact that forearm blood flow is extremely sensitive to water temperatures between 34-36°C suggests that slight increments of water temperature in this range may reduce $[NE]_p$ by diminishing the thermal stimulation of peripheral vasoconstriction. On the other hand, ambient water pressure may have reduced the $[NE]_p$ by stimulating volume receptors. The reductions of vital capacity during water immersion indicated that residual gas was displaced from the lung by translocation of peripheral blood into the thorax. Subsequent distension of the heart could stimulate the volume receptors.

The hypothesis of this study was incorrect since $[NE]_p$ was not lower in cold water than in cold air. Therefore, stimulation of volume receptors did not inhibit the norepinephrine response to cold receptors during cold-water immersion.

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MUSCULARITY AS A FUNCTION OF SPECIES, SEX AND AGE IN SMALL MAMMALS

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The size of individual skeletal muscles in mammals is well known to change with the degree of mechanical loading imposed on the muscle. Less clear has been the effect of changes in loading of the whole organism on size of the total skeletal musculature. Pitts and coworkers have provided valuable insights by dissection and weighing of the skeletal musculature of animals subjected to various chronic centrifugation, exercise and dietary regimens, and their recent findings are reported at this meeting. However, questions remain (7).

Independently, our laboratory several years ago embarked on a systematic definition of body composition and energy metabolism changes as a function of sex and age in 5 species of small laboratory mammals, the mouse, hamster, rat, guinea pig and rabbit (4,6). Six animals of each sex were examined in 8 age cohorts ranging from 1 to 24 months for each of the 5 species.

RESULTS

Among the body composition parameters measured was body creatine mass by analysis of the dry, fat-free, whole-body powder prepared from each animal (5), to provide an estimate of the skeletal muscle mass of the body. The results of the creatine analyses are shown in Fig. 1.

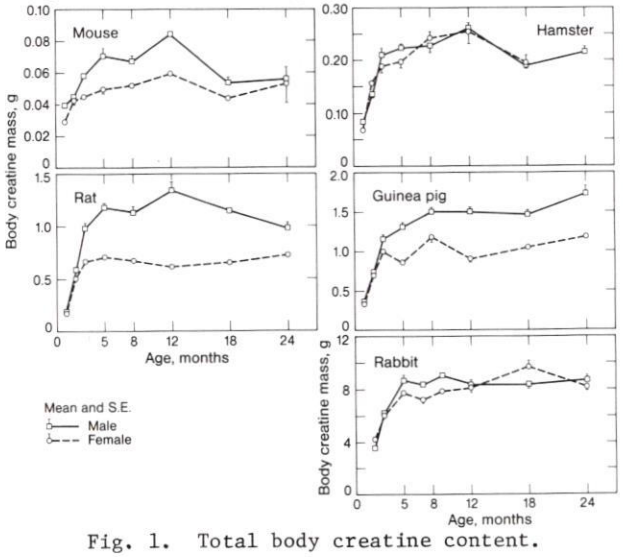

Fig. 1. Total body creatine content.

It may be seen that in the smaller species, the mouse, hamster and rat, creatine mass tends to reach a peak value at about 1 yr of age; whereas, in the guinea pig and rabbit there seems to be no diminution in creatine mass in the older animals. Furthermore, as shown by 2-way analysis of variance there is a marked dimorphism between the sexes in body creatine mass for the mouse, rat and guinea pig, while the hamster and rabbit appear to be monomorphic in this respect.

Examination of fat-free body mass of the same animals by analysis of variance revealed a similar marked sexual dimorphism for the mouse, rat and guinea pig, and sexual monomorphism for the hamster. However, the rabbit displayed a small, but statistically significant dimorphism. Also, there was little to no diminution in fat-free body mass in the older animals such as that noted for creatine mass in the mouse, hamster and rat.

We have previously described a procedure for estimation of body skeletal muscle mass from body creatine mass which depends upon the finding that about 97% of the body creatine mass is contained in the skeletal muscle cells (3). Body skeletal muscle mass may be computed by the relationship

$$\text{Fat-Free Skeletal Muscle Mass, g} = \frac{\text{Body Creatine Mass, g} \times 97}{\text{Creatine, g/100g Fat-Free Muscle.}} \qquad (1)$$

However, it was pointed out that intracellular muscle creatine concentration may vary as a function both of species and age of animal (2). Accordingly, we have now measured the creatine content of fat-free skeletal muscle samples from animals of various ages for our 5 species with the result shown in Fig. 2.

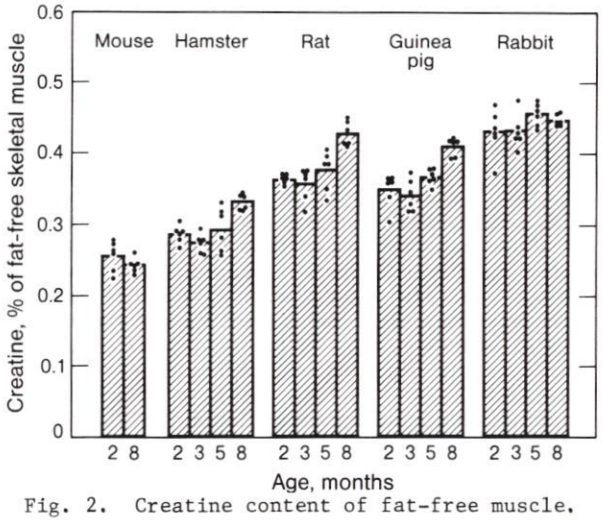

Fig. 2. Creatine content of fat-free muscle.

Two-way analysis of variance of the data reveals that both species and age affect muscle creatine concentration significantly, and therefore must be taken into account when estimating skeletal muscle mass from body creatine mass. In order to accomplish this we derived a least squares regression of muscle creatine concentration on age for each of the 5 species for computation of the appropriate value of muscle creatine concentration in animals of particular species and age. An alternate procedure might be to measure creatine content of muscle biopsies obtained before total body creatine content is determined.

The body skeletal muscle mass was computed for each animal in our series by means of Eq. 1, and it was found that fat-free skeletal muscle mass for the 5 species varies as a function of sex and age in the same manner as does body creatine mass shown in Fig. 1. Again the mouse, rat and guinea pig display a strong sexual dimorphism, whereas the hamster and rabbit statistically are monomorphic. Furthermore, the 3 smaller species seem to attain a maximal skeletal muscle mass at about 1 year of age, while the guinea pig and rabbit exhibit no diminution in skeletal muscle mass out to age 2 years.

It remains now to consider how the proportion of skeletal muscle mass to fat-free body mass varies as a function of species, sex and age. This proportion, the percentage of the fat-free body mass represented by the skeletal musculature of the body, we term the *muscularity* of the animal and the results of our examination of this major body composition parameter are shown in Fig. 3.

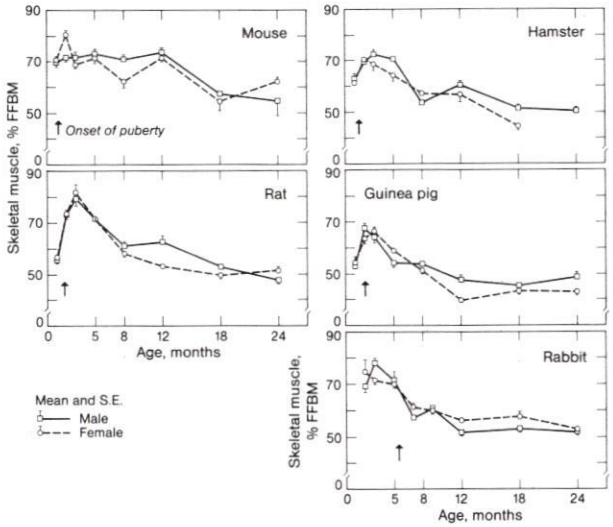

Fig. 3. Skeletal muscle percentage of fat-free body.

Little to no difference in muscularity between sexes is apparent in these 5 species of small mammals. Only the hamster and guinea pig exhibit a small, statistically significant, sexual muscular dimorphism. On the other hand, there is a pronounced species difference in muscularity as shown by 3-way analysis of variance. The mouse emerges as the most muscular species with an overall mean skeletal muscle mass of 69% of fat-free body mass. The guinea pig seems to be the least muscular species, 54%, and the rat, rabbit and hamster exhibiting intermediate muscularity, 62%. It is of interest to note that the degree of muscularity in these 5 species is in conformity with the subjective impression that the mouse is exceptionally kinetic, in contrast to the more lethargic mode of movement displayed by guinea pigs.

There is also a marked change in muscularity with age in both sexes of all 5 species. It is low in the young animals 1 month of age, reaches a peak at 2-3 months of age, and then declines in exponential fashion out to 2 years of age. The onset of puberty (1) is shown by an arrow in Fig. 3 for each species, and it may be seen that there is good correspondence with the attainment of maximal muscularity. In contrast, as shown by us earlier (6), metabolic maturity is not reached in these species until age 6-8 months and mature body size is not attained until the animals are about 1 year of age.

DISCUSSION

Some generalizations may be made from the present results. It is evident that among these small mammals there is no indication of scaling of muscularity to body size, despite the 100-fold difference in body mass represented. Thus, it appears that in this size range of mammals mechanical loading by Earth gravity has not been as important a natural selection factor for muscularity as other requirements on the animal. For example, the correspondence noted between muscular maturity and sexual maturity may well indicate a primary value of optimal mobility for species survival. Nevertheless, it is equally clear that gravitational loading has probably been an important determinant of muscularity among the larger mammals, for which allometric hypergonic scaling of skeletal muscle mass on total body mass has been demonstrated (8).

The observation that the proportion of the fat-free body mass represented by the skeletal musculature seems to reach a pronounced peak value at age 2-3 months in these small mammals carries the implication that the fraction of the fat-free body represented by other components must increase compensatorily in older animals. We suggest that in all likelihood it may be the supporting components of the body, the connective tissue and skeleton, which increase as muscularity diminishes. Future analysis of additional body composition data from our 5-species series is expected to provide a test for this hypothesis.

Finally, we may conclude that under normal circumstances muscularity in small laboratory mammals displays large, systematic variation as a function of both species and age. Therefore, it is important to take this variation into account when such animals are subjects of experiments dealing with the effects of altered gravitational loading on the skeletal musculature of the mammal.

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ACTIVITY OF CALCIUM ACTIVATED PROTEASE IN SKELETAL MUSCLES AND ITS CHANGES IN ATROPHY AND STRETCH

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The reduction of protein content in skeletal muscle undergoing disuse-induced atrophy is correlated with accelerated rates of protein degradation and reduced rates of protein synthesis (1). It is not known in what manner myofibers are partially disassembled during disuse atrophy to fibers of smaller diameter; nor is it known which proteases are responsible for this morphological change in contractile protein mass. Dayton and colleagues (2) have suggested that the Ca^{2+}-activated protease (CaP) may initiate myofibril degradation. The discovery of a form of CaP that is activatable by nanomolar concentrations of Ca^{2+} indicates that CaP activity may be regulated by physiological concentrations of Ca^{2+}(3). The enhancement of proteolysis by the Ca^{2+} ionophore A23187, reported by Etlinger (4), is consistent with a significant role for CaP in protein degradation. It was of interest, therefore, to measure the levels of CaP activity and the CaP inhibitor in extracts obtained from skeletal muscles of rat and chicken limbs undergoing disuse atrophy or stretch hypertrophy, respectively.

METHODS

Hindlimb atrophy was induced in male Sprague-Dawley rats weighing 300 g by the suspension method of Holton (5). Muscles were removed after ether anesthesia and cervical dislocation and stored in liquid N_2. CaP was extracted for assay with 9 volumes of solution containing 100 mM KCl, 50 mM Tris-acetate pH 7.8, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 1 mM NaN_3 per unit volume of muscle, homogenization being accomplished by means of a Polytron. After centrifugation (28,000×g), CaP was separated from its inhibitor by absorbing the protease from 1 ml of supernatant solution by passage through an equilibrated 5.5 x 25 mm phenyl-Sepharose column. The adsorbed CaP was eluted with 1.5 ml of 1 mM EDTA, 5 mM 2-mercaptoethanol and 1 mM NaN_3. Total and low CaP were assayed at Ca^{++} concentrations of 4.5 mM and 0.15 mM, respectively. The inhibitor activity was assayed by the inhibition of a standard preparation of purified, inhibitor-free, rat CaP (6). The proteolytic activity was measured on a casein substrate labeled covalently with the fluorescent adduct 4-methyl umbelliferone (7). The protease activities are all expressed in μmoles of 4-methyl umbelliferone released in a 30 min period at 25°/g wet muscle.

RESULTS

The distribution of total CaP activity, i.e., the sum of the high and low Ca^{2+} requiring forms, is summarized in Fig. 1. The soleus muscle contains about three times more CaP activity than the extensor digitorum longus (EDL), plantaris, or gastrocnemius muscles, the last three muscles being about equal in CaP activity. It is also evident that the inhibitor activities are roughly equal in concentration, being high enough in concentration so that a large portion of the total CaP activity is suppressed. Hence, it is essential to separate the inhibitor from the protease to obtain a true estimate of the protease activity.

The CaP activities found in some of the muscles of the rat whose hindlimbs were maintained load-free, but isotonically active for 5 days, is summarized in Table 1. The suspended muscles which sustained the greatest increase in CaP activities were, in rank order; soleus >> EDL > plantaris, whereas the gastrocnemius showed no change. Both the soleus and plantaris sustained weight losses of 30% and 22%, respectively. The EDL muscle, in contrast, did not incur a significant weight loss (3%), although it displayed a 26% increase in CaP inhibitory activity. However, the increased CaP activity of the EDL was offset by a substantial increase in CaP inhibitory activity, and the absence of a weight loss may be related to this increase of inhibitor.

The low Ca^{2+} protease activity increased most sharply in the load-free soleus (142%) in keeping with the increase in total CaP activity. A substantial increase in low Ca^{2+} activity was evident in the plantaris, and a relatively small increase in low Ca^{2+} activity was evident in the gastrocnemius. Notable as before is the EDL which showed no changes in low Ca^{2+} activity or in wet weight.

Stretch-induced hypertrophy (8) of the patagialis muscle in the chicken wing produced a two-fold increase in the total CaP activity per gram of wet muscle. The low Ca^{2+} activity which comprised less than 10% of the total did not show significant change. The CaP inhibitor activity, however, showed an equally large increment in direct proportion to the total CaP. This finding

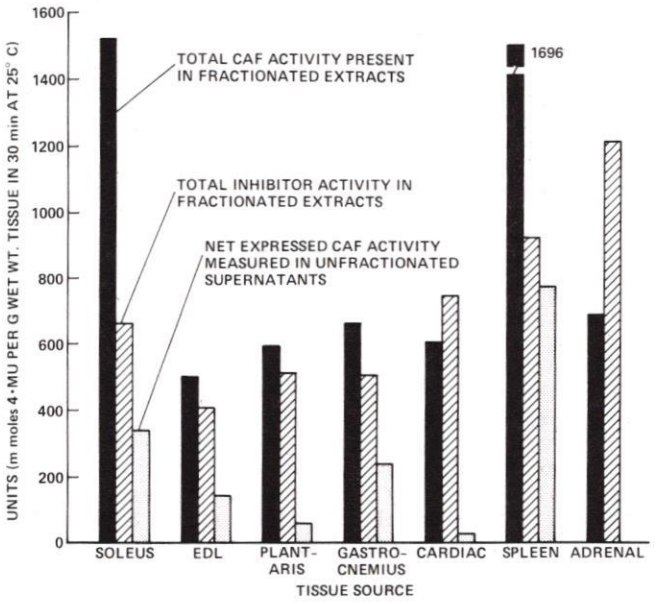

Fig. 1. Specific activities of CaP inhibitor, and net expressed calcium-activated protease activity in some hindlimb muscles and organs of the adult male rat.

Table 1. Changes in calcium-activated protease and inhibitor-specific activities in rat hindlimb muscles maintained load-free for 5 days.

MUSCLES[1]	TYPE	WEIGHT mg/100 g	TOTAL CaP μ mole g^{-1}	LOW Ca^{2+} ACTIVITY μ mole g^{-1}	CaP INHIBITOR units/g^{-1}
SOLEUS	CONTROL SUSPENDED Δ	43.0 ± 3.0 30.3 ± 3.1 (-30%)	1020 ± 114 1484 ± 242 (+42%)*	31 ± 16 75 ± 16 (+142%)*	782 ± 74 754 ± 158 NS
EDL	CONTROL SUSPENDED Δ	50.1 ± 3.5 48.7 ± 1.7 (-3%)	416 ± 27 526 ± 74 (+26%)*	98 ± 41 135 ± 11 (+38%)†	254 ± 105 398 ± 69 (+49%)†
PLANTARIS	CONTROL SUSPENDED Δ	102 ± 6.9 88.8 ± 6.4 (-13%)	466 ± 62 569 ± 35 (22%)†	55 ± 38 120 ± 29 (+118%)*	393 ± 66 434 ± 60 NS
GASTROCNEMIUS	CONTROL SUSPENDED Δ	499 ± 23 498 ± 33 NS	533 ± 60 581 ± 55 NS	173 ± 21 215 ± 38 (+24%)†	367 ± 75 362 ± 227 NS

(PN 1-17-84)

*$p \leq 0.01$, †$p \leq 0.05$

[1]5 CONTROL AND 4 SUSPENDED RATS (300 g, ♂)

[2]4-METHYL UMBELLIFERYL-CASEIN SUBSTRATE

[3]BY INHIBITION OF PURIFIED RAT MUSCLE CaP

indicates that the net activity in the hypertrophying muscle remains essentially constant.

DISCUSSION

The finding that the total CaP activity is highest in the soleus muscle is consistent with the higher rate of protein turnover reported for this muscle relative to other leg muscles (9). Moreover, the largest increase in specific CaP activity occurred in the soleus muscle, which also sustained the largest drop in weight after the 5 days during which time the hindlimbs were in a load-free state.

The low Ca^{2+} protease activity increased to the greatest extent in the soleus muscle and to smaller degrees in the plantaris, EDL, and gastrocnemius muscles. The occurrence of the largest increase in the atrophying soleus and plantaris muscles is consistent with the proposal that the low Ca^{2+}-requiring form of CaP may be the physiologically active form which is involved in protein turnover (6).

In contrast to the increases in CaP activity found at 5 days of hindlimb unloading, the changes in CaP inhibitor activity were insignificant except for the EDL, in which a 50% increase was observed. Although it is not known whether the inhibitor does in fact inhibit CaP in vivo, it is noteworthy that the EDL sustained no weight loss despite a significant increase in CaP activity. It may be speculated that the increased CaP activity was offset by the elevated level of inhibitor.

Increased CaP activity was also demonstrable in hypertrophying muscle, induced in the chicken patagialis muscle by stretch (8). However, in this rapidly growing muscle the elevated CaP activity was offset by a proportionately increased CaP inhibitor activity. The significance of the increase in these two factors is unclear at present.

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EFFECTS OF CHRONIC CENTRIFUGATION ON MICE

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Previous studies have shown that exposure to excess gravity in vitro alters the developmental sequence in embryonic mouse limbs and palates (Duke, Janer and Campbell, 1984; Duke, 1983). The effects of excess gravity on in vivo mammalian development was investigated using a small animal centrifuge. Four-week old female mice exposed to excess gravities of 1.8-3.5G for eight weeks weighed significantly less than controls. Mice were mated after five weeks of adaptation to excess G, and sacrificed either at gestational day 12 or 18. There were fewer pregnancies in the centrifuged group (4/36) than in controls (9/31), and crown rump lengths (CRL) of embryos developing in the centrifuge were less than CRLs of 1G embryos. These results show that although immersed in amniotic fluid, embryos are responsive to ΔG.

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Introduction

Previous studies have shown that mice can grow and reproduce under chronic acceleration (Oyama and Platt, 1967; Briney and Wunder, 1960). Animals born and reared in an increased G environment were found to be normal, except for having relatively smaller body mass than their 1G counterparts. However, detailed analyses of embryonic and fetal development were not performed.

The studies reported here differ from previous ones in that large numbers of animals were examined and teratological techniques were used to detect gravitationally induced changes in developmental processes.

Materials and Methods

The small animal centrifuge in which the mice were exposed was based on the design of Walters, Wunder and Smith (1959) with two cageholders containing four cages each. The cage holders have one degree of freedom, allowing the holders to swingout at an angle determined by the rpm. The G force generated is perpendicular to the cage floor, and is determined by the equation:

$$G = (.00001118)\ (radius)\ (rpm)^2$$

When the centrifuge is operated at 40 and 45 rpm, the linear accelerations generated range from 1.8 to 3.5G (101-155 cm "swingout" radii).

In the first experiment, seventy female mice were placed in the centrifuge at four weeks of age, twenty at 1.8, 2.1 and 2.3, and ten at 2.8G. Controls consisted of seventy female mice housed in the centrifuge room. The centrifuge was stopped 30 minutes every other day for ad libitum feeding of Purina laboratory chow, and maintenance, and 2.5 hours weekly for weighing. Water was available through a nuzzle valve system. Since the mice were not housed individually, pairfeeding was not attempted. To avoid effects of maternal stress on the embryos, mice were allowed to adapt to the above mentioned excess G forces for five weeks prior to breeding and were weighed weekly during this period. After eight weeks of centrifugation, mice were returned to 1G for two weeks.

During the experiment, mice in the top cage (1.8G) were found to weigh less than mice at higher G's. Since this could have been due to translucency of the top of the acrylic cageholders, the tops were covered 46 days into the experiment. After adaptation, estrus was induced by replacing the bedding material in the female cages with bedding material from male cages. Two days later, five females from each G level were each paired with a male at 1G for four hours (9 AM-1 PM). (Males were not exposed to excess G.) They were then returned to the centrifuge and sacrificed by cervical dislocation on gestational day 12.

The second experiment was conducted similarly, using different G levels. Cage tops were covered throughout the experiment. The seventy four-week-old female mice were divided as follows: ten at 2.3, and twenty at 2.6, 2.9 and 3.5G. In this experiment, pregnant females were sacrificed on gestational day 18.

Embryos from both experiments were fixed in 3% glutaraldehyde in cacodylate buffer and crown rump lengths (CRL) measured.

Results

Weight Studies

Experiment I: Mean weights of centrifuged mice were lower than controls at all time periods, significantly so for 2.1, 2.3 and 2.8G for weeks one to three, and for 1.8G mice throughout the experiment (Fig. 1). After 8 weeks of acceleration, mice were returned to 1G for two weeks. Results were varied: 1.8 mice were unaffected, 2.1 and 2.3G mice lost weight, and 2.6G mice continued to gain.

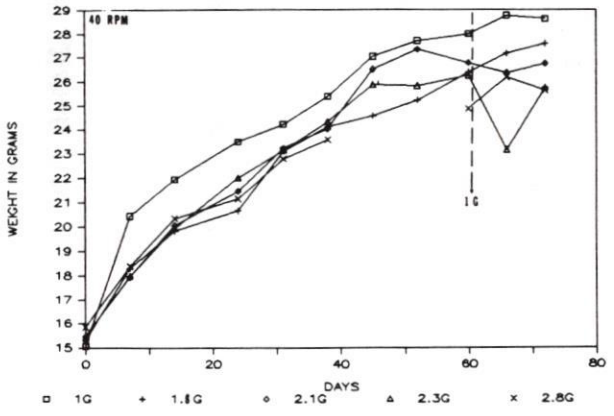

Figure 1: Weight Results Experiment I

Experiment II: In this experiment mean weights of centrifuged mice were again lower than controls at all time periods (Fig. 2 and 3). This difference was significant throughout the entire experiment for 2.6, 2.9 and 3.5G.*

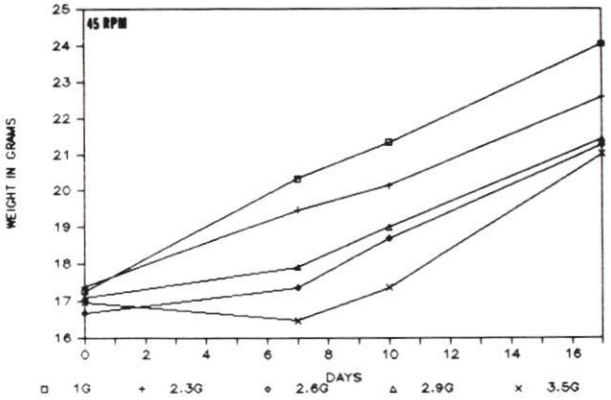

Figure 2: Weight Results Experiment I; 0-17 days of centrifugation.

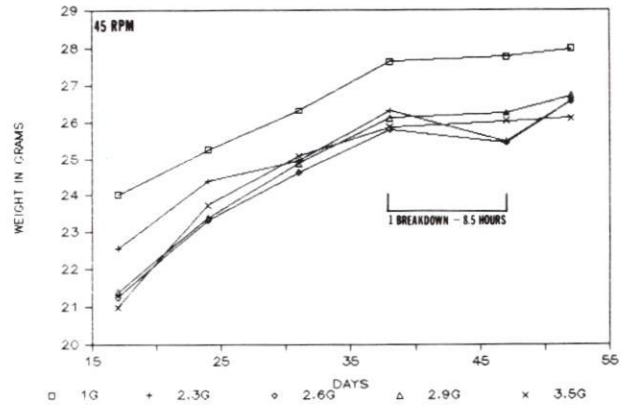

Figure 3: Weight Results Experiment II; 17-52 days of centrifugation.

Reproductive Studies

Results of reproductive studies are shown in Tables I and II. The number of pregnancies in control females was higher (5/15-Exp I; 4/16-Exp II) than in females exposed to excess G (3/20-Exp I; 1/16-Exp II), but there was no effect on litter size or number of resorptions/litter. Embryos exposed to excess G were smaller than controls (Fig. 4), significantly so in most cases (see Tables).

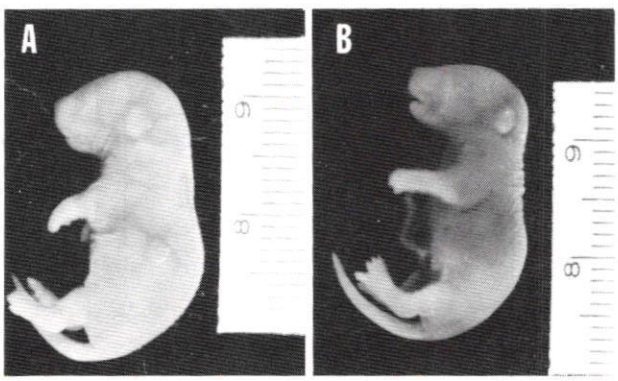

Figure 4: 18 day old embryos; A= Control, B= Centrifuged.

* An 8.5 hour breakdown (Fig. 3) resulted in loss of weight in mice exposed to 2.3 and 2.6G. Mean weight of mice exposed to higher G's (2.9 and 3.5G) remained the same.

Table I: Effects on Reproduction (Experiment I)

G	No. Mated	No. Pregnant	Avg. No./Litter	Avg. No. Resorptions/Litter	Mean CRL (mm) Gest. Day 12
1	15	5	8	1	8.48
1.8	5	1	11	0	8.19
2.1	5	1	8	0	8.04***
2.3	5	0	--	-	----
2.8	5	1	7	2	8.10**

Table II: Effects on Reproduction (Experiment II)

G	No. Mated	No. Pregnant	Avg. No./Litter	Avg. No. Resorptions/Litter	Mean CRL (mm) Gest. Day 18
1	16	4	11	0	23.80
2.3	4	1	10	0	23.13*
2.6	4	0	--	-	-----
2.9	4	0	--	-	-----
3.5	4	0	--	-	-----

* P<.05 ** P<.02 *** P<.01

Discussion

The results of the weight studies are consistent with those of Oyama and Platt (1967) and Briney and Wunder (1960), demonstrating the adequacy of the centrifuge design, and of the female mouse as a model for gravitational studies. The low weights in the 1.8G mice are probably not due to a rotation effect, but to their being able to orient on the overhead lights. When the cage tops were covered, the mice began to gain weight more rapidly. No such effect was observed in the second experiment. Responses of animals to return to 1G (one deliberate and one accidental) were varied. 1.8G animals were not affected; their rate of weight gain remained the same. Animals at 2.1, 2.3 and 2.6G lost weight, and animals at 2.8, 2.9 and 3.5G continued to gain. The results suggest that 1.8G animals were not unduly stressed by exposure to excess G nor by return to 1G. Animals at intermediate G's were stressed by return to 1G, resulting in weight loss. Animals at higher G forces were stressed by return to 1G, but were also released from some stress of exposure to higher G's. In future experiments levels of corticosteroids will be measured so that stress levels can be accurately determined.

Time mating of mice depend on induction of estrus at specific times. The fewer pregnancies in the centrifuged mice may have been due to (a) changes of timing in the estrus cycle under excess G, (b) stress produced by return to 1G for matings, especially in mice exposed to higher G forces, (c) an effect of excess G on the establishment or maintenance of pregnancies, and/or (d) differences in the fecundity of the individual male or female. These possibilities are now being investigated.

The smaller CRLs of centrifuged embryos cannot be explained simply by relation to smaller maternal size. In experiment I, 1.8G mice weighed less than the rest when matings were performed, but CRLs of these embryos did not differ significantly from controls. These results, coupled with those of Serova et al (1984, 1983) on development of rat embryos under hyper-and hypogravity, show that mammalian embryos, even though immersed in amniotic fluid, are responsive to ΔG.

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ACCELERATION OF FUSION IN MOUSE PALATES BY *IN VITRO* EXPOSURE TO EXCESS G

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Palatal shelves from 13- and 14-day mouse embryos were excised and cultured in contiguous pairs. Experimental cultures were exposed to 2.6 G in a culture centrifuge; controls were in the same incubator. After 24 hours, palates were prepared for light or electron microscopy. Scoring of paraffin sections according to the stage of fusion seen in the medial epithelial edges (MEE) showed that palates exposed to excess G were in more advanced stages of fusion than were controls. Ultrastructurally, control MEE had tightly apposed cell membranes and numerous desmosomes; in centrifuged MEE, desmosomes had been removed and there was much intercellular space. Nuclear membranes were intact in control MEE, but showed marked deterioration in MEE of centrifuged palates. Few lysosomes and no necrosis were seen in control MEE; centrifuged MEE had numerous lysosomes as well as necrotic cells. Basal lamina were intact in controls, but interrupted in centrifuged palates. The results confirm the hypothesis that gravitational increases speed up the differentiative process.

Introduction

In about 10 years or so, men and women from Earth will be longterm inhabitants of a space station and possibly a lunar base, both of which are currently in the planning stages. Plants and animals will be growing and developing in gravities other than that of Earth. How these gravitational changes will affect development is indicated by a number of experiments involving a variety of organisms. Some of these experiments are outlined in Table 1. Their results indicate that differentiation is speeded up under excess G and slowed in low or null G. The studies reported here were undertaken to determine what effect excess gravity would have on another mammalian system undergoing development--the secondary palate of embryonic mice.

Vertebrate secondary palate development begins with the palatal shelves growing downward on either side of the tongue (7). In the mouse, this begins on day 12, Theiler stage 20. Subsequently, the mandible grows down and forward, pulling the tongue out of the way. The shelves elevate to a horizontal position above the tongue and begin the process of fusion (7), the stages of which are shown in Figure 1. During fusion, the shelves first adhere by means of glycoproteins appearing along the medial epithelial edge (MEE). The contacting epithelia then reorganize and undergo programmed cell death, allowing the underlying mesenchymes to come in contact. The process of cell death in the MEE occurs *in vitro*, and does not require contact with another shelf (Figure 1C). In this series of experiments, the effects of excess G (2.6 G) on *in vitro* fusion of embryonic mouse palates was studied.

TABLE 1.

EFFECTS OF ΔG ON DIFFERENTIATING TISSUES

Delayed differentiation in bones of growing rats aboard Cosmos 1120 (1).

Morphogenetic depression in mammalian limb buds developing *in vitro* under excess G (2).

Increased lymphocyte activation under 2, 4, and 10 G *in vivo* (3, 4) and *in vitro* (4).

Decreased lymphocyte activation under simulated or actual μG conditions *in vivo* or *in vitro* (5, 6).

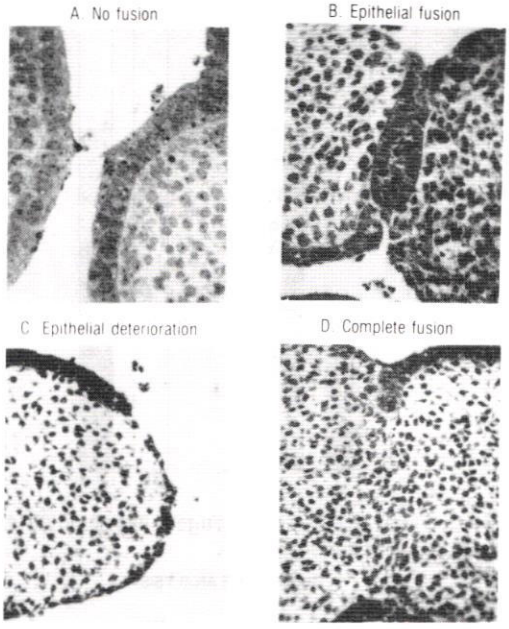


FIGURE 1. Stages of Palatal Fusion.

Materials and Methods

Timed matings of ICR mice were used to provide 13- or 14-day embryos-Theiler stages 21 and 22 (8). After decapitation of embryos and removal of the lower jaw, palatal shelves were excised and placed in culture in contiguous pairs. The culture system used is a modification of that of Kochhar and Aydelotte (9) with the palates being placed on a Millipore membrane filter on top of a Millipore depth filter in a 35mm culture dish. The medium was BGJ_b with 50μg/ml gentocin as the antibiotic. Medium was added to the level of the upper filter.

Experimental cultures were placed in swingout cups on a culture centrifuge and exposed to 2.6 G (16cm; 120rpm). Control cultures were placed in the same incubator. After 24 hours, palates were removed and processed for light or electron microscopy.

For TEM studies, palates were fixed overnight in 1.5% glutaraldehyde, postfixed in 1% OsO_4 and embedded in Araldite. Thick sections were taken for orientation purposes. Thin section of the MEE were taken from the center of the palate-the last area to become fused in vitro.

Formalin-fixed, paraffin-embedded palates were used for light microscopy analyses of fusion stages. Initially, cross-sections from the first, middle and third regions of the palate were scored as to the most advanced stage of fusion seen. Later, serial transverse sections were taken of the entire palate, and scored according to the stage(s) of fusion present in each section. Chi-square analysis was performed on the frequencies obtained.

Results

Palates exposed to excess G were found by light microscopy to be in more advanced stages of fusion than control plates (Figures 2 and 3). Ultrastructurally, cells of the medial epithelial edge of centrifuged palates were more degenerated than those of control palates (Figure 4).

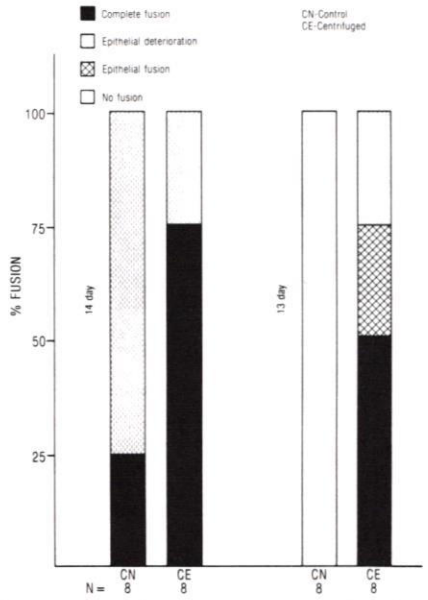


FIGURE 2. Fusion in Centrifuged Palatal Cultures (cross sections).

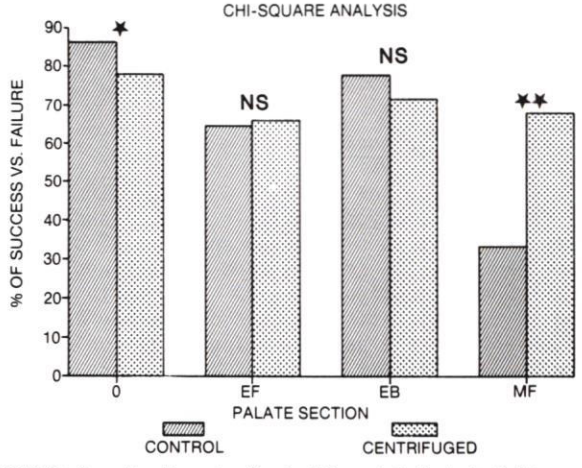
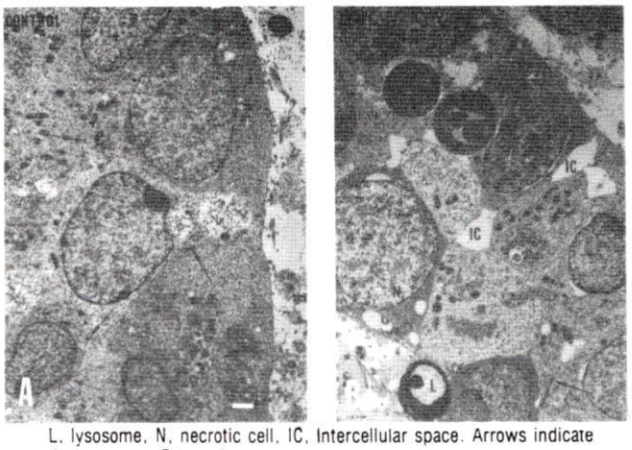


FIGURE 3. Fusion in Centrifuged Palatal Cultures (serial transverse sections). O=no fusion; EF=Epithetial Fusion; EB=Epithelial Breakdown; MF=Mesenchymal Fusion.
*P < .05
**P < .01



L, lysosome, N, necrotic cell, IC, Intercellular space. Arrows indicate desmosomes. Bar = 1 μm.

FIGURE 4. Electron microscopy.
A=Controls; B=Centrifuged.

Conclusion

This study shows that palatal fusion is accelerated under 2.6 G. The results are consistent with the theory that gravitational increases speed up the differentiation process. Excess gravity's effect is assumed to operate by compresion induced changes in cell shape (2). In the case of palate, exposure to excess gravity may mimic the changes in cell shape that occur during elevation. Changes in cell shape may trigger a switch from division to differentiation, possibly mediated by cyclic AMP.

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READAPTATION OF RAT'S MUSCLES FOLLOWING SPACE FLIGHT

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In the course of the Cosmos-1129 experiments a detailed study was performed on the atrophy of rat muscles with different functions and, at the same time, on the changes in muscle weight, extent and velocity of contraction. The results of these experiments have been reported in several publications /1, 2, 3/. The purpose of the current work is to study the way of readaptation of the muscles of animals used in the experiments.

METHODS

In the Cosmos-1129 biosatellite experiments male Wistar rats weighing about 300 g were used. After their return from the flight the animals were divided into three groups. Those in group I were immediately sacrificed /R0 group/. The animals in group II /R6 group/ were exsanguinated on the 6th, those in group III /R29/ on the 29th day after return. These rats constituted the so-called flight group /F/. A double control was applied in the experiments. The so-called synchronous /S/ group lived on the ground under identical conditions as the flight group /cage of the same size, similar temperature, air pressure and humidity, identical feed, etc./ The third part of the rats lived under the usual terrestrial conditions in the so-called vivarium /V/.

In these experiments four muscles were examined: the soleus muscle which contains mainly slow fibres, the m. extensor digitorum longus /EDL/ consisting overwhelmingly of fast fibers, as well as the mixed muscles, brachial and triceps muscles /medial head/ containing both types of fibre. The transport, storage and prolonged examination of the muscles were facilitated by glycerol treatment of the fibres at 0°C /4/, then followed storage in 50% glycerol at -20°C. The preparation can be regarded as an organised actin-myosin-tropomyosin-troponin system which maintains its permeability to ATP as well as cations and anions. The myofibrils 0.25-0.4 mm in diameter were placed in a transducer and ATP-calcium^{++} contraction were produced.

RESULTS AND DISCUSSION

The results obtained with the soleus muscle are summarized in Fig. 1.

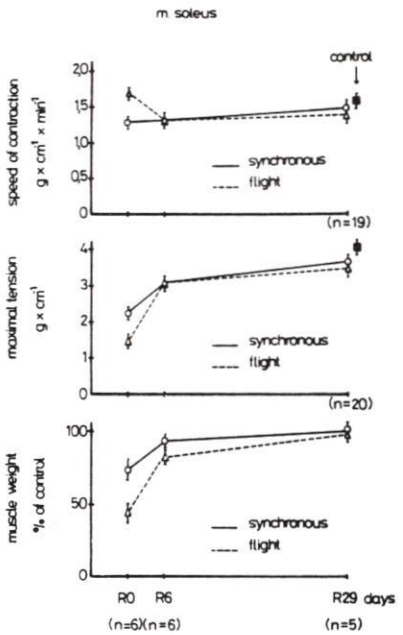

Fig. 1.

Muscle weight in the flight group /F/ decreased by about 55% whereas weight loss in the S group was only 20%. The muscle of the six rats that participated in the flight still showed a decrease of 20% on the 6th day after landing. The data of the five rats sacrified on the 29th day indicated complete recovery of muscle weight. Maximum tension significantly decreased after landing both in the S and F groups, whereas the velocity of contraction significantly increased in the animals participating in the flight. According to the data of group R6 and R29, complete recovery did not take place even on the 29th day, thus, in this period there was a decrease in the contraction velocity on the soleus muscle, too.

The weight of the EDL muscle, as shown Fig. 2, decreased by about 35% during the flight. Decrease in group S was round 12%. Readaptation of this muscle also took place quickly, since the muscle weight of the control animals was reached as early as on the 6th day. It is worth mentioning in group R6 both maximum tension and contraction velocity were higher than in the control animals.

The weight of the brachial muscle /Fig. 3/ decreased by round 30% during the flight, decrease in the S group was only 12%. There was a corresponding decrease in maximum tension and contraction velocity. In the course of our experiments all parameters measured essentially recovered as early as the 6th day.

There was a 40% loss of weight in the triceps muscle /Fig. 4/ in the F group, whereas loss was only 20% in the S group. In group F muscle weight loss was still significant on the 6th day, on the other hand, maximum tension and contraction velocity had recovered by that time.

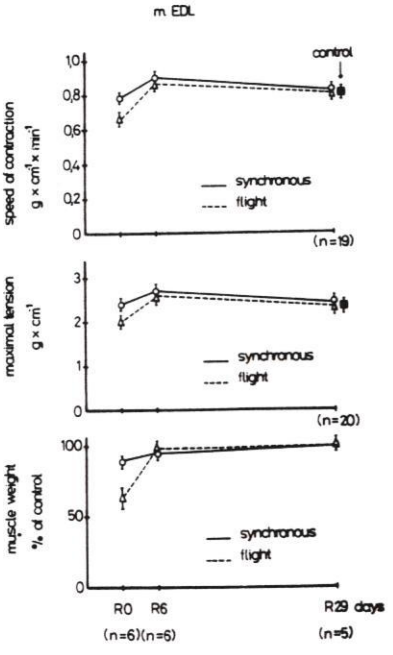

Fig. 2.

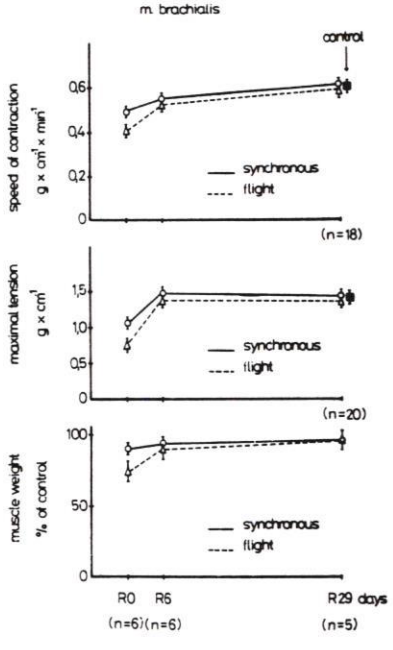

Fig. 3.

Our experiments have proved that the muscles, depending on their function, behave in different ways under conditions of weightlessness. The highest degree of atrophy occurs in the mainly slow-fibre containing soleus muscle, atrophy is much slighter in the EDL muscle which contains fast fibres. The readaptation is not identical in the muscles with different functions: the EDL muscle regains its weight in a few days, whereas that of the soleus muscle, similarly to the triceps /medial head/, is regenerated only after weeks. Maximum tension also shows a different tendency: the soleus does not reach the control value even in 29th days, the EDL "overshoots the mark" just as in respect of contraction velocity. These phenomena can be explained by the too fast recovery of the EDL muscle. Another interesting point is that the contraction velocity of the soleus muscle increase as a result of weightlessness. This phenomenon, also observed under conditions of immobilisation, can be accounted for by the assumption that by the enhancement of the myosin LC3 chain the slow muscles are transformed into fast ones /2, 3/. It should also be mentioned that Booth and Seider /5/ performed experiments to study how the soleus muscle of the rat is recovered after 3 months of plaster cast immobilisation. These authors found that muscle weight returned to normal earlier than the value of maximum tension.

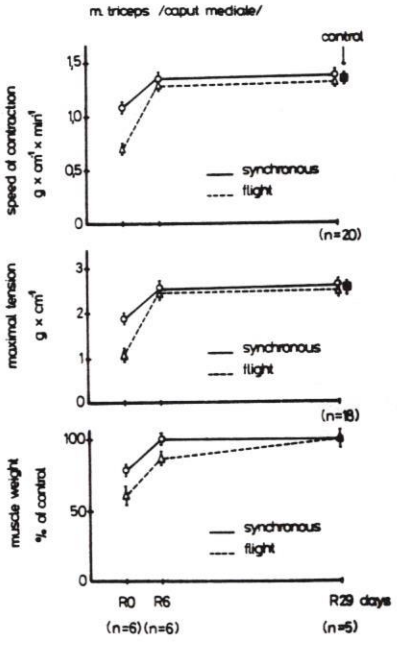

Fig. 4.

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Alterations In Bone Forming Cells Due To Reduced Weight Bearing.

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ABSTRACT

A reduction in new bone formation occured as a result of space flight (Cosmos 1129) and in the suspended animal model of Morey-Holton. Our results indicate that alkaline phosphatase activity of the bone forming cells is also reduced under these conditions, and the cells in the diaphysis are more affected than those in the metaphyseal region. In addition, these cells show (1) reduced proline incorporation into bone matrix, and (2) increased intracellular lysosomal activity. A change in the cytoskeleton could be the common factor in explaining these results. This suggestion is futher supported by our previous observations that colchicine injections result in decreased osteoblastic function.

INTRODUCTION

Results from the Cosmos series of space flights and from animals suspended in an antiorthostatic hypokinetic non-weight bearing position, indicate that there is a significant reduction in new bone formation (1). Using the Morey-Holton model for suspending animals in a non-weight bearing mode, we have investigated the mechanisms responsible for this effect.

The bone forming cell, the differentiated osteoblast, demonstrates two phenotypic expressions: (a) The synthesis of collagen which becomes part of the extracellular bone matrix, and (b) The synthesis and release of alkaline phosphatase enzyme. The functions of alkaline phosphatase are several (2) however it is an excellent marker of new bone formation and we measure its activity for that reason. Because osteoblasts have different degrees of activity, depending on their location within the skeletal system, it is not particularly useful to homogenize bone samples and make biochemical measurements. Therefore this study will also correlate osteoblastic function with the skeletal distribution of these cells.

METHODS

Adult male Wistar or Sprague-Dawley rats, 180-250 grams, were suspended by the Morey-Holton method (3) for 1-14 days. Femurs or tibias were removed from sacrificed animals and treated with 2% paraformaldehyde plus 0.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 18 hrs at 4°C. These tissues were prepared for routine electron microscopy or were decalcified in preparation for histochemistry.

Histochemical methods for demonstration of alkaline and acid phosphatase at the electron microscopic level have been described (4).

Quantitative alkaline phosphatase determinations were made by incubating frozen sections of metaphyseal or diaphyseal bone in appropriate media at 37°C for 1 hour. The media consisted of 4 mM 4-methyl umbelliferone phosphate, in 0.077 M glycine buffer, pH 10.0, and 0.46mM magnesium chloride (5). The fluorescent 4-methyl umbelliferyl was liberated by the enzyme activity and measured fluorometrically. This data was corrected for variation in tissue size and thickness by quantitation of total DNA (6) on similar numbers of adjacent sections which were used for the alkaline phosphatase determinations.

Autoradiography was carried out on plastic embedded metaphyseal and diaphyseal bone, at the light microscopic level (7). Tritiated proline was administered 18-24 hrs prior to sacrifice of suspended and control animals. Quantitation of silver grains within the osteoid volume was corrected for the amount of surface area included in the counting procedure.

RESULTS

Electron microscopic histochemistry indicated that alkaline phosphatase, which was localized to the external cell membrane of the osteoblast, was reduced in activity as a result of suspension. However, the osteoblasts along the osteoid surfaces within the diaphysis were more obviously affected than similar cells within the metaphyseal region of the same bone. In contrast to this finding, lysosomal and Golgi associated acid phosphatase activity appeared to be increased in those osteoblasts along the diaphyseal bone surfaces. This effect was not so obvious in metaphyseal osteoblasts.

Quantitation of alkaline phosphatase on frozen sections of bone has indicated the following: (a) When metaphyseal and diaphyseal bone are incubated as one complete piece, the alkaline phosphatase

activity is only slightly reduced as a result of 14 days suspension. One study of 7 controls and 7 experimentals showed a reduction in alkaline phosphatase activity of 11.7% ± 2.6% in the suspended animals. (b) When diaphyseal bone is measured alone, reduction in alkaline phosphatase can be as much as 2X lower than comparable control tissues. There is variation here depending on how long the animals have been suspended. We are presently relating the changes in alkaline and acid phosphatase to the length of time of the suspension.

Quantitative counts of the hydroxyproline incorporation into new bone substantiates the alkaline phosphatase results. The greatest decrease in incorporation occurs in diaphyseal bone, but this is dependent on length of time of suspension. The decrease can be as much as 50% less than controls. Incorporation into metaphyseal bone has not yet shown a significant change as a result of suspension.

DISCUSSION

The mature osteoblast, the cell responsible for bone formation, demonstrates two highly specific activities, (a) collagen synthesis and release to form the extracellular matrix, and (b) the synthesis of alkaline phosphatase enzyme. The present study shows that both these functions are reduced as a result of suspension in the Morey-Holton model. However, the osteoblasts along the surfaces of diaphyseal bone were much more affected than similar cells in the metaphyseal trabecular bone. Osteoblasts in both skeletal areas are morphologically and metabolically similar but the distribution of weight bearing forces, differences in the vascular supplies, and the absence of nerve fibers in the metaphysis but their presence in the periosteum of the diaphysis could all contribute different influences over the functional activity of osteoblasts in each of these areas.

An increase in acid phosphatase activity seems to correlate with the decrease in alkaline phosphatase activity. This could be explained by a possible increased degradation of collagen peptides within the osteoblast. Other cell types have shown significant reduction in collagen synthesis mediated through an increase in intracellular degradative activity(8). Whatever the mechanism, the decrease in tritiated hydroxyproline incorporation into matrix indicates that new collagen synthesis is suppressed by absence of weight bearing.

These diverse results may be explained by the suggestion that the cytoskeletal system of the osteoblast plays a critical role in many metabolic activities. We have demonstrated previously that colchicine injections inhibit alkaline phosphatase activity of osteoblasts (9). Other cytoskeletal disrupters can affect cellular differentiation (10). And intracellular lysosomal activity can be altered by microtubule disrupters (11). Therefore we suggest that the cytoskeletal system (ie, microtubules, actin, microtubule associated protein, vinculin, etc) could be the hypogravity or reduced weight bearing "sensor" within the bone forming cells. In future studies, using immunocytochemical methods for determining the distribution of the cytoskeletal elements, we hope to determine why the osteoblast in the metaphysis responds differently than those cells in the diaphysis.

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FACTORS INFLUENCING MUSCLE MASS IN THE RAT AT 1.0 AND MULTIPLE G'S

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ABSTRACT

Using 16 samples of rats, 13 at terrestrial gravity and 3 subject to chronic centrifugation, we have calculated product-moment correlation coefficients among individuals between mass of muscle and A. (live mass - FFBM) and B. (FFBM - muscle mass) where FFBM is fat-free body mass. Parameter A represents the inert load to be carried and yielded no significant correlations with mass of muscle. Correlations with parameter B test whether muscle maintains a constant mass relationship to the rest of the fat-free compartment and yielded significant positive correlations in 8 of the 13 samples at terrestrial gravity and in none at elevated chronic accelerations. It was concluded that the mass of muscle was not significantly correlated with the inert load being carried, even at 4.15g, but was correlated with the masses of other components in the FFB and that physical exercise and changes in diet did not disrupt these relationships but increases in chronic acceleration probably did disrupt them.

INTRODUCTION - Studies of muscle hypertrophy and atrophy carried out at terrestrial gravity have influenced investigators to expect a loss of muscle mass in weightless animals and a gain in those subjected to chronic centrifugation. Although responses to acceleration are directly related to body size, the laboratory rat is not too small to respond. Rather the rat shows clear responses but they are not the expected ones. Chronic centrifugation reduces muscle mass (8) and, while the results of weightlessness are not well established, there is evidence that weightless rats gain in muscle mass (10).

Because muscle hypertrophy in response to physical overload with its consequent stretch is so firmly established (12), we have searched for a possible occult effect of load on muscle mass in the chronically centrifuged rat (7), but without success. These results leave causal mechanisms as remote as ever.

Consequently, it was decided to search for an effect of the inert physical load carried by individuals, all at the same level of acceleration. This load appeared adequate for the purpose since it ranged as widely as 3.3-fold within a sample (Table 1) and amounted to as much as 40% of live mass. Using correlation techniques we have tested whether individual differences in mass of total muscle are influenced by the inert load or by constant genetic relationships with other total components.

METHODS - Sixteen samples of laboratory rats were examined, most being previously described. The method for manual separation of total musculature and other components has been described (4). Other details in treatment of the individual samples are summarized in Table 1.

As first approximations, A) live mass minus fat-free body mass (LM-FFBM) was regarded as a measure of inert load to be carried by the individual, stored fat being its major component in most cases, and B) fat-free body mass minus mass of total fat-free muscle (FFBM-Musc) represented the other components in the fat-free body to which muscle mass might be quantitatively related. Total mass could not be used for the purposes of A nor muscle mass as a percent of FFBM for the purposes of B because the occurrence of the major component muscle in both of the parameters being tested insured a significant correlation which was arithmetical rather than physiological.

Correlations of muscle mass with A and B were tested using Fisher's product-moment correlation coefficient (11). The use of this statistic was valid since all the variables appeared normally distributed, and where any correlation existed, it appeared to be rectilinear.

RESULTS - As shown in Table 1, muscle mass is not correlated with inert load, the low coefficients being nearly evenly distributed between positive and negative values with only one sample showing a significant ($P<.05$) correlation. By contrast parameter B shows 8 correlations with muscle mass in the 13 studies at terrestrial gravity (four with $P<.01$, one $P<.02$, and three $P<.05$) and no significant correlations in the 3 studies at elevated accelerations. Our interpretation is that probably there are genetically determined quantitative relationships between mass of muscle and mass of other components which are not disrupted by the exercise and dietary factors employed but may be disrupted by changes in level of chronic acceleration.

DISCUSSION - Physical exercise of intact rats fails, with rare exceptions, to produce hypertrophic gain in mass of skeletal muscles (e.g., 3,9) probably because the strain developed in the active muscles is inadequate. The animals represented in Table 1 probably failed to show hypertrophy in response to an inert load for the same reason. However, if the massive gastrocnemius muscle in the exercising rat is tenotomized or denervated thereby multiplying the load on the small synergistic soleus or plantaris, the latter two consistently gain in mass (e.g., 1,2). The failure of our rats centrifuged at 4.15g to show muscular hypertrophy of either total musculature or of a separate sample of antigravity muscles (8) implies either that the muscular strains developed still fell below some threshold necessary for hypertrophy or that the load effect was present but completely overbalanced by another factor. We prefer the latter interpretation since it is difficult to explain why the load effect would <u>not</u> be present in the centrifuged animal.

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Table 1. Correlations between muscle mass and two body compartments.

Sample Description	Muscle Mass (g)	Compart Mass (g)	r
I. At terrestrial gravity			
1. ♀, 77, 370 da, ad lib reg (13)	126±11	A 59±9	-.164
		B 94±6	.342*
2. ♀, 8, 4 mo, ad lib reg (8)	179±19	A 69±12	-.632
		B 123±8	.393
3. ♀, 10, 343 da, ad lib reg (6)	156±14	A 95±34	.269
		B 100±7	.727*
4. ♂, 15, 141 da, hifat diet, exer (5)	169±20	A 61±23	.566*
		B 128±13	.642*
5. ♂, 15, 141 da, hifat diet, no exer (5)	211±13	A 183±40	-.178
		B 163±10	.287
6. ♂, 10, 141 da, chow diet, exer (5)	157±17	A 51±9	-.036
		B 125±8	.708*
7. ♂, 15, 141 da, chow diet, no exer (5)	197±14	A 91±13	.038
		B 151±12	.770*
8. ♂, 9, 293 da, chow diet, exer (5)	208±11	A 102±14	.038
		B 190±13	.642
9. ♀, 27, 6 mo, ad lib reg (13)	122±12	A 65±12	.022
		B 85±6	-.073
10. ♀, 26, 6 mo, endurance exer (13)	125±11	A 43±11	-.171
		B 83±7	.670*
11. ♀, 26, 6 mo, exhaustive exer (13)	117±11	A 49±13	-.253
		B 84±7	.388*
12. ♀, 10, 5 mo, ad lib reg (9)	121±9	A 60±11	-.028
		B 90±5	-.080
13. ♀, 10, 4 mo, ad lib reg (13)	117±11	A 47±10	-.614
		B 86±5	.685*
II. At elevated chronic accelerations			
14. ♀, 8, 4 mo, 2.76g (8)	166±16	A 45±5	.381
		B 111±7	.685
15. ♀, 8, 4 mo, 4.15g (8)	151±9	A 38±4	.566
		B 107±3	.229
16. ♀, 9, 343 da, 4.15g (6)	135±12	A 38±6	-.071
		B 99±9	.441

Sample description provides: sex, number of rats, age, details of regimen, and reference in that order. Masses in means ± standard dev. r = product-moment correlation coeff, reg=regimen, exer=exercise. Body compartment A=live mass minus fat-free body mass, B=fat-free body mass minus mass of fat-free total skeletal muscle.
*means significance at P<.05.

Gravity Receptors: An Ultrastructural Basis for Peripheral Sensory Processing

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ABSTRACT

Our ultrastructural study of serial sections has shown that type II hair cells of the anterior part of the utricular macula are integrated into the afferent neural circuitry of type I cells, which are arranged in clusters. Additionally, there exists a complex system of intramacularly originating efferent-type nerve fibers and terminals. The findings taken together suggest that, on morphological grounds, complex processing of sensory information occurs in gravity receptors. Asymmetry of such a complex system may contribute to motion and Space-motion sickness.

INTRODUCTION

Mammalian gravity receptors are composed of two kinds of hair cells, type I and type II, and intervening supporting cells (see diagram). Type I hair cells have a rounded cell body and a constricted neck region. The neck ends apically as the cell expands into its flared upper end. This type hair cell is innervated by a cup-like nerve ending, called a calyx, that encloses the hair cell body and neck. The type II hair cell, in contrast, has a more tubular shape which, however, is deeply indented in places by nerve endings. The nerve endings are either button-shaped (boutons), finger-like, or demi-calyces.

Traditionally, type I and type II hair cells have been considered to be largely functionally distinct. That is, although several type I hair cells might share a single calyx, or several type II hair cells might be innervated by branches of the same nerve fiber, each cell type on the whole was depicted as communicating with the central nervous system independently of the other. On some occasions, however, calyces sent collaterals to neighboring type II hair cells, or leaned against them (Ades and Engstrom, 1965). This suggested that the afferent innervation might be more complex than was commonly realized.

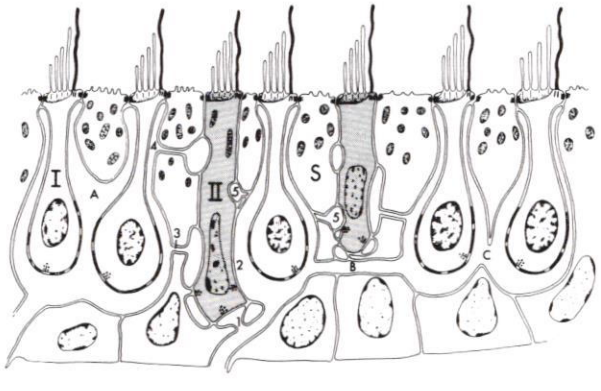
In addition to the afferent terminals, vesiculated boutons of efferent-type were described. These synapsed on the calyces, particularly at the base where the calyx emerged from the parent nerve fiber, and directly on type II hair cells (Engström, 1958). The vesiculated boutons were said to be terminals of, or boutons en passant along, nerve fibers of central origin (Gacek, 1960). The basic plan, then, was that afferents were largely segregated according to cell type innervated, with each cell type having a direct line to the central nervous system; and that calyces and type II hair cells were under central regulation through an efferent system arising in the brain stem.

Recently, we undertook an ultrastructural investigation of rat gravity receptors through the use of serial sections mounted on Formvar membranes. Study of montages prepared from the series revealed that integration of type II hair cells into the afferent neural circuitry of the type I cells was the rule rather than the exception, at least for the anterior part of the utricular macula (Ross, 1984b). The saccular macula was less completely studied, but its inferior portion appeared to show comparable integration of type I and type II hair cells (Ross, 1984b). Additionally, calyces and afferent nerves terminating as calyces served as the origins for beaded, vesiculated collaterals that corresponded morphologically to the efferent nerve fibers described previously as terminals of nerves of central origin. The findings taken together indicate that, on morphological grounds, neural innervation patterns of rat graviceptors are complex and that integration and processing of linear acceleratory information begins at the periphery.

MATERIAL AND METHODS

Inner ear tissues were obtained from a total of 35 Sprague-Dawley rats and prepared for study according to methods detailed in Ross, 1984b. The following account is based chiefly on the study of two long series, one cut vertically (91 sections) and another horizontally (96 sections) through the anterior part of the utricular macula; and on several shorter series (20-30 sections each) through the inferior part of the saccule.

RESULTS



This diagram illustrates the integration of type I (I) and type II (II, stippled) hair cells observed in the anterior portion of the utricular macula. S, supporting cells. For further description, see text below.

Type I hair cells in the regions examined were organized into clusters (Ross, 1984a,b). The above diagram illustrates the point that type I hair cells were often grouped in shared calyces (A,C) which sometimes were interconnected with others through nerve-like connections or bridges (B, lower center.) Type I clusters, then, were sampling vectoral information from a wider region of

the macula than previously considered. Additionally, type II hair cells were integrated with neighboring type I cell clusters through calyceal sharing (2, in diagram), calyceal collaterals from portions of the calyx over the hair cell body (3) or neck (4), or through collaterals arising from the parent nerve fiber (1). Each type II hair cell in this portion of the utricular macula was innervated by 2 and sometimes 3 calyces, directly or through collaterals. Sometimes the same afferent branch supplied two nearby type II cells (5 in diagram). The findings correlate well with the proportion of type I to type II cells in the series, ~2.3:1. Thus, type II hair cells were integrated into neural circuits of more than one adjacent type I cell cluster. Moreover, our results indicated that the polarizations of the hair cells in the integrated groups were not identical (not illustrated).

The intramacularly originating efferent system was complex in its distribution, with many of its fibers extending over long distances in the case of the utricular macula. In the present series, therefore, it was not possible to determine the precise circuitry for diagrammatic purposes. In the short saccular series available it could be shown that efferent-type collaterals sometimes synapsed on neighboring type II hair cells (Ross, 1984b). Aside from terminals on type II hair cells, vesiculated boutons ended on calyces, near the origin of the calyx and at sites of afferent collateral branching; on neighboring boutons en passant; and on nerve fibers of both afferent and efferent-type.

Synaptic boutons on calyces, at calyceal roots of afferent-type collaterals, and along nerve fibers in the neuroepithelium were of asymmetric type. The boutons were filled largely with small, round vesicles ~40-60 nm in diameter. A few vesicles were of the same size lengthwise but were flattened; and others were large, round vesicles ~70-90 nm in diameter that contained dense cores ~50-70 nm across. There was a post-synaptic density and electron-opaque material filled the intercellular compartment. Synapses on the type II hair cells were different in that there was an extensive subsynaptic cistern closely applied to the opposing hair cell membrane. The cistern was a portion of the transitional endoplasmic reticulum (TER) that connects the rough endoplasmic reticulum and the Golgi apparatus. Thus, synapses on the type II hair cell not only potentially modulate membrane excitability but also the functioning of the Golgi apparatus. To make the situation even more complex, subsynaptic cisterns also occurred opposite afferent-type nerve endings on type II hair cells.

A similar cellular modulation is possible in the case of the type I hair cell. Here, TER was closely applied to the hair cell membrane at sites where no intercellular material filled the space between the calyx and the hair cell. Very often, smooth endoplasmic reticulum (SER) spanned the calyx directly opposite. In some cases such transcalyceal smooth ER occurred in relation to efferent terminals on the calyces, offering the possibility of modulation of type I hair cell activity by the efferent system.

Finally, it should be noted that our study of utricular serial sections would suggest that not all calyces gave rise to efferent-type collaterals while some gave off more than one that passed in different directions.

DISCUSSION

The findings of hair cell clustering and of an intramacular system of efferent-type collaterals provides morphological support for the concept of peripheral sensory processing in graviceptors. Because each hair cell in a linked population has a different dynamic polarization, each would be expected to have a different but correct response to the same vectoral stimulus. Afferent nerve activity must reflect an analysis (or interpretation) of the differing signals subsequent to their modulation by the efferent-type system.

The intramacularly originating efferent system might function to focus signal to noise, through lateral inhibition. However, this system (or parts of it) might be facilitatory; the nature of its modulatory effects can only be determined experimentally. A further possible function is in adaptation to a constant stimulus, or to one that is novel (i.e., microgravity).

The innervation patterns we have described are complex and would suggest to us that they cannot be identical for the two sides. This, in turn, might indicate that a certain degree of asymmetry exists in the vestibular system as some of the experimental research of Bárány (1906) originally suggested. Just as we are right or left-eyed or -handed, we may also be right or left-eared. Such naturally occurring macular asymmetry might explain differences in susceptibility to motion and Space-motion sickness. Whether asymmetry normally exists, and whether lesser or greater asymmetry might be protective in a novel environment, are amenable to testing both on the ground and in the Space environment.

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THE EFFECTS OF GRAVITY ON THE VERTICAL VESTIBULO-OCULAR RESPONSE OF THE MONKEY

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INTRODUCTION

Benson (1, 2) studied the interaction of nystagmus produced by dynamic linear acceleration and that produced by angular acceleration. He examined the human horizontal vestibulo-ocular response (HVOR) to sinusoidal yaw oscillation under two experimental conditions. In the first, the oscillation axis was horizontal and in the second, the oscillation axis was vertical. He calculated gain and phase between peak eye velocity and peak head velocity over the frequency range from 0.01 to 2.00 Hz. A comparison of the Bode plots of gain and phase relations for each of the two experimental conditions demonstrated that during horizontal axis oscillation there was a significant reduction in phase advance re head velocity between 0.01 and 0.05 Hz but between 0.2 and 0.5 Hz the phase was advanced by about 5°. Also, over the frequency range from 0.01 to 0.50 Hz the gain was greater for horizontal axis oscillation. Finally, at the lowest frequency (0.01 Hz), not only was there a reduction in phase advance of eye velocity but there was also a modulation of eye velocity which was related to the orientation of the left-right head axis relative to gravity. Maxima and minima of the modulated cumulative slow phase velocity waveform occurred when the subject was near the left or right side down position.

Several authors have studied the constant velocity, off-vertical axis response in monkeys (8, 11). Young and Henn (11) studied the off-vertical axis vertical vestibulo-ocular response (VVOR) in two monkeys. The tilt angle of the axis of rotation was varied from 0-50°. At 45° tilt, VVORs were studied at a range of speeds from 15 to 140°/s. Vertical nystagmus was produced, for example, at a tilted axis angle of 45° and a constant rotational velocity of 67°/s. The nystagmus was unidirectional and showed a constant DC bias component and cyclic modulation component. The peak slow phase velocity was slightly phase advanced relative to the head-down (inverted) position. It was noted that the nystagmus modulation varied sinusoidally with axis tilt angle and when the peak-to-peak modulation of the vertical nystagmus exceeded the bias component (generally for tilt angles above 40°), the nystagmus showed a reversal once per revolution as observed for humans (3). As Young and Henn (11) pointed out, the relationship of the nystagmus modulation magnitude to the component of gravity in the plane normal to the axis of rotation supports the notion that the modulation component is dependent solely on the applied linear acceleration whereas the bias component is relatively independent of the magnitude of the rotating gravity vector.

As part of a larger study, we sinusoidally oscillated monkeys about an earth-vertical axis and about an axis tipped 45° off-vertical. We prepositioned the animals so that angular acceleration acted in the sagittal head plane and produced a vertical nystagmus. During both on- and off-vertical axis rotation we oscillated the animals under two conditions: 1) with eyes open and viewing a well lit room (EOL condition) and 2) with vision obstructed (eyes open in the dark-EOD condition). We calculated gain and phase relations between head velocity and eye velocity and we asked the question: does simultaneously acting optokinetic stimulation (OKS) and changing gravitational linear acceleration modify gain and phase relations between head velocity and vertical eye velocity (extracted from vertical nystagmus) produced by angular acceleration? Our preliminary results indicate that the answer to the question is yes. These results will be presented below.

METHODS

Seven cynomolgus monkeys (Macaca fascicularis) were implanted with head restraint devices and a pericorneal search coil in one eye (4, 5). Postoperative strabismus was not noted in any of the monkeys.

Four generator coils were mounted on an animal restraint device in which the animal was placed on-board a Contraves-Goertz Model 823 rotator. Vertical and horizontal eye movements were phase detected (9) and tape recorded along with a channel of rotator tachometer voltage and a channel of linear accelerometer voltage. Each monkey received a standard rotational paradigm twice, once with EOD and once with EOL. Vision was occluded by covering the monkey, for brief periods of time, with a light-tight but ventilated bag. Amphetamine was administered during the test sessions (0.1 mg/kg/hr IM) to maintain an elevated level of arousal. The rotational paradigm consisted of 18 sinusoidal rotations separated by a one-minute rest period. For all rotations, the animal was placed on his left side. Four additional rotations (velocity steps) were provided. Velocity step rotations to a constant velocity of 20°/s were used as an OKS so that the resulting vertical optokinetic nystagmus (VOKN) could be scored and a calibration factor derived to relate slow phase eye velocity to eye coil voltage output. During the step rotations, the animal was permitted to view a well lit room. It was assumed that nystagmus recorded from one to two minutes following acceleration to constant velocity had slow phase velocities equal to the rotation velocity of 20°/s. The assumptions of this method have been verified in the squirrel monkey (7). The standard paradigm consisted of a randomized presentation of six intensities of oscillation (± 5, 10, 30, 60, 100, and 150°/s) at three frequencies (0.01, 0.10, and 1.00 Hz). The standard paradigm was administered during both vertical axis rotation and with the axis of rotation tipped 45°. Vertical nystagmus was analyzed by scoring the upward slow phase velocity (SPVU) and the downward slow phase velocity (SPVD) and by determining gain and phase between

peak SPV and rotator velocity derived from the tachometer voltage. Slow phase velocity of up-beating and downbeating nystagmus was obtained using a computer program, implemented on a DEC PDP 11/20 and CSPI MAP array processor. The details of this program have been reported elsewhere (6) but the essentials of data acquisition will be presented.

Pretaped vertical eye movement and tachometer voltages were filtered (DC-35 Hz) to prevent alaising and digitized using 10-bit bipolar A/D converter at 80 Hz. Segments of 2048 samples of both vertical eye movement signal and the tachometer signal were obtained. The vertical eye movement signal was differentiated, saccades were detected and removed, and the remaining data points were fit by the fundamental frequency of the sinusoidal stimulus using a least square algorithm. Offset voltages were removed from both the eye velocity array and the tachometer array and both arrays were digitally low pass filtered from DC to 2 times the fundamental frequency. Both the signals were then presented on a video terminal display for selection of segments for analysis. Segments of eye velocity were selected which appeared to have the most consecutive cycles with the largest amplitude peak-to-peak eye velocity. In no case was the number of cycles chosen less than two and the average number of cycles for each frequency was: three at f=0.01; three at f=0.10; and seven at f=1.00. Amplitude ratio (AR) and phase values were calculated between peak SPVU and CW rotation as well as peak SPVD and peak CCW rotation for each half cycle. Also, AR and phase values were obtained using the first harmonic of a Fourier analysis of the eye velocity and tachometer voltage signals.

RESULTS AND DISCUSSION

No statistically significant difference (Mann-Whitney U-test, $P < 0.05$) was found between SPVU and SPVD AR and phase values. Therefore, they were combined. Mean AR/phase pairs ($\pm$ 1 SD) for six monkeys (EOD condition) at a peak velocity of $\pm$ 60°/s and frequencies of f=0.01; f=0.10; and f=1.00 Hz were: 0.21 ($\pm$ 0.14)/-101 ($\pm$ 12) deg; 0.72 ($\pm$ 0.22)/-175 ($\pm$ 14) deg; and 0.98 ($\pm$ 0.13)/-177 ($\pm$ 6) deg, respectively.

Table 1 presents AR and phase values for one of the less sensitive monkeys (monkey F) tested using both earth-vertical and off-vertical axis rotation and both EOD and EOL conditions.

Table 1. AR and phase values for MK F oscillated at 0.01, 0.10, and 1.00 Hz ($\pm$ 60°/s) about either an earth-vertical or off-vertical axis with (EOD) and without vision obstructed (EOL).

Condition		Frequency (Hz) 0.01	0.10	1.00
EOD 0 deg tilt	AR	0.125	0.70	0.80
	Phase	-72	-161	-176
EOL 0 deg tilt	AR	0.63	1.10	1.27
	Phase	-158	-176	-179
EOD 45 deg tilt	AR	0.24	0.79	0.98
	Phase	-159	-167	-180
EOL 45 deg tilt	AR	0.93	0.99	1.14
	Phase	-183	-183	-181

The 0° tilt values in Table 1 for monkey F, suggest that at 0.01 Hz, the EOL condition causes a further 86° phase lag of the eye velocity signal when compared to the EOD condition (but VOKN plus VVOR does not produce perfect compensation at 0.01 Hz and the phase is still 22° phase advanced).

However, a comparison of the values for the EOL 0° tilt condition and EOL 45° tilt condition suggests that reorientation of the head relative to gravity plus VOKN plus VVOR increase the gain to near one and the phase to near -180° over the entire bandwidth 0.01-1.00 Hz. It should be pointed out that no attempt was made to control the angle of rotation of the gravity vector in the sagittal head plane. During low frequency rotation (0.01 Hz), a component of the gravity vector rotated throughout the sagittal head plane. An analogous rotation of a component of the gravity vector occurred at the lower frequencies in Benson's (1) study of the human HVOR.

The preliminary results in Table 1 seem to suggest that both vision and gravity are necessary to achieve perfect vertical ocular compensation of head movements in the frequency range f=0.01 to 1.00 Hz. Moreover, there seems to be a much more dramatic effect at lower frequencies which is not unexpected since this seems to be the range where the optokinetic system (10) and gravity sensitive system (otoliths) are most effective in complementing the dynamic response of the semicircular canal system.

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FAR-FIELD BRAINSTEM RESPONSES EVOKED BY VESTIBULAR AND AUDITORY STIMULI EXHIBIT INCREASES IN INTERPEAK LATENCY AS BRAIN TEMPERATURE IS DECREASED

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INTRODUCTION

The effect of decreasing brain temperature upon the transmission of neural signals along the brainstem auditory pathway has been well documented in cats [6] and mice [2]. The increase in the absolute and interpeak latencies of components of the brainstem auditory evoked response (BAER) has indicated that a progressive slowing occurs along the pathway as the signals ascend toward higher brainstem areas. Therefore to fully describe BAERs, both peak latencies and temperature are measured, especially in anesthetized preparations when brain temperature can be labile.

In comparison to the numerous studies on the auditory system there are few studies that relate far-field responses evoked by angular acceleration to the vestibular system [1]. Moreover the temperature dependence of such responses has apparently not been investigated.

In this study we performed experiments designed to examine whether interpeak latencies of the BAER in rats depended upon temperature. This led to experiments designed to examine whether interpeak latencies of responses evoked by an angular acceleration show a dependence on temperature.

METHODS

Six Long-Evans male rats (250-500 grams) were used in this study. All surgical and recording procedures were performed on animals anesthetized with sodium pentobarbital. Recording electrodes (five stainless steel screws) were implanted in the skull at locations previously described by Jones et.al. [4]. A recording screw was placed low in the mastoid bone on each side. A vertex recording screw was placed midway between lambda and bregma, and two ground screws were placed over the frontal sinus rostral to bregma. To measure brain temperature a calibrated bead thermistor (VECO) was placed subdurally opposite the vertex screw. Wire leads were secured to each recording screw and the thermistor. A special nut was also placed upon the skull surface. This nut was used to attach a piezoelectric crystal for delivering auditory clicks, and it also provided a reference point for angular accelerations. The nut was stereotaxically positioned equidistant between the external auditory meatus. Finally, the exposed skull surface, implanted hardware, and nut were fixed in place with dental acrylic.

The neural activity evoked by each stimulus modality was analyzed in a similar manner. The electroencephalographic signal was amplified (Grass P511 preamplifier) and sampled immediately after each stimulus. Final waveforms were the average of 128 or 512 responses using a laboratory microcomputer (Sierra Data Sciences). Brain temperature was recorded at the onset and at the completion of averaging each waveform. After recording responses to both stimulus modalities at approximately 37°C brain temperature was allowed to fall to approximately 34°C. Responses were then recorded at the low brain temperature.

BAERs were recorded in response to bone-conducted stimuli using a piezoelectric crystal secured to the special nut [5]. Voltage pulses (+92 volts, 10 pps) applied to the crystal induced vibrations that were conducted to the skull and stimulated the auditory system.

For recording responses to angular acceleration the head position of the anesthetized rat was maintained over the axis of platform rotation. This was accomplished by securing the nut (fixed in dental acrylic upon the animal) to a head holder, which in turn was fixed to the platform. The fixed location of the nut assured consistent and repeatable placement of each animal with reference to the auditory meatus. The animal's body was held on the platform within a plexiglass tube, and dead space was filled with foam rubber to limit body movement. Brief, rapid rotatory movements of the platform (1.3° in less than 15.5 msec, 0.5 stimuli per second) were used, and responses to this angular acceleration were recorded as detailed above. All recordings were made on anesthetized rats.

RESULTS

Figures 1 and 2 are typical responses recorded from one of the six animals used in this study. Responses to both stimulus modalities were recorded from the same animal.

A graphic representation of the effect of decreasing brain temperature upon the BAER in rats is shown in figure 1. The lines between the BAERs recorded at approximately 37°C and 34°C were drawn to indicate shifts in peaks. Variations in the slope of these lines away from vertical indicate changes in peak latency. It can be observed that the peaks of the BAER recorded at a brain temperature of approximately 34°C occur later in time than the peaks from the 37°C waveform. Furthermore, the later peaks of the BAER recorded at the low temperature are more delayed than the earlier peaks. This indicates that an increase in interpeak latency occurs as brain temperature is decreased. For example, peaks identified by the line labelled with the arrow showed an increase in interpeak latency of 0.55 msec at the lower brain temperature.

The responses to angular acceleration shown in figure 2 are representative of waveforms recorded at brain temperatures of 37°C and 34°C. As in figure 1, the lines between the responses recorded at the two temperatures connect physiologically similar peaks, and graphically illustrate changes in peak latency. A progressive deviation in the lines away from vertical in the later peaks indicates that increases in interpeak latencies are associated with decreasing brain temperature. For example, the peaks identified by a line labelled with the arrow showed an increase in interpeak latency of 0.20 msec at the lower brain temperature.

DISCUSSION

It has been previously reported in studies on cats and mice that the increases in interpeak latency of the BAER observed when temperature is decreased indicates that central conduction along the auditory pathway is slowed. We have observed that decreasing brain temperature results in an

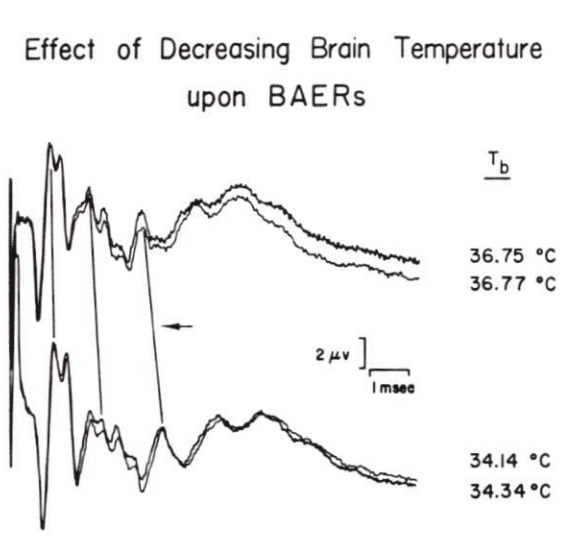

Figure 1: The effect of decreasing brain temperature upon the BAER. These waveforms were recorded from one animal (Rat 40), and are the result of averaging responses from 128 successive auditory stimuli. The recordings were made in duplicate to demonstrate reproducibility, and the brain temperature during the recording of each waveform is indicated at right. The lines inserted between the waveforms indicate physiologically similar peaks.

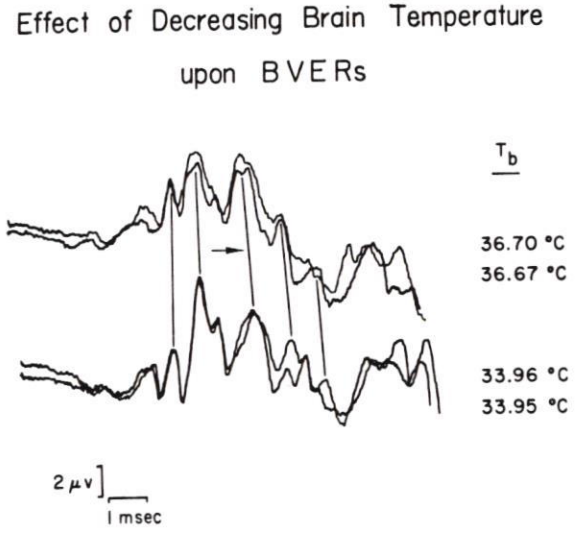

Figure 2: The effect of decreasing brain temperature upon the BVER. These waveforms were recorded from the same animal that produced the BAERs in figure 1. They are the result of averaging responses from 128 successive angular accelerations, and are presented in duplicate indicating reproducibility. Brain temperature during the recording of each waveform is shown at right. Physiologically similar peaks are indicated by the lines inserted between waveforms.

increase in interpeak latency of the BAER recorded from rats (figure 1). This study demonstrates that peak latencies of the BAER recorded from rats are sensitive to temperature changes, as has been previously shown in other species [2, 6].

In this study we recorded responses to brief, angular accelerations. However, additional experiments are necessary before one can with certainty attribute these responses to the vestibular system, and not the auditory or somatosensory systems. Experiments involving masking with white noise appear to exclude the auditory system as the generator of these responses [3]. Preliminary experiments controlling for possible somatosensory input were also performed. No appreciable response was observed when the animal's head was fixed in place and the animal's body moved with the platform. Therefore, we tentatively conclude that these responses may be attributed to the vestibular system, and may be denoted brainstem vestibular evoked responses (BVERs).

We observed increases in interpeak latency of the BVER in response to lowering brain temperature in the rat. The increases in interpeak latency of the BVER waveforms are consistent with the proposal that these waveforms are neural in origin. In addition, this study indicates that the peak latencies of the BVER are sensitive to changes in brain temperature, as has been shown for the BAER.

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THE CHEMORECEPTOR TRIGGER ZONE FOR VOMITING IS NOT ESSENTIAL FOR MOTION SICKNESS IN CATS

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The emetic chemoreceptor trigger zone (CTZ) in the area postrema of the medulla oblongata is generally thought to be indispensable for the mediation of motion sickness. We compared the susceptibility to motion-induced vomiting of normal cats with cats in which the area postrema was chronically ablated. Effective destruction of the CTZ was established by loss of emetic sensitivity to i.v. deslanoside and by postmortem histological confirmation of the lesion. The cats were subjected to vertical sinusoidal motion on a spring-suspended platform driven manually at about 30 cpm through excusions of 30 in for no longer than 30 min. With repeated testing, vomiting occurred in 7 of 8 postrema-ablated cats (mean latency of 8.5 min) by comparison with 13 of 26 normal cats (mean latency of 12 min). Two of the lesioned cats vomited in all of 4 and 7 trials, respectively. We found no remarkable difference of responsiveness between operated and normal cats. Hence, the CTZ is not indispensable for motion-induced vomiting.

Central emetic coordinating mechanism and the sensory role of the chemoreceptor trigger zone (CTZ).

The vomiting center in the medullary reticular formation coordinates the performance of vomiting initiated by all causes. However, the neural coordinating mechanism is not directly activated by chemical agents (Borison and Wang, 1953). The CTZ in the area postrema serves as the origin of sensory input to the center for a variety of emetic agents injected into the blood stream and cerebrospinal fluid. Thus, ablation of the area postrema permanently renders the operated animal selectively unresponsive to those chemical substances without disturbing the reflex responses ordinarily evokable through other sensory inputs to the vomiting center as, for example, through gastrointestinal afferent nerves.

Prevailing view of CTZ involvement in motion sickness.

All authoritative sources in the literature (e.g., Money, 1970; Reason and Brand, 1975) convey the belief that the CTZ is an essential link in the emetic reflex pathway of motion sickness. This perception is based on the work in dogs by Wang and Chinn (1954) supported by Brizzee et al. (1980) with experiments on squirrel monkeys. An indispensable role of the CTZ in motion sickness further implies that a humoral factor is responsible for activating the chemosensitive element. Pursuing this rationale, the humoral transmission step at the CTZ becomes the logical focus of attack for therapeutic intervention in motion-induced vomiting (Borison, 1983). The present work was undertaken to critically examine the mediation of motion sickness by the CTZ in another animal species. Our new observations in the cat disagree with the prevailing point of view.

Surgical ablation of the area postrema.

Lesions of the area postrema are made under sterile conditions in the pentobarbital-anesthetized cat by means of hand-held pencil-type cautery, with direct visualization through an operating microscope, while the cat is maintained on artificial respiration as a precautionary measure. Full convalescence takes approximately 3 weeks. Each cat is then tested for emetic responsiveness with a normally effective dose of deslanoside (0.16 mg/kg, i.v.), a digitalis glycoside, to which it is invariably found to be unresponsive as a result of the CTZ obliteration. Figure 1 shows histologic sections of the caudal medulla from two such lesioned cats in comparison with a section taken at the same level from a normal brain. Both operated cats were among those susceptible to motion sickness, described below.

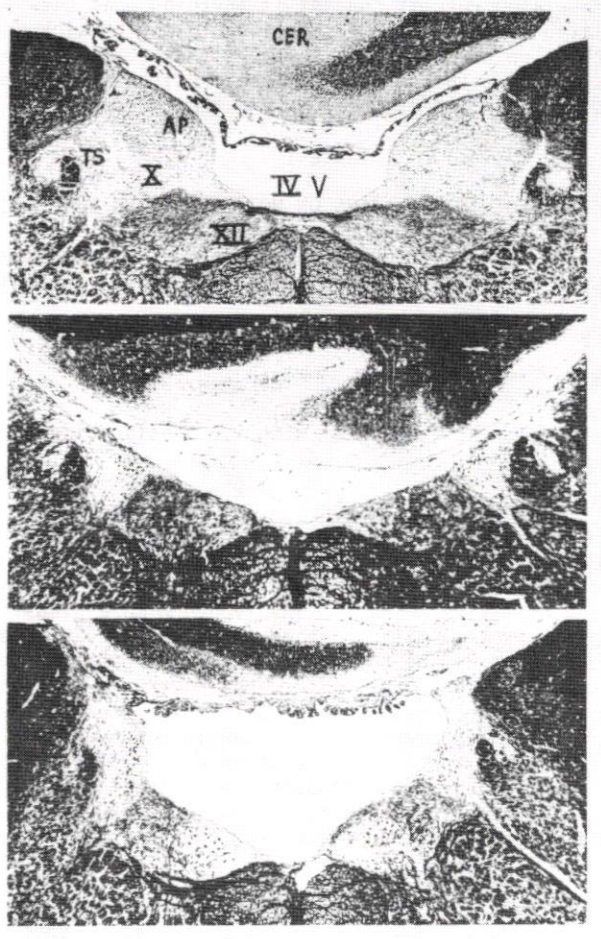

Fig. 1. Coronal sections of the medulla oblongata in the cat at the middle level of the area postrema. Top section: normal specimen. Center and bottom sections: cases of chronically ablated area postrema. Abbreviations: cer - cerebellum; IV V - fourth ventricle; AP - area postrema; TS - tractus solitarius; X - nucleus of the vagus nerve; XII - nucleus of the hypoglossal nerve.

Motion sickness in normal and CTZ-ablated cats.

Vomiting was induced in susceptible cats by subjecting them to vertical sinusoidal motion on a manually driven spring-suspended platform previously used in the NASA-Ames Research Laboratory and made available to us by courtesy of N. Daunton. The tested cat was confined in a plexiglas box but it was unrestrained and easily able to turn around. The motion stimulus consisted of approximately 30-inch excursions at the rate of 30 cycles/min terminated after 30 min or earlier if and when vomiting resulted. Repeated motion tests on a given animal were usually separated by a week or longer, though highly susceptible cats showed no apparent development of tolerance with more frequent testing. Vomiting never occurred while a cat was on the platform at rest. Our experience is consistent with the observations reported from the NASA-Ames Laboratory (Suri et al., 1979). Results of the present study on the effects of CTZ ablation are summarized in table 1. Fifty percent of unselected normal cats vomited on at least one occasion in repeated tests with an average latency of 12 min. By comparison, 88% (7 of 8) CTZ-ablated cats vomited under the same test conditions with an average latency of 8.5 min. Two of the operated cats responded in all of 4 and 7 trials, respectively. The one lesioned cat that was unresponsive to the motion stimulus manifested locomotor ataxia as a consequence of the operation. The tabulated results suggest if anything that CTZ-ablated cats are more susceptible to motion sickness than are normal cats. However, these data are insufficient to demonstrate a real difference in emetic responsiveness between the two groups.

Table 1

EFFECT OF CTZ-ABLATION ON MOTION-INDUCED VOMITING

		Incidence of vomiting				Latency
Cats	N	Test-1	Test-2	Test-3	All tests	Av. min.
Normal	26	38%	36%	18%	50%	12.0
CTZX*	8	63%	43%	66%	88%	8.5

*No vomiting was evoked with i.v. deslanoside.

Comments and conclusions.

The present study in the cat contradicts earlier work in the dog and squirrel monkey. Species difference is an improbable explanation because of the primitive mechanism involved. A more plausible explanation is likely to be found in the variable degree of damage to nearby structures caused in the process of fulgurating the area postrema. We conclude, therefore, that the CTZ in the area postrema is not indispensable for motion-induced vomiting. It follows that the postulation of a related humoral factor is no longer tenable. A truly indispensable element for the mediation of motion sickness may exist close to but separate from the area postrema.

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THE PRIMATE CIRCADIAN TIMEKEEPING SYSTEM IN A HYPERDYNAMIC ENVIRONMENT

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This study was designed to examine the possible effects of hyperdynamic fields (+G) on the circadian timekeeping system of the squirrel monkey. Eight monkeys were sequentially exposed to 1.0, 1.5, 2.0 and 1.0 G. During the two 1 G phases and the 2.0 G phase, the animals were exposed to constant light (LL) in addition to the 24-hr light-dark cycle (LD 12:12) present during the rest of the experiment. All animals were housed simultaneously on an 18 ft diameter centrifuge. The animals were allowed food and water ad lib, the intake of which was monitored electronically every 30 minutes. In all three acceleration fields, the animals demonstrated prominent circadian rhythms in both feeding and drinking. In the LD cycle, both rhythms were entrained with 24 hr periods. A phase delay occurred between the rhythms and the LD synchronizer in the +G enviroment. Moreover, when the animals were exposed to LL at 2.0 G, the mean free-running period was longer than when the animals were at 1 G either before or after the hyperdynamic exposure. In summary, this study demonstrates that the circadian timekeeping system of these primates is functional in the hyperdynamic environment, however, some of its components appear to be regulated at different homeostatic levels.

During their evolution on earth, mammals have been exposed to a unique dynamic component of the environment -- the 24-hour day resulting from the geophysical rotation of the earth. This temporal aspect of the environment has supplied the selective pressure for the evolution of a circadian timekeeping system. This physiological control system is capable of modifying the homeostatic regulation of virtually all physiological and behavioral systems within the organism over the course of the 24-hr day. These circadian rhythms normally demonstrate a 24-hour periodicity when the animals are exposed to the ambient light-dark cycle or other temporal aspects of the environment. However, endogenous pacemakers within the system will maintain this rhythmicity in the absence of all the temporal cues from the environment, with a period close to but no longer equal to 24 hours.

In contrast, however, these same mammals have evolved in the presence of a constant gravitational field (1 G on earth). Yet, many of these homeostatic systems which display circadian rhythmicity are sensitive to altered dynamic environments. For example, regulation of body temperature in rodents and primates is altered where animals are exposed to either a hypodynamic (4) or hyperdynamic (1,3) environment. These temperature responses in the hyperdynamic environment are also modified depending upon time of day (2). Primates exposed to 2 G during the day show a depression in body temperature, while the same animals exposed to 2 G during the night showed no changes in body temperature.

This study was designed to examine the influence of the hyperdynamic environment on the homeostatic regulation of the circadian timekeeping system itself. The pacemakers within this system are relatively insensitive to many environmental parameters. In the squirrel monkey, for example, the only two environmental factors recognized to be strong temporal cues are the light-dark cycle and cycles of food availability (6). All other environmental time cues studied have been ineffective in entraining the circadian timekeeping system.

Eight adult male squirrel monkeys weighing between 900 and 1200 grams were used in this study. Unrestrained animals were individually housed in Plexiglas cages which were in turn housed inside light-tight modules on an 18-foot diameter centrifuge. Animals were allowed food and water ad libitum throughout the study. Food and water intake were monitored electronically and recorded every 30 minutes by a computer throughout the study.

The animals were exposed to two hyperdynamic fields (1.5 and 2.0 G) via chronic centrifugation. The first 15 days on the centrifuge the animals were at 1.0 G. This was followed by 12 days at 1.5 G and 35 days at 2.0 G. This study was concluded with a final phase of 18 days at 1 G. Two lighting conditions were used in the study. Most of the time, the animals were exposed to a 24-hour light-dark cycle consisting of 12 hours of 600 lux light and 12 hours of darkness (LD 12:12). The lights were on from 08:00 to 20:00 and off from 20:00 hours to 08:00. The second lighting environment was a constant 600 lux illumination (LL). The animals were exposed to LL during 1.0 G before and after centrifugation and during centrifugation at 2.0 G. Ambient temperature was maintained at $26^{\circ} \pm 1^{\circ}C$.

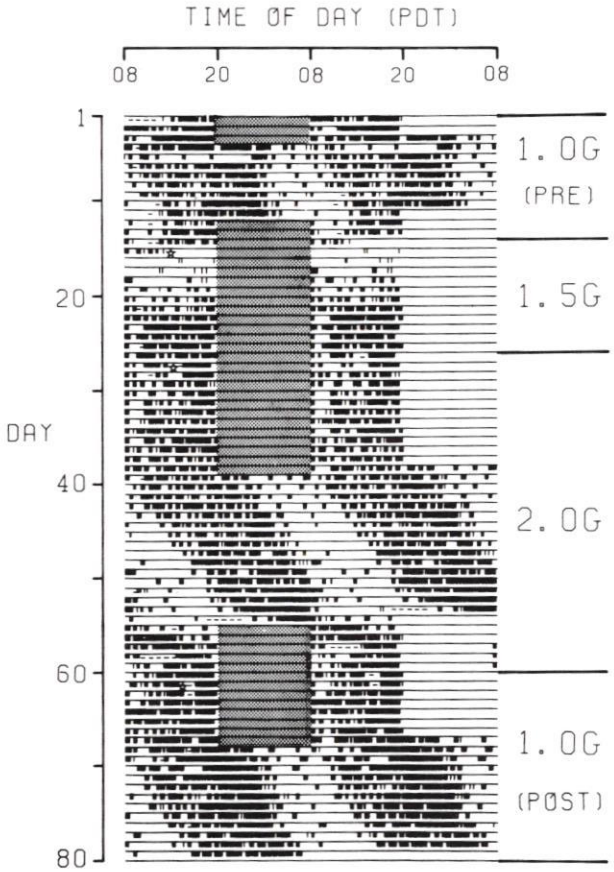
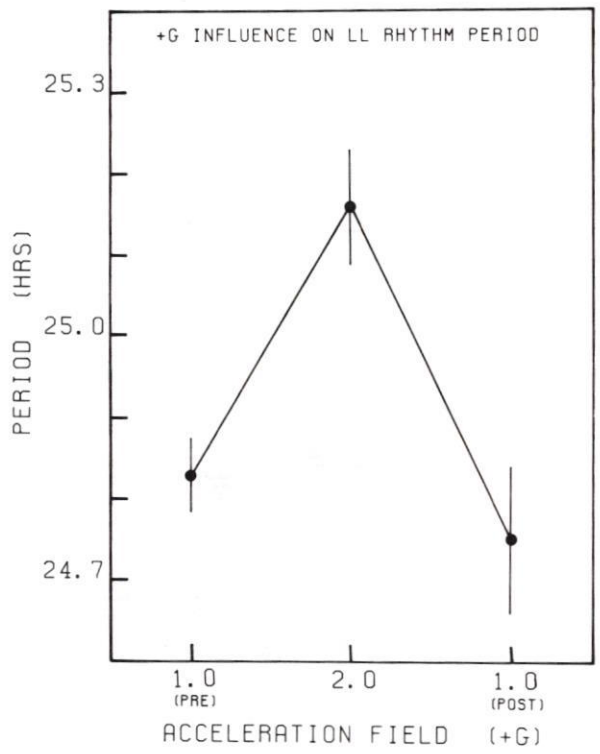


Figure 1 shows a digital actogram of the feeding data of one animal. This preliminary report only describes the results from the feeding data, however, the drinking showed similar responses. In Figure 1, the data are plotted with succeeding days (indicated on the left)

below each other and the graph double plotted, that is, each 24-hr day is duplicated on the right for ease of visual analysis. The data are plotted such that each downward stroke of the pen on this event record indicates the consumption of a food pellet by the animal. The shading in the left-hand portion of the record shows where darkness occurred during the light-dark cycles. The acceleration field intensities are indicated on the right side of the figure. When the animals were exposed to the light-dark cycle, at all G levels, the feeding rhythm persisted and was entrained to the 24-hour LD cycle. Further, it can be seen that when the animals were in constant light the rhythms persisted with a free-running period, greater than 24 hours.

On average, during centrifugation there was no significant change in the 24-hour level of food intake of the animals, nor was there a significant change in the amplitude of the rhythms of food intake during any of the phases of the experiments except during the initial 3-5 day transition into each hyperdynamic environment.

There was, however, a significant change in the phase relationship of the feeding rhythm compared to the light-dark cycle. In the hyperdynamic fields there was a phase delay of the feeding rhythm (i.e. the time of computed maximal feeding was later in the day). This was a significant effect ($p < .02$) and was greater at 2 G than at 1.5 G. Further, when the animals were returned to 1.0 G, the phase of the rhythms advanced to the previous 1 G control phase. Thus, at 1 G before and after the hyperdynamic exposures, the maximal feeding occurred at around 14:00. At 1.5 G this maximum was computed to be around 14:30 and at 2.0 G was still later in the day (approximately 15:00). This response would be consistent with an increase in the pacemaker period.



Indeed, this is what we found when we examined the animals in constant light. Figure 2 shows the average free-running periods (± SEM) of all the animals in LL. In this case the animals demonstrated an increase in free-running period from 24.8 hours to approximately 25.2 hours at 2.0 G. This response was also significant ($p < .01$) and reversible as the period returned to 24.8 hours during the 1.0 G postcentrifugation.

Thus, this study has demonstrated that the hyperdynamic environment does indeed influence the homeostatic regulation of the circadian timekeeping system. At present, the only other example of biological rhythms in a primate in altered gravitational fields is that of the biosatellite monkey (4). In this animal, although other stress responses were involved, there was a change in the entrainment of the rest/activity cycle and the body temperature rhythms in-flight. The primate data from the recent Cosmos flight also suggests a non-24-hour temperature rhythm, however, the flight was too short to make any definitive statement (unpublished observations).

At present, the mechanism for this response is not clear. It could be hypothesized that changes in pacemaker metabolism and therefore the endogenou pacemaker period were induced. These animals d become hypothermic, at least transiently, under these conditions (1) which may provide a stimulus for increase metabolism. However, the squirrel monkey is no recognized to be sensitive to the temporal cues induce by alterations in ambient temperature (6), which als induce changes in metabolic levels. Rather, it woul appear that a more specific and longer lasting influenc of the gravitational fields is producing these changes i the circadian timekeeping system.

The magnitude of the period changes in the 2 G fiel are important considering the insensitivity of th circadian timekeeping system to other environment influences (6). Light has the most prominent perio modifying influence that we are aware of at present. O previous observations of changes in light intensity ha shown that a similar range of period changes (i.e. 24.8 25.3 hours) is observed when changing light intensity fro 600 to 6 lux (5).

It thus appears that gravitational field strength h significant influences on a variety of homeostat regulatory systems in primates and other mammal Further, this study has shown that these systems not on respond differently at different times of day, but t expression of the circadian pacemakers themselves is al modified.

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PRIMATE BODY TEMPERATURE AND SLEEP RESPONSES TO LOWER BODY POSITIVE PRESSURE

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Cephalic fluid shifts, induced by lower body positive pressure (LBPP) are known to influence various physiological systems (i.e. cardiovascular and renal). In earlier experiments, we observed an apparent change in the arousal state of primates in such LBPP conditions. This study was designed to examine the effects of LBPP on arousal state and body temperature level which is normally correlated with sleep. Chair restrained male squirrel monkeys were exposed to 40 mmHg LBPP for 90-100 minutes between the daytime hours of 13:00-15:00. Each monkey was placed in a specially modified restraint chair to which they were highly trained. Deep body temperature (DBT) was collected from 10 animals. Sleep parameters were obtained from 6 animals chronically implanted for sleep recording. A video camera was used to observe each animal's apparent state of arousal. LBPP resulted in an approximate 0.9°C decrease in DBT. During video observation some animals appeared drowsy during LBPP, however, sleep recording revealed no significant changes in the state of arousal. Thus, LBPP is capable of inducing a mild hyperthermia. Further, the mechanisms underlying the observed lowering of body temperature appear to be independent of arousal state.

The absence of gravitational forces experienced in spaceflight constitute an important and unique environmental challenge to the physiology of an organism. To investigate the nature of the physiological responses to weightlessness, a number of ground based human and animal experimental models have been employed. These include antiorthostasis, water immersion, and lower body positive pressure (LBPP), to name only a few. In these studies the extracellular fluid, which normally pools in the lower body, is displaced cephalid. The headward fluid shift in space has been hypothesized to be responsible for a number of physiological responses, including space motion sickness, changes in fluid, electrolyte and hormonal balance, and muscular function.

Previous studies (4) have employed LBPP in squirrel monkeys as a means of inducing cephalic fluid shifts. A number of renal and cardiovascular responses to LBPP have been elucidated in these studies. However, absent from these studies are measurements of thermoregulatory responses, which may be coupled to LBPP induced cardiovascular adjustments.

There is a growing body of evidence to suggest that thermoregulation is influenced by variations in gravitational forces. For example, hyperdynamic environments produce transient hypothermia in both rats (2) and monkeys (1). Further, antiorthostasis can lower body temperature in man (5) and rodents (Musacchia, personal communication). Finally, and perhaps most importantly, data from space flight studies suggest that zero gravity may induce hypothermia in both rats (6) and primates (3).

In preliminary studies, video observation of squirrel monkeys revealed that some monkeys become drowsy or sleepy during LBPP. That sleep is normally highly correlated with changes in body temperature prompted us to investigate this phenomenon in greater detail. Thus, in this study we monitored body temperature and polygraphically determined arousal states in squirrel monkeys prior to, during, and following the administration of LBPP. We report here that LBPP results in a significant lowering of body temperature due, at least in part, to physiological factors independent of arousal state.

Ten chair restrained male squirrel monkeys (Saimiri sciureus) weighing 950-1100 g were exposed to 40 mmHg of LBPP for 90-100 minutes between the daytime hours of 13:00 and 15:00. Each of these monkeys was placed in a restraint chair to which they were highly trained. The LBPP system enclosed the lower portion of the animal's body. A customized neoprene rubber belt worn above the hips sealed a partition isolating the lower half of the body. Each animal was given several hours to accommodate to the isolated recording environment prior to data collection. Monkeys in the same apparatus without the rubber belt, were subjected to equivalent airflow, but no pressure, served as controls. Both temperature and pressure of the air supplied to the LBPP apparatus were regulated and constantly monitored. A video camera was used to monitor the monkey's behavior at all times.

Body temperature was monitored by two means: either using a colonic temperature probe (YSI 401) which was inserted 6 cm beyond the anus, or via a chronically implanted intraperitoneal temperature probe. Four animals were anesthetized with a combination of valium (0.7 mg/kg) and pentobarbitol (13 mg/kg). A temperature transducer was surgically placed intraperitoneal 1 cm anterior to the umbilicus, with transducer leads externalized dorsally at the mid-scapular region. All animals were allowed to recover a minimum of four weeks prior to experimental use.

Arousal states were objectively determined from six chronically implanted animals in which we continuously recorded the electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG) patterns. The implant hardware consisted of a microminiature receptacle with stainless steel leads. Monkeys were anesthetized as above, their heads shaved, and EEG ad EOG leads affixed into position with stainless steel screws. Two EEG leads were positioned bilaterally over the frontal cortex of the brain and two leads placed over the occipital cortex. The EMG leads were routed subcutaneously and positioned bilaterally in the neck musculature. The connector was affixed to the skull with dental acrylic. Monkeys were permitted in excess of 6 weeks recovery prior to any LBPP manipulations.

Body temperature data were collected automatically using a micro-computer based data acquisition system. Polygraph sleep data and video assessed indices of activity were manually scored by two technicians, and all data were entered into a mini-computer for analyses and plotting. Sleep states were determined using criteria modeled after the guidelines set by the UCLA Brain Information Service, and subsequently modified for application in squirrel monkeys. Our scoring technique differs in that we divide slow wave sleep into only two categories: light slow wave sleep (SWS1) and deep slow wave sleep (SWS2).

In this study, the administration of LBPP resulted in a marked lowering of body temperature (Figure 1). Body temperature was lowered by an average of $0.93^{\circ}C \pm 0.13$ ($X \pm SEM$) in response to LBPP. The decrease in body temperature was significant (p .01) using a one tailed student's t-test. Each animal studied showed a latency in the thermoregulatory response to LBPP which lasted several minutes. The decrease in body temperature observed is consistent with that reported in

antiorthostasis studies. For example, Kleitman (5) noted a decline of 0.5°C in human body temperature upon the assumption of a horizontal body position from that of standing. In rats, head down tilt also results in a similar depression of body temperature (Musacchia, personal communication).

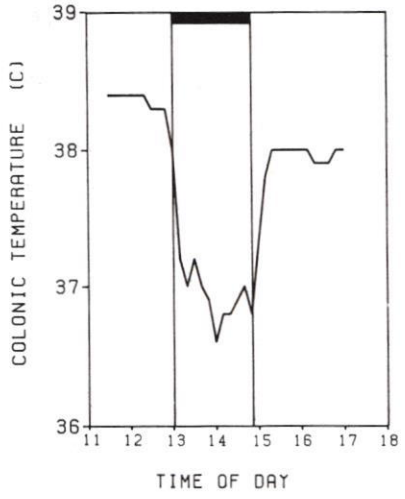

Figure 1.

That some of the monkeys appeared sleepy during earlier LBPP studies prompted us to question whether a decrease in heat production resulting from sleep would account for the observed hypothermia. Polygraphic sleep recordings confirmed that some animals did indeed show slow wave sleep patterns consistent with sleep patterns we have observed in other monkeys. However, the majority of the animals in this study showed no significant change in arousal state upon the administration of LBPP even though body temperature was depressed. For example, in Figure 1, 100 minutes of LBPP induced a 1.5°C hypothermia. However, this animal demonstrated no behavioral or electrophysiological correlates which would suggest a change in arousal state. In each animal hypothermia persisted throughout the presentation of LBPP, and body temperature returned to pre-experimental control levels within 15-20 minutes post-LBPP. Figure 2 shows the influence of LBPP on the arousal states of four representative monkeys. During LBPP one monkey (top) showed a demonstrable increase in SWS1 sleep. The other monkeys, however, did not show a noticeable change in arousal state. Each of the animals in Figure 2, however, became hypothermic in response to LBPP.

Presently the mechanisms underlying the LBPP induced hypothermia are unclear. In light of the congruence between our findings and those of antiorthostasis and space flight studies, it appears that the cephalic fluid shift, which occurs using these techniques, may be a contributing factor to the observed hypothermia. Cephalic fluid shifts could potentially influence central thermoregulatory centers. Certainly other environmental factors are capable of influencing temperature regulation. For example, light can exert direct influences on body temperature, resulting in variations in temperature much like that seen during LBPP. Further, these direct influences of light can also be independent of arousal state.

Hypothermia may also be the result of cardiovascular adjustments to which thermoregulation is coupled (i.e. cutaneous heat loss resulting from peripheral vasodilatation). Cephalic fluid shifts have also been shown to activate a wide variety of cardiovascular responses, including a decrease in heart rate, increase in blood pressure, decrease in total peripheral resistance, and a decrease in the circulating levels of ADH, renin, and aldosterone to name only a few. An increase in

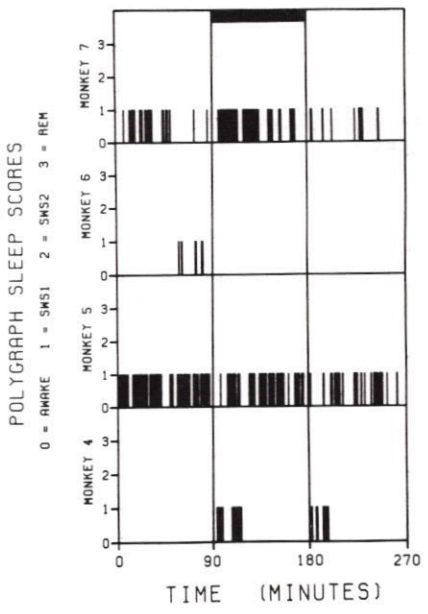

Figure 2.

cutaneous blood flow induced by any or all of the above mentioned cardiovascular responses could account for heat loss during LBPP.

In summary, a number of environmental factors can influence temperature regulation. This study further implicates gravity as one such factor. When LBPP is used to induce a cephalic fluid shift, body temperature is lowered. This decrease in body temperature is not explained on the basis of a change in arousal state, which was observed in some animals, but appears to be dependent on other physiological mechanisms to which temperature regulation is coupled.

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CORE TEMPERATURE OF TAILLESS RATS EXPOSED TO CENTRIFUGATION

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INTRODUCTION

Although it comprises only about 5% of the total body surface area of the rat, the tail can dissipate about 17% of the animal's body heat (6). In the present study, we have investigated the role of the tail in the altered thermoregulation of rats during acute hypergravic exposures (achieved by centrifugation). Such exposures produce a rapid fall in core temperature (T_c) leading to a sustained hypothermia (5). In addition to the T_c changes, there is a significant, but transient increase of tail temperature, indicative of an accelerated rate of heat loss (1,4).

To determine the extent to which increased heat loss from the tail affects the hypothermic response, rectal temperature changes were measured in both tailless and intact rats subjected to centrifugation. Results from this study indicate that the increased heat loss from the tail per se does not contribute in a measurable way to the hypothermia induced by centrifugation stress.

METHODS

Two groups of male, Sprague-Dawley rats were used in this study. In one group, the rats were 31 days old and in the second group, they were 114 days old. Both groups were maintained on a 12 hr light, 12 hr dark cycle with food and water provided ad libitum.

The tails of 12 rats (6 from each age group) were surgically removed while the rats were under ether anesthesia. Twenty-four days later (when the rats were 55 or 138 days old), the 12 tailless and 12, age-matched intact rats (controls), were individually placed in restraining devices (conically-shaped, plexiglas half-cylinders attached to flat plates). The restrained rats were placed on a centrifuge with a 4 ft radius.

To measure T_c, a thermistor (Yellow Springs Instruments Series 400) was inserted into the rectum. To measure foot temperature (T_f), a thermistor was taped to the skin over the foot pad of the right hind foot. T_c and T_f were recorded every 5 minutes with an Acurex Autodata 9 recorder.

So that they were exposed to the same experimental conditions, tailless and intact rats were simultaneously exposed to centrifugation.

In all experiments, baseline T_c and T_f were measured for 1 hr at 1G. This first hour was followed by a 1 hr period at 2.8G. Rats were then returned to 1G for an additional 1 to 3 hrs. Ambient temperature throughout the experimental trials was maintained close to 22 °C.

RESULTS

T_c and T_f of 55-day-old rats. As illustrated in the upper part of figure 1, for the 1G period prior to centrifugation, T_c of both intact and tailless rats remained close to 38.2 °C. During centrifugation, T_c of intact and tailless rats fell by about the same amount ($p > 0.1$, Student's t-test) so that by the end of 1 hr at 2.8G, T_c of intact rats was 35.4 ± 0.2 °C (mean ± S.E.M.) and T_c of tailless was 35.7 ± 0.7 °C. For the next 2 hrs and 40 mins (when the rats had been returned to 1G), T_c increased but not to pre-centrifugation levels.

As illustrated in the lower part of figure 1, at the onset of centrifugation, T_f increased to 32.3 ± 0.8 from 30.2 ± 0.9 °C in intact rats and from 30.3 ± 0.8 to 35.0 ± 0.3 °C in tailless animals. The T_f increase in tailless rats was greater ($p < 0.05$) than the T_f increase in intact animals. After approximately 10 mins of centrifugation, T_f of tailless and intact rats fell rapidly and remained below the pre-centrifugation level for the remainder of the centrifugation period as well as for the period of 1G recovery.

T_c and T_f of 138-day-old rats. Similar to the response of 55-day-old rats shown in figure 1, there was a marked fall in T_c of 138-day-old rats during centrifugation (figure 2). The magnitude of the T_c fall in tailless rats was not significantly different ($p > 0.1$) from the magnitude of the T_c fall in the intact animals. Also, there was a transient increase of T_f at the onset of centrifugation. However, in contrast to the response of 55-day-old rats, the magnitude of the T_f increase was not significantly greater ($p > 0.1$) in the tailless relative to the intact rats. After the initial increase, T_f of intact and tailless rats fell to a level that was within 1 or 2 °C of the pre-centrifugation level.

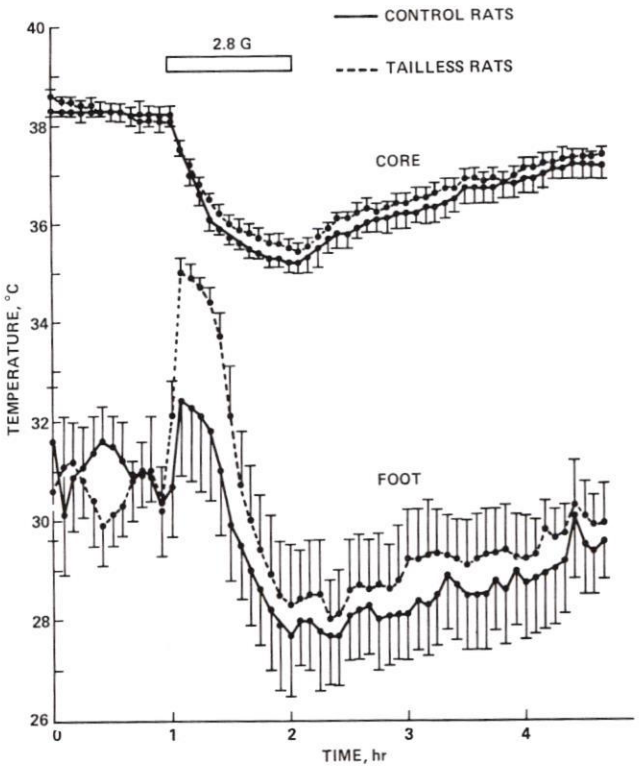

Figure 1. Core and foot temperatures of 55-day-old rats before, during and after centrifugation at 2.8G. Each point is the mean (+ S.E.M.) temperature response of 5 rats.

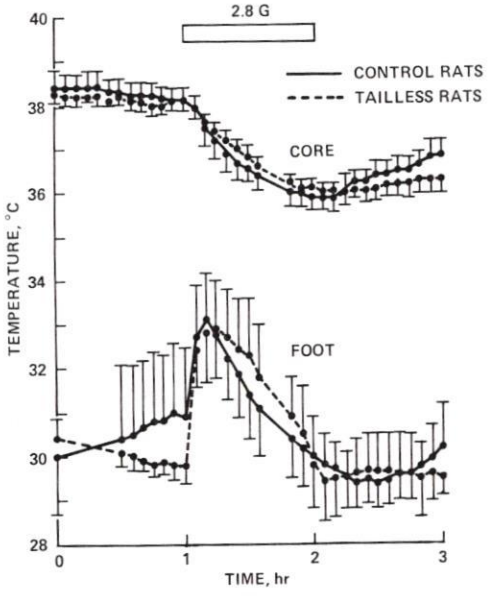

Figure 2. Core and foot temperatures of 138-day-old rats before, during and after centrifugation at 2.8G. Each point is the mean (+ S.E.M.) temperature response of 5 rats.

DISCUSSION

From a comparison of the core temperatures in tailless and intact rats, we conclude that at 22 °C, the loss of body heat from the tail per se does not measurably contribute to the hypothermia induced by centrifugation at 2.8G. Loss of heat from other parts of the body-- such as the feet, ears and face-- may have a measurable effect on the hypothermic response.

Our T_f measurements provide a qualitative indication of heat loss from areas of the body other than the tail. For example, the transient increase of T_f at the onset of centrifugation indicates an increased loss of heat from the feet to the environment. Also, for 55-day-old tailless rats, the significantly greater (relative to the intact rats) increase of T_f indicates that heat loss from the feet during during centrifugation is greater in these animals than in the intact rats of comparable age. By contrast, in 138-day-old rats, the T_f increase at the onset of centrifugation was not significantly different in tailless compared with intact rats. Thus 55-, but not 138-day-old rats may compensate for the loss of their tails with an increased blood flow to the feet resulting in an increased heat loss from the feet.

Finally, the inhibition of heat production mechanisms is probably a significant factor in the hypothermia of centrifuged, tailless rats. Previous studies (2,3,4) have shown the inhibition of heat production mechanisms to be a significant factor in the hypothermia of intact rats exposed to centrifugation. The fact that the tailless rats of our study were hypothermic during centrifugation suggests heat production in these animals is also inhibited.

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EFFECTS OF CLINOSTAT ROTATION ON MOUSE MEIOTIC MATURATION IN VITRO

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The effects of microgravity on meiosis, fertilization, and early embryonic development in mammals are being examined by using a clinostat to reorient the cells with respect to the gravity vector. A clinostat capable of supporting mammalian cells in tissue culture has been developed. Initial studies have focused on examining the effects of clinostat rotation on meiotic maturation in mouse oocytes. Oocytes recovered from ovarian follicles were subjected to clinostat rotation on a horizontal or vertical axis or to static conditions for a 16 hr period. No gross morphological changes and no effects on germinal vesicle breakdown were observed under any rotation conditions (¼,1,10,30,100 RPM). Success of meiotic progression to Metaphase II was comparable among experimental and control groups except at 100 RPM, where a slight inhibition was observed.

In an effort to understand the role of gravity in basic cellular processes, we are examining the cells upon which development of the adult organism is absolutely dependent: the gametes and cells of the early embryo. In order to simulate a microgravity environment under laboratory conditions, a clinostat was developed which is suitable for mammalian tissue culture. The clinostat, based upon the design originally developed by Tremor and Souza (Space Life Sciences 3: 179-191, 1972), is depicted in Figure 1.

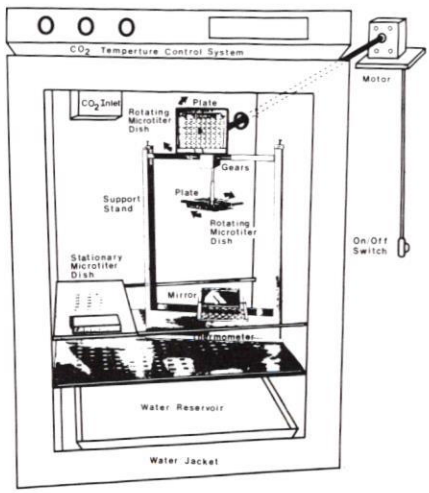

Figure 1. Diagram of the clinostat suitable for use in mammalian tissue culture.

The major adaptations that were necessary were due to the unique in vitro culture requirements of mammalian oocytes and embryos, including a temperature of 37°C, a constant atmosphere of 5% CO_2 in air, and 100% humidity. A second major technical consideration lay in the selection of an appropriate culture vessel made of tissue culture grade plastic, of small diameter, and allowing for exchange of gases. Sterile 96-Well Micro Test III tissue culture plates (Falcon 3072) were chosen. Culture media was placed in the center well and overlaid with paraffin oil. The paraffin oil allowed an exchange of gases, provided a barrier against contamination, lowered the evaporation rate of the media, and prevented the media from draining out of the center well when the plate was placed in a horizontal position.

A final modification was the addition of 2 bevel gears set at a perpendicular angle to each other, which allowed simultaneous horizontal and vertical rotations (note Figure 1). We believe this has been a most important addition in that it permits exposure of cells to conditions very close to that of the experimental series, except for reorientation relative to the gravity vector.

Use of the Clinostat for Studies on Meiotic Maturation

Young, sexually mature, female Swiss Webster mice were used for all the experiments. Ovaries were dissected free of adhering tissues and oocytes were freed from follicles by puncturing the follicles and teasing apart the ovary tissue. Oocytes were pooled and aliquots were placed into the experimental or control culture system. Care was taken to minimize the time interval between oocyte collection and the start of rotation (ideally ≤10 min). Cultures for rotation were mounted onto the metal support plates of the clinostat. The center well, containing the oocytes in culture, was carefully positioned at the center of the axis of rotation. Cultures were incubated for 16 hrs at 37°C under 5% CO_2 in air. In the majority of experiments, the experimental series had, in addition to the static control and the clinostat rotated cultures, cultures which were rotated on the clinostat with a vertical axis of rotation (an axis parallel to the gravity vector).

After the 16 hour culture, the oocytes were removed from the culture system and immediately observed under the dissecting microscope for polar body formation and gross morphology, including any obvious cellular abnormalities such as granular or necrotic appearance of the cytoplasm, fragmentation, rupture of the zona pellucida, clumping of cumulus cells, etc. No consistent morphological changes were observed at any of the rotation speeds and culture conditions used.

Oocytes were then processed for cytogenic analysis according to a modified protocol of Misguchi and Dukelow (J. med. Primatol. 10:180-186). Cells were stained for immediate observation with 2% Toluidine Blue in resin. Such preparations can be scored for germinal vesicle breakdown and progression through meiosis to Metaphase I or Metaphase II. In addition, preliminary cytogenetic assessment can be made, particularly with respect to chromosome number and obvious chromosomal fragmentation or structural rearrangement.

The results of the analysis of germinal vesicle breakdown on the control and clinostat-rotated oocytes are presented in Table 1.

TABLE 1

GERMINAL VESICLE BREAKDOWN UNDER CLINOSTAT ROTATION

Conditions	# of experiments	# of oocytes analyzed	Average % GVBD ± SD	*Significant
¼ RPM - control	6	124	90 ± 9.4	No
¼ RPM - exp	6	169	83.5 ± 12.4	
¼ RPM - conrol	2	42	95 ± 1.4	No
(vert) - exp	2	45	93 ± 1.4	
1 RPM - control	11	177	96.6 ± 4.4	No
1 RPM - exp	11	214	79.5 ± 14.3	
10 RPM - control	11	175	86.7 ± 8.2	No
10 RPM - exp	11	233	83.6 ± 10.4	
30 RPM - control	7	245	84.9 ± 6.0	No
30 RPM - exp	7	210	82.1 ± 11.7	
30 RPM - control	5	104	90.2 ± 7.6	No
(vert) - exp	5	94	89.2 ± 11.0	
100 RPM - control	11	371	85.6 ± 7.3	No
100 RPM - exp	11	219	84.7 ± 11.5	
100 RPM - control	12	419	86.0 ± 7.1	No
(vert) - exp	12	248	82.5 ± 12.6	

*As assessed by Chi-square analysis

Rates of germinal vesicle breakdown were comparable in both the horizontally and vertically rotated cultures as well as in those that were static. This was not surprising since it is believed that the stimulus responsible for triggering the resumption of meiosis under in vitro culture conditions is elicited very soon (less than 30 min) after release from the follicle. In this situation, effects of gravitational force changes would have had to be implemented within approximately 20 minutes.

In most mammalian oocytes, fertilization occurs with the oocyte arrested in the second meiotic metaphase. Progression to this stage in a timely sequence is thus a prerequisite for normal fertilization and subsequent completion of chromosomal disjunction to ensue. Oocytes in the present experiment were examined for their progression to Metaphase II, after 16 hours under control or experimental conditions; the results are summarized in Table 2.

TABLE 2

PROGRESSION TO METAPHASE II OF MEIOSIS UNDER CLINOSTAT ROTATION

Conditions	# of experiments	# of oocytes analyzed	Average % M^{II} ± SD	*Significant
¼ RPM - control	5	82	85.4 ± 11.3	No
¼ RPM - exp	5	115	67.2 ± 18.3	
¼ RPM - control	2	37	79.0 ± 11.3	No
(vert) - exp	2	41	82.5 ± 0.7	
1 RPM - control	8	91	96.1 ± 5.5	No
1 RPM - exp	8	113	76.8 ± 15.0	
10 RPM - control	9	117	81.9 ± 6.5	No
10 RPM - exp	9	148	70.7 ± 14.7	
30 RPM - control	5	144	95.0 ± 6.2	No
30 RPM - exp	5	113	71.8 ± 19.2	
30 RPM - control	4	75	88.0 ± 6.7	No
(vert) - exp	4	66	91.0 ± 3.6	
100 RPM - control	9	224	88.9 ± 8.2	Yes
100 RPM - exp	9	152	56.9 ± 30.4	
100 RPM - control	10	265	89.3 ± 7.9	No
(vert) - exp	10	147	86.7 ± 11.1	

*As assessed by Chi-square analysis.

The efficiency of Metaphase II formation was similar among control (static) oocytes, vertically-rotated oocytes at ¼, 30, and 100 RPM, and experimentally (horizontal) rotated oocytes at ¼, 1, 10, and 30 RPM. At 100 RPM, however, a significant (by Chi-square analysis) decrease in the number of oocytes reaching Metaphase II after 16 hours in culture was observed. Whether this is due to an irreversible block of a proportion of oocytes in the Metaphase I stage or to a lag in the timing of the progression of meiotic disjunctive events is not known.

Regardless of the mechanism involved, such an inhibition would be expected to affect the reproductive potential of these cells, since achieving Metaphase II is essential for normal fertilization to occur. These results are also of interest in light of recent observations of an inhibition of a mitotic response to the mitogen Conconavalin A of cells cultured in vitro in both the microgravity environment of space(Cogoli, A. et al., Science 225: 228-230, 1984) as well as in the simulated microgravity environment of the clinostat (Cogoli, A., et al., Aviat. Space Environ. Med. 51: 29-34, 1980).

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METABOLIC EFFECTS OF VARIOUS INTENSITY OF MAGNETIC FIELDS ON THE ALBINO RATS UNDER DIFFERENT DYNAMICS

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This paper concerns to the comparison between the bio effects of different intensity of magnetic field on the biological body under different dynamics, for an approach to clarify the physiological characteristics of gravity through the interaction with bio-magnetic factors. Following to the last two reports on low, geo and high magnetic fields (LM. 4mOe, GM around of 600mOe, and HM. 200 Oe) under hypodynamics (othostasis) (LD), in this report, results of the experiments on the same magnetic conditioning under normodynamics, terrestrial vivarium life (ND) and some kind of hyper dynamics (HD) will be reported. Using rats, several physiological parameters, were measured before, during, and after the 3-4 weeks of conditioning. Results: 1) Body weight in LD decreased in all groups, but the effect of LM and HM groups were observed. 2) Resting metabolic rate of LM and HM groups strongly decreased in ND. 3) LM acts to increase the urinary volume more in ND but produce a oligouresis in LD. 4) K+ and Na+ excretions have a tendency to decrease in HM group and increase in LM group in ND. In ND, such relation is inverted. 5) The Ca^{2+} excretion rate in LM during LD decreases strongly. This is a typical LM effects attenuating the action of LD. The relation between magnetic field intensity and urinary excretion rate of Ca^{2+} in ND and LD animals can be expressed as the theoretical curvilineal regression line of 2 degree, and be able to suggest the reversion of the relation of Ca^{2+} excretion rate between LD and ND at the special intensity of magnetic fields.

INTRODUCTION

We had already reported about the experimental results in comparison of the responces of many physiological functions of mice and rats during and after low magnetic field (LM, 4mOe) exposure, under simulated hypogravic conditions (LG) by orthostatic suspension technique, to those of geomagnetic fields exposure (NM, 600mOe) under normodynamic terrestrial life (NG). And the results of the same experiment by high magnetic fields (HM, 200 Oe) under the same LG conditions was also reported.

In this report, the comparison on the effects of the some low and high magnetic forces exposure during normodynamics in vivarium life was reported. This data during normodynamics in vivarium life will be also an valuable information as the standard control for any other conditions.

EXPERIMENTAL PROCEDURE

Through all the experiments in this report, body weight, resting metabolic rate, daily urinary volume, daily urinary excretion rate of K+, Na+, and Ca^{2+}, blood pressure, pulse rate, rectal temparature, during 3-4 weeks of orthostasis and of vivarium life were measured. Resting metabolic rate and blood pressure as respiratory and cardiovascular parameters, were recorded at the same time of the day with the body weight once a day by indirect calorimetry and electro sphygmomanometer (PE-300 Narco Bio-Systems Inc.) at the tail artery. As the metabolic parameters, urine volume urinary excretion of K+, Na+ and Ca^{2+} were determined by Mosher's flame spectrophotometry, Shimazu's Atomic absorption spectrometry on the urine of whole day. Data of 3 different intensity magnetic fields exposure groups were compared with each other. In the experiments under hypodynamics condition, a kind of covering case for the sustainment of ferrite magnet were used. This case effects to make a constant environment for each subject and to serve for good conditioning during the experimental run. In the apparatus for the magnet exposure, two ferrite magnets of 12 cm by 20 cm in size fixed on the aluminum case at 9 cm interval. The case was set over the suspended animal in parallel to the body axes (Fig.1). Such magnets produce about 200 Oe at the center between the two magnets, where the center of the animal body were fixed. For GM and LM groups, the aluminum case of the same size were used. They held the bakelite plates in stead of the magnet. We have found that the resting metabolic rate of such cased animals were significantly lower than that of without the case, so that we performed all our experiments, under such covered condition. In this experiments under normodynamics, those are vivarium condition, the cages of 18x18x18cm were placedin bigger cases of 21.5x35x35cm (Fig.2). Wall of each side held a ferrite magnet of 22x5x30cm size, the average intensity of the magnetic fields of each part of each cage is 200-300 Oe. 2-4 Wister King Strain albino rats of 2-3.2 months age were kept in a upper mentiond cage that were set in the upper mentioned case for vivarium life and also were suspended in a static orthostatic suspension apparatus in the small case for hypodynamics exposure. For the normodynamic condition in the low magnetic field, that was prepared by active shielding using with Helmhorz coils.

RESULTS AND DISCUSSION

1) Body weight: The body weight curve of ND group and hypodynamic group, were expressed as the percent of pre-experiment control value during vivarium life for 4 weeks. In the GM group, after a sharp but transient decreasing, very slow increasing of the body weight were observed. But in LM and HM groups,the clear decreasing were observed after the maximum point that is located at around of the 10 days of the exposure.

2) Resting metabolic rate: The resting metabolic rates of each group in vivarium control group, slow decreasing were observed, and this seems to be representing the effect of growth or aging. In this group and the hypodynamics control group , on the contrary, the values of HM and LM groups, after a transient decreasing, increased slowly but steady and have a tendency to come back to the pre-experiment value. But the values of GM group had no such tendency at least during 3 weeks observations.

3) Urine volume: This was expected to increase at the case of hypodynamics. Results in all the groups of vivarium control have a significant increase of urinary excretion, following the growth, and in the LM group, this tendency was larger than in the other groups. In the hypodynamics condition , there was the same tendency, but no significance was observed statistically, but LM group showed a strong oligouresis throughout the hypodynamics ex-

posure periods. These findings show definitely the special influence of LM to the function of urinary excretion system, and the action is different following the kinetic or gravitational conditioning of the animal body.

4) Urinary excretion of K+ and Na+: On the vivarium control, urinary excretion rate of K+ per standard body weight, have no special tendency during the experimental run, it seems to have some rhythmical flucutuations in each several days but through all the process of the exposure, highly significant decreasing were observed in the case of HM group. In the hypodynamics condition, GM group showed a typical type, fluctuation, that had reported by us on same animals under the same hypodynamics procedure already. The HM group have also similar tendency but the levels were not so high as that of GM group. HM seems to act to attenuate the increasing of the urinary excretion rate of K+ by hypodynamics. And at the case of LM group, they were decreased significantly, especially during the 3rd week of experimental run. On the case of Na+, almost same result to the case of K+ were noticed in both normo and hypo dynamics lives.

5) Urinary excretion rate of Ca^{2+}: Carcium is one of the most interesting substances in connection with the clinical treatment in gerontology, valutudinarian medicine and space medicine, On the urinary excretion rate of Ca^{2+}, no changes were found in both cases of LM and GM in the case of vivarium control group. But only the HM group has a special clear characteristic of strong decreasing. The same tendency were observed also in the case of K+ and Na+, but the decreasing levels were largest in the case of Ca^{2+} . HM seems to be very favorable for the saving Ca^{2+} in the body at the case of vivarium life. And on the case of hypodynamics, the results were very similar with that of the case of K+ and Na+, and increasing or decreasing levels were largest in the case of Ca^{2+}. HM seems to be better condition for hypodynamic life than GM condition. But LM seems to be best condition for such a life.

The diagram in Fig.3 shows the relation between Ca^{2+} excretion level and magnetic force level, in the case of normodynamics vivarium control life and hypodynamics life. The thin lines, medium lines and thick lines are representing the Ca^{2+} levels of the 1st, 2nd and 3rd week of the experiment, respectively for the vivarium life. The thin dotted medium dotted and thick dotted line show the Ca^{2+} levels of 1st, 2nd and 3rd week of the experiment, respectively for the hypodynamic exposure. They are able to be expressed as the thoretical curvilineal regression line and its regression equation is expressed on 2nd degree. As seen in this curves, for the vivarium control life, the HM is a most favorable, and for hypodynamic life including hypogravic life, LM seems to be most favorable, but the around of GM is not so good condition from the Ca^{2+} metabolic point of view, and between 0.5 Oe and 200 Oe, the worst point is expected to be.

From the results of Nahas et al[2], 200 Oe - 1200 Oe seems to be safety from the growth rate of rats, and by Barnothy[3] , the animals damage was reported only after prolonged exposure to magnetic fields exceeding 40000 Oe^3 , anyway, from the clinical or pathological point of view, there seems to exist the clitical points of the magnetic force for each species of biological body. On such intensity of damageous level for the each higher species of animal, further investigation must be needed

SUMMARY

On the findings, next points will be noticed concering the biomagnetic differences of dynamic cinditioning of the biological body.

1) Resting metabolic rate conditionated in abnormal intensity of magnetic fields decrease in ND but not LD.

2) LM acts to increase the urine volume more in ND but that is in verse in LD.

3) K^+ and Na^+ excretions have a tendency to decrease in HM group , and increase in LM group in ND, but that is reversed in LD.

4) Ca^{2+} excretion rate in LM during LD strongly decrease. This is a typical LM effects attenuating the action of LD.

The relation between the intensity of gravity and magnetic field from the physiological point of view, under such limitation of the biologically favorable range of the intensities of magnetic forces, seems to be worth to investigate.

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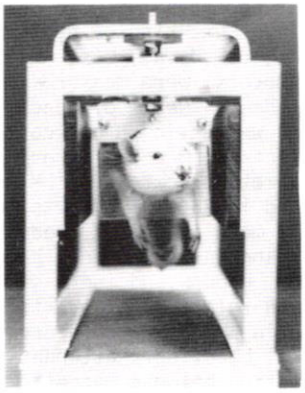

Fig.1. Animal suspended in the case.

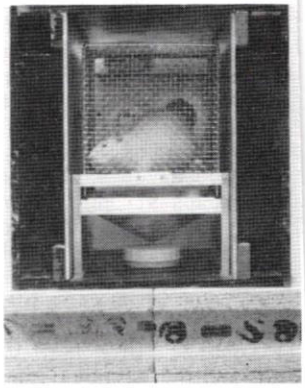

Fig.2. Vivarium animal placed in HM.

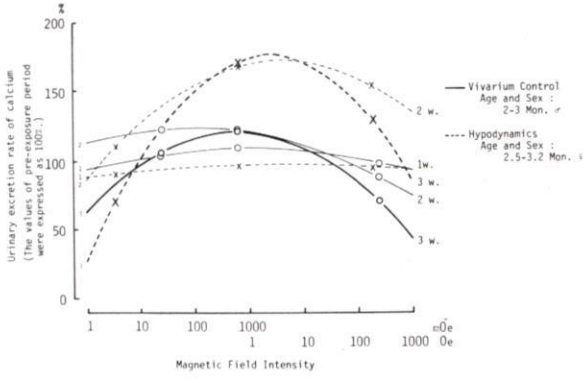

Fig.3. Relation between magnetic field intensity and urinary excretion rate of calcium of the vivarium control rats and hypodynamics exposure rats.

SOME PHYSIOLOGICAL CONSEQUENCES OF G-INDUCED BODY FLUID SHIFTS AND MUSCLE LOAD REDUCTION

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Actual or simulated microgravity produce cephalic congestion, changes in the low pressure system, and extra/intracellular electrolyte shifts. Taking this into account it is possible to overcome the major drawbacks of the fluid shift theory to explain some physiological consequences of adaptation to microgravity. Based on the results of 0-g simulations and those obtained during space flight we have to consider the following mechanisms: 1. The cephalic fluid shift influences central neuronal processes. We observed changes of cerebral reflex loops as well as an increase of the parasympathetic tone. 2. Reduction of left ventricular dimensions has been observed during 0-g simulations and after space flights. During the remobilization phase of 0-g simulations we always observed an increase of left ventricular dimensions above control, provided that total blood volume had been reestablished. This indicates cardiac deconditioning. 3. Besides the muscular disuse the intracellular fluid and electrolyte shift offers an additional explanation of the imbalance between blood glucose and insulin. 4. As a result of sustained reduced loading of the vascular walls in o-g textural changes in the vascular walls are likely to occur.

Adaptation to microgravity is a complex process involving multiple organ systems (4). Parallels can be drawn from simulation models of weightlessness, for example immersion or bedrest, both in horizontal or head down tilt position. The validity of the model, however, must still be fully exploited. Some of the physiological events develope rapidly, others more slowly, and there is some concern that continuing processes may result in irreversible pathophysiological changes.
When we consider a schematic diagram of the fluid shift - fluid shift induced through weightlessness or through head down tilt (HDT) simulation - we see that marked changes are to be anticipated in the cephalic area and in the lower body area (Fig. 1). Since also intrathoracal changes were observed, the heart and the lung cannot remain unaffected by the adaptation process considering the changed conditions in terms of volume shift and pressure.

Hence in the following, physiological observations will be presented from 3 areas: 1. from the cephalic area, reflex changes and changes of the autonomic nervous system; 2. changes concerning the heart itself as the organ responsible for propelling unoxygenated blood to the lungs and delivering oxygenated blood to the peripheral tissues in accordance with their metabolic requirements, and 3. changes in metabolism perfusion, elasticity of the lower extremities as example for the circulatory periphery.

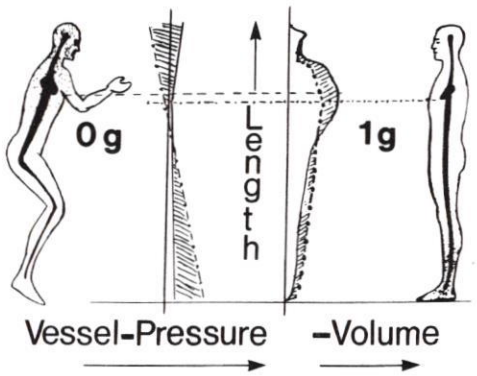

Figure 1. Schema of g-induced fluid shift.

As consequence of changes in the low pressure system during microgravity and micro-g simulation, there occurs blood rush to the head with congestion of nose and ears, feeling of heaviness of the head and sensation of pressure in the eyeballs.
The visible venous stasis of the external head veins indicates a similar stasis of the intracranial veins. An increase of pressure in the venae jugularis leads to a venous stasis, which expands into the capillary system. This cephalic redistribution of fluids may disturb the neural transmission and/or integration of the signal generated by the peripheral sensors or by the cortex itself.

During simulation we have studied the nystagmus reflex loop. All caloric tests were performed in 6 HDT including the control ones. This measurement allows to examine the neural transmission and the integration of signals in the area of this reflex loop.
The most remarkable changes in the nystagmus parameters during the 7 days period of HDT were found in the maximum slow phase velocity (Fig. 2). Compared with the control values there was a significant response decline on the first day similar to those observed during first Spacelab payload (FSLP). From the 3rd day on, we observed an increase which became significant on the last HDT day, sometimes with an overshoot above control. In the recovery phase values approached control levels.

Without sensory conflict, the changes in vestibular function, found in this study, may be considered as a consequence of a disturbed circulation on the cerebral structure. Possible explanations are:
- The pressure changes in the receptor itself. The venous flow could be responsible for changes of the inner ear function. It is well possible that venous stasis results in an increased endolymphatic pressure which then could be responsible for the feeling of pressure in the ear.
- A slight cerebral edema, which possibly develops, may affect the vestibular-ocular and vestibular-spinal reflex arc.
- The third possibility could be the assumption of alterations in the autonomic nervous system, eventually caused by regulation mechanisms evoked through fluctuation of the intracranial pressure.

In the vestibular and cochlear parts of the inner ear, too, afference and efference exist. The origin of the efference, coming from central, are probably autonomic (1).

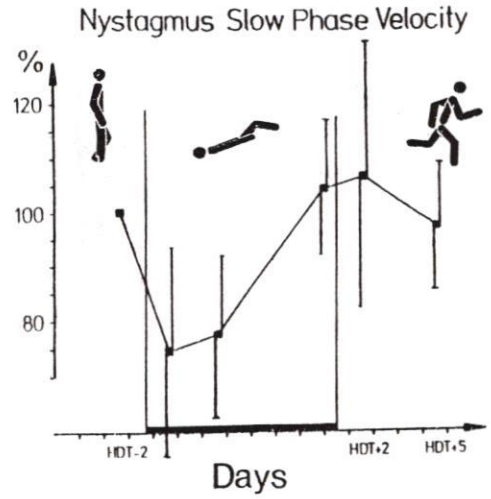

Figure 2. Time course (mean+sd) of nystagmus slow phase velocity changes during microgravity simulation (n=6).

In parallel the respiratory/circulatory centre is influenced by the autonomic nervous system, too. When passing from 1 g to weightlessness, the autonomic nervous system is most probably shifted to a different level of activity.

The observation of the heart rate, monitored over 24 hour periods, shows significant decrease during the first days of simulated weightlessness (Fig. 3, top pannel). This is particularly obvious when the minimum of heart rate of each day is considered, which is found between 0100 and 0400 a.m. At this time all influences from daily activities are largely eliminated and it can be seen that the heart rate stays at this reduced level over a period of more than 2 or 3 days.
In Fig. 3 the mean resting heart rate section is shown together with the excretion rates of adrenaline, noradrenaline and 17-hydroxycorticosteriods.
The heart rate decreases at the beginning of HDT and increases distinctly at the end of our simulation of weightlessness.
17-hydroxycorticosteroids show a similar response as the heart rate: Increase by 25% of control values in the first 3 days of HDT, normalization during the last 3 days of bedrest, a rapid decrease of 25% after HDT, and shift back to normal values towards the end of the experiment. There was a trend that noradrenaline shifts to values 25% higher than normal during the first 4 days of HDT, however, the difference was not significant.
With the beginning of HDT period the concentration rates of adrenaline decreased over 3 days to 50% of control values; they returned to normal immediately at the first day after HDT.
Together with the decrease of heart rate, this indicates a shift to a more parasympathetic tone during the first 2-3 days of adaptation, since the unique difference between the first HDT night and the previous ones is the additional fluid shift.

This time course of heart rate changes during the micro-g simulation period allows us to compare the heart size at identical heart rate, that is the heart rate of the controls.

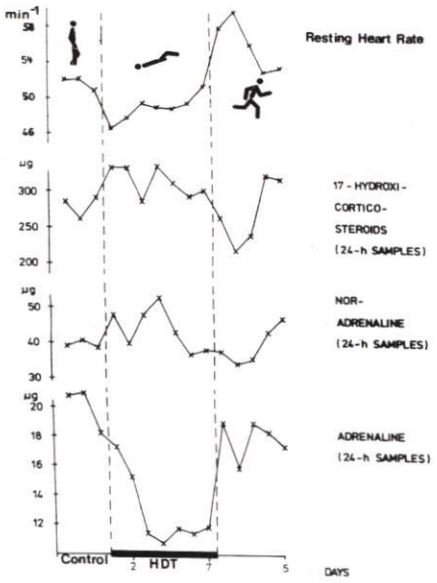

Figure 3. Time course of heart rate and hormone excretion rates changes during a 0-g simulation.

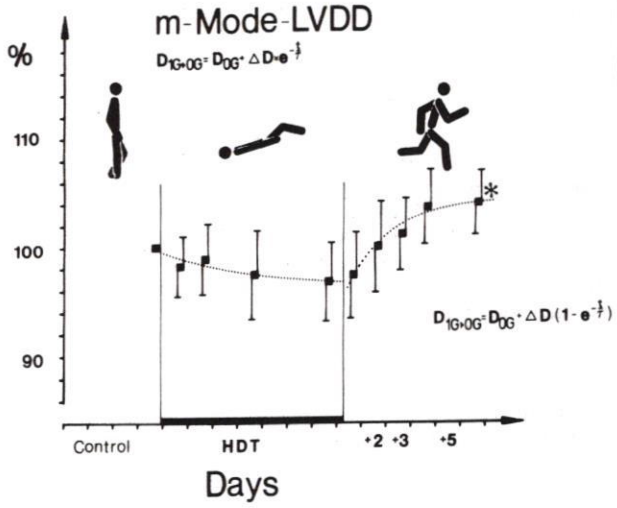

Figure 4. Left ventricular diastolic diameter changes during a 6 HDT 0-g simulation (n=12).

If this is done, all left end-diastolic diameters and calculated left ventricular volumes show a reduction during HDT and an increase to higher than control values during recovery (Fig. 4). For the assessment of standard diameter, the distance between septum and posterior wall was used. The fitted curves demonstrate an exponential decrease of the left ventricular standard diameter which often becomes more pronounced with progressing micro-g simulation time. After termination of HDT position the reverse is true: The diameter increased also, exponen ially, but with a higher speed.
Observations of the heart contractility under micro-g conditions, sofar, have been based on the assumption that there is no change in cardiac performance (6).
From the clinical point of view, an impaired heart function cannot be excluded, when heart dimensions increase.
A preliminary conclusion from these observations is that there, indeed, has been a reduction of cardiac

contractility during the simulation. For the increased cardiac output requirement during recovery, the heart is making use of the Frank Starling mechanism and, in order to compensate for the loss of contractility, it is performing with a higher preload. The higher preload is reflected by the increase in volume of the left ventricle which was observed in all the simulation studies even if the blood volume reached control levels again.
To confirm this conclusion it would be important to get more information about heart contractility during and after weightlessness exposure.
For the dynamic testing of the heart we use the lower body negative pressure (LBNP)-device. The objective of this test is to produce ventricular function curves (VFC) indicating changes in contractility and in ventricular performance. The shift of the VFC indicated a diminished contractility at the end of the microgravity simulation period. After five days of recovery control values were not reached yet (2, 3).

But we used LBNP also in the classic way to measure orthostatic tolerance time. For orthostatic tolerance testing we used a stepwise LBNP protocol before and after micro-g simulation. The orthostatic tolerance was reduced by 47%. No significant differences between trained or untrained test subjects could be observed (see Table 1).
The physical performance capacity was studied before and after our simulation of weightlessness (8). From this point of view 7 of 17 test subjects were highly endurance trained athletes, with an average oxygen uptake of 69 ml/min/kg bodyweight and the other 10 on average had a maximum oxygen capacity lower than 40 ml/min/kg bodyweight.
Before bedrest the 4 minimol/liter level of arterial lactate acid concentrations was reached at a mean workload of 230 W. This workload had decreased by about 8% after our simulation of weightlessness. Maximum oxygen uptake did not change significantly (Table 1).

	ORTHOSTATIC TOLERANCE TIME MIN	WORKLOAD at 4mmol/l LACT. W	MAX. OXYGEN UPTAKE ml/min/kg BODYWEIGHT	INSULIN RESPONSE 60 min after GLUCOSE INTAKE µU/ml
CONTROL	19 ± 2.7	231 ± 33	56 ± 8.3	48 ± 18
7 DAYS 6° HDT	10 ± 3.5	212 ± 28	55 ± 15.6	120 ± 15
CHANGE	-47 %	- 8.2 %	ns	+150 %

Table 1

This relatively small amount of reduction does not correspond to the subjective findings from trained athletes that they need more than 2 weeks to overcome the difficulties after our bedrest simulation.

We also observed inappropriately high insulin responses to standardized glucose loads during simulation. The changes occur already after two days and are more enhanced with increasing duration. Obviously insulin becomes relatively ineffective in lowering blood glucose, otherwise glucose levels would be consistently lower than under control conditions, but there is a small increase in blood glucose. The excessive insulin responses to glucose loads (OGTT) during bedrest are caused by higher secretion rates from the B-cells of the pancreas, since C-peptide concentrations in plasma exhibit similar patterns of enhancement in response to the glucose tolerance tests.
There can be no doubt that also responses to glucose tolerance tests differ in dependency on the physical training status of the subjects. Physical training and physical activity must be considered as a factor that significantly reduces insulin responses to glucose loads and consistently is effective in lowering insulin elevations in blood plasma (9). It is obvious that these findings are of relevance for space flights. Hyperinsulinism in response to provocative glucose loads can be predicted: either by direct effects of microgravity on the fluid and semifluid compartments of the body, or by influences of the reduction in physical loads causing muscular disuse and atrophy.

Last but not least, the lower extremities play an important role with respect to orthostatic tolerance. The wall tension of the lower extremities have to withstand the hydrostatic pressure, generated by the gravity field. In weightlessness there is a probability of disuse athrophy of the vessel musculature and the surrounding supporting tissue. For measuring elasticity we have to deal with the ratio: pressure variation/leg volume variation.
In our laboratory we developed a new technique for measuring leg volume by means of ultrasound device. Computations of leg volume are made in regions with high muscle mass, and in the ankle region where muscle is practically absent.

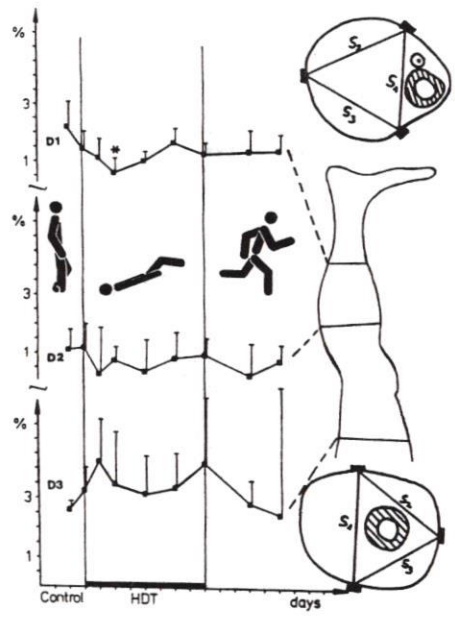

Figure 5. Ultrasound plethysmographic measurements of leg volume during microgravity simulation. Triangle areas at ankle (D1), at calf (D2), and at thigh (D3) level.

Fig. 5 shows the time course of the leg volume response during the change of LBNP box pressure from -20 to -40 mmHg. The higher the position on the leg, the greater the response to the box pressure. No significant differences between trained and untrained subjects could be observed. Only in position D1, where the striated muscles are nearly absent, we found a significant increase of rigidity

during the first 2 days of the HDT period. The comparison with the blood volume demonstrates that plasma volume loss goes in parallel to this increase of rigidity (5).
The unstressed lower extremities loose interstitial fluid during simulation of weightlessness and as a consequence the rigidity increases.

Blood volume, calculated from hemoglobin concentration and hematocrit, decrease by 14% during the simulation period. Fluid shift evokes, also, an electrolyte shift between the extra- and intracellular space. Potassium concentration in leucocytes increases during the total period of 0-g simulation, conti uously. The increase lasts into the recovery period in sofar as on the 5th day in ambulatory period no trend for normalization could be observed (Fig. 6). Since the leucocyte is representative of other tissues, similar changes in other cell populations must be considered (7).

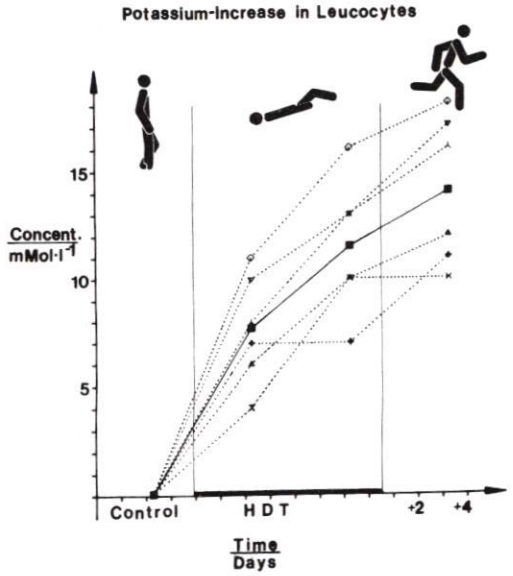

Figure 6.

We found, at least, two long lasting changes after 7 days of HDT:
1. The extra/intracellular electrolyte shift and
2. the increased insulin response to OGTT.

This indicates that metabolic changes last considerably longer than most of the cardiovascular changes. Ventricular function changes may be an exception. These changes could be seen as a support for the subjective symptoms, reported by athletes.

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THE EFFECT OF MICROGRAVITY ON THE PRENATAL DEVELOPMENT OF MAMMALS.

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The experiment designed to study the effect of microgravity on the prenatal development of mammals was for the first time performed on the Soviet biosatellite Cosmos-1514. In preparing the experiment, we assumed that the effect of microgravity on the developing fetus would be mediated via the maternal body, depending on its metabolic parameters and hormonal status.

Previous studies of rats flown on Cosmos biosatellites revealed changes in the adult animals that might produce an adverse effect on the fetus. They include osteoporosis, involution of lymph organs, inhibition of erythropoiesis, etc. (1).

Fetal development requires a significant activation of anabolic processes. To what extent is it possible in microgravity against the background of increased catabolism in an adult animals. What will be the pattern of organogenesis, particularly bone development, against the background of calcium deficiency in the maternal body—these and many other questions that remained obscure formed the program of the rat embryology experiment on Cosmos-1514. We viewed the pregnancy state as a specific provocative test, as an experimental model that can be used to examine the reserve capabilities of the animal body in microgravity.

We used virgin adult female rats of the Wistar strain borrowed from the Stolbovaya (USSR) nursery. We designed the experiment taking into consideration the most vulnerable stages of the prenatal development (2); in view of the fact that the purpose of the first flight experiment was to demonstrate the possibility of fetal development in microgravity as such, we concentrated on a very stable stage: the rats were fertilized preflight and exposed to microgravity from gestation day 13 through gestation day 18.

Throughout the flight ten rats were kept as a group in a BIOS-Vivarium cage of 66 x 22 x 16 cm equipped with automatic feeders, water bowls, ventilation and waste management systems, and lamps. In order to discriminate between the effects of microgravity and other space flight factors, we employed several control groups, viz. vivarium control, synchronous control in a biosatellite mockup (3), preflight control sacrificed at the launch day. All the animals were fed with a paste diet specially prepared for biosatellite studies (4).

Exposure to microgravity for 5 days during the last term of gestation produced a distinctly adverse effect on the mothers. During the flight - gestation days 13 through 18 - the flight dames gained only 5 g each, while the synchronous rats gained 65 g which is a normal weight gain of rats at this term of gestation. Food consumption by flight and synchronous rats was essentially identical: on the average 54 and 57 g per rat per day.

Hemoglobin concentration in the flight females was significantly lower than in the vivarium and synchronous rats: 9.8 g% versus 12.8 and 13.4 g%, respectively.

The flight rats showed a decreased thymus weight: 238 mg versus 338 mg in the vivarium rats and 273 mg in the synchronous controls ($p < 0.05$). Similar variations were seen in the total count of thymocytes: 735 mln versus 1149 mln in the vivarium controls and 1044 mln in synchronous controls.

The flight rats showed a slight increase of the adrenal weight to 92.8 mg versus 82.3 mg in the vivarium and 79.8 mg in the synchronous controls ($p>0.05$).

Mention should be made of a significant decrease of the liver weight in the flight rats to 10.3 g versus 13.3 g in the vivarium and 14.6 g in the synchronous controls.

In spite of the fact that the flight females showed a far smaller weight gain and other adverse changes, their reproductive function remained essentially unchanged. The preimplantation and total embryonic mortality rate of the flight, vivarium and synchronous females sacrificed on gestation day 18 was very close. The average number of alive fetuses per mother was 13 in the flight group versus 13 in the synchronous and 12 in the vivarium controls. There were no stillborns in either group. The number of fetuses and placentas with hemorrhages was increased both in the flight and synchronous groups, probably, due to the effect of impact acceleration.

The average fetus weight was 0.84 g in the flight group versus 0.92 g in the vivarium and 0.94 g in the synchronous controls ($p<0.05$); the water content of the flight fetuses was significantly higher than that of the controls. This can be regarded as a sign of delayed development because as the fetus grows, its water content progressively declines. The concentrations of DNA, RNA and protein per g of the wet tissue of the flight and control fetuses were identical.

The flight animals showed a reduction of the placenta size and weight. Histological examinations demonstrated that the changes involved the labyrinthine compartment of the placenta, i.e., the site of the most active mother-fetus exchange, and that the thickness of the spongy layer did not change. In all groups the level of placenta hydration was identical.

Skeleton morphometry of the flight fetuses demonstrated delayed development that involved 5-20% reduction of the ossified areas of virtually every bone as compared to the controls; it is interesting to note that the most drastic changes were seen in the least mature segments of the skeleton.

The results of this portion of the experiment demonstrated that the mammalian fetus can grow and develop, if mothers were exposed to the effects of microgravity during the last term of gestation. The flight fetuses showed no overt anomalies, except hemorrhages that were also seen in the synchronous animals and were evidently induced by impact acceleration. The flight fetuses lagged behind the controls with respect to the body weight, ossification level, and water content; these differences were, however, small. It is interesting to note that on gestation day 18 the total weight of the fetuses of each mother was very similar in the flight and vivarium groups, i.e., 11.40 and 11.47 g , respectively. In other words, in spite of significant weight losses, the flight rats were capable to stimulate anabolic processes associated with fetal growth and development to the same extent as the controls.

Five pregnant females of the flight group were allowed to survive until natural delivery. It should be noted that beginning with the first postflight day they gained weight rapidly: if throughout the flight the weight gain was 5 g versus 65 g in the controls, during the first 24 h after recovery the weight gain was 35 g and during several days prior to the birth it was 60 g, i.e., it was very close to the weight gain in the controls within the same time period.

Out of the five females four gave birth to alive pups, each to 12 pups, on the average, versus 12 pups in the synchronous and 10 pups in the vivarium controls. Mention should be made of more prolonged labor of the flight rats. This may be associated with a decline of their general resistance and muscle changes. The weight of the newborns in the flight group was 5.9 g, being lower that in the vivarium controls (6.4 g) and higher than

n the synchronous controls. The appearance of the flight newborns corresponded to their calendar age. Visual examinations of the newborns and studies of their viscera according to Wilson (5) revealed no abnormalities. The newborns of the flight and synchronous groups showed no hemorrhages seen previously in the fetuses. This suggests that the hemorrhages were small and the tissue changes they induced were reversible.

Morphometric measurements of the skeleton of the flight newborns demonstrated a 10-17% increase of the ossified areas as compared to the controls, whereas the flight fetuses showed a delayed skeleton development. Consequently, during readaptation the growing fetuses not only caught up with the controls but even surpassed them.

It is important to note that the flight newborns had a significantly reduced weight of the liver ($p<0.001$) and an unchanged weight of the thymus, spleen, kidneys, and the heart. Their hemoglobin concentration was lower than in the vivarium and synchronous controls: 10.6 g% versus 11.4 and 11.7 g%, respectively.

One of the flight mothers delivered stillborns. This can be explained by hard labor and a very large fetus in the litter. All the newborns were of normal weight and had no visible anomalies, except for the large one. It showed a fresh hematoma on the head which could develop during labor. Microanatomical examination revealed forebrain hydrocephalus.

The most distinct differences between the flight and control groups were detected during the first week of their postnatal development. They included a high percentage of the mortality rate of the flight animals (up to 19%) as compared to the vivarium (0%) and synchronous (2.5%) controls. There were appreciable individual differences between flight females: cases of postnatal death were seen only in two of four animals, while in controls all the litters were essentially identical. Bearing in mind the fact that the organogenesis of the flight pup proceeded in part in the microgravity state, we were afraid that the differences between the groups would increase as the pups would grow and develop and as the working load on the organs that developed inflight would enhance. Fortunately, we were wrong and the death of the newborns occurred only within the first week after birth.

During the first 20 days of their postnatal life the pups were weighed every morning; their weight remained 1 - 5 g lower than in vivarium controls but higher than in the synchronous controls. This permits us to attribute the lower weight gain of the flight pups to the effect of other than microgravity flight factors. In spite of the delayed growth, the flight pups with respect to other parameters developed in parallel and sometimes ahead of the controls. For instance, the hair cover developed within identical time intervals in the flight and vivarium pups, while the eye opening occurred on days 13-15 in the flight pups versus days 14-16 in the controls.

Postmorten examinations of the flight pups, i.e., the pups whose prenatal development occurred in part in microgravity, did not reveal any differences in the absolute and relative weights of the thymus, spleen, adrenals, liver, kidneys, when sacrificed on days 15, 30, or 100 of their postnatal life. On day 30 the flight males showed a significant increase of the epididymis weight: 121 mg versus 83 - 86 mg in the controls. The difference was not observed on day 100; this can be interpreted as an accelerated development of the epididymis in the time period that preceded puberty.

The reproductive function of the males and females that developed in microgravity was evaluated when they reached 3 months: they were mated with one another and with intact animals according to the scheme that did not allow intrafamilial contacts. Vivarium and synchronous rats were exposed to a similar test. There were no differences with respect to the

total number of alive and dead newborns, weight at birth and during the first months of life, time of eye opening and time of ear detachment. Nevertheless, the postnatal mortality rate in the group obtained from flight males and females was as high as 12% and in the other groups it was very low, if any. Analysis of the data showed that the increased mortality rate was associated only with the offspring of the two flight rats who themselves delivered newborns which died soon after flight. In other words, this abnormality occurred both in the mothers and in the offspring and needs further study. It can hardly be incidental because the high postnatal mortality rate was found only in one group out of the three, i.e., the flight group (during postflight examinations) and only in one group out of the six, i.e., in the pups obtained from mating of the males and females that developed in microgravity (during reproductive examinations of F_1 generation). Most pups who died after birth were produced by the females from the families which had a similar history.

The results obtained give evidence that the mammals exposed to microgravity have retained their ability to activate adaptive processes and to mobilize reserves in response to pregnancy. In spite of serious changes in the maternal body, the fetuses that were generated on the ground continued to grow and develop in microgravity slightly lagging behind the controls. After recovery they completed their development: they were born, they reached sexual maturity and, in turn, produced their own offspring.

It should be emphasized that characteristics of the pups who grew in microgravity and on the ground were different. Some of them are transient, occur at individual stages of development and produce no effect on the viability of animals. Conversely, the fact of an increased postnatal mortality rate seems to be important.

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HE EFFECT OF WEIGHTLESSNESS ON MOTOR AND VESTIBULO-MOTOR REACTIONS

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Deterioration of the voluntary movements control is a consistent consequence of the lowered gravitational load. Physiological measurements that were performed in real or simulated weightlessness indicate a longer time taken to perform motor acts, a lower accuracy of voluntary muscle control, a large number of errors associated with voluntary movements (1,2,3). The data obtained suggest that the gravitational unloading acts as a trigger initiating significant changes in the motor control system. It is traditionally assumed that muscle atrophies play a major role in the development of coordinational and motor disorders in weightlessness. However, the status of the motor control system changes drastically at an early stage of orbital flight when atrophic processes are in incipiency. At this stage spinal synergy and central integration processes are disturbed. It is beyond doubt that one of important factors responsible for disorders in the integrated activity of the motor control system is disturbance of the sensory function due to changes in the vestibular, proprioceptive, and interoceptive afferent inputs.

These observations give a strong impetus to a careful study of sensory interactions in the systems that ensure integrated reactions and mechanisms of its adaptation to real and simulated weightlessness.

As it was shown earlier the elimination of support loads due to 7-day exposure to both weightlessness and immersion caused a rapid development of gravitational musculature hypotension and a decrease of the thresholds of sole vibration sensitivity, i.e., hyperreactivity of the support input (2). Modifications of the afferent flow towards the motor centers (functional deafferentation) in turn gave rise to increased reflex excitability of gravitational muscles motoneuronal pools and to disorders in the function of interlimb interactions. Alterations in the afferent flow, new conditions for reflex realization, decrease of the tone and strength-velocity abilities of the gravitational muscles in combination resulted in a drastic decline of accuracy of the motor control systems (3). At the same time both exposures - to weightlessness and immersion led to an increased excitability of the vestibular input that became apparent by an increase of the vestibulo-ocular reflex gain and a decrease of precision of gaze fixation control. Comparison of the changes induced by gravitational unloading in the vestibulo-oculomotor interaction and in the musculo-skeletal system brings us to two distinguishable processes: hypersensitivity and ataxia.

It should be borne in mind that all the facts that allowed this conclusion were obtained in the examinations carried out at different time intervals after the above exposures. However it is obvious that symptoms observed on different postflight days may reflect the transient processes inherent in adaptation to 1 g rather than flight traces. This is a fundamental problem that has been clarified by the primate experiment on Cosmos-1514.

During the 5-day space flight one of the two primates performed specific motor acts which he was trained to do in order to get juice as a reward. In the course of selected time intervals parameters of gaze fixation reaction and precise lever pressing were measured. The flight data were compared with the results obtained 24 (for the former) and 2 h (for the latter) prelaunch.

Gaze Fixation Changes during 5-day Flight

The gaze fixation reaction on a visual object that appears unexpectedly in the peripheral field of vision has been examined in details in primates (4) and man (5). It was shown that in both species the speed and precision of this biologically important reaction are ensured by an automatic and highly coordinated interaction of its three constituents: saccade towards the target, head movement in the same direction, and compensatory eye movement. This coordination that leads to the formation of a functional complex consisting of independent programs of rapid eye and head movements and compensatory eye movement, works through a feedback look that involves proprioceptive, optic and, primarily, vestibular afferentation. Thus analyzing kinematic parameters of the reaction we could evaluate the excitability of the canal part of vestibular apparatus and particular features of vestibular signals utilization. During each session the primate performed all 256 conditioned-reflex complexes with no errors. The major portion of the complex was a rapid gaze fixation on the targets that were randomly presented at a distance of 40 degrees to the right and left from the center. In the study kinematic parameters of eye and head movements were recorded.

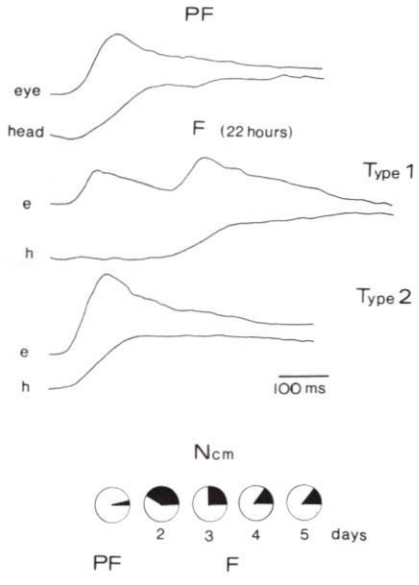

Figure 1. Fragments of experimental records, obtained preflight (PF) and in-flight (F). Type I - fragment of gaze fixation with corrective saccade. Below - number of corrected movements.

In orbital flight the amplitude and velocity parameters of gaze fixation changed significantly while time parameters varied but slightly. During the first flight day (22 h after launch) the saccadic amplitudes and velocities increased markedly (Fig. 2). The high amplitudes and velocities of the saccadic movements were recorded at later flight stages. Simultaneously the velocities of eye compensatory movements increased significantly (Fig. 2). These movements provide in normal conditions fixation the gaze in the course of the head turn since catching the target.

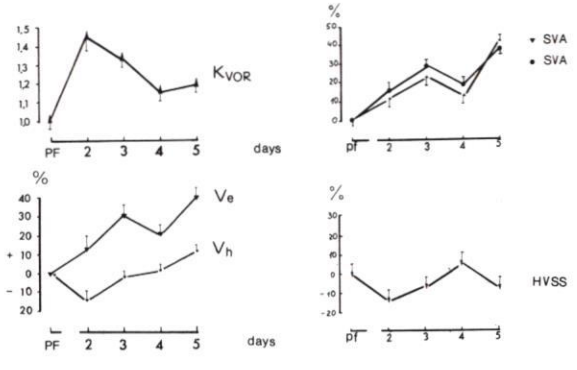

Figure 2. Changes in the characteristics of the gaze fixation reaction in the primate during "Cosmos-1514" flight. On the left-coefficient of gain of the vestibulo-ocular reflex (K_{VOR}) and maximum velocity of eye counterrolling (V_b) and head movement (Hd); on the right - amplitude and maximum velocity of the saccadic movements (above) and head velocity that supresses saccade (below).

The excessive inadequate velocities of the compensatory eye counterrolling that resulted from the excessive vestibular signals about head movements brought about disorders in gaze fixation, led to the target loss and generation of a large number of adjusting saccadic movements (Fig. 2). They occurred only in 4% of gaze fixation reactions 2 h prelaunch and in 30-40% 22 h after launch.

The coefficient of gain of the vestibulo-ocular reflex (the ratio of the maximum velocities of eye counterrolling and head turn) is normally equal to unity. At 1 g these velocities are identical, thus providing gaze fixation during head movements. At 0-g the maximum velocity of eye counterrolling was significantly greater than that of head turn. As a result, the gain coefficient of the vestibulo-ocular reflex increased to 1.4 on mission day 2, slightly decreased later on, but always remained higher than the normal value. This is indicative of enhanced vestibular excitability in the weightless state.

The increased vestibular excitation and excessive canal signals were also evidenced by changes in other parameters of the gaze fixation reaction and modifications of the motor behavior on the whole. In flight the thresholds of saccadic inhibition that were determined by the minimum velocity of head movement which provides inhibition and arrest of the saccadic movement were decreased (Fig. 2). During the first mission days the amount of head movements was also lower. The maximum amplitude of voluntary head movements that are performed spontaneously, in addition to gaze fixation, also decreased.

The changes in the gaze fixation parameters in flight suggest two major pathways that can compensate for gaze fixation disorders under the conditions when the vestibular signals are no longer adequate. These pathways can be termed behavioral and neurophysiological. The behavioral pathway is the first to be initiated and is controlled by the mechanisms of voluntary movement control. This pathway leads to a decrease of the head movements velocities and to an increase of their amplitudes. It is obvious that the interaction of gaze fixation components is markedly facilitated. The pathway of neurophysiological compensation develops in parallel but with a certain time delay. This pathway brings about inhibition of vestibulo-oculomotor transmission: throughout the flight the gain of the vestibulo-ocular reflex decreased to 1.1-1.2.

Having in view the identity of mechanisms involved in gaze fixation in humans and nonhuman primates (4,5), the data on the vestibular excitability of obtained with monkeys in weightlessness can be easily extrapolated to man. These findings contribute to our knowledge of the vestibular system derived from postflight examinations of cosmonauts.

)luntary Movement Changes during 5-day ,ight

Motor tests were performed at different stages of study and every day in-light. During the tests the primate made tereotyped conditioned-reflex movements f the foot: he pushed the stick whose gonist is the triceps muscle. Electro-yograms of muscles of the working leg nd mechanograms of the actograph were ecorded.

In preflight control studies the leg EMG recorded during a series of physical novements was a sequence of bursts of agonist and antagonist activities (Fig. 3). In the course of the plantar flexion of the ankle, i.e., against the action of the actograph spring, the triceps muscle of the calf worked and the other muscle remained inactive. Dorsal flexion involved bursts of the activity of the anterior tibial muscle.

When performing the motor program in flight, the primate sometimes did not respond to conditioned signals and did not do what he was supposed to do. As the flight continued, the animal refused to do the job more frequently, and the number of faultless acts that were rewarded with juice diminished.

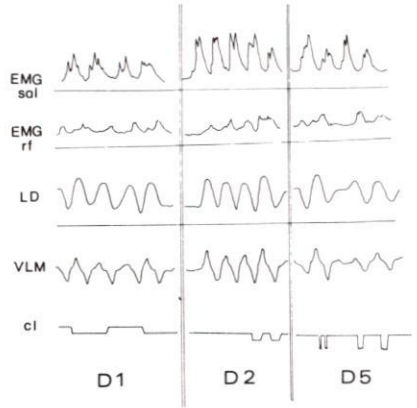

Figure 3. Fragments of experimental records, obtained on different days of flight. There are seen: 2 integrated EMG (above), velocity and amplitude of movements.

Analysis of recordings made on different flight days (Fig. 4) showed distinct changes in the pattern of movements, their myographic and kinematic parameters. At L + 4 h the pattern of movements remained unchanged although their velocities, especially in plantar flexion, were far lower (by 30-50%) than in the control. The amplitude of EMG of the calf rear muscles did not change, but the movement amplitudes decreased slightly (Fig. 4).

On mission days 2-4 the movement velocities increased again, even exceeding the preflight level. The EMG amplitude of the rear calf muscles that predominate in the motor act increased drastically, exceeding several time the preflight level.

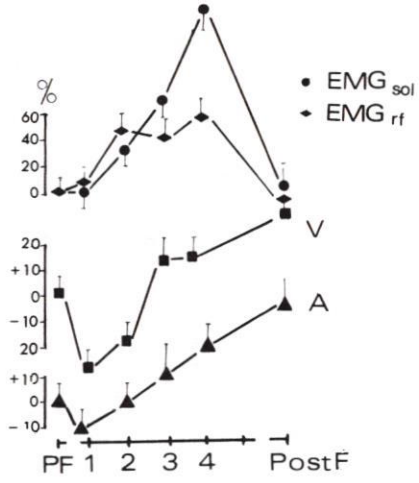

Figure 4. Changes of the characteristics of conditioned-reflex movements of monkey in flight shown in percentage to the preflight level.

The activity of femoral muscles that are not directly involved in the motor act also increased by about 20%. The muscle activity was grouped in distinct spikes. The movement amplitudes increased gradually.

On mission day 4 the electromyographic activity of the rear muscles of the calf remained unchanged and the movement amplitude of the ankle increased significantly. The amplitude, velocity and duration of sequential movements were no longer stereotyped and varied from one motor act to another. The pattern of the femoral muscle function changed drastically. They showed a high tonic activity, the EMG amplitude grew by over 50%, and the activity was no longer synchronized in bursts.

It should be emphasized that on those flight days when the primate performed the motor program with no errors, the latent times of responses to conditioned signals remained short. The movements were performed in a rhythmic manner. No intersignal reactions occurred. All this indicates that the primate developed adequate responses to the situation, and that the above changes were associated with disorders in the motor function and muscle periphery during the first days and central mechanism at later stages.

All these changes were functional and disappeared to a large extent upon return to the Earth.

The data accumulated in the flight study helped to distinguish three types of motor disorders occurring at different time intervals. The first-type disorders seen within several hours after launch reflected a drastic decrease of muscle efforts, particularly in muscles of the calf rear group, with slight changes of the amplitude of electromyographic activity. The second-type disorders develop-

ing on the second flight day were associated with rearrangement of the muscle response: EMG amplitude and duration increased significantly and kinematic parameters of the motor response return to norm. At later flight stages changes of the third type develop which can be characterized as atactic.

Comparison of the flight data with the results of man hypokinesia studies suggests that the changes that are the first to be discerned in flight are caused by a decline of the muscle tone developing within the first hours of orbital flight. In this case the second-type changes seem to reflect the processes meant to compensate for the deficiency of muscle efforts due to involvement of a large number of myofibers in the contraction function. The third-type changes that occur at a later stage and develop gradually are brought about by central processes; they can be related to a poorer functioning of central program mechanisms due to disturbances in the proprioceptive afferent inflow.

This suggestion was supported by the results of H-reflex testing, that was performed postflight on day 2 and revealed a marked increase in spinal mechanisms excitability. As it is demonstrated by Fig. 5, H-reflex of a large amplitude (more than mv) was evoked easily by a stimulation of a comparable

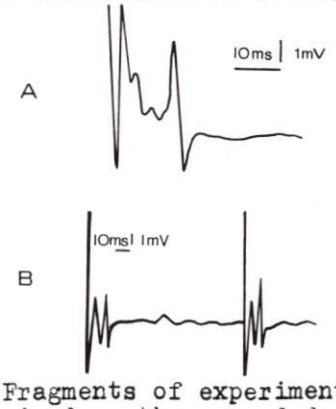

Figure 5. Fragments of experimental records, obtained on the second day postflight. In A - a single H-reflex, in B - two H-reflexes separated by 100 ms time interval.

weak shock. When conditioning procedure was used the amplitude of the second response of a pair at less than 100 ms time-interval was not inhibited but rather enhanced. Usually H-reflex in monkey could be elicited only when conditioned by pyramidal or rubral stimulation.

Analogous changes of characteristics of voluntary and reflex motor reactions in monkeys were recorded also during and after exposure to bed-rest hypokinesia.

The results of the experiments seem to confirm the real hypogravitational nature of motor disturbances, recorded after exposures to real and simulated weightlessness, and point out to the great importance of sensory and reflex mechanisms in their pathogenesis.

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STATOCYTES AS GRAVIPERCEPTORS

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Orientation of plant parts in relation to the gravity vector is achieved by the operation of asymetric growth in the immature regions. When negatively gravitropic organs (shoots) are displaced from the vertical, growth is enhanced on the lower side with respect to gravity and/or reduced upon the upper side until the organ is returned to the preferred position. The reverse is true for positively gravitropic organs (roots). In higher plants the gravity sensing tissues possess a limited number of specialised cells (statocytes) containing sedimentible membrane-enclosed amyloplasts (statoliths). Statocytes are differentiated only in precise locations in the plant and in close positional association with certain other cell types. Statoliths normally occupy the lowest part of the statocyte with respect to the gravitational force. On re-orientation of an organ the movement and/or repositioning of statoliths is transduced to biochemical messages through changes in the electrical potential across perturbated membranes. These events set in train a local control of hormone production and cell growth that leads to the preferred gravipositioning of the organ.

Perception of Gravity

Plants precisely orientate their various parts with respect to the force of gravity. Displacement from the preferred orientation causes some movement or perturbation within the cells that is recognised and transduced into a differential growth on the two sides of the organ, so that the organ is restored to the preferred position with respect to the gravity vector.

One thing is certain, however, not all plants operate the same mechanism for achieving this.

In some lower plants perception appears not to be linked to a sedimentation of particles (statoliths) in the 1 x g field. For example, in the multicellular stipes of basidiomycetes an ultrastructural study by ourselves has failed to reveal sedimentation of particles in horizontally placed and negatively gravitropically responding stalks of the fruit bodies. Similarly, in the unicellular fruiting stalk of Phycomyces no sedimenting particle is observed although there is a negative curvature response to the gravity stimulus. Some other perturbation must therefore be involved in the sensing of gravity and the nature of this, so far, is unknown.

Most plants, however, perceive gravity through specific cells (statocytes) by a linked perception-response system involving the sedimentation of particles or organelles under the force of 1 x g. In the rhizoids of the alga Chara, sedimentable 1-2μm particles of barium sulphate crystals function as the statoliths. On placement of the rhizoids in a horizontal position the distribution of the statoliths on the plasmamembrane of the lower side of the wall is believed to block the deposition of the new cell wall material so that growth at that position is arrested. The upper side of the rhizoid continues to elongate so that the tip becomes re-orientated to the normal downward direction (Sievers A. & Schröter K. Planta 96: 339-353. 1971).

In higher plants much evidence links the presence of cells with sedimentable amyloplasts to the ability of the organ to respond to gravity by a period of asymmetric growth. Amyloplasts normally sediment within the graviperceptive statocytes within 2-30 min. They are the only organelles to show significant sedimentation before the onset of differential growth.

Positional Differentiation of Statocytes

Whereas almost all cells of aerial parts contain chloroplasts which are not sedimentable at 1 x g, cells with sedimentable amyloplasts (statocytes) are precisely positionally differentiated. Very few such cells are present in any graviperceptive tissue. Statocytes develop first as normal chloroplast-containing cells. On differentiation to a statocyte, thylakoids are attenuated and the storage starch grains enlarge from a typical 1-2μm in a chloroplast to 3-5μm of an amyloplast.

In roots, statocytes are restricted to the columella region of the root cap. In stems of dicotyledons, statocytes are normally present only in a ring of cells surrounding the vascular bundles and correspond to the endodermis or starch sheath. In monocotyledonous stems the statocytes are confined to a cap of cells adjacent to the phloem in the vascular bundles (eg. in nodal tissue in flowering stalks of grasses and in the base of coleoptiles.)

Is the presence of functional statocytes with sedimentable statoliths essential for geoperception?

(a) Roots. We know that if the root cap is removed graviperception is lost. Perception and response is not restored until a new cap is regenerated with new statocytes containing sedimentable statoliths (Juniper B.E. Ann. Rev. Pl. Physiol. 27: 385-406, 1976).

(b) Coleoptiles. The slow rates of gravicurvature of the mutant "amylomaize" can be related to the small amyloplasts (4.0μm) which sediment more slowly than those of the wild type maize containing amyloplasts of 5.2μm (Hertel R, de la Fuente R.K. and Leopold A.C. Planta 88: 204-214, 1969).

(c) Grass nodes. These do not respond to gravity when placed horizontally until the stage of development is reached at which a critical number of statocytes, containing sedimentable amyloplasts, are differentiated (Wright M. 1984 in preparation).

(d) Where functional statocytes have been depleted of their amyloplast starch by cold treatment, hormone treatment or by starvation, graviperception is either impaired or lost and is restored only on reappearance of sedimentable statoliths (Juniper, B.E. Ann. Rev. Pl. Physiol.27: 385-406, 1976).

Asymmetry in transduction

In order for a gravity displaced or sedimenting organelle to effect a differential growth on the two sides of the organ destined to

respond, some asymmetry must be built into the transducing system that converts graviperception to a biochemical response. Assuming that the gravity sensing cell of higher plants is the statocyte, then the perturbation of amyloplasts within the statocyte is the initiating event that sets in train the gravitropic response. Apart from co-ordinating its own activities a statocyte must then act in concert with neighbour cells. It can relay information to them in one of two ways:-
(a) by transmitting an electrical stimulus. This could occur when a statolith makes contact with the membrane of an adjacent organelle or with the plasmamembrane.
(b) by transmitting some specific chemical messenger. This could be released either from the statolith or the organelle with which it makes contact.

For transduction to occur the electrical stimulus or chemical messenger must be picked up by cell surface receptors of the appropriate neighbour cell and then transduced to the functional chemical signal that leads to the regulation of cell growth. The specific neighbour cell that produces the signal is therefore a "transducer" cell. The signal it emits must either activate or inhibit a biochemical (i.e. an enzyme) reaction at some rate-limiting step. We know already that the rate-limiting step in any gravitropic response is the elongation growth of cells on the upper and lower sides of the graviresponding organ.

For the graviperception-response system studied by us in the nodes of flowering stalks of grasses we propose that the rate-limiting step could be the conversion of IAA-conjugates to free IAA.

Activation of the enzyme(s) concerned with the production of free IAA can occur when the statocyte, the statoliths and the neighbouring transducer are in a specifically orientated association with respect to gravity.

Evidence in support of transducer cells and the control of IAA levels in graviperception-response in grass nodes

The cells of the nodes (the leaf sheath base) do not elongate when the intact shoot is maintained in the vertical position. However, the cells retain the capacity to elongate and will do so if (a) they are supplied with IAA or (b) placed in the horizontal position. In the latter situation only the cells of the lower side of the leaf sheath base will extend.

If excised segments of the leaf sheath base are orientated in different positions through 360° with respect to the gravity vector for 24h, only those segments which are placed in the lower side position will show the maximum elogation (c. 45%), whilst those placed in the upper side position do not grow ($\lesssim$ 3%). Excised segments of leaf sheath base maintained in the vertical position will show a small growth increment (c. 15%). (Wright M. & Osborne, D.J. Biochem. Physiol. Pflanz. 171: 479-492, 1977).

Analysis of the free IAA levels in excised segments of leaf sheath bases placed in vertical, upper or lower positions has shown that IAA levels increase within 15 min in the lower side reaching a maximum at 60 min. Levels in the upper side decline and levels in the vertical position show no significant change (Wright, M., Mousdale D.M.A. and Osborne D.J. Biochem. Physiol. Pflanz. 172: 581-596, 1977).

It is pertinent that enhancement of cell growth rate and the commencement of curvature is first discernible within 20 min of placement in the horizontal position i.e. 10 min after the increase in free IAA levels can be detected. In segments re-orientated through 180° from the lower to the upper side position, the level of free IAA falls c. 50% within 60 min. In segments re-orientated through 180°C to a lower side position IAA levels increase. Corresponding with the changes in free IAA, rates of elongation of the cells in these segments are arrested or enhanced accordingly.

Segments of leaf sheath base tissue from the nodes of grasses can therefore be "switched on" for IAA production and cell growth or "switched off" for IAA production and growth by positioning them appropriately in the gravitational field.

How is the switching system for IAA production and cell growth achieved in grass nodes?

Amyloplasts (like chloroplasts) possess negatively charged envelopes of high Ca^{++} content with an associated Mg^{++} dependant ATP-ase (Gaynor J.J. & Galston A.W. Pl. & Cell Physiol. 24: 411-421, 1983; Sack F.D., Priestley D.A. & Leopold A.C. Planta 157: 511-517, 1983). Repeated contact of amyloplast membranes with those of the statocyte plasmamembrane could initiate depolarization events and the emission of electrical or chemical signals. When this occurs on the side of the statocyte next to the neighbour transducer cell (in this tissue, a bundle sheath cell) the signal could be received and transduced into the activation of enzymes converting IAA-conjugate to free IAA. When vertical (Fig 1a) only a small proportion of the statocyte plasmamembrane can be activated to transmit a signal to the bundle sheath cell. In this position, the level of free IAA maintained is insufficient to sustain more than 15% cell elongation in an excised leaf sheath base. In the upper side position (Fig 1b) all contact of amyloplasts with the transducer cell side of the statocytes is precluded - free IAA production is reduced and growth is "turned-off". In the lower side position (Fig 1c) contact of amyloplasts with the transducer cell side of the statocytes is maximal, IAA formation is highest and cell elongation greatest (c.45%). The cap of statocytes internal to each vascular bundle may serve to amplify the signal that is emitted to the bundle sheath cells.

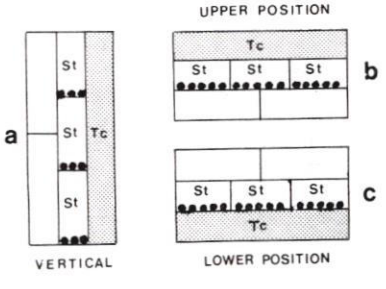

Fig.1. Positional association of statocytes (St) and transducer (Tc) cells (bundle sheath cells) in grass nodes.

Where a single ring of statocytes is formed as in dicotyledon stems, the position of the transducer cells is unknown but they could lie internally or externally to statocytes. In roots, where the statocytes lie on the root cap columella, cells external to the columella could be involved (the so-called peripheral cells) and a role for cells of the quiescent centre can also be envisaged for transducing signals from the root cap. Whereas in grass nodes, a model involving a control of free IAA levels only has been considered, growth inhibitor as well as growth promotor regulation may be involved in other graviperceptive-responding organs.

DISTRIBUTION OF EXTENSION GROWTH ALONG GRAVIREACTING ROOTS OF ZEA MAYS

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Abstract

Horizontal primary roots of Zea mays were photographed during the course of their gravireaction and during a preceding growth period in the vertical orientation. The displacement of marker particles on the root surface permitted elemental growth rates to be calculated. These show that in the temperature range 20-25°C there is a stimulation of growth along the upper side and a reduction along the lower side. At 30-35°C the stimulation is absent though the growth reduction is still present. Elemental growth rates have also been related with the development of curvature. The results are discussed in terms of the changed balance of endogenous growth regulators believed to regulate gravitropism.

Introduction

The primary root apex of seedlings of a number of plant species has been an object of gravitropism experiments for many decades. Most of these works have been concerned with gravi-perception and the physiology of the tropism (which in general is positive - the roots bend downwards). Less is known of the actual process of growth during the tropism apart from the self-evident fact that there is relatively less growth in the lower half of the root than in the upper half. This differential growth could be the result of either stimulation, reduction, or abolition of growth in various parts of the growing tip (see Table 1 in (1)). One of us (2,3) has indicated that a profile of relative elemental growth rates (REGRs) along the upper and lower surfaces of a horizontal gravireacting root would be a useful way of representing the distribution of growth, and hence visualising how the gravity-induced curvature actually comes about (see Fig. 1).

Methods for determining REGRs of vertical roots were devised about 30 years ago, notably by Erickson and Sax (4). We have adapted these methods to define profiles of REGRs along opposite sides of vertical roots and along the upper and lower sides of the same roots reoriented to the horizontal during the period when they begin to show a gravity-induced curvature.

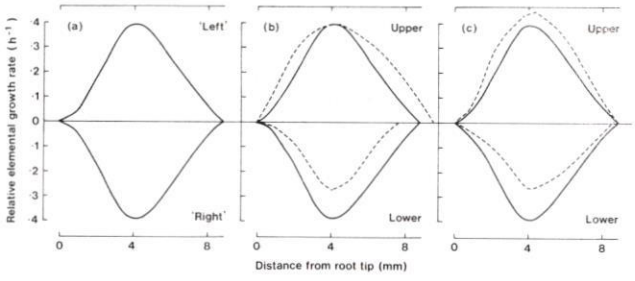

Figure 1. Distribution of REGRs along the root axis. (a) REGR along two opposite sides of a vertically-growing root (from ref. (4)). (b) and (c) Notional distributions of REGR along the upper and lower sides of a horizontally-oriented root some time during gravitropism (broken lines). In (b) the growing zone is extended on the upper side (the maximum REGR value is unchanged) and curtailed on the lower side with a reduction in the REGR. In (c) the maximum REGR value is increased on the upper side and decreased on the lower side but the length of the growing zone is unchanged. The solid lines in (b) and (c) indicate the REGR of the root in its previous vertical orientation (as in (a)).

* * * *

REGRs are most informative when growth is steady. REGR profiles obtained for a gravitropic root are obtained during a period when growth is obviously not steady; they are then really an average representation of the growth that occurs during the observation period. Nevertheless, the information they give is sufficient to reveal the major features of growth redistribution along the horizontal, bending root following a period of growth in the vertical orientation.

Material and Methods

Caryopses of Zea mays cv. LG 11 were germinated in darkness so that the primary roots emerged and grew vertically downwards. For each determination of a REGR profile, a young seedling with a straight, vertical root 1-3 cm long was selected. Carbon particles were lightly sprinkled on the surface of the root. The seedling was fixed against a pad of moist paper with the root oriented vertically (tip down) and enclosed in a Perspex container maintained at a constant temperature between either 20-25°C or 30-35°C. In each case the light intensity at the level of the root was 5 $\mu E\ m^{-2}\ s^{-1}$.

A camera fixed to a microscope was focussed on the root at right angles to its tip through the side of the container. After a 30 min 'acclimatization' period in a vertical orientation the root was photographed every 5 min for 30-40 min. The root was then turned to the horizontal and photography continued. Enlarged prints of known magnification were made of the root tip and the distances from the apex of selected carbon particles on the two opposite 'sides' of the root were measured at each time.

The first step in determining an REGR for a particular 'side' of a root is to calculate the rate of displacement away from the apex of as many carbon particles as can be conveniently distinguished. Then, the displacement rate of each particle is plotted against the position

that that particle occupied halfway through the observation period ($t_{1/2}$) and a logistic curve is fitted to these data. Slopes at points along this curve yield a smoothed REGR distribution for the growing zone at time $t_{1/2}$. REGR is expressed in mm mm^{-1} min^{-1} or % h^{-1}.

From the photographs it is also possible to estimate (i) the rate of root extension when the root is growing vertically and later when horizontal; (ii) the evolution of curvature of the root; (iii) the distance from the apex at which a convexity and concavity first appeared on the upper and lower sides, respectively; (iv) the position of the distal and proximal limits of the convexity and concavity in relation to (ii).

Results and Discussion

We analysed a number of roots growing first vertically and then horizontally. Each root showed different graviresponses, the two most marked variables being (a) the period before a bend developed (no root began to curve in less than 10 min, a few required up to 60 min before doing so), and (b) the rate of root extension. This natural variability of root growth highlights the uniformity of certain events directly associated with the bending itself when it did occur. These are as follows:

1) In the temperature range 20-25°C gravitropic bending was accompanied by an increase in the maximum value of the REGR (as compared to the maximum found in the same root when vertical) on the upper side (Table 1), and by a reduction of the REGR on the lower side. A typical result is shown in Fig. 2. Extension growth was therefore stimulated on the upper side and depressed on the lower side. The longitudinal extent of the growing zone did not seem to be affected on either side, though a differential in this variable is one way in which the tropism could occur without any change in the maximum of the REGR (see Fig. 1b).

2) When roots were grown at 30-35°C much less stimulation of growth was found on the upper side (Table 1) but a reduction still occurred on the lower side. We suggest that this contrast with the pattern of differential growth at 20-25°C is because at the higher temperature root growth rate has reached a maximum and no further stimulation is possible on the upper side. At

* * * *

Table 1. The mean maximum Relative Elemental Growth Rates of horizontal roots during the major bending period estimated at two different temperatures. The mean REGRs of the roots in their previous vertical orientation are also given.

Expt.	Temp °C	Max. REGR (mm mm^{-1} min^{-1} x 10^{-3}) Vert.	Horiz.	Horiz. as % Vert.	n
1	20-24	3.0±0.6	3.5±0.3	126±17	5
	30-35	4.5±0.2	4.3±0.3	95± 3	5
2	21-25	5.7±0.7	7.3±0.6	133±10	5
	30	8.1±1.1	8.5±1.0	105	1

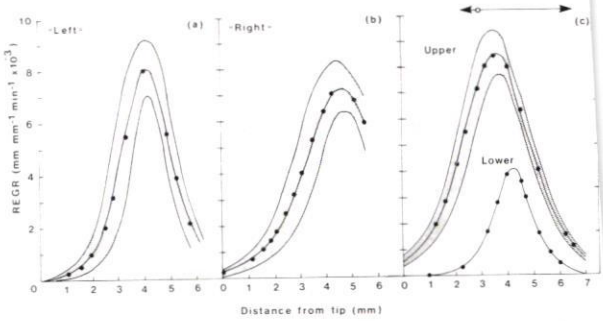

Figure 2. REGR profiles along (a) the 'left' and (b) the 'right' side of a vertical root and along (c) the upper and lower sides of the same root reoriented to the horizontal during the first 44 min of its tropic response. In this period the root curved 37°. The tips of the arrows at the top of (c) indicate the distal and proximal limits of the convexity after 44 min; the circle shows where the curvature was first seen (at 10 min). Error bands indicate SE.

* * * *

the lower temperature there is a potential for enhanced growth which can be released by a gravity-induced change in the balance of endogenous growth regulators. Enhancement of growth on the upper side could be the result of either an increase in the level of growth promoter or a decrease in the level of growth inhibitor. There is evidence that both types of regulators are present in root tips (5), so differential growth across the root axis (and perhaps along the axis too) could be the result of a change in their relative (although not necessarily their absolute) amounts.

3) The position on the upper side where the curvature first appears was always just distal to the position of the maximum REGR (eg. Fig. 2c). The curvature increases with time: during the tropism the proximal limits of the convexity (upper side) and concavity (lower side) move back along the root considerably further than the distal limits move towards the tip. The mean rate of movement of the proximal limit of the convexity was 6.9 ± 1.1 mm h^{-1} (at 21-25°C) which was faster than the rate at which any marker particle was displaced away from the tip. Clearly, differential growth propagates basipetally.

We suggest that a root becomes able to curve soon after it is placed horizontally. A curvature only becomes evident when a signal, propagated basipetally from the root apex and which is associated with a side-to-side change in the balance of endogenous growth regulators, arrives at a point where the local growth rate is sufficiently large for a differential to be manifested. This point is just distal to where the maximum REGR is located.

The nature of the signal that triggers differential growth is uncertain. The rate at which differential growth propagates basipetally exceeds the rates of basipetal transport of either abscisic acid (6) or indole acetic acid (7), two regulators which have been implicated in the control of root growth (5).

Acknowledgements

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GRAVITY AND CELL DIFFERENCIATION IN THE LENTIL ROOT

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ABSTRACT

The target cells which react to a 2 h gravitropic stimulus are located in the distal part of the meristem and in the proximal part of the cell elongation zone. The length of the parget cells of the upper and lower halves of roots which had been stimulated for 2 h was compared to that in roots which were kept vertically. It is shown that the gravitropic curvature occured in the distal part of the meristem because of greater elongation of the cells in its upper half. In contrast, in the cell elongation zone bending occured because of an inhibition of cell growth in the lower half of the horizontally stimulated roots. The ultrastructural features of the two types of gravirecting cells were analysed using semi quantitative criteria and compared to the model of differentiation of cortical cells in vertical roots. We have shown that differentiation was modified in meristematic gravireactig cells. For elongating cells involved in gravi-response, cell differentiation appeared to be slower in the lower part than in the upper part of stimulated roots. It is therefore proposed that the growth inhibitor (s) which are responsible for graviraction also inhibit (s) celle differentiation.

INTRODUCTION

It is generally accepted that the differential growth of the upper and lower sides of a gravistimulated root is responsible for its bending. However, it is not well established whether bending occurs because of an inhibition of the lower part of the root or because of an in crease of growth in its upper part. There is a general agreement (1-4), except for Veen (5), that the overall growth of stimulated roots is lower than that of vertical controls. However, when the elongation of the upper and lower halves of gravistimulated roots is compared to that of the sides of vertical roots, the results obtained are more divergent (1-5).

The comparison of cell length : - 1 in the upper and lower halves of stimulated roots and - 2 in the sides of vertical controls lead to the following conclusion. Cell length is less in the lower than in the upper half (6-11). Cell length in the sides of vertical roots was found to be similar to that of the lower part of stimulated roots (9-11). It is clear that there is an inconsistency between the results dealing with root elongation and cell growth in gravi-stimulated roots. The aim of this study therefore was to analyse the nature of the target cells which respond to a gravitropic stimulus and to analyse their elongation in vertical roots and horizontally stimulated roots.

RESULTS

I - The nature of the target cells which respond to gravity.

The location of the fonctional zones (meristem and cell elongation zone) was analysed (Fig.1) : - 1 by examining the mitotic index as a function of the distance D from the root extremity (Fig. 1, A) ; - 2 by studying cell length as a function of D (Fig. 1, B). The results obtained lead to distinguish five different zones in the root tip (I to V). The location of the zone of curvature determined directly on photographs is represented by a bar on figure 1 B. It is clear that the bending zone takes place in the distal part of the meristem and in the proximal part of the cell elongation zone.

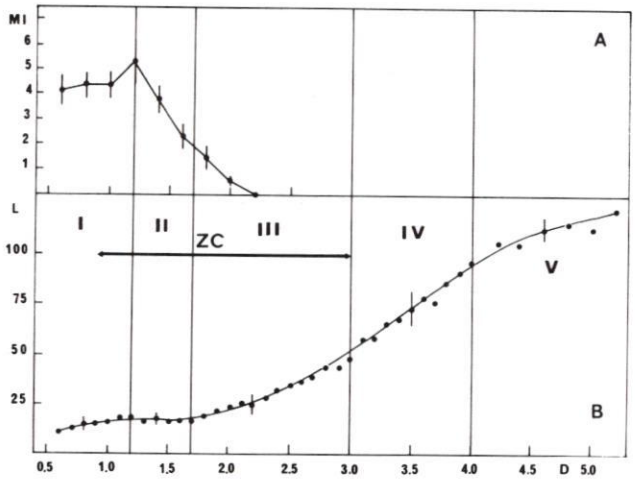


Figure 1 - Localisation of the meristem and of the cell elongation zone in lentil roots.
A. Mitotic index as a funtion of the distance D (in mm) from the root extremity.
B. Cell length (in µm) as a function of the distance D (in mm) from the root extremity.
Typical confidence intervals are indicated by vertical bars on certain points.

II - Response of the two types of gravireacting cells.

A mark was made with eyeliner on vertical roots at a distance of 1.3 or 1.8 mm from the root extremity. These marks correspond to the distal part of the meristem or to the proximal region of the cell elongation zone. Cell length, at the level of the mark, was studied on semi thin sections in roots stimulated for 2 h. From the results shown in table I, it is clear that curvature occurs because of greater elongation in the upper half than in the lower half of stimulated roots. However, it must be noticed that the curvature is due to an increase of cell length in the upper part of the meristematic zone and by an inhibition of cell elongation in the lower part of the proximal region of the cell elongation zone. Thus, the two types of target cells do not respond similarly to a gravistimulus.

Location of the mark	Vertical roots	Stimulated roots	
		Upper half	Lower half
1.3 mm t=0	15.6 ± 1.1 (µm)	18.5 ± 1.9	15.8 ± 1.5
1.8 mm t=0	22.2 ± 1.2	23.2 ± 0.9	20.6 ± 1.4

Table I - Cell elongation (in µm) in vertical roots or in horizontally stimulated roots. A mark was made at 1.3 or 1.8 mm from the root extremity and cell length was studied at the level of the mark after a 2 h period of growth of the roots in the vertical or in the horizontal position. Each mean is followed by its confidence interval at the 5 % level. Means with asterics are significantly different from the others.

III - Cell differentiation in the gravireacting zone

The ultrastructural features of organelles in the cortical cells of vertical roots were analysed for each region (I to V, see Fig. 1) using semi-quantitative criteria (12). A model for cortical cell differentiation was thus elaborated and made it possible to detect slight variations occuring in ultrastructural features.

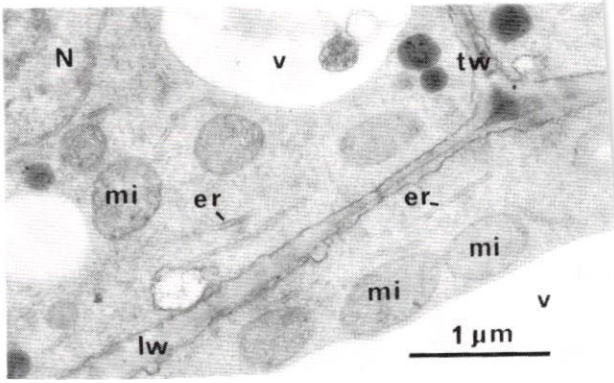
Differentiation of cortical cells in roots which reacted to a gravitropic stimulation was also analysed. The ultrastructural features in gravireacting cells, located at 1.3 or 1.8 from the root extremity, were also analysed using the same semi-quantitative criteria and compared to the model of differentiation of cortical cells in vertical roots (13).

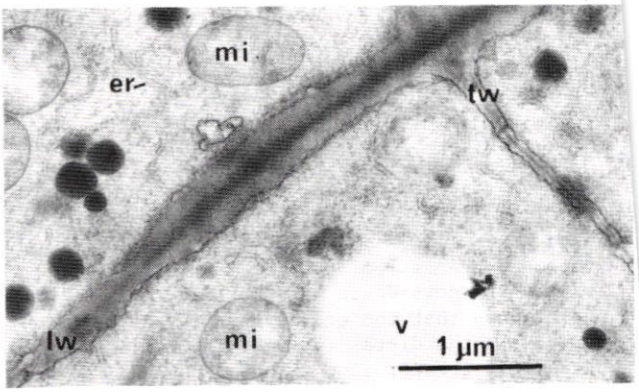
For the target cells of the meristem, their differentiation is greatly modified in the upper half of stimulated roots. As shown in Figures 2 and 3 cell elongation is accompanied by a dramatic modification of the mitochondria which have a dense stroma and swollen cristae in the upper half of stimulated roots. The upper half differ also from those of the lower half by the presence of more voluminous amyloplasts and more developed endoplasmic reticulum.

For the target cells of the cell elongation zone, cell differentiation appears to be slightly delayed in the lower half of the stimulated root.

DISCUSSION

The results obtained in our analysis are in agreement with the hypothesis of a lateral transport of an inhibitor in the gravistimulated root (14-18). The stimulation of the meristematic cells of the upper half of stimulated roots could be due to a decrease in concentration of the inhibitor in these cells, while the inhibition of cell growth in the lower half of the elongation zone could be due to an increase of in concentration of this inhibitor. Mitotic activity and cell differentiation in the primary root could be at least partly regulated by the mechanism responsible for the gravitropic response.





Figures 2 and 3 - Cortical cells of the upper and lower halves of roots stimulated for 2h. These cells were located 13 mm from the root extremity before the stimulation. lw, longitudinal wall ; mi, mitochondria; N, nucleus ; er, endoplasmic reticulum ; tw, tranverse wall ; v, vacuole.

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GRAVITY-INDUCED ASYMMETRIC DISTRIBUTION OF A PLANT GROWTH HORMONE

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ABSTRACT

H E. Dolk demonstrated that gravistimulation induced an asymmetric distribution of auxin in a horizontally-placed shoot. We wish to determine where, and how that asymmetry arises and have demonstrated that the endogenous auxin, indole-3-acetic acid, becomes asymmetrically distributed in the cortical cells of the Zea mays mesocotyl during 3 min of geo-stimulation. Further, indole-3-acetic acid derived by hydrolysis of an applied transport form of the hormone, indole-3-acetyl-myo-inositol, becomes asymmetrically distributed within 15 min of geo-stimulus time. From these and prior data we have developed a working theory that the gravitational stimulus induces a selective leakage, or secretion, of the hormone from the vascular tissue to the cortical cells of the mesocotyl.

INTRODUCTION

H. E. Dolk demonstrated almost 50 years ago that gravistimulation induced an asymmetric distribution of the auxin diffusing from a horizontally placed shoot such that about 40% of the hormone diffused from the upper half of the shoot and 60% diffused from the bottom half (1). Our laboratory extended that finding by demonstrating with a gc-ms assay that the endogenous indole-3-acetic acid (IAA) was, in fact, asymmetrically distributed with about 40% of the IAA in the upper half of the shoot and 60% in the lower half (2). Other laboratories (e.g. 3) showed that externally supplied labeled IAA also became asymmetrically distributed. Now, with more complete knowledge of the inputs to, and outputs from the IAA pool, it may be possible to better understand how and where that asymmetry arises (e.g. 4). A satisfactory theory must account for the asymmetry in diffusable auxin, the asymmetry in endogenous IAA, and the asymmetric distribution of applied labeled IAA. As a working theory we propose that the gravitational stimulus induces selective leakage, or secretion, of IAA from the vascular stele into the surrounding cortical cells, thus accounting for the hormone asymmetry and, possibly, the resultant growth asymmetry. This is a more explicit statement of the lateral transport theory of Went and Cholodny (e.g. 5) in what we hope is a more readily testable form.

RESULTS AND DISCUSSION

IAA metabolism:

Fig. 1 summarizes our current knowledge of the inputs to and the outputs from the IAA pool. We had earlier developed quantitative data for the rates of these reactions in the endosperm (6, 7, 8) and are currently developing quantitative data for the pool of IAA in the vegetative shoot (7, 8, 9, 10, 11, H. Nonhebel, unpublished). It is only with quantitative knowledge of the rate constants for all the inputs and outputs, together with knowledge of rates of transport of IAA and its conjugates within the tissue, that we will be able to precisely account for the IAA asymmetry. As an approximation, we believe that the input to the shoot IAA pool from conjugate hydrolysis is about 10 pmol shoot^{-1}•h^{-1} and that the output from the IAA pool from oxidation catabolism is also about 10 pmol• shoot^{-1}•h^{-1}. We have not yet satisfactorily evaluated the input to the IAA pool from aromatic biosynthesis. Our studies of the incorporation of deuterium into non-exchangeable positions when the seedlings are grown on deuterium oxide can not yet be quantitated (12). Our present concept is that inputs to the IAA pool, other than by conjugate hydrolysis, are small in the case of the conjugate-rich shoots of sweet corn. Aromatic biosynthesis would, of course, be the major route in mature plants and also in the case of conjugate-poor varieties of maize.

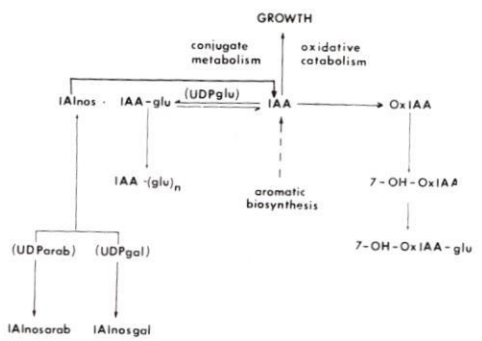

FIGURE 1. A summary of the anabolic and catabolic reactions of IAA in seedlings of Zea mays sweet corn. The input to the IAA pool from aromatic biosynthesis is shown as a dotted arrow since it is known only by deuterium incorporation studies (12) which could represent exchange and have not been quantitated.

IAA transport:

IAA, or any of the compounds shown in Fig. 1 could be transported. Thus, movement of IAA or its conjugates could result in more diffusable endogenous IAA in the lower half of the stimulated tissue thus accounting for the observation of Dolk (1) and many others. Such movement could also account for the greater amount of endogenous free IAA observed in the lower half of the tissue as measured by mass spectrometric methods (2). However both observations (1, 2) could also be accounted for by a shift in the equilibrium between free and conjugated IAA and indeed, such a shift in free to bound equilibria has been observed for the case of photo-inhibition of growth (13). For the case of tropisms it is difficult to separate transport from changes in the relative amounts of free and bound IAA.

The asymmetry in distribution of applied labeled IAA (e.g. 3) must have involved a lateral transport of the radioactivity (14) especially when the amount of radioactivity in the tissue is summed with that accumulating in the receiver block. A variant of the lateral transport theory has been proposed in which it would be the velocity of transport which the tropic stimulus affected (15).

Our working theory, involving selective leakage, or secretion, from the vascular stele into the surrounding cortical cells, would involve an "L" shaped movement of the applied labeled IAA from the stele (16) into the surrounding cortical cells, there to be used, conjugated, or catabolized as countable radioactivity. We believe this is consistent with available results.

Gravity-induced asymmetry in the endogenous free and ester IAA:

We had earlier observed that both free and ester IAA are compartmentalized in the mesocotyl of Zea mays seedlings with 89% of the IAA of the stele as free IAA and 81% of the IAA in the cortex as ester IAA (17). Thus, it was desirable to study the effect of the gravitational stimulus on the amount of free and ester IAA in the cortical cells of the mesocotyl in the region of the developing tropic bend. To accomplish this required a very sensitive assay and we chose to do an isotope dilution assay, using tritium labeled IAA as an internal standard and an electron capture detector to measure the pentafluorobenzyl ester of the IAA. This is a modification of the method previously described (18) and employed a capillary gc column, as shown in Fig. 2. The method was, of course validated by comparison with our selected ion monitoring mass spectral method (19).

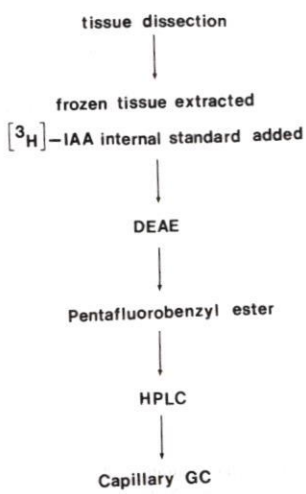

FIGURE 2. Flow sheet for the gas chromatographic IAA assay employed.

The results of these experiments together with some earlier results (18, 20) are shown in Fig. 3. As can be seen the free IAA asymmetry can be detected in 3 min and the free plus ester IAA in 15 min. As yet incomplete data indicate that there is also an asymmetry of free plus ester IAA in 3 min. It is this almost simultaneous asymmetry of both free and ester IAA together with the high concentration of free IAA in the stele that suggests to us that it is the free IAA of the stele that leaks out into the cortex, there to be rapidly esterified to regain the normal ratio of free to ester IAA.

It is, of course, not too meaningful to compare the onset of curvature with the onset of IAA asymmetry, since the methods may have widely different sensitivities. However, one can say that the detectable asymmetry in IAA is at least simultaneous with, a possibly precedes, the growth asymmetry. It will be of interest follow the exact time course of hormone asymmetry since that may informative concerning the gravity-sensing mechanism.

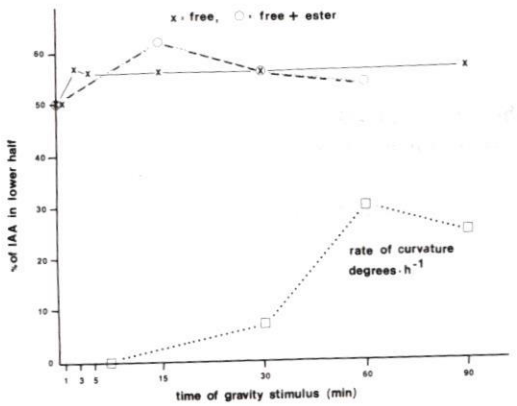

Fig. 3. The time course of changes in the free and free plus ester IAA in the cortex of the mesocotyl of the geo-stimulated corn shoots as a function of time of geo-stimulation. The time course of rate of tropic curvature is also shown (18).

Gravity-induced asymmetry in the distribution of labeled glucose and indole-3-acetyl-myo-inositol (IAInos) applied to the kernel and transported to the shoot:

We also studied the rate at which an asymmetry could be detected in substances being transported from kernel to shoot (21) and these data are presented in Fig. 4. For this experiment, the labeled

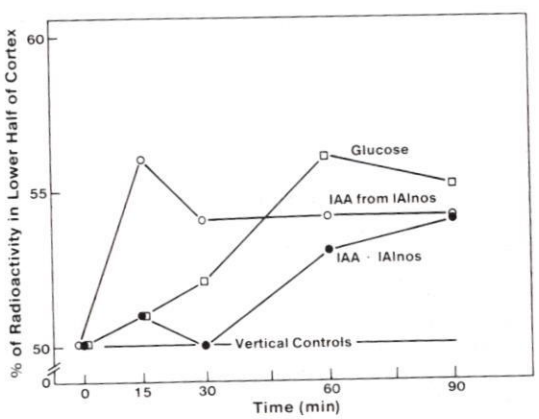

Fig. 4. Time course for the development of an asymmetric distribution of labeled glucose and indole-3-acetyl-myo-inositol applied to the kernel and transported to the shoot. The indicated geo-stimulus time was given after a 2 h loading period while the seedlings were still in a vertical position.

compound was applied to a cut endosperm surface, the seedling incubated for 2 h to permit loading of the shoot, and the geo-stimulus then given for the indicated time. Two things are apparent, first both glucose and IAInos develop an asymmetric distribution, and 2) there is a more rapidly developing asymmetry in the free IAA derived by hydrolysis of IAInos.

This experiment indicates two kinds of transduction of the geo-stimulus, first, the same change in stele leakage, or secretion, that we deduced from experiments on endogenous IAA, and, second, a temporary perturbation of the ratio of free to free plus ester IAA such that an asymmetry of free IAA distribution can be seen before there is an asymmetry of total IAInos radioactivity. This observation deserves further study since it may indicate enhanced IAInos hydrolysis on the rapidly growing lower side of the mesocotyl.

DISCUSSION

There are reports that IAA asymmetries are not always observed following tropic stimulation (22). Certainly however they have been sufficiently frequently observed to warrant study. Questions have also been raised as to whether the observed 60-40, or even, 55-45 asymmetry is sufficient to explain the observed growth asymmetry (23). Since, there are only a few data available, where it is possible to relate growth and an accurately measured IAA content (24, 25), we believe this question can be separately studied from the important question of how and where the hormone asymmetry arises.

A working theory for the mechanism of the gravity-induced hormone asymmetry:

This hypothesis is based upon work from this laboratory with seedlings of Zea mays sweet corn (e.g. 26). Modifications would be required concerning especially the source of the IAA in older plants, or in seedlings with different, or even, no conjugates. The theory is consistent with experiments involving endogenous IAA and those involving application of radio-labeled IAA.

1. Indole-3-acetyl-myo-inositol (IAInos) diffuses from the endosperm to the scutelum and is actively taken up into the stele (7, 10, 11, 27);
2. IAInos moves up the stele to the region of either the coleoptile tip, or the node between the coleoptile and mesocotyl (10, 21, 27);
3. The IAInos is there hydrolyzed and the IAA then transported downward, still within the stele (16, 21, 28);
4. During the course of downward movement, the IAA is secreted from the stele into the surrounding cortical tissues (21);
5. The amount of secretion from the stele, through the endodermis, is under metabolic control and changes in response to the gravitational stimulus so that more IAA is secreted from the stele on the lower side of the tissue (18, 20, 21, 29);
6. The IAA, immediately upon secretion into the cortex (plus epidermis) is converted to an equilibrium mixture of free to ester IAA. These enzyme mediated changes are rapid relative to the rates of transport and secretion and thus transitory disequilibria between free and ester IAA may occur (21);
7. Both free and ester IAA are now present in larger amounts in the lower half of the cortex plus epidermis, the IAA binds to its receptor, and the cascade of reactions resulting in growth ensues (20, 21,30).

A diagrammatic representation of this theory is presented in Fig. 5.

The above theory is presented as a working theory and the literature citations are only to indicate relevant literature and not that the point is established. We present the theory since it does provide many testable points.

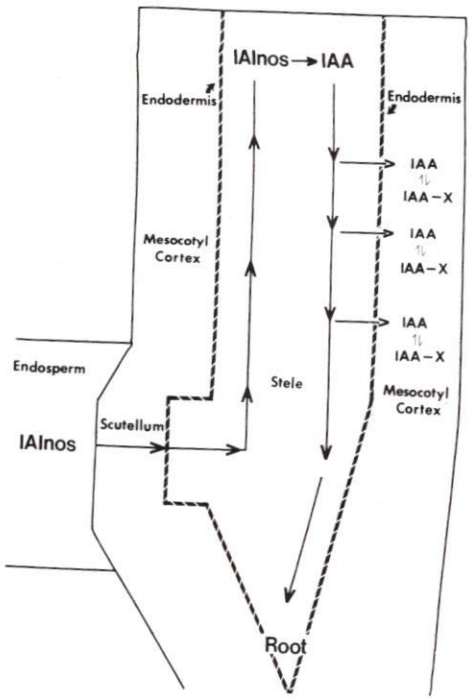

Fig. 5. A theory for the mechanism of the induction of an IAA asymmetry by gravity in a geo-stimulated Zea mays shoot.

ACKNOWLEDGEMENTS

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THE EFFECT OF CLINOSTAT ROTATION ON PROTOPLAST REGENERATION

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Abstract

Cell wall free plant cells - protoplasts - have been rotated for varying periods of time on a slow (4rpm) and fast (50rpm) rotating horizontal clinostat. After rotation the protoplasts have been cultivated in a nutrient medium and differences in mitotic activity have been found between protoplasts rotated on the slow and fast clinostat. During regeneration to mature plants there are apparently no morphological differences between the test groups and the non-rotated control. Preliminary results from a cytogenetical analysis and electrophoretic determination of the isoenzyme pattern in mature plants indicate minor changes as a result of the clinostat rotation of the protoplasts.

Introduction

The totipotency of plant cells i.e. the ability to regenerate a mature plant from one single cell, makes the plant cell a valuable tool for differentiation studies. Enzymatical removal of the plant cell wall gives protoplasts which are protected from the environment only by the labile plasmamembrane and therefore represent a highly sensitive system with respect to external stimuli e.g. gravity. The aim of the present study has been to study regeneration of tobacco leaf protoplasts exposed to a simulated microgravity environment on a clinostat.

Protoplasts - isolation and regeneration

Tobacco can easily be regenerated from protoplasts via callus cultures to flowering plants and is therefore useful as a model for differentiation studies. Fig.1 shows the isolation process for tobacco mesophyll protoplasts. The yield of chloroplast-containing tobacco-leaf protoplasts is normally very high (Fig.2). Viability has been estimated to 80-90%. After isolation the protoplast starts regenerating a new cell wall - a process which normally takes 2-4 days. This is followed by the first cell division and a mature flowering plant is obtained via a callus culture after app--roximately 24 weeks (Fig.3). It is important to use freshly isolated, highly sensitive protoplasts to test environmental factors, before cell wall regeneration is completed.

The use of clinostats

In earth-based experiments, gravity cannot be eliminated, but by rotating plants with their main axes paralell to the rotating horizontal axis of a

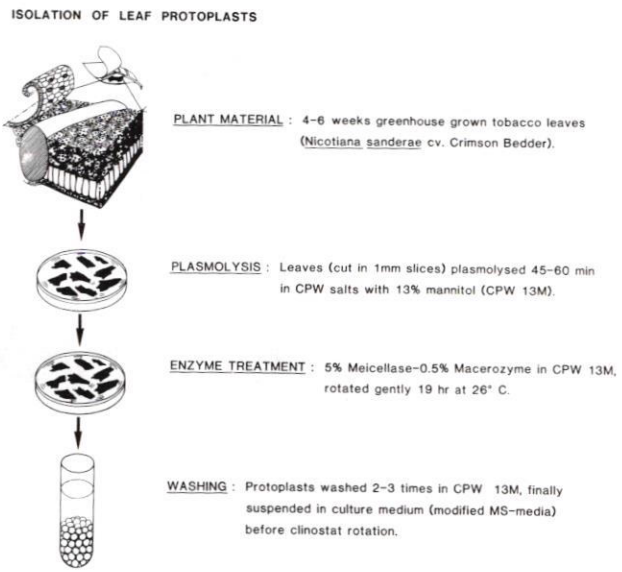


Figure 1. The procedure for isolation of protoplasts from tobacco leaves.

clinostat, it is possible to compensate the directional influence of gravity. For more than 200 years now clinostats have been used as tools in plant physiology. The classical type being the slow rotating clinostat with rates of up to 2-4 rpm (Fig.4 a).

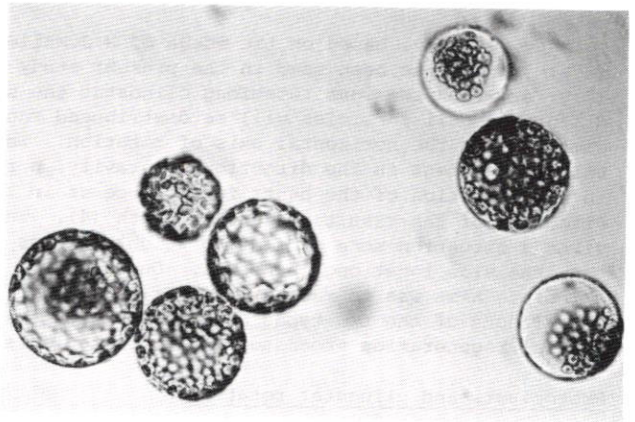
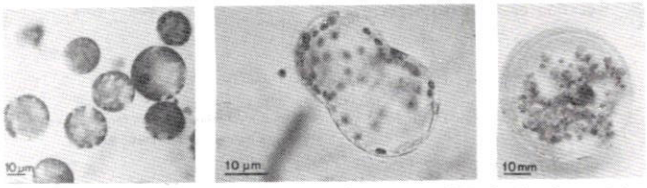


Figure 2. Freshly isolated tobacco protoplasts filled with chloroplasts.

Certain limitations to clinostats have been pointed out by several investigators (e.g.1,2). Especially the rotation rate has been questioned repeatedly and found to be critical for some effects (2). On the slow rotating clinostat, the sedimentable cell particles will move along circular paths during rotation, thus exerting a certain degree of mechanical stress on the cells. However, the diameter of these paths can be made as small as desired by increasing the rotation frequency. One must, on the other hand adjust the distance from the rotational axis to the outer limits of the system very carefully, so that the centrifugal force does not exceed a certain value.

These criteria have been fulfilled in the development of the socalled fast rotating clinostat. A prototype of such an instrument was developed by Dr. W. Briegleb and coworkers at DFVLR,



1.Freshly isolated protoplasts. 2.First cell division - 3 days. 3.Early developmental stage in callus formation - 6 weeks.

4.Established callus cultures - 8 weeks. 5.Ready for differentiation - 10 weeks. 6.Shoot and root differentiation 12 weeks.

7.Young tobacco plant transferred to soil - 14 weeks. 8.Regenerated tobacco plants - 20 weeks. 9.Flowering plants - 24 weeks.

Figure 3. Regeneration of tobacco plants from protoplasts.

Cologne, Germany, based on the ideas of H.J.Muller (3). A copy has been used in the present study (Fig. 4b). On the fast rotationg clinostat the sedimentable cell particles will be distributed randomly along the horizontal axis of rotation. They neither sediment in the direction of gravity, nor in the direction of the centrifugal force or in the direction of the resultant force of both (4). This gives a situation more similar to weightlessness than the slow rotationg clinostat. One of our principle aims was to make a comparative study of the effects of the two types of clinostats on the initial regeneration processes in the protoplasts.

Protoplasts and clinostat rotation

The two types of horizontal clinostats used are shown in Fig. 4. A slow rotating clinostat (Fig.4a;2rpm) equipped with two narrow tubes (diam. 4mm) and the fast rotating clinostat (Fig.4b;50rpm) with one narrow tube (Fig.4c; diameter 2mm) positioned exactly along the horizontal axis of the clinostat.

The sterile, freshly isolated protoplasts were diluted to 5×10^4 per ml nutrient medium and rotated in darkness for 2,4,6,18 and 24h. Control groups were kept in similar tubes under the normal influence of gravity. After rotation both the test and control groups were transferred to small Petri dishes with nutrient medium in which they were cultivated for up to 6-7 weeks. At this stage small callus cultures had been established and these were transferred to solidified nutrient medium in flasks. After 8 weeks differentiation was initiated by placing the cultures on fresh medium containing the cytokinin BAP (benzyl-adenin-purin; 1.8mg/l) and the auxin NAA (naphtyl-acetic acid (0.2mg/l)). After 12 weeks roots and shoots appeared and the seedlings were transferred from the

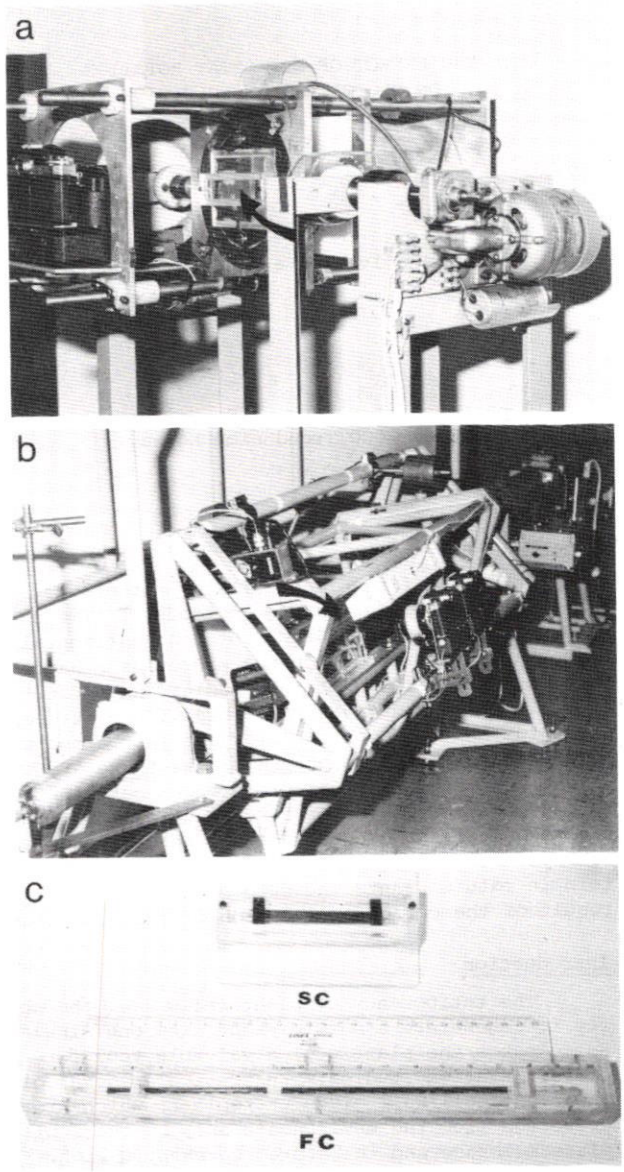


Figure 4. The slow (a) and fast (b) rotating clinostat with (c) the plant chambers (SC and FC). The positions of the chambers on the respective clinostats are indicated (arrows).

sterile cultivation medium to soil and allowed to develop to mature plants.

Effects of clinostat rotation

To study the effects of clinostat rotation the following parameters were estimated:

- mitotic activity of the protoplasts,
- light and electron microscopical analysis of the protoplasts and callus cultures,
- morphological observations of the protoplasts, callus culture and maturing plants,
- electrophoretic isoenzyme pattern,
- cytogenetical analysis.

As shown in Table 1 a slight decrease in the number of cell divisions can be observed after rotation on the clinostats. It is also noteworthy that only 7-12% of the total number of the original protoplasts - both in the control and test groups - divide and establish a tissue culture. Regeneration of the protoplasts follows the same pattern for both rotated and control groups. The first

Rotation period		FAST CLINOSTAT 6 days	FAST CLINOSTAT 8 days	SLOW CLINOSTAT 6 days	SLOW CLINOSTAT 8 days
2 hours	Control	10.3 ± 1.3	11.3 ± 1.0	10.4 ± 1.2	11.3 ± 1.0
	Clinostat	8.9 ± 0.6	12.0 ± 0.8	8.4 ± 0.7	9.4 ± 0.7
4 hours	Control	11.3 ± 1.4	12.0 ± 0.8	9.2 ± 1.5	8.0 ± 1.4
	Clinostat	10.2 ± 1.3	9.8 ± 0.9	7.3 ± 1.6	6.9 ± 1.1
6 hours	Control	10.7 ± 1.1	12.3 ± 0.6	8.1 ± 1.9	10.6 ± 1.7
	Clinostat	9.8 ± 0.9	11.9 ± 0.6	7.2 ± 2.2	8.4 ± 1.1

Table 1

The effect of rotation on the fast and slow clinostat on the initial cell divisions of tobacco protoplasts. The values are given as percentage of the total number of cells. Mean errors indicated.

cell division in both groups can be observed after 3 days and after 8 weeks the established callus cultures are ready for differentiation. After 14 weeks no obvious morphological differences can be seen between the rotated and control group (Fig.5a) It has not been possible to find any morphological changes in the regenerated flowering plants (Fig. 5b).

Although the morphology of the mature plants apparently is not influenced by rotation on the clinostat, rotation periods exceeding 6 hours seem to have an effect at the cellular level. The content of starch grains in the chloroplasts has e.g. been found to decrease after rotation for 24 hours on the slow but not on the fast clinostat.

Examination of cellular effects can also be detected by testing the electrophoretic mobility

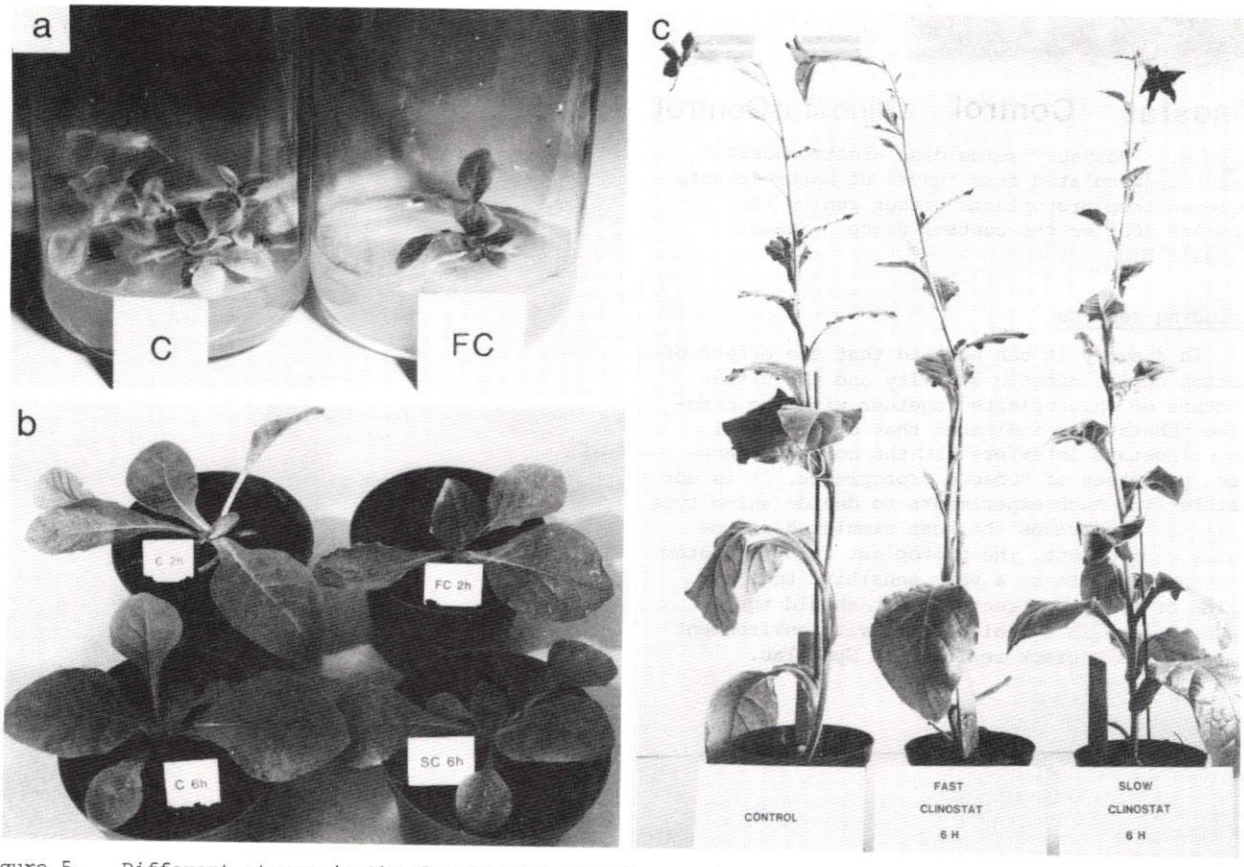


Figure 5. Different stages in the development of tobacco plants from protoplasts rotated on the slow (SC) and fast (FC) for 2 and 6 hours. C= control plants. a; 12 weeks old, b; 14 weeks old transferred to soil c; 24 weeks flowering plants.

of isoenzymes (esterases, peroxidases, amylases etc.) Experiments on these lines have been started. Separation of acidic and neutral leaf proteins using disc polyacrylamide electrophoresis has shown that there are minor qualitative differences in protein pattern between the rotated and control group (Fig. 6)

It is a well known fact that plants regenerated from callus cultures often exhibit genetic diversity - socalled somaclonal varation. In the present study it was assumed that clinostat rotation would increase the genetic diversity. After conventional cytological analysis a certain degree of disturbance was found both during mitoses and meioses in the control plants developed from protoplasts. Abnormalities found were mainly in the form of anaphase bridges and fragments of chromosomes. A detailed and systematic comparisom between the rotated and the control plants is in progress.

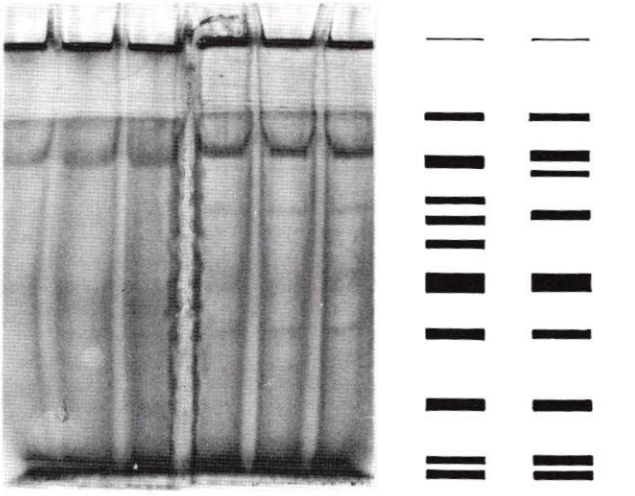


Figure 6. Polyacrylamide disc electrophoresis of proteins isolated from leaves of mature plants developed from protoplasts either run on the clinostat (6h) or the control group. Stain: Coomassie blue.

Concluding remarks

In summary it can be said that the effect of rotation on the mitotic activity and the ultrastructure of chloroplasts together with the chromosome abberations indicates that conventional slow clinostats interfere with the normal regeneration processes of tobacco protoplasts. It is not possible from such experiments to decide which type of clinostat provides the best simulated microgravity environment. The protoplast culture system seems, however, to be a very sensitive tool for studies of gravity effects and it should therefore be tested in a real microgravity environment e.g. during a Biorack reflight in Spacelab.

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LIGHT-INDUCED GRAVITROPIC RESPONSIVENESS IN ZEA PRIMARY ROOTS

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The induction by light of gravitropic response in Zea primary roots was governed by the "all-or-none law". The response was induced by light energies above a threshold value. The action spectrum for this light effect showed a large peak at 650 nm. The effect of red light was not reversed by far-red light. Some reducing agents lowered the threshold value up to about one-third of the control. These results indicated that light may exert 2 functions in the gravitropic response of Zea roots, one being the photochemical transformation of a photoreceptor (not phytochrome) and the other being the induction of a reduction state in the tissue.

The content(activity) of an unidentified growth inhibitor was highest only in the lower halves of horizontally oriented roots which had been irradiated with red light. This growth inhibitor increased the content of hydroxyproline proteins in the cell wall. This inhibitor may cause the downward curvature by increasing the level of hydroxyproline proteins in lower halves.

The guidance systems by which the young growing organs in plants achieve their orientation in a gravitational field have been studied for more than a century. Gravitropism can be regarded as a catenary process, a chain of reactions causally linked in sequence. The first step is the immediate action of gravity on some component mass of the system causing therein a physical change. This has been called susception. The second step is the transformation of the physical information into biochemical information. This has been called reception. The third step is the transmission of the information from the site of perception to the region of response. This step is reasonably certain to be mainly one of differential transport or supply of growth inhibitor(s). The final step is the response of the reacting zone to this differential level of growth inhibitor(s).

Another type of gravity-sensing orientation system is found on the primary roots of some plant species where, in addition to gravity, light is indispensable to the induction of gravitropic response, so this response is called the light-inducing gravitropic response. Although little is known on the photo-sensing mechanism, some results have suggested that light makes available a critical component in the gravitropic response mechanism, and the simplest possibility is that a specific amount of an important growth inhibitor is synthesized or released in the cap following irradiation, and then transported basipetally and exerts its effect in the growing zone of roots.

This system would have a merit for the analysis of gravitropic response, since the roots cannot show the gravitropic curvature even under a gravitational field, if the light is not given to them.

Spectral Dependence

Caryopses of Zea mays L., cv. Golden Cross Bantam 70 were imbibed for ca. 12 h in a Petri dish in the dark in running tap water covering the grains, and were then planted, with the embryo pointing upward, on moist vermiculite in continers covered with a nylon sheet. The primary roots were allowed to elongate horizontally on the vermiculite at 26° ± 2°C in the dark, except that during transfer and other operations they were exposed to very weak green safe light. Caryopses with primary roots 2.0-2.5 cm in length, obtained ca. 35 h after the transfer were used for the experiments.

Zea roots were exposed to equal quantum fluxes of 1.42×10^{-9} E cm^{-2} s^{-1} at different wave lengths and the minimum exposure time for inducing a response to 4 h gravistimulation was determined for each wave band. The action spectrum thus obtained shows a large peak at 650, a small peak at 410, and a shoulder at 663 nm. Curvature started about 1 h after the light exposure.

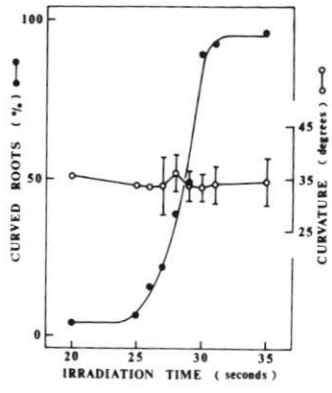

Figure 1. Effects of light on the percent of curved roots and the gravitropic curvature of curved roots. The roots were exposed to 2.43×10^{-10} E cm^{-2} s^{-1} of red light (663 nm). Results were determined after keeping them for 4 h the horizontal position. (From Suzuki, T. and T. Fujii, Plant & Cell Phyiol. 142 275-279, 1978)

continued for the following 1.5 h, and then ceased, irrespective of fluence level.

Zea roots were exposed to 2.43 x 10^{-10} E cm^{-2} s^{-1} of red light at 663 nm for various periods of time. The results, obtained after 4 h gravistimulation, are shown in Fig. 1. Curvature is hardly observed at exposure times shorter than 26 s; above this exposure period, the percentage of curved roots increases drastically with increasing exposure time, to reach a plateau at 31 s. However, the degree of curvature of those roots that do undergo curvature is constant, irrespective of the exposure time, with a mean of ca. 35°. Thus, the gravitropic responsiveness in our material appears to be induced at light doses exceeding a definite energy value, whereas the actual response (the degree of curvature) does not seem to depend on the light dose above that threshold value. In other words, the induction of the gravitropic responsiveness in Zea roots seems to be an all-or-none response.

To determine the minimum light energy needed to induce gravitropic response, Zea roots were exposed to different fluences of red light at 663 nm for various times, and then given 4 h gravistimulation. The minimum fluence was calculated by multiplying the light energy by the minimum exposure time for inducing the response. The minimum exposure time at a given energy of light was determined by finding the intersection point of a tangent line in the steepest part of each curve and the horizontal line in the lag part of the curve. The minimum light fluences were very similar, regardless of light energy, the mean value being 6.51 x 10^{-9} E cm^{-2}. These result confirm the conclusion that there is a threshold light energy value for the induction of gravitropic responsiveness in Zea roots. Moreover, the results show the validity of Bunsen-Roscore reciprocity law, in that identical responses are produced by identical products of light energy and duration. This fact indicates that the response is initiated by the photochemical transformation of a photoreceptor.

Zea roots were exposed to red light (663 nm), far-red light (729 nm), or red followed by far-red, and then subjected to 4 h gravistimulation. The results are shown in Table 1. Although the energy of a 6-s exposure to the red light at 1.52 x 10^{-9}E $cm^{-2}s^{-1}$was only slightly above the threshold value for inducing the response, both the percentages of curved roots and the magnitude of the curvatures were not affected by far-red.

Table 1. The gravitropic response in Zea roots exposed to the sequence of red and far-red light. Quantum fluxes of both red (663 nm)and far-red (729 nm) light were 1.52 x 10^{-9}E $cm^{-2}s^{-1}$. (From Suzuki, T. and T. Fujii, Plant & Cell Physiol., 142, 275-279, 1978)

Light treatment	Curved roots (%)	Curvature
R (10s)	92.5	34.5±5.10
R (6s)	88.9	36.4±4.21
FR (5 min)	6.7	17.5
R (10s)→FR (5 min)	91.7	34.7±3.39
R (6s)→FR (5 min)	91.2	32.6±3.49

In another experiment, Zea roots were exposed for various times to red light (649 or 663 nm, 2.04 x 10^{-10}E $cm^{-2}s^{-1}$) or to red light mixed with far-red (729 nm, 1.12 x 10^{-9}E cm^{-2} s^{-1}) light. As can be seen from Fig. 2, no significant difference was observed in the gravitropic responsiveness. With red light of 649 nm quite similar results were obtained as with red light of 663 nm. These results imply that the induction of gravitropic responsiveness in Zea roots is not controlled by phytochrome.

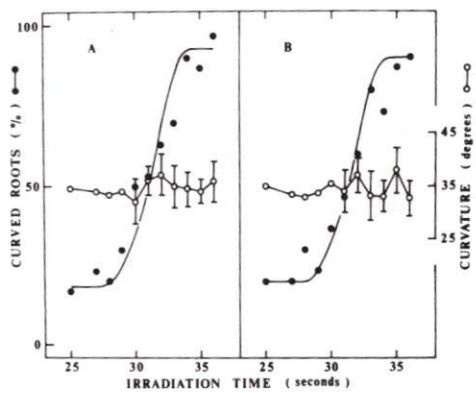

Figure 2. The effect of far-red light on red-light-induced gravitropic responsiveness in Zea roots. The roots were exposed to red light at 663 nm only (A) and to red light mixed with far-red light at 729nm(B) for various lengths of time. Results were determined after keeping them for 4 h the horizontal position. (From Suzuki, T. and T. Fujii, Plant & Cell Physiol., 142, 275-279, 1978)

Dual Functions of Red Light

Two-mm root tips, including the root cap which is the site of light perception in primary maize root, were excised from 1.5- to 2.0-cm Zea primary roots and exposed to 1.97 x 10^{-10}E cm^{-2} s^{-1} red light at 649 nm for various periods of time, and the amount of nicotinamide nucleotides was determined. A preliminary experiment showed that the minimum exposure time for the induction of graviresponsiveness in Zea primary roots was 15 s in this light intensity and that the minimum light energy (the threshold energy value) was 2.96 x 10^{-9}E cm^{-2}. The light doses just above the threshold energy value caused a drastic rise in the NADPH level and a drop in the NADP level. The data indicate that the ratio of NADPH/NADP rose sharply immediately after the irradiation with light doses above the threshold energy (Fig. 3). These results suggest that light somehow increases the reducing capacity of the cells by increasing NADPH, and that the increase in reducing power is someplace

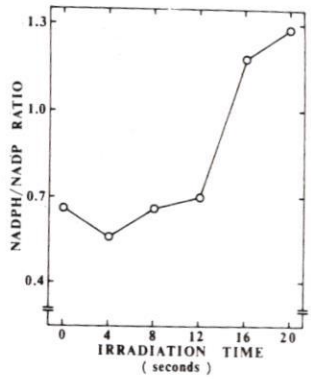

Figure 3. Effect of light on NADPH/NADP ratio in 2 mm root tips of Zea. Two-mm root tips excised from the primary roots were exposed to 1.97 x 10^{-10}E cm^{-2} s^{-1} red light at 649 nm for various lengths of time.

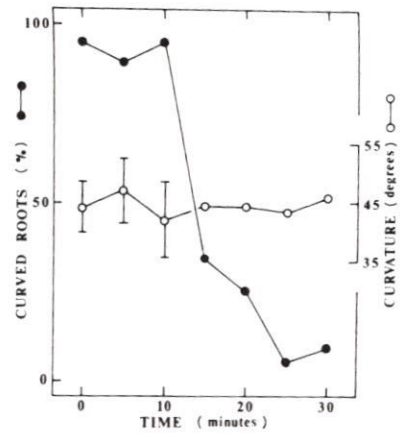

Figure 4. Effect of ascorbate on the gravitropic responsiveness in Zea primary roots. The primary roots pretreated for 15 min with buffer (pH 5.8) with or without 1 µM ascorbate were exposed to red light at 649 nm for various lengths of time. Results were determined after keeping roots for 4 h in a horizontal position. Arrows indicate the minimum exposure time. (From Suzuki, T. and M. Tanaka, and T. Fujii, Plant Physiol., 67, 225-228, 1981)

along the chain of events leading to induction of the gravitropic response.

Zea primary roots pretreated with 20 mM KH_2PO_4-Na_2HPO_4 buffer (pH 5.8) with or without 1 µM ascorbate for 15 min were exposed to 1.97 x 10^{-10}E cm^{-2} s^{-1} red light at 649 nm for various periods of time and then horizontally oriented in darkness. After 4 h, gravitropic responsiveness was recorded (Fig. 4). The minimum exposure time for inducing the response was shortened by the pretreatment with ascorbate up to about one-third of the control, the threshold energy value being 8.86 x 10^{-10} E cm^{-2}. Significant changes in the degree of curvature of roots that do undergo curvature could not be observed, regardless of the presence of absence of ascorbate, the mean value being about 45°. These results indicate that the effect of red light for the induction of gravitropic responsiveness in Zea primary roots can be partially substituted by adding exogenous ascortate.

Other reducing agents, dithiothreitol (DTT), glutathione, and sodium hydrosulfate, also lowered the threshold energy value up to one-third of the control value at the optimum concentration of each, but did not change the mean angle of curvature. Data in Table 2 shown the threshold energy values for inducing the gravitropic response in the roots pretreated with these reducing agents. The threshold energy values were very similar for all reducing agents used.

In another experiment, the primary roots were exposed to 1.97 x 10^{-10}E cm^{-2} s^{-1} red light for 9 s, and then treated with 1 µM ascorbate for 15 min after various dark periods. After 4 h gravistimulation, the gravitropic responsiveness was recorded. Ascorbate was effective in inducing the response only when it was applied within 10 min after the light irradiation, and its effect was gradually decreased with increasing length of the inserted dark period between light and ascorbate treatments

Table 2. Effect of some reducing agemts on threshold energy value. (From Suzuki, T., M. Tanaka, and T. Fujii, Plant Physiol. 67, 225-228, 1981)

Reducing Agent	Threshold Energy Value
	x 10^{-10}E cm^{-2}
None	29.6
Ascorbate, 1 µM	8.86
DTT, 1mM	8.26
Glutathione, 0.1 mM	8.48
Sodium Hydrosulfite, 10 nM	8.48

(Fig. 5). These results, together with the results in Table 2 and Figure 4, imply that reducing agents would exert their effects on the amplifying mechanism for light stimulus but not on the perception mechanism of light, resulting in the stimulation of gravitropic responsiveness of Zea primary roots. These results suggests that the induction of gravitropic response in Zea primary roots is initiated by a dual function of light, one being the photochemical transformation of a photoreceptor and the other being the induction of a reduction state in the tissue.

Implication of A Growth Inhibitor

In a preliminary experiment it was found that a substance having an Rf value of 1.6 upon thin layer chromatogram with toluene-ethyl acetate-acetic acid (40:5:2) strongly inhibited the growth of Zea roots. The position of this substance on the thin-layer plates could be easily detected by UV radiation; fluorescence was quenched in this zone and this quenching served as a marker for the inhibitor. Zones corresponding to this substance were scraped

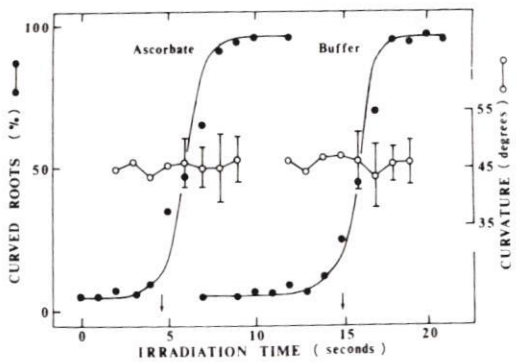

Figure 5. Timing of ascorbate treatment in the induction of gravitropic responsiveness in Zea primary roots. The primary roots exposed to red light at 649 nm were treated with 1 µM ascorbate for 15 min after the various dark period. Results were determined after keeping the roots for 4 h in a horizontal position. (From Suzuki, T., M. Tanaka, and T. Fujii, Plant Physiol., 67, 225-228, 1981)

off the plates and eluted twice with water saturated ethyl acetate. After drying in an evaporator, each extract was dissolved in a volume of 0.02 M phosphate buffer (pH 5.8) equivalent to the fresh weight of the root halves, and the growth-inhibiting activity was assayed with Zea roots.

One h after the light treatment, the apical segments, 7 mm long and including the elongating zone, were cut from the roots and were carefully bisected longitudinally with a steel razor blade into the upper and lower halves. Immediately after cutting, the upper and lower halves of 1,500 segments were frozen with liquid N_2 and crushed to powder in a chilled mortar. The acidic dichloromethane extract was evaporated to dryness and analyzed by thin-layer chromatography.

The growth of Zea roots was strongly inhibited by the amount of this substance present in the lower halves and slightly by that in the upper halves of irradiated roots; only slight inhibition was caused by the inhibition both in the upper and lower halves of roots kept horizontally in complete darkness.

The quantitative estimation of auxin in our material showed that the ratio of IAA (indole acetic acid)-like activity content in the lower and upper halves of horizontally oriented, red-light treated and dark-grown Zea roots was 3.4 and 2.9, respectively, in favour of the lower halves; on the other hand, however, the slight increase in IAA-like activity in the lower halves of roots exposed to light was not reflected in the biological activity as determined by the Zea root-growth test. These results imply that asymmetrical redistribution of IAA does occur upon gravistimulation but is not an essential element for gravitropic curvature, occurring also in gravistimulated roots which had not been exposed to light and thus not undergone curvature.

Table 3. Inhibition of Zea root growth by an unidentified compound extracted from Zea roots and located at Rf 0.16 on thin-layer plates. For the root-growth-inhibition assay, 20.5-mm apical segments were cut from 54-h-old Zea primary roots of the same cultivar, and floated for 2 h on a volume of 0.02 M phosphate buffer (pH 5.8) equivalent to the fresh weight of the tissue and containing the unidentified compound. The control length after 2 h was 5.53±0.031

Condition	Root halves	Inhibition (%)
Dark	Upper	25.0
	Lower	17.0
5 min red light	Upper	21.0
	Lower	43.0

The upper halves of horizontally oriented roots contained 1.6 times as much abscisic acid (ABA; a growth inhibitor) as the lower ones, while no marked difference in the content of ABA was observed in both the upper and lower halves of non-irradiated one. These results also strongly indicate that ABA is not the growth inhibitor necessary for the gravitropic curvature at least in our material.

Proteins, characterized by high levels of hydroxyproline, are firmly associated with the cellulose microfibrils in plant cell wall. The nature of these proteins is still unknown but a correlation between the increase in the hydroxyproline content of the wall and the decrease in cell elongation has been deomonstrated. If the increase in wall hydroxyproline is one of the factors which causes the cessation of cell elongation in roots, then the asymmetrical redistribution of the growth inhibitor in gravistimulated roots should regulate the contents of wall hydroxyproline in the upper and lower part of the horizontally oriented roots, it being lower in the former.

Five-mm apical segments of Zea roots labeled with ^{14}C-proline were treated for 2 h, with the inhibitor or without it. The radioactivity incorporated into the SLS-insoluble cell wall fraction in segments treated with the inhibitor increased greatly. (Fujii, T., T. Suzuki, and R. Kato, Plant & Cell Physiol., 22,1185-1190, 1981). These results suggest that the inhibitor is one of the factors that cause differential growth between the upper and lower halves and downward curvature by increasing the level of hydroxyproline proteins rigidly bount to the cell wall in the lower halves of gravistimulated and red light-treated Zea primary roots.

ABSCISIC ACID IS NOT NECESSARY FOR GRAVITROPISM BY PRIMARY ROOTS OF ZEA MAYS

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We did not detect any abscisic acid (ABA) in roots or leaves of carotenoid-deficient mutants of Zea mays. Similarly, we did not detect any ABA in roots or leaves of seedlings treated with Fluridone, an inhibitor of carotenogenesis. Primary roots of untreated, Fluridone-treated, and mutant seedlings were strongly graviresponsive. These results indicate that ABA is 1) synthesized via the carotenoid pathway, and 2) not necessary for positive gravitropism by primary roots of Zea mays.

Many investigators have suggested that abscisic acid (ABA) is involved in or directly responsible for root gravitropism (see reviews by Juniper, 1976; Moore, 1984; Pilet, 1982; Wilkins, 1984). Basipetal transport of ABA from the root cap to the elongating zone (at greater concentrations in the lower half than in the upper half) of the root presumably results in a more rapid growth of the upper half of the root, thereby accounting for the positive gravitropism characteristic of primary roots. However, much of the evidence for this mechanism for root gravitropism has come from experiments involving exogenous application of ABA to root tips. These treatments probably result in non-physiological levels of ABA in the experimental tissues, thereby complicating interpretation of results. Nevertheless, this problem in experimental design is understandable, since there has been no way to completely inhibit ABA synthesis in plant tissues (Milborrow, 1984; Quarrie, 1983).

As pointed out by Milborrow, our knowledge of the pathway for ABA biosynthesis is noteworthy for its deficiencies (Milborrow 1984). One suggested pathway for ABA biosynthesis is the "direct synthesis pathway", by which ABA is synthesized via the terpenoid pathway from mevalonic acid. An alternate pathway for ABA synthesis (i.e., the "carotenoid pathway") is via the photolysis or photosensitive enzymatic oxidation of carotenoids. There is currently no decisive evidence against either pathway (Milborrow, 1984; Quarrie, 1983).

If ABA is synthesized via the carotenoid pathway, then inhibiting carotenogenesis should also inhibit ABA synthesis. In this study we treated seed and seedlings of Zea mays with Fluridone, an inhibitor of carotenogenesis (Bartels and Watson, 1978). We subsequently measured the ABA content and graviresponsiveness of the Fluridone-treated roots in order to determine if ABA is necessary for root gravitropism. We complimented these studies with similar analyses of the viviparous vp-5 and w-3 mutants of Zea mays, both of which are carotenoid-deficient (Fong, Koehler, and Smith, 1983; Fong, Smith, and Koehler, 1983).

Materials and Methods - Seeds of Z. mays cv. I.O. Chief were soaked in Fluridone (1-methyl-3-phenyl-5-(3-trifluoromethylphenyl)-4-(1H)-pyridinone, concentration = 100 mg/l) for 20 h and then planted in vermiculite. Seedlings were watered daily with Fluridone (100 mg/l). Untreated seeds were soaked and watered with water only.

Seeds of the w-3 and vp-5 mutants of Zea mays used in this study were planted at the Texas A&M University Farm, grown under normal cultural conditions, and self- or sib-pollinated. Heterozygous mutants were identified by pleitropic effects of homozygous mutants. The endosperm of mutant seeds was shite, while that of normal seeds was yellow. Further descriptions of these mutants are provided elsewhere (Fong, et al., 1983). Seeds of the I.O. Chief cultivar of Z. mays were obtained from Empire Seed Co., Waco, Texas.

Endogenous ABA was extracted and quantified using high performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) procedures (Ciha, Brenner, and Brun, 1977; Dorffling and Tietz, 1983). Quantification of endogenous ABA was made using calibration plots derived from a series of standards subjected to extraction and purification procedures. Analysis sensitivity was approximately 2 ng ABA/g fresh weight (FW). A minimum of 3 extractions were performed for each treatment. Gravicurvature was measured by shadowgraphing horizontally-oriented roots (20 ± 5 mm long) in a closed, humid (relative humidity = 95%) chamber in white light (fluence = 310 uW/cm^2).

Results - The ABA content of untreated, Fluridone-treated, and carotenoid-deficient mutant seedlings of Z. mays are presented in Table 1. Gravicurvatures of horizontally-oriented primary roots of these seedlings are presented in Table 2.

Table 1. Abscisic acid (ABA) content (± standard deviations) of untreated, Fluridone-treated, and carotenoid-deficient mutant seedlings of Z. mays.

	ABA content, ng ABA/g fresh weight	
Cultivar	Leaves	Roots
I.O. Chief		
Untreated	678 ± 74	398 ± 64
+ Fluridone	N.D.	N.D.
w-3		
Normal	594 ± 122	379 ± 71
Normal + Fluridone	N.D.	N.D.
Mutant	N.D.	N.D.
vp-5		
Normal	402 ± 77	309 ± 32
Normal + Fluridone	N.D.	N.D.
Mutant	N.D.	N.D.

N.D. = none detected, with an analysis sensitivity of 2 ng ABA/g fresh weight.

Discussion - We did not detect any ABA in roots or leaves of Fluridone-treated seedlings of Z. mays (Table 1). Consistent with this observation is the fact that Fluridone-treated seeds of Z. mays are viviparous (Fong, et al. 1983), a characteristic long associated with ABA content (Taylorson and Hendricks, 1977). Significantly, ABA treatment reverses this Fluridone-induced vivipary of Z. mays seeds. Also, Fluridone-treat-

Table 2. Gravicurvatures (± standard deviations) of primary roots of untreated, Fluridone-treated, and carotenoid-deficient mutant seedlings of Zea mays.

Cultivar	Time, h	
	3	6
I.O. Chief		
Untreated	66 ± 15	80 ± 16
+ Fluridone	68 ± 12	84 ± 18
w-3		
Normal	41 ± 9	74 ± 13
Normal + Fluridone	38 ± 7	72 ± 11
Mutant	36 ± 10	66 ± 9
vp-5		
Normal	29 ± 7	54 ± 8
Normal + Fluridone	33 ± 6	60 ± 12
Mutant	27 ± 9	57 ± 10

ed seedlings of Z. mays are hypersensitive to gibberellic acid, a plant growth regulator that usually causes responses antagonistic to ABA (Devlin, Kisiel, and Kostusiak, 1980). Taken together, these results indicate that Fluridone completely inhibits ABA synthesis in Z. mays seedlings.

Fluridone inhibits carotenogenesis in seedlings of Z. mays (Bartels and Watson, 1978). Therefore, the apparent absence of ABA in Fluridone-treated seedlings correlates positively with carotenoid deficiency. Correspondingly, we could not detect any ABA in roots or leaves of the carotenoid-deficient vp-5 and w-3 mutants of Z. mays, which, like Fluridone-treated seed, are viviparous (Fong, et al. 1983). These results indicate that ABA is synthesized via the carotenoid pathway.

Although apparently lacking ABA, primary roots of Fluridone-treated and mutant seedlings of Z. mays were strongly graviresponsive (Table 2). These results indicate that ABA is not necessary for positive gravitropism by primary roots of Z. mays. Consistent with this conclusion is the observation that although ABA is absent from tips of primary roots of Z. mays cv. Orla 264, these roots are nevertheless strongly graviresponsive (Rivier and Pilet, 1981).

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ARABIDOPSIS SEED PRODUCTION LIMITED BY CO_2 IN SIMULATED SPACE EXPERIMENTS

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Arabidopsis thaliana (L.) Henyh. plants were successfully grown many times in simulated space experiments from seed to seed in our laboratory. Attempts were unsuccessful when closed cultures were used. Only when gas exchange was permitted between culture atmosphere and ambient or when atmospheric volume was large did Arabidopsis produce seeds. Some factor(s) in the atmosphere limited seed production. CO_2 was examined because of its nutrient status and is amenable to analysis. Plants were grown axenically in closed test tubes modified for gas sampling. CO_2 levels decreased in the first week. Unexpectedly in the following weeks, CO_2 increased to about 10 times ambient. Studies are needed to identify the reason for this increase. Sealing cultures to isolate plants from crew environment may affect seed production and compromise a life support system. Appropriate cultural conditions and methods must be determined and incorporated to support and enhance biological space experiments and systems.

Studies were initiated to obtain baseline data appropriate for flight experiments investigating the effects of microgravity on the reproductive processes of higher plants. The initial phase of these studies was to define cultural methods compatible with the growing of higher plants through several consecutive life cycles and still be within the constraints of the limited available power, volume and the environmental conditions found in available space vehicles. A cultural method had to be developed that would permit the plants to grow under light of low flux density and if feasible after seed planting, to grow without attention until mature viable seeds of the following generation were formed. With these restrictions our attention turned to the small culture systems and plant species used by various investigators in space science. In vitro culture system and environmental condition similar to those used by Brown et al., 1979 and Merkis et al., 1976 were selected. Arabidopsis thaliana was chosen for its very short life cycle of about 30 days and its small size of about 20 cm height at maturity. Under natural conditions the plant will produce viable seeds in 24 to 35 days. Surprisingly, plants grown in closed in vitro cultures do not produce seeds even after 124 days of growth. Only when there is a gas exchange between ambient and culture atmosphere will seeds be produced (Hoshizaki, 1982). In these experiments, plants of Arabidopsis were grown in test tubes containing an agar nutrient media. Culture tubes were left uncovered, covered with a metal cap that permitted air circulation or tightly covered with vinylidene polymer film which did not permit air or moisture exchange with the ambient atmosphere. The open and metalcapped plants produced seeds in 26 and 29 days, respectively. On the other hand, plants growing in tubes tightly sealed with the polymer film were without seeds on the 40th day after seed planting. On this day the film was deliberately pierced permitting gas exchange between the culture and ambient atmosphere. Seeds were recorded on these plants 9 days later. It was surmised that some factor(s) in the closed culture atmosphere suppressed seed production. Thus efforts were directed to study the role of in vitro culture atmosphere and its components on the seed production of in vitro grown Arabidopsis plants.

Arabidopsis thaliana (L.)Henyh.,"Columbia strain" plants used in this study are traceable to Dr. G. P. Redei, University of Missouri. Plants were grown following aseptic procedures modified from Mirsche and Brown, 1965 and Brown et al., 1976. The agar medium was supplemented with 2.0 percent sucrose and 0.002 percent glutamic acid. Containers ranging in size from 20 x 150 mm test tubes to one quart jars were used (Fig. 1). Of the five sizes used, each had four replicates and a single surface sterilized seed was planted in each replicate. The culture tubes were aseptically sealed with a sterile vinylidene polymer film which was held tightly in place with rubber bands. Cultural conditions were 700 ft. c. of light from fluorescent lamps, 23 $\pm$1°C and 50-55 percent relative humidity. Plants grown in the larger containers developed earlier. All plants grown in one quart jars had flowers by the 18th day and had seed pods by the 23rd day after planting while in the 20 x 150 mm test tubes, none of the plants had flowered by the 32nd day (Fig. 1). In fact no seeds were observed on the 60th day on any plants grown in test tubes. An exception was one plant in a 20 x 150 mm tube which probably had a seal leak. This plant had 3 small pods yielding a total of 15 seeds as compared to plants in the one pint jars which produced on an average of 112 seeds. These results appear to indicate that some atmospheric factor was in short supply in the smaller cultures and only when the volume is large enough can plants complete their life cycle and produce viable seeds. The most prominent candidate appeared to be CO_2.

A study was then initiated to determine CO_2 concentration levels and to observe responses of plants grown through a complete life cycle in a closed system. Culture size of 25 x 200 mm was selected to optimize the depletion of atmospheric CO_2 by the plants. On the other hand, this volume is still sufficient such that a significant volume change would not be imposed on the culture system when many 40 μl aliquots were removed for analysis. Culture tubes were modified with short side arms to receive rubber septums for gas analysis. Arabidopsis seeds were aseptically planted on sucrose glutamate supplemented nutrient agar and grown under conditions previously described. Atmospheric gas was sampled weekly except for the 5th and 7th week. Concentrations of around 6000 ppm of CO_2 were found during the 3rd to 6th week in weekly sampled cultures while concentrations as high as 140,000 ppm were measured in cultures that were sampled only at the

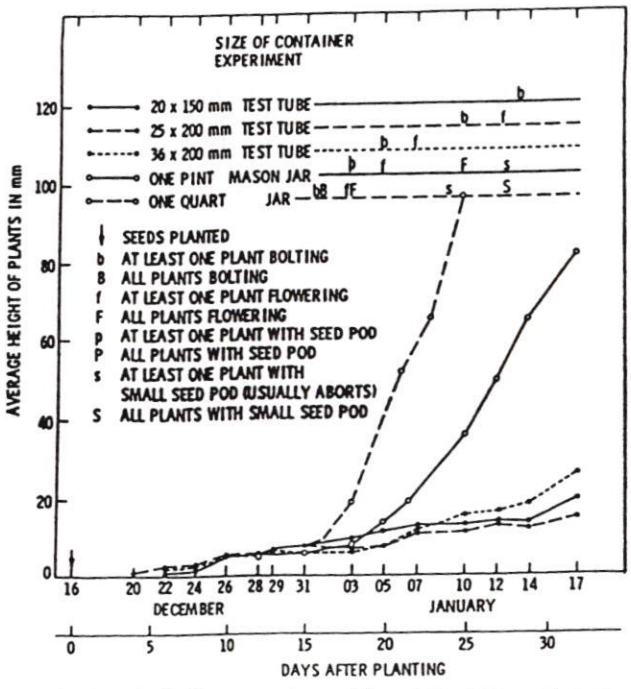

Figure 1. Arabidopsis plants grown from seed in sealed containers. Each container had 20 ml of media except for the 20 x 150 mm tubes which had 10 ml. Atmospheric volumes after addition of nutrient agar media were 27, 55, 160, 455 and 950 ml. Horizontal lines at top of figure depict the time of various developmental stages as indicated on the abscissa. Time of bolting and rate of stem elongation appears to be respectively, delayed and suppressed as size of container decreased. Also see text.

beginning and end of the experiment. No seeds were found on these plants while seeds developed in cultures where CO_2 had dropped to ambient levels or in our controls which had adequate gas exchange with ambient (Table 1).

High CO_2 levels in the atmosphere may have suppressed growth and development of seeds in Arabidopsis. No other gas was measured. Since these cultures were grown axenically and contaminants were ruled out by microbiological plating tests, the CO_2 is more than likely a metabolic product of the plant. Although ethylene was not measured, it is tempting to speculate that by the end of the second week, cotyledons entering the senescence stage would release ethylene into the culture atmosphere with a concomittent release of CO_2. Furthermore, this release of CO_2 could induce an additional increase of ethylene (Grodzinski et al., 1982) which in turn would hasten the senescence of other tissues. Dhawan et al., 1981 have reported increase or decrease of CO_2 levels from that of ambient would increase or decrease respectively the production of ethylene in intact sunflower plants. Under this scenario, an accumulation of CO_2 in closed cultures could occur.

Production of Arabidopsis seeds in small closed containers may be limited by the accumulation of CO_2. Sealing or isolating plants from the crew area and culturing plants in a minimal atmospheric volume may not be the ideal strategy for growing plants in a controlled ecological life support system. Furthermore, a minimum ratio between plant biomass volume and growth chamber atmospheric volume may exist above which plants will grow normally and complete their life cycle. Conversely, at volume ratios below this limit, plants may metabolize abnormally as a response to unusual concentrations of gases such as ethylene.

TABLE 1. AVERAGE CO_2 CONCENTRATION, PPM, IN CULTURE ATMOSPHERE AND AVERAGE NUMBER OF SEED PODS PER PLANT

	CULTURES SEALED BY VINYLIDENE POLYMER FILM					CULTURES PLUGGED WITH POLYURETHANE FOAM				
	With Plant				Less Plant	With Plant				Less Plant
	Atmosphere Sampled:									
	Weekly		Twice		Weekly	Weekly		Twice		Weekly
WEEK	CO_2	PODS	CO_2	PODS	CO_2	CO_2	PODS	CO_2	PODS	CO_2
0	962±172*	0	1193±81	0	1331±102	485±3	0	427±5	0	477±8
1	270±43	0	--	0	1340±39	586±7	0	--	0	567±14
2	885±188	0	--	0	1635±11	458±14	0	--	0	484±4
3	6720±2265	0	--	0	1866±57	478±9	**	--	**	461±5
4	5841±1180	**	--	0	1956±78	519±7	2.7	--	6.0	494±15
5	--	0	--	**	--	--	9.3	--	9.3	--
6	5741±2023	0	--	0	1914±156	499±8	14.0	--	16.3	486±13
7	--	0.3	--	0	--	--	21.3	--	19.0	--
8	431±102	2.0	137,300 ±6073	0	1571±187	497±8	27.3	512±11	26.3	459±10
n	3	3	2	2	2+	2	3	2	3	2+

* High initial CO_2 due to flaming culture tube rim with a bunsen burner.

** Appearance of first flower.

+ Four replicates were sampled at 0 and 8th week. CO_2 concentrations are expressed as ppm ± S.E. Pod counts are expressed as average per plant.

The implications of these findings are that in a controlled ecological life support system envisioned for a space station, various components of the atmosphere surrounding the higher plants may have to be maintained within prescribed limits. Otherwise as indicated by our results, an abnormal response and drastic upset of the atmosphere may occur. These results may also imply that for each species of higher plant, an optimal atmospheric volume would be required. The recent success of culturing Arabidopsis from seed to seed in space (Merkis 1984) may have been enhanced by the use of ventilated growth chambers where variations of the atmospheric constituents would have more than likely been kept to a minimum and their concentrations thus kept within normal range.
(Supported by NASA Contract NAS7-918.)

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INFLUENCE OF CLINOSTAT ROTATION ON FERTILIZED AMPHIBIAN EGG PATTERN SPECIFICATION

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Pattern specification in fertile Xenopus eggs rotated on horizontal clinostats was monitored with respect to primary embryonic axis formation, subsequent morphogenesis, and compartmentalization of the cytoplasm. At the speeds of 1 to 24 rpm (which are believed to simulate microgravity) a large percentage of eggs developed normal axial structures. Eggs clinostated at 12 rpm showed a randomization of dorsal/ventral polarity. The cytoplasmic compartments showed some clinostat effects but no abnormal mixing, disruption or dislocation of compartments. It is predicted that Xenopus eggs fertilized and allowed to develop in space will retain normal cytoplasmic density compartments, establish primary axes and undergo normal morphogenesis in space. Their dorsal/ventral polarity may not, however, be determined by the sperm entrance site (as is the case for 1g eggs).

The amphibian egg provides an excellent model system to study the effects of gravity on early pattern specification because of its large size, external development, ease of experimental manipulation and its clear gravitational response (rotation of fertilized eggs with respect to the gravity vector). The mature freshly spawned (unfertilized) as well as fertilized early cleavage Xenopus egg cytoplasm can be subdivided on a cytological and experimental basis into distinct density compartments (Neff et al, 1984). Although several more compartments have been described (Neff et al, 1984; Dorfman and Cherdantsev, 1977), for this study four cytoplasmic compartments were analyzed. Figure 2a diagrams them. The small yolk mass (SYM) takes up the major portion of the animal hemisphere and consists primarily of small yolk platelets (1-4 um diam.). This compartment contains the nucleus. The intermediate yolk mass (IYM) is found in the equatorial zone of the egg and consists predominantly of intermediate sized yolk platelets (4-8 um diam.) The large yolk mass (LYM) takes up a large proportion of the vegetal hemisphere and consists primarily of large yolk platelets (8-15 um diam). The subcortical vitelline layer (SVL) is about 35 to 65 um deep and is found in the vegetal hemisphere between the LYM and the cortex. It contains, in addition to large, intermediate, and small yolk platelets, pigment granules and the germplasm. These compartments possess unique densities and possibly also unique viscosities. They have the potential to localize information which is important for the establishment of the primary embryonic axis and subsequent morphogenesis. These compartments flow predictably as integral masses in gravity-orientated eggs. Figure 2d shows the flow of density compartments in an inverted T=0.75 (75% of the time interval to first cleavage T=1.0) egg. The LYM and IYM flow with the gravity vector, the SYM flows against the gravity vector and the SVL does not respond. The compartment flow has been correlated with egg polarity (Neff et al, 1984) and may play an active role in establishing the primary embryonic axis as well as subsequent morphogenesis.

To test the role density compartments may play in early embryonic pattern specification of amphibian eggs the following questions were asked. Can fertile Xenopus eggs establish normal primary axes and undergo normal subsequent morphogenesis under simulated microgravity conditions? What happens to density compartments of fertile eggs exposed to microgravity conditons?

Microgravity conditions were simulated with horizontal clinostats. Several unique features were incorporated into the experimental designs. The clinostat speeds for simulating microgravity conditions was determined empirically. In one series of experiments eggs were fertilized while rotating on clinostats. The polarity of clinostat rotated eggs with respect to the sperm entrance site (SES) and involution site (dorsal/ventral (D/V) polarity) was monitored. Because of the unknown side effects of clinostats (Brown, 1979) emphasis was placed on normal development rather than the historical interest in abnormal development (Dorfman and Cherdantsev, 1977; Tremor and Souza, 1977).

The eggs were placed on two types of clinostats. FreRo (free rotation) - eggs in their jelly coats were loaded into cellophane bags (Nalgene cat. # 500-0100) and fertilized. The bags were filled with 20% Steinberg's solution (pH 7.5), heat sealed and attached to the clinostats (rotation began about T=0.1). Early microgravity effects were monitored. In several experiments eggs were fertilized on rotating clinostats (sperm suspension and 20% Steinberg's solutions were injected with syringes into the bags containing eggs). NaRReO (natural rotation, reorientation) - eggs were fertilized, allowed to rotate, dejellied (2.5% cysteine-HCl) oriented with respect to SES and immobilized in 6% gelatin (2 parts 175 Bloom and 1 part 300 Bloom). In the NaRReO mode eggs were on the clinostats by T=0.35. The proper clinostat speeds for microgravity simulation were determined to be between 1 and 24 rpm. The natural rotation rate of fertile eggs in the perivitelline space was determined to be less than 1 rpm (1.6-32.0 rph). At speeds over 24 rpm FreRo eggs oriented with respect to the centrifugal field. Controls consisted of non-clinostated eggs and vertically clinostated eggs. All experiments were carried out at 15 C.

A large percentage (> 50%) of FreRo eggs horizontally clinostated established normal primary embryonic axes and developed normally to the tadpole stage. Figure 1 shows the percentage of FreRo eggs clinostated at 1, 2, 8, 12 and 24 rpm until T=1.0 which underwent normal morphogenesis to the tailbud stage (average = 82% of controls). Similar results were obtained with eggs clinostated until T=3.0 or blastula stages, and when eggs were fertilized on the clinostats.

To determine the effects of simulated micrgravity on egg polarity, NaRReO eggs were

horizontally clinostated at 1, 2, 8 and 12 rpm with known initial polarity (normally oriented eggs were placed on the clinostats with their SES perpendicular to the axis of rotation such that the side of the egg with the SES rotates up at the beginning of clinostat rotation). At the initiation of gastrulation the location of the dorsal lip was determined with respect to the SES. At 1, 2, and 8 rpm the polarity of the gastrulae correlated with the SES. At 1, 2, and 8 rpm 46, 41, and 40%, respectively, of the involution sites were located in the quadrant opposite the SES. At 12 rpm the involution site showed no preference for any quadrant (28, 26, 26 and 21% in each of the 4 quadrants) indicating a randomization of the D/V polarity from the SES.

The composition (yolk platelets) and topology of density compartments subjected to simulated microgravity were analyzed for the following reasons: (1) to test the density compartment model of amphibian egg primary axis formation by attempting to disprove the model: Cases were searched for in which the compartments were clearly disrupted, mixed and/or displaced despite normal development. (2) to see if there is a cytological basis for the randomization of polarity at 12 rpm. (3) in order to collect background information for a detailed morphological analysis of density compartments.

2, 6, 12 and 24 rpm horizontally clinostated FreRo eggs were fixed at T=1.0, embedded in paraffin, and serially sectioned (5 um) parallel to the first cleavage furrow. They were stained in a modification of Heindenhain's azan stain (Neff et al, 1984) which stains yolk platelets yellow/orange and the animal hemisphere cytoplasm, nucleus, and germplasm blue. A second set of eggs was clinostated until T=3.0 and monitored for normal morphogenesis to the tail-bud stage. Non-clinostated eggs served as controls. 128 eggs from 4 spawnings were analyzed for disruption, mixing, and/or gross dislocations of the density compartments. Since many inverted eggs develop normally (Neff et al, 1984) density compartments were not considered abnormal unless they were more disrupted than that routinely observed in typical inverted eggs. In general, compartments were identifiable in all experimental eggs. Little mixing with their neighboring compartments was observed. The stability of the compartments was as follows: SVL (includes germplasm) > LYM, > IYM=SYM. Figure 2b shows the density compartment topology of a typical clinostated egg (61% of the cases). Some movement of the periphery of the IYM and LYM into the SYM is shown. In 18% of the cases the clinostated eggs could not be distinguished from the controls. In 21% of the cases (more prevalent at the higher clinostat speeds of 12 and 24 rpm) the mass movements of the compartments mimiced the movements seen in rotated and inverted eggs (see Fig. 2c). No correlation between density compartments and randomization of polarity of 12 rpm was evident. Variations seen in the fine details of density compartment composition (yolk platelet size) and topography (size and shape) between different spawnings will complicate fine, detailed density compartment analysis. We therefore feel that the fine analysis of density compartments should be directed towards identifying molecular markers for the density compartments or subcompartments. We are presently engaged in making monoclonal antibodies to SVL and germplasm proteins.

From these results it is predicted that Xenopus eggs fertilized and allowed to develop in the weightless environment of space will retain normal density compartment composition and topographical relationships between compartments, establish normal primary embryonic axes, and undergo normal morphogenesis. Dorsal/ventral polarity may not, however, be linked to the SES.

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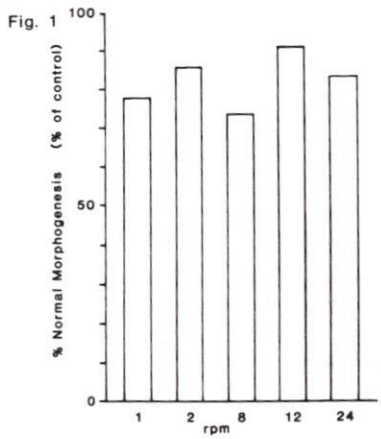
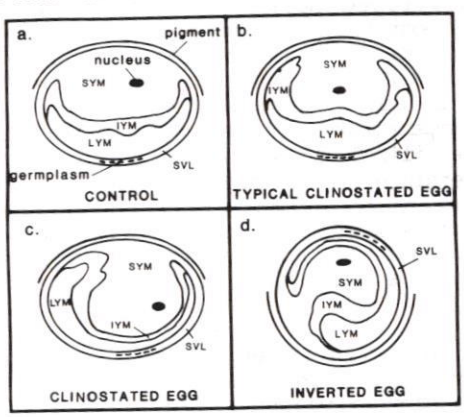
Fig. 1



Summary of the data from FreRo clinostated eggs. Clinostats were stopped at T=1.0. Normal morphogenesis was monitored at the tailbud stage. Approximately 1000 eggs (5 experiments) are included in this data.

Fig. 2



Diagramatic representation of results of density compartment investigation. Diagrams a, b and c depict cytological sections of eggs cut parallel to the first cleavage furrow through the nucleus of one of the blastomeres. Diagram d depicts a midsaggital section of a T=0.75 inverted egg. Abbreviations are described in the text.

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TISSUE DENSITIES IN DEVELOPING AVIAN EMBRYOS

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Changes in the weight:mass ratio provide the physical basis for the biological responses of terrestrial organisms to alteration in the ambient acceleration field (5). Where organisms such as aquatic animals occupy dense media, changes in the gravitational environment produce compensating changes in the weight:mass ratios of organism and medium, such that little net load is imposed upon the organism. This relationship also applies to organs of terrestrial animals. Changes in the ambient acceleration field produce compensatory changes in surrounding tissues so that the organ may not develop a significant net load. This relationship has been investigated in the case of the vertebrate brain (2). However, density gradients within the organ/ organism will produce a local gravitational loading, which may lead to biological responses. In fact, a significant density gradient would be an essential character for a gravity receptor.

Prenatal development, both in mammals and birds, occurs characteristically in a buoyant condition. In both cases a volume of amniotic fluid develops and surrounds the embryo while it is still of microscopic size. This situation prevails until the latest stages of prenatal development. In mammals the amniotic fluid is lost immediately prior to parturition through rupture of the sac. In chick embryos the amniotic fluid is ingested, beginning on the 13th day with the process being completed by the 18th day of development, just prior to the pre-hatch reorientation of the embryo. Consequently, a net load upon the embryo/fetus is not considered to be a major factor in gravitational experiments of prenatal development.

Prenatal development includes marked changes in chemical composition as well as changes in size. This is readily apparent from extensive and detailed examination of the chemical growth for the chick embryo (4). These chemical materials vary in density, as well as in distribution among the tissues of the developing organism. Consequently, the existence of density gradients, and changes in them may be anticipated during prenatal development.

METHODS:

The densities of egg and embryo materials were measured by observing buoyancy when they were placed in standard $CuSO_4$ solutions. This procedure is commonly utilized by hematologists in determining specific gravities of blood and plasma (3). The solutions were prepared by diluting saturated solutions of $CuSO_4$, and adjusting the specific gravity to a standard value by the addition of water or saturated $CuSO_4$. This could be done within 0.0004 sp. gr. units with the use of sensitive hydrometers (1 cm stem = 0.0038 sp. gr. units). A series of standard solutions was prepared in increments of 0.003 sp. gr. units. Where desired, solutions with intermediate specific gravity could be prepared by mixing equal quantities of standard solutions.

Small samples of embryo tissue or egg material were placed in a tube containing 50 ml of a standard solution, dropping it from several cm height to avoid adherence to the meniscus. By doing this serially, noting the tendency of the sample to rise or sink in the solution, the specific gravity could be estimated to an accuracy of 0.001 sp. gr. units. The material added to the $CuSO_4$ solution is isolated by a thin layer of protein precipitated by the copper, so that one volume of solution could be used for a dozen or more samples before there was any detectable change in specific gravity. This method depends upon some minimal protein content. For low protein materials, the procedure can be adapted with other non-aqueous solutions. Such an adaptation has been reported for measuring density of cerebrospinal fluid, which normally contains no protein (2).

Densities of embryos, tissues and egg materials were calculated as the product of specific gravity and water density at the temperature of measurement. All densities were calculated to a standard 20°C. This calculation assumes that the coefficient of thermal expansion of embryo and egg materials is the same as that for water, which appears to be a reasonable assumption (6).

Fertile eggs for these experiments were obtained from a high hatchability commercial line. Eggs were incubated by standard procedures and withdrawn at intervals for experimentation. All embryos utilized were viable and appeared to be developing normally.

Fairly accurate estimates of the density of a material also can be made if its proximate chemical composition is known. A review of physiochemical tables, which are available in a variety of handbooks, indicates that there are fairly uniform densities for compounds within a particular chemical category (proteins, fats, etc.). So it is possible to establish a mean density for each chemical category, for example: water, 1.00; ash, 2.00; protein, 1.38; carbohydrate, 1.54; and fat, 0.92. From these one can derive an equation for estimating density from proximate composition (Eq. 1).

RESULTS:

The contents of the fertile hen eggs undergo substantial quantitative change over the period of embryo development (fig. 1). Initially the eggs are comprised of yolk (37%) and albumen (63%), but as embryogenesis proceeds these are utilized to produce the embryo and the transient extra-embryonic fluids (allantoic and amniotic). This is most obvious for albumen, which is reduced to about half its initial volume in 8 days of incubation. The embryo does not become a significant component (5%) until 10 days of incubation, about midway in the developmental period.

Volumetric changes in the egg contents are accompanied by changes in their density (fig. 2). This is particularly noticeable in albumen,

Eq. 1. Estimation of density from chemical composition.

$$\text{Density} = \frac{\text{gm component}}{\text{gm } H_2O + (0.725 \text{ gm protein}) + (1.0827 \text{ gm fat}) + (0.65 \text{ gm carbohydrate}) + (0.5 \text{ gm ash})}$$

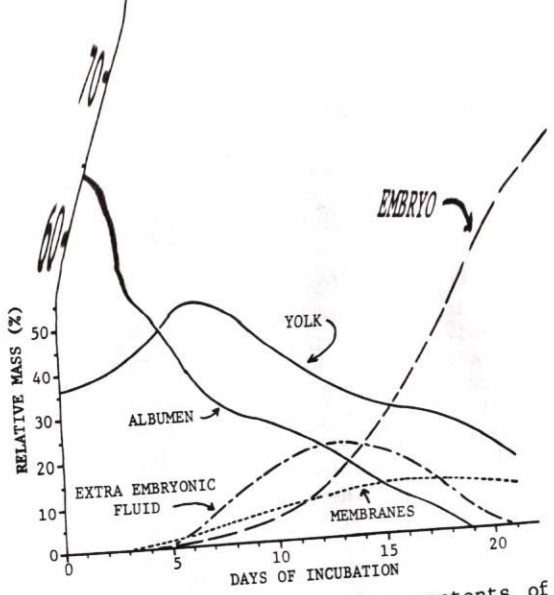

Fig. 1. Mass distribution among contents of incubating eggs (4). The relative sizes of components are calculated as % of total egg contents.

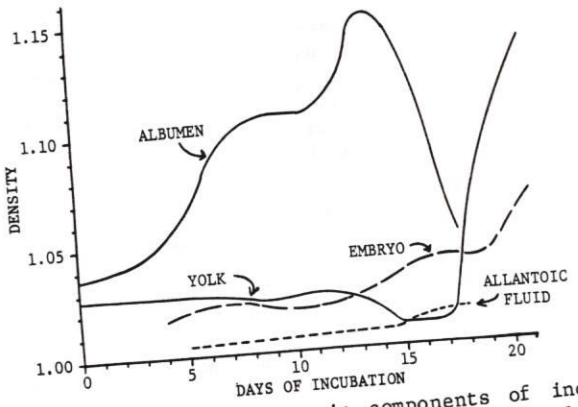

Fig. 2. Density changes in components of incubating eggs. Density, gm/cc at 20°C, was calculated from their chemical composition, except for allantoic fluid which was directly determined.

which has a marked increase in density between the 4th and 14th days of incubation. In the first two-thirds of incubation, there is a sequence of increasing density among egg contents: amniotic fluid, embryo, yolk, and albumen. As a result the embryo is located at the bottom of the amniotic fluid, providing mechanical protection, but at the top of the albumen. This provides a minimum diffusion distance between the egg's air cell and the embryo, facilitating oxygen transport. This is particularly important before the allantois is functional in respiration. The allantoic membrane begins its rapid development after 5 days of incubation (1).

Embryo density increases exponentially, as does embryo mass, but the density kinetics are much lower. The changes in embryo density are not symmetrical, and parts of the embryo have patterns of changing density that are dissimilar from that of the intact embryo (figs. 3 and 4). Undoubtedly the progressive development and calcification of the skeleton is a factor.

CONCLUSION:

It is evident that the developing embryo undergoes density changes, as well as an asymmetry of these changes among body parts. Some of these density changes and perhaps all of them are functionally related. However, density distributions are particularly important in gravitational investigations of embryogenesis since they will produce forces tending to dislocate parts of the embryo. Consequently internal density gradients, should be measured and utilized in the interpretation of gravitational investigations of avian embryogenesis.

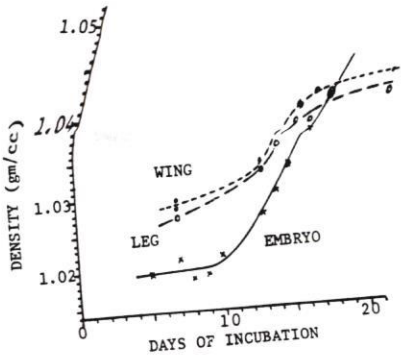

Fig. 3. Density of whole embryo and limbs. These were measured by observing buoyancy of tissue in standard $CuSO_4$ solutions, and calculated to 20°C.

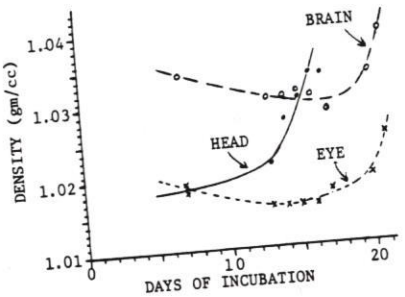

Fig. 4. Density (20°C) in the head region of developing embryos, measured by buoyancy of tissues in standard $CuSO_4$ solutions.

ACKNOWLEDGEMENT:

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SELECTION OF JAPANESE QUAIL FOR THE HIGH EGG PRODUCTION UNDER HYPODYNAMY

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ABSTRACT

We have been selecting Japanese quail line with high egg-laying during hypodynamy. At selection of hens we apply 50% selection pressure, it means that we select from population one half of individuals with the highest egg-laying during 14 days long hypodynamy. In the fourth filial generation the level of egg-laying increased, and during hypodynamy it reaches: a. 24% at time of its minimum (5th day), compared with 5% in parental population. b. 52.3% at time of its maximum (14th day), compared with 16% in parental population. c. 40.9% of initial egg-laying as the average egg-laying during 14 days long period, compared with 25.1% of initial egg-laying in parental population of quails. In second filial generation we also tested the changes in behaviour (using the open-field test) in addition to the egg-laying characteristics. Results indicate the differences in motor activity and frequency of distress calls between control and selected lines of animals.

INTRODUCTION

Specific conditions of space flights such as weightlessness and limited space constitute for living organisms situations to which organism must be promptly adaptable (2). One of the ways of adaptation, which has been applied first of all in the case of man, is systematic long-lasting preparation by ingenious training simulating states similar to stay in space. In animal species with fast reproduction cycle it is much more profitable to respect individual variability in reaction on this nonphysiological state and by way of inheritance to obtain individuals with the most advantageous characters in given situations. Japanese quail is because of it favourable conversion coefficient of received food to first-quality egg protein placed on the one of the prominent places when we are reasoning about the structure of alimentary links of closed ecosystem in space ship. Considering the fact that we found out in the preceding experiments with short-term (4, 6), medium-term (6) and long-term (5) hypodynamy that Japanese quail is a species with good adaptation abilities we have decided to select population of animals with high egg-laying under simulated weightlessness - hypodynamy.

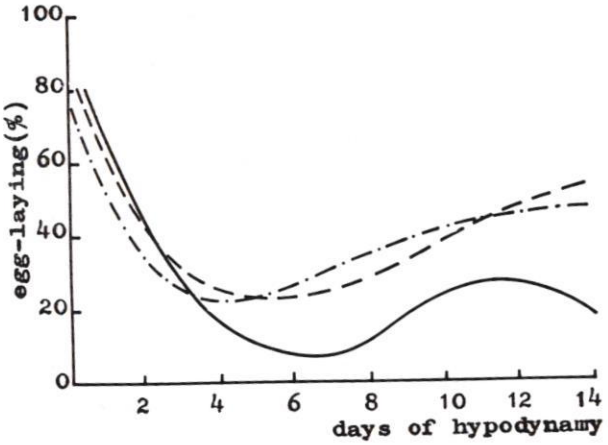
MATERIAL AND METHODS

The parental population for the initial selection was obtained by crossing the randombred and imbred lines. One half of the animals was kept in randombred population as the control line. To form the line of quails resistant to hypodynamy the following approach was used. Eight weeks old hens were exposed to 14 days hypodynamy in jackets. During this time egg-laying was recorded. The egg-laying level during hypodynamy served as the criterion for selection. On the base of these results 50% of hens with the highest level of egg-laying were chosen from each generation and mated to cockerels. The presented work demonstrates results of the quail selection up to the fourth filial generation. Though the selection of individuals for the formation of a further generation was made on the basis of the level of egg-laying during the hypodynamy, the other parameters such as fertility and hatchability of eggs as well as the growth rate of Japanese quail were studied too. We were interested whether selection affected also behaviour of animals. In the second filial generation the fearfulness of 1, 28, 70 days old animals (immediately after 14 days lasting hypodynamy), 72 days old animals (2 days after hypodynamy) and 112 days old animals was studied using the open field test (3). The locomotor activity was recorded in 10 minutes test. Immediately after the termination of hypodynamy, respectively 2 days after hypodynamy one of four possible postures, i.e. laying, sitting, getting up on shanks and standing was registered. In one day-old chicks peeping was also recorded. The egg-laying dynamics was analysed by using the mathematical model (1) and body weight increaments by using the phenomenological growth equation (7).

RESULTS

In the parental generation of Japanese quail it was found out that the most expressive decrease of egg-laying occured on the 6.2 day from the start of hypodynamy and the level of egg-laying achieved only 5% of the initial egg-laying level (Fig. 1). On the 14 day i.e. at the termination of the experiment, egg-laying reached 16%. The average egg-laying during hypodynamy represented 25.1% of the potential egg-laying. In the first, and particularly in the second filial generation the overall course of egg-laying curve was better. The most expressive decrease of egg-laying during hypodynamy was recorded on the 3.7 day when the egg-laying level reached 20.2%. On the 14 day of hypodynamy egg-laying raised up to 46.2%. The average egg-laying during hypodynamy represented 36.3%. After two further generations the course of egg-laying curve was improved. The lowest egg-laying level was recorded on the 4.8

day when it reached 24% of the potential egg-laying. At the termination of hypodynamy the level of egg-laying raised up to as much as 52.3%. The average egg-laying during the 14-days lasting hypodynamy was 40.9%.



Generation	X_{MIN} (%)	T_{MIN} (day)	X_{14} (%)	$\bar{X}$ (%)
P	5.0	6.2	16.0	25.1
F_2	20.2	3.7	46.2	36.3
F_4	24.0	4.8	52.3	40.9

Figure 1. Effect of selection on course of egg-laying and on some egg-laying parameters in quail during hypodynamy. —P - parental generation, - - F_2 - 2nd filial generation, – – F_4 - 4th filial generation; X_{MIN} - minimal egg-laying, T_{MIN} - time of reaching the minimum, X_{14} - level of egg-laying at time of hypodynamy termination, $\bar{X}$ - mean of egg-laying during hypodynamy.

The second filial generation of quails bred for the resistance to hypodynamy was compared with the parental population as well as with the control one, i.e. the non-selected filial generation. In the fertility and hatchability of quails no significant changes were recorded. The body weight of quails hatched from the selected line was significantly higher as well as the body weight increament during maturation and adultness (Fig. 2). The 14-days hypodynamy led to a temporary decrease of body weight. In case of the selected line the body weight remained after the termination of hypodynamy still higher.

The frequency of peeping of one day-old quails from the selected line was in comparison with the control group significantly lower (Fig. 3). The locomotor activity of one day-old quails was in both lines approximately the same. The test with 4-weeks old females from the selected group showed that these were significantly more active. The quails exposed to hypodynamy in the control as well as in the selected group had the same locomotor activity immediately after its termination. Significantly increased locomotor activity was observed 2 days after the termination of hypodynamy in selected line. The differences again disappeared in the final test in the age of 16 weeks.

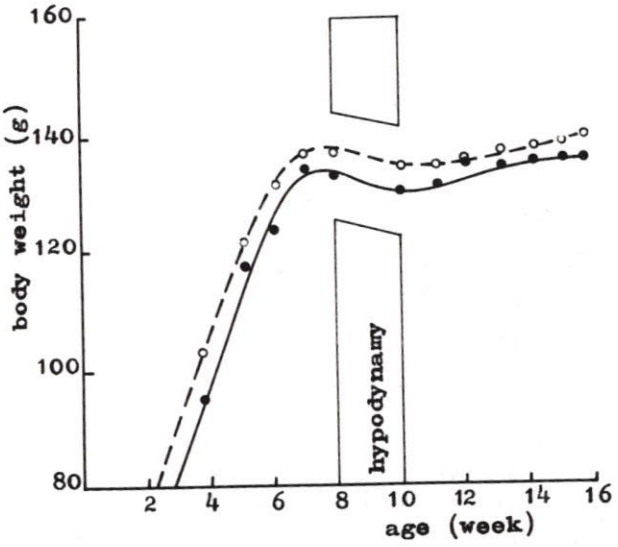


Figure 2. Growth curves of 2nd filial generation of quails selected for egg-laying during hypodynamy. ●—● control, ○– –○ selection.

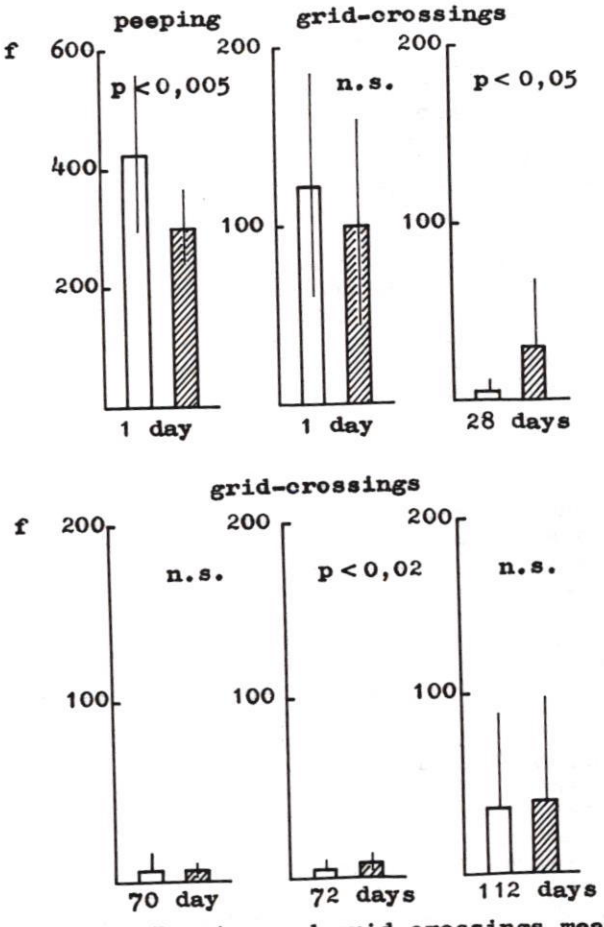


Figure 3. Peeping and grid-crossings mean frequency in control and selected lines of quails of 2nd filial generation. □ control, ▨ selection.

The behaviour of the quails from the both groups was immediately after the hypodynamy nearly the same, i.e. - laying, sitting and getting up on shanks prevailed (Fig. 4). Two days after hypodynamy the getting up on shanks and standing occured more frequently in both groups. However, the differences between the control and selected populations were not significant.

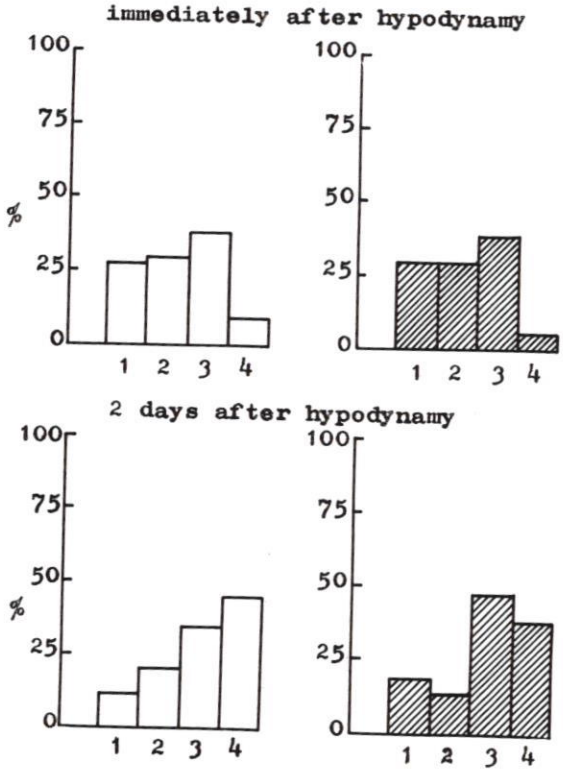


Figure 4. Percentual representation of quails that reached after hypodynamy posture: 1 - laying, 2 - sitting, 3 - getting up on shanks, 4 - standing. ☐ control, ▨ selection.

CONCLUSIONS

Summarizing the results from the first four generations of the line selected for the increased level of egg-laying during hypodynamy shows that the selection has a positive effect on the parameters of egg-laying and simultaneously refers to the improving adaptability of quails to the changing physiological conditions due to hypodynamy. An accompanying feature of the selection pressure on a higher egg-laying during hypodynamy is the increased growth intensity. The behavioural tests show that quail begins to be less fearful.

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INHIBITION OF THE SPIDER HEARTBEAT BY GRAVITY AND VIBRATION

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ABSTRACT

The rate and vigor of the spider heartbeat is controlled by an external pacemaker. A mechanical feature of the spider cardio-vascular system is the production of high serum pressure in the prosoma and the legs. This appears to be the source for leg extension. The lyriform organ on the patella of the leg is sensitive to vibratory and kinesthetic stimuli. This sensitivity depends upon the degree of leg extension. Thus the activity of the heart and the response characteristics of the sense receptor are related. The effect of a supra-threshold vibratory or gravitational stimulus is to produce an inhibition and a tachycardia of the spider heartbeat.

INTRODUCTION

Parry and Brown(1954) demonstrated that the legs and prosoma of the spider were under considerable pressure. They proposed an hydraulic mechanism for active extension of the leg. They maintained that the pressure was due to the heart. This implies that if the heartbeat is modified, the" pressure head" will change and consequently the position of the legs. For example, if the heartbeat is reduced by a minute flow of CO_2 the legs slowly fold into the prosoma. Upon removal of the gas and the consequent increase in cardiac activity the legs extend outward with pulsatile movements (Finck 1984, unplb observations). The activity of the heart and the movements of the legs of the spider appear related.

The spider heart is neurogenic. Contractions of the myocardium are produced by impulses from an external pacemaker(Bursey & Sherman 1970). Wilson (1967) suggested that the heartrate of the spider could be modified by sensory stimuli. He also commented on the possibility of nervous inhibition. Sherman (1983) reported that the inherent rhythm of the cardiac ganglion is modulated by cardio-acceleratory and -inhibitory neurons which arise from the subesophageal ganglion. The subesophageal portion of the spider nervous system is devoted to sensory/motor representation of the legs and contains association centers (Babu 1965). The physiological and anatomic literature therefore indicate a basis for sensory control over the spider heart.

Finck(1982) reported that following a change in the intensity of the gravito-inertial field the heartbeat decreased then increased in rate. He attributed this change to stimulation of the lyriform organ on the patella of the leg. The lyriform is known to respond to vibration. Therefore it was decided to examine the effect of sensory influences upon the heart with vibratory stimuli. There are several good reasons to engage in this procedure: 1) the lyriform is very sensitive to compression of the exoskeleton, (Barth 1981), 2) vibration thresholds are low (Walcott & Van Der Kloot 1959, Finck 1972), 3) vibratory stimuli can be precisely controlled in the laboratory and 4) since gravity and vibration stimuli appear to compete for the same sense input the effects of either stimulus might be comparable.

METHODS

Female spiders of the species Nuctenea sclopetaria Cl. were employed in this research. Cardiograms were taken by transilluminating the heart with a cool Helium-Neon laser and detecting the light change by photocell. The animals were unrestrained and never removed from their home cages (culture dishes). Vibrations were applied to the culture dish (substrate) by a Ling-Altec vibrator. Stimuli were 2 seconds duration with a rise/decay time of 50 msecs. Attenuators controlled the stimulus amplitudes which were calibrated in terms of RMS displacement. The inter-trial interval was 5 minutes. When spiders were given tilt stimuli a computer controlled tilt-table was substituted for the vibration delivery system. In order to learn more about the influence of the lyriform organ on the heart, resting cardiograms were taken (in a separate group of animals) before and after varnishing the patellae. A non-toxic glue (Elmers) was used in this procedure.

RESULTS

The median heartrate was recorded 2 days before and after varnishing the site of the lyriform organs. Six spiders were used in this removal experiment and all had increased heartrates post-varnishing. The mean rates were 47 BPM-before and 67 BPM-after the procedure. The +41 percent increase was statistically significant (Wilcoxon Matched Pairs-Signed Ranks, non-parametric test). Unvarnished controls showed normal heartrates. These data offer initial evidence that the spider heart is influenced by the sense receptor.

Fig.1 shows cardiograms of a spider given a 90 Hz sinusoid at different amplitudes of vibration. The general nature of the response is a bradycardia, some vigorous beats and a tachycardia. A stimulus of .056 microns (lower record) was below the sensory threshold. The response to a 1.76 micron displacement (top record) was the cessation of the heartbeat for 10 seconds. Recovery to this stimulus was not complete for at least 2 minutes. In this example as in all our observations, response latency decreased with stimulus intensity. The legs in this animal were not well extended from the prosoma. This accounts for the relatively high absolute threshold.

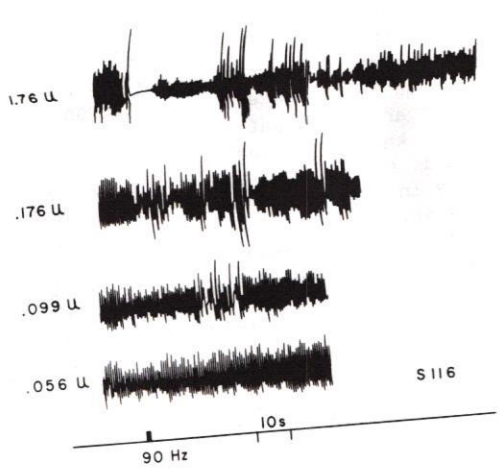

Fig.1. Spider cardiograms in response to 90 Hz substrate vibration. The parameter is RMS amplitude in microns.

Fig.2 shows cardiograms of 3 spiders all with their legs well extended. Responses to vibration is on the left and to tilt on the right. The upper left record contains a cardiotac display which demonstrates the bradycardia soon after the stimulus onset. The lower left record shows a just supra-threshold response; a bradycardia followed by a tachycardia. Note the very low stimulus displacement. Tilt stimuli (right) were 1,2 and 3 degrees from the horizontal over a 6 second interval. These correspond to .166,.33 and .5 degrees per second. The 1 deg tilt is a apparently ineffective. The record labelled 3 degs. shows a bradycardia followed by a tachycardia. These are typical responses to either vibration or tilt.

DISCUSSION AND CONCLUSIONS

The sensory modulation of the heartbeat in the unanesthetized and unrestrained spider has been demonstrated. Therefore the anatomic/physiological mechanism of the cardiac ganglion reported in the literature has a correlate in the normal behavior of the spider heart. The neural center in the subesophageal ganglion which integrates the signals from the legs and delivers an output to the pacemaker has yet to be identified.

How the cardiac mechanism and sensory receiver function in the gravity system of the spider is the subject of a theoretical model. This model proposes an hydraulic feedback system which can change a 'set-point' for Gz and other mechanical stimulus events. This set point is the position of the spider leg. A simple approach is to assume that the leg functions like a lever converting displacement into pressure. Thus small displacements of the tarsus (leg tip) are amplified so as to deform the stiff exoskeleton near the lyriform organ. One can think of this as an impedance matching method to get the organ stimulated. Or,alternatively, to isolate the very sensitive transducer from non-relevant stimuli (noise). von Bekesy (1967) observed that such sensory inhibition is a method used effectively in living systems to improve the signal-to-noise ratio.

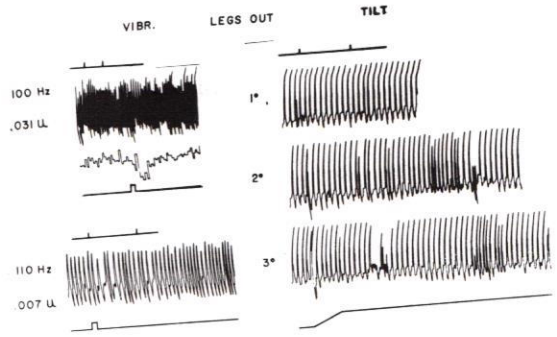

Fig.2. Spider cardiograms in response to vibration (left) and tilt (right). The hack marks are 10 sec intervals.The legs were extended in the 3 spiders.

AN HYPOTHESIS

The activity of the mechanical sensory system is important in maintaining inhibitory control over the spider heart. If weight is removed as a proprioceptive stimulus in orbital flight the inhibitory output from the subesophageal ganglion will decrease.This should result in an abnormally fast heartrate. A corollary hypothesis suggests that long periods of leg extension obtain when the spider is in the micro-gravity of space.

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IUPS Commission on Gravitational Physiology

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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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