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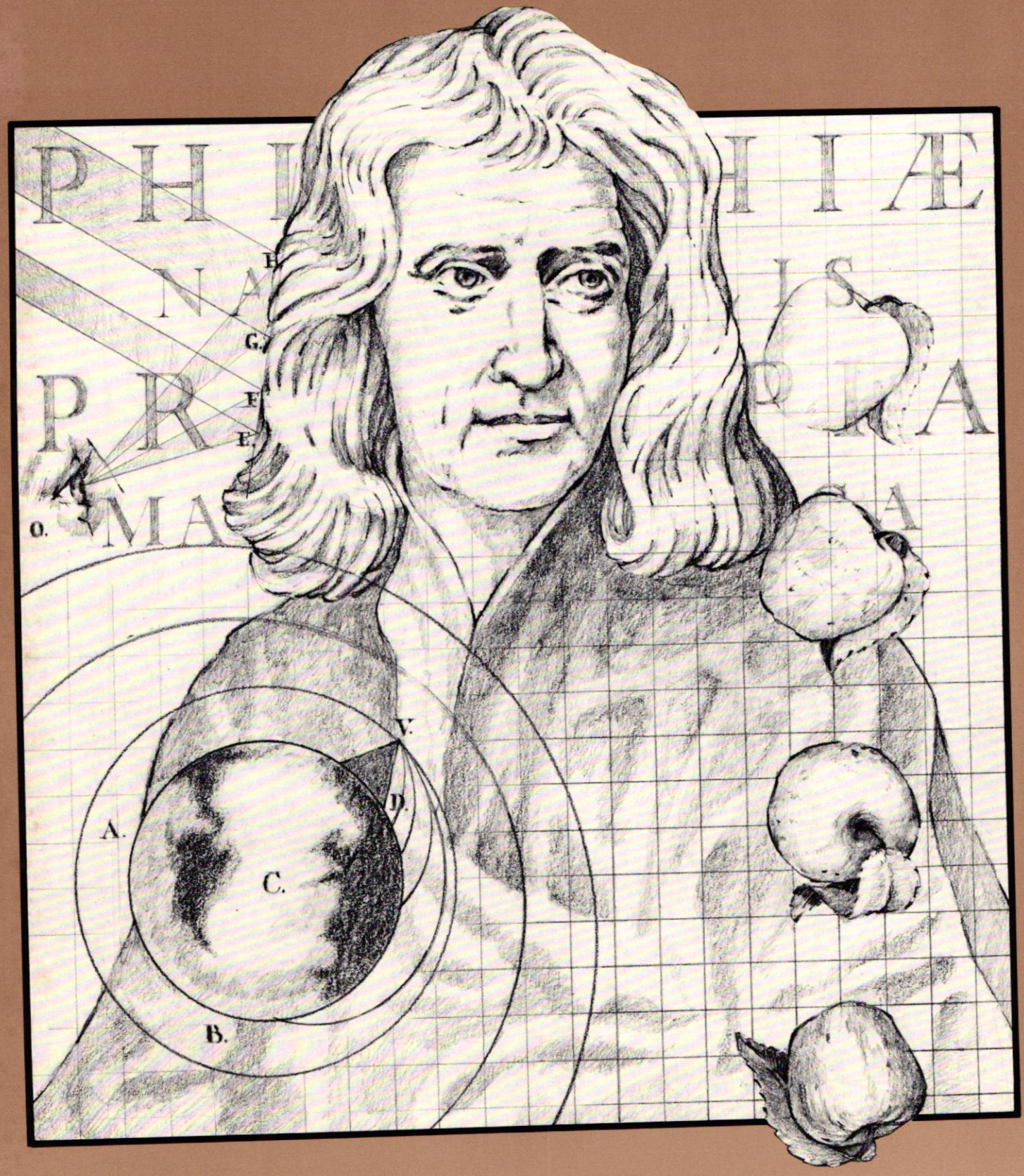

Proceedings of the Fourth Annual Meeting of the IUPS Commission on Gravitational Physiology

10-15 October 1982
San Diego, California

International Union of Physiological Sciences
Commission on Gravitational Physiology

DEDICATION

The Fourth Annual Proceedings of the Commission are dedicated to Sir Isaac Newton in gratitude for his fundamental and insightful contributions to the basis for gravitational physiology.

Isaac Newton was born on a farm near Woolsthorpe, Linconshire, England on Christmas Day 1642 (Julian) the year of Galileo's death. His father died 2 months before, and in 1645 his mother remarried leaving young Newton in the care of her mother. When Newton was 12, he was sent to a nearby public school. He was a poor student, spending his time on matters of his own interest rather than the assigned subjects. Two years later, Newton's mother was again widowed, and he returned to the farm. He did not do well as a farmer, spending time reading and studying mathematics. Fortunately for the world, an uncle was a member of Trinity college, and in 1660 he saw to it that Newton returned to school to prepare for Cambridge. He matriculated at Trinity college in 1661 and received the B.A. degree in 1665. In that year the university was closed for fear of the plague and Newton again returned to Woolsthorpe for 18 months. It was during this period that Newton germinated his prodigious discoveries in mathematics and physical sciences. In the first few months he developed the binomial theorem and an early form of differential calculus. From experiments with prisms he discovered the composite nature of white light, and he conceptualized the universality of the gravitational force. The apocryphal anecdote relating the fall of apples to the development of the theory of universial gravitation was reported by Voltaire (*Philosophie de Newton*, 1738). By Newton's later account, during these months "I was in the prime of my age for invention, and minded Mathematics and Philosophy more than any time since."

When Cambridge reopened (1667), Newton returned, having been elected a fellow of Trinity college. A year later he succeeded his teacher, Isaac Barrow, as professor of mathematics, a position that he held for 34 years. By all accounts, Newton does not appear to have been a popular or a successful teacher. Newton's scientific contributions of importance to gravitational physiology should be well known to anyone interested in the field, particularly the Laws of Motion and the Universal Law of Gravitation, which are elucidated in the *Principia* (1687). The law of gravity defines the constant loading force impinging on all Earth organisms throughout the course of evolution. The laws of motion are largely a clarification and formalization of concepts dealt with by Galileo (and this was acknowledged by Newton). Newton also defined the nature of similar systems, those exhibiting proportional scale effects (Newton's Law of Similarity). He also had many other interests, not all of which were modern (alchemy, astrology and spontaneous generation), and was profoundly occupied by religious ideas. In 1689, Newton was elected a member of Parliament for the university but kept the seat only until the next year and never played a prominent political role. In the years 1692-94 he suffered a mysterious illness. Huygens in a letter to Leibniz described the condition as phrenitis and added that his friends had to keep him shut-up for a period of time. However, he seems to have made a good recovery, publishing his *Optiks* in 1704 and two subsequent editions of the *Principia* in 1713 and 1726.

Newton became a national hero and was knighted by Queen Anne in 1705. In 1703 he was elected president of the Royal Society, and each year thereafter for the rest of his life. In 1695, Locke, Pepys and others called Newton's poverty to the attention of Lord Halifax, and he was made Warden, and later Master of the Mint. He undertook his duties at the Mint as seriously as his scientific studies, applying his knowledge of metallurgy to the development of new coinage. Unfortunately this displaced his scientific activity. In 1721, at an age of 79, Newton's health began to fail, and he died in 1727. He was buried with honors in Westminister Abby, and vertically, a usual space-conserving practise perpetually memorializing $+G_z$.

Newton was totally devoted to his science, and is described by the Durants as a "monk of science." His unusual powers of concentration have been reported by many. He would consider a problem for weeks on end until a solution was achieved. His personality has been described as shy and aloof. However, he was very sensitive, particularly to criticism and is reported to have responded poorly to it. He was irregular in sleeping and eating, and indifferent to his personal care. He had little, if any, recreation activity, and his life was virtually devoid of humor or romance.

Newton's early formative years were in a time of great social upheaval in England -- the Civil War (1642-48), the Commonwealth (1648-60) and restoration of the monarchy (1660). Contemporary science depended upon modified principles of Plato and Aristotle. In Newton's lifetime, science became modern, and his role in the transformation was major. Although a quantitative approach to science had been used earlier by Galileo, Copernicus and Kepler, it was Newton who consolidated the quantitative character and precision of modern science.

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

10-15 October 1982, San Diego, California

The Fourth Annual Meeting of the International Commission of IUPS on Gravitational Physiology was held in San Diego, CA on 10-15 October 1982 in conjunction with the 33rd Annual Fall Meeting of the American Physiological Society, at their kind invitation. The Gravitational Physiology activities included a one-day symposium of 16 invited papers on a variety of topics. Four days of open sessions in gravitational physiology were also held, during which 68 voluntary papers were given.

The following 170 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. We also express appreciation to the Society for their gracious hospitality and provision of excellent facilities for the meeting.

The Fifth Annual Meeting of the IUPS Commission on Gravitational Physiology will be held in the USSR, at the kind invitation of the USSR Academy of Sciences and the Ministry of Health. A Preliminary Announcement giving details appears on the next page, and a welcome to participate is extended to all interested scientists.

PRELIMINARY ANNOUNCEMENT

IUPS Commission on Gravitational Physiology
Fifth Annual Meeting
July 1983, Moscow, USSR

The Fifth Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences is being planned for the USSR, tentatively in Moscow in July 1983. The meeting will be held at the invitation of the Soviet Academy of Sciences and the Ministry of Health, USSR.

The Commission Meeting will comprise open sessions for slide presentations of contributed 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 role of gravity in the manifestations of scale effects in animals and plants. The Commission Meeting will also comprise a one-day symposium by invited speakers on several topics in gravitational physiology.

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

Your participation in the Commission Meeting is welcomed. If you are interested in the particulars, please complete the form below and send it to Dr. Orr E. Reynolds, Commission Business Officer, by 15 February 1983.

Airmail to: Dr. Orr E. Reynolds
American Physiological Society
9650 Rockville Pike
Bethesda, MD 20814 USA

☐ I wish further information about the 5th Annual Meeting of the IUPS Commission on Gravitational Physiology to be held in the USSR, July 1983.

☐ I plan to attend.

☐ I plan to present a paper at the Meeting.

☐ I cannot attend but wish to be kept on the Commission mailing lists.

Name (Please Print) _____

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THE EFFECT OF GRAVITY ON PLANT CELLS

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INTRODUCTION

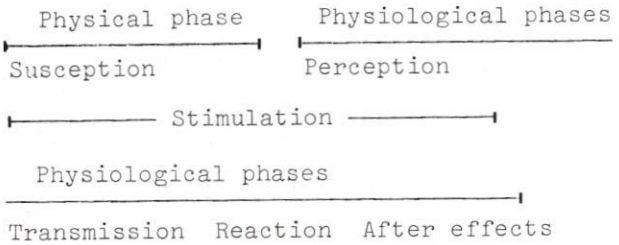
The direction of growth of plant organs and to some extent also the internal structure of the organs is determined partly by the action of gravity on the plants. When the various organs (roots, stems, leaves) are turned away from their normal position with respect to gravity, they perform gravitropic (geotropic) movements which enable them to approach their normal orientation. Main roots show a positive response, main stems a negative response and leaves orientate themselves at a certain angle to the direction of gravity in a plagiotropic reaction.

THE STATOLITH THEORY

Two alternative theories have been put forward to explain the primary action of gravity:
The statolith theory; sedimentation of movable cell organelles (statoliths) enables the cell to perceive gravity (6,9).
The geoelectric theory; gravity induces an electrical potential difference between the upper and lower sides of plant organs placed horizontally (2).

More recent results (see e.g.12) have shown that the latter theory is no longer valid since the measureable potential difference is a purely physical phenomenon appearing under anoxia and in dead tissue.

It is generally accepted that the gravitropic movement is a result of a reaction chain, similar to that applicable to a number of other physiological processes. In the chain a theoretical distinction can be made between various phases:



The term stimulation is commonly used to comprise the physical phase and the first physiological phase called perception. In plant gravitropism the physical phase consists of the direct action of gravity on the statoliths. The mechanical effect performed by the statoliths creates a certain "excitation" which is forwarded in the transmission phase to the site of reaction where the reaction proper - the gravitropic curvature - takes place. The curvature which appears in the elongation zone of the plant organ is a result of a transversal redistribution of plant growth regulators. This unequal distribution of such regulators as indolyl-acetic acid and/or abscisic acid in the upper and lower part of a horizontally stimulated stem or root gives rise to differential growth which ultimately leads to curvature.

SITE OF PERCEPTION

As yet there is insufficient experimental evidence to locate the perception site in negatively gravitropic organs (coleoptiles, hypocotyls, shoots and grass nodes).

In hypocotyls of sunflower the site of perception is thought to be in the peripheral cell layers (3), and the gravitropic response appears simultaneously in all parts along the length of the organ.

In roots the site of perception is in the root cap - the extreme outermost part of the root tip. Experiments have shown that if the cap is removed surgically, the root is unable to perceive gravity although the rate of elongation of the root remains unaltered. In addition, the cap is also the site of production and/or release of plant growth regulators. From the cap the signal or regulators are transmitted to the subapical elongation zone where curvature takes place as a result of differential growth.

GRAVITY-PERCEIVING CELLS

Fig.1 shows a longitudinal median section of the root cap of garden cress stained with PAS (perchloric acid and Schiff's reagent) which stains carbohydrates including starch grains in the amyloplasts. In the normal vertical position the amyloplasts are located in the bottom end of each of the cells in the central portion of the root cap (Fig.1a). In a similar section of a root kept

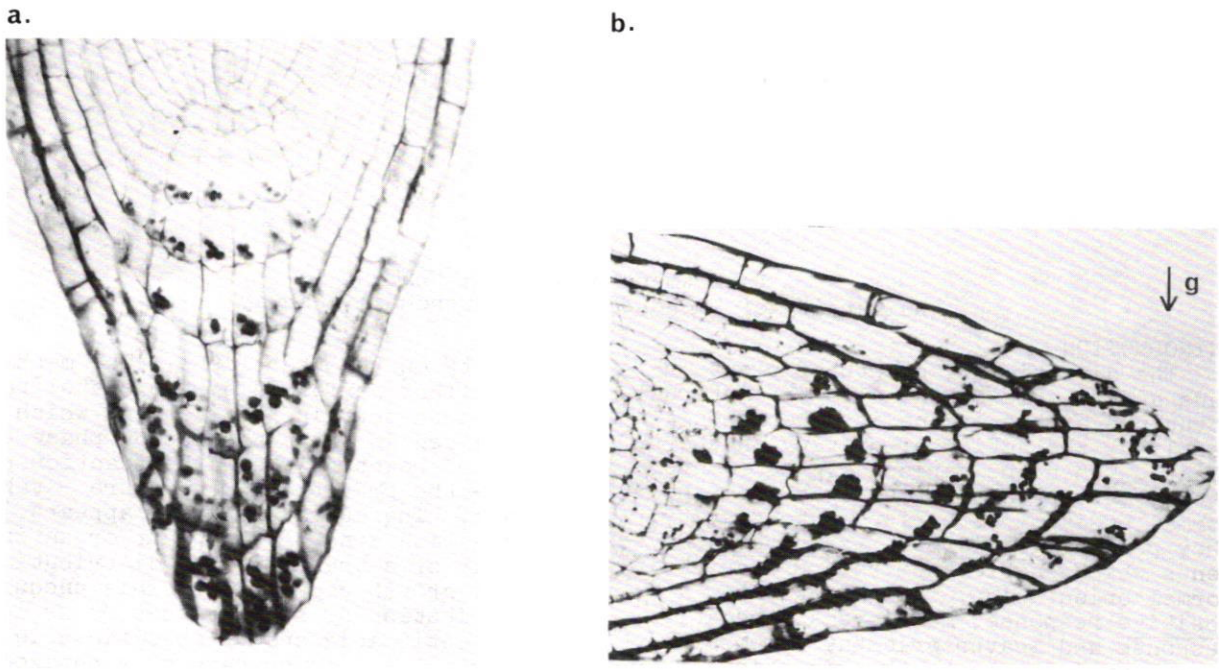


Fig.1. Longitudinal section of the root tip of garden cress (Lepidium sativum) stained by the PAS method. a: The amyloplasts in the youngest (most proximal) root cap cells are close to the floor of each cell. Root kept in the normal position. b: The amyloplasts close to the lower, longitudinal cell wall. Root kept horizontal for 15 min.

horizontally for 15 min before fixation, the amyloplasts have slid to the lower, longitudinal walls of the cells (Fig.1b). The central root cap cells - statocysts - are generally accepted to be the gravity sensing organ in plant roots and the amyloplasts are sensors for the perception (statoliths). In coleoptiles the statoliths are usually located in a "starch sheath" around the central cylinder and in young stems in specific layers.

Several attempts have been made over the years to demonstrate the dependence of the gravitropic reactions on the presence and movements of statoliths. So far the evidence obtained supporting the statolith theory is convincing but indirect:

- There is a close correlation between gravitropic response and assumed or observed movements of statoliths (8).
- Statocysts from which statolith starch have been removed do not show gravitropic responsiveness (7).

It has been suggested that other particles might function as statoliths e.g. mitochondria, microsomes or protein molecules. According to calculations of Audus (1) and Gordon (4) the mitochondrion and the amyloplast are the only organelles that, after stimulation of the root, could settle in statocysts at rates compatible with known minimum stimulation times.

MECHANISMS OF GRAVIPERCEPTION

What is the primary mechanism of statolith action within the statocysts? Volkmann and Sievers (12) put forward three alternative suggestions as to the primary action of statoliths after horizontal stimulation:

1. The new distribution of statoliths after gravity - induced translocation is the important factor.
2. The sliding of the statoliths along cytoplasmic structures during translocation is the intracellular stimulus.
3. Whether or not true translocation occurs, the critical factor is the change in amount and direction of pressure exerted on the original site of sedimentation i.e. on a sensitive structure.

At present the third model is the most interesting and its relevance can be most clearly demonstrated in cress statocysts. In these cells a striking polar arrangement of cell organelles can be found; near the distal cell pole a multiple layer of cisternae of rough endoplasmic reticulum (ER) is formed onto which the starch statoliths sediment (Fig.2). The nucleus is located near the proximal wall while all the other cell organelles are distributed at random. The ER-complex is considered to be the sensitive substratum; in the normal vertical position the statolith pressure on the ER-complex is equal in

the statocysts. Any deviation from this normal orientation e.g. a horizontal stimulation, causes a change in pressure. This may be the basis for the asymmetrical signal which results in the gravitropic curvature.

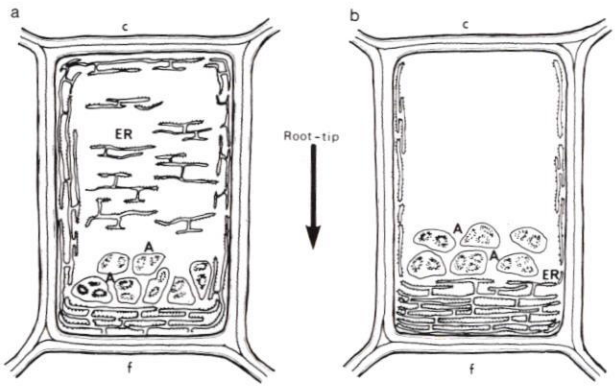


Fig.2. Semi-schematic representation of statocyte cells in ageotropic (a) and normal (b) pea root. The distribution of endoplasmic reticulum (ER) and amyloplasts with starch grains (A) in statocyte in the normal, vertical position. The root tip direction (arrow) and the lower (f) and upper (c) part of the statocyte are also indicated.

Further support for the idea of interaction between statoliths and ER comes from work on ageotropic plants (10). In a mutant of pea which does not react gravitropically it has been found that the ER is distributed at random in the cell (Fig.2b).

It has been demonstrated experimentally that membranes are affected under defined pressure e.g. changes of $2 \cdot 10^6$ dyne$\cdot$cm^{-2} induce action potentials (13). Enzyme activities are also influenced; acetylcholinesterase is activated by 10 dyne$\cdot$cm^{-2} (11), a value comparable to the pressure of 2-4 dyne$\cdot$cm^{-2} exerted by amyloplasts. Interactions between the amyloplast membrane and the ER-complex may also result from electrostatic forces.

At present experiments on these lines are in progress in our laboratory. Cell wall-free statocysts, protoplasts, have been isolated and it is hoped that changes in charges on the individual protoplasts after subjection to different g values can be detected by electrophoresis.

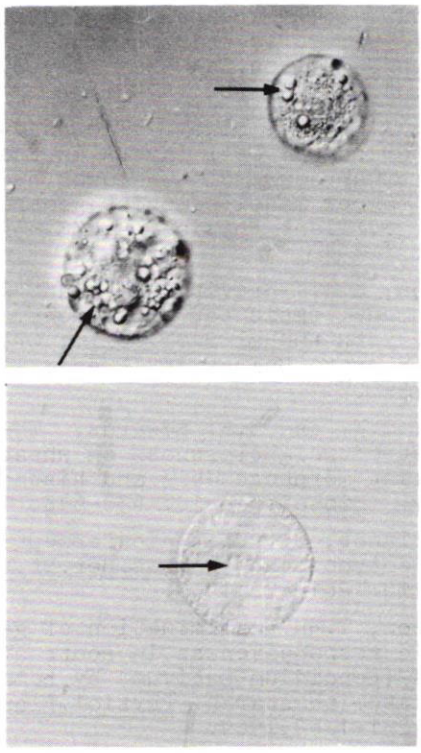


Fig.3 Protoplast (cell wall free statocyst) isolated from a root cap cell of cress. The amyloplasts are indicated (arrows). Interference contrast microscopy.

PLANT GRAVITROPISM AND SPACE RESEARCH.

In earth-based experiments, gravity cannot be eliminated, but by rotating plants with their main axes parallel to the rotating horizontal axis of a clinostat, it is possible to compensate the directional influence of gravity. Ground based experiments with clinostats may well yield results qualitatively similar to those obtained in an orbiting satellite (5), but for quantitative data on threshold accelerations a space laboratory seems indispensable.

The Microgravity Programme under the European Space Agency offers such an opportunity. A number of experiments, chosen from proposals submitted by European biologists, are to be performed in the Biorack in Spacelab. Among these are

plant biology experiments which may provide conclusive evidence for the statolith hypothesis and endomembrane interactions in the statocysts.

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THE FIRST PLANTS TO FLY ON SHUTTLE

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Sunflower seedlings were the first plants to make a journey for scientific purposes in earth orbit on board a NASA Shuttle. All scientific test objectives were achieved although not until our third attempt to gain useful results. The test was called HBT (Heflex Bioengineering Test) because its chief purpose was to validate the culture system we plan to use in a Spacelab-1 experiment called Heflex now scheduled for flight on 30 September 1983. Thus HBT was an empirical verification test and its scientific interest lay only in that it was important support for Heflex.

It is necessary to explain some features of Heflex in order to make clear the purpose of HBT. Heflex (Helianthus Flight Experiment) will measure in microgravity the growth kinetics of 4 day old sunflower seedlings. On earth plant organ growth is invariably a cyclic process induced by differential growth which leads to oscillations at a frequency of about ½ cycle per hour. The process is called circumnutation, often shortened to nutation.

Scientific interest in this ubiquitous phenomenon of oscillating growth stems from physiologists' desire to understand the mechanism that controls or drives the oscillations. Gravitational physiologists in particular are concerned because, according to one biophysical theory (4), the process should be gravity dependent yet, according to a different view (3), gravity is not the exclusive driver for the oscillations. In spite of various kinds of ground based experimentation the matter remains unresolved (5). However, the nearest approach to a consensus has been the recognition that a free fall (satellite orbit) experiment would test directly whether circumnutation could persist in the absence of a g-force as driver. That is the essential design feature of Heflex (1,2).

We shall use sunflower seedlings in the Heflex experiment only because Helianthus annuus has been the test subject for much recent work on the circumnutation process. As background for Heflex we have examined in depth the environmental parameters that affect the kinetics of hypocotyl nutation and have standardized plant age, temperature, growth medium, and other conditions that were found to affect either amplitude or frequency of the oscillations. Plant age, shoot height, and growth rate are correlated. Older (taller) plants characteristically nutate more slowly but oscillations have greater amplitude. Changes in water content of the soil mixture used as growth medium alter the relationship between plant age and shoot height. We standardized on a water content of 70 weight %, which is near the value that supports most rapid growth. (Fig. 1).

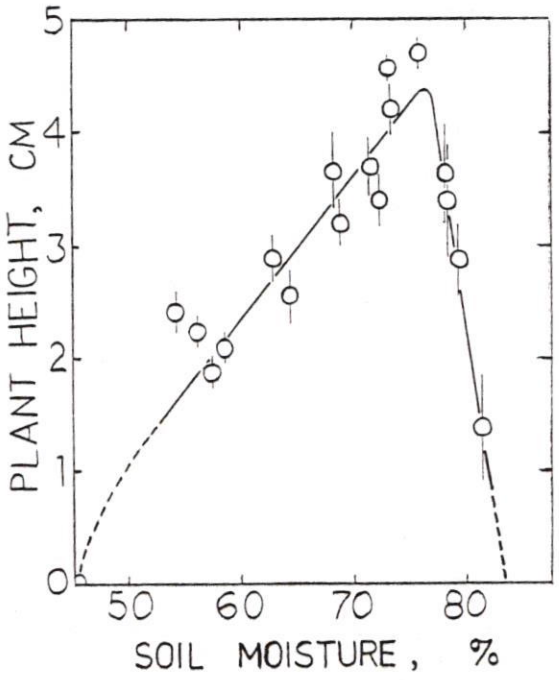

Fig. 1. Heights of 96 hr old plants in relation to soil water % at 1 g.

However, we probably do not understand all factors that account for growth retardation below the optimum water content nor the much greater retardation above that optimum. One may reason that the growth/moisture function of Fig. 1 reflects inhibition from water deficiency toward one extreme and suppression perhaps from anaerobiosis of the flooded root system at the other extreme. It seems possible that distribution of water throughout the soil may be critical for establishing just where the inhibitory influence dominates the stimulatory effect of increasing water content. At 1g the relationship of Fig. 1 obtains, but in

weightlessness the water, subject only to the influence of surface tension, could move by capillarity to assume a different distribution, conceivably more (or less) conducive to plant growth. The curve might then shift to the left or to the right in a free fall environment.

While pondering that possibility we learned that early Soviet tests of seed germination and plant development in weightlessness had yielded poor results initially. This was attributed to the "wicking effect" of the material used for holding the seeds and seedling root systems which evidently had worked well on earth but had "drowned" the plants in orbit (6).

One cannot simulate on earth the condition of protracted weightlessness for studies of moisture distribution in a porous medium like soil but the matter was testable in satellite orbit. We proposed a test to be performed during a 4 day flight of the Columbia in which a series of culture modules would be prepared in which seeds would be planted in our standard soil mixture covering a wide range of water contents. We hoped to obtain a curve comparable with Fig. 1 although with fewer data points due to limitation of space in the Shuttle middeck locker allocated for the bioengineering test, HBT.

Test flights of the Columbia were planned for 2,4 and 7 days duration. To be most nearly comparable with plants to be grown for the Heflex experiment, 4 or 5 day old plants would be ideal. HBT was scheduled by NASA as part of the OSTA-1 payload on the STS-2 mission that was expected to last 5 days.

The HBT flight package was a suitcase-like Plant Carry-on Container (PCOC), exactly the same as will be used for Heflex. It accommodated 85 germinating seeds. Eight were in modules just like those to be used in Heflex; the soil in these was at 70% water content. The remaining 77 seeds were in narrower "long modules" but the glass-lined aluminum "pots" containing the soil mixture were the same for all 85 seeds.

Thus, the PCOC, 8 modules, all pots, and all soil mixtures were high fidelity simulations of Heflex flight hardware. Soil in the 77 long modules had water contents in which the range from 55% to 77% was represented.

In Heflex the temperature can be controlled actively to maintain 24°C but HBT was thermally passive. A small battery operated temperature recorder included in the PCOC was capable of electronic recording of temperature at 15 min intervals throughout the mission. The resulting thermal profile would be used later for a 1g simulation of a successful flight test that was not achieved until our third attempt to acquire useful HBT data.

Planting seeds, loading the PCOC, activating and inserting the temperature recorder, transporting the flight package to the launch pad, up the elevator, through the white room (crew access passageway), and into the assigned locker was accomplished shortly before midnight on 3 Nov. '82. The launch scheduled for the next moring did not occur due to a Shuttle equipment malfunction; it was rescheduled for 12 Nov.

For a second time we went through all the same preparatory steps the night before launch and this time the Columbia lifted off successfully. We learned almost immediately that problems with the Shuttle might dictate a drastically shortened mission and by mid-afternoon that was confirmed. By accelerating our travel plans we arrived before the Columbia at the NASA Dryden Flight Research Facility, adjacent to the landing site, Edwards Air Force Base. The altered mission time, 54 hr, was insufficient for HBT to provide useful data on soil moisture effects.

A reflight of HBT, now referred to as HBT-II, was recommended and was scheduled for STS-3 although that mission, planned for 7 days, was longer than ideal for HBT. On the night of 21 March for the third time we planted seeds, loaded the PCOC, etc. and the following morning we watched another perfect lift-off. This time no problems with the Shuttle seriously threatened the completion of the mission although meteorological factors beyond NASA control forced the use of an alternate landing site, a dry lake bed on the White Sands Missle Range in southern New Mexico, and extension of the mission for an extra day because high winds and blowing dust had made a Shuttle landing on the scheduled day unacceptable risky.

On the morning of 30 March weather at White Sands was perfect, the Columbia landed, and 70 min after touchdown we opened our PCOC and began measuring 213 hr old Helianthus seedlings. All HBT objectives were achieved although a 1g control test was required since the flight had been substantially longer than any of our previous ground based tests and the temperature had varied considerably from the too-much-to-hope-for constant 24°C.

The control test was performed at our home laboratory several weeks later (Fig. 2). This

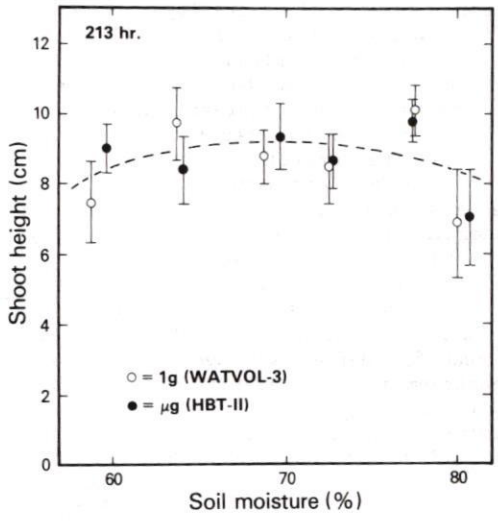

Fig. 2. Comparison of flight results (HBT-II) compared with those of 1 g control (WATVOL-3).

imitated the duration and the temperature profile we had recorded in the PCOC on STS-3. There was no significant difference between the data trend of shoot height vs soil water % as measured on STS-3 and on the ground. Results also confirmed that flight plants in high fidelity Heflex modules growing in soil at 70% water content were as acceptable for use in the Heflex experiment as were those grown at 1g. Therefore we do not propose any change in the soil water content we planned for Heflex.

Because they may be of practical importance to future Shuttle experimeters, two kinds of environmental observations (g-force and temperature) made in connection with the STS-2 and STS-3 Missions are worth discussing.

The NASA Shuttle's capability to achieve a protracted hypogravity condition is not absolute. At the orbital altitudes to be anticipated there is not a perfect vacuum. Atmospheric drag will provide a low but finite g-force on the vehicle. Drag is a function of altitude and attitude of the vehicle. We may expect the force to be between 10^{-4} and 10^{-6} g. We know of no evidence that suggests that plants' (or animals') bioaccelerometers can detect such weak forces.

Prior to reentry a velocity decrement must be induced by a brief retrorocket firing. This amounts to a small g-pulse in the same vector direction with respect to the payload as occurred during launch acceleration. On the STS-3 Mission this occurred at exactly 15:13:29.8 (Greenwich Mean Time in hr:min:sec). The retropropulsion was established very quickly with a slower tail-off to zero during engine shutdown. A worst-case calculation assumes -0.152 m s^{-2} for the entire 149 s of the "deorbit burn" which amounts to -2.3 g·s. For the few species of higher plants that have been studied carefully this application of an accelerating force would be below the plants' level of detection.

Deceleration caused by atmospheric drag increasing during reentry was not detected by the on-board accelerometers until 21 min after completion of deorbit burn. Deceleration then increased progressively for 15 min, achieved a maximal value of 1.6 g, then declined toward 1.0 g for the remaining 12 min before touchdown. Integration under the g-force vs time profile yielded a g·t product of 26.5 g min. (Fig. 3).

Although the literature on plants' g perception thresholds provides information on only a few species, chiefly monocots, and reveals between 1 and 2 orders of magnitude disagreement even on the best studied species, Avena sativa, a conservative upper limit may be taken as about 5 g·min for the minimal accumulated g-force x time product needed to cause a detectable plant response. It is, therefore, quite significant that a Shuttle-borne microgravity experiment can be expected to return to the botanical investigator plant subjects that have been exposed, even before the Shuttle lands, to at least five times the g-force experience that would be expected to cause a gravitropic response by his test plants.

That is not all the bad news; subsequent to landing there are safeing procedures that must be carried out on the vehicle, medical certification of crew members' conditions, crew egress accompanied by ceremonial photographic and other activities---all before an experiment package can be removed from the Shuttle middeck stowage locker and transported to the Principal Investigator's field laboratory. On both the STS-2 landing at Dryden Flight Research Center and the STS-3 landing at White Sands Missile Range our HBT flight package was received by the P.I. team 70 to 75 min after touchdown. Conceivably that time might be shortened in future, perhaps to as little as 30 or 40 min.

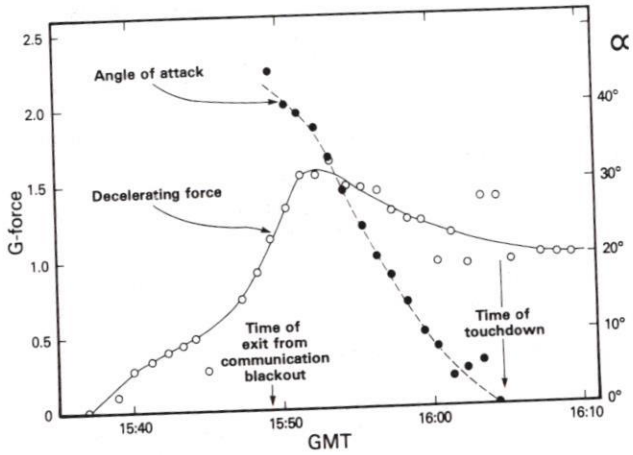

Fig. 3. G-Force profile of STS-3 during reentry to landing.

Nevertheless, it now seems unlikely that the post-landing g-experience could be reduced below about 35 min. Accordingly, under the best circumstances, an experimental payload carried in the mid-deck of the Shuttle probably would "feel" about 1 g·hr of gravitropic or gravimophogenic influence after leaving orbit but before the Principal Investigator Team would have access to it.

One g·hr is at least an order of magnitude greater than our most conservative estimate of the threshold stimulus for a gravitropic response. The literature on gravimorphic responses of higher plants does not include sufficient information on g-threshold values to support a usefully precise generalization. Most authorities cite a threshold value applicable to the gravitropic response mechanism and only tacitly assume its applicability to gravimorphic responses. In any case, it seems evident that, for those experiments for which such an in-flight and post-flight stimulus would be unacceptable, the experimental protocol must include in-flight preservation of the test material. Possibilities are: cytological fixation by chemical reagents or by deep freezing. Either method is feasible in principle. Latter is more attractive for several important but scientifically trivial reasons.

Another aspect of the Shuttle environment worthy of attention of prospective scientific customers for flight opportunities is the temperature that may be expected to prevail in ths middeck of the Shuttle. There is a bank of lockers---each a little smaller than the overhead luggage racks of our larger transcontinental or transoceanic passenger planes. Some of these lockers have been used and in future will be used to house experimental flight packages. It seems that NASA could identify no pressing operational reason for recording with good time resolution the temperature profile within a middeck locker on the first one or two orbital missions of the Shuttle vehicle, Columbia. But on the Columbia's 2nd and 3rd test flights we were able to record four times each hour the temperature within our test package (PCOC).

In the case of STS-2 when the PCOC was installed prelaunch in a locker its recorded temperature was 23.4°C. By the time of launch the temperature had risen to 24.8°C. Four hr after launch the temperature began rising until, 16 hr after launch, it was 27.4°C. For the remainder of the mission only minor flunctuations occurred. During reentry, removal of the PCOC from the locker, and transportation by van to the field laboratory the temperature was 26.7 ± 0.2°C. The relatively high in-flight temperatures that were recorded came as an unwelcome surprise since our desired temperature was 24.0 ± 0.2°C.

When HBT was reflown on STS-3, we again included a temperature recorder in the PCOC. Due to malfunction of part of the spacecraft cooling system on STS-2 but not on STS-3, to differences in spacecraft maneuvers on the two missions, and to the presence of an intermittent heat source from an experiment housed in a locker adjacent to the one that held our PCOC, we could not have expected temperature profiles recorded on the two missions to be in close agreement and they were not. On STS-3 the PCOC exhibited diurnal fluctuations in temperature of about 1.5°C. Also there was a progressive warming trend of 0.6°C per day. For more than 90% of the mission the daily mean temperature was above our desired upper limit of 24.2°C. For the last 4½ days of this mission the recorded temperature never was below 24.2°C (Fig. 4). Because temperature in the middeck will be important for most biological test subjects located there, we hope NASA will quickly adopt a policy of monitoring this on all future Shuttle missions. Data accumulated in that way should be very useful for experiment planning.

In retrospect we may note that HBT achieved a number of "firsts". It was the first scientific test on a NASA Space Shuttle to use plants as test subjects. It was the first opportunity for a non-NASA experimenter to make use of the late access capability to load a test package into the Columbia while on the launch pad a few hr. before lift-off. It was the first test of the post-flight early recovery procedure which on future missions will be in great demand by biological investigators. Although not planned to fly more than once, HBT was the first biological test which was reflown after it was unable to provide useful data from an earlier mission. Finally it was the first biological test to be carried in an Orbiter middeck locker and therefore it was the first to acquire data on relevant environmental conditions, especially the temperature profile, within the locker throughout the mission.

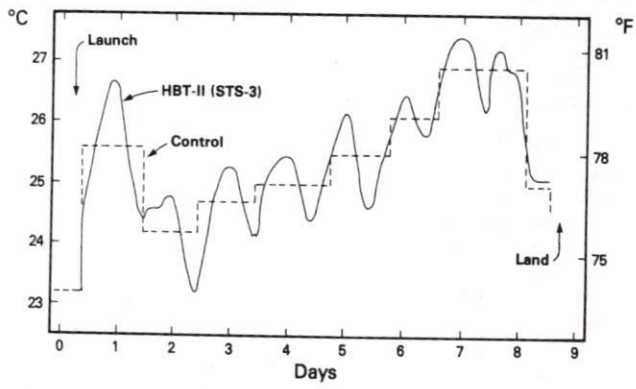

Fig. 4. Temperature profile recorded within PCOC during STS-3 compared with the profile used for 1 g control.

ACKNOWLEDGEMENTS. The HBT efforts and related ground studies were supported by NASA grants and contracts NGR-39-030-010 and NAS-9-15340 to the University City Science Center and by NGR 39-010-149 and NAS 9-15531 to the University of Pennsylvania.

Data on STS-3 reentry were provided by Mr. F. W. Brizzolara, Jr. of the Johnson Space Center. We are grateful for his help.

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THE EFFECT OF WEIGHTLESSNESS ON THE REPRODUCTIVE FUNCTION OF MAMMALS

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The development of life on the Earth included at least three periods when its inhabitants had an opportunity to feel a gravitational change: transition of living creatures onto the land, return of part of them to the ocean, and penetration into outer space. The striking diversity of the plant and animal kingdoms gives evidence that in the first two cases living organisms were capable to get well adapted to an altered gravity, having retained the ability to grow and to reproduce their like. However, it should be borne in mind that we are witnessing the result of adaptation being totally unaware of the losses which might occur at different stages.

The experiments in actual space flights are significantly shorter than the natural "experiments" that took place in the course of evolution of life on the Earth.

Onboard biosatellites of the Cosmos series Wistar rats were exposed to space flight effects for approximately 1/50 of their life time. They did not show marked decrease of their resistance or pathological visceral changes. However, it is still impossible to answer the questions as to how long mammalians of different species may fly in space and whether they may reproduce and grow the viable offspring in flight.

That these events are in principle possible suggest the data by Smith and Burton (11) who succeeded in growing over 20 generations of birds chronically centrifuged at 2 g. It is important to note that the initial population included a certain portion of birds with an increased tolerance to acceleration; the selection proceeded via the mating of the most resistant specimens which survived chronic centrifugation. In other words, the selection was performed, as in natural conditions, at the population level, using the specimens best adapted to a modified environment.

In real weightlessness experiments of this kind will be performed via a number of complex intermediate stages. One of them is the conduct of embryological studies to be carried out in space flight.

The embryological experiments on insects, fish and amphibians carried out in actual and simulated weightlessness are well known. The experiments on Drosophila melanogaster demonstrated no embryonic changes in space flight (4, 6). It is interesting that the Drosophila development in weightlessness was followed by no changes in the ultrastructure of muscles, particularly wing muscles. The experiment with fish eggs (Fundulus heteroclitus) exposed to the combined effect of clinostating and then space flight demonstrated a marked influence of zero-g on the early developmental stages - up to 52 hours (1). Zero-g produced an inhibitory discoordinating effect on the epiboly, gastrulation and differentiation of the axial complex of an embryo. Similar changes were seen in the clinostating experiment with amphibian eggs (Rana temporaria, Rana esculenta). The authors attribute these changes to the inhibition of segregation-transport processes in the ovoplasm (1, 3).

The effect of weightlessness on the prenatal development of mammals was not studied. The embryological experiments on invertebrates and lower vertebrates mentioned above can hardly be cited even for tentative prediction of the development of mammals due to significant differences in their resistance and reactivity.

It is known that as living organisms ascend the evolutionary staircase their resistance to environmental effects, including radial acceleration, goes down (9, 10). The high resistance of invertebrates and cold-blooded vertebrates to acceleration is associated with their metabolism and nervous function rather than with their size (9). For instance, large reptiles and amphibians show greater resistance to hypergravity than small birds and mammals (9). A similar correlation between resistance and the level of biological organization was also demonstrated in hypoxic studies (9). In regards to weightlessness it is more difficult to determine the dependence of total resistance on the position the species occupies in the evolutionary row. However, the dependence obviously exists, which determines the fact that insects show essentially no response to weightlessness (4, 6), whereas mammals exhibit distinct changes in the musculo-

skeletal system, red blood, lymph organs, and heart.

The effect of weightlessness on the prenatal development of mammals is likely to be mediated via the maternal organism. This may be accompanied by both specific changes associated with zero-g and nonspecific changes developing as a stress-reaction. Among the disorders in adult mammals osteoporosis, involution of lymph organs, suppression of erythropoiesis may be most important for fatal development in space flight (2). Fetal development requires significant activation of anabolic processes. To what extent may it occur in weightlessness, when total catabolism in an adult animal is enhanced? How will the organogenesis, particularly the development of bones in the case of calcium deficiency in the maternal body proceed? These are the questions which are still open. They will constitute the program of the embryological experiment to be flown on the upcoming biosatellite Cosmos. The experiment of 7 days in duration will be carried out on 10 female rats fertilized preflight.

There are many objective problems associated with the conduct of the embryological experiment in flight. Figures 1 and 2 show developmental abnormalities found in fetuses that resulted from the exposure of pregnant rats to the effects accompanying the launch and recovery, i.e. vibration, acceleration and impact acceleration. Such abnormalities, although rare, make it necessary to plan the flight experiment, taking into consideration the critical stages of prenatal development. In the first experiment the purpose of which is to determine whether a mammalian fetus may develop in weightlessness we will use the most stable developmental phase (gestation days 13-20). However, later this strategy would be changed because it is obvious that when we expose the most resistant specimens and developmental stages rather than the population as a whole we devoid ourselves of the possibility to follow the responses to zero-g in their extreme manifestations.

From the prospective point of view we will have to study the entire cycle of mammalian development in space flight: from fertilization to delivery and sexual maturation. However, such research may have many problems both technical and physiological related to stress situations at different flight stages.

An attempt to assess the fertilization capacity of rats in weightlessness was made in the Cosmos-1129 flight. As the animals produced no offspring, the events that took place in flight can be reconstructed only with respect to the indirect index - weight of flight females as compared to the controls, weight during normal gestation and gestation with resorption. On the basis of these data we can assume that in the 18.5-day flight 2 out of 5 females experienced fertilization and early stages of gestation (against 100% in the vivarium controls). The gestation could be discontinued due to the effect of impact acceleration which followed weightlessness-induced decrease of total resistance. In other females the factor responsible for the prevention of gestation could be a disorder in oestral cycles and fertilization caused by the stress effects of the early flight stages. Although these data are tentative, it is interesting to note that a similar situation was seen in the simulation studies in which female rats were exposed to the immobilization stress immediately before males were placed into the cage.

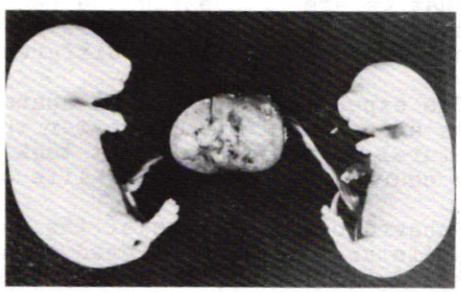

Fig. 1. Fusion of two fetal placentae of the rat exposed to vibration and acceleration on gestation day 8.

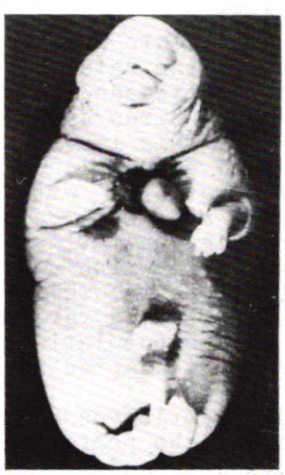

Fig. 2. Cardiac ectopy in the fetus of the rat exposed to impact acceleration on gestation day 11.

No matter how long space flights will be, they will always end by return to the Earth. Therefore, one of important aspects of the study of mammalian development in zero-g is to evaluate space flight aftereffects: growth, development, age-related changes in resistance and ageing rate of the animals after different stages of prenatal and postnatal life in zero-g.

The pertinent experimental data are so far scanty and controversial.

Two and a half month after the Cosmos-605 flight a study was carried out in which flight males were mated with vivarium females. The offspring did not differ from the control litters in the total number of newborns, weight at birth, weight gained during the first postnatal month, weight of different organs, blood parameters, and hypoxia tolerance (9). Similar results were obtained in the study in which flight males were mated with control females 2.5-3 months after the Cosmos-1129 flight. When the mating was done 5 days after flight, no increase in the pre- or post-implantation death rate of the offspring was seen either.

These findings suggest that the level of dominant lethal mutations in mature spermatozoids as well as in stem cells of spermatogenesis in the male rats exposed to zero-g for 1/50 of their life time was close to these parameters in the controls.

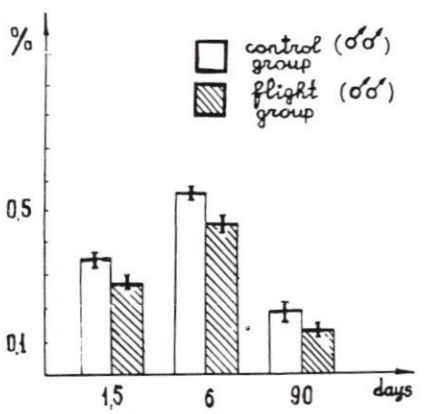

Fig. 3. Spleen mass (as a percentage of body mass) of the offspring of flight males mated with control females 5 days after recovery.

However, the state of the offspring produced by the nonflown females fertilized by the flight males soon after recovery differed from that of the controls. The flight offspring showed transient manifestations of physiological immaturity, e.g. delayed growth in the postnatal period, delayed eye opening and hearing initiation. At different stages of postnatal development (2, 6, 90 days) the flight offspring showed a decrease in the absolute and relative spleen weight (Fig. 3), and a significant decrease in the counts of lymphocytes and neutrophils in blood (Fig. 4). The thymus weight remained unchanged. The examination of flight offspring at the age of 90 days showed smaller changes in the lymphocyte and neutrophil counts in response to a single immobilization stress and a smaller decrease in the spleen weight during repeated immobilization. The thymus reaction to the stress did not alter (Fig. 5).

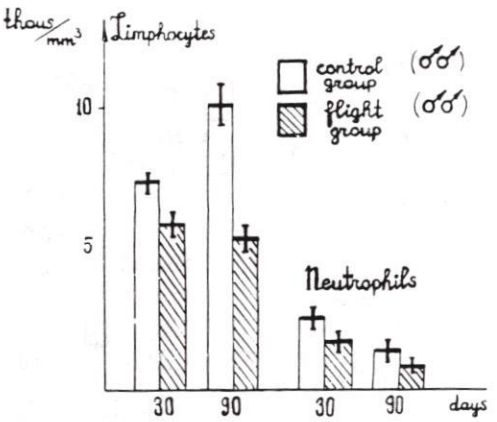

Fig. 4. Lymphocytes and neutrophils in the offspring of flight males mated with control females 5 days after recovery.

The changes seen in the offspring obtained after early mating may be associated with the general asthenization of flight rats and decline of their resistance (7). This hypothesis finds support in the fact that the time interval between the placement of males till their mating with females in the experiment was 2.5 days longer than in the controls. Our data is actually the first attempt to assess the reproductive function of animals mated at different time intervals after flight. The conclusions from these observations should be drawn with great caution. But it is clear today that further investigations should address potential aftereffects.

Embryological experiments with mammals in space flight are still at the earliest developmental stages. Nevertheless, we hope that the study of the effect of weightlessness on different ontogenetic stages of mammals beginning with fertilization and early developmental

stages and ending with the rate of postnatal transformations and ageing may become an interesting model for the investigation of general mechanisms of the physiological effect of zero-g. The outstanding Russian physiologist L.A. Orbeli stated that "the correct understanding of the evolution of functions is possible when the same researchers use four procedures, basically different but leading to the same purpose," They are: use of comparative physiology, study of ontogenetic material, use of clinical data, and special experiments (6). One can hardly doubt the fact that the application of the ontogenetic approach to the exploration of the effects of weightlessness will provide a better understanding of the mechanisms of its action.

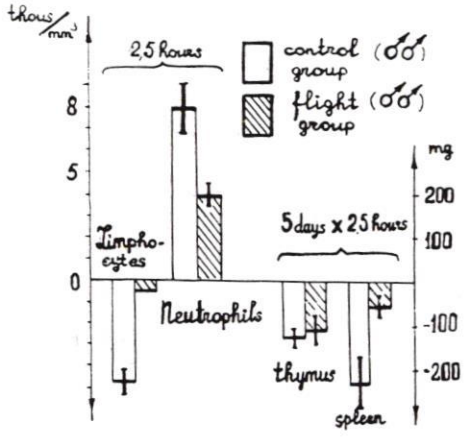

Fig. 5. Reaction to immobilization stress (variations in the parameters in comparison to the initial values) in the 3-month old litter of flight males mated with control females 5 days after recovery.

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EFFECTS OF CHRONIC ACCELERATION ON BODY COMPOSITION

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ABSTRACT - These studies were made possible by three technical factors: 1) the availability of chronic centrifugation of rats for prolonged periods of time, 2) the fat-free basis for expressing composition, and 3) success in routinely separating all muscle from bone. Centrifugation of adult rats revealed an unexpected decrease in mass of fat-free muscle and bone, in spite of the added load induced by centrifugation. During centrifugation the lower but constant fat-free body mass was probably regulated because under different circumstances it was approached from either above or below. Rats weightless for 18.5 days, also unexpectedly, give indirect but strong evidence that muscle had increased in mass. Other changes in the weightless rat included: a smaller fraction of skeletal mineral, a smaller fraction of water in the total fat-free body, and a net shift of fluid from skin to viscera. Adult rats centrifuged throughout post-weaning growth showed smaller masses of bone and central nervous system (probably attributable to slower growth of the total body) and a larger mass of skin than controls at 1g. Efforts at simulating the effects of weightlessness or centrifugation by regimens at terrestrial gravity were unsatisfactory.

In our efforts to apply the concepts of regulatory physiology to body composition we have been facilitated by three technical or methodological factors:

(1) the fat-free basis for expressing composition.
(2) the availability of chronic acceleration which will perturb the ordinarily highly stable fat-free body mass (FFBM) in rats of all age groups.
(3) our success in routinely separating all muscle from bone.

The realization that neutral fat, being primarily an inert store of fuel, dilutes and obscures significant regularities in body chemical composition (3) is still poorly appreciated and frequently ignored in the collection and analysis of data. More recently it has been shown that body fat also obscures regularities in organ and tissue composition of the body (4,5).

It would have been difficult to study regulation of mass and composition of the fat-free body without the ability to perturb it, and chronic centrifugation enabled us to do so in a predictable and reversible manner even in adults where it is imperturbable by most factors (8).

Finally, the separation of total skeletal musculature (the largest component of the fat-free body) from bone was largely a triumph of determination over tedium. However, it enabled us to study mass changes on musculature as a whole rather than on selected individual muscles.

CHRONIC CENTRIFUGATION

Adult free-moving rats were centrifuged 24 hours a day every day with one or two 1-hour service stops per week. Most of our studies were carried out at accelerations between 2.76 and 4.15 g, within which range rats are judged normal and healthy by several lines of evidence. Rats reach a new steady state in body mass by 10-14 days after the initiation of centrifugation (8).

A priori it appeared reasonable that during centrifugation muscles would have to develop much more tension for maintenance of posture and locomotion leading to an increase in mass (hypertrophy). However, the contrary was observed, the inverse rectilinear relationship being obvious in Fig. 1. No hypothesis to explain this unexpected result has been advanced.

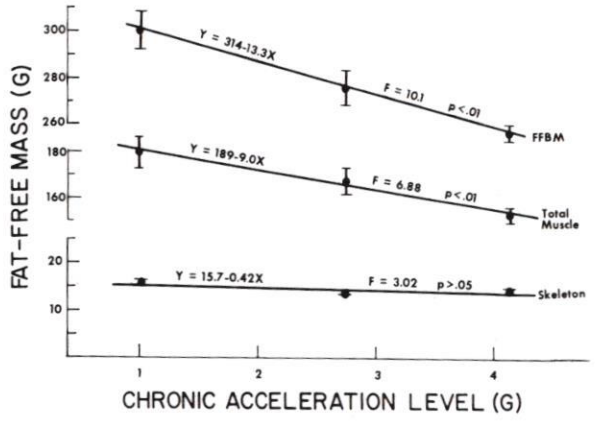

Fig. 1. Some effects of 60 days centrifugation at 3 g-levels, 8 rats per group.

The new steady state values observed at hypergravic states are completely reversible upon returning to terrestrial gravity (6).

The failure of bone to respond is also obvious in Fig. 1. This too was unexpected because although the mass of muscle exerting stress on the skeleton was reduced by ∿15%, the weight of the body supported by it during centrifugation was increased 4.15-fold which certainly caused increased strains in the skeleton.

Among other components unaffected by centrifugation were gut, skin and central nervous system.

There are several lines of evidence that the lower but parallel fat-free body mass during centrifugation is regulated. Perhaps the principal evidence is that animals experimentally displaced from that parallel level will approach it from either above or below, as in Fig. 2.

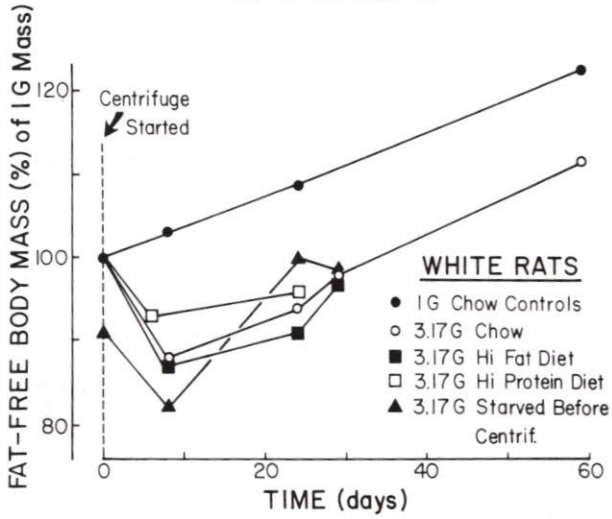

Fig. 2. Effects of centrifugation on fat-free body mass of groups (N=6 to 8) experimentally displaced upward or downward.

WEIGHTLESSNESS

Unrestrained rats in individual cylindrical compartments were exposed to 18.5 days of weightlessness in the Soviet Cosmos Mission 1129 (9). After recovery their bodies were dissected and analyzed chemically.

Muscle and bone were not separated in this study. However, the total body content of nitrogen, potassium and creatine was each significantly higher in the exposed animals than in the controls. It is difficult to escape the conclusion that there was a statistically significant increase in muscle mass of the rats returned from space. In an earlier study of rats returned from 19.5 days of weightlessness a statistically significant increase in the mass of the quadriceps muscle was reported but not commented upon (10).

The rats in the exposed group also showed the following statistically significant differences from the controls: a smaller fraction of mineral in the skeleton, a smaller fraction of water in the total fat-free compartment, and a net shift of fluid from skin to viscera. These corroborate findings on astronauts (2, 11).

GROWTH DURING CENTRIFUGATION

On rats introduced to centrifugation (4.15 g) at 35 days of age samples for body composition analysis were taken at seven age intervals up to 360 days of age (6). The centrifuged group showed the following statistically significant differences from controls at terrestrial gravity: smaller masses of FFBM, muscle, bone, CNS, heart and kidneys and a larger mass of skin. Obviously, the principal differences from the results of centrifuging adult rats were the changes in bone, skin and CNS which were unchanged in the centrifuged adults. These differences were probably all attributable to the interaction between centrifugation and the growth process.

Further insight into the relative growth of individual components can be obtained from a log-log plot which linearizes the data (Fig. 3).

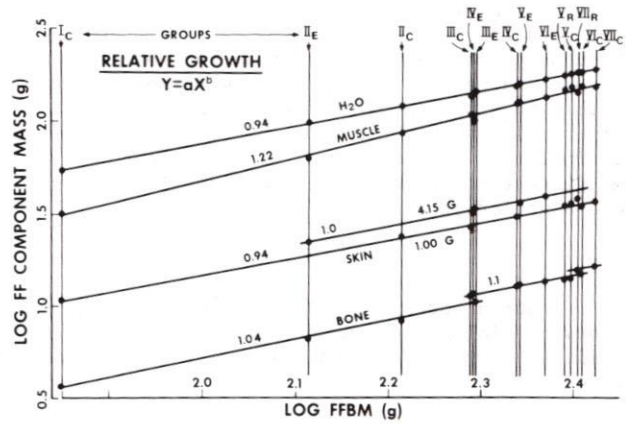

Fig. 3. The allometric plot of relative growth. The slope (b) is given above each line. Roman numerals identify sacrifice groups and subscripts C, E and R indicate 1 g, 4.5 g, and retired from 4.15 g respectively. Eight rats/group.

The slope of the straight line fitted to each component is the exponent b of the simple power function

$$Y+aX^b \qquad \log Y=\log a+b\log X$$

which has been termed the allometric equation and closely describes a wide variety of data on relative growth.

In Fig. 3 the growth of each component is seen to be quantitatively related to growth of the total fat-free body by the parameter b. Skin alone, besides its relationship to the FFBM (b is ∿1) also shows two populations (1 g vs 4.15g). The response of bone in Fig. 3 is clarified by reference to an earlier study (5) which showed log bone mass in mature rats to be independent (b=0) of log FFBM and determined solely by age. In these less mature rats bone appears to be in transition between growth associated with FFBM and with age.

EFFORTS AT SIMULATION

A regimen carried out at terrestrial gravity which replicates the body composition effects of Δg might help identify physiological mechanisms common to the two conditions besides having valuable practical applications. Simulation of either weightlessness or hypergravic states in the adult rat is difficult because these Δg conditions alter size and composition of the adult FFBM whereas the only way reported to do so at 1 g is by fasting. The body composition effects of starvation (1) differ in important respects from either ±Δg. However, the effects of an exercise regimen initiated soon after weaning is compared with those of centrifugation in Table 1 because the physical stress of increased activity at 1 g might simulate that of normal activity during centrifugation.

The study in table 1, column 2 was made on adult rats whereas those in columns 3 and 4 were started at 35 days of age (growth studies). The two centrifugation studies are in good agreement except for skin which is larger in the growth study, and central nervous system (CNS) which is smaller in the growth study. CNS is relatively insensitive to Δg per se and the smaller values in both growth studies (Col. 3 and 4) are probably due to the lower growth curves followed by rats exercised or centrifuged starting at an early age.

In the table we see three tissues of the eight which respond differently to treadmill exercise and to centrifugation: bone, heart, and skin. Clearly treadmill running is only modestly successful as a simulator of hypergravic states. This supports our earlier conclusion from rats exercised during centrifugation that the physical load induced by centrifugation has little effect on body composition (7).

Table 1. - Body composition effects of chronic centrifugation compared with exercise [(Control-Experimental)/Control] x 100

Exptl. regimen	4.15g	4.15g	Treadmill[3]
Start at	120 da	35 da	35 da
End at	180 da	243 da	141 da
Organ \ Ref	(8)	(6)	(5)
FFBM	86.0[1]	89.8[2]	82.2[1]
Muscle	84.4[1]	85.1[2]	80.9[1]
Bone	91.6	91.4[2]	97.7
Heart	85.4[1]	90.6[2]	99.3
Gut	101.7	101.7	98.9
Skin	96.5	105.4[2]	77.7[1]
Liver	82.8[1]	91.9	82.6[1]
CNS	100.4	93.8[2]	92.1[1]

Controls were 1g (col. 2&3) and sedentary (col.4). Statistically significant difference between experimental and control groups: [1] by t test ($P<.01$); [2] by analysis of variance ($P<.01$) on the last three time points - 140, 214 and 243 days of age. [3] Forced running 18 m/min, 14% grade, 2x30 min/day, 7 days/week. CNS = brain and spinal cord.

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ADH RESPONSES TO VOLUME SHIFTS IN THE LOW PRESSURE SYSTEM

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ABSTRACT

Antidiuretic hormone (ADH) responses to blood volume shifts in the low pressure system depend on the state of hydration of the subject and the interaction between the osmoreceptors and the vascular receptors of the low and the high pressure systems. In the hydrated subject, vascular fluid becomes hyposmotic as the result of water immersion. ADH release is inhibited by the osmoreceptors. In hydropenia, immersion does not produce a hyposmotic plasma and ADH is inhibited through action of the low pressure system receptors. There is a further interaction between the high and low pressure system receptors, the former exerting greater influence on ADH release in hypotension. Vascular receptors adapt, as has been shown in the case of atrial receptors, and buffer acute changes rather than serve the role of absolute regulators.

The state of hydration does play a role in determining the ADH response to blood volume shifts in the low pressure system. Both osmotic and volume factors become primary. Schrier et al in 1979 (1) thoroughly reviewed the osmotic ADH release. They further provided a schema for the interaction of osmoreceptors and vascular receptors on the neurosecretory cells of the supra optic and paraventricular nuclei of the hypothalamus. (Figure 1) The vascular receptors can be those located in either the high or low pressure system. There is a further interaction to consider, that occuring between the receptors of the high pressure system and those of the low pressure system.

Of particular note is the work of Menninger (2) who demonstrated that directly stretching the left atrium inhibited the firing rate of over 70 per cent of the antidromically identified secretory cells in the supra optic nucleus. He extended his observations to include the right atrium (3) and found that both right and left atrial stretch inhibited ADH release although the left atrium gave the predominant response.

By following changes in plasma ADH while sequentially sectioning the innervation of the aortic and carotid baroreceptors and the cardiopulmonary receptors, Thames et al (4) concluded that the cardiopulmonary receptors with vagal afferents tonically inhibited ADH release. In a subsequent study, these same authors (5) demonstrated an interaction between the two receptor groups by following plasma levels of ADH as carotid sinus pressure was varied with and without cold block of the vagus nerve. Arnauld et al (6) have studied the interaction between the high and low pressure system receptors in experiments on the unanesthetized monkey using hemorrhage as the stimulus. Although these authors conclude that simultaneous blood volume and arterial pressure reductions are necessary for ADH release, their data on this point are not convincing. Weitzman et al (7) have re-examined the data and concluded that an improper statistical assessment of their data was used. Similar experiments on dogs reported by Rocha e Silva, Jr. et al (8) led to the conclusion that control of vasopressin secretion shifts from low to high pressure system receptors as the arterial pressure falls below 80 mm. Hg. This later view is in keeping with data obtained in our laboratory on the response of atrial and aortic baroreceptors in dogs (9). The firing rate of atrial receptors fell by 50 per cent after a 10 per cent blood volume loss while aortic receptor firing declined less than 10 per cent after a 20 per cent decrement in blood volume.

The interaction of osmotic and volume factors in the control of ADH release has been assessed in men by Robertson and Athar (10) using orthostasis, hydropenia and a hypertonic saline infusion as the means of osmotic and volume stimuli. They conclude that while moderate increase in blood volume influences osmotic regulation, the influence is small and in effect causes changes in the set point of the osmoreceptor, a view that is in detail different from that of Schrier et al (1). Weitzman et al (7), using salt retention and salt depletion in man, concluded that with salt depletion volume factors appear to override osmolar regulation of AVP (arginine vasopressin) secretion. Even so,

osmolar homeostasis was maintained more effectively than volume homeostasis. It must be remembered, however, that mechanisms affecting osmotic control other than those supported by ADH were operating in these experiments.

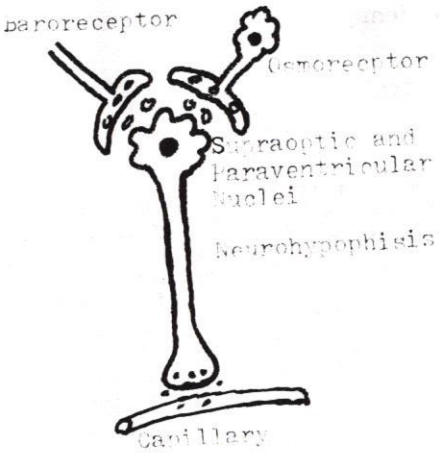

Figure 1: Schema representing osmotic and non osmotic control of ADH release. Redrawn from Schrier et al.(1)

A careful assessment of the effect of osmolality on vasopressin release after hemorrhage by Weitzman et al (7) provides a view of the interaction of osmotic factors with neural sensory input from both the high and low pressure systems. These investigators performed graded hemorrhage on conscious dogs which were either euhydrated, dehydrated, water loaded, or infused with saline. They noted that although the plasma osmolality influences base line ADH levels, the logarithmic response of ADH to hemorrhage was the same regardless of plasma osmolality. Furthermore, their data support a role for both high and low pressure system receptors in the control of ADH release.

A number of experimental procedures have been used to simulate the blood volume shifts that occur in the weightless condition. Water immersion has been used in this regard. A comprehensive review by Epstein(13) brings together many of the pertinent hemodynamic and humoral results seen in man. Epstein (13) studied the urinary excretion of ADH in mildly hydropenic subjects and noted an increased excretion rate as the result of immersion. In a later study (14) he confirmed that plasma levels of ADH fell as the result of immersion to the neck of a mildly hydropenic subject. Plasma osmolality did not decrease in the course of these observations.

In examining the mechanism of diuresis on the immersed dog, Davis and DuBois (15) concluded that hemodilution was the responsible factor. They expected, among other things, a direct correlation between left atrial transmural pressure and the amount of diuresis observed. This is an unwarranted conclusion in view of the well established receptor electrophysiological responses to atrial filling previously noted. (Figure 2).

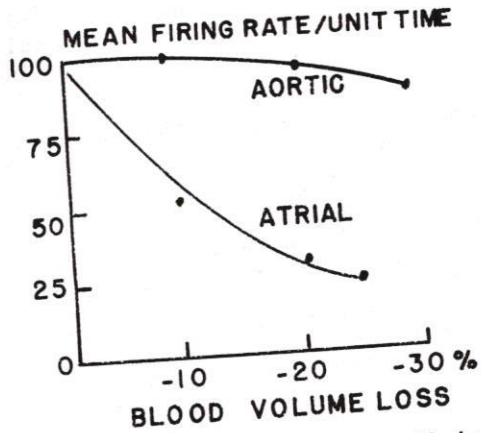

Figure 2: Comparison of mean firing rate per second of six aortic and six atrial fiber preparations during stepwise reduction of blood volume. Adapted from Gupta et al (9).

Koshla and DuBois (16, 17) extended these studies to man. They confirmed the fact that the hydrated individual recruited a hyposmotic fluid when first immersed. The hypotonicity was magnified when the expected diuresis was blocked by injections of vasopressin. The observations of Epstein (13), noted above, on hydropenic subjects indicate that in their experimental design hyposmotemia is not a factor and that vascular receptors are the primary sensors. In the case of the well hydrated subject, it may be surmised that the first fluid mobilized is hyposmotic and that this may account for the initial diuresis. Later, as osmotic pressure falls, volume factors supercede.

Detailed studies of the fluid shifts occuring in water immersion have been reported from NASA Ames laboratories by Greenleaf et al (18,19). Both studies used well hydrated subjects and reported that a hyposmotemia occured in water immersion. These studies included extensive measures of concerned fluid volumes and the possible source of the plasma dilution were carefully investigated. Similar extensive studies have not been reported for the hydropenic subject who, according to Epstein's observations (13), do not develop hyposmotemia as the result

of neck immersion. None the less, plasma ADH was suppressed. This latter observation is of particular interest to gravitational physiologists since, as stated by Greenleaf et al (18), diuresis may not have been experienced by the space lab astronauts because of intentional preflight dehydration followed by nausea during the early phases of their respective flights. Loss in plasma volume was, however, a uniform finding that increased with increasing flight durations.

Epstein et al (10) have reported an eight hour immersion study using his neck immersion model and somewhat less hydration than that used by Greenleaf (19). Diuresis persisted throughout the eight hours while plasma osmolality showed no significant change between the beginning and conclusion of the experimental period. He queries whether or not central hypervolemia was restored. For reasons to be discussed, the central hypervolemia undoubtedly persists and new functional relationships become established with respect to control of the cardiovascular system.

Kass and Moore-Ede (11) have reported on the renal responses to central volume expansion of four days in the squirrel monkey induced by lower body positive pressure. It is of interest to note the plasma osmolality increased by by an average of 12 mosmol/Kg H_2O; yet a diuresis persisted throughout the four days of experimental observations.

There is adequate evidence to involve the low pressure system receptors in providing controlling inputs for the release of ADH. These receptors will adapt to chronic distention as has been shown in studies of experimental heart failure. Greenberg et al (22), reporting from our laboratories, showed that firing frequency from the classical type B receptors of Nonidez was markedly reduced in dogs with experimental heart failure induced by tricuspid avulsion and pulmonary artery stenosis (Figure 3). Zucker et al (23) confirmed this observation using A-V fistula to produce heart failure. They further demonstrated that closure of the fistula reversed the impaired atrial receptor firing towards normal. Congestive heart failure produced by these methods is associated with increased plasma ADH levels presumably due to the reduced activity of the atrial receptors. Heart failure is also associated with increased sympathetic drive to the vascular system--something not seen in space flight. One can speculate, however, that chronic centralization of the blood volume as seen in weightlessness could result in atrial distension and hence an altered sensitivity of the atrial receptors. The interaction between volume and osmotic factors in the control of ADH would be altered. On return to earth, a reverse adaptive process would take place. These are matters that have not yet received directed investigation.

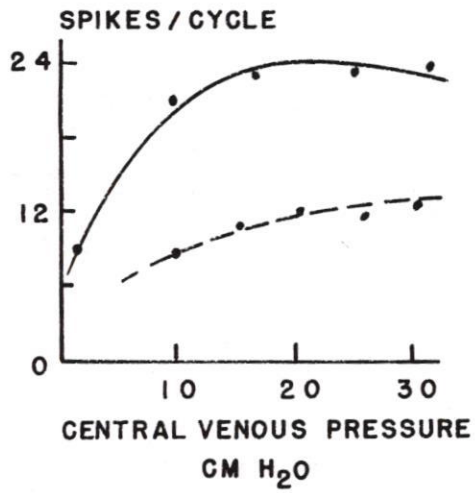

Figure 3: Frequency of atrial receptor discharge plotted against mean central venous pressure. Solid line = control group; broken line = group with congestive heart failure. Redrawn from Greenberg et al (22).

The lymphatic system must not be overlooked in considering fluid volume shift affecting the vascular system. Active pumping mechanisms return capillary filtrate to the central circulation. As reiterated by Aukland et al (24) in their review of interstitial fluid volume (IFV) the IFV represents a balance between capillary filtration and lymphatic removal of the filtrate along with any plasma proteins that may have escaped through the capillary wall. The lymphatic channels are affected by gravity gradients. The absence of gravity would make the lymphatic system particularly effective in reducing the size of the IFV. This additional method of shifting fluid into the low pressure system will play a role in the control of ADH release. As Aukland et al further pointed out, there are no receptors controling IFV. Rather, local mechanisms and the well known Starling forces and lymphatic drainage strike a balance that determines the IFV. This balance is certainly altered in the absence of gravity.

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EFFECTS OF WEIGHTLESSNESS ON PULMONARY FUNCTION

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INTRODUCTION

At the present time there is considerable interest in the effects of weightlessness on human physiology. Much of the work centers on the vestibular, cardiovascular, bone, and blood systems because of the known deterioration in these organs as a result of weightlessness.

However it should be emphasized that the lung is exceptionally vulnerable to gravitational forces and that weightlessness has a profound effect on many aspects of pulmonary function. For example it is clear that gravity plays a major role in determining the distribution of ventilation, bloodflow, gas exchange, alveolar size, intrapleural pressures, and mechanical stresses within the lung. Moreover experiments in which it has been possible to measure the distribution of ventilation and bloodflow during short periods of weightlessness show that marked changes do indeed occur. For these reasons it seems appropriate to review present knowledge of the effects of gravity and increased acceleration on the lung, to summarize available data on the effects of short periods of weightlessness, and to indicate future directions of research which are planned for Spacelab.

Effects of Gravity on the Lung

Early measurements of the distribution of ventilation and regional lung expansion were carried out using radioactive xenon (12). It was shown that in the normal upright human lung, the ventilation of the base was greater than that of the apex. In addition, regional functional residual capacity (FRC) and regional residual volume (RV) as a proportion of regional total lung capacity (TLC) were less at the base than the apex. When the subjects were examined in the supine position, the differences between apex and base were virtually abolished.

Measurements of the uneven distribution of bloodflow in the upright human lung using radioactive carbon dioxide (16) showed that bloodflow decreased strikingly from base to apex in the normal lung reaching very low values at the apex. In the supine position, these differences between apex and base were abolished. These topographical differences of ventilation and bloodflow result in regional differences of ventilation-perfusion ratio, the variable which determines gas exchange in any lung unit. This ratio increases strikingly from base to apex of the upright human lung. As a result there are large local differences in alveolar P_{O_2}, P_{CO_2}, pH and other gas exchange variables (15).

Large regional differences in alveolar size in experimental animals were demonstrated by freezing dogs in the head-up position (5). It was found that the apical alveoli were four times larger by volume than those at the base. However when the animals were frozen in the supine position, the size of the apical and basal alveoli were the same confirming that gravity was responsible for the differences. Regional differences of intrapleural pressure were first demonstrated in anesthetized dogs (8) and subsequently consistent findings were reported in man by measuring esophageal pressure at different levels (11).

Finally, there is also good reason to believe that the mechanical stresses transmitted through the lung parenchyma are considerably greater at the apex than the base of the upright human lung. Although it has not been possible to measure these directly, the stress distribution has been analyzed by finite element techniques (17). These regional differences of stress are caused by gravity and are consistent with the measured regional differences of intrapleural pressure.

Increased Acceleration

Several studies have shown that these regional differences of structure and function are exaggerated by increased acceleration, thus providing additional evidence for the role of gravity. For example, headward acceleration of human volunteers at +2g and +3g on a centrifuge exaggerated the normal regional differences of lung expansion at RV and FRC (2). Furthermore, the preferential ventilation of the base which is normally seen at 1g was exaggerated at 2g. However, at 3g, the ventilation to the bases was reduced because of dependent airway closure.

An increase in the unevenness of distribution of bloodflow during headward acceleration was reported using radioactive xenon (1). Indeed, during headward acceleration of 3g, it has been shown that the upper half of the lung may be completely unperfused (4). Additional studies on subjects exposed to +1gx through +5gx (eyeballs in) have shown changes in the distribution of ventilation and bloodflow in the expected direction though of smaller magnitude than for +gz (headward) acceleration. Exaggeration of the normal differences in alveolar size during +3gz acceleration in dogs has also been demonstrated (6). A full discussion of all these studies on the effects of gravity and increased acceleration on pulmonary function has been published (14).

Short Periods of Weightlessness

The effects of short periods of weightlessness on pulmonary function have been studied by flying a jet aircraft through the appropriate parabolic trajectory. Measurements were made on four subjects during 112 weightless periods lasting up to 27 seconds each on board a Learjet aircraft at NASA Ames Research Center (9). Weightlessness was obtained during all or part of each test by varying the aircraft flight profile.

The inequality of ventilation was measured from single breath nitrogen washouts which were performed with the test inspiration containing an initial bolus of argon at RV. During the expiration, the concentrations of nitrogen and argon were measured with a mass spectrometer, and expiratory flow, volume, and acceleration were also recorded. Figure 1 shows a typical example of a control measurement made during straight and level 1g flight. Note the cardiogenic oscillations on both the N_2 and argon tracings, and also the abrupt terminal rises in N_2 and argon concentrations. The cardiac oscillations are evidence that the upper and lower regions of the lung have different N_2 and argon concentrations, and that they empty at different rates as a result of the movement of the heart within the chest. The terminal rises in N_2 and argon concentration are caused by preferential emptying of the upper lung regions toward the end of expiration when the small airways of the lower lung regions close. Thus, both the cardiogenic oscillations and the terminal rises are evidence that during the test inspiration, regional differences of lung expansion occurred thus resulting in regional differences of tracer gas concentrations.

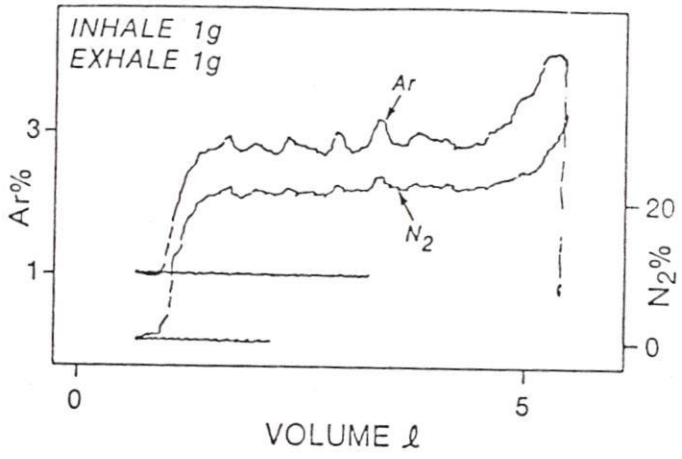

Figure 1. Typical single-breath N_2 washout obtained during straight and level flight in the Learjet. From (9).

Figure 2 shows an example of a tracing obtained when the test inspiration was made at zero g followed by a test expiration at 2g. This sequence is particularly informative because the positive g load during the test expiration ensures that the lung empties sequentially so that any regional differences in the concentrations of N_2 and argon will be revealed both as cardiogenic oscillations on the alveolar plateau and as terminal rises. However, as the tracing clearly shows, the cardiogenic oscillations were greatly diminished and there was a striking absence of terminal rises for both N_2 and argon. These tracings provide very strong evidence that during these short periods of weightlessness, the topographical differences of lung expansion and ventilation are virtually abolished. It is of interest that the marked changes in the distribution of ventilation as a result of weightlessness occurred in spite of very small alterations in chest wall shape (10). This finding has important implications about the way in which gravity distorts the lung.

Information was also obtained on the distribution of pulmonary bloodflow during weightlessness. For these tests, the subject first hyperventilated for about 5 seconds and then held his breath at TLC for approximately 15 seconds. Expired P_{O2} and P_{CO2} were then measured with the mass spectrometer during a steady flow exhalation to RV. By hyperventilating, the subject lowered the P_{CO2} and raised the P_{O2} throughout the lung. During the subsequent breathhold (and exhalation) CO_2 was added to, and O_2 removed from the various lung regions at a rate which depended on the local perfusion per unit lung volume. The resultant concentration differences, if any, were evident in the expired gas tracings as cardiogenic oscillations.

Figure 3 shows examples of results measured at 0, 1 and 2 g (the last obtained during a constant banked turn). Note that whereas cardiogenic oscillations of both P_{O2} and P_{CO2} were obvious at 1g and exaggerated at 2g, they were virtually absent at zero g. This is strong evidence that the topographical inequality of bloodflow is very nearly abolished under these conditions of brief weightlessness.

Plans for Studies in Spacelab

Two possible avenues of approach have been considered for measuring the effects of weightlessness on pulmonary function during space flight. The first is to use a radioactive gas to measure the topographical distribution of ventilation and bloodflow. This technique is the most direct

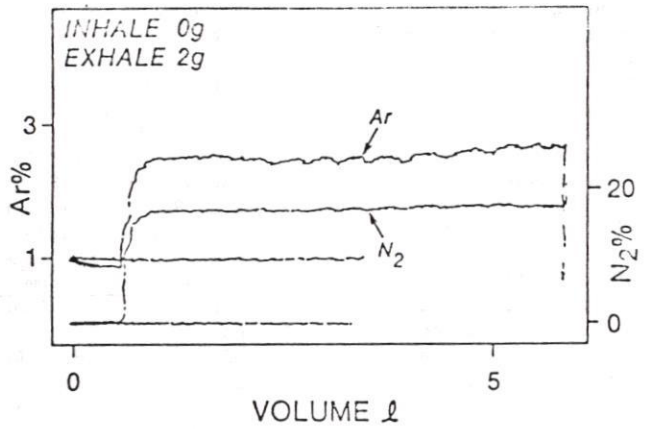

Figure 2. Single-breath washout obtained when the test inspiration was taken during zero g but the expiration was during 2g on the Learjet. From (9).

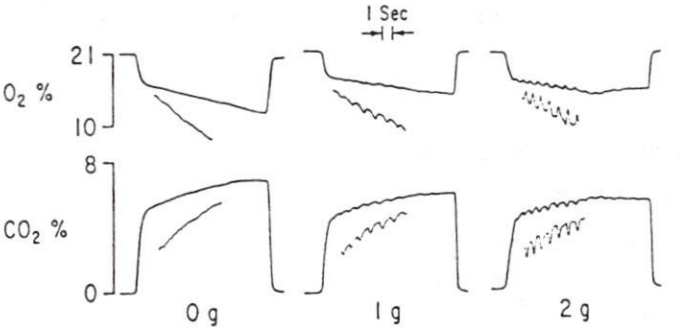

Figure 3. Examples of expired P_{O_2} and P_{CO_2} tracings obtained following breath-hold periods at zero, 1 and 2 g. From (9).

method of determining how weightlessness alters these functions of the lung. However there are two serious shortcomings in this approach. The first is that although these radioactive gas studies give very direct information about the distribution of ventilation and bloodflow in the lung, they give very little other information. A second practical disadvantage is that the use of large volumes of radioactive gas in a spacecraft raises the possibility of contaminating the spacecraft atmosphere.

For these reasons, a second avenue of approach was preferred. This was to design a battery of non-invasive, pulmonary function tests which would give information about the distribution of regional function in the lungs, but also give much other information as well. Indeed, most of the tests are modifications of standard respiratory function tests which are used extensively in clinical pulmonary function laboratories. Important criteria in the selection of tests were that they should be non-invasive, be simple for the astronaut to carry out, be suitable for automated operation, and that the data should be amenable to analysis by computer. As an aside, we believe that such a package might have considerable potential in clinical pulmonary function laboratories as a screening test for the detection of early lung disease (7).

The basic group of tests is as follows:

1. Single breath nitrogen washout. This test gives important information about the distribution of ventilation. Figures 1 and 2 show the striking changes which occur as a result of a short period of weightlessness.
2. Inequality of the distribution of pulmonary bloodflow. This is the test which gives tracings of the kind shown in Figure 3. Note that a short period of weightlessness caused a marked change in

the pattern.

3. Single breath diffusing capacity for carbon monoxide. This test, which will be carried out during air and 100% O_2 breathing, gives information about the diffusing capacity of the blood-gas barrier and the volume of blood in the pulmonary capillaries. A redistribution of blood volume to the thorax during zero g will increase both these variables.

4. Forced expiration spirometry. This is a standard clinical function test. Decreases in vital capacity were reported in Skylab (13) and reductions in maximal expiratory flow rate have also been seen during zero g (3).

5. Rebreathing measurement of residual volume and cardiac output. RV may decrease at zero g because of the increase in thoracic blood volume, and there may be an increase in resting cardiac output as a result of a rise in central venous pressure.

6. Resting gas exchange and ventilation-perfusion inequality. It is very likely that the normal inhomogeneity of ventilation-perfusion ratios will decrease during weightlessness.

Present plans are to fly this package in Spacelab 4 during the latter part of 1985. These studies will be carried out several times during the seven day mission. We believe that these experiments will throw a great deal of light on the important changes in pulmonary function caused by weightlessness.

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CIRCADIAN VARIATIONS IN TOLERANCE TO +Gz ACCELERATION

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ABSTRACT

The possibility of a diurnal variation in human tolerance to sustained +Gz acceleration was examined in 8 normal subjects. Greyout thresholds were determined by a standard technique at two rates of acceleration onset (0.1 and 1.0 Gs^{-1}), at 6 times of the day spread over a period of 4 weeks. Heart rate was also monitored during a 15s centrifuge run at +3Gz with a 1.0 Gs^{-1} onset rate. Other factors investigated included blood pressure, intra-ocular tension, body temperature, serum cortisol and serum glucose. Significant circadian rhythmicity ($P<0.05$) was found in slow onset greyout threshold and in the +3Gz heart rate response. Whilst diurnal changes were also seen in the other measures they were, with the exception of cortisol, either too small in magnitude or of the wrong phase to account for the rhythm in G-tolerance. However, this change was small - amplitude 0.115G, acrophase time 0407 hr - and not considered to be of operational significance.

INTRODUCTION

Circadian rhythms have been demonstrated in a number of physiological parameters which can affect man's physical and mental performance (3). Thus, performance can be degraded when man cannot choose the optimum hours for a given task - for example, shift work, time zone changes, extended operations, or the exigences of war (4).

One aspect of performance relevant to military aviation is man's tolerance to accelerations induced by aircraft manoeuvres, particularly in air-to-air combat. With the advent of newer, higher performance aircraft, forces can be generated which, in terms of both level and duration, are well beyond normal human tolerance. Any factor which alters tolerance, for example diurnal variation, will, therefore, be of operational importance.

Exposure to sustained acceleration in the headwards direction (+Gz, footwards acting inertial force) produces a hydrostatic fall in blood pressure at head level and, consequent upon the pooling of blood in more dependent parts of the body, a fall in cardiac output. Depending upon the G-levels, exposure time and individual susceptibility, these cardiovascular changes produce visual symptoms and loss of consciousness.

The mechanisms responsible for the visual and cerebral effects differ in that the first is due simply to the lowering of blood pressure at eye level. For retinal blood flow to be maintained, the pressure of blood in the retinal artery must exceed the extra-vascular pressure resistance offered by the normal intra-ocular pressure of some 20 mm Hg. At a lower pressure than this the vessels collapse, flow ceases and, once the available oxygen has been consumed, retinal function is lost. Owing to a further pressure drop from the central to the peripheral retinal vasculature, the subject initially experiences a greying of his peripheral visual fields which progresses (if the G-level is gradually increased) to tunnelling of vision and complete visual loss (blackout). By use of test lamps placed in appropriate parts of the visual field, the subject's greyout tolerance can be assessed to an accuracy of about 0.2G (1.0G being equivalent to an acceleration of 9.91 ms^{-2}).

Loss of consciousness results from a reduction in cerebral blood flow, also consequent upon the fall in blood pressure at head level, but reflex dilatation of cerebral vessels and concomitant falls in cerebrospinal fluid and jugular venous pressures ensure that flow is maintained to a higher level of +Gz acceleration than in the retina. Thus on average, a relaxed subject will greyout after 5s at +3.6Gz, lose all vision at +4.0Gz, but only become unconscious around +5.0Gz.

A further factor which influences tolerance to +Gz is the rate at which the acceleration is applied. If the rate of acceleration onset is low ($0.1Gs^{-1}$, or less), then a baroreceptor induced reflex increase in heart rate and peripheral resistance can become effective as the stress develops. Tolerance is then some 1.0Gz greater than with a rapid onset of acceleration ($1.0Gs^{-1}$).

It should be clear from the foregoing that greyout tolerance is likely to be modified by a number of factors which are known to undergo diurnal change - blood pressure and intra-ocular tension, for example - and might also be affected more subtly by other rhythms such as those in body temperature, and blood glucose and cortisol levels. Thus, in one study (9), tolerance to +Gz acceleration in female subjects was found to correlate well with the amplitude of the diurnal variation in serum cortisol.

The present study was undertaken in order to investigate the possibility of a circadian rhythm in G tolerance. Other factors which could affect tolerance and could have their own rhythm were also studied.

METHODS

Eight healthy volunteer subjects (7 male, 1 female) were used as subjects. They underwent

daily centrifuge runs for the week preceding the experiments for familiarisation and to eliminate subsequent effects of training on G tolerance. Experiments were conducted over a 4 week period at a rate of two per subject each week. Each subject underwent one experiment at 0800, 1200, 1600, 2000, 0400 and 0800 hr, four of them in the order given, the other four in the reverse order. Subjects refrained from taking caffeine or alcohol for 12 hr prior to an experiment, ate a standard meal (chosen by the subject and kept constant) 5 hr before an experiment, consumption then being restricted to 50 ml water per hour. Heavy exercise was avoided. The measures made in each experiment were: serum glucose; serum cortisol; resting blood pressure; oral temperature; ambient temperature; tolerance to slow onset ($0.1Gs^{-1}$) acceleration; heart rate response to +3Gz at $1.0Gs^{-1}$; tolerance to rapid onset ($1.0Gs^{-1}$) acceleration; and intra-ocular pressure.

On arrival for an experiment the subject had 10 ml of venous blood withdrawn for analysis. The sample was allowed to clot for 30 min in an iced and Parafilm covered tube before being centrifuged and the serum divided into four aliquots. Cortisol was estimated in duplicate using a competitive protein binding kit (Cortipac, The Radiochemical Centre, Amersham). Glucose was measured on an autoanalyser by the neucuproine method, also in duplicate.

Electrocardiogram electrodes were then applied, and the subject moved to the centrifuge gondola for an initial 10 min rest period. The subject was strapped into a standard aircraft ejection seat (back angle 15° reclined from the vertical) with his eyes 76 cm from a horizontal bar carrying a central 2.5 cm diameter red light and two similar sized green lights which subtended a 50° visual angle. The surroundings were well illuminated and the subject was observed using closed circuit TV. During the rest period, blood pressure was measured at least twice by clinical sphygmomanometry, environmental and oral temperatures were determined and an ECG recording was made for the final three minutes and throughout the subsequent centrifuge runs.

The subject was instructed to remain relaxed and to fixate on the central red lamp as acceleration in the +Gz axis was applied at a rate of $0.1Gs^{-1}$ to a maximum of +6Gz. The medical observer illuminated the peripheral green lamps in a random fashion every 1-3 sec and the subject extinguished them as soon as seen using a hand held button. The cetrifuge was stopped as soon as the subject failed to respond to a green signal and the G value at this point was measured from a pen recording of acceleration and response.

After the heart rate had returned to within 5 bpm of the resting value, a standard +3Gz run with onset rate of $1.0Gs^{-1}$ and 15 sec plateau was carried out. Thereafter, runs were conducted at selected levels (to the nearest 0.2G) until a level was found at which definite, but temporary, peripheral visual loss occurred with no central light loss. Repeat runs were kept to a minimum using a standardised approach in order to avoid undue fatigue to the subjects.

Following the centrifuge runs, intra-ocular pressure measurements were made in triplicate on the right eye only using a Haag-Streit series 900 applanation tonometer. As with all other determinations which could be subject to observer bias, care was taken that a subject had the same observer on all occasions.

All individual data were analysed by computer fitting of both a sine wave of 24 hr wavelength and a straight line by least square regression. Pooled data were examined in the same way and diurnal variation was accepted when a significantly better fit was achieved by the sine wave at a P value of less than 0.05.

RESULTS

A summary of the sine wave analyses is given in table 1 in terms of P value, mesor (mid-point of the curve), amplitude (distance from mesor to peak) and acrophase (time of the peak value). When the P value was greater than 0.05, the sine wave fit was not considered significant and only an average value is given. Significant circadian rhythms are illustrated in figure 1 to show their relative amplitudes and time relationships.

The resting measures show clear cut rhythms in serum glucose and cortisol, and in systolic blood pressure. Diastolic blood pressure does not show a significant rhythm but, despite this, the rhythms in pulse pressure (systolic minus diastolic pressures) and mean pressure (diastolic plus one third pulse pressure) are highly significant. Oral temperature also showed a clear diurnal rhythm with a peak to trough range of 0.4°C.

TABLE 1. SUMMARY OF RESULTS

Variable	P Value	Mesor (average)	Amplitude	Acrophase time (hr)
Serum glucose (m mol/l)	0.001	5.371	0.173	0808
Serum Cortisol (n mol/l)	<0.001	261.700	160.000	0852
Systolic BP (mm Hg)	<0.001	120.800	6.472	1828
Diastolic BP (mm Hg)	0.866	(77.90)		
Pulse pressure (mm Hg)	<0.001	42.900	5.871	1821
Mean BP (mm Hg)	0.001	91.920	4.496	1827
Oral temperature (°C)	0.005	36.58	0.203	1610
Tolerance to slow onset acceleration (G)	0.046	4.256	0.115	0407
Increase in HR at threshold (Δ bpm)	0.725	(22.06)		
Increase in HR at +3Gz (Δ bpm)	0.009	15.600	3.363	1259
Tolerance to rapid onset acceleration (G)	0.332	(3.483)		
Increase in HR at threshold (Δ bpm)	0.849	(21.52)		
Intra-ocular pressure (mm Hg)	0.002	11.600	1.344	0901

Of the two greyout tolerance measures, only tolerance to slow onset rate acceleration was significant, and that by only a small margin from the selected cutoff P value of 0.05. Heart rate changes at greyout (both slow and fast onset rate) did not reveal circadian variation, but the increase in heart rate measured over the last 10 sec of a standard 15 sec run at +3.0Gz (rapid onset rate) did. Finally, intra-ocular pressure showed a well marked rhythm with an overall variation of 2.7 mm Hg.

DISCUSSION

The observed circadian rhythm in tolerance to slow-onset-rate acceleration was of low amplitude (±0.1G, or 2.7%) and peaked in the early morning (0407 hr). No significant rhythm was detectable in tolerance to acceleration of fast onset, and this suggests that the observed rhythm is related to the body's compensatory response to the stress. Thus, at an onset rate of $1.0Gs^{-1}$, there is inadequate time for compensatory reflexes to come into effect and greyout tolerance is more directly related to the increased vertical gradient of hydrostatic pressure within the cardiovascular system. There has been one other (unpublished) study of circadian variation in acceleration tolerance which showed a peak in the early afternoon, almost 180° out of phase with the present findings (Vogt, quoted in 4 and personal communication). However, in the Vogt study, the tolerance criterion was complete visual loss of a low intensity lamp in dark adapted subjects. Since the light threshold shows a similar periodicity without centrifugation (4), the observed rhythm may not have represented an alteration in acceleration tolerance *per se*.

The rhythms in glucose, cortisol, oral temperature, blood pressure and intra-ocular pressure are comparable in both magnitude and phase with other values reported in the literature. Thus, Möllerström (6) reported minimum values for blood sugar at around midday (0808 hr in our study for the pooled results, but 1247 hr in the one subject to show an individually significant rhythm at P = <0.01). Obviously, much depends on the experimental protocol, and our subjects had all eaten a standard meal at a fixed time prior to sampling regardless of the time of day - hence the rather small range of values seen. Conroy and Mills (3) have reviewed cortisol changes and found most peaks occurring between 0400 and 0800 hr (0852 in our study); and body temperatures which tend to peak at around 1600 hr (1610 hr in the present study). The pattern of blood pressure changes reported tends to be critically dependent upon rest and activity. In the present study subjects were always in a comparable physical state and the rhythm found approximates to that described by Richardson (8) who also found peak values in the early evening.

Of the rhythms so far discussed, two can be directly related to greyout tolerance and the others implicated. Thus, it can be calculated that a rise in mean blood pressure of 9 mm Hg (the overall variation found in our pooled results) will, if maintained during centrifugation, cause a direct increase in greyout tolerance of some 0.4G - the hydrostatic fall in pressure at eye-level being of the order of 25 mm Hg per G. The time relationship between blood pressure and greyout tolerance (and some of the other measures) is illustrated in figure 1 and it may be seen that the two rhythms are almost 180° out of phase. Thus, an increase in tolerance occurs despite a concomitant *fall* in blood pressure. Similarly, a 2.7 mm Hg fall in intra-ocular pressure will allow perfusion (and effective vision) at an equally lowered retinal arterial pressure, equivalent to an increase in greyout tolerance of about 0.1G. Examination of figure 1, however, shows that these events occur at a time separation of some seven hours.

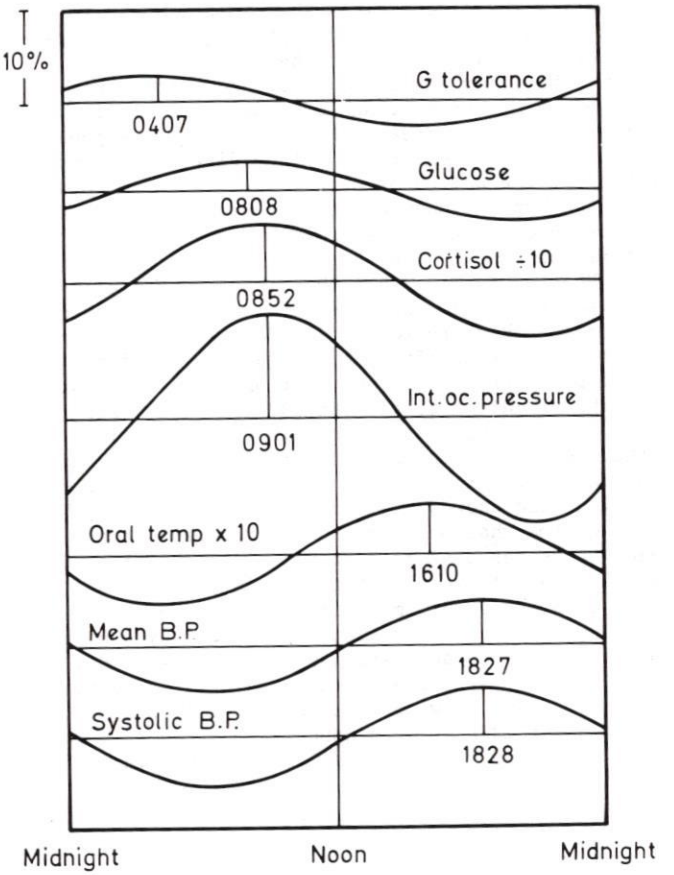

Fig. 1. Significant circadian rhythms and their time relationships. The best-fit 24 hr period sine-waves are scaled on the basis of percentage change. Cortisol and oral temperature have scale factors of ÷10 and x10 respectively.

It has been reported that halving the blood glucose level by insulin injection causes a 20% reduction in G-tolerance (2), and hypoglycaemia has been suggested as a probable cause of unconsciousness during flight (7). However, it is unlikely that the small change seen in the present study (±0.17m mol/l) could be responsible for the change in tolerance, even though the peaks are only 4 hr out of phase. Similarly, whilst a rise in core temperature to 37.8°C causes a 0.5Gz reduction in greyout threshold (1), this is related to a reduction in peripheral resistance, a lowering of resting blood pressure and diversion of cardiac output to the skin in an attempt to lose excessive heat.

A change in oral temperature of only 0.4°C due to normal circadian variation is not associated with heat stress and would not induce peripheral vasodilation: no change in G-tolerance would be expected. Thus, the time relationship between these two factors (figure 1) is probably fortuitous.

Serum cortisol levels have been shown to increase in response to +Gz acceleration, attributed to activation of the pituitary - adrenal axis (5). A relationship has also been shown between the G tolerance of female subjects and the amplitude of their individual cortisol rhythms (9). Whilst the action of cortisol in causing sodium retention and increasing the circulating blood volume would tend to counteract the hypotension produced by +Gz acceleration, the time course of the response is too slow to have any immediate effect on G-tolerance. However, a higher resting level due to circadian variation could increase the body's acute response to +Gz acceleration stress either directly, or by enhancing the action of catecholamines as it does with the hyperglycaemic effect of adrenaline. It may be noted that the two rhythms have a phase difference of only four hours.

Bearing in mind that the circadian variation in G tolerance that we have shown could have been of considerably greater amplitude were it not for the counteracting effect of rhythms in resting blood pressure and intra-ocular pressure, to what may the variation be attributed? The only relevant factors appear to be cortisol (and possibly other related hormones) and the observation that the heart rate response to a constant +3Gz acceleration also showed a significant rhythm (table 1). At the time that G tolerance was greatest, the heart rate response to +3Gz was close to its nadir, suggesting that acceleration was indeed seen as less of a stress at that time. On the evidence available, circadian variation in the body's hormone response to acceleration stress is the most likely cause for the observed findings, and merits further study. However, the small amplitude of the rhythm must make it of secondary importance in factors affecting +Gz acceleration tolerance in military aviation.

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HUMAN TOLERANCE TO ROTATION AT DIFFERENT G's

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INTRODUCTION

In order to resolve the problem of generating artificial gravity in space vehicles, it is necessary to study man's physiology in a rotating environment (1). Many aspects of man's life in such an environment on the Earth are studied fairly well (2, 3). Some of the data obtained in these studies can be with good reason extrapolated to space vehicles in which artificial gravity of 1 g will be generated. It is, however, expected that in space flight the gravity will be lower than 1 g (4). Hence, it is important to study man's life in a rotating environment at artificial gravity differing from 1 g (5). This study deals with man's life and work when rotated at 1.1, 1.6 and 2 g.

METHODS

The experiments were carried out in the cabin mounted on the centrifuge arm at different distances from the axis of rotation. The cabin was placed so that at 15.3 rpm the resultant of the centrifugal and gravitational forces was perpendicular to the cabin floor. The angle of the cabin inclination to the horizontal plane in every position was determined by calculations.

The experiments were performed in 3 series. In the first series the resultant of the forces (the gravity magnitude) was 1.1 g, in the second and third series it was 1.6 and 2.0 g. In each series the rotation time was 1 hour.

During rotation the test subjects were in the chair and performed the vestibular test - they nodded their heads in the radial direction to the axis of rotation at a rate of a nod per sec (to 100 rapid movements). This simulated active work during rotation. The tolerance to rotation under such conditions was evaluated with respect to the occurrence and level of symptoms of motion sickness. Clinical symptoms of motion sickness were estimated in scores according to Galle (6). During 1-hour rotation the estimates were made 7 times. The sum of scores was used as a quantitative index of rotation tolerance.

During rotation heart rate, blood pressure and respiration rate were recorded. When the subjects performed slow head movements (2 nods at a rate of 1 movement per 3 sec), their eye nystagmus was recorded.

The study was performed on 22 healthy males, aged 23-40, who participated in each series separated by an interval of at least a week.

RESULTS AND DISCUSSION

The experiments showed that at 1.1 g (1st series) the test subjects developed 10-15 min after the onset of rotation the so-called vestibulo-autonomic symptoms: increased salivation, perspiration, fever, nausea and sometimes vomiting. During the second 30 min of rotation they displayed sleepiness, inactivity, heachache and other "central" symptoms of motion sickness.

The study of the level of motion sickness with respect to one of the important symptoms (nausea) demonstrated that it occurred in 15 test subjects and ended in vomiting in 7 of them. Due to a marked deterioration of the health condition (enhanced symptoms of motion sickness) the vestibular test was interrupted by 8 subjects.

When artificial gravity was increased to 2.0 g symptoms of motion sickness declined: the number of the test subjects who complained of nausea went down to 13 and that of those who developed vomiting to 2. The number of cases when the vestibular test was interrupted decreased twice.

Figure 1 shows the relationship between the rotation tolerance and the magnitude of gravity. As the latter increased, this index declined (curve 1). Since the intensity of motion sickness varied significantly in different test subjects, two polar groups of 6 in each were selected. The first group consisted of the subjects susceptible to motion sickness. When performing the vestibular test during rotation at 1.1 g, they developed noticeable symptoms of motion sickness. The 2nd group was comprised of the test subjects who developed insignificant manifestations of motion sickness under the same condi-

tions.

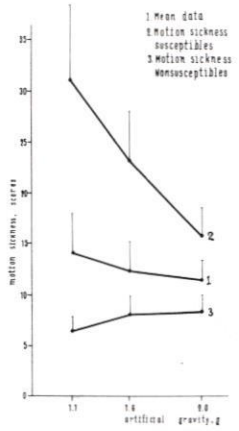

Fig. 1. Tolerance of 1-hour rotation as a function of gravity.

The susceptibles displayed a distinct improvement of rotation tolerance as gravity increased: the resultant estimate of clinical symptoms decreased significantly (curve 2). The same parameter in the nonsusceptibles remained virtually unchanged as the gravity grew (curve 3).

Thus, the nonsusceptibles maintained high tolerance to 1-hour rotation at 2.0 g. At the same time the susceptibles displayed a significant decrease in the level of clinical symptoms and, therefore, an improved rotation tolerance.

During rotation heart rate, blood pressure and respiration rate varied insignificantly. Mention should be made of a slight increase in heart rate at 2.0 g: at 1.1 g this parameter varied in the range 72-80 beats/min and at 2.0 g it was 70-90 beats/min. It is important to note that in all experimental runs there were no cardiovascular reactions to the vestibular test. In other words, the changes in heart rate and blood pressure as compared to the levels recorded at 1.1 g were a reaction to increased gravity (7) and were not caused by vestibular effects. Thus, an analysis of cardiovascular and respiratory parameters yielded no additional information on the relations between vestibular reactions and increased gravity.

The examination of nystagmograms showed that in all experimental runs as the centrifuge arm grew the horizontal component of the nystagmic reaction increased noticeably, especially with respect to the number and amplitude of nystagmic beats. When interpreting this phenomenon, it is important to take into consideration the fact that in our study the direction of the resultant of the centrifugal and gravitational forces coincided with the long axis of the body. At the same time the body orientation relative to the axis of centrifuge rotation varied from 0° at the minimal arm length to 60° at the maximal arm length. Therefore, when the subjects made nods in the direction of the axis of rotation the frontal semicircular canals were primarily stimulated with the minimal arm used and the horizontal canals were mainly involved with the maximal arm employed (8). This was indirectly confirmed by the nystagmograms of eye movements. It was therefore expected that with an increase in the arm of rotation the symptoms of motion sickness would aggravate (9). We, on the contrary, observed an alleviation in motion sickness. It can be inferred that the factor which caused a significant reduction in the level of motion sickness and improvement of rotation tolerance by the susceptibles in the 2nd and 3rd series was increased gravity rather than a change in the stimulation of semicircular canals.

At 2 g all test subjects reported a marked sensation of increased weight of the head and limbs; they also mentioned that it was difficult for them to get up from the chair. During head movements, chair turns and other movements they complained of fatigue. When standing they reported the feeling of blood rush to the legs. During rotation these feelings did not weaken and their fatigue increased. By the end of rotation some subjects showed single ectopic beats. After completion of rotation at 2.0 g every test subject without any exception reported considerable tiredness.

Evaluating their rotation tolerance, most test subjects noted that at 1.1 g symptoms of motion sickness were predominant and at 2.0 g the feeling of heaviness and fatigue were in prevalence. From the subjective point of view the vestibular test was best tolerated at 1.6 g; in this situation symptoms of motion sickness were as if in the background. This is the reason why the susceptibles evaluated 1.6 g as something really good (two subjects estimated the exposure as much better than, three as better than and one as similar to that at 1.1 g), whereas the nonsusceptibles estimated 1.6 g as an additional effect and indicated that they tolerated the rotation slightly worse (three test subjects noted deterioration of tolerance and three reported no appreciable difference).

It is known that different physiological systems are involved in mechanisms of motion sickness (10). The inhibitory effect of increased gravity on the level of motion sickness demonstrated in our study is mediated via the motor sensors, the functional state of which varies (11, 12) with gravity. There are data concerning the inhibitory effect of the excitation of the motor sensor on the motion sickness manifestations (13).

The manned experiments were supplemented with animal studies. The experiments were carried out on 30 noninbred male rats weighing 180-235 g. The animals were divided into 3 identical groups: the rats that were exposed to 21-day rotation (with an angular velocity of 33.3 rpm) and were housed in groups at 1.1 and 2.0 g (1st and 2nd groups), with the 3rd group being controls.

The physical state of the rats was assessed using such indexes as static and dynamic endurance. The static endurance was measured as the time during which the animals remained holding to the pole (14), the dynamic endurance was measured as the time during which they were swimming (in water of 33-36°C) with a weight (making 20% of their body weight) attached.

The vestibular function was evaluated with respect to the changes in the nystagmic reflex in response to the angular acceleration $30^{\circ}/sec^{2}$. The nystagmic reaction was determined on the basis of the latent period, number of beats, duration and average frequency.

The animals, both experimental and control, were examined before and at different time intervals after the 21-day rotation.

The study demonstrated that after 21-day rotation the rats did not show any significant differences in their static and dynamic (Fig. 2) endurance as compared with the controls. Consequently, a prolonged exposure of animals kept in groups in a rotating environment at 2.0 g does not impair their health state.

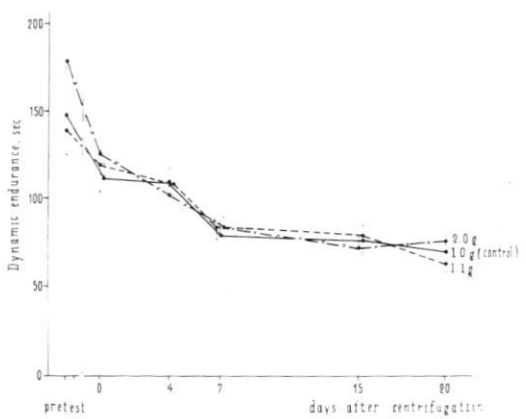

Fig. 2. Rat dynamic endurance after 21-day rotation at different g's.

Changes in the nystagmic reaction during readaptation (Fig. 3) were similar to those we described earlier (15). They seem to reflect alterations in the state of the nystagmogenic center caused by prolonged stimulation of the semicircular canals due to cross-coupled acceleration together with additional stimulation of the otolith apparatus by increased gravity. It should be noted that in the first days after exposure the number of beats, duration and frequency of nystagmus in the animals rotated at 2.0 were slightly less than in the animals rotated at 1.1 g. This tendency was, in all likelihood, caused by the effect of increased gravity on the otolith apparatus (16).

Our findings may be of practical importance for the medical support of future space flights in which artificial gravity will be generated. The people who tolerate well a prolonged exposure in a rotating environment on the ground can hardly be expected to show a decreased tolerance to the rotation at an artificial gravity less than 1 g. Nevertheless, bearing in mind the hypothesis about the qualitative similarity between human reactions to gravitational effects varying within a certain range of magnitudes and duration (17), it can be thought that the people susceptible to motion sickness on the ground will poorly tolerate rotation at smaller g's. This means that the crewmembers of space vehicles in which artificial gravity of low values will be generated should be selected with greater care from the viewpoint of their vestibular tolerance.

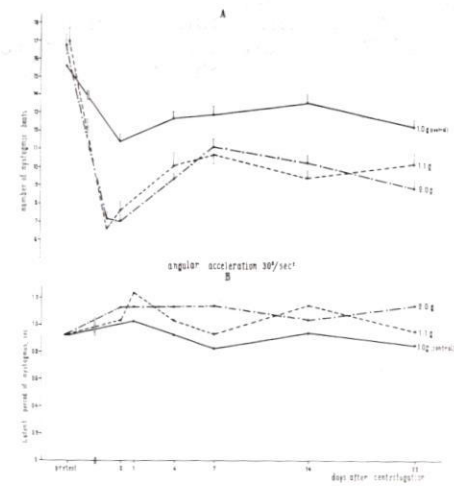

Fig. 3. Number of beats (A) and latent period (B) of nystagmus in rats after 21-day rotation.

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STATIC AND DYNAMIC MECHNISMS OF SPACE VESTIBULAR MALAISE

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ABSTRACT:
Previous experience in manned spaceflight showed that space sickness can be provoked or increased by dynamic stimuli such as head movements or locomotion. However, in susceptible persons spacesickness also occured in the weightless state without such provocative stimuli. Experiments with unilateraly destated goldfish showed that these fish although well compensated on the ground, show acute signs of vestibular asymmetry when flown in parabolic flight. It is suggested that crew members who were susceptible to static spacesickness might have a latent well compensated asymmetry of their otolith system.

After many manned space flights during the last 3 decades, it is well documented that dynamic stimuli, like head movements and locomotion in weightlessness, can provoke or increase symptoms of space sickness in susceptible crew members (8,10). Furthermore, it became obvious, that the incidence of space sickness augmented with increasing size of the spacecapsules, i.e. with greater possibility for free locomotion of the crew.

Movements of the head are connected with optokinetic as well as mechanical stimuli. During the latter linear acceleration changes (m/sec^3) on the basis of 0-g. Angular accelerations do not seem to be very provocative in space as the vestibular experiments in skylab have already proven (6). One of the main differences of horizontal linear acceleration in space as compared to the ground is, that in weightlessness the acceleration vector during reciprocating movements goes through zero and reverses its direction through 180°, while on the ground it only goes through 1-g, thereby rotating it's direction only slightly.

Another important difference is, that the amplitude of the linear acceleration vector changes on the basis of zero-g in space during locomotion and head movements to a higher degree than on the basis of 1-g on the ground. Furthermore, it appears likely that the otolith membranes, when floating loosely without any friction on the macular cilia would display larger excursions in response to a given near horizontal stimulus tangential to the utricular macula than when under the strong perpendicular gravitational pull on the ground. Last, but not least, it is possible that intracentral neurophysiological mechanisms would further increase the differential sensitivity according to Weber-Fechner's law.

For all these reasons, linear movements of the crew in space would lead necessarily to "unusual" vestibular information, which very probably is neither confirmed by the canal system, nor the visual system nor by the expectations about the actual orientation of the body (2). Such "mismatch" is known to lead to motion sickness (9,11).

It is less clear yet, whether the microgravity environment of space per se, without much movement of the subject can cause space sickness in susceptible persons. The pilot in the skylab 4 mission was already severely spacesick while resting on the couch in the command module prior to entering the skylab workshop (6,8).

While vestibular malaise, as we know on the ground, is most often triggered by overstimulation of the vestibular mechanoreceptors, static spacesickness seems to be caused by understimulation. Considering the lack of external stimuli, it appears to be logical to search for an internal stimulus which could cause the vestibular malaise.

In pathological cases of vestibular disorder in humans and in laboratory animals on the ground two facts are prominent:

1. Vestibular malaise often occurs in cases of acute bilateral asymmetry of the vestibular organs. This can be caused by a wide range of pathogenic reasons ranging from unilateral deficiencies of blood supply to Menieres disease or acute unilateral impairment of the 8th cranial nerve.

2. Such bilateral asymmetries, if continuing are spontaneously being compensated by internal rearrangement of nervous acti-

vity within days and weeks, so that the symptoms of vestibular dizzines are alleviated or even disappear on the ground (4,5).

It appears probable, that also static spacesickness represents temporary vestibular asymmetry that would occur only in persons who already on the ground have a latent asymmetry but normaly do not suffer from it because of near perfect compensation. The question arises, why the compensation on the ground would not help them any more in the state of weightlessness.

One possible reason is, that any bilateral differences in the weight of the otoliths that are compensated on the ground do not exist any more in weightlessness, but that the unilateral compensatory activity is still carried on for a period of several days in space, causing vestibular discomfort (2,3,13). An alternative explanation would be that unilateral deficiency of the tonic activity of the macular epithelium is compensated only for ground conditions. New asymmetry would occur, when in the state of weightlessness the gain of the remaining statoreceptors goes up on both sides because of the general lack of external input. A comparable "recruiting" mechanism is long known in the case of failing cochlear mechanoreceptors engaged in hearing.

Trying to distinguish between these two possibilities, we performed experiments with unilaterally destated goldfish.

We removed in 13 Goldfish on the left side the utricular otolith (at the same time probably damaging the utricular epithelium (12). After the operation these fish violently spun around their longitudinal axis to the left side. When flown on the same or the next day in their tanks in parabolic-weightlessness flight in light aircraft, they continued to spin in the same direction. Even fish, that already had recovered and appearently fully compensated on the ground began to spin into the old direction again, when weightlessness occured in parabolic flight. (see Fig. 1 and 2).

These experiments proved, that compensated otolith weight differences alone cannot explain vestibular asymmetries in space. If this would be the case, the freshly operated fish would not have rotated in weightlessness, and the fish that had already compensated on the ground would have rotated to the other side, which was only rarely observed (1).

Our fish experiments support rather the other hypothesis, that in weightlessness the general gain of the otolith system is raised by the same factor on both sides. However, when one assumes that the otolith system was partially

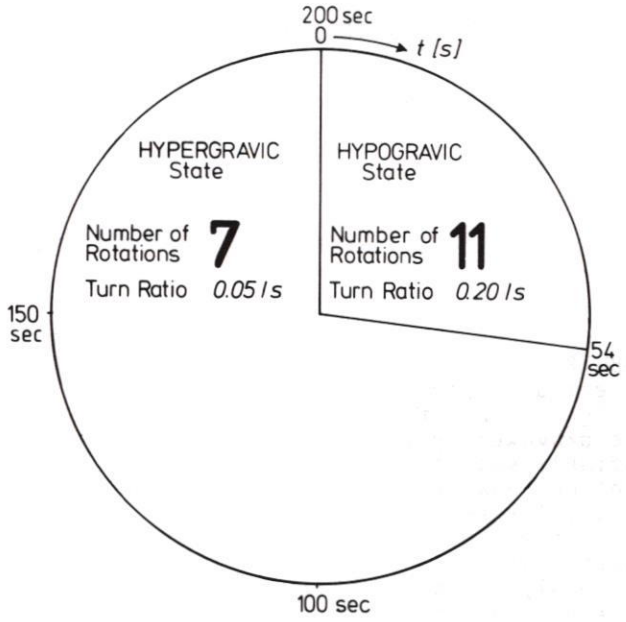

FIG. 1:
BEHAVIOUR OF UNILATERALLY DESTATED GOLDFISH IN PARABOLIC AIRCRAFT FLIGHT. SUM OF ALL FISH ROTATIONS PERFORMED DURING ONE EXPERIMENTAL FLIGHT CONTAINING 10 PARABOLAS. ALL ROTATIONS OF THE FISH WERE AROUND THE LONGITUDINAL AXIS TOWARDS THE OPERATED SIDE. NOTE THAT THE FISH ROTATED MORE OFTEN AND FASTER DURING THE SHORT HYPOGRAVIC PHASE THAN DURING THE LONG HYPERGRAVIC PHASE OF THE FLIGHT.

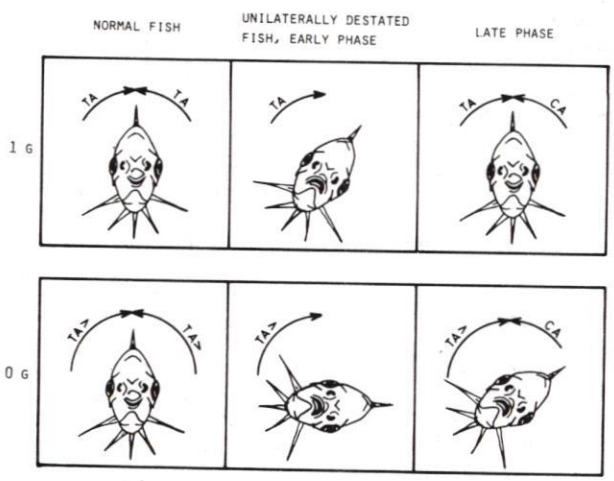

FIG. 2:
VESTIBULAR BEHAVIOUR OF UNILATERALLY DESTATED GOLDFISH IN PARABOLIC FLIGHT.
TA = TONIC ACTIVITY OF UTRICULAR EPITHELIUM
TA> = INCREASED TONIC ACTIVITY IN WEIGHTLESSNESS
CA = COMPENSATORY ACTIVITY

deficient on one side, any proportional augmentation of the gain would lead only to increased asymmetry. The preponderance of the activity of the intact side cannot be fully neutralized by the still weak compensatory activity of the other side which was carried over from the ground. Eventually additional compensation would occur in space.

Our experiments may offer a possibility to explain better than before static as well as dynamic space sickness. Both might be enhanced by an early temporary increase of the gain of impulses from the otolith apparatus. While persons with a perfectly symmetrical otolith system would be affected in space only during strong dynamic stimuli, man possessing slight vestibular asymmetries would suffer already from static space sickness when resting immobile on the couch.

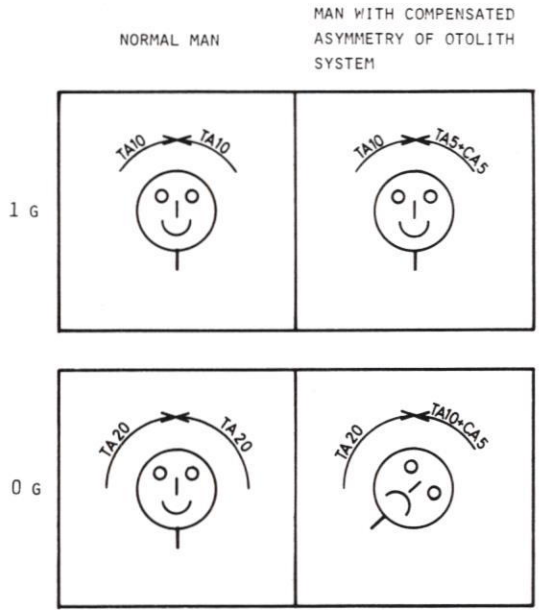

FIG. 3:

POSSIBLE MECHANISM OF STATIC SPACE SICKNESS IN MAN WITH COMPENSATED ASYMMETRY OF OTOLITH SYSTEM.

TA10 = TONIC ACTIVITY OF OTOLITH SYSTEM, VALUE OF 10 ASSUMED
TA5 = CHRONIC UNILATERAL VESTIBULAR DEFICIENCY WELL COMPENSATED ON THE GROUND
TA20 = ASSUMED TWOFOLD INCREASE OF TONIC ACTIVITY IN WEIGHTLESSNESS
CA5 = COMPENSATORY ACTIVITY

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THE ASSOCIATION BETWEEN CANCELLOUS ARCHITECTURE AND LOADING IN BONE: AN OPTICAL DATA ANALYTIC VIEW

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The nature of the relationship between the cancellous architecture of bone and the stresses that bones bear during function is an old problem. Starting with the observations of Culman and Meyer in the middle of last century, see the historical survey in Murray (10), and extending through a large number of findings until about the time of the masterly review by Evans (2) a great deal of evidence was assembled suggesting that there is a one-to-one relationship between (a) the orthogonal network of principal stresses that is one way of describing the stress situation within a bone under load, and (b) the orthogonal network of bony trabeculae that seems evident in a section or radiograph of a bone.

In the earlier half of this century, this concept was more fully demonstrated by stress analyses of bone form, both theoretical and experimental, and by architectural investigations of bone sections and radiographs (e.g. 6, 9, 15). Although the idea was disputed over the same period of time by other investigators (e.g. 8) the argument was never fully settled. But the apparent value of the idea as applied in more recent investigations (e.g. 7, 18) has left it as the conventional wisdom.

Yet a number of studies, usually undertaken for other reasons, supply data that, obiter dicta, throw some doubt upon the concept, and, indeed, suggest that it requires modification. Thus detailed studies of cancellous architecture using both light microscopy (e.g. 17) and scanning electron microscopy (e.g. 19) indicate that trabecular networks are not orthogonal in their details. Yet such findings do not necessarily deny that these architectures may be orthogonal in their overall average patterns.

In the same way, studies of stresses within the ends of long bones using experimental and theoretical stress analyses (3, 5, 16) demonstrate that the real stress patterns existing in cancellous regions during function are not yet fully determinable. Again, however, this does not necessarily mean that the orthogonal stress patterns that have hitherto been used in the theory are incorrect.

But, in summary, these findings do suggest that it is not certain whether or not the architectural patterns are orthogonal, nor which particular orthogonal stress networks are correct stress descriptors. It is, therefore, not yet possible to be certain that these architectural and stress arrangements are associated in a one-to-one relationship with each other.

Recent studies in my laboratory (11, 12, 13, 14, and 20), originally undertaken as a preliminary to study of cancellous patterns in fossils, have shown that the details of the cancellous network in sections and radiographs of vertebrae can be analysed so as to reveal further information about the many bony elements, both their size and direction, in a way that is not so readily achieved using the more customary visual or densitometric scanning methods. And in so doing, these investigations are presenting information that specifically denies the conventional relationship between stress and architecture in cancellous bone.

OPTICAL DATA ANALYSIS

These new studies depend upon the examination of architectural patterns in sections, tomograms and radiographs of bones using optical data analysis. This is a technique that produces a Fourier analysis of the information in any two-dimensional representation emphasizing details about size and orientation of picture elements.

The method uses LASER light in an Image Analyser 3000 to produce Fourier transforms of transparencies of sections, tomograms or radiographs of bones (Figure 1). The transforms them-

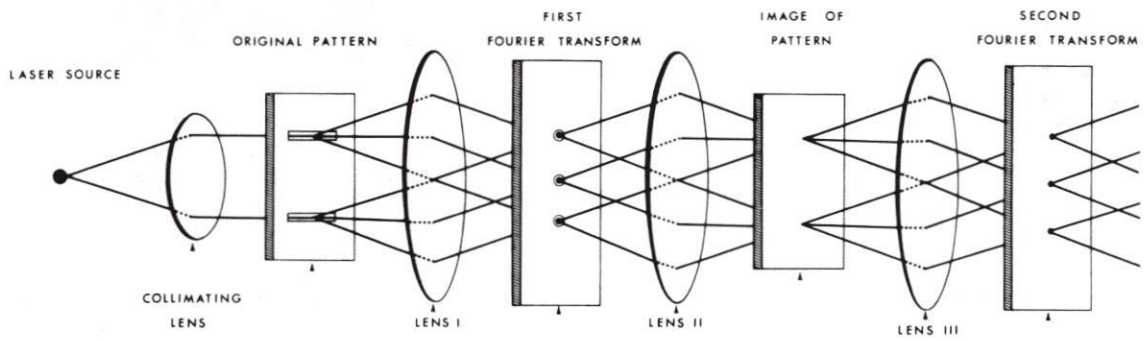

Figure 1

selves frequently contain such clear pictorial information that they supply immediate qualitative data. In addition, however, they are further studied with a variety of ancillary techniques (densitometry, measurement, statistics) to obtain quantitative results.

VERTEBRAL CANCELLOUS PATTERNS

Analyses of sections, laminographs and radiographs of the bodies of human lumbar vertebrae using this technique provide Fourier transforms from a specific vertebral level (the fourth lumbar) showing that the basic pattern of cancellous network at this level is, indeed, orthogonal. This was first implied by study of sections of fourth lumbar vertebrae using optical data analysis a decade ago (11, 12) and has been confirmed recently in my laboratory (13) for sections, tomograms and radiographs at that level (in an effort to make sure that the findings were not artefacts of the plane of examination). The finding is illustrated in Figure 2 in which visual inspection of a radiograph of a human fourth lumbar vertebra shows mainly vertical and horizontal radiographic shadows. The horizontal and vertical cruciate form of the Fourier transform of that radiograph confirms that, indeed, mainly vertical and horizontal shadows predominate. Similar cruciate transforms are figured by Heuck and colleagues in a study from a German laboratory that also utilizes exactly this technique (4).

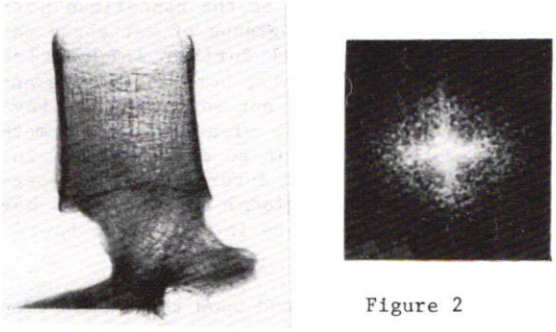

Figure 2

In contrast to this finding, however, analysis at other vertebral levels (e.g. the second lumbar vertebra in humans) demonstrates unequivocally that off-orthogonal elements are present (Figure 3) as first suggested ten years ago (11, 12) and now confirmed (13, 14). Although visual inspection of this radiograph confirms the presence of mainly vertical and horizontal radiographic shadows, visual inspection of the transform reveals that, in addition to its horizontal and vertical cruciate form, additional elements lie in off-orthogonal directions. Further quantitative study of the transform confirms this visual impression.

This finding alone, however, does not necessarily deny the original hypothesis because these off-orthogonal elements are only a small part of an overall orthogonal arrangement. The technique may be finding and displaying merely those local perturbations in architecture such as we already know exist from the microscopic studies of cancellous architecture. The "grand average" view may still be an overall orthogonal pattern.

As our studies have progressed, however, to include (for purposes of testing certain evolutionary hypotheses) radiographs of vertebrae from our closest living relatives, chimpanzees and gorillas, it is apparent that the existence of off-orthogonal elements in cancellous architectural patterns is the rule rather than the exception (14, 20). Even here, however, a basic orthogonality is still discernible. Radiographs of chimpanzee vertebrae seem to contain mainly vertical and horizontal shadows. Their transforms are consistent with this in having a primary horizontal and vertical cruciate form; they do always show, however, a number of off-orthogonal elements (Figure 4).

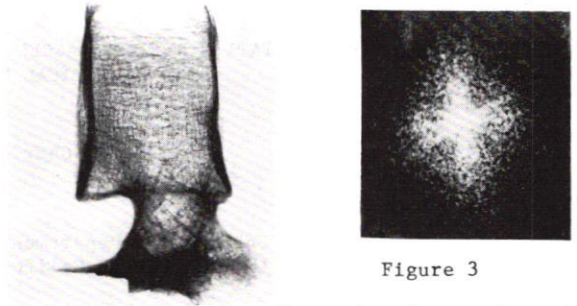

Figure 3

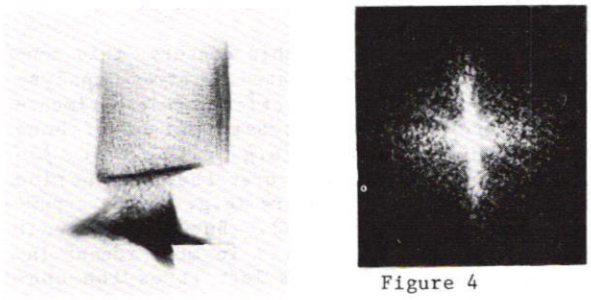

Figure 4

The basic orthogonality can be confirmed by examination of saggital sections of vertebrae (Figure 5); it is quite difficult to see the off-orthogonal elements in figure 5 without careful inspection using a hand lens.

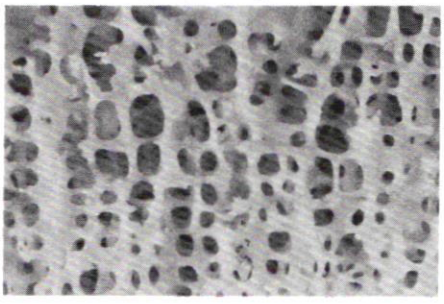

Figure 5

But when, finally, the studies progress as far as examining radiographs of orang utans, though visual inspection of radiographs shows no apparent major difference from chimpanzees and humans (Figure 6), the new method of analysis reveals a total lack of orthogonality in the radiographic shadows that are one measure of the cancellous pattern. Orang utan Fourier transforms are star-shaped (Figure 6). This indicates that the radio-

graphic shadows, and therefore the cancellous materials, lie at many different angles within the vertebra.

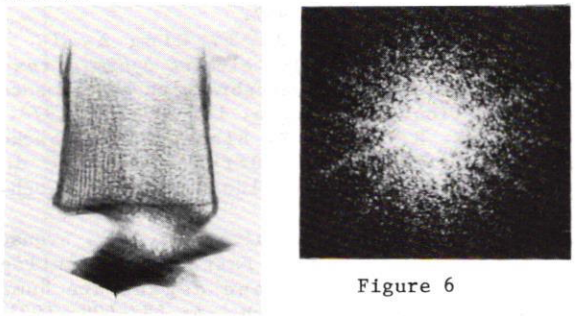

Figure 6

There are several different patterns that might, at first sight, give such a transform. For instance, an orthogonal pattern, but one that markedly changed direction from point to point within the radiograph (such as in a radiograph of an irregular region like the femoral head and neck, or the calcaneum), or a pattern of individual star-shaped nodules (such as are sometimes seen within osteosarcomata), or even an essentially random trabecular network (such as might be found in woven bone) could all give transforms something like that found in figure 6. In fact, however, that particular transform stems from radiographic shadows reflecting a trabecular arrangement that is spongiform or honey-comb-like.

So unlikely did this result appear to be that it was felt especially necessary to check it through the examination of sections of vertebrae. Saggital sections of orang utan vertebrae display a generally honey-comb appearance (Figure 7) in which no orthogonal network can be discerned, even though, of course, there are materials laid down in the vertical and horizontal directions as well as at all other angles.

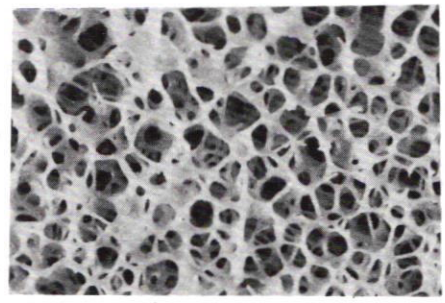

Figure 7

IMPLICATIONS FOR BIOMECHANICS

The generally orthogonal cancellous patterns in vertebrae in both humans and chimpanzees are compatible with the original concept of Culman and Meyer, corroborated by so many investigators since, that there is a one-to-one association between orthogonal networks of principal stresses and orthogonal trabecular architectures.

The extra off-orthogonal elements found in some lumbar vertebrae in humans and in all such vertebrae in chimpanzees and gorillas throw some doubt upon that concept, but could be relatively easily explained by a slightly modified hypothesis stating that the orthogonal stress network was associated, not with an exact orthogonal architectural arrangement, but with an overall "average" architectural arrangement that was basically orthogonal. Such smaller elements as were non-orthogonal might be explained as related to minor perturbations of stress at small localities within the bones, or to stresses due to less frequently or less powerfully operating activities of the species. For instance, the additionally angled elements found in the second lumbar vertebra in humans could be associated with additional local stress due to that vertebra being located away from the average line of gravity acting on the vertebral column; this is to be compared with the lack of these elements in the fourth lumbar vertebra which is placed in such a way that the average line of gravity passes more or less directly through it (11, 12). Additionally orientated elements in chimpanzees and gorillas might well be associated with some of the acrobatic arboreal activities that these basically quadrupedal creatures undertake on occasion.

But the absolute absence of an orthogonal architectural network in even only a single species, the orang utan, denies the concept entirely. Presumably a different relationship exists. The nature of the functional difference between orang utans and the other hominoids (humans, chimpanzees and gorillas) suggests what that may be.

Humans, whatever they do over time, are primarily bipedal. Whatever differently orientated stress networks there may be in a particular vertebra as a result of a human standing on one hand on the parallel bars, or swinging by the hands on the high bar, or even supporting the entire body during one arm press-ups (all unusual activities for average humans), most of the time stress networks will be related to the variety of activities that have in common a basically vertical position of the trunk relative to gravity in a creature that is generally bipedal. Most of the time, therefore, stress networks will have a similar general orientation, and thus will be readily borne by a single approximately orthogonal architectural pattern.

In the same way, though chimpanzees and gorillas carry out a wide variety of activities including swinging under branches, most of their actions relate to a single bodily posture: a more or less horizontal trunk, relative to gravity, in creatures that are mainly quadrupedal. Most of the time, again, stress networks will have a similar general orientation, and again, they will be borne readily by a single orthogonal architectural pattern.

In the case of both humans and chimpanzees the smaller number of off-orthogonal elements may relate to special stresses associated with those fewer activities (with a non-erect trunk on occasion in humans, with a non-horizontal trunk on occasion in chimpanzees and gorillas) that each species also carries out from time to time.

The orang utan, in contrast, does not habitually carry the trunk in any single position relative to gravity. It may be supported or suspended from one, two, three or four limbs, from vertical, horizontal or inclined branches, in any and all postures and movements.

No single position of the trunk is average during the mechanical stress-bearing portion of its day. Any one angulation is about as equally likely as any other. The stress networks to be added over time must surely include orthogonal networks at a wide variety of angles. Their summation is surely a star. And it is a honey-comb pattern that might be best fitted for bearing, sequentially, such a wide array of stress networks.

If this idea is correct, then we can predict that honey-comb networks might be found in the vertebrae of other large animals that hang, spider monkeys, and sloths, for instance. And we would even predict non-orthogonal networks in large aquatic mammals such as whales and dolphins which, living in a yet different gravitational milieu, might be expected to have a wide variety of stress patterns acting upon their vertebrae at different times. Pilot examination of vertebral sections in some of these species (spider monkeys and dolphins) confirms these predictions.

Some studies of osteoporosis in humans depend upon the conventional idea of the relationship between stress and architecture (e.g. see 1 which specifically suggests, in this context, that vertebral trabeculae form an orthogonal network). But the proven existence of off-orthogonal elements, together with the fact that these elements are usually smaller than the orthogonal ones, suggests that incipient osteoporosis might be rather readily detected using this technique at a stage long before it is detectable by conventional methods. The smaller, off-orthogonal elements will be the ones to disappear first in any osteoporotic process, and though loss of such small elements is generally difficult to detect by conventional radiographic methods, it is easily detected using the optical Fourier transform. Certainly interesting sectional and radiographic pictures exist in the vertebrae of older people who, for a variety of reasons, may have stress networks that have become changed, perhaps in part in relation to lack of exercise and changed posture over many years.

Whether or not the more or less orthogonal cancellous networks in human vertebrae would be modified in individuals working for long periods of time in zero gravity may be of some interest, although, presumably, it may be many years before that matter can be fully examined. That caveat notwithstanding, small changes may well occur in astronauts and the differential magnifying effect of this technique suggests that it may not be impossible to discover what they are. The findings described here do suggest that a re-investigation of some of these problems may be in order.

ACKNOWLEDGEMENTS

I am indebted to my former graduate student, Dr. H. C. L. Yang who carried out some of these analyses. The work was supported by NIH grant: AM 16805, by NSF grant: DEB 24366, and by research funds from the University of Southern California.

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CHANGES IN WEIGHTLESSNESS IN CALCIUM METABOLISM AND IN THE MUSCULOSKELETAL SYSTEM

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Since 1965 several observations have been made in man and in animals of the derangements in calcium metabolism in the weightlessness of space flight. The purpose of this presentation is to review and summarize the several studies reported over this period until quite recently. On the muscle metabolism side of changes in the musculoskeletal system, relatively few observations have been resported, but enough to indicate that significant changes occur.

BACKGROUND

The likelihood that weightlessness would bring about significant alterations in mineral metabolism was predicted from the experience of various clinical and experimental conditions involving decreased activity of muscle and bone. One of the classic clinical settings for atrophy of bone and hypercalciuria is in the patient convalescing from bone fractures of a degree necessitating considerable body immobilization. In experimental animal studies development of osteoporosis has been observed in direct correlation with the degree of disuse.

In an early experimental study of inactivity in human beings (1), immobilization of four healthy young men in pody plaster casts for 6-7 weeks led to marked increases in urinary calcium and to significantly negative calcium balances (Fig. 1), and there were related losses of nitrogen and phosphorus. Several subsequent bed rest studies of normal subjects confirmed these substantial metabolic derangements. The longest observation (2) showed that although the elevated urinary calcium subsided partially during the third and fourth months of bed rest, it nevertheless remained significantly higher than control levels for as long as bed rest was continued (for seven months) and did not fall to normal until the subjects were put back on their feet. Calcium balance remained on the average at -200 mg/day during bed rest.

EARLY SPACE FLIGHT OBSERVATIONS

The first attempt at controlled metabolic observations in space flight was performed in conjunction with the 14-day Earth-orbital U.S. Gemini-VII flight in 1965. That relatively short study (3) revealed quite modest losses of calcium and phosphorous and varied changes in the metabolism of other elements. During the Apollo series of flights to the Moon, urine and stool specimens were collected, but dietary intake was not controlled for calcium, and the flights were too short (up to 11 days) for any calcium metabolic changes of significance.

Early Soviet reports (4) (1966) of metabolic observations indicated that elevated urine and plasma calcium levels had been observed in periodic measurements on two cosmonauts during an 18-day flight of a Soyuz spacecraft.

SKYLAB STUDIES OF CALCIUM METABOLISM

The first opportunity to study in a comprehensive manner the possible effects of weightlessness on calcium metabolism came in the U.S. Skylab program of 1973-74. The series of three manned flights in Skylab were successively for 28, 60 and 84 days. The mineral and nitrogen metabolic balance study (5-8) required of the three astronauts on each flight nearly constant dietary intake, continuous 24 hour urine collections and total fecal collections for 21-31 days fefore each flight, throughout each flight and for 1718 days postflight for a total of 909 man-days of metabolic study. Only by this exacting technique can one observe a week to week pattern of change in calcium or nitrogen excretion and balance and obtain a near-accurate determination of the quantitative degree of change in the whole body content of the element being measured.

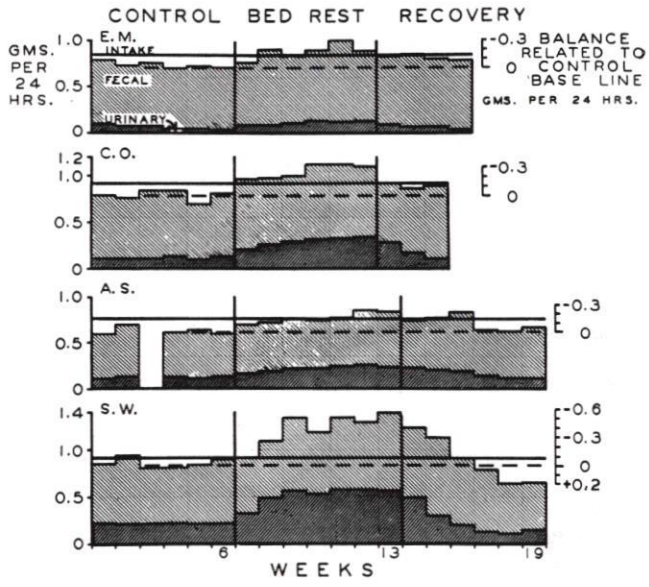

Figure 1. Effect of immobilization on the calcium metabolism of four normal male subjects. In each subject the daily calcium intake was kept constant throughout all periods of the experiment. For each subject, the control baseline (interrupted horizontal line) is an average of the total outputs of the last four control weeks.

As previously reported, in flight calcium excretion in the urine increased quite consistently in a pattern of gradual rise over the first 2 to 4 weeks to a plateau level varying from 60% to more than 100% greater than the control level. Figure 2, displaying the data for the 60 day flight, shows the pattern and also the individual variation, and Figure 3 in addition indicates the persistence of a high level of calcium excretion over the nearly three months of weightlessness in Sl-4.

Fecal calcium excretion showed an unusual but fairly consistent pattern of a decrease during the first few weeks in flight followed by a gradual rise to levels greater than in the pre-flight control phase. This pattern presumably reflected changes in intestinal absorption of calcium; the later stage higher fecal calcium levels were in line with the isotropically measured decrease in intestinal absorption of calcium in long term bed rest studies.

Figure 4 showing balance data for SL-3 is illustrative of the shift in calcium balance from positive during preflight to negative in flight (with much inter-individual variation).

Urinary hydroxproline increased inflight with considerable inter-individual differences; the mean increase for the nine crewmen was 30 percent. There was also a significant increase in total and nonglycosylated hydroxylysine.

The increase in plasma calcium in flight averaged 0.6 mg/dl and was very consistent from subject to subject. Plasma phosphorus was increased on the average 0.4 mg/dl.

Bone mineral content measurements by photon absorptiometry were made before and immediately after flight under the directions of John Vogel. The significant losses were limed to the os calcis and occurred in the science pilot of SL-3 and science pilot and pilot of SL-4 (-7.4 percent, -4.5 percent and -7.9 percent, respectively). These three astronauts had the greatest increases in urinary calcium and greatest negative shifts in calcium balance.

Phosphorus balance data showed a distinct increase in flight in urinary phosphorus, a small increase in fecal phosphorus, and negative balance in all crewmen.

SKYLAB OBSERVATIONS RELATIVE TO MUSCLE METABOLISM

Nitrogen balance data revealed during flight a pronounced increase in urinary nitrogen excretion, while fecal nitrogen remained characteristically unchanged. The increase in urinary excretion, consistent in all three flights, was 20% and averaged 3.1 gm/day. Figure 5 shows the nitrogen balance data for the three astronauts of the 84 day flight. After the distinct losses of the first month, balance was held close to zero only by protein intakes of about 120 gm/day and probably to some extent by vigorous muscular excercise. The muscles were not fully protected, however, by diet and exercise because the average decreases in leg volume were 7 to 11% in the three flights and in leg muscle strength 10 to 25%; decreases in leg volume and strength were less in SL-4 on which there was a vigorous muscle exercise program. The average daily absolute or actual negative nitrogen balance in weightlessness for all 9 astronauts was -0.9 gm, equivalent to a loss per month of a little less than 2 pounds of muscle protoplasm.

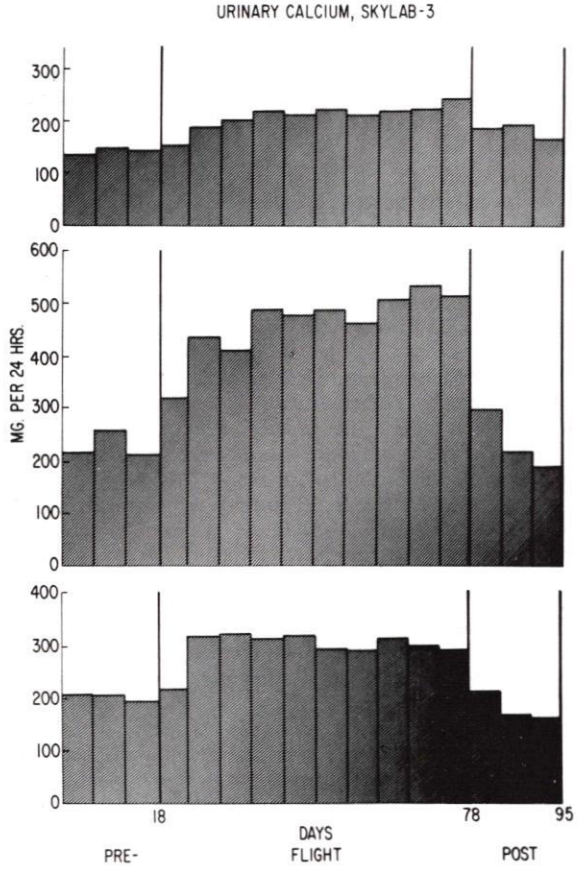

Figure 2. Effect of space flight on urinary calcium excretion in astronauts on the 60-day flight (SL-3).

STUDIES ON KOSMOS FLIGHTS

In the series of flights of Kosmos program, which the Soviets began in 1966, various animals have been sent into space. As reported in 1976, (9) Wistar rats abord the 22-day Kosmos-605 when returned to Earth revealed in their femora, tibiae and humeri decreased metaphyseal trabecular bone mass. Results of later, additional studies on Kosmos flights will be reported by the Soviets at this meeting.

The invitation of the Soviets to put aboard some of their Kosmos biosatellite flights a number of U.S. animal experiments has led to very interesting results with respect to efffects on bone. Kosmos-782 in November 1975 and Kosmos-936 in August 1977, both orbiting for about 19 days, carried two studies on 50-day old, growing rats of special interest. Morey and Baylink (10) in histological observations of bone, including tetracycline labeling, immediately after flight noted almost complete cessation of bone formation on the periosteal surfaces of the tibiae. Flight rats allowed to readapt to Earth gravity for 25 days resumed bone formation at a rate similar to that of control animals. In the second of these two Kosmos flights a group of flight rats was subjected to artificial gravity by centrifugation at 1G; in this group, decrease in bone formation was not quite as great as in the weightless group, recovery of bone mass after flight was more rapid, and decrease in femur breaking strength was prevented.

In subsequent Kosmos studies, Morey and associates (11) have seen cessation of bone formation in the humerus as well as the tibia, decreases in bone mass greater in trabecular bone than in cortical, and decreases in vertebral crush strength and in tibial trabecular bone mass that did not return to normal in 29 days after the flight. Simmons (12) has found changes in the mandible which indicate a defect in maturation in bone formation, and Roberts (13) similarly has noted a defect in conversion of progenitor cells to pre-osteoblasts.

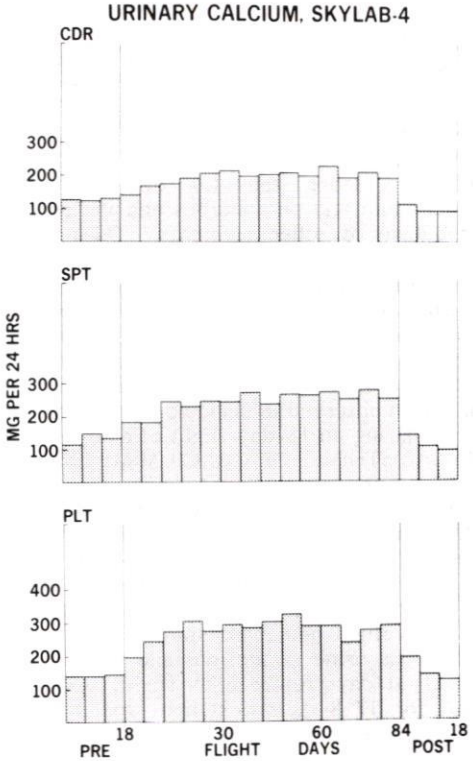

Figure 3. Effect of space flight on urinary calcium excretion in astronauts of the 84-day flight (SL-4).

RECENT SOVIET MANNED FLIGHTS

Recently, the Soviets have been conducting some measurements of changes in bone mass before and after their recent flights of up to six months duration. Decreases in bone mineral content of the os calcis, by photon absorptiometry, have been about 5 to 8% (14).

This past year a cooperative study has been initiated between the Institute of Biomedical Problems, Moscow, and the Department of Radiology, U.C.S.F., San Francisco, to measure by quantitative computed tomography the bone mineral content of the vertebrae of cosmonauts before and after the long flights (approximately six months usually) of the current Salyut-Soyuz series.

SIGNIFICANCE OF CALCIUM AND NITROGEN LOSSES

The increases in urinary calcium of astronauts in space flight were strikingly similar in pattern to those observed in immobile bed rest and nearly as great in degree. The negative shift in calcium balance also indicated slightly greater loss from immobilization, -280 mg/day versus -184 mg/day for the six astronauts of the first two Skylab flights. In studies of ordinary bed rest, calcium losses have been about the same as in the space studies.

The total calcium loss rate generated by the second month in space amounted to approximately 5.5 g/month or about 0.4% of total body calcium per month. Although this rate seems small in relation to the whole skeleton, the general similarity to bed-rest calcium losses (particularly the failure to show any tendency toward abatement in 3 months' time) suggest that mineral loss will continue for a very long time, presumably many months. After 6 months of weightless flight, the calcium loss would amount to about 2.5% of total body calcium. This is slightly more than the amount of loss which in studies of paralytic poliomeylitis resulted in X-ray visible rarefaction in the distal tibiae. Assuming that such excess excretion would continue in weightlessness, the calcium loss rate of 0.4% per month observed in Skylab takes on potentially serious significance in realization of the fact that the loss is predominantly local in the lower extremities and in view of the fact that flights to Mars and back (for example), will probably take in the range of 1.5-3 years.

The mechanism for these calcium losses and changes in metabolism is likely complex. The increases in urinary hydroxyproline excretion along with the increases in urinary calcium are indicative of increased resorption of bone. Supportive of this thesis are isotopic kinetic data which show increased bone resorption in patients immobilized by paralytic disease. On the other hand, the tetracycline labeling studies of Morey and Baylink in Kosmos flight growing rats indicated definite interruption of bone

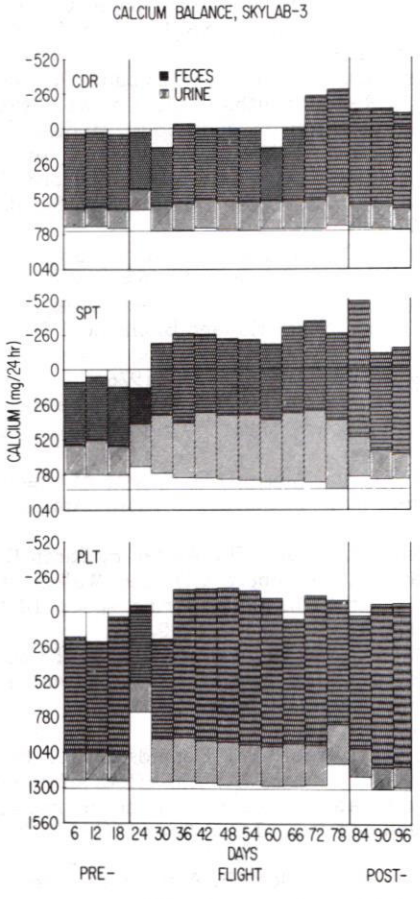

Figure 4. Calcium balance before, during, and after space flight in astronauts of the 60-day flight (SL-3). In this balance graph the data are plotted in conventional Albright-Reifenstein style--the intake downward from the zero base-line, then urinary (light shading) and fecal (heavy shading) excretion upward from the intake lines. Shaded areas above the zero base-line indicate negative balance loss.

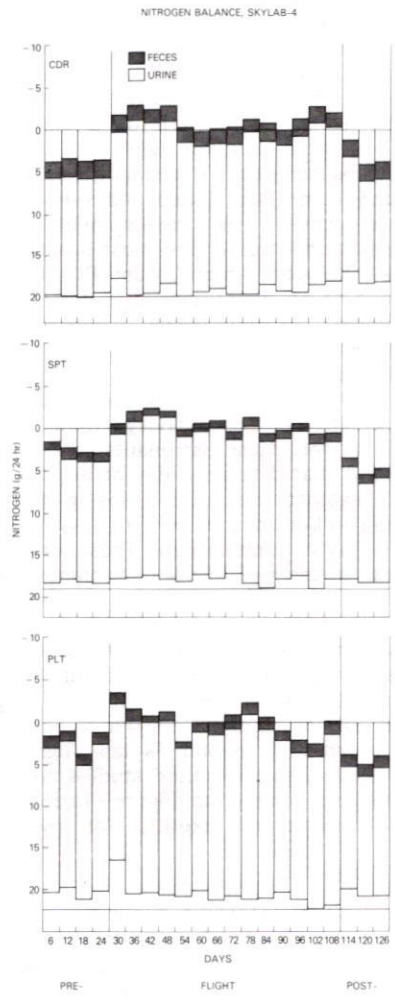

Figure 5. Nitrogen balances before, during and after space flight in astronauts of the 84-day flight (SL-4).

formation, a phenomenon also noted by us by X-ray examination of the tibiae in patients with recent paralysis by poliomyelitis. It is quite possible for both processes to be taking place at the same time, increased resorption and decreased formation, representing a clear disruption of the normal state in which these processes are consistently coupled.

The increased excretion of nitrogen and phosphorus, also similar to that observed during bed rest, reflected the substantial loss of muscle tissue which was clearly observed in the astronauts' legs. Both muscle and mineral loss occurred despite an exercise regimen which was followed on all flights and which was especially vigorous on the third flight.

EFFORTS TO FIND COUNTER MEASURES

The conclusion drawn from these data was that it is reasonable to predict musculoskeletal safety in space flight for something like 8 to 10 months, but that musculoskeletal function might be seriously impaired in crews on space flights of extreme duration unless protective measures are developed.

NASA has been sponsoring studies at the Public Health Service Hospital in San Francisco to find some means of protecting the skeleton against the negative calcium balance of bed rest which would be applicable to retarding or preventing calcium loss in space. Using normal human subjects and bed rest, as the best analogue for weightlessness, Hulley, Schneider and associates (15-17) have studied the following procedures or agents: physical exercise, compression of skeleton by pressure on the shoulders and heels (both continuously and intermittently), lower body negative pressure, intermittent rapid impact-loading at the heels, salmon calcitonin, high phosphate dietary intake, high calcium-high phosphate dietary intake, and diphosphonates. Thus far, all of the physical techniques have essentially failed and salmon calcitonin also failed to prevent the development of negative calcium balance in bed rest. The most promising protective measures have been 1) high Ca and PO_4 intake, which enabled balance to be maintained well for 3 months but apparently allowed "escape" during the fourth month of bed rest (as fecal calcium kept on increasing), and 2) EHDP di-phosphonate.

In an effort to determine how strong an equivalent measure to weight-bearing activity would have to be devised for long weightless astronauts, the PHS investigators (18) found that 3 hours a day of ambulation had a partial effect against the calcium-losing action of the remaining hours in bed, but to prevent loss of mineral, it took 4 hours a day of ambulation. Thus, some procedure suitable for use in the weightless environment equivalent to 4 hours of ambulation will have to be devised to give complete protection of the skeleton.

With regard to the decrease in muscle function in weightlessness, vigorous exercise against resistance appears to afford some protection, at least as judged by the contrast in loss of leg muscle strength and leg volume between the third and second Skylab flights. To my knowledge, however, no controlled studies have yet been done to test more precisely the degree of effectiveness of protection of muscle by exercise or by any other procedure.

SUMMARY

As a summarizing statement, a number of studies and observations of the effects of weightlessness on man and animals has indicated inpairment of musculoskeletal function and derangement in calcium and nitrogen metabolism, disturbances of a degree that might be hazardous on flights of approximately 9 to 12 months of more. Studies under way in humans using bed rest as the nearest Earth analogy to weightlessness have yet to develop a fully or even a largely protective counter measure.

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CALCIUM-PHOSPHORUS METABOLISM AND PREVENTION OF ITS DISORDERS IN HYPOKINETIC RATS

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The first manned space flights demonstrated physiological changes, including disorders in fluid-electrolyte metabolism: decrease in the circulating blood volume, negative fluid and electrolyte balance (1-3) and shifts in calcium metabolism (4-6). The importance of these variations increased with flight time. For instance, no signs of stabilization of calcium metabolism were seen in the crewmembers of prolonged Skylab and Salyut flights (7-11). The above shifts may cause changes in bone density, cardiovascular function, blood coagulation, urinary calculus and other disorders (12-14). One of the best approaches to the study of mechanisms of physiological changes developing in space flight is to fly mammals onboard biosatellites.

The investigations of rats flown for 18.5-22.5 days on Cosmos biosatellites demonstrated different bone changes, most important of which were shifts in bone formation (15-17) and bone metabolism (18). The measurements of the body composition of Cosmos-1129 rats showed that their total skeletal mass declined, and body calcium and phosphorus decreased by 22.1 and 13.5%, respectively (19).

Delayed growth of skeletal bones and their mineralization, decreased strength and increased sensitivity to loads are caused, among other factors, by variations in calcium balance. In this context of interest is the study of calcium balance carried out on rats flown on Cosmos-782, Cosmos-936, and Cosmos-1129 and exposed to hypokinesia.

The technical difficulties associated with calculations of food consumption made it difficult to compute calcium balance in space flight. It was, however, found that calcium excretion was elevated (22).

Calcium balance was measured in flight, synchronous and vivarium rats. The data give evidence that calcium balance was positive in the three groups, with the flight rats showing a high calcium retention which persisted till the end of observation. This suggests an increased calcium excretion inflight. The hypothesis is supported by the results of hypokinetic experiments. On hypokinetic days 9-11 calcium balance was negative and after exposure a high calcium retention was seen. By the end of hypokinesia (28 days) hypocalcemia, slightly increased phosphorus and lowered alkaline phosphatase were observed. The specific weight of femoral diaphyses and epiphyses, their content of calcium, phosphorus and oxyproline decreased significantly.

Thus, examinations of calcium-phosphorus metabolism in flight and hypokinetic animals have demonstrated its consistent changes.

Calcium and phosphorus metabolism is to a large extent controlled by vitamin D. This vitamin functions in its hormonal (active) form, i.e., 1,25-hydroxycholecalciferol ($1,25(OH)_2D_3$, or calcitriol) which produces a direct stimulating effect on bone resorption. On the other hand, one can think about the specific function of 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$), which is actually stimulation of bone formation and mineralization. This stimulated our interest in the formation of the metabolites from their precursor $25(OH)D_3$ in hypokinetic rats.

The experiments were performed on Wistar rats weighing 215±2.4 g that were kept in small-size cages for 7 and 28 days. The rats were fed diets containing physiologically normal quantities of calcium, phosphorus and vitamin D. Labelled precursor - 25-hydroxy/26(27)-methyl-^{3}H/cholecalciferol with a specific activity of 7.7 Curie/mMole was injected intraperitoneally in the ethanol-propylene glycol mixture. Vitamin D_3 labelled metabolites were isolated by high-performance liquid chromatography from serum, kidneys, intestinal mucosa and bones.

On hypokinesia day 7 no significant changes in the metabolism of ^{3}H-$25(OH)D_3$ were seen, except for its slight decrease in kidneys. The prolonged hypokinesia exposure (28 days) resulted in a decrease in ^{3}H-$1,25(OH)_2D_3$ and an increase in ^{3}H-$24,25(OH)_2D_3$ in serum and kidneys. In intestinal mucosa and femoral bones the content of both metabolites diminished and that of ^{3}H-$25(OH)D_3$ increased. These findings indicate a decline in the synthesis of $1,25(OH)_2D_3$ in kidneys (by 21.3 and 35.3% after 7-day and 28-day hypokinesia,

respectively). The intestinal mucosa and bones accumulated ^{3}H-25(OH)D_3, thus suggesting the synthesis of 24.25(OH)$_2D_3$ from its precursor.

These data together with variations in calcium metabolism give evidence that metabolites of 25-hydroxycholecalciferol are required to maintain calcium homeostasis and to regulate bone status in hypokinetic rats. The reduction in the concentration of circulating 1,25(OH)$_2D_3$ leads to a decrease of the calcium absorption in the small intestine and hypocalcemia which may limit the mineral content of newly formed bones. The decrease of both metabolites in bones may point to a strong inhibition of bone formation during hypokinesia whereas enhanced resorption appears less probable. These data suggest that active metabolites of vitamin D_3 can be used to prevent and treat disorders in calcium metabolism during hypokinesia.

It was previously shown that 1,25(OH)$_2D_3$ in adequate doses stimulated efficiently calcium absorption in the small intestine, normalized its calcium absorption in blood and enhanced bone formation and mineralization during hypokinesia (20), whereas 24,25(OH)$_2D_3$ did not increase calcium absorption as compared to the effect of 1,25(OH)$_2D_3$ and stimulated ossification in a larger degree than 1,25(OH)$_2D_3$ (21).

The combined effect of both metabolites was examined using male Wistar rats weighing 200-250 g which were kept in hypokinetic cages for 28 days. The rats were given a semi-synthetic casein-starch diet that contained 0.6% calcium, 0.6% phosphorus and adequate doses of vitamin D. Vitamin D_3 metabolites (1,25(OH)$_2D_3$ and 24(R,S),25(OH)$_2D_3$) were given to rats per os in 0.1 ml propylene glycol in the following doses: 1,25(OH)$_2D_3$ - 30 and 150 mg, and 24,25(OH)$_2D_3$ - 250 and 1250 mg. The pharmacological activity of D_3 metabolites was determined, measuring calcium, phorphorus and alkaline phosphatase in blood, specific weight of femoral diaphyses, the content of calcium, phosphorus and oxyproline in diaphyses and epiphyses, and the labelled calcium (^{45}Ca) absorption in the small intestine.

The greatest effect on bone composition was produced by the combined administration of both metabolites in physiological doses or 1,25(OH)$_2D_3$ in the physiological dose combined with 24,25(OH)$_2D_3$ in the 5-fold physiological dose. In this case the content of calcium, phosphorus in blood, absorption of labelled calcium in the small intestine, as well as the calcium and phosphorus content in femoral diaphyses and epiphyses differed from the controls but insignificantly (Fig. 1).

The calcium and phosphorus content was high in the epiphyses. The content of oxyproline (collagen) decreased in diaphyses and slightly increased in epiphyses (Fig. 2, 3).

Thus, the data obtained point to distinct changes in calcium-phosphorus metabolism and bone characteristics in animals after flight and prolonged hypokinesia. Our preliminary results indicate a 30-40% decrease in the active transport of calcium in the small intestine during hypokinesia. The changes in bone chemical composition and specific weight may be associated with enhanced calcium resorption and osteoporosis-like changes. The comprehensive evaluation of bones during prolonged hypokinesia allows the conclusion about their delayed growth.

The prolonged hypokinetic exposure caused changes in 25(OH)D_3 metabolism in spite of its adequate dietary intake. This was paralleled by a decline in ^{3}H-1,25(OH)$_2D_3$ in serum, kidneys, intestinal mucosa and bones. The normal reciprocal relations between 1,25(OH)$_2D_3$ and 24,25(OH)$_2D_3$ were seen in serum and kidneys, whereas in intestinal mucosa and bones ^{3}H-24,25(OH)$_2D_3$ was decreased and nonmetabolized 25(OH)D_3 increased. These data give evidence for a reduced synthesis of 1,25(OH)$_2D_3$ and an enhanced synthesis of 24,25(OH)$_2D_3$ in kidneys. In intestinal mucosa and bones ^{3}H-25(OH)D_3 accumulated, thus indicating a decreased synthesis of 24,25(OH)$_2D_3$ from this precursor (by 20.5% in mucosa after 28-day hypokinesia, and by 22.6 and 43.9% in bones after 7- and 28-day hypokinesia, respectively). The decreased decline of ^{3}H-24,25(OH)$_2D_3$ in bones occurred despite an enhanced circulating level of this metabolite. It may be assumed that this decline is in part associated with a lowered binding of the metabolite by receptors, while the proportional decrease in ^{3}H-1,25(OH)$_2D_3$ appears to be caused only by the decline of the metabolite production in kidneys.

The results of the drug testing give evidence that 1,25(OH)$_2D_3$ and 24,25(OH)$_2D_3$ in physiological doses may prevent changes in calcium-phosphorus metabolism during hypokinesia, thus indicating their involvement in the regulation of vitamin D metabolism. A more effective action of 24,25(OH)$_2D_3$ on the maintenance of the femoral composition may suggest the specific effect of this metabolite on bone mineralization. The effect of 24,25(OH)$_2D_3$ may in part be attributed to the stimulated synthesis of 1,25(OH)$_2D_3$ (22), its preliminary 1α - hydroxylation (23, 24) as well as its direct stimulating action on calcium absorption in small intestine when taken per os (25). The uptake of active metabolites of vitamin D_3, particularly their combinations, may also modify the secretion of parathyroid hormone and calcitonin (26, 27).

These findings can be viewed as an

indication that in order to prevent changes in calcium-phosphorus metabolism active metabolites of vitamin D_3 can be used in the weightless and hypokinetic states.

FIG. 1. Effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on Concentration of Ca, P, Alkaline Phosphatase Activity in the Serum and in Vitro Ca^{45} Transport by Small Intestine in Hypokinetic Rats

Group of animals	Dose, ng	Calcium, mg/100 ml	Phosphorus, mg/100 ml	Alkaline phosphatase U/l (37°)	Ca^{45} transport nmol Ca/cm 60 min
Control	-	10.4±0.2	8.2±0.2	71.8±4.4	88.8±4.6
Hypokinesia	-	9.3±0.2	8.6±0.3	44.8±2.7	61.1±3.3
+ $1,25(OH)_2D_3$	30	9.9±0.1	9.0±0.4	38.0±2.4	76.2±7.4
+ $1,25(OH)_2D_3$	150	11.2±0.3	10.0±0.2	35.6±2.8	82.1±7.1
+$24,25(OH)_2D_3$	250	9.6±0.2	8.4±0.4	44.6±2.3	70.2±3.8
+$24,25(OH)_2D_3$	1250	10.2±0.1	8.6±0.3	49.2±2.0	74.2±8.7
+$24,25(OH)_2D_3$ and $1,25(OH)_2D_3$	1250 + 30	10.1±0.3	8.5±0.4	47.4±2.2	73.6±3.1
+ $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$	30 1250	10.3±0.2	9.0±0.4	51.8±1.6	1.6±9.0

FIG. 2. Effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on Chemical Composition of Femoral Epiphyses in Hypokinetic Rats

/mg/g Dry Matter/

Group of animals	Dose, ng	Calcium mg/cm³	Phosphorus mg/cm³	Hydroxyproline mg/cm³
Control	-	220 ± 2.3	129 ± 1.2	24.7 ±1.0
Hypokinesia	-	186 ± 2.6	116 ± 2.7	21.8 ± 0.9
+ $1,25(OH)_2D_3$	30	199 ± 4.3	120 ± 2.6	23.5 ± 0.5
+ $1,25(OH)_2D_3$	150	181 ± 3.0	112 ± 2.5	22.0 ± 0.6
+$24,25(OH)_2D_3$	250	208 ± 2.9	119 ± 1.6	23.5 ± 0.8
+$24,25(OH)_2D_3$	1250	206 ± 4.0	121 ± 3.2	23.8 ± 1.0
+ $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$	30 + 250	214 ± 3.3	127 ± 2.7	25.1 ± 1.0
+ $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$	30 + 1250	217 ± 3.2	125 ± 2.6	25.9 ± 0.9

FIG. 3. Effect of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on the Specific Weight and Chemical Composition of Femoral Diaphyses in Hypokinetic Rats

Group of animals	Dose, ng	Specific weight: g/cm³	Calcium, mg/cm³	Phosphorus, mg/cm³	Hydroxyproline mg/cm³
Control	-	1.68 ± 0.01	477 ± 7	260 ± 3	37.1 ± 1.0
Hypokinesia	-	1.48 ± 0.02	381 ± 8	225 ± 5	28.3 ± 1.0
+ $1,25(OH)_2D_3$	30	1.57 ± 0.03	424 ± 10	233 ± 4	29.9 ± 0.8
+ $1,25(OH)_2D_3$	150	1.43 ± 0.01	387 ± 11	223 ± 5	27.4 ± 1.0
+$24,25(OH)_2D_3$	250	1.61 ± 0.01	439 ± 9	247 ± 4	31.7 ± 1.0
+$24,25(OH)_2D_3$	1250	1.63 ± 0.01	435 ± 10	241 ± 4	32.3 ± 0.9
+ $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$	30 + 250	1.65 ± 0.02	447 ± 8	245 ± 6	32.4 ± 1.0
+ $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$	30+1250	1.65 ± 0.02	454 ± 12	250 ± 6	32.7 ± 1.1

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EXPERIMENTAL ANALYSIS OF MOTOR EFFECTS OF WEIGHTLESSNESS

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In the multicomponent motor system zero-gravity transforms into several effects - muscle unloading, reduction of support reactions, change in movement biomechanics, etc., each of which may be responsible for a dysfunction of its components. In space flights of different duration the weight of the above factors is obviously different. For instance, in short-term flights the reduction of support reactions may make the major contribution to the development of motor dysfunctions.

As known, the stimulation of support receptor zones plays an important role in the organization of muscle tonic reactions developing like reflex grossynergies; support afferentation is important in activation of extensor motor centers (1). In the absence of support loads the tone of gravitational muscles decreases (2-3). According to the clinical observations, this decrease is in turn the factor that initiates changes in different components of the motor system, viz. muscle afferents and motoneuron entities, reflex mechanisms and trophic apparatus whose activity, as shown recently, is also determined by the motoneuronal activity (4).

In order to identify the role of support unloading in weightlessness-induced motor disorders, it was necessary to compare changes in the motor system after exposure to weightlessness and immersion adequately simulating the elimination of support loads. That was the purpose of the present investigation.

MATERIALS AND METHODS

The motor effects of weightlessness and immersion were studied in 17 members of Salyut-6 visiting crews and 22 participants of simulation experiments. The support unloading was simulated by dry immersion. According to (5), the method reproduces entirely the hypokinetic effects in the absence of discomfortable phenomena. In both cases the exposure was, as a rule, 7 days. (The time of two short-term flights was 4 days and 14 days with 3 crewmembers in each).

The experimental protocol was close to that used in previous Salyut-6 long-term flights (6) and consisted of procedures and tests allowing quantitative evaluations of different parts of the motor system. The function of the main proprioceptive inputs - muscle and support - was assessed with reference to the recruitment functions of T- and H-reflexes of the triceps surae muscle and thresholds of the vibrosensitivity of the sole support areas; the strength-velocity parameters of leg muscles - using isokinetic dynamometry; the support system of the feet - with the aid of X-ray examinations; motor control systems - by stabilographic characteristics and postural correction responses. These methods were described in detail elsewhere (6). Having in view the purpose of the study, variations in the tone of leg muscles at rest and at maximum contraction were also carefully examined during immersion. This was done measuring transverse stiffness. The use of an electromyotonometer of a new design that can record independently the force with which the transducer is attached and the tissue responses in a wide range of deforming forces (Fig. 1), as well as high standardization of measurement conditions (stability of the range of forces with which the transducer is attached, maintenance of the limb position, and control of the relaxation and tension of the muscle under study by means of an electromyographic signal used as a feedback) have increased significantly the accuracy and reproducibility of the data and the reliability of the method.

The application of the identical battery of tests supplied with more sophisticated techniques in simulation studies has yielded comparable data which may contribute to our understanding of the weightlessness-induced changes.

RESULTS

The study demonstrated a close similarity between the effects of short-term exposure to zero-g and immersion.

Sensory systems. The changes in the T-reflex after 7-day space flight were similar to those seen previously in the long term crews. As shown in Fig. 2, zero-g was followed by a decrease in the reflex thresholds and maximum amplitude; it also altered interlimb relations: the flexion of

the other foot which normally inhibits the T-reflex produced a smaller effect postflight.

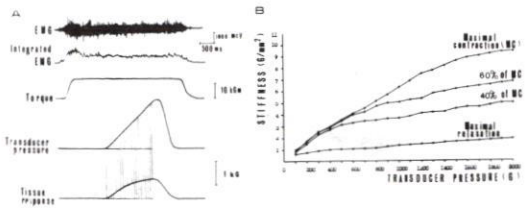

Fig. 1. Sequence of recordings during electromyotonometric studies.

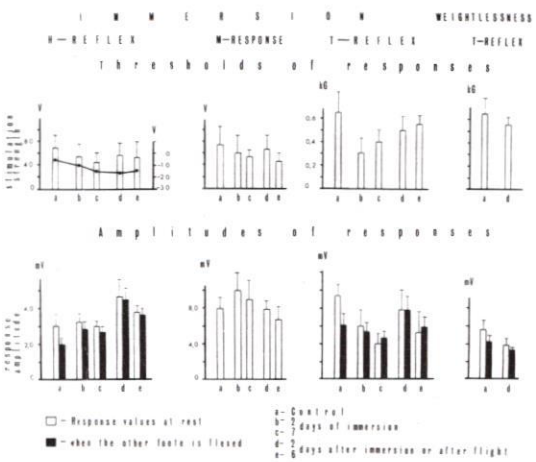

Fig. 2. Parameters of evoked reactions of m. soleus during immersion and space flight. The thick line in H-reflex sequence shows the relative threshold; 0 is the threshold of M-response.

Similar changes in the T-reflex were observed during immersion. The shifts developed on day 1 and progressed as the exposure continued. On immersion day 1 the reflex thresholds were 350±44 deg (versus 617±30.0 deg in the controls). As was the case in spaceflight, the decrease of the thresholds was followed by a decline of the maximum amplitude and disorders of interlimb synergies. After exposure the reflexes recovered in a wave-like manner, the process being, however, incomplete by the 7th day of observation. The changes were the largest during the first day of the onset and completion of the immersion study. Although the general trend of the effects was identical, their intensity in short-term flights was lower than in long-term flights and immersion.

Similar changes of the thresholds of the H-reflex, which is generally viewed as a "receptor-free" analog of the T-reflex, and less distinct changes of the thresholds of the direct muscle (M) response suggest that the muscle hyper-reactivity that accompanies support unloading is of complex nature and involves both central (increased motoneuronal excitability) and peripheral mechanisms. The hypothesis concerning changes in the neuro-muscular periphery during immersion is confirmed by the alterations of the pattern and time characteristics of the M response seen in the experiments (Fig. 4).

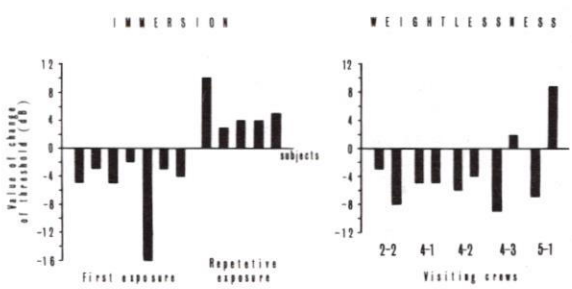

Fig. 3. Vibrosensitivity of soles during immersion and space flight.

Space flight and immersion caused distinct hyper-reactivity of the support input as well: 8 out of 10 crewmembers and all test subjects first exposed to immersion showed a marked decrease in the vibrosensitivity thresholds of the sole during the post-exposure examination (Fig.3).

The muscle apparatus. After immersion every subject showed a noticeable decline in the strength characteristics of the triceps surae muscle. The decline, that averaged 30-40%, was significant during both dynamic and isometric contractions. The strength capability of the anterior tibial muscle remained virtually unchanged during dynamic contractions and fell (to 30%) during isometric ones

Similar changes in the strength-velocity properties of leg muscles were found in the visiting space crews. As follows from Fig. 5, they exhibited a significant

decrease (by 20-30%) of the maximum strength of leg extensors at all velocities including isometric contractions; they did not show any significant changes in the strength characteristics of the anterior tibial muscle that remained practically unaltered also during isometric contractions, which was not the case with immersed subjects.

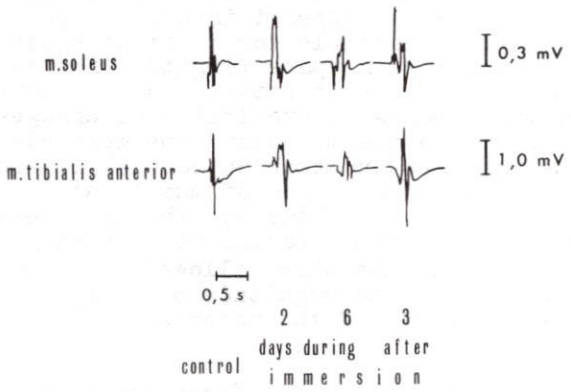

Fig. 4. M-response of m.soleus during 7-day immersion.

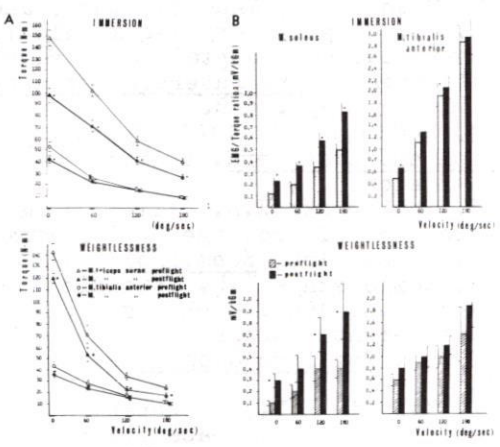

Fig. 5. Properties of leg muscles before and after 7-day immersion (above) and space flight (below). Panel A shows velocity-strength characteristics, Panel B-ratio between EMG amplitude and the torque.

An analysis of the ratio between the EMG maximum amplitudes and the muscle efforts revealed its increase in leg extensors after space flight and immersion (Fig. 5,B). This was another indication of the decline in the functional capability of the muscles; as to the anterior tibial muscle, its EMG effort ratio did not change after both exposures.

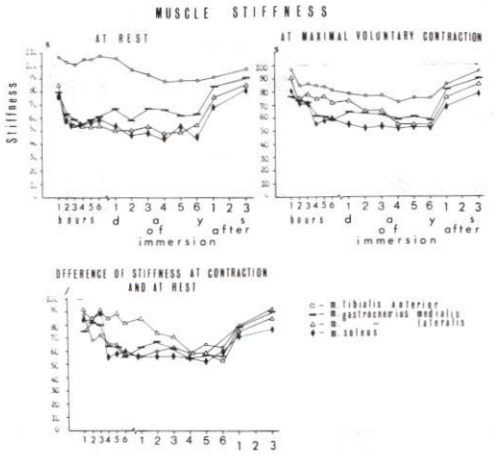

Fig. 6. Stiffness of leg muscles at rest and contraction during 7-day immersion.

The myotonometric measurements during immersion showed that the stiffness of the triceps surae muscle decreased at rest and during contraction, the decrease reaching maximum during the first hours of immersion (Fig. 6). The stiffness change in the three caputs of the muscle was different: it was the highest in the soleus muscle and the lowest in the caput mediale, which tended to restore its stiffness beginning with immersion day 2 (Fig.6). The variation in the stiffness of the anterior tibial muscle at rest was insignificant and variable. During maximum voluntary contractions the decrease in the extensors was greater than at rest, the anterior tibial muscle also losing its stiffness.

Movement control system. Space flight and immersion produced noticeable modifications in the characteristics of tracking movements, stabilograms and cyclograms of the gait which pointed to disorders in the mechanisms of the accuracy control of movements, posture and locomotion, their lesser efficiency and stability. When performing the tracking task, the subjects showed a 2.5-3-fold increase in the number of errors and variability. When the subjects were standing in the comfortable and Romberg positions, their stabilograms displayed a far larger portion of high-frequency oscillations of the tremor type (Fig. 7A). This indicates a greater involvement of active muscle mechanisms in the postural maintenance. Disorders in the upright stability were also distinct in the perturbation tests: the thresholds of correction responses decreased, and their electromyographic cost increased drastically (Fig. 7B).

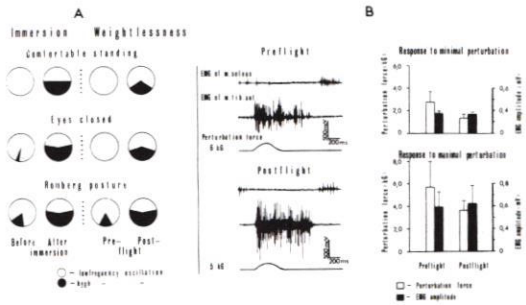

Fig. 7. Characteristics of stabilogram (A) and EMG postural correction responses to perturbation (B and C) before and after immersion and space flight.

CONCLUSION

In conclusion, it is important to discuss some problems of theoretical and practical interest.

1. As follows from our study, 7-day exposure to zero-g or immersion brings about changes in different parts of the motor system that are close to those seen in longer-duration flights (6). However, the level of changes is different: after short-term flights reflex and sensory effects are less pronounced, whereas changes in muscle properties are more marked and stable. The specific feature of disorders associated with short-term effects is their close correlation which suggests that they are produced by one or several related factors; as mentioned above, there was no relationship of this sort in long-term flights. The latter may be attributed to a regular use of different countermeasures which were never employed in short-term flights.

2. Following immersion the level of motor disorders was slightly greater than after space flights of equal duration. It can be due to the summation (and probably potentiation) during immersion of the support unloading effects with the effects of hypokinesia, which is substantial in simulated and negligible in actual space flights. It is well-known that onboard space station crewmembers are very busy performing various duties and operations. The related motor activity seems to be the factor responsible for the difference in the level of the above changes.

3. Obviously, the reduction of motor capabilities seen after short-term flights and immersion may be caused by disorders in any parts of the motor system, e.g. change in the feed-back activity, decrease in support reactions, reduction of the strength of gravitational muscles, etc. However, the high correlation between the tone and strength decrease in the antigravitational muscles after 7-day space flight and immersion, as well as the distinct relationship between postural changes and body harness rigidity (they disappeared when the number of degrees of freedom was decreased and enhanced when the number was increased) suggest that the tonic shift due to the lower support inputs is the main factor responsible for motor disorders during short-term space flights. The ideas about a relationship between motor disorders in response to gravitational changes and shifts in the muscular tone were advanced earlier by other authors (7,8) who detected clinical signs of muscle atony in space crewmembers. However, the hypothesis needed experimental verification based on quantitative data which allowed a reliable comparison of the magnitude of changes in different parts of the motor system.

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THE NASA SPACE BIOLOGY PROGRAM

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Introduction

Space biology has many definitions. Inclusively, space biology is defined as the various biological sciences concerned with the study of living things in the space environment. Space biology can also be defined by the inherent physical factors of space and space travel--biophysical, biodynamic, and aerospace medical factors--and their effects on living organisms. For example, biophysical factors result from the environment of space, such as weightlessness, radiation, temperature, and decreased atmospheric pressure; biodynamic factors are due to the flight dynamics of the spacecraft, including acceleration, vibration, and noise; and aerospace medical factors are created by the involvement of human beings in the artificial environment of the spacecraft, including nutrition, toxicology, isolation, and breathing in the artificial atmosphere. All of these factors are of concern to space travel and experimentation and must be addressed in the design of the spacecraft, the medical operation program, and the biological flight experiments to ensure the safety of crew members and the integrity of biological payloads.

In the United States, supporting humans in space as well as using the space environment to advance basic biological research is the responsibility of the Life Sciences Division of the National Aeronautics and Space Administration's Office of Space Science and Applications. In fact, effective utilization of the space environment to push back the frontiers of biological knowledge is one of the major reasons for a human presence in space. The NASA Life Sciences Division's Space Biology Program is responsible for assuring that the unique features of the space environment will be utilized effectively for basic biological research, as well as for assuring that basic biological science contributes effectively to a human presence in space.

Research Opportunities

Among the effects of spaceflight, the phenomenon of weightlessness provides the greatest research opportunity both because of its uniqueness to space and because of the importance of gravity to life on Earth. Hitherto, biological studies with altered gravitational fields were limited almost exclusively to increases in gravity, since weightlessness could be maintained for only a matter of seconds on Earth. Access to space provides an opportunity to conduct research under weightless conditions and to manipulate gravity from its norm of one down to zero, effectively providing the full spectrum of gravitational research capability for the first time. This, plus the stability and pervasiveness of gravity on Earth, its obvious impact on biological evolution, and its continuing effect on the morphology, physiology, and behavior of living organisms, has led the Space Biology Program to concentrate its efforts and resources on investigating the biological significance of gravity.

Program Objectives

The objectives of the Space Biology Program are to understand how gravity affects and how it has shaped life on Earth, to use gravity as a tool to investigate relevant biological questions, and to understand how near-weightlessness affects both plants and animals, thereby enhancing our capability to use and explore space. These objectives specifically exclude biomedical problems encountered by man in space.

Much of the knowledge gained through the Space Biology Program will also have application in designing controlled ecosystem life support systems to maintain animal and plant health in the space environment. While the applications of this research to future long-term manned spaceflights are obvious, there will also be a considerable "pay off" in our understanding of the nature of terrestrial

ecosystems and the basic principles of biology.

Program Scope

The research in the Space Biology Program is divided into three broad areas:

1. The role of gravity in development. Specifically, the effect of gravity on reproduction, development, maturation, and evolution.

2. Gravity receptor mechanisms. This includes the identification of the organ or site of gravity reception and the biological systems and mechanisms that transmit the information to a responsive site.

3. The physiological effects of gravity. This includes the use of gravity's physiological effects to explore biological problems; an understanding of how gravity affects and controls the physiology, morphology, and behavior of organisms; how gravity and other environmental stimuli and stresses interact in this control; and the biological mechanism by which living systems respond and adapt to altered gravity, particularly that of the space environment.

Scientific Approach

With the proven feasibility of the Space Shuttle, we now have the capability of performing biological experiments in space. Ultimately, when a station is established in space, we can expect to conduct research that will provide long-term exposure to near weightless conditions. However, such opportunities are and probably will continue to be limited by cost. Consequently, ground-based studies will continue to be important.

Although spaceflight is necessary to provide the final validation for many experimental hypotheses tested in ground-based research, gravitational experiments on Earth help to hone the questions, provide the necessary baseline data, and develop spaceflight experimental protocol.

The experimental approach of the ground-based studies in the Space Biology Program is to manipulate gravity on Earth and develop weightless simulation models to: (1) develop and test gravitational hypotheses, (2) identify gravity-sensitive biological systems and interacting environmental response mechanisms, (3) analyze biological systems and mechanisms known to be gravity sensitive, (4) analyze flight experiment data and iteratively expand ground research capability, and (5) plan and design future space experiments.

In addition, research is conducted to understand how the uncontrollable biodynamic factors of the spacecraft will affect the results of the various flight experiments.

Weightlessness, and physiological conditions similar to those induced by weightlessness, are partially simulated in the laboratory by techniques that include immobilization and water immersion of animals, and clinostat rotation of plants and some animal cells.

In animal research, the attempt is to simulate the reduction in apparent weight and alteration in hydrostatic pressure that occurs under weightlessness conditions. Simulation is accomplished in the Program's research by the use of a unique immobilization method--an animal suspension system--that successfully induces some of the physiological changes associated with weightlessness. This method has been developed as well as used by scientists associated with the Space Biology Program.

The immobilization procedure involves suspending a rat in a harness or by its tail so that the hind limbs are free but non-load-bearing while the front limbs are used for propulsion, eating, and grooming. The animal is both hypokinetic and hypodynamic. Further, the animal is usually positioned to affect a head-down tilt. This antiorthostatic positioning appears to produce fluid shifts similar to those associated with zero gravity (1,2). Animal research within the Space Biology Program has concentrated on the use of rats because rats have been and promise to continue to be the animal most used in spaceflight experiments.

The clinostat is an apparatus which, by its motions, modifies or equalizes the directional input from gravity to an attached organism. The rotation of experimental material on a clinostat is useful only for those physiological phenomena that have relatively long exposure thresholds (or presentation times), so that it is possible to rotate the organism slowly enough to avoid the complications produced by centrifugal acceleration, but fast enough so that the time spent in any one position does not result in an induced georesponse (3).

To achieve hypergravity conditions, gravity loads above one are produced by centrifugation and are especially useful in identifying gravity-sensitive biological systems and mechanisms. Changing the directional input of gravity to an organism has also proved to be a successful technique to study the biological effects of gravity.

Present Focus of Program

The research focus of the Space Biology Program is dependent upon several dynamic factors: NASA requirements, the characteristics of flight experiment opportunities, the sensitivity of specific biological systems to gravity, the scientific value of the research, the state of knowledge and technology in the specific scientific areas, the interest of scientists in studying the biological questions, and the availability of funds to support the research.

Within the scope of the Space Biology Program, the current Program is focused on answering the following basic scientific questions:

1. Does gravity influence plant and animal fertilization and early development, and can fertilization and early development proceed normally in a near zero-gravity 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 effect on the parent or the direct effect on the embryo itself?
2. What is the role of gravity in the formation of structural elements such as lignin, cellulose, silica, chitin, and bone calcium phosphates at the molecular level 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?

Current Research

Current developmental research includes the following:

1. Plant studies to characterize seed-to-seed growth processes of select higher plants and to establish whether or not the degree of sensitivity or responsiveness to gravity varies at different degrees of organization or stages of development.
2. A baseline of functional development in the mammalian vestibular and proprioceptive systems is being established in preparation for a space experiment to determine whether sensory deprivation of a gravity stimulus during embryogenesis leads to altered neural patterns or affects an organism's capability to perceive and respond to gravity.
3. A four-species model of laboratory mammals has been developed for metabolic scale effect studies to examine the effects of change in ambient temperature and gravitational loading on metabolic energy expenditure.
4. The extent to which novel gravity orientation influences normal pattern formation in amphibian eggs is being analyzed, as well as the effects of simulated weightlessness on mammalian meiosis, fertilization, and early embryogenesis.

Biochemical, physiological, and morphological changes in organisms exposed to simulated hypogravity or hypergravity are delineated and quantified, to understand the physiological effects of gravity. Much of the animal work is directed toward elaborating the regulatory factors in homeostatic adaptation to and deconditioning from the metabolic perturbation associated with a change in the gravity field. Specific research is directed toward: defining gravity-induced alterations in hormone secretion and/or function; identifying changes in calcium fluxes due to altered gravity; defining alterations in cell proliferation at different gravity levels; identifying in bone and skeletal muscle the biochemical and morphological alterations induced by the reduced load-bearing and antiorthostasis produced using the rat model system; clarifying the neurophysiological mechanisms underlying the gravity-sensitive regulation of temperature; and evaluating the anti-orthostatic rat as a model for cardiovascular and hematopoietic effects of weightlessness, and using it to study mechanisms involved in changes encountered.

Understanding animal, and especially mammalian, organs of equilibrium and how they function in both a one-G and a weightless environment is not only of significant scientific interest but highly relevant to the space program. Only the most basic research is supported by the Space Biology Program, however, because of extensive research in other Life Sciences Programs directed toward solving the medical problem of space motion sickness. Current Space Biology research is directed at: (1) elucidating the role of otoconial calcium in inner ear function, calcium involvement in mechanisms of transduction in mammalian gravity receptors, and gravity receptor cell interaction; and (2) investigating the gravity receptors and biotransducers in simple animals exemplified by an insect and arachnid.

Plant research to understand not only gravity perception but the mechanism involved in the response includes studies to: disclose the biological mechanisms by which mechanical perturbation affects growth and blocks gravitropism; identify those plant responses to clinostating caused by gravity compensation and those caused by the mechanical stresses of horizontal rotation; isolate amyloplasts and define their physical and chemical nature and examine the dynamics and kinetics of amyloplast movement in response to gravity to evaluate the interaction of amyloplasts with other cytological elements; determine changes in growth patterns induced by gravitational stimulation and relate these changes to altered distribution of chemical (including hormonal) signals in the responding organ; understand the cellular functions that are altered by gravity and light to produce gravitropic growth and identify the role of the light stimulus in the response; and unravel the responses of individual cells to gravistimulation and understand the role of gravity in nutation.

Future Focus of Program

In the immediate future, research will continue to focus on the five main areas identified under Present Focus that include the mechanisms of gravity perception and information transmission and the early effects of gravity on the structure and physiology of higher plants and mammals during their normal life span. Because calcium ions are widely recognized as second messengers for regulating the adaptive responses of plants and animals to different sensory stimuli, more emphasis will be placed on investigating the potential role of calcium in regulating gravitational responses in organisms. These areas as well as studies of the interaction of gravity with other stresses and stimuli can in general be tested on the Space Shuttle and have application to long-term manned flight as well as providing additional insight into biological mechanisms on Earth.

There is reason to believe that opportunities will become available for relatively self-contained biological experiments to be flown in the middeck lockers of the Shuttle orbiter during many of the Shuttle missions. Research that supports or can be tested by such an experiment will also be considered of high priority.

As longer flight missions become available, biological questions that require longer periods of micro-G, such as multigeneration and radiation experiments, will increase in importance. It has been recognized for some time that ionizing radiation causes cumulative damage in all living things, but knowledge of the biological effects of the cosmic radiation encountered in space, which contains high energy, heavy particles, is poorly understood. Furthermore, the biological consequences of the interaction of this unique radiation and gravity are less known. Since long-term space exposure is needed to adequately investigate the effects of radiation, such research is well suited for the spaceflights of six months or longer that a space station will provide.

Conclusion

Despite being hampered by limited access to the space environment, the research carried out by the NASA Space Biology Program since its inception has increased our knowledge of both plant and animal physiology in general as well as gravitational biology. The Space Shuttle and future Space Station promise a bright future for space biology research on the ground as well as in space. Ground and space research are mutually dependent, and the accomplishments of each are dependent upon an opportunity to do both. These efforts will contribute not only to our knowledge of the biological effects of the space environment and our ability to use space, but to a better understanding and improved life on Earth.

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STATUS OF JOINT US/USSR EXPERIMENTS PLANNED FOR THE COSMOS '83 BIOSATELLITE MISSION

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INTRODUCTION

Cooperation between the USA and the USSR in Space Biology and Medicine began in 1971 with the signing of the US/USSR Science and Applications Agreement. Annual meetings between the two countries were initiated to discuss problems and areas of mutual scientific interest a result of which was the creation of a Joint US/USSR Biological Satellite Program. Under the aegis of this program, a variety of US biological and radiation physics experiments were flown on three unmanned Soviet satellites, Cosmos 782, Cosmos 936 and Cosmos 1129, launched in 1975, 1977, and 1979, respectively. The spaceflights were each approximately three weeks long and contained payloads of rats, plants, insects and a variety of other organisms. The principal objective of the biosatellite flights was to determine how the stresses of spaceflight affect biological systems with particular emphasis on biomedical problems common to man and animals. As a consequence of these three flights, much has been learned about how organisms are affected by, and adapt to, orbital spaceflight (1). A fourth mission which will fly nonhuman primates, Macacca mulatta, has been planned to continue such joint studies. This mission marks the first time the Soviet Union will orbit nonhuman primates, and will enable scientists to extend the results of previous experiments using an animal surrogate much more closely related to man than the rat. The purpose of this article is to describe the plan for this latest mission and the status of the joint US/USSR experiments included in the payload.

Mission Description

The Cosmos '83 mission is scheduled for launch during the last half of 1983 with a nominal orbital duration of seven days. A modified Vostok Spacecraft will be employed similar to that used for previous biosatellites. It is a spherical craft approximately 2.4 meters in diameter with a 900Kg payload and a 2300Kg gross weight (Fig. 1). During the flight, the atmosphere within the craft will be maintained at approximate sea level conditions with gaseous impurities removed by circulating cabin air through cannisters of absorbent materials. The light:dark cycle will be set at 16:8 hours during the flight with a nominal ambient temperature of 24+2°C. Relative humidity will be maintained within the range 50-85%.

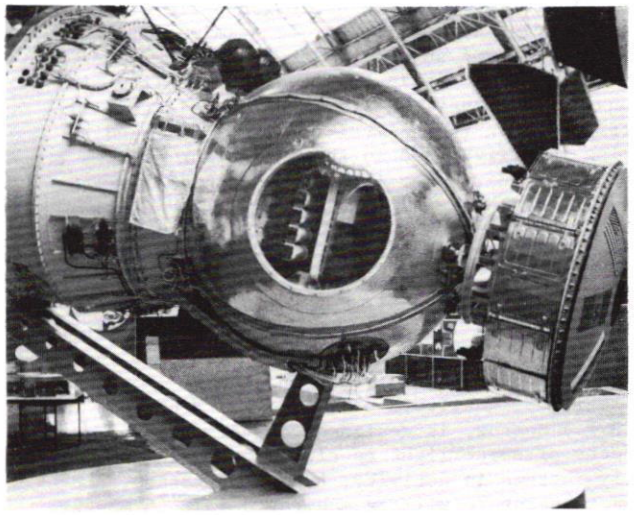

Fig. 1. Cosmos 782 Spacecraft on display in the Soviet Space Museum, Astakeno, USSR.

The primary objective of this mission is to establish the capability to utilize nonhuman primates for space-flight biomedical research. Therefore, the health and safety of the animals is of the utmost importance. Care has been taken to minimize the instrumentation applied to the animals on this first mission, to reduce the complexity of the experiments conducted and to make the environment of the animals as comfortable as possible. Secondarily, scientific studies are planned which focus on the effects of spaceflight on the vestibular, musculoskeletal and cardiovascular systems, as well as animal behavior and performance. Two male Rhesus monkeys, 3-4 years old, and 3.5-5.0Kg will be housed in individual pods and restrained in fur-lined chairs. The pods will be oriented so that the monkeys can make visual contact with one another during the flight and they will in turn be monitored on the ground by remote video. The animals will be fed a paste diet twice daily and will be provided with a fruit juice reward for performing behavioral tasks. In addition to video, ECG, body temperature, respiration rate, diet and liquid consumption will be telemetered to the ground during the flight.

The monkeys will be thoroughly trained before the mission and adapted to the spacecraft environment. Approximately eighteen animals will be prepared for flight and, on the basis of their behavior, performance, health and quality of physiological sensor signals, two will be selected for flight, two will serve as backups and two will be placed in a spacecraft mockup and provide ground-based control data.

Sharing the cabin with the monkeys will be a variety of plant and radiation biology experiments as well as 10 female Wistar strain rats, 8-13 days pregnant at launch. The latter will provide the first information on mammalian embryogenesis during spaceflight. Upon landing a recovery team will fly to the site and establish a field laboratory where the animals will be examined, tested and prepared for shipment back to Moscow for detailed studies. As in previous biosatellite missions, an international team of investigators will participate in the analysis of data collected during the flight and in the postflight studies of the animals during their readaptation to terrestrial conditions.

Joint US/USSR Experiments

There are four joint US/USSR experiments being developed for the Cosmos '83 mission (Table 1). The first of these, an experiment by F. M. Sulzman S.U.N.Y., Binghamton and V. Ya. Klimovitsky, Institute of Biomedical Problems (I.B.P.) Moscow, is designed to evaluate the effects of spaceflight on circadian rhythms. Daily rhythms in activity, body temperature, and skin temperature will be monitored in both monkeys during the flight and during the pre and postflight periods. The data will permit evaluation of both external sychronization of the rhythms to the 16:8 hour light:dark cycle, and internal synchronization of the rhythms to each other. To monitor animal activity, a special noninvasive piezoelectric strip was developed which will be mounted at one of several body locations now under study. Each time the piezoelectric strip is deformed, as when the animal moves, a voltage spike occurs and, if above a preset threshold, the spike will be registered as a count in a battery powered solid state memory device. Counts are summed over 16 minute intervals and stored for postflight data analysis. Skin temperature will also use a noninvasive transducer located on the right shin, affixed by glue and a porous tape and recorded at sixteen minute intervals. Body core temperature will be obtained with a small temperature transmitter implanted in the right armpit and, like activity and skin temperature, recorded at sixteen minute intervals. Ambient temperature in each monkey pod will be recorded although at 30 rather than 16 minute intervals. Heart rate and calf muscle myography will also be available for postflight analysis, however these two parameters will be recorded for only 5 minutes each 2 hours during the flight. A representative sample of data obtained with the flight biorhythm hardware from a monkey instrumented and restrained as in flight, is given in Figure 2.

The second joint experiment planned for Cosmos '83 is designed to investigate the loss of body calcium experienced by both men and animals during spaceflight (2,3,4). C. E. Cann of the University of California, San Francisco and Yu. Kondratyev, I.B.P., Moscow will enrich, with Calcium-40, the paste diet provided to the monkeys before, during and following the flight. Food intake will be monitored and exreta collected during the flight. By eliminating the naturally occurring Ca-48 from the diet, the only significant source of this isotope in the exreta will be from bone. Analysis of the Ca-40/Ca-48 ratio in the diet, excreta and serum, will provide information on calcium turnover in the body during the

TABLE 1. COSMOS '83 JOINT US/USSR EXPERIMENT PAYLOAD

	INVESTIGATOR	TITLE
1.	F. Sulzman, S.U.N.Y., Binghamton V. Klimovitsky, Institute of Biomedical Problems, Moscow	Synchronization Of Primate Circadian Rhythms During Spaceflight
2.	C. Cann, U. California, San Francisco Yu. Kondratyev, Institute of Biomedical Problems, Moscow	Calcium Metabolism And Correlated Endocrine Measurements
3.	J. Keefe, Biospace Inc., Cleveland L. Serova, Institute of Biomedical Problems, Moscow	Behavioral And Neuroanatomical Studies Of Neonatal Rats Following In Utero Exposure To Spaceflight
4.	V. Krotov, Institute of Biomedical Problems, Moscow H. Sandler, NASA-Ames Research Center	"Krovotok" - Cardiovascular Studies Of Nonhuman Primates During Spaceflight

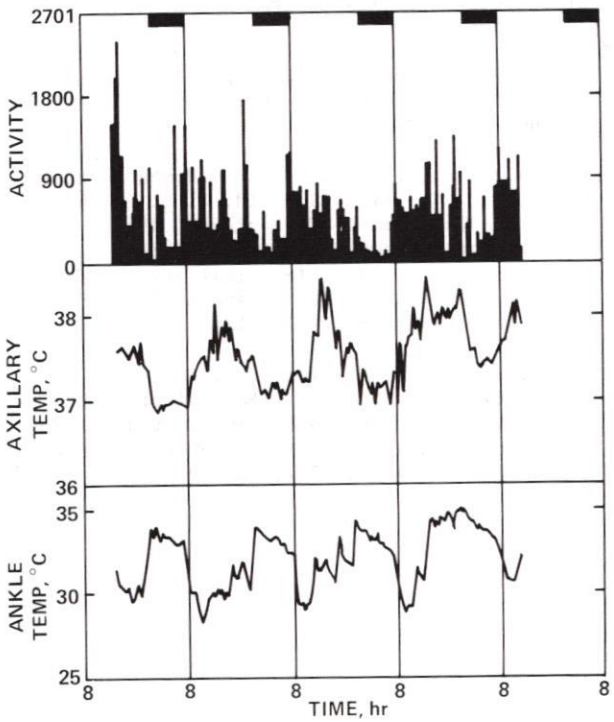

Fig. 2. Biorhythm data obtained from a restrained male rhesus monkey instrumented with flight (skin temperature and activity) and nonflight (axillary temperature) hardware during a mission simulation test. The light:dark cycle was 16:8 hours with the solid horizontal bars along the top indicating the dark period.

preflight, inflight and postflight periods. To facilitate the interpretation of the Ca-40 data and to increase our understanding of calcium homeostasis, pre and postflight analyses of sera will be performed for Ca, Mg, P, calcitonin, parathyroid hormone and the vitamin-D metabolites.

The third joint experiment planned for Cosmos '83 is a cardiovascular study proposed by V. Krotov, I.B.P., Moscow with support from H. Sandler, NASA-Ames Research Center. The objective of this experiment is to monitor blood flow and pressure in the left common carotid artery and to correlate blood flow and pressure with other cardiovascular (CV) parameters obtained during spaceflight, i.e., ECG, and chest impedance plethysmography. Data obtained from this experiment will be used to assess the changes which occur in the CV system during the early stages of exposure to hypogravity and during the stress of returning to earth. Such spaceflight data has not been obtained before in large nonhuman primates.

To measure carotid blood pressure and flow velocity (CPF), a small implantable cuff was designed to fit around the artery. The CPF cuff contains both a strain gauge pressure transducer and a Doppler flow crystal system. It is connected to a battery powered signal processor which in turn is connected to the onboard data tape recorder. Cardiovascular data will be recorded for 5 minutes at 2 hour intervals throughout the flight and during a series of pre and postflight antiorthostatic tests. Representative data obtained during a two week mission simulation are shown in figure 3. Analysis of the flight CV CPF

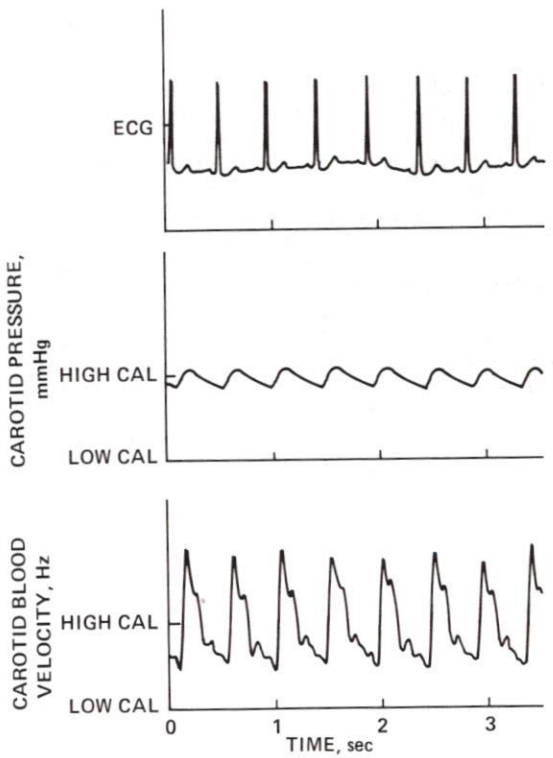

Fig. 3. Cardiovascular data from a restrained male rhesus monkey implanted with a CPF cuff during a mission simulation test.

cuff data will provide maximum, minimum and mean blood pressure and flow velocity as well as pulse wave velocity.

The fourth joint experiment included in the Cosmos '83 payload is a mammalian embryology study proposed by J. R. Keefe, Biospace Inc., Cleveland, and Dr. L. Serova, I.B.P., Moscow. Ten young female Wistar strain rats will be 8-13 days pregnant at the time of launch. During the flight, they will be housed in a sound proof chamber with a 16:8 hour day:night cycle and fed a paste diet with water provided ad libitum.

Following the flight, five of the animals will be autopsied at the recovery site and both the adults and fetuses examined and prepared for subsequent histological and biochemical studies by an international team of scientists. The remaining five females will be returned to Moscow to complete their gestation period whereupon a battery of postnatal tests will be conducted. The primary focus of the Keefe and Serova experiment is the histomorphometric analysis of vestibular, olfactory and visual organs and their central nervous system pathways. Complementary studies of neonatal behavior and the development of various sensory responses (tactile, olfactory, visual, auditory) will be conducted and correlated with the histological data.

Summary

A one week flight of an unmanned Soviet spacecraft is secheduled for the last half of 1983. On board the craft will be two restrained Rhesus monkeys, 10 pregnant rats and a variety of small plant and radiation biology experiments. Four joint US/USSR investigations are included in this mission, three of which will use the monkeys for studies of biorhythms, calcium homeostasis, and the cardiovascular system. The fourth experiment will study rodent embryogenesis and neonatal behavior and development following in utero exposure to spaceflight. Specialized sensors and battery powered hardware have been designed, fabricated and qualified for flight. Baseline data is being collected in the investigators' laboratories and final planning among the science teams is underway.

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COMPARISON OF CARDIOVASCULAR EFFECTS OF SPACE FLIGHT AND ITS ANALOGS USING COMPUTER SIMULATIONS

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INTRODUCTION

The mechanisms underlying the physiologic response to weightless exposure which leads to cardivascular deconditioning are not clear at the present time. Efforts to elucidate these mechanisms have included investigations using ground-based analogs of space flight such as water immersion, supine bed rest, and head-down tilt. More recently, such efforts have been aided by computer simulations employing mathematical models of the cardiovascular system (9). The simulation studies are aimed at testing the various hypotheses that have been advanced to explain the cardiovascular effects of space flight and its analogs, as well as integrating them so as to provide a composite picture. In this paper, we will present the results of a few of our cardiovascular model simulations and compare them with available experimental data in an attempt to test some suggested hypotheses of space flight and its analogs.

THE MATHEMATICAL MODELS

Two different mathematical models of the cardiovascular system provided the framework to compare the results of various investigations. One is a long-term model describing the circulatory, fluid, and electrolyte regulation and is a modification of a basic model developed originally by Guyton (4). It consists of five vascular compartments connected to an interstitial fluid compartment and a cellular fluid compartment. With the addition of leg vascular and tissue compartments and gravity-dependent circulatory elements, it has been used to simulate water immersion, head-down tilt, and supine bed rest, ranging in duration from a few hours to a few weeks (6). The other is a short-term model based on the assumption of a closed vascular system with no provision for fluid filtration into the extravascular space. It is a beat-to-beat model intended to simulate responses lasting less than 30 minutes and has been used successfully to simulate exercise, lower-body negative pressure (LBNP) and tilt experiments under one-g conditions (3).

RESULTS AND DISCUSSION

We have performed various simulation studies with the aid of the above two models. The results of two of these studies are presented and discussed here.

I. Long-term Cardiovascular Model Simulation

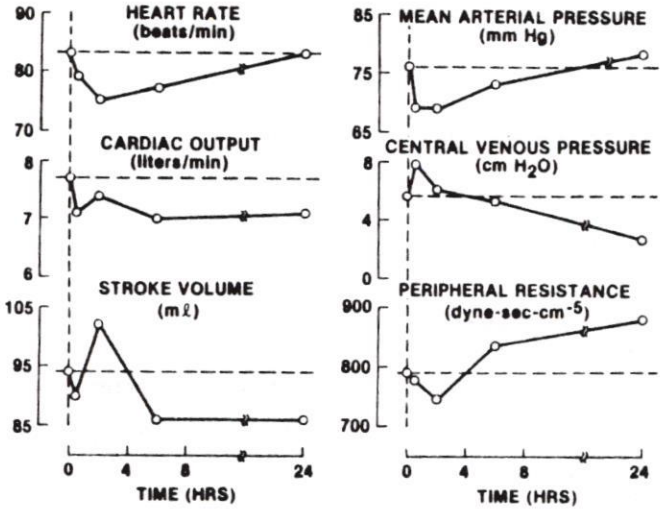
The long-term cardiovascular model was used to simulate a 24-hour study of head-down tilt at -6°. The gravity-dependent elements of the model produced fluid shifts from the legs to the thoracic region, resulting in a net loss of blood volume. This is in agreement with the observations made in head-down tilt as well as in supine bed rest, water immersion, and space flight. The comparison of the simulation results with experimental data of Nixon et al. (7) is shown in the figure on the following page. Both show an increase in peripheral resistance as discussed below in the simulation of cardiovascular deconditioning using the short-term model.

II. Short-term Cardiovascular Model Simulation

Several hypotheses were required to simulate the cardiovascular deconditioning effect. Those which were consistent with long-term simulation results discussed above and which produced the most realistic model response included: (a) a loss of blood volume as compensatory response to headward fluid shifts and central hypervolumia; (b) a redistribution of the diminished blood volume with resultant lower values of mean pressures and volumes in the leg veins and venules, and (c) a higher resistance to flow in all parts of the circulation.

The blood volume loss in our simulation is 10 percent or 500 ml. This figure is close to the mean loss of 450 ml calculated from the published results of several different studies on supine bed rest. We attribute a significant proportion of this loss in blood volume to the loss from venous compartments of the lower extremities. The reduction in leg volume observed in Skylab astronauts (1) and in supine bed rest studies (2) supports this hypothesis. The simulation yielded a leg blood volume loss of 50-200 ml which is consistent with the observed total leg volume change.

Leg blood volume reduction results in diminution of mean pressure and mean volume and a lower operating point on the pressure-volume (P-V) relationship. A shift of the operating point toward the nonlinear part of the P-V curve implies an increase in leg venous compliance as has been reported to occur during space flight (1). It does not imply, however, a fundamental change in the shape of the P-V relationship. Also, increasing compliance per se was not very effective in reproducing the experimental observations in our simulation.



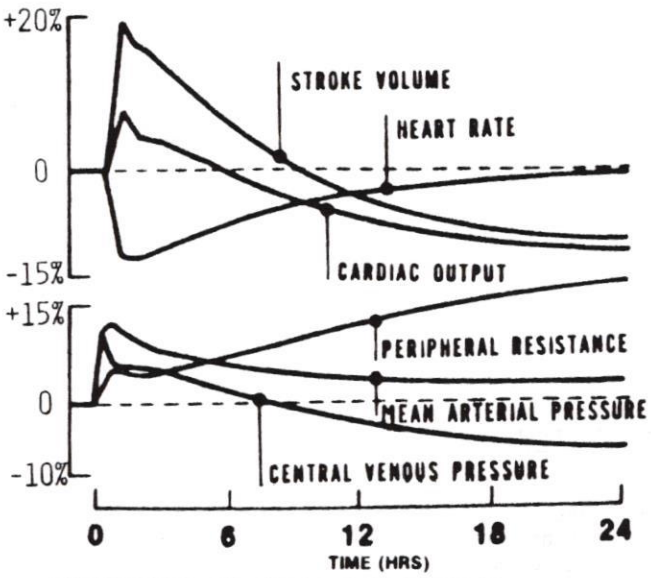
RESPONSE TO HEAD-DOWN TILT EXPERIMENT (Nixon, et al.)

Our hypothesis on the increase of flow resistance is based directly on available experimental data and model simulation results. An increase of peripheral resistance has been observed both in supine bed rest (5,8) and, to a greater degree, in head-down tilt (7). Measurements in Skylab astronauts showed a postflight decrease in cardiac output, and an increase in mean arterial pressure (1), indicating a rise of total peripheral resistance.

The table below compares some simulation results with measured data on the cardiovascular response to 50 mmHg LBNP. (The bed-rest data are the average values from 1 to 5 different studies in which the number of bed-rest days ranged from 7 to 35; the Skylab data are the average values from the three manned missions). We found no gross disagreement between experimental and simulated data for either LBNP response, or 70° tilt response following bed rest when simulated with the same set of hypotheses. Thus, the above hypotheses provide one coherent explanation of the measured changes in a number of cardiovascular variables.

Cardiovascular Parameter	Source	Change from Resting to 50 mmHg LBNP Pre-	Post-
Heart Rate (beats/min)	Bed Rest	23	39
	Skylab	11	25
	Model	13	28
Pulse Pressure (mmHg)	Bed Rest	-12.6	-14.3
	Skylab	- 9.1	-21.0
	Model	- 9.4	-16.1
Leg volume (ml)	Skylab*	570	698
Leg Blood Volume (ml)	Model	423	556

* Calculated assuming a combined leg volume of 15 liters preflight



RESPONSE TO HEAD-DOWN TILT: MODEL

CONCLUSIONS

- The cardiovascular response to orthostatic stress following exposure to weightlessness or any of its analogs cannot be explained fully on the basis of blood volume loss alone.
- Peripheral resistance changes appear to play a significant role in producing the observed cardiovascular responses.
- The lowering of mean pressures and volumes in the legs seems to be more dominant than any fundamental change in leg venous compliance.

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FLUID SHIFTS IN VASCULAR AND EXTRAVASCULAR COMPARTMENTS OF HUMANS DURING AND AFTER SIMULATED WEIGHTLESSNESS

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INTRODUCTION

Physiological responses to weightlessness in space travelers include the following fluid shift alterations: decreased leg volume, cephalic fluid shift, plasma volume loss, and facial edema (6,10). Upon return to Earth's gravity, severe orthostatic intolerance combined with pain and possible edema of lower legs are reported after relatively long space flights (11). Head-down tilt at 5° is considered a good procedure to simulate many of these responses in a normal gravity environment (2,4,8). The objectives of this study were to measure transcapillary pressures and to calculate possible fluid shifts in muscle and subcutaneous tissue of the lower leg before, during, and after head-down tilt.

METHODS

After obtaining approval from local Human Use Committees at the V.A. and U.C.S.D. Medical Centers, 6 normal male subjects were tilted at 5° head down for 8 hours. Fluid intake and output parameters were continuously monitored one day before, during, and one day after tilt. Transcapillary pressures (interstitial fluid pressure P_t by the wick technique, interstitial-fluid colloid osmotic pressure π_t by an empty wick catheter, venous-blood colloid osmotic pressure π_v) were measured by methods described elsewhere (3). Soleus muscle (an antigravity muscle) was sampled by needle biopsy (1) and dried to determine changes in muscle water content (4). Blood pressure and heart rate were recorded every 2 hours. Urine was collected in a graduated cylinder and rates of output were calculated. Finally, lower-leg volume was measured by water displacement (temperature corrected) every 4 hours.

RESULTS

The 6 volunteers in this study experienced facial edema, headache, nasal congestion, diuresis, and decreased lower-leg volume in response to 8 hours of 5° head-down tilt (Fig. 1).

There were significant decreases ($p<0.05$) in systolic and diastolic pressures 2 hours after initiation of tilt but blood pressures normalized thereafter. Lower-leg volume, urine output and interstitial fluid pressures of tibialis anterior muscle and overlying subcutaneous tissue also changed significantly (Fig. 2).

No significant change was detected in colloid osmotic pressures of blood or interstitial fluid. A trend to soleus muscle dehydration was suggested by lower water content, although the change

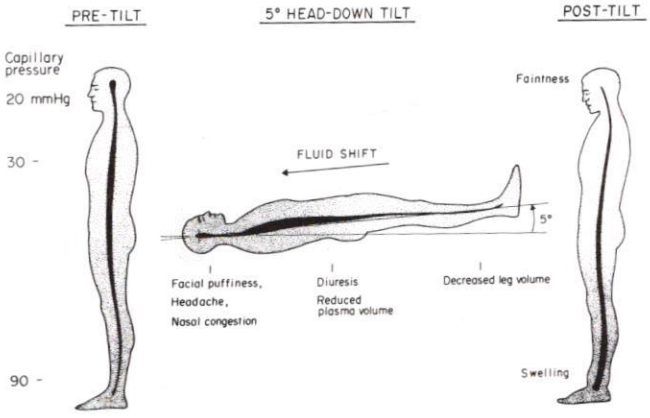

Fig. 1-Summary of physiologic responses to weightlessness simulation by 5° head-down tilt. Most of the responses depicted were documented in our 8 hour study. A previous investigation of head-down tilt (8) found reduced plasma volume and many long-term bedrest studies describe orthostatic intolerance (faintness and leg swelling) during readjustment to upright posture. The steep gradient of capillary blood pressure from head to foot during standing is probably lost during head-down tilt, based on previous data by Levick and Michel (7). Venous blood is immediately shifted cephalad upon exposure to head-down tilt (Reproduced with permission from A.R. Hargens. Fluid shifts in vascular and extravascular spaces during and after simulated weightlessness, Med. Sci. Sports Exercise, in press, 1983).

detected was only significant at $p<0.10$. Based upon previous studies of interstitial compliance (9,12), interstitial fluid was shifted continuously from the lower legs at 12 $ml \cdot hr^{-1}$ during head-down tilt. Total and interstitial-fluid volumes of the lower leg returned to baseline values during the 24 hour post-tilt recovery period.

DISCUSSION

Most of the 300 ml fluid shift from the lower legs during the first 4 hours of 5° head-down tilt is probably a passive redistribution of blood to high-capacitance, thoracic veins. However, interstitial fluid is continuously lost from muscle and subcutaneous tissue during 8 hours of head-down tilt. Upon assumption of supine position, capil-

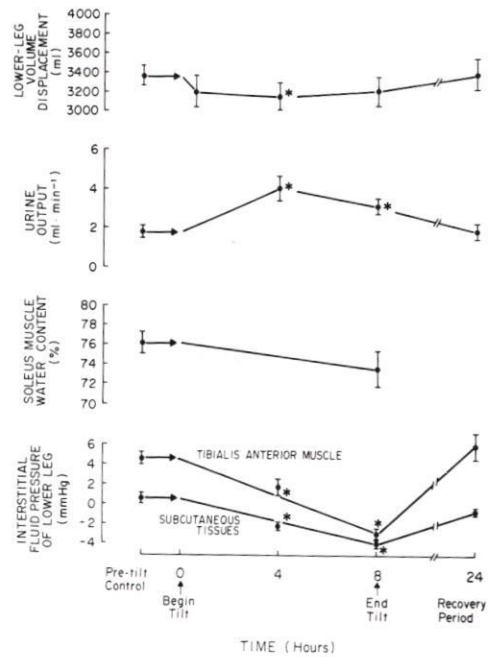

Fig. 2-Significant fluid shifts during tilt are indicated by reduced volume of one lower leg, elevated urine output, trends to decreased water content of soleus muscle, and decreased interstitial fluid pressures in tibialis anterior muscle and overlying subcutaneous tissue (means ±S.E. for 6 subjects). *Denotes significant change ($p<0.05$) from pre-tilt, control value as evaluated by ANOVA procedures for repeated measures (Reproduced with permission from A.R. Hargens. Fluid shifts in vascular and extravascular spaces during and after simulated weightlessness, Med. Sci. Sports Exercise, in press, 1983).

lary blood pressure falls from 90 to 30 mm Hg in human feet (7), and therefore, decreased capillary pressure may represent the mechanism for tissue fluid loss in the legs during head-down tilt. Kirsch and associates (5) measured a relatively rapid loss of tissue thickness over the tibia during 5° head-down tilt. However, their observations using ultrasonic techniques may primarily reflect a blood shift from veins in the lower leg during the tilt maneuver. Long periods of weightlessness in space travelers or bedrest in patients may produce similar responses.

During upright posture in humans, edema in the lower leg is prevented by precapillary myogenic control of blood flow and blood pressure as well as segmental variations in capillary vessel morphology. Countermeasures to maintain precapillary-muscle tone may be necessary during long space flights to prevent swelling of lower-leg tissues upon readjustment to Earth's gravity.

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COMPUTER SIMULATION ANALYSIS OF THE BEHAVIOR OF RENAL-REGULATING HORMONES DURING HYPOGRAVIC STRESS

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The alterations of body fluids and their electrolyte composition that occur in weightlessness are controlled in large part by hemodynamic, neural and hormonal regulators. In particular, a group of renal-regulating hormones, consisting of anti-diuretic hormone (ADH), aldosterone (ALDO), and renin-angiotensin (R-A), have been the focus of many space flight related studies. However, the findings from space flight have been difficult to interpret or to reconcile with endocrine data obtained from one-g analogs of weightlessness such as water immersion, head-down tilt, and bed rest. The purpose of the present study was to examine these data, describe the major characteristics of the hormone response, and to assess the controlling mechanisms.

Figure 1 organizes the endocrine data from a number of hypogravic studies, whether performed in one-g or zero-g, into a qualitative, composite description. All of the stresses considered have the common characteristics of a reduction in hydrostatic gradients and result in an acute headward shift of fluid, while longer-term, these maneuvers lead to reductions in body water, plasma volume, and electrolytes. Based on water immersion and head-down tilt studies (1,2), there is a suppression of the plasma or urine levels of each hormone of interest during the acute period where comparable space-flight data are lacking. Following this acute phase, only the R-A system demonstrates a consistent behavior; i.e., a significant elevation in plasma concentration. During space flights lasting more than one month ALDO was found to be elevated and ADH suppressed (3), although during intermediate periods, data from various bed-rest studies and space flight are not always in agreement (blank areas in Fig 1). It is the general behavior shown in Figure 1 that we seek to understand. In particular, it is desired to reconcile the differences between the acute and chronic responses as well as the differences among the separate studies.

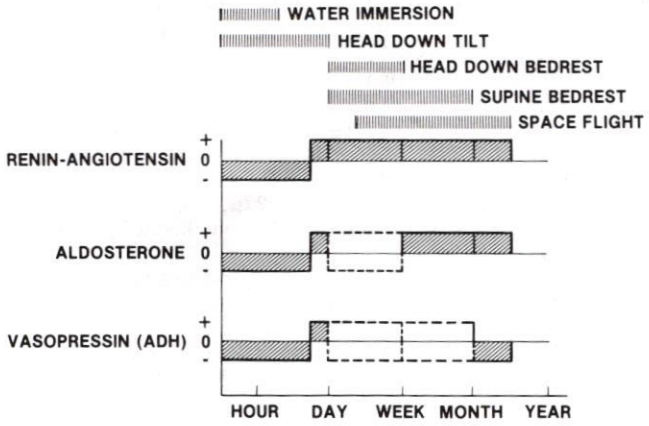
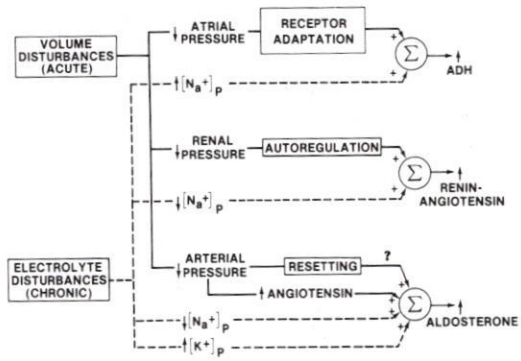


FIG 1. HORMONAL CHANGES DURING HYPOGRAVIC STUDIES: A COMPOSITE

The primary technique used in this analysis was the computer simulation of a mathematical model developed by Guyton (4) and modified for application to space-flight stresses (5). This model contains many elements which represent the dynamic interactions between acute and long-term adaptive control of the body fluids and the cardiovascular system. Previous studies have established the validity of the model for simulating several one-g analogs of weightlessness and space flight itself (5,6,7).



INFLUENCE OF VOLUME (——) AND ELECTROLYTE (-----) CONTROLLERS ON HORMONAL SECRETION

FIG 2.

A schematic description of the factors which influence the three hormones in the model is depicted in Figure 2. As shown, each hormone is responsive to two general types of controlling stimuli: volume disturbances (as reflected by atrial, renal, or arterial pressures) and electrolyte disturbances (plasma sodium, Na, or potassium, K, concentrations). The volume stimuli may provide control only during acute disturbances, either because of the existence of several types of adaptive mechanisms indicated in Figure 2, or because volume disturbances are fully corrected by various volume-regulating mechanisms. The influence of the electrolyte disturbances are not known to adapt, however, and it has been proposed that they are the primary long-term controllers of these hormones

(8). All of the stimulating factors shown in Figure 2 are known to change at one time or another during hypogravic maneuvers. For example, during the onset of weightlessness, blood pressures likely become elevated. (Unfortunately, the long-term responses of either venous or renal pressures have not been studied.) Also, plasma Na is frequently reported to be depressed sufficiently to suppress ADH secretion, while plasma K is variable, dependent in part on muscle atrophy, metabolic intake, and excretion.

Figure 3 illustrates the hormonal responses obtained in the mathematical simulation of a 7-day head-down (-6^0) bed-rest study (9). The dynamic behavior of these responses reflects the presence of multiple and competing pathways and the interactive nature of the renal-endocrine and fluid-electrolyte systems. This simulation of head-down tilt was accomplished by imposing the hydrostatic forces associated with -6^0 on the appropriate circulatory elements. The model automatically responds to this stress by redistributing fluid to the upper body volume segments and initiating compensatory reactions to alleviate the central hypervolemic condition. The acute changes in plasma hormone levels were not measured in the experimental study, but the model exhibited a significant decrease in all three hormone levels during the first several hours in accord with the data summarized in Figure 1. Acute hormone suppression was predicted, in this instance, as a result of elevated blood pressures, and was obtained both with a normal diet of water and electrolytes (dashed line) and with a restricted diet (~ 2/3 normal intake) similar to that consumed by the bed-rested subjects (solid line). The dietary reductions were crucial to the accuracy of the long-term simulation responses because they permitted the plasma electrolytes (Na and K) to be reduced to levels found in the experimental study. As indicated in Figure 2,

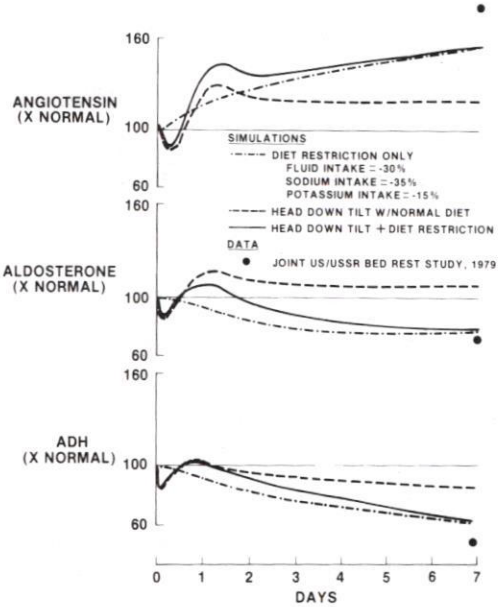


FIG 3. SIMULATED PLASMA HORMONE RESPONSES DURING 7-DAY HEAD-DOWN BED REST

a reduction in plasma Na tends to reduce ADH and increase R-A; also, since changes in plasma K levels exert a much more powerful effect on ALDO than equivalent changes in other stimuli, a reduction in plasma K was the predominant factor causing long-term ALDO suppression. In all cases the model's chronic responses to head-down tilt plus diet changes (solid line) converge toward the responses where dietary changes alone were assumed (dotted line), indicating that the long-term hormone behavior is a result solely of the dietary impact on the plasma electrolytes. The addition of the dietary effects to the simulation protocol also improves the agreement between the long-term model responses and experimental data (solid circles). In these simulations, pressure disturbances had nearly normalized, and had only a minor effect after the second day.

This analysis suggests that the renal-regulating hormones represent a tightly coupled system that responds acutely to volume disturbances and chronically to electrolyte disturbances. During hypogravic maneuvers this leads to an initial suppression of hormone levels and a long-term effect which varies depending on metabolic factors (such as diet, sweat loss, physical activity, and muscle atrophy) that can alter the plasma electrolytes. In addition, the simulations reveal that if pressure effects rapidly normalize, a transition phase may exist which leads to a dynamic multi-phasic endocrine response (Fig 1 and 3). The directional changes of these hormones found during Skylab and specific ground-based analog studies can be qualitatively accounted for by these mechanisms. Also, the discrepancies between the different studies (Fig 1), may be a result of competing and time-varying stimuli which differ between subjects and test conditions. Verification of this hypothesis requires, in part, the collection of data which is currently lacking, including measurements of endocrine behavior during the acute phase of space flight and measurements of various circulatory pressures during the longer-term periods of hypogravity.

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ALTERATIONS IN GLOMERULAR AND TUBULAR DYNAMICS DURING SIMULATED WEIGHTLESSNESS

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ABSTRACT

There are few studies pertaining to the functional adaptation of the kidney to fluid and electrolyte shifts induced by prolonged weightlessness. In the present study, we examined the effects of 7 day 20-25° head-down tilt to simulate weightlessness on the dynamics of glomerular ultrafiltration and reabsorption. The head-down tilt method was utilized in 6 Munich-Wistar rats and the 25° head-down tilt was maintained during measurements of glomerular dynamics and proximal tubule reabsorption. The results demonstrate a significant reduction in nephron filtration rate during head-down tilt. The reduction in nephron filtration was due to an increase in efferent arteriolar resistance which decreased nephron plasma flow. Despite the reduction in nephron filtration, absolute proximal fluid reabsorption remained constant resulting in a significant reduction in the delivery of sodium and water to distal portions of the nephron.

INTRODUCTION

An experimental model of head-down tilt in the rat has been utilized to simulate weightlessness in a variety of studies which have included examination of factors affecting salt and water excretion (2,4,5). The model of head-down tilt in rats for simulating weightlessness had been shown to yield results similar to those which have been observed in actual weightlessness studies relating to fluid shifts and the musculoskeletal system (4). Since there are profound fluid shifts as well as alterations in fluid and electrolyte balance due to elimination of the gravity environment, the kidneys must exert a major contribution to the changes that occur. In the present study, this model was utilized to examine alterations in renal function after 7 days of head-down tilt. Specifically, we examined the effects of prolonged 20-25° head-down tilt on the dynamics of glomerular ultrafiltration and tubular reabsorption.

METHODS

Experiments were performed on male Munich-Wistar rats weighing 200-295 gms at the time of experimentation. Experimental rats were positioned in a head-down tilt apparatus (20-25°) for 7 days as described by Morey-Holton and Wronski (4) prior to the actual micropuncture or blood volume measurements.

The rats were anesthetized with Inactin (100 mg/kg body wt intraperitoneally) and surgical preparation for the head-down tilt rats was performed with the rats maintained in a 25° head-down position. All measurements of glomerular dynamics and proximal tubular reabsorption were performed while the rat was maintained at a 25° head-down tilt (n=6). The control group was maintained in a horizontal position during preparation, surgery and measurement period.

In another group of rats (n=4 control and n=4 7 day, 25° head-down tilt) the same surgical preparation was performed and once the animal was stabilized, measurement of blood volume was performed.

Glomerular and single nephron filtration rates were calculated as previously described utilizing C^{14} inulin (1). Pressure measurements were obtained with a servo-nulling device with 1-3 μm tip pipettes. Calculations for oncotic pressure, plasma and blood flows, and glomerular permeability coefficient are as previously described (1). All data are expressed as mean ± standard error of the mean.

RESULTS

After 7 days of continuous head-down tilt, the rats decreased in body weight by 18±4 gms. This loss is significantly different from the normal weight gain during the same time period of 16±2 gms. Serum potassium and urinary potassium excretion were significantly elevated in the measurement period during the head-down tilt position compared to the control group ($p<0.05$) (Figure 1).

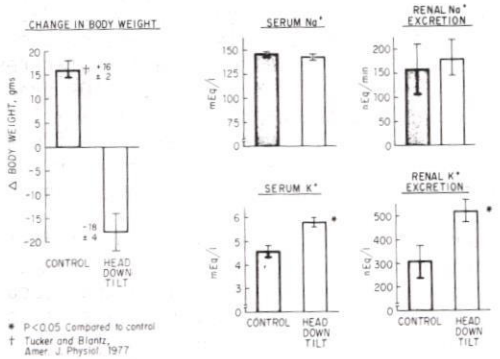

Figure 1. Effect of 7 day head-down tilt on body weight, serum electrolytes, and electrolyte excretion.

Mean arterial pressure was significantly decreased during prolonged head-down tilt (105±3 vs. 116±3 mmHg in the control group, $p<0.05$). Plasma renin activity (PRA) was measured in both control and head-down tilt rats. Control PRA was 15.9±1.7

ng $ml^{-1} \cdot hr^{-1}$ during the measurement period. In the head-down tilt rats, PRA was significantly decreased to 6.4±0.8 ng·$ml^{-1} \cdot hr^{-1}$ ($p<0.01$). This reduction in PRA is different from the response found in chronic volume depletion in rats, a condition also characterized by fluid and electrolyte losses. In a previous study from this laboratory, PRA was 17.2±0.9 ng·$ml^{-1} \cdot hr^{-1}$ (7) in chronic volume depleted rats that were surgically prepared in the same fashion.

Blood volume in head-down tilt was not significantly decreased in the prolonged head-down tilt rats compared to normal controls (10.8±0.5 vs. 11.4±0.1 ml, respectively).

There was a significant decrease in single nephron glomerular filtration rate (SNGFR) in head-down tilt rats compared to control (36±1 vs. 40±1 nl/min g kidney wt, respectively, $p<0.05$). While kidney glomerular filtration rate was 1.0±0.05 ml/min g kidney wt in the head-down tilt rats and 1.2 ±0.05 ml/min g kidney wt in the control group. APR was 19±1 nl/min·g kidney wt in the head-down tilt rats and 18±1 nl/min·g kidney wt in the control rats. With the unchanged APR and a decrease in SNGFR during prolonged head-down tilt, late proximal tubule flow rate (LPFR) was decreased from 22±1 nl/min·g kidney wt in controls to 17±1 nl/min·g kidney wt ($p<0.01$) reflecting a reduction in the delivery of sodium and water to distal portions of the nephron. The urine flow was 1.5±0.1 µl/min in the head-down tilt rats compared to 1.9±0.1 µl/min in the control group.

There was no significant difference in the glomerular hydrostatic pressure gradient (ΔP) observed (35±0.9 mmHg in control and 37±0.4 mmHg during head-down tilt). There was also no significant change in either systemic oncotic pressure or the glomerular permeability coefficient in the head-down tilt rats at 94±4 nl/min·g kidney wt compared to a control value of 117±7 nl/min·g kidney wt ($p<0.02$) and this alteration in SNPF was the major factor for the reduction in SNGFR.

Afferent arteriolar resistance (pre-glomerular) did not significantly change in the head-down tilt rats compared to control (22±2 vs. 22±1 x 10^9 dynes·sec·cm^{-5}, respectively). However, there was a significantly higher efferent arteriolar resistance in head-down tilt rats (14±1 x 10^9 dynes·sec·cm^{-5}) compared to the control rats 11±1 x 10^9 dynes ·sec·cm^{-5}) ($p<0.05$). This increase in efferent arteriolar resistance was the major contributing factor to the decrease in single nephron blood flow.

DISCUSSION

Changes in both the distribution of fluid volume between vascular and extravascular compartments and the overall fluid volume status that occurs during weightlessness must produce alterations in renal function. There are very few studies in the current literature investigating the contribution of glomerular and tubular dynamics in fluid volume readjustments that occur during weightlessness. After 7 days of head-down tilt, Deavers et al. found an overall diuresis and natriuresis (2) during the time course of the head-down tilt. We have observed a reduction in glomerular filtration rate monitored at the single nephron level. The reduction in SNGFR found in head-down tilt rats in this study indicates that changes in glomerular ultrafiltration contribute to the maintenance of fluid and electrolyte volume in this condition. Despite the significant reduction in SNGFR, APR remained constant such that there was a significant reduction in LPFR, reducing the load of fluid and electrolytes to distal portions of the nephron. These events may provide a mechanism contributing to the retention of fluid and electrolytes.

The reduction in SNGFR was due to an increase in efferent arteriolar resistance which decreased nephron plasma flow. The increase in efferent arteriolar resistance was probably not due to activity of the renin-angiotensin system since PRA was significantly diminished. The decrease in PRA during head-down tilt is significantly different from that which has been observed during chronic volume depletion in which PRA is not different from control levels but increases in efferent arteriolar resistance which were observed were in part due to activity of renin-angiotensin system (7).

We observed an increased potassium excretion in the head-down tilt rats which may be due to the increase in serum potassium levels. Despite the reduction in SNGFR the filtered potassium load increased due to the increase in serum potassium concentration. This increase in filtered load of potassium plus a possible increase in intracellular potassium concentration could be sufficient to increase renal potassium excretion even if aldosterone levels were depressed as have been indicated in other models of simulated weightlessness (6).

In summary, prolonged head-down tilt has reduced SNGFR and late proximal tubule flow rate, while APR has remained constant which contributed to a retention of fluid. Efferent arteriolar resistance increased, and is the major factor producing the reduction in nephron plasma flow. There was an elevated potassium excretion as observed by others (3). Plasma renin activity was significantly reduced indicating potential involvement of renal adrenergic system, or other vasoactive systems in increasing renal vascular resistance. There were no changes in either hydrostatic or colloid osmotic forces, and no change in the glomerular permeability coefficient. However, significant alterations in glomerular and tubular dynamics do occur which are undoubtedly produced by changes in the distribution of fluid and electrolytes outside the kidney which have been observed in this model of simulated weightlessness.

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CLONIDINE AS A COUNTER MEASURE FOR METABOLIC STUDIES DURING WEIGHTLESSNESS SIMULATION.

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Montastruc (Europ. J. Pharmacol., 1981, 72, 372-376) shows that Clonidine (C) inhibites the diuretic response elected by left atrial distension in chloralose anesthetized dogs. Furthermore the increase in the intrathoracic volume observed in man, during space flight provokes a diuretic response. Our work involves the effects of C, in man during a simulation of weightlessness.
6 young, healthy volunteers were placed in bedrest and in antiorthostatic position at -4° during 7 days ; 3 of these received 0,450 mg of C during the bedrest. Before, during and after the experiment we took blood and urine samples for the determination of the hormonal and metabolic indices involved in blood volume regulation.
In the subjects without C, we noted increased diuresis, sodium depletion and aldosterone outflow ; plasma renine and aldosterone rose after the 24th hour. In the subjects with C the diuresis was inhibited and the sodium depletion was stopped after the 2nd day ; plasma renin and plasma aldosterone don't rise significantly : finally the ADH concentration in urine was reduced. We conclude that it's interesting to use C as a counter measure for metabolic and hormonal studies during the experiments of weightlessness simulation.

Experiments in prolonged bedrest in antiorthostatic position aim to reproducing on earth the hydroelectrolytic and hormonal modifications observed in man under weightlessness conditions. This method results in a redistribution of blood volume from the infracardiac regions toward the thoraco-cephalic regions with change then provokes stimulation of the auricular receptors. Consequently, antidiuretic hormone (ADH) and renin secretions are inhibited resulting in a decrease of volemia, and this phenomenon in a second stage, stimulates these same system (1). However, Montastruc (2,3) has shown that Clonidine suppresses diuresis induced in dogs by breathing under negative pressure (a technique with results in stimulation of the auricular receptors) ; he suggests the use of this drug in aerospatial medicine ; for example, in order to protect the astronauts from the well know acceleration induced dehydratation. The aim of this study is to verify this hypothesis in more during an experiment of weightlessness simulation.

Materials and methods : 6 young, healthy volunteers (average age) were chosen after satisfactorily completing psychological and medical tests. The period of bedrest was set at 7 days and the antiorthostatic position was maintained at -4° head down. Control blood and urine samples were taken the day before starting the experiment. 2 daily blood samples were taken during the 7 days of the bedrest. Urinary collections were made on every 24 h. Daily sodium and potassium intake was constant before, during and after the period of bedrest. Blood pressure, pulse, diuresis and the sensation of thirst were carefully noted.The subjects were weighed before and after the experiment.
The following plasma determinations were made : plasma renin activity, aldosterone, total neurophysines, sodium, potassium, hematocrit and plasma osmolality.
Sodium, potassium, aldosterone and the antidiuretic hormone were measured in the urines.
3 of these subjects received 0,450 mg of Clonidine daily.

RESULTS :
+ Clinically, the subjects presented the usual subjective signs (feeling of head fulness ...) and the classical objective manifestations (facial and palpebral oedema).
+ There was no significant modifications in blood pressure. We noted a decrease in the heart rate principally in subjects with Clonidine.
Plasma analyses in patients without Clonidine revealed a significant increase of hematocrit ($p < 0,01$), decreased natremia and increased kaliemia, a marked increase of Plasma Renin Activity ($p < 0,002$), increased aldosterone levels ($p < 0,01$) and a little variation of neurophysines. The volunteers receiving Clonidine had, by the third day, considerably less Plasma Renin Activity rise (50 % instead of 150 %, $p < 0,002$). This lesser increase also occured in hematocrit and natremia ($p < 0,02$). Urine analyses evidenced ;
- in volunteers without Clonidine, increased diuresis, with any significant change in ADH excretion and outflow of Na+ ($p < 0,01$) and K+.
- in the subjects with Clonidine urinary excretion of ADH was significantly decreased ($p < 0,01$), the polyuria was stopped after 24 h in the subjects with Clonidine.

DISCUSSION AND CONCLUSION
In the subjects without Clonidine the modifications recorded are significant particularly during the first 4 days. It is clear then that if, in a first stage, the blood volume is redistributed from the periphery toward the cardio pulmonary system, adjustements very quickly occur to establish a new equilibrium : loss of water and sodium, volemia

decrease causing an intensive activation of the renin aldosterone system.
In the subjects with Clonidine we see a definite difference during the first 3 days. It seems clear that when a new equilibrium is established with decreased volemia, Clonidine is involved in reducing ADH and renin secretions. The results obtained in dogs thus stand confirmed and should be discussed in relation to the sympathicomimetic properties of Clonidine. We have been able to point out the very different hormonal and humoral reaction in treated and untreated subjects. Is therefore seems valuable to further study the mechanisms of this substance in weightlessness simulations in view, like it is proposed by Montastruc, of its use by astronauts as protection against problems in space flights. The use of this drug can also constitute a counter measure for the experiments of weightlessness simulation.

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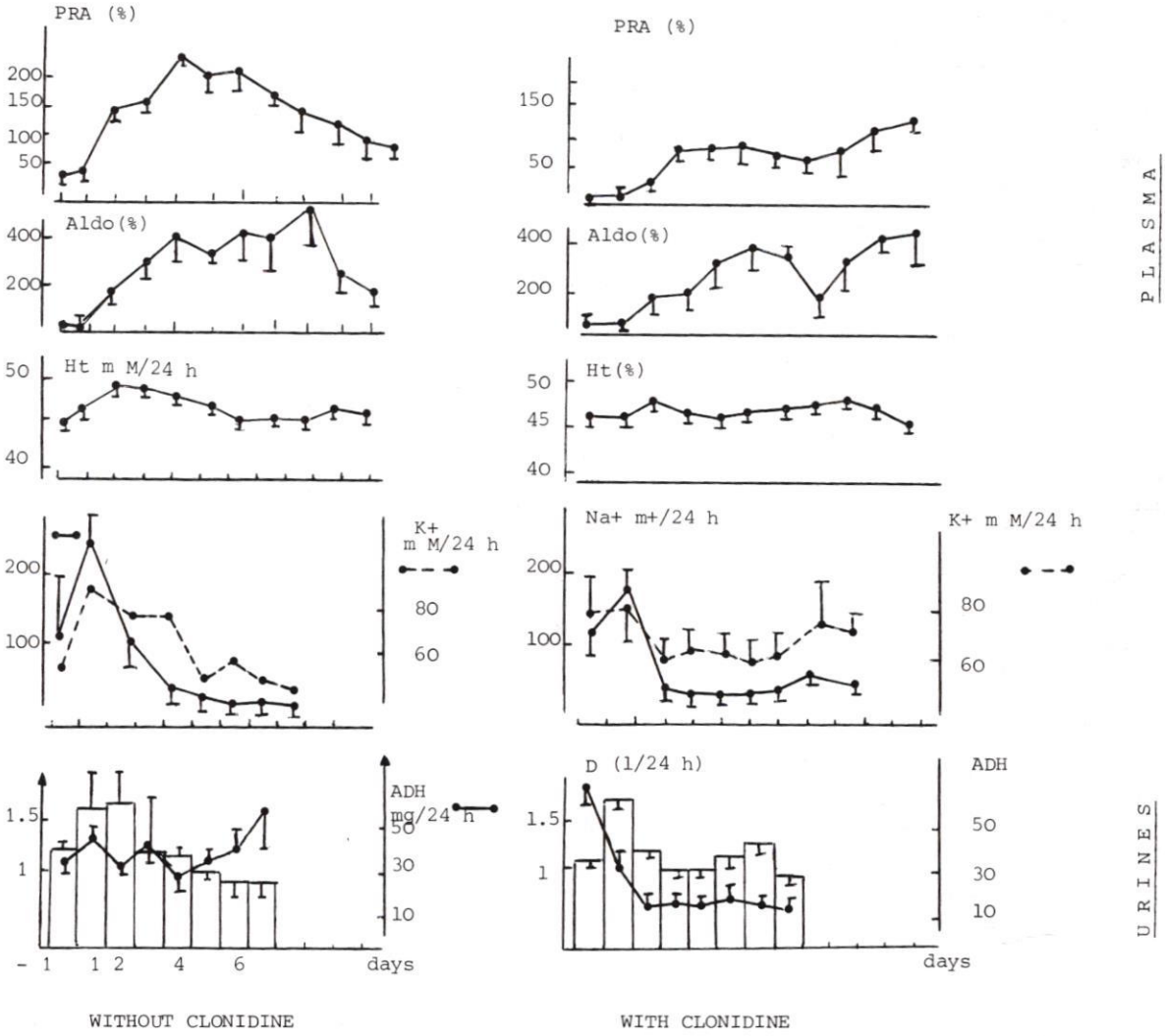

Figure 1 : Effects of Clonidine on the different parameters implicated in the regulatic of the volemia.

EFFECTS OF ANTIORTHOSTATIC POSITION AT-4° ON HYDROMINERAL BALANCE.

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Experiments of bedrest in antiorthostatic position are conducted to simulate cardio-circulatory, metabolic and hormonal modifications observed during space flight. 4 young healthy volunteers were placed in strict bedrest and in antiorthostatic position at -4° during 7 days. 1 day before, during and 2 days after the experiment, we took 2 samples daily of blood for the détermination of the plasma concentration of Na+, K+, creatinine, urea, renin activity, aldosterone ; in the urines the same parameters were studied. The haematocrit rose from 43.5±1.5 to 46.8±0.9 ($p < 0,001$) ; the plasma sodium fell from 138.1±0.5 to 136.3±0.7 mmol ($p < 0.01$) : plasma renin activity and plasma aldosterone rose significantly after the 24th hour. We also noted increased diuresis, sodium depletion and aldosterone out flow ; the volunteers presented with the classic clunical picture of cephalic congestion. Blood pressure was not significantly modified ; heart rate decrease by 22 % until the 4th day. These reactions were most striking during the first 3 or 4 days, and result from a redistribution of the body's fluid volume toward the cardiac cavities and the head away from the lower part of the body.

INTRODUCTION : it is presently well accepted by all researchers that periods of weightlessness result in significant metabolic and hormonal modifications in man (2,5,11,13). These modifications are the most significant consequence of the redistribution of blood mass from the infra-cardiac regions toward the thoracic region (3). Better understanding of these phenomena should enable men to spend longer, more confortable periods of time in weightlessness.

On earth it is possible to simulate weightlessness ; nevertheless, certain conditions for example immersion (4) or prolonged bedrest results in redistribution of blood mass (9,10).

Ten years ago, a new method appeared : its consists of having the subjects oberve not only bedrest, but bedrest in antiorthostatic position (head down tilt) the tilt varying from -2° to -6° depending on researchers.

The aim of this study is to confirm the value of such a position in order to study and better understand the cardio-vasculair (6,8,12) metabolic and hormonal modifications occuring during manned space flight, and to be able to prevent the problems related to the first phase of the weightlessness period.

MATERIALS AND METHODS

Four young, healthy volunteers (average 25 yr =3.2) where chosen after satisfactorily completing psychological and medical tests. The period of bedrest was set at 7 days and the antiorthostatic position was maintained at -4° (head down)/

Control blood samples were taken during the 24 hr preceding the beginning of the experiment : during this phase the subjects led normal lives. They were then hospitalized in the Department of Neurology of the CHU Toulouse Rangueil, and placed in bed in antiorthostatic position. They were not permitted to lift their heads from the bed, but they could turn over on their sides.

During the first 5 days of the experiment, and before its beginning, 2 daily blood samples were taken (8 a.m and 6 p.m). The first 3 samples thus serve as control values. Then 1 sample daily (8 a.m) was taken the remaining 2 days and during the readaptation phase.

These different blood withdrawals were immediately centrifuged and frozen in order to determine the different parameters all at once.

Urinary collections were made on every 24 hr. Daily sodium and potassium intake was constant before, during and after the experiment (120 mEq/day of sodium and 60 mEq/day of potassium). Caloric intake ranged from 2000-2500 calories per day. Water was unrestricted, and the sensation of thirst was carefully noted.

The subjects were weighed before and after the investigation.

Blood pressure, heart rate, and samples of electrocardiographic tracings were taken every 2 hr throughout the experiment.

As for plasma, we determined plasma renin activity, aldosterone (1), sodium, potassium (IL 243), creatinine (Technicon). The value of hematocrit was obtained by micro-methods (Clay-Adams).

Sodium, potassium, creatinine and aldosterone content were determined in the urine.

The different results presented correspond to the mean value of the 4 subjects (mean ± SD)

RESULTS

Clinically, the subjects complained of a feeling of head fullness, nasal congestion, headache, visual disturbances ; lastly, marked facial puffiness, predominantly palpebral, occured in all 4 subjects. These different reactions reached a maximum at about the 36th hr after the beginning of the experiment and tended to disappear thereafter.

There was no significant variation is blood pressure.

No anomaly was found in the electrocardiographic tracings ; lastly, heart rate decreased significantly during the period of bedrest compared to the pre and post experiment phase (-22 ± 7.8 %) As regards weight, a mean loss of 1.8 kilos ± 0.6 was noted.

Plasma determinations : a significant increase in hematocrit value from the 25th hour after the beginning of the experiment which lasted throughout the first 48hr. A decrease in plasma sodium during the first 3 days : from 138.25 mEq ± 0.43 in the control state to 136.25 + 0.33 at the 36th hour. Potassium and creatinine were not modified. Plasma renin activity decreased at the 6th hr (-53 % ± 19 %); 2 peaks were noted : at the 48th hr and the morning when the subjects were to get up (fig.1). Finally, the value of plasma aldosterone progressively increased to reach a maximum the 4th day (+ 127 ± 18 % at the 60th hr) (fig. 1).

Urine : increased diuresis was noted within the first 24 hr after the beginning of bedrest. It reached a maximum at the 48th hr (+45 ± 23%) return to the control state from the 5th day. This polyuria was present in spite of the absence of thirst and without having drunk water (fig.2).

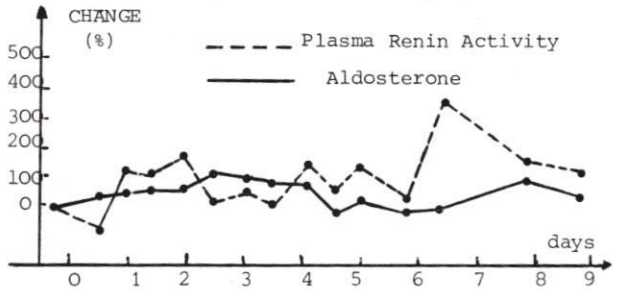

Fig. 1 : Responses of plasma renin activity and plasma aldosterone to bedrest.

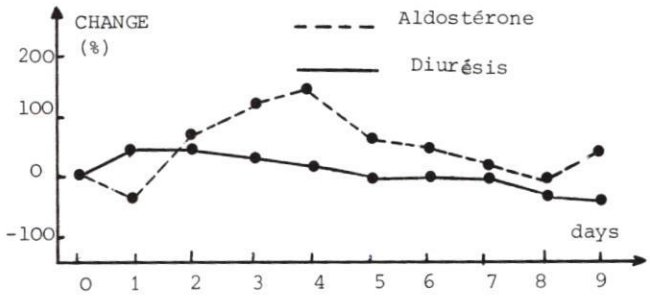

Fig. 2 : Responses of diuresis and urine aldosterone concentration to bedrest.

Also noted was a very significant loss of potassium and sodium during the first 48 hr (+49 ± 11 % and + 136 ± 19 % respectively). Potassium loss persisten until the end of the experiment. Finally, regarding hormones, the aldosterone level progressively increased to peak at the 96th hr (+ 138 ± 6.7 %) (fig. 2)

DISCUSSION

From the clinical standpoint, we observed that prolonged bedrest in antiorthostatic position resulted in symptoms identical to those experienced by astronauts in manned space flights. Significant hydroelectrolytic and hormonal modifications were observed. Sodium and potassium losses were related to a redistribution of blood volume with increased thoracic blood volume and stimulation of atrial receptors ; this process inhibited renin and aldosterone secretion for a short time at the beginning. The inhibition of this system appears related to a decrease of sympathetic activity ; indeed, in the course of studies on immersion and on prolonged bedrest, a decrease of urinary noradrenaline excretion and an increase of catecholamine metabolite excretion have been noted.

This phenomenon was observed on the return of the SKYLAB missions.

The increase of plasma renin activity and aldosterone value from the 4th day of the experiment are an answer to the sodium and plasma volume depletion observed during the first phase of the experiment. From these results, it seems evident that studies of prolonged bedrest in antiorthostatic position constitue an effective method of simulating on earth the metabolic and cardio-vascular modifications observed during manned space flight.

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CARDIOVASCULAR AND ENDOCRINE EFFECTS OF GRAVITATIONAL STRESSES (LBNP). THE INFLUENCE OF ANGIOTENSIN-CONVERTING ENZYME INHIBITION WITH CAPTOPRIL.

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INTRODUCTION

The neuro-hormonal control of the cardiovascular system has been studied more intensively during the last decade, and is of prime importance to gravitational physiology. Several studies, mostly on isolated tissue have demonstrated an interaction between the renin-angiotensin system and the sympathetic nervous system (1, 2). In dogs it has been shown that the pressure response to procedures that caused release of noradrenaline was enhanced during the infusion of angiotensin II (3). Hatton et al. (4) found that angiotensin II interacts with the sympathetic nervous system to maintain arterial blood pressure, in a study using lower body negative pressure (LBNP).

In the present study we used LBNP to challenge the processes regulating blood pressure. In order to examine the significance of the renin angiotensin system in relation to sympathetic nervous activity and plasma vasopressin concentration, we compared the hemodynamic and hormonal responses to LBNP before and after captopril, a converting enzyme blocking agent.

METHODS

Nine healthy males were studied during 10 min of LBNP at -20 and -40 mm Hg, respectively. After the experiment and the risks involved had been explained to the subjects oral accept was obtained.

Arterial pressure was recorded by a capacitance manometer connected to a catheter inserted percutaneously in the right brachial artery.

In the same arm a long venous catheter was introduced into the superior vena cava for monitoring of the central venous pressure (CVP) by an electric manometer.

Cardiac output (CO) and lung tissue volume (LTV) was measured by a rebreathing technique using a mass spectrometer as previously described (5). Heart rate (HR) and ECG were monitored throughout the experiment.

Forearm blood flow (FBF) was measured by venous occlusion plethysmography using an air filled rubber cuff (6).

Total peripheral (TPR) and forearm vascular (FVR) resistances were calculated as TPR=MAP/CO and FVR=MAP/FBF, where MAP is the mean arterial pressure averaged by electrical integration.

Blood samples were drawn from the arterial catheter for analysis of plasma concentrations of renin, angiotensin I and II and vasopressin, and epinephrine and nor-epinephrine. These hormones were measured according to current radioimmunologic and radioenzymatic methods, respectively.

PROCEDURE

The subjects reported to the laboratory at 8 AM after a light breakfast which did not include coffee nor tea. The catheters were placed in the artery and vein. He was then placed horizontally in the LBNP box to the level of the iliac crest. After 60 min in this position control resting values were taken, and LBNP applied for 10 min at -20 mm Hg. After another 15 min new resting values were taken and another period of 10 min LBNP was applied at -40 mm Hg. He then received 100 mg Captopril per os, and rested for 30 min. Then the protocol was repeated. Except for an occasional hematoma no side effects were observed. A few of the subjects did not support the LBNP for the total length of the -40 mm Hg LBNP period. This trend was even more pronounced after medication. In any case of pre-syncope, the LBNP was terminated.

RESULTS

Before captopril: As a result of LBNP there were expected decreases in CO, SV, CVP and MAP and concurrent increases in HR (table 1). The changes were most pronounced at -40 mm Hg. FBF decreased at the onset of LBNP but increased during the LBNP to attain higher than resting values at the end of -20 mm Hg LBNP, while it did not completely reach that value during -40 mm Hg. The calculated changes in FVR showed the opposite pattern, first increasing, and then after 4-6 min decreasing towards resting values. An increase in LBNP pressure from -20 to -40 mm Hg accentuated this tendency. TPR, however, tended to increase during the entire period of LBNP. Nor-epinephrine increased both at -20 and -40 mm Hg, renin, angiotensins I and II only at -40mm Hg and epinephrine and vasopressin not at all.

After captopril: The changes in CO, SV, CVP, and MAP during LBNP were more pronounced, whereas FBF, FVR, and TPR showed changes similar to those observed before captopril. Increases in nor-epinephrine, renin and angiotensin I were significantly greater after captopril. Marked increments in epinephrine and vasopressin were observed only after captopril. Unchanged, low angiotensin II values during LBNP demonstrated an effective blockade of converting enzyme.

TABLE 1. Cardiovascular and hormonal parameters measured during LBNP at-20 and -40 mm Hg with and without Captopril medication. The table brings mean values and SE (SE only for n≥6). Number of subjects measured is indicated by the digit behind the SE/. As some of the analyses were not performed when this abstract was written, the number of subjects investigated varies for the hormone concentrations. In the card.vasc. parameters the number of subjects varied because some could not sustain the full protocol for LBNP.

	CONTROL						CAPTOPRIL					
	Rest	-20 mm Hg LBNP		Rest	-40 mmHg LBNP		Rest	-20 mm Hg LBNP		Rest	-40 mmHg LBNP	
	60 min	2 min	8 min	15 min	2 min	8 min	60 min	2 min	8 min	15 min	2 min	8 min
CVP;mm Hg	6.60	5.32	5.05	6.95	3.48	2.78	6.55	3.67	4.20	6.98	3.08	2.25
	1.22/6	1.20/6	1.15/6	1.38/6	-/5	-/5	1.22/6	1.31/6	-/4	-/5	-/5	-/4
CO;l/min	6.39	5.51	5.05	6.19	4.42	3.80	6.47	4.60	4.20	6.35	4.13	3.61
	0.30/9	0.47/9	-/4	0.36/9	0.27/8	.33/8	0.51/9	0.31/9	0.30/9	0.52/8	0.23/8	-/5
LTV;l	1.11	1.00	0.88	1.07	0.85	0.86	1.13	1.01	0.92	1.06	0.85	0.88
	0.07/9	0.04/9	-/4	0.08/9	0.09/8	.06/8	0.11/9	0.09/9	0.09/9	0.07/8	0.04/8	-/5
HR;bpm	59.6	65.2	69.9	58.9	71.0	72.8	59.8	67.3	67.8	62.0	81.9	83.3
	3.0/9	2.7/9	4.0/9	2.6/9	2.6/9	4.4/8	2.5/9	2.7/9	3.6/9	3.2/8	5.9/8	4.9/6
MAP;mm Hg	88.9	91.0	88.0	91.0	89.6	85.9	91.0	88.8	83.7	88.8	85.3	81.7
	2.3/9	1.8/9	2.9/9	2.3/9	2.5/9	3.8/8	1.7/9	1.9/9	3.3/9	2.0/8	3.0/8	3.1/6
TPR;MAP/CO	14.2	17.3	18.2	15.1	20.4	23.5	14.6	19.9	20.6	14.5	20.9	22.3
	0.9/9	1.3/9	-/4	1.0/9	0.9/8	1.8/8	0.8/9	1.2/9	1.5/9	1.1/8	1.0/8	-/5
SV;ml	111.1	86.6	71.4	107.4	63.7	54.1	109.7	69.3	64.2	104.4	52.5	43.8
	10.5/9	9.1/9	-/4	8.6/9	4.8/8	7.1/8	9.7/9	5.3/9	6.4/9	9.7/8	4.9/8	-/5
FBF;ml/min/l	26.3	21.9	26.0	29.5	19.1	19.8	25.1	19.0	22.6	25.4	15.3	19.1
	3.5/9	3.4/9	3.4/9	3.9/8	3.2/9	2.1/8	3.6/9	2.2/9	4.0/9	6.4/8	4.4/8	6.4/6
FVR;MAP/FBF	3.9	4.9	4.0	3.6	5.3	4.7	4.2	5.2	4.8	4.5	7.5	5.9
	0.5/9	0.7/9	0.6/9	0.6/8	0.5/9	0.5/8	0.6/9	0.6/9	0.8/9	0.7/8	1.2/8	1.1/6
Epineph;ng/ml	0.072	0.077	0.068	0.053	0.095	0.080	0.080	0.125	0.220	0.074	0.148	0.316
	0.018/6	0.014/6	0.017/6	0.015/6	0.025/6	-/5	.019/6	.034/6	.109/6	-/5	-/5	-/5
Nor-epi;ng/ml	0.200	0.243	0.268	0.200	0.302	0.372	0.255	0.313	0.337	0.242	0.352	0.486
	0.013/6	0.033/6	0.018/6	0.015/6	0.021/6	-/5	.026/6	.025/6	.044/6	-/5	-/5	-/5
Renin;μGU/ml	20.7		23.2	23.2		46.7	49.2		91.0	70.4		138.4
	3.9/6		4.4/6	2.6/6		15.4/6	12.9/6		-/5	-/5		-/5
Angio I;pg/ml	20.5		21.2	16.3		32.5	67.7		123.0	104.4		225.6
	1.9/6		1.8/6	3.5/6		7.7/6	16.2/6		-/5	-/5		-/5
AngioII;pg/ml	15.7		19.7	20.0		34.0	12.2		14.4	10.2		13.8
	4.1/6		6.3/6	5.8/6		12.8/6	5.1/6		-/5	-/5		-/5
Vasopr;pg/ml	1.9		2.4	1.5		3.6	1.6		14.2	1.7		77.6
	-/3		-/3	-/3		-/2	-/3		-/3	-/2		-/2

DISCUSSION AND CONCLUSION

MAP remained stable before captopril during -20 mm Hg LBNP. The unchanged plasma concentrations of renin and angiotensins I and II before captopril might indicate that the renin angiotensin system does not participate in the maintenance of the blood pressure in this situation. However, the fall in MAP at -20 mm Hg LBNP after captopril indicates active involvement of the system already at this low gravitational stress. The ensuing increments of epinephrine and vasopressin, demonstrated only after captopril, suggest reflex activation of these hormones, although a direct interaction with the renin angiotensin system could be another possible mechanism.

Although there was an activation of these vasoconstrictor hormones and the sympathetic nervous system after captopril the fall in blood pressure was not compensated by an enhanced increase in TPR, pointing to a central role of the angiotensin system in normal blood pressure homeostasis.

ACKNOWLEDGEMENTS

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HORMONAL AND RENAL RESPONSES TO PLASMA VOLUME EXPANSION AFTER HORIZONTAL RESTRAINT IN THE RHESUS MONKEY

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INTRODUCTION

It has been determined that horizontal restraint in rhesus monkeys results in a decrease in plasma volume, while arterial pressure is unchanged (2). These animals also exhibit an orthostatic intolerance and a reduced resistance to $+G_Z$ stress. Another study has shown that a 25% blood volume expansion (BVE) activates the renal compensatory mechanisms and decreases circulating levels of antidiuretic hormone (ADH) and aldosterone (ALDO) in the ketamine-anesthetized monkey (1). The purpose of the present study was to repeat the 25% BVE after 7 and 14 days of horizontal restraint in order to discover any changes in the renal and hormonal responses that may have contributed to the changes found with horizontal restraint.

METHODS

Subjects for this study were five male rhesus monkeys (Macaca mulatta) weighing 6-12 kg. Blood volume (BV) was determined using $R^{125}ISA$ and ^{51}Cr-tagged red blood cells (5). Control studies of BVE were then performed according to the method described in Billman et. al. (1).

Following the control studies, the monkeys were placed in horizontal body casts (2). After 7 days, the 25% BVE studies were repeated, and the animals were returned to their cages. An 8 week recovery period followed, then 3 of the monkeys were casted again, and the BVE studies were run after 14 days.

Plasma and urine samples were then analyzed (3,4). The control values were averaged, and the control and 7-day data were analyzed using an analysis of variance for repeated measures. Due to the small n, the 14-day data were not statistically analyzed.

RESULTS

The renal and hormonal responses to 25% BVE before and after 7 & 14 days of horizontal restraint are shown in Tables I-IV.

Plasma ADH levels were found to decrease significantly after BVE both before and after casting. After 7 days of restraint, the ADH levels were higher than the control values. A similar trend was shown after 14 days casted.

Plasma ALDO was also significantly decreased after BVE. The levels were reduced in some subjects after 7 days casting, but the differences of the means were not statistically significant. Again, the data after 14 days restraint showed similar results.

The renal function data after BVE is also similar for the control, 7 and 14-day restraint periods. Urine output and clearances of creatinine PAH, Na and K all had similar responses to BVE after casting, except for K clearance after 7 days, which decreased slightly.

Resting urine output decreased from control in some animals after 7 days casting, but this was not found after 14 days and was not statistically significant. The other parameters showed no consistent changes between control and the restraint periods, except that sodium clearance appeared to be decreased in some animals after casting.

DISCUSSION

A 25% BVE in ketamine-anesthetized monkeys leads to a decrease in plasma ADH and ALDO with a diuresis and a natriuresis (1). These findings are duplicated in this study after 7 and 14 days of horizontal restraint. The renal clearances and plasma flow were also similar before and after restraint. Thus, changes in the response of the renal system to BVE alone could not account for the conditions associated with horizontal restraint.

A major change was the increase in control plasma ADH, ADH can increase in response to a decrease in arterial pressure or a decreased BV. It has been demonstrated that arterial pressure is unaffected by horizontal restraint while BV drops (2). The ADH shift could be an attempt to maintain blood pressure or BV in the face of a decreasing BV. The cause of this BV loss is undetermined at this time.

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TABLE I

Plasma Hormone Levels ± SE
BVE Before and After 7 Days Casting

CONTROL

	C	50
ADH	2.64 ± .32	1.38 ± .05**
ALDO	12.96 ± 7.90	3.12 ± .62*

7 DAYS

	C	50
ADH	6.62 ± 1.16*	3.02 ± 0.54**
ALDO	6.70 ± 3.19	2.06 ± 1.03*

TABLE II

Plasma Hormone Levels ± SE
BVE Before and After 14 Days Casting

CONTROL

	C	50
ADH	3.1 ± .21	1.33 ± .03
ALDO	19.70 ± 11.60	4.80 ± 2.22

14 DAYS

	C	50
ADH	10.16 ± 1.84	4.47 ± .72
ALDO	13.43 ± 5.12	3.00 ± .28

TABLE III

Renal Responses to BVE ± SE
Before and After 7 Days Casting

Control

	C	50
$\dot{V}$	.32 ± .09	.40 ± .09*
Ccr	21.64 ± 2.91	39.85 ± 13.49*
Cpah	80.39 ± 7.55	118.52 ± 17.71*
$UNa\dot{V}$	7.75 ± 3.94	18.13 ± 4.27*
$UK\dot{V}$	11.23 ± 3.92	13.58 ± 4.28

7 Days

	C	50
$\dot{V}$	.16 ± .02	.32 ± .04*
Ccr	28.13 ± 2.21	38.24 ± 5.20*
Cpah	115.19 ± 23.43	174.90 ± 26.11*
$UNa\dot{V}$	2.02 ± 1.15	16.38 ± .05*
$UK\dot{V}$	18.25 ± 7.05	17.05 ± 3.39

TABLE IV

Renal Responses to BVE ± SE
Before and After 14 Days Casting

Control

	C	50
$\dot{V}$	.31 ± .10	.48 ± .13
Ccr	20.24 ± 4.54	21.29 ± 5.05
Cpah	81.33 ± 10.96	113.00 ± 24.50
$UNa\dot{V}$	4.21 ± 1.68	18.30 ± 6.54
$UK\dot{V}$	12.55 ± 6.25	16.14 ± 6.58

14 Days

	C	50
$\dot{V}$	.35 ± .23	.51 ± .28
Ccr	26.09 ± 6.67	57.58 ± 16.62
Cpah	48.63 ± 11.61	151.23 ± 34.67
$UNa\dot{V}$	1.83 ± .46	17.82 ± 9.95
$UK\dot{V}$	5.94 ± .84	11.07 ± 1.06

BVE = 25 % Blood Volume Expansion
ADH = plasma antidiuretic hormone, pg/ml
ALDO = plasma aldosterone, ng/100 ml
$\dot{V}$ = urine output, ml/min
Ccr = creatinine clearance, ml/min
Cpah = PAH clearance, ml/min
$UNa\dot{V}$ = sodium excretion, uEq/min
$UK\dot{V}$ = potassium excretion, Eq/min
* = $p < .05$; ** = $p < .01$

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HORMONAL CHANGES IN ANTIORTHOSTATIC RATS

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ABSTRACT

Hypokinesia, especially hypokinesia with negative tilt ("antiorthostatic hypokinesia"), mimics some of the effects of weightlessness. We have shown that cardiac output is increased during early exposure of rats to antiorthostatic hypokinesia. The increase of the stroke volume and of the cardiac output observed in the antiorthostatic hypokinetic rats is probably the consequence of a blood volume shift toward the chest brought forth by head-down positioning of the animals. It is also possible that struggling of the animals to escape from the harness and an increased metabolism contribute to the elevation of cardiac output. In order to study this hypothesis "stress hormones" were measured in the antiorthostatic rats. Plasma levels of ACTH, corticosterone and prolactin were measured in the arterial blood (0.3 ml) sampled before, during and after hypokinesia from chronic aortic cannulas of the rats.

INTRODUCTION

Gravity has a profound effect on mammalian organisms. The effect of gravity on the cardiovascular system is especially great. It has been reported that certain circulatory adaptations occur during exposure of man to weightlessness. New circulatory readaptations have been observed after return to earth. Changes induced by weightlessness include shift of blood and body fluids, overloading of atria of the heart and consequent neurohumoral stimulation, excessive water loss and a blood volume decrease. These circulatory changes lead to orthostatic intolerance and to a decreased work ability after return to earth.

Hypokinesia, especially hypokinesia with negative tilt ("antiorthostatic hypokinesia"), mimics the effect of weightlessness. This has been shown in human experiments (4) and in animal experiments (1-3). Antiorthostatic hypokinesia simulate the effect of weightlessness on cardiovascular systems as well. We have reported that antiorthostatic hypokinesia leads to a slightly elevated heart rate and mean arterial blood pressure (from 100 to 112 mm Hg) and an increased cardiac output (5). These changes lasted only one or two days. The increase of stroke volume and of cardiac output observed in antiorthostatic hypokinesic rats is probably the consequence of a blood volume shift toward the chest brought about by the head-down position of the animals. It is possible that struggling of the animal to escape from the harness and consequently an increased metabolism contribute also to the elevation of cardiac output. In order to study this hypothesis, levels of "stress hormones" were measured in blood of the antiorthostatic rats. The determination of stress hormones represents thus means to measure level of the stress imposed to the rats and adaptation of the animals to the new (hypokinetic) situation.

METHODS

Sixty four unanesthetized, unrestrained adult female Spraque-Dawley rats (200 ± 10 g) with chronically implanted aortic catheters (6) were used in the experiments. Morey-Musacchia system for inducement of antiorthostatic hypokinesis was used (2, 3). The rats were able to exercise using only front limbs. The hind limbs were unloaded. The head of the animals was tilted down 20°. The animals were exposed to seven day long head-down hypokinesia. The blood (0.3 ml) was sampled from the chronic aortic cannula two times prior to antiorthostatic hypokinesia, three times during hypokinesia (first, third, and seventh day), and three times after release of the animals from the harness. Blood was withdrawn from the animals that were at rest for at least 10-15 min. Levels of plasma hormones were determined by radioimmunoassays.

RESULTS

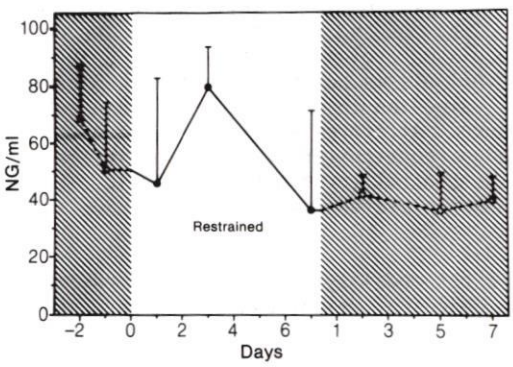
Antiorthostatic hypokinesia brougth forth on day one and day three an increase in plasma ACTH level. Plasma corticosterone level was also increased on day one and somewhat less on the day three while plasma prolactin was elevated only on day three. All plasma hormone levels were back to the control values on the day seven of the hypokinetic exposure. After removal from the harness plasma level of corticosterone and of prolactin stayed at the control (prehypokinetic) values but plasma ACTH was elevated for a few days.

DISCUSSION

Using a rat model system that mimics physiological consequences of weightlessness we have shown that antiorthostatic rats have an increased stroke volume and an increased cardiac output (5). This increase is probably the consequence of a blood volume shift toward the chest (central venous pressure decrease from o to -5 mm Hg) brought forth by head-down position of the animals. But struggling of the animal to escape from the harness and a consequent increase of metabolic

rate might also contribute to the elevation of the cardiac output. In order to study the level of "stress" induced by harness and by head-down position the ACTH, corticosterone and prolactin were measured in the blood of the antiorthostatic hypokinetic rats. Selection of these hormones is based on their common link to the existence of exogenous stressors. It is well known that plasma levels of ACTH, corticosterone and prolactin are readily affected by stress. Our results have shown that the level of all three plasma hormones increased during the first few days of exposure to antiorthostatic hypokinesis. This indicates that the animals were "stressed" and that their metabolic rate was increased in beginning of the hypokinesia. This explains the augmentation of the cardiac output. But the animals adapted to the new environment rather rapidly and the plasma levels of three measured hormones returned to the control value. The continuous decrease of the cardiac output observed during antiorthostatic hypokinesia (beginning with an elevated value) might be explained on the basis of these findings. Similar hormonal changes were found in rats exposed to 18.5 day long weightlessness of the Cosmos 936 flight. Tigranyam et al. (7) have studied plasma levels of some of the hormones studied in our experiment. The investigation was done six hours after the space flight. The authors found plasma content of norepinephrine significantly increased but plasma epinephrine or plasma ACTH level unchanged.

On the basis of the results we conclude that stress induced during early exposure to antiorthostatic hypokinesia is one of the reasons for reported increase of the cardiac output (5). However, the animals adapt to the new situation quickly. After a few days of exposure the increased plasma hormonal levels return to control values. (Supported by NASA Grant #NAG 2-87)



Plasma prolactin before, during and after head-down hypokinesia.

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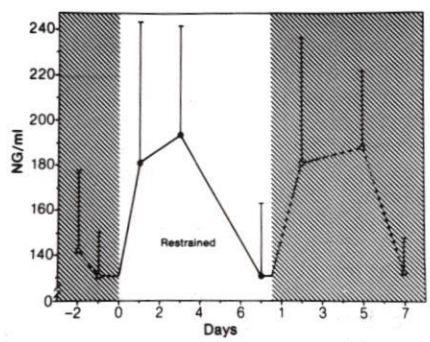
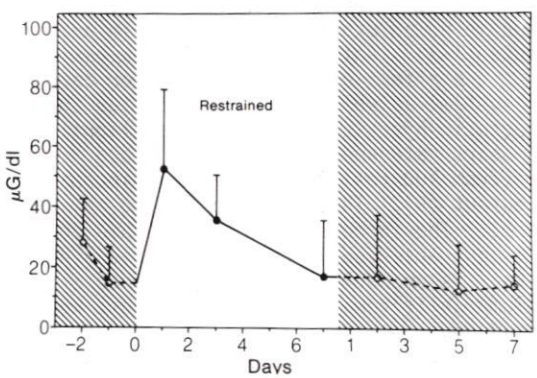


Plasma ACTH.



Plasma corticosterone

Fluid Shifts and Erythropoiesis: Relevance to the "Anemia" of Space Flight

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To model the fluid shifts thought to occur in man during space flight, cephalic fluid shifts have been induced in man subjected to horizontal or headdown bedrest, in squirrel monkeys exposed to lower body positive pressure, and in rats subjected to antiorthostatic hypokinesia. The influence on erythropoiesis of such fluid redistribution has been studied. Only in man did a cephalic fluid shift consistently and significantly lead to a plasma volume reduction and an increased hematocrit. Although there was evidence for erythrosuppression and the subjects were "anemic" at the end of the study, serum erythropoietin titers remained normal throughout bedrest. The erythrosuppression probably did not arise due to the increased hematocrit but may have been related to P50 shifts or the loss of body weight. Each model appeared to reproduce different parts of man's physiological response to weightlessness and promises to be useful in unraveling the etiology of the "anemia" of space flight.

INTRODUCTION

An increased rate of hemolysis of red blood cells (RBC) due to hyperoxic cabin atmospheres undoubtedly contributed to the "anemia" of space flight in the Gemini missions. However, most data from later flights is compatible with the concept that the primary cause of this "anemia" is suppression of RBC production(1). A major cause of this erythrosuppression has been considered to be the increased hematocrit (Hct) which follows the reduction in plasma volume (PV) after a cephalic fluid shift(1). It has been considered that this increased Hct (which is due only to a relative increase in red cell mass (RCM) and is hypovolemic) suppresses RBC production by a mechanism similar to that which occurs when the Hct is increased in hypervolemic situations with an absolute increase in RCM such as post-hypoxia or following RBC transfusion. The object of the present studies was to assess the erythropoietic effects of a cephalic fluid shift in a variety of "models" for space flight.

MATERIALS AND METHODS

Cephalic fluid shifts were induced in men by chronic horizontal (Ho) or 6° head-down (HD) tilt bedrest for either 7 or 30 days. Two cm Hg lower body positive pressure (LBPP) was applied to male squirrel monkeys for up to 7 days(2) and gravity was used to induce a cephalic fluid shift in antiorthostatic, hypokinesic rats(3). Isotopic methods (51Cr and 125I) were used to assess RCM and PV(1); CBC were obtained using a Coulter Model S; reticulocytes were enumerated on blood films stained with New methylene Blue; peripheral Hct in the animal studies were routinely obtained by the standard semi-micro method; P50 values were derived from oxyhemoglobin dissociation curves generated by a Hem-O-Scan and serum Ep quantitated by the incorporation of 59Fe into heme of fetal mouse liver cells in vitro(4).

RESULTS

The results of two 7-day bedrest investigations showed no statistical differences between studies or between Ho and HD bedrest. Therefore, results from these studies have been combined. Changes in peripheral Hct in the three space flight "models" are documented in Figure 1. The increases in Hct in humans was significant ($P<0.05$) on all study days. The Hct increase in rats on Day 1 was significant but statistically variable results between experiments were apparent. All other data points did not differ significantly from pre-exposure values. In all "models," reticulocytes (either % or number) were subnormal for all or part of the study periods but in no instance were these changes statistically significant. Serum Ep titers showed no significant changes in either humans or monkeys (results from rats are pending).

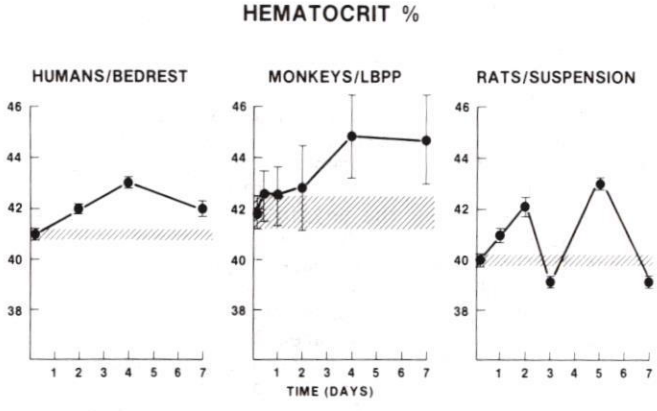

Figure 1; Peripheral hematocrit changes during 7 days of induced cephalic fluid shifts.

Both RCM and PV were significantly below pre-exposure values at the end of bedrest but have yet to be determined in the other two "models."

Hemoglobin P50 values were 5%-10% above pre-exposure levels in both monkeys and rats throughout 7 days although at no time point were the changes statistically significant. While monkeys showed no significant weight loss during 7 days LBPP, both humans in bedrest and suspended rats either lost weight or did not gain weight at the control rate (Figure 2). In a 30-day Ho bedrest, Hct did not differ significantly from control after day 8-13, reticulocytes continued to be moderately suppressed, serum Ep titers showed no significant changes (Table 1) and RCM and PV continued to decline to the end of the exposure period.

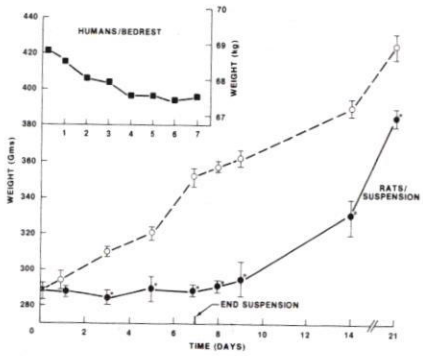

Figure 2; Changes in body weight during induced cephalic shifts (O=control rats, ●=rats exposed to antiorthostatic hypokinesia).

TABLE 1; Serum erythropietin titers during bedrest

	Serum erythropoietin titers, U/ml	
	7 day studies	30 day study
Pre	0.08±0.01	0.24±0.04
Day 2	0.14±0.04	
4	0.19±0.07	
7	0.08±0.02	
8		0.21±0.10
14		0.22±0.06
21		0.24±0.08
28		0.23±0.05

DISCUSSION

A cephalic fluid shift and its influence or erythropoiesis is being studied in three species, each with different endogenous hydrodynamic gradients. With these systems, questions can be addressed regarding the physiological significance of fluid shifts generally as well as their relevance to the "anemia" of spaceflight. Only in man did a cephalic fluid shift consistently and significantly result in an elevated Hct due to a PV decrease. Monkeys appeared to compensate for a comparable shift by a "fast-through" mechanism - an increased fluid excretion balanced by increased fluid intake(2), while a compensatory mechanism in rats, if it exists, has yet to be firmly identified. In none of the "models" was the evidence very convincing for erythrosuppresion although humans in bedrest consistently demonstrated a significantly reduced RCM at the end of both 7 and 30 days. Isotopic methods to more accurately measure RBC production are clearly warranted to assess what appears to be a small and, presumably, chronic suppression of erythropoiesis. In the two animal "models," P50 shifts were observed of a magnitude and direction predicted(5), on the basis of mathematical modeling, to markedly suppress RBC production. Another possible cause of the RCM loss during bedrest is the loss of body weight or, more specifically, of lean body mass (LBM). Such a loss in body weight occurs in man in space and in suspended rats on earth and if it causes a direct reduction in hematologically-active bone marrow, a potential mechanism for suppression of RBC production exists which might occur independently of peripheral mechanisms involving Ep. Such a concept agrees with that developed from a fourth "model" (mice with a reduced PV due to water deprivation) which has been supported by mathematical modeling(5). In conclusion, PV reductions may not be a species-independent response to a cephalic fluid shift. The "anemia" which accompanies the cephalic fluid shifts in man does not appear to result from the elevated Hct as the predicted mechanism for such an effect, i.e., a decrease in serum Ep titers was not observed. Absence of changes in serum Ep would also tend to argue against a significant role for P50 shifts. However, the weight loss which also accompanies cephalic fluid shifts in humans and rats and, after the acute phase, is not entirely due to the fluid loss, may be of significance to the "anemia" in view of the strict correlation between RCM and LBM. A unified study with these three "models," each of which appears to isolate different factors potentially involved in the "anemia" of space flight, promises to be particularly rewarding in attempts to unravel the etiology of this change.

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THE RELATIVE CONTRIBUTIONS OF GRAVITY, BUOYANCY, AND COLD TO THE CHANGES OF HUMAN PLASMA VOLUME DURING SIMULATED WEIGHTLESSNESS

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Cardiac volume receptors may reduce plasma volume (V_p) during immersion by initiating diuresis. Other authors suggested that cold temperature and hydrostatic pressure (buoyancy) translocate sufficient blood into the central blood volume to distend the heart and stimulate volume receptors. Accordingly, body fluid responses in 6 men were attributed to either the forces of buoyancy (immersion, 35°C water), cold (14.8°C air), or cold buoyancy (29.8°C water) when the responses differed significantly from control (27.6°C air). Reduction of vital capacity ($p<.05$) indicated expansion of central blood volume by the forces of buoyancy and cold buoyancy. Buoyancy and cold buoyancy caused diuresis ($p<.05$), but only cold reduced the V_p ($p<.05$). Shifts of plasma into the tissues probably resulted from cold-induced vasoconstriction because decrement of V_p was independent of urine volume. We conclude that V_p is maintained during continuous expansion of central blood volume (6 hours) in spite of a diuretic response to stimulated volume receptors.

INTRODUCTION

Body fluid responses to immersion are models for the diuresis and loss of plasma volume during weightlessness. Head-out immersion may reduce plasma volume (V_p) by causing diuretic loss of plasma water. The hydrostatic forces of immersion distend the left atrium by expanding the central blood volume. Volume receptors presumably suppress the secretion of antidiuretic hormone by responding to distension of the left atrial wall (1, 2). The vasoconstrictive response to cold water may potentiate immersion diuresis by additively filling the central blood volume. If diuresis reduces V_p during immersion in thermoneutral water (35°C), then cold water (30°C) may further reduce V_p by enhancing the diuretic response to thermoneutral water.

METHOD

Six men sat for 2 hours in 27.6°C air before 6-hour sittings in 27.6°C air (gravity, control), 14.8°C air (cold +gravity), 35°C water (buoyancy + gravity) and 29.8°C water (cold buoyancy +gravity). The subjects were hydrated to the same extent before their 4 experiments. Fasting and upright sitting were maintained during the 8-hour experiments. Each man's exposures occurred in random sequence at 10-21 day intervals. Statistical significance ($p<.05$) was assigned to environmental effects by 1-way ANOVA and to effects of environment and time by 2-way ANOVA. Significance ($p<.05$) between the stressful exposures (14.8°C, 29.8°C, and 35°C) and control was judged by the Tukey test.

Changes of baseline plasma volume (ΔV_p) were computed from baseline blood volume (CO rebreathing technique) using serial hematocrits and hemoglobin concentrations ([Hb]). Vasoconstriction was measured by plasma norepinephrine responses ($[NE]_p$, radioenzyme assay). Urine volumes (Vu) and vital capacities (V. C.) were measured at comparable times every 90 minutes. Water clearance (C_{H_2O}) and osmolar clearance (C_{osm}) were derived from vapor pressure osmometry of plasma and urine. Metabolic rate (M) and rectal temperature (T_{re}) were indicators of cold stress.

RESULTS

Comparable thermoregulatory responses to cold stress in 14.8°C air (T_{re} = 35.5°C, M = 68 $W \cdot m^{-2}$) and 29.8°C water (T_{re} = 36°C, M = 73 $W \cdot m^{-2}$) exceeded the thermoregulatory response to control (T_{re} = 36.8°C, M = 45 $W \cdot m^{-2}$) ($p<.05$). The thermal response to 35°C water (T_{re} = 36.9°C, M = 54 $W \cdot m^{-2}$) was comparable to control. Peak $[NE]_p$'s in 14.8°C air and 29.8°C water (1.5 and 1.1 $ng \cdot ml^{-1}$) exceeded peak levels in 35°C water and control (.34 and .36 $ng \cdot ml^{-1}$) ($p<.05$).

Vu was closely related to V.C. (fig. 1). Only immersions in 35°C and 29.8°C water reduced V.C. (5.30ℓ, 5.05ℓ) below control (5.72ℓ) ($p<.05$). The tendency toward lower V.C. in 14.8°C air (5.62ℓ) was insignificant. Baseline Vu's (.1 - .15ℓ) were comparable and Vu did not significantly change during control (fig. 1). The comparably high Vu's in 35°C and 29.8°C water were significantly greater than control ($p<.05$). The Vu's in 14.8°C air were not significantly elevated. The total Vu's in 29.8°C water (1.45ℓ) and 35°C water (1.11ℓ) were comparable and both exceeded control (.47ℓ)($p<.05$). Total Vu in 14.8°C (.8ℓ) was not significant. C_{osm} was significantly elevated in 35°C and 29.8°C water ($p<.05$). Negative C_{H_2O}'s were not significantly altered by the stressful environments.

Higher [Hb]'s occurred in 14.8°C air and 29.8°C water ($p<.05$). Body dehydration did not cause hemoconcentration. Body weight loss in 29.8°C

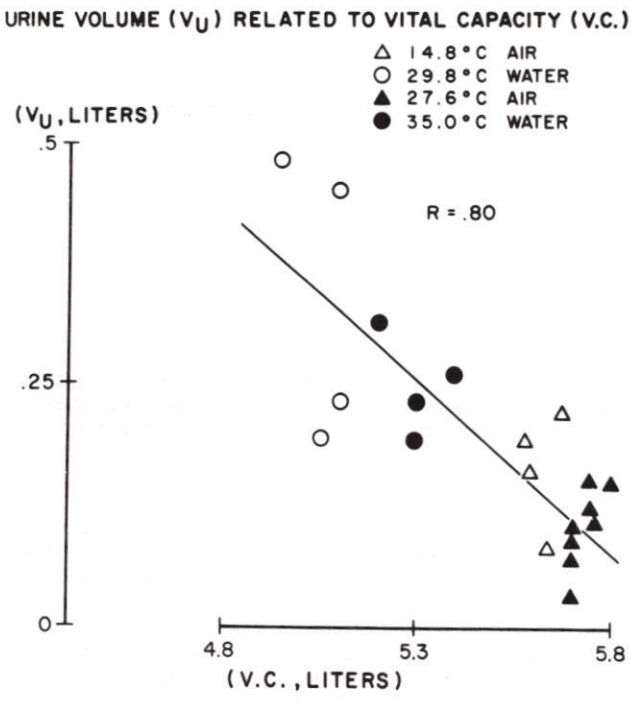

Figure 1

water (-1.76 kg) exceeded losses in 14.8°C air (-1.25 kg) and control (-1.00 kg)($p<.05$). The weight loss in 35°C water (-1.38 kg) was not significant. Maximal ΔV_p (+.11ℓ) during control was insignificant with respect to time. The effects of buoyancy, cold, and cold buoyancy on ΔV_p and cumulative Vu were computed by subtracting control responses from responses to 35°C water, 14.8°C air, and 29.8°C water (fig. 2). Buoyancy initially raised V_p (+.31ℓ) before V_p returned toward baseline (+.14ℓ and +.12ℓ) at higher Vu's

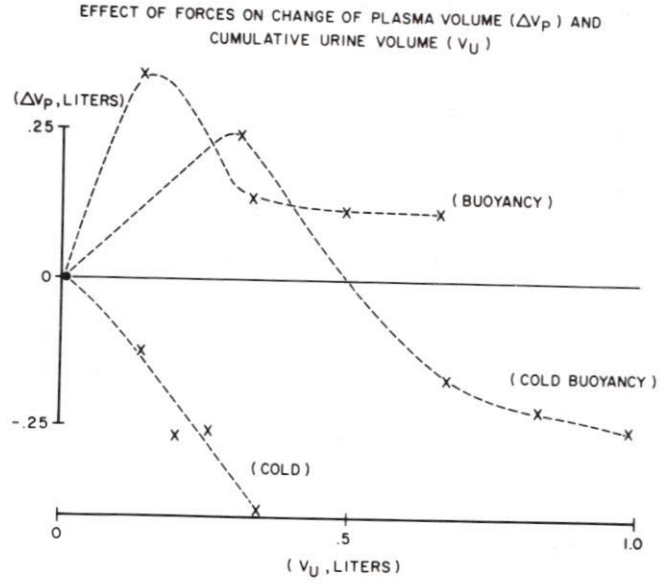

Figure 2

(.33ℓ and .65ℓ). Cold caused loss of V_p (-.38ℓ) as cumulative Vu reached .3ℓ. The ΔV_p was biphasic during cold buoyancy (ΔV_p = .+24ℓ at Vu = .31ℓ; ΔV_p = -.25ℓ at Vu = .99ℓ).

DISCUSSION

Decrements of V.C. during immersion indicated that buoyancy expanded the central blood volume (fig. 1). The diuretic reponses to 35°C and 29.8°C water, also attributable to buoyancy, were characterized by significant elevations of Cosm and negative values for CH_2O. Volume receptors did not suppress secretion of antidiuretic hormone because the changes of CH_2O were insignificant. However, cardiac volume receptors may raise Cosm by regulating sodium excretion (2). It is clear from the results that neither cold receptors nor vasoconstriction stimulated a reflex diuresis.

Diuresis did not reduce V_p below baseline during simulated weightlessness (35°C immersion). Loss of V_p during cold stress (fig. 2) must partially occur by plasma shifts into the extravascular space because dehydration and diuresis did not occur during exposure to 14.8°C air. Strict maintenance of the upright posture for 8 hours probably prevented occurrence of cold diuresis (3). The elevated $[NE]_p$'s in 14.8°C air and 29.8°C water were indicative of vasoconstriction during cold stress. Cold may reduce V_p during immersion by vasoconstrictive reduction of vascular capacity (3)(4).

CONCLUSION

Volume receptors may precipitate an osmotic diuresis, but the diuresis does not reduce plasma volume within 6 hours of onset of central blood volume expansion.

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ESTIMATION OF SKELETAL MUSCLE MASS FROM BODY CREATINE CONTENT

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The skeletal musculature of the body responds to changes in loading by increase or decrease in mass, as indicated by the long-recognized phenomena of disuse atrophy and exercise hypertrophy. More recently, evidence has been obtained of selective atrophy of antigravity muscles such as the soleus in chronic weightlessness (3), and of selective hypertrophy of the leg adductors in chronic centrifugation (2). Hence, a method for measuring skeletal muscle mass of the body is useful in studies of the effects of change in gravitational loading.

As part of our continuing study of gravitational scale effects among mature mammals we have previously described a 4-species model, comprising the hamster, rat, guinea pig, and rabbit, which provides sufficient range of body size for assessment of allometric parameters (8). We have also developed a standard dissection and analytical procedure for making organ mass and body composition measurements in the individual animals, which permits examination of the distribution of biochemical entities among the three major body components, skin, viscera and skinned, eviscerated carcass (7).

The composition of the carcass is of particular interest in gravitational physiology because it represents the principal weight-bearing organ of the body (6). In general terms, it is possible to dissect out and weigh the skeletal muscle mass, as has been done by Pitts and Bullard (9) for example, but the method is tedious and relatively inaccurate especially in smaller mammals.

Another approach to determination of skeletal muscle mass has been through the measurement of body creatine content. Numerous investigators starting with Bürger (1) have pointed out that the body creatine is distributed 97% to the skeletal musculature and only 3% to the remainder of the body. Hence, if the total body creatine content and muscle cell creatine concentration are known, muscle mass may be computed. *In vivo* methods for estimating skeletal muscle mass in man have been described which depend upon isotopic dilution of 14-C labeled creatine (5), or on measurement of urinary creatinine excretion rate or total plasma creatine mass (10). However, these methods are somewhat cumbersome, yield results with substantial variance, and are not well suited for use with small mammals.

As documented by Hunter (4), intracellular muscle creatine concentration varies among species, and with age within a given species. Hence it was necessary to establish the concentration values for metabolically mature individuals of the 4 species of interest here.

A series of 6 male animals, 8 months of age, of each of the 4 species was used to provide samples of skeletal muscle and of smooth muscle for creatine analysis. The sample of skeletal muscle analyzed consisted of hindlimb muscles with tendons trimmed away. The smooth muscle sample consisted of the stomach, intestinal tract and lower third of the esophagus stripped of mesentery and cleaned of contents. The samples were dried and then extracted with petroleum ether to measure water and fat content. The dried, defatted tissue was comminuted to yield a powder for creatine analysis. Values obtained for creatinine concentration expressed as grams creatine per kilogram of fat-free fresh muscle are shown in Table 1.

Table 1. Fat-free muscle creatine concentration. Mean ± SD for 6 animals in each group.

Species	Skeletal Muscle (g/kg)	Smooth Muscle (g/kg)
Hamster	3.33 ± 0.11	0.67 ± 0.04
Rat	4.28 ± 0.17	0.68 ± 0.10
Guinea Pig	4.10 ± 0.13	0.75 ± 0.05
Rabbit	4.48 ± 0.08	0.67 ± 0.04

In agreement with Hunter (4) it is evident that skeletal muscle tissue contains 5-7 times as much creatine as does smooth muscle tissue, and that significant differences exist between species. Hence, it is important to use the appropriate species value for skeletal muscle cell creatine concentration in computing skeletal muscle mass from creatine mass.

A second series of 6 animals of each of the 4 species was dissected for organ mass measurements and determination of water, fat and creatine contents of the carcass, skin and viscera. The distribution of the total body creatine among the three major body components is shown in Table 2, and it may be seen that 80-89% is found in the carcass, 6-11% in the skin, and 5-14% in the viscera, again with significant differences between species.

Table 2. Creatine content of major body components. Mean ± SD for 6 animals in each group.

Species	Carcass (g)	Skin (g)	Viscera (g)	Total (g)
Hamster	0.145	0.012	0.024	0.181
	±0.033	±0.002	±0.003	±0.037
Rat	0.822	0.074	0.062	0.958
	±0.054	±0.007	±0.003	±0.053
Guinea Pig	1.096	0.148	0.099	1.343
	±0.110	±0.028	±0.015	±0.144
Rabbit	6.056	0.379	0.333	6.768
	±0.482	±0.051	±0.037	±0.519

Inasmuch as the bulk of the skeletal muscle mass of the body is associated with the carcass component of the animal, *a priori* one would expect the bulk of the body creatine likewise to be found in the carcass. Less expected, however, was the finding that as much as 11-20% of the creatine is distributed in the skin and viscera.

As prepared by our dissection procedure, the skin is readily stripped off the carcass along the

plane of the deep fascia, except for the region of the face where separation of skin and the musculature is difficult. As a result, some skeletal muscle is removed with the skin. Also, the widespread panniculus carnosus is included with the skin as additional skeletal muscle. The only other skin tissue containing creatine is the smooth muscle represented by the erector pili; however, the quantity represented is quite small.

For practical purposes, therefore, carcass and skin creatine contents are entirely ascribable to their skeletal muscle contents. The viscera component, however, includes all the organs other than skeletal muscle which contain appreciable quantities of creatine; in particular, digestive tract, liver, spleen, testes, heart and brain (1). Since these account for 3% of the total body creatine, that amount was subtracted from the viscera creatine content for each animal. The remainder was ascribed to skeletal muscle tissue contained by the viscera component such as in the tongue and upper third of the esophagus, entities included in our dissection of the digestive tract.

The fat-free skeletal muscle mass of each of the three major body components could now be calculated by dividing creatine content of carcass and skin, and corrected creatine content of viscera, by the appropriate species fat-free skeletal muscle creatine concentration listed in Table 1. This was done for each animal, with the mean results shown in Table 3.

Table 3. Skeletal muscle mass of body components. Mean ± SD for 6 animals in each group.

Species	Carcass (g)	Skin (g)	Viscera (g)	Total (g)
Hamster	43.6	3.5	5.6	52.7
	±10.0	±0.5	±0.8	±10.8
Rat	192.0	17.4	7.8	217.2
	±12.7	±1.6	±0.7	±11.9
Guinea Pig	267.4	36.0	14.2	317.6
	±26.8	±6.9	±3.3	±34.2
Rabbit	1,351.7	84.6	28.9	1,465.2
	±107.7	±11.4	±9.3	±112.3

It was also possible to compute the fat-free skeletal muscle mass of the body as a percentage of total body mass and fat-free body mass for each animal, as summarized in Table 4.

Table 4. Total and fat-free body masses, and per cent skeletal muscle in each. Mean ± SD for 6 animals in each group.

Species	Total Body (kg)	Fat-Free Body (kg)	Skeletal Muscle (% TBM)	(% FFBM)
Hamster	0.155	0.122	33.7	43.1
	±0.026	±0.021	±2.2	±2.2
Rat	0.587	0.437	37.1	49.7
	±0.020	±0.014	±2.1	±2.6
Guinea Pig	1.008	0.735	31.6	43.2
	±0.116	±0.057	±2.6	±2.8
Rabbit	3.794	2.780	38.7	52.8
	±0.267	±0.192	±3.2	±3.3

There seems to be relatively little variability in the proportion of skeletal muscle present in the fat-free body, although there are significant species differences -- the rat and rabbit tending to be more muscular than the hamster and guinea pig.

Finally, the fat-free skeletal mass of the carcass could be computed as a percentage of total carcass mass and fat-free carcass mass for each animal, as shown in Table 5.

Table 5. Total and fat-free carcass masses, and per cent skeletal muscle in each. Mean ± SD for 6 animals in each group.

Species	Total Carcass (g)	Fat-Free Carcass (g)	Skeletal Muscle (% TC)	(% FFC)
Hamster	74.5	67.1	58.1	64.6
	±14.6	±13.6	±3.7	±3.7
Rat	310.1	268.4	61.9	71.5
	±7.7	±7.7	±4.1	±3.9
Guinea Pig	464.3	397.9	57.7	67.1
	±43.3	±21.1	±4.5	±5.0
Rabbit	1,980.0	1,720.3	68.4	78.6
	±157.1	±118.4	±5.3	±3.8

It may be seen that skeletal muscle represents 2/3-3/4 of the fat-free carcass of the animal, the remainder comprising principally bone and connective tissue.

In summary, we conclude that the procedures outlined here provide a valuable adjunct to study of the effect of change in gravitational loading on skeletal muscle mass. Of particular utility is the option of examining changes in skeletal muscle content of the animal carcass because of its weight-bearing function, as well as measuring total body skeletal muscle mass.

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ENHANCEMENT OF CHRONIC ACCELERATION TOLERANCE BY SELECTION

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If a group of animals is exposed to a stressful environment it becomes apparent that all are not equally stressed, nor are they all equally successful in becoming physiologically adapted. In several cases it has been demonstrated that reproduction of animals which exhibit a superior capacity for physiological adaptation (e.g., by survival) will yield progeny with an increased frequency of superior adaptational capacity. The mechanism for this improvement in environmental response is one of phenotypic selection. Elimination of susceptible individuals modifies the gene frequency of the group -- decreasing the frequency of susceptible individuals. In physiological terms, the selection process concentrates the adaptates found in a group into individuals.

In 1955, a research program was initiated by C.F. Kelly and A. H. Smith at Davis to investigate the physiological consequences of chronic acceleration. This involved the exposure of animals to moderate acceleration fields and for long periods, such that the animals could become physiologically adapted. The principal experimental animals were domestic fowl, bipeds sharing many appropriate physiological characteristics with humans (e.g., a well-developed vasomotor function). Centrifuging birds became stressed in fields greater than 1.5 G, and the stress-syndrome has been described (2). The degree of stress and progress of physiological adaptation could be evaluated hematologically (1). Modifications observed in animals physiologically adapted to chronic acceleration also have been reviewed (3).

It became convenient to reproduce the survivors of one experiment to provide subjects for a succeeding experiment. Selection criteria (in addition to survival) included freedom from "breast blister" (a sternal callosity which indicates excessive sitting) and freedom from postural abnormalities. This acceleration-selection procedure was continued through 23 generations, and the progressive improvement in chronic acceleration tolerance is indicated by the following survivorship curves. These generally are exponential and described by the equation:

$$S_t = 100e^{-kt} \quad [r; p]$$

Where: S_t indicates the survivors, as % of the initial group, at time t (days);
$-k$ is the mortality rate, as % S_t per day;
r is the correlation coefficient of the relationship; and,
p indicates the random probability (<0.05 being statistically significant).

Following is a table or representative survivorship relationships for males. Not all experiments yielded useful data because of age of the animals, duration of treatment at one field strength or additionally imposed treatments. Where the centrifugation proceeded for a significant period at a lower field, the exposure time in the survivorship equation is modified as (t-n), merely to indicate the treatment time when $S_o = 100$. The survivorship for unselected stock, and for the early selected generations, is a simple exponential function of the treatment time. However, with continued selection (e.g., S_7 and later) the curve becomes complex and the exponential decrease in survivorship proceeds only to a limit. This indicates the presence of a segment of the treatment group that does not contribute to the observed mortality kinetics.

ACCELERATION SELECTION AND ACCELERATION TOLERANCE

Exposed to 2 G Field:

Unselected: $S_t = 100e^{-0.016(t+2)}$ [r = -0.983; p<0.001]

S_3: $S_t = 100e^{-0.012t}$ [r = -0.942; p<0.01]

S_7: $S_t = 78e^{-0.023t+20}$ [r = -0.979; p<0.001]

S_{23}: $S_t = 32e^{-0.018t+66}$ [r = -0.991; p<0.001]

Exposed to 2.5 G Field:

Unselected: $S_t = 100e^{-0.023(t-1)}$ [r = -0.994; p<0.01]

S_{21}: $S_t = 75e^{-0.028(t-19)+25}$ [r = -0.994; p<0.001]

Exposed to 3 G Field:

Unselected: $S_t = 100e^{-0.037(t-3)}$ [r = -0.997; p<0.001]

S_8: $S_t = 83e^{-0.033(t-24)+17}$ [r = -0.998; p<0.001]

S_{13}: $S_t = 80e^{-0.030(t-121)+20}$ [r = -0.987; p<0.001]

It is apparent that the mortality rates at each field strength are rather uniform, and that the mean mortality rate increases with field strength:

Field Strength (G)	$-\bar{k}$ (± SD)
2.0	0.017±0.005
2.5	0.026±0.004
3.0	0.033±0.004

A regression of mean mortality rate ($-\bar{k}$) upon the increased field strength (G-1) indicates that the effect is arithmetic and, statistically, highly significant:

$$-k = 0.017\ (G-1) \qquad [r = 0.999;\ p<0.001]$$

These results indicate that the acceleration selection did not affect the mortality rate of the susceptible individuals. The effect of the selection was to increase the magnitude of the mortality limit -- the segregation of a progressively larger G-tolerant group from the initial population. These kinetics indicate that the development of the G-tolerant group resembles an all-or-none response, suggesting that the establishment of G-tolerance depends upon very few (genetic) factors. The varying size of the G-tolerant group at different field strengths (e.g., S_{23} at 2 G and S_{21} at 2.5 G) demonstrates that the factors or combination of factors that are protective at one field are not equally protective in more intense fields. The relationship between mortality rate (of susceptibles) and increased field strength (G-1) provides evidence that the mechanisms responsible for this mortality are not affected by Earth-gravity. In view of the segregation of a tolerant group by acceleration-selection, this relationship may indicate that evolution under Earth-gravity had a similar effect, producing modern gravity-tolerant organisms.

Although the nature of the demonstrated enhancement in chronic acceleration tolerance by selection would appear to apply generally, the quantitative aspects are less certain. The initial objective in the development of the acceleration-selected line was to increase the numbers of animals completing an experiment. Since development of the selected line was not a primary objective, the animal age, field strength and duration of the treatment varied according to the requirements of the experiments being conducted.

Equally as important as the selection kinetics are physiological modifications apparent or absent in the acceleration-selected line. No qualitative change in the response to acceleration has been observed. Individuals of the selected line which go through chronic acceleration sickness exhibit all of the signs seen in similar individuals of unselected stocks. Tolerant individuals of unselected stock are likewise indistinguishable from tolerant individuals of the selected line -- the latter merely has more of them. This is consistent with the concept that the selection merely leads to the segregation of a tolerant group. Also, no great changes in the body composition are evident in controls of the selected line -- although this is one of the major changes observed during chronic acceleration (4). The only consistent change seen in controls of the selected line is a progressively smaller (-20% at S_{23}) body size. This reduction in size may have a beneficial effects, since the severity of acceleration effects is inversely related to body size.

The observed improvement in acceleration-tolerance with serial selection is fairly rapid. Generally it is considered that processes which exhibit a rapid selection progress have a metabolic basis -- because of the directness of the sequence: metabolic process-enzyme-gene (5). However, no characteristic metabolic changes have been identified, so far, in the acceleration-selected line.

In conclusion, it is apparent that acceleration selection is effective in improving chronic acceleration tolerance. However, the variable selection procedure employed in developing this acceleration-tolerant line limits the confidence in quantitative evaluation of the procedure. It would be worthwhile to repeat this serial acceleration selection with standard selection criteria (animal age, field strength, and selection pressure) to identify the relationships that apply. Such information would have some importance regarding the evolutionary aspects of gravitation.

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INCREASED GLUCONEOGENESIS IN HYPER-G STRESSED RATS

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INTRODUCTION

Spaceflight animals are exposed to hyper-G as a result of the rapid transition from weightlessness to 1G upon returning to earth. Exposure of rats to hyper-G by the use of the centrifuge is stressful and effects numerous metabolic alterations (4). Ground based studies on hyper-G stress are needed to help differentiate the effects induced by spaceflight weightlessness to those induced upon returning to 1G. Investigations from our laboratory show that exposing rats to hyper-G stress leads to increases in plasma glucose and liver glycogen. This study was undertaken to investigate the role of gluconeogenesis in the altered carbohydrate metabolism. Blood levels of substrates and hormones involved in gluconeogenesis were determined.

MATERIALS AND METHODS

Overnight fasted, male Sprague-Dawley rats (250-300 g) were exposed to 3.1G for various time periods (0.25 to 24 hr). After each exposure period, centrifuged and control rats were decapitated and blood collected for various analyses; liver samples were removed for glycogen determination. Plasma glucose was determined by the ortho-toluidine method (Dow Diagnostics); enzymatic methods were used for glycerol (2) and lactate (Sigma Kit); liver glycogen by the method of Good et al (3). Plasma catecholamine was assayed radioenzymatically (Upjohn Diagnostics). Radioimmunoassay was used to determine plasma insulin (Becton-Dickensen Kit) and glucagon (1). Gluconeogenic activity in centrifuged and control rats was determined by measuring the incorporation rates of ^{14}C-(U)-labeled alanine, lactate and glycerol into plasma glucose and liver glycogen. ^{14}C-(U)-labeled substrates (5 μCi/rat) were injected, i.p., into centrifuged and control rats just prior to centrifugation. MICA (5-methoxyindole-2-carboxylic acid), a gluconeogenic inhibitor (5), was used to block the hyperglycemic response in hyper-G stressed rats; 5 mg/100 g b.w. was injected, i.p., into centrifuged rats and controls 30 min before the start of the centrifugation.

RESULTS AND DISCUSSION

The responses in rats to 3.1G as a function of exposure time on plasma glucose, insulin, gluconeogenic hormones and substrates, and liver glycogen are summarized in Table 1. Hyper-G stressed rats showed an immediate increase in plasma glucose at the onset of centrifugation. This increase persisted throughout all the exposure periods. A substantial part of the initial rise in blood glucose can be attributed to an increased rate of gluconeogenesis. This conclusion is based on the observations that the incorporation rates of ^{14}C-(U)-labeled alanine, lactate and glycerol into plasma glucose and liver glycogen were all increased in hyper-G stressed rats (Table 2) and MICA blocked the hyper-G induced hyperglycemia and liver glycogen deposition (Table 3).

An enhanced rate of hepatic glycogenolysis cannot account for the hyperglycemic response since liver glycogen levels were depleted or reduced to a low level in the overnight fasted rats prior to their exposure to centrifugation. On the contrary, there was an increase in liver glycogen deposition in centrifuged rats as early as 0.50 hr exposure time, with progressively larger amounts accumulated as the exposure time was extended to 24 hr (Table 1).

Availability of glucose precursors are known to regulate gluconeogenesis in the liver. This study shows that the increase in gluconeogenic activity of hyper-G stressed rats is due to an increase in the mobilization of gluconeogenic substrates from peripheral tissues to the liver as a result of increases in circulating catecholamines and glucagon. As seen in Table 1, plasma catecholamine was transiently increased at 0.25 and 0.50 hr exposure times, with maximum levels at 0.25 hr; blood lactate paralleled this response. Lactate, arising from the action of epinephrine on muscle glycogenolysis, is probably the major source for the initial rise in blood glucose and liver glycogen deposition.

Another effect of epinephrine is the stimulation of lipolysis, and in a previous study (4), centrifuged rats showed increases in plasma free fatty acids, indicating increased lipolysis. In this study, plasma glycerol showed a sustained decrease (Table 1). This suggests that there is an accelerated utilization of glycerol, produced by lipolysis, via gluconeogenesis in hyper-G stressed rats.

Alanine, another important gluconeogenic substrate, was also decreased in the plasma of centrifuged rats (4). This decrease is probably attributable to the stimulatory effect of glucagon on hepatic alanine uptake and conversion to glucose since glucagon was increased in hyper-G stressed rats (Table 1).

The reason for the sustained hyperglycemia in spite of increases in plasma insulin levels in hyper-G stressed rats was not delineated in this study and is a topic for further investigations. There is the possibility that the catecholamines

TABLE 1. Effect of exposure time to 3.1G on plasma glucose, insulin, gluconeogenic hormones and substrates, and liver glycogen in rats

Exposure Time	.25 hr		.50 hr		1.0 hr		4.0 hr		24 hr	
	Control	3.1G	Control	3.1G	Control	3.1G	Control	3.1G	Control	3.1G
Plasma Glucose mg/100 ml	107±2	143±3§	108±3	158±7 δ	104±3	189±10§	110±4	145±8†	113±5	184±8§
Liver Glycogen g/100 g	0.07± 0.01	0.14± 0.02	0.10± 0.02	0.40± 0.03†	0.10± 0.01	0.67± 0.07§	0.16± 0.10	2.05± 0.17§	0.19± 0.10	2.54± 0.29§
Plasma Total Catecholamine (NE + E), ng/ml	7.1± 1.2	15.2± 2.5**	6.6± 0.8	10.9± 1.0**	7.5± 0.8	8.1± 1.0	5.0± 1.0	6.1± 0.8	7.5± 1.9	6.6± 1.2
Plasma Insulin µU/ml	7.8± 1.2	25.4± 1.4**	12.0± 3.0	52.3± 5.5§	8.6± 2.0	35.4± 2.5§	8.7± 1.7	24.7± 5.6**	10.6± 1.0	33.1± 4.8§
Plasma Glucagon pg/ml	155±13	195±17*	157±12	220±20**	151±9	207±19**	146±9	197±18**	158±7	190±9**
Blood Lactate mg/100 ml	7.0± 0.2	17.1± 1.9§	5.6± 0.3	11.1± 0.9**	6.4± 0.3	7.7± 0.4	6.2± 0.5	5.4± 0.4	7.5± 0.7	7.5± 0.7
Plasma Glycerol µmoles/ml	0.23± 0.01	0.19± 0.01*	0.22± 0.01	0.16± 0.01**	0.22± 0.01	0.18± 0.01**	0.22± 0.01	0.16± 0.01§	0.23± 0.01	0.15± 0.01§

Level of significance of difference from control groups: *P<0.05; **P<0.025; †P<0.01; δP<0.005; §P<.001

Table 2. Incoporation of ^{14}C-(U)-labeled gluconeogenic substrates into plasma glucose and liver glycogen in rats exposed to 3.1G for 1.0 hr

Substrate	Treatment	^{14}C Incorporation Plasma Glucose	Liver Glycogen
L-Alanine	Control	0.11±0.01	0.09±0.01
	3.1G	0.19±0.01 (.005)	0.56±0.07 (.001)
L-Lactate	Control	0.10±0.01	0.04±0.01
	3.1G	0.17±0.02 (.010)	0.37±0.06 (.001)
Glycerol	Control	0.13±0.01	0.13±0.03
	3.1G	0.19±0.01 (.025)	0.49±0.08 (.005)

Plasma Glucose: percent dose incorporated/ml
Liver Glycogen: percent dose incorporated/g
Numbers in parentheses = P values

TABLE 3. Blockage of hyperglycemic and liver glycogenesis responses in hyper-G stressed rats (3.1G - 1.0 hr) by 5-methoxyindole-2-carboxylic acid (MICA)

Treatment	Plasma Glucose mg/100 ml	Liver Glycogen g/100 g
Control + Saline	111±3	0.12±0.04
3.1G + Saline	182±10	0.57±0.10
Control + MICA	97±6	0.02±0
3.1G + MICA	108±6	0.02±0

might promote insulin counterregulation by inhibiting the insulin-stimulated glucose utilization by peripheral tissues.

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RESTRAINT HYPOTHERMIA IN COLD-EXPOSED RATS AT 3 G AND 1 G

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INTRODUCTION

This study focuses on the relationship between heat loss, heat production and the hypothermia observed in rats exposed to centrifugation (6). Experiments were specifically designed to determine if hypergravity affects heat production (i.e., oxygen consumption) and if restraint modifies the ability of rats to activate thermogenic mechanisms after cold exposure (10°C) in a hypergravic field.

METHODS

Ten male Long-Evans hooded rats (400-500 g) were maintained at 23°C on a 12 hr light - 12 hr dark cycle with food (Simonsen's White Diet) and water ad libitum. The rate of oxygen consumption was measured using a closed system (4) modified for use at hypergravity. Because of the sensitivity of the closed system to temperature changes, chamber temperature was recorded throughout the experiments. Thus corrections could be made for changes in oxygen volume (and apparent oxygen consumption) due to changes in chamber temperature rather than actual respiration.

Throughout the experimental protocol, the rat being examined was housed in a plexiglass chamber either free or restrained in a plexiglass holder (a half cylinder over a flat plate) that prevented him from standing. In the restrained rats, core (T_c) and tail (T_t) temperatures were measured with thermistors (Veco 32/A).

Both restrained and unrestrained rats were exposed for 1 hr periods to 1 G and 3 G at ambient temperatures of 24 or 10°C. Differences in the rates of oxygen consumption between the two groups of rats were analyzed with Student's t-test.

RESULTS AND DISCUSSION

Measurements at 24°C in 1 G and 3 G fields on restrained rats. At 1 G, restrained rats consumed oxygen at the rate of 20 ± 2.0 mls·min^{-1}·kg$^{-0.67}$ (mean ± S.E.). Over the first hour of restraint, core temperatures of rats fell 0.9 ± 0.3°C from an initial value of 37.1 ± 0.1°C. Thus the restraint hypothermia observed for the five rats at 1 G appears to be due to increased heat loss rather than decreased heat production, an observation consistent with the experiments of Bartlett and Quimby (1) and Nagasaka et al. (5). {In contrast, using other types of restraint that confined rats with a neck stock and elastic band, decreased heat production rather than increased heat loss was thought to be the primary cause of the hypothermia (8).}

When the restrained rats at 1 G and 24°C were exposed to a 3 G field (Fig. 1), there were no

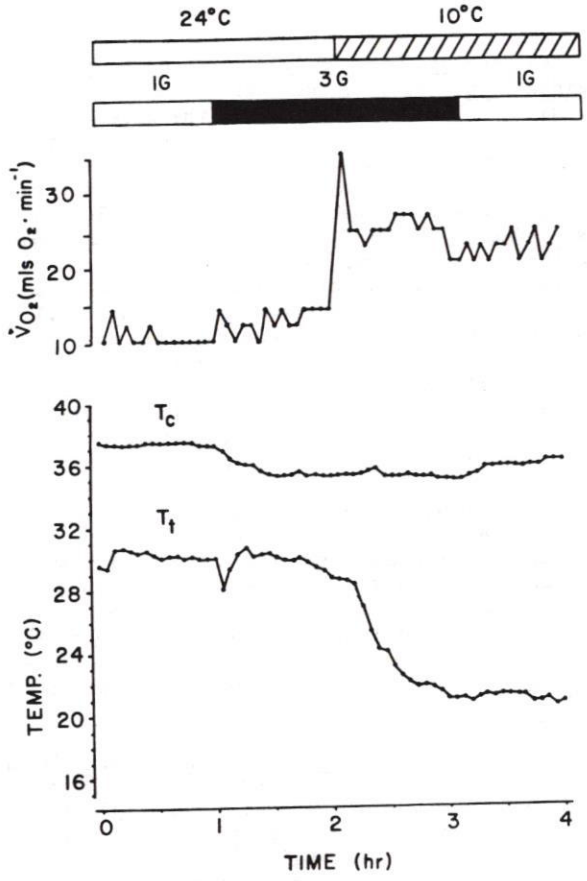

Figure 1. Effects of exposure to cold (dashed bar) and a 3 G field (solid bar) on the rate of oxygen consumption, core temperature, T_c, and tail temperature, T_t, of a restrained rat. (The brief peak in the $\dot{V}O_2$ curve immediately following the onset of cold exposure is an artifact due to gas contraction in the chamber. Control measurements without an animal in the chamber showed that gas contractions following a change from 24°C to 10°C gave a transient peak that died away within 15 minutes.) The temporal pattern of applying the cold exposure and the 3 G field was that followed in all experiments.

significant changes in the rate of oxygen consumption ($p > 0.1$). The average rate of oxygen consumption of the five rats at 3 G and 24°C was 19.2 ± 1.7 mls $O_2 \cdot min^{-1} \cdot kg^{-0.67}$ measured over a 30 min period after the rats had been at 3 G for half an hour. However, in these 5 rats there occurred a 1.2 ± 0.4°C fall in core temperature. This fall in the T_c of rats exposed to the hypergravic fields has been noted in several previous studies (2,3,6, 7); and it has been interpreted as indicating impairment of the thermoregulatory system by the hypergravic fields. The drop in T_c without a significant decrease in oxygen consumption indicates that the hypothermia at 3 G is most likely due to increased heat loss rather than decreased heat production.

Measurements at 10°C in 3 G fields on restrained rats. At 3 G, when the ambient temperature was lowered from 24 to 10°C, the rates of oxygen consumption of the five restrained rats almost doubled, increasing to 34.2 ± 3.1 mls $O_2 \cdot min^{-1} \cdot kg^{-0.67}$ measured over a 30 minute interval after the rats had already been in the cold for 30 minutes. (During the first few minutes of cold exposure the gas within the chamber was contracting; but after 30 minutes, volume changes in the closed system reflected oxygen consumption by the rat.) This increased oxygen consumption of the cold-exposed rats in the 3 G field indicates that the neurocontroller for temperature regulation can still be appropriately activated by low ambient temperature. However, the core temperature of the 5 rats continued to fall, dropping from a value of 33.4 ± 1.1°C to 32.7 ± 1.0°C at the end of 1 hour of combined stressors -- cold exposure and a 3 G field. Thus, even though oxygen consumption was dramatically increased, the thermoregulatory system was incapable of defending core temperature. The fall in T_c despite increased oxygen consumption suggests that the hypothermia observed in rats in 3 G fields at 10°C is due to increased heat loss, not decreased heat production -- effects similar to those at 3 G and 24°C.

Measurements during re-entry to 1 G at 10°C on restrained rats. When the rats were transferred from a 3 G to a 1 G environment at 10°C, their rates of oxygen consumption remained about the same (32.1 ± 3.6 mls $O_2 \cdot min^{-1} \cdot kg^{-0.67}$). In addition, during the first hour after the 5 rats were returned to 1 G, their T_c increased an average of +0.4 ± 0.8°C. In a previous study of unrestrained rats, Giacchino *et al.* (3) had observed a similar increase in core temperature upon re-entry to 1 G after a 3 G exposure. With the concurrent measurement of oxygen consumption and T_c, the increased T_c of the restrained rats in the present study can be interpreted as reflecting decreased heat loss rather than increased heat production.

Comparison of restrained and unrestrained rats at 24°C and hypergravity. The rates of oxygen consumption of unrestrained rats were also measured at 1 G and 3 G at an ambient temperature of 24°C. For 5 rats, this rate averaged 15.6 ± 1.2 mls $O_2 \cdot min^{-1} \cdot kg^{-0.67}$ at 1 G and 14.7 ± 1.7 mls at 3 G. The lack of any significant change in the rates of oxygen consumption (and hence heat production) in these unrestrained animals despite a fall in T_c (3) indicates the occurrence of an impaired thermoregulatory system. Thus both unrestrained and restrained rats (as described above) display a reduced ability to appropriately activate thermogenic mechanisms to maintain a normal body temperature when exposed to hypergravic fields at 24°C; in both cases, this impairment appears to reflect increased heat loss and a lack of activation of heat production.

In conclusion, based on concurrent measurements of T_c, T_t and oxygen consumption, the present study indicates that thermoregulatory mechanisms are impaired when rats are exposed to 3 G fields. At 24°C as well as at 10°C this impairment results in an inappropriate increase in heat loss.

This research was supported in part by NASA research grant NSG-2234. CBM was a recipient of a training grant NGT 05-004-008 during the period of this research. The use of the Chronic Acceleration Research Unit at the University of California, Davis is appreciated.

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SHORT HYPERDYNAMIC PROFILES INFLUENCE PRIMATE TEMPERATURE REGULATION

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Primates have been shown to be sensitive to hyperdynamic fields. That is, when exposed to + $2G_z$, body temperature falls. The purpose of this study was to examine the relative sensitivity of these animals to short centrifugation profiles which mimic the gravitational envelope seen on the space shuttle during launch (8 minutes, 2.9 G_z max) and re-entry (19 min, 1.7 G_z max). Four loosely restrained squirrel monkeys, isolated from additional external stimuli, were exposed to these profiles. During launch simulation, the temperatures never fell markedly below control levels. However, subsequent to return to 1G, the recovery phase showed decreases in body temperature in all four animals averaging 0.4°C over the next 10 to 15 minutes. The two animals exposed to the re-entry profile showed decreases in body temperature within five minutes of the onset of centrifugation. Maximum fall in body temperature was reached by the end of the centrifugation phase and averaged 0.7°C. Thus, the temperature regulation system of this primate is sensitive to short hyperdynamic field exposures.

Experimentation in the zero G environment of space, such as occurred in Skylab and will occur in the future in the Space Shuttle, are complex in that the initial transition from the earth gravity environment includes a hyperdynamic environment as a result of injecting the spacecraft into orbit. Thus we not only are dealing with a zero G environment for the duration of the flight but we must contend with conflicting physiological responses as a result of sensitivity of biological systems to this short hyperdynamic environment. A similar problem may arise upon return to earth as a result of the re-entry forces.

Many biological functions have been shown to be affected by hyperacceleration and zero G environments. Among these are temperature regulation in a variety of organisms (1,2,3,4,5). To begin examining the influences of different segments of space flight on such physiological systems, we have studied the body temperature regulation of the squirrel monkey (Saimiri sciureus) to short hyperacceleration profiles mimicking the launch and re-entry environments of the Space Shuttle. In this study we report that such hyperdynamic fields are capable of reproducibly producing depressions in body temperature.

Four loosely-restrained squirrel monkeys were exposed to hyperdynamic fields. The centrifugation consisted of either: a) an 8 minute biphasic field simulating launch in which a maximum of 2.9 G_z was reached at the end of the field or, b) a 19 minute centrifugation resembling re-entry in which a 1.7 G_z maximum field strength was reached. These animals (extensively conditioned to restraint) were allowed to acclimate to centrifuge for as much time as necessary for the body temperature to stabilize prior to centrifugation. Body temperature was measured by a thermistor inserted 6 cm past the anus and taped to the base of the tail. Temperature information was recorded continuously on a strip chart recorder. The data was subsequently digitized at one minute intervals, and average responses were computed.

All animals consistently demonstrated depressions in body temperature in both hyperdynamic environments. Figure 1 shows the response of one animal to the launch profile simulation. The temperature did not show any depression until the centrifuge was turned off. During that time the temperature fell 0.6°C. The average decrease in body temperature of all four animals during the launch profile was 0.4°C.

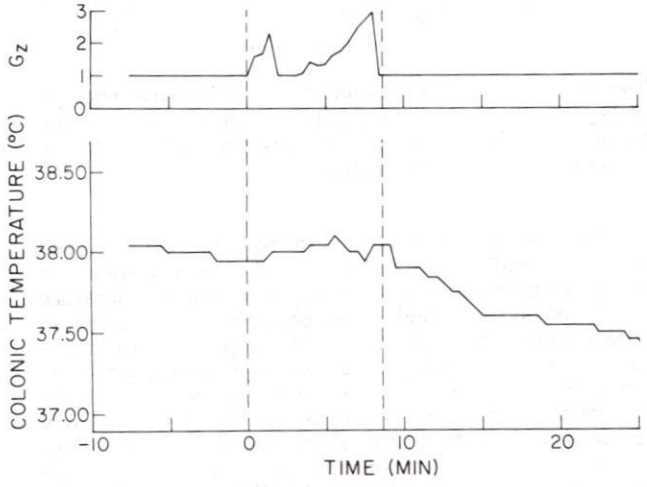

Figure 1. The top panel shows the launch profile as produced in the G_z axis. The bottom panel shows the body temperature response of a squirrel monkey exposed to the launch profile.

Figure 2 shows the body temperature response of an animal to the re-entry profile envelope. In this profile, the centrifuge cage yoke was fixed at a set angle allowing the cage zero degrees freedom of movement. This exposed the animals to changes in both the G_z and G_x axis. In this environment, the two animals centrifuged both showed a marked fall in body temperature averaging 0.7°C. The onset of the temperature response was within the first five minutes of centrifugation and persisted throughout the profile. Upon completion of the re-entry profile, the body temperature began to rise.

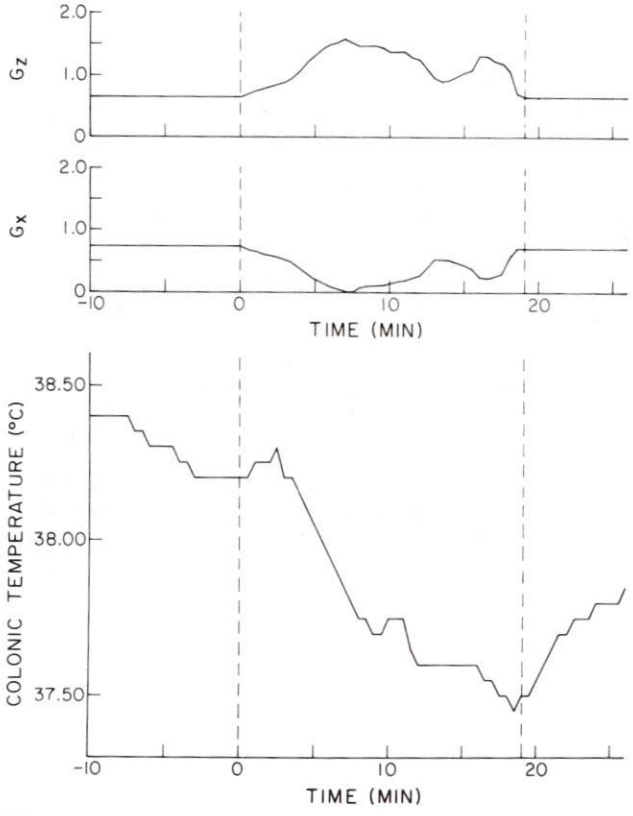

Figure 2. The top two panels show the relative changes of G_z and G_x during the re-entry profile. The bottom panel shows the temperature response of a squirrel monkey to the re-entry profile.

The sensitivity of this primate to both the launch and re-entry profiles demonstrate the importance of recognizing these responses for this, the temperature regulation system, and other physiological systems in understanding the adaptation of organisms to space flight. For example, an experiment measuring body temperature in space must be concerned with the effects of launch on the subsequent changes in body temperature. To fully understand the initial responses to the zero G environment, one must be able to compensate by knowing the effects of lift-off. Similarly, with return to earth gravity after adapatation to zero G, it is necessary to know both the influence of zero G and that of re-entry.

At this point in time, we still do not understand the mechansism behind these responses of the temperature regulation system. In rodents, similar responses in temperature regulation have shown increases in heat loss and decreases in heat production in a 2 G_z environment (cf. 2).

We have also not yet fully described the complete interaction between the temperature regulation system and the hyperdynamic environment. When exposed to 2 G_z for 60 mins, the body temperature falls for the duration of that period of time and starts to recover immediatly after centrifugation is ended (1). However, during the launch profile responses, the animals on average did not start showing a fall in body temperature for the first five minutes (similar to the 60 minutes centrifugation), but upon cessation of the centrifugation the body temperature still continued to fall for a period of at least 10 to 15 minutes even though the animals were returned to 1G. Also, the role of posture or orientation of the field, has not been delineated. It was suggested in rats that a $-G_z$ axis may reduce or eliminate the response (2). The results presented in this study also suggest posture may play a role. The re-entry profile exposed the animals to a mixed acceleration field with components in the G_z and G_x axes. The first ten minutes of this profile, which was milder than the launch profile, induced a larger physiological response than the launch profile.

Thus, we have shown that temperature regulaton in the squirrel monkey is sensitive to mild hyperdynamic fields mimicking the launch and re-entry profiles of the space shuttle. Such responses indicate that careful planning must be given to space flight experimentation to compensate or account for experimental results due to establishing the zero G environment in space. Further, work is needed to define these responses more completely in these and other systems and determine the mechanisms involved.

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ALTERED AUDITORY FUNCTION IN RATS EXPOSED TO HYPERGRAVIC FIELDS

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INTRODUCTION

Recent studies suggest that central thermoregulatory mechanisms are impaired during exposure to modest orthodynamic hypergravic fields of 1.5 G to 3 G (1,6). The exact nature and anatomical locus of the dysfunction is not known. At 3 G, however, impaired function may be limited to regions rostral to the brainstem since auditory projections through the pons and midbrain remain unaltered while thermoregulation is substantially impaired (4). The present study was undertaken to determine if the affected region might expand to include brainstem auditory projections when rats are exposed to 6 G fields.

METHODS

Seven Long-Evans adult rats were used in the present study. Under general anesthesia (sodium pentobarbital) each rat was implanted with vertex, mastoid, and frontal sinus stainless-steel skull screws for recording electroencephalographic (EEG) activity. Calibrated thermistors were placed in the diencephalon and used to monitor brain temperature. As described in detail elsewhere (3), bone-conducted auditory stimuli were produced by the vibrations of a polycrystalline ceramic which in turn was driven by an applied voltage pulse (+8.0 to +60.0 V in amplitude; 0.06 msec in duration). Coupling of the vibrations to the skull was accomplished by bolting the crystal to a dental acrylic platform which covered the skull surface and screws. All animals were allowed to recover from surgery at least 5 days before recording auditory potentials.

Auditory brainstem responses (ABRs) were used to monitor auditory function. ABRs consist of a series of electrical waves occurring within the first 6 milliseconds following a click. ABRs are produced by the brainstem and auditory periphery and can be recorded from the scalp (2). The waves are numbered 1 through 5, where wave 1 represents peripheral auditory nerve activity. Wave 2 is thought to be generated in the region of the cochlear nucleus while waves 3 through 6 represent activity at successively higher stations in the pons and midbrain along auditory pathways.

EEG activity was amplified ($x10^4$), filtered (LF 10^2Hz, HF $3x10^3$Hz: -6dB points) and recorded on magnetic tape. A stimulus timing pulse was recorded on a separate channel so that the successive evoked potentials could be averaged to obtain the ABRs.

In awake, freely moving animals, ABRs were recorded at 1 G for 1 hour prior to acceleration, during approximately 1 hour of 6 G orthodynamic acceleration and then again for a variable time immediately following acceleration. The animals remained in the same chamber throughout the procedure. During 1 G control periods, centrifugation noise (previously recorded in the chamber during acceleration) was recreated to assure equal ambient noise conditions throughout the experiments.

Interpeak latencies were used to indicate changes in central conduction or transmission times and were calculated by subtracting the latency of the auditory nerve potential (the first positive peak, 1P) from each later peak in the ABR.

All statistical comparisons were made using the paired Student t-test. Immediately after a change in the gravitational field there were only small changes in brain temperature (0.20 ± 0.24°C). Periods before and after acceleration were compared to periods of acceleration where brain temperatures were similar.

RESULTS AND DISCUSSION

All seven animals showed ABR changes indicating impaired conduction/transmission of brainstem auditory signals during exposure to the 6 G acceleration field. Significant increases in central conduction time occurred for peaks 3N, 4P, 4N, and 5P ($P < .05$, N=negative, P=positive). The absolute latency values for these same peaks were also significantly increased. Figure 1 illustrates the nature of these delays for one animal. In this case delays are clearly apparent in the negative-going peak of wave 3 (3N). Substantial delays are also present in peaks 4 and 5.

In addition to prolonged latencies in peaks 3N through 5P, in some animals earlier peaks showed a tendency to decrease in latency. Moreover, in three animals the amplitudes of waves 3 and 4 tended to increase during acceleration. In one animal there was a doubling of the amplitude of waves 3 and 4. These effects indicate variability of ABRs in individual trials; however they were not observed in all animals, and suggest the possible coexistence of conduction enhancement along with a more significant conduction impairment. The underlying mechanisms are unknown and merit further study.

The results of this study clearly establish that brainstem auditory processing is altered during 6 G acceleration in the rat. Latency changes

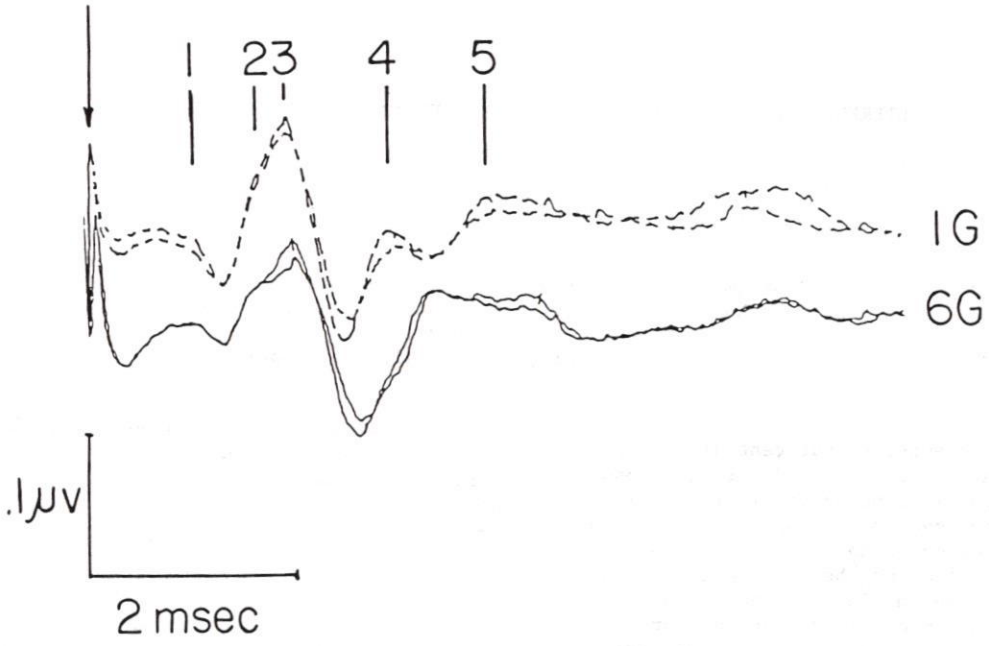

FIGURE 1. ABR traces recorded for a rat in 1 and 6 G fields: the two dashed traces were recorded at 1 G, the two solid traces at 6 G. Positive peaks are numbered 1 through 5. Negative polarity is plotted downward. Each trace represents the summation of 1024 responses. The arrow at the left of the traces indicates the onset of the stimulus. Substantial delays in the traces recorded at 6 G may be seen in the negative-going edge of peak 3 (3N) and can also be seen in peaks 4 and 5. Two traces were recorded and superimposed for each of the two conditions to illustrate the stability of the ABR waveform and the reproducibility of the effects produced by the 6 G field. For this animal the difference in brain temeprature for the two G-field conditions was 0.02°C. Calibration marks are shown for voltage (vertical bar in microvolts) and time (horizontal bar in milliseconds).

cannot be accounted for by changes in brain temperature since the mean difference in temperature between the two conditions (1 and 6 G) was -0.20 ± 0.24°C in these experiments. Temperature differences in this range are too small to produce delays seen in these unanesthetized animals (4,5).

The delay of peaks later than 3P suggest that structures above the midpontine level were impaired. We may therefore rule out the occurrence of substantial dysfunction of the auditory system in the periphery and lower brainstem. The findings reported here for 6 G experiments along with those reported elsewhere for fields below 4 G (4) are consistent with the hypothesis that impaired function proceeds in a rostro-caudal progression as field strength is increased.

ACKNOWLEDGEMENTS

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INFLUENCE OF ABDOMINAL RESTRICTION ON GAS EXCHANGE DURING +Gz STRESS IN DOGS

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INTRODUCTION

It has been generally agreed that acceleration atelectasis occurs only when +Gz stress is accompanied by both 100% oxygen breathing and use of a G-suit (3). The effects of G-suit abdominal bladder inflation, per se, on gas exchange during +Gz stress have not been clearly defined. Barr et al. noted little change in arterial oxyhemoglobin saturation during +Gz exposure when abdominal counterpressure was applied in dogs breathing air. When counterpressure was not applied, arterial oxyhemoglobin saturation fell (1). Glaister, on the other hand, reported a progressive fall in oxyhemoglobin saturation when dogs with an abdominal binder were exposed to +Gz stress (2). Our study was designed to examine the time course of gas exchange detriment resulting from +Gz stress and the influence of G-suit abdominal bladder inflation on that detriment.

METHODS

Seven adult mongrel dogs weighing 19.9 ± 2.6 kg were anesthetized with 30 mg/kg pentobarbital sodium and intubated with a cuffed endotracheal tube. A Millar, catheter-tip pressure transducer was introduced through the right femoral artery and positioned in the thoracic aorta for arterial blood pressure monitoring. A 7 Fr catheter was introduced through the left femoral artery and positioned in the thoracic aorta for arterial blood sampling. Because blood sampling from the pulmonary artery is not always possible at high +Gz levels, a 7 Fr side-holed catheter was introduced through the right external jugular vein and positioned in the right ventricle for "mixed venous" blood sampling.

A standard G-suit abdominal bladder (CSU-12P) was placed around the animal, and the animal was secured to a V-board restraint in the supine positon.

Prior to the first +Gz exposure, the animal was heparinized with 3000 units of heparin sodium injected intravenously, and control arterial and mixed venous blood samples were drawn for blood-gas analysis.

Each animal was exposed to +3,4, and 5 Gz with an onset rate of 0.1 G/sec. Exposures were made with and without G-suit abdominal bladder inflation. The standard G-suit inflation scheme was followed in which the suit begins to inflate at +2.2 Gz with 1.5 psi/G. When the desired +Gz test level was reached, arterial and mixed venous blood samples were drawn over approximately 18 seconds. At +40 seconds of the exposure, a second set of blood samples was drawn. The exposure was terminated immediately after the second set was complete (total exposure=60 seconds).

Three minutes after reaching 0 G (centrifuge stopped, animal supine), a third set of blood samples was drawn. The animal's lungs were then inflated several times with a large volume using an Ambu bag, and a 5-15 minute rest period was allowed prior to the next exposure.

RESULTS

Little change in blood-gas status was noted regardless of G-suit status when animals were exposed to +3 Gz stress. The arterial Po_2 response to +4 and +5Gz is shown in Figure 1. When the standard G-suit inflation scheme was used, arterial Po_2 decreased significantly as the exposure continued ($P<0.05$, unpaired t-test). After a 3 minute post-G recovery period, arterial Po_2 when the G-suit was used was 5-10 Torr lower than control values (i.e., 0 Gz, pre-exposure) ($P<0.05$, unpaired t-test).

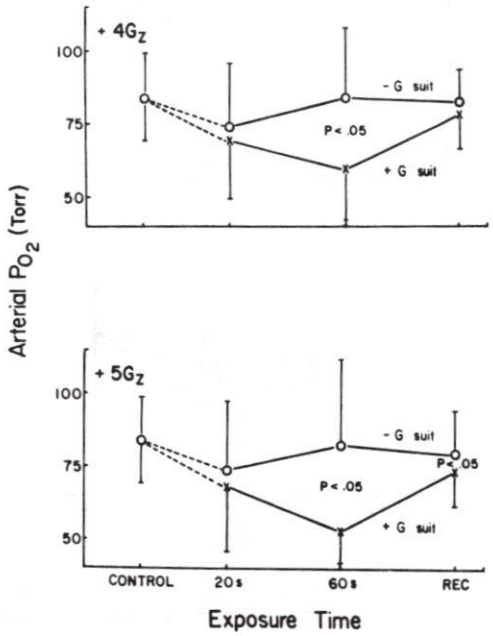

Figure 1. Arterial Po_2 at +4 and +5Gz as a function of time without (O) with (X) G-suit bladder inflation. Bars indicate standard deviation. Statistical significance was determined by Student's unpaired t-test.

Arterial Pco_2 data are shown in Figure 2. At +4Gz, abdominal bladder inflation did not affect CO_2 exchange significantly. During the exposure, arterial Pco_2 remained nearly constant. Arterial Pco_2 after 3 minutes of recovery, however, rose ($P<.01$, unpaired t-test) indicating a decreased alveolar ventilation relative to the rate of CO_2 arrival to the alveoli. During exposure to +5Gz without G-suit inflation, arterial Pco_2 fell indicating increased alveolar ventilation relative to CO_2 arrival at the lung. This pattern was not evident when the abdominal bladder was inflated.

Estimates of cardiac output changes during the exposures were made by assuming that CO_2 production remained constant and examining the venous-arterial CO_2 content differences at each sampling point. These estimates suggest that early in the exposure, abdominal bladder inflation did not help maintain cardiac output, but, as the stress continued, cardiac output in trials without G-suit inflation became compromised to a greater extent than in those with G-suit inflation. Cardiac output fell 36% more at +4Gz and 39% more at +5Gz in trials without the G-suit than in trials with G-suit inflation.

DISCUSSION

Although the results of this study are subject to several interpretations, we believe that major changes in ventilation-perfusion relationships resulting from airway closure were associated with abdominal bladder inflation.

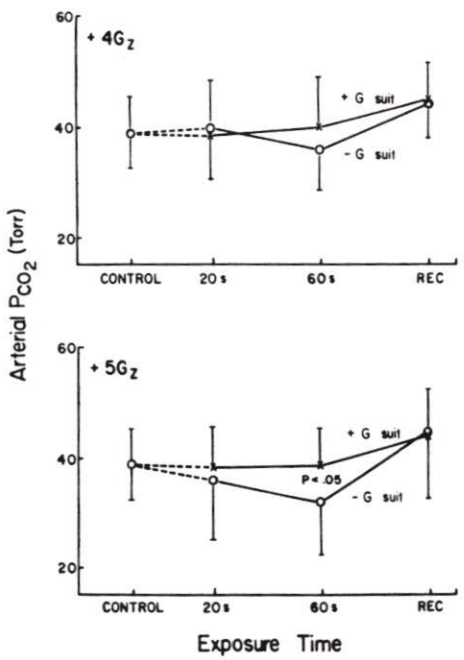

Figure 2. Arterial Pco_2 at +4 and +5Gz as a function of time without (O) with (X) G-suit bladder inflation. Bars indicate standard deviation. Statistical significance was determined by Student's unpaired t-test.

The continued decrease in arterial Po_2 seen with abdominal bladder inflation (Fig. 1) indicates a greater distribution of blood flow to low ventilation-perfusion areas. Studies in our laboratory of regional intrapleural pressure changes during +Gz stress (unpublished data) indicate that G-suit inflation results in intrapleural pressures high enough to cause lung compression over a significant portion of the lungs. Hence, as +Gz stress is imposed with G-suit inflation, the action of the G-suit bladder may promote airway closure in a larger lung region that similar exposures without an abdominal bladder. Increased airway closure would lead to areas of very low ventilation-perfusion ratio which would be reflected by a significantly lower arterial Po_2 (Fig. 1). The fact that arterial Po_2 did not return to control values after +4 and +5Gz exposure with the G-suit, but did return after similar exposure without the suit, provides further evidence that airway closure or atelectasis was associated with bladder inflation.

The proposed mechanism of acceleration atelectasis is alveolar collapse due to oxygen absorption (3) from areas with airway closure caused by mechanical forces. In air breathing animals, presence of nitrogen prevents alveolar collapse due to oxygen absorption. Glaister (2) examined the lungs of dogs exposed to up to +4 Gz stress. An abdominal binder was used, and the animal breathed air throughout. Glaister described the appearance of most alveoli in sections taken from the lung base as "closed off vacuoles in an otherwise solid tissue" suggesting airway closure rather than atelectasis.

Although our blood gas data are consistent with progressive atelectasis during +Gz exposure with G-suit inflation, we feel that airway collapse is a more likely explanation since the detriment was easily removed by rapid reinflation of the animal's lungs with an Ambu bag. We attribute the persistence of airway closure to the absence of large inspiratory efforts by the anesthetized animal following the +Gz exposure.

ACKNOWLEDGMENTS

The author thanks the personnel at the centrifuge facilities at USAFSAM and SUNY at Buffalo for their cooperation and assistance and Mr. Paul Beeman for his technical assistance.

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CHARACTERISTICS OF STATOLITHS FROM ROOTCAPS AND COLEOPTILES

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Amyloplasts with intact envelopes have been isolated from corn coleoptiles. Amyloplasts were found to have a mean zeta potential of -19.4 mv. Their charge was confirmed by the binding of cationised ferritin (CF) and fluorescein isothiocyanate (FITC)-CF. Membrane bound calcium, indicated by chlorotetracycline (CTC) fluorescence, was detected throughout the cytoplasm of rootcap cells. The presence of CTC fluorescence around amyloplasts isolated from corn coleoptiles suggests that calcium binds to the amyloplast envelope.

INTRODUCTION

Although amyloplast sedimentation is generally thought to be an essential feature of plant graviperception, the nature of the intracellular signal given by sedimentation is unknown. One hypothesized signal is an intracellular charge redistribution resulting from the sedimentation of statoliths bearing a strong surface charge (7), but it is not known if amyloplasts have such a charge. A net negative charge on isolated amyloplasts is demonstrated here through electrophoretic measurements and labelling with CF.

Previous research using the ion microscope shows rootcap statoliths to be rich in calcium (2). We employed CTC to determine if any of this calcium was membrane bound.

METHODS

The techniques and results involving amyloplast isolation, microscopy and CF are detailed elsewhere (4). Briefly, amyloplasts were isolated from corn coleoptiles by gentle homogenization and low g centrifugation. The isolation medium contained 0.3 M sucrose, pH 7.6, with the addition of 1 mM HEPES, and 10 mM KCl for electrokinetic measurements and 50 mM Tris for all other purposes. Zeta potentials were determined using a vertically oriented Northrup-Kunitz electrophoresis cell. Amyloplasts were incubated either with CF (750 µg/ml) and then fixed for electron microscopy or with FITC-CF (CF concentration, 250 µg/ml) and examined with epifluorescence optics.

CTC (100 µM in distilled HOH or buffer) was applied to Vibratome sections of corn root tips or to coleoptile amyloplast suspensions. A UG 1 exciter, a 420 nm beam splitter and 520 -560 nm barrier filter were used for fluorescence work. N-phenyl-1-naphthylamine (NPN), a lipophilic fluorochrome, was used as in (5) except that it was diluted with buffer for plastid incubation (UG 1 exciter, 395 nm reflector, 410 nm splitter).

RESULTS

The low g pellet contained individual starch grains and a small proportion of intact amyloplasts. In the electron microscope, both membranes of the organelle envelope were seen to be present and continuous.

Amyloplasts from plants of different ages (5-9 d) and degree of exposure to light (0-9 d) all had negative zeta potentials ranging from -8 mv to -30 mv (mean of -19.4 mv). Isolated starch grains had comparable values.

The net negative surface charge was confirmed ultrastructurally by the binding of CF to both amyloplasts and starch grains. However, binding to the starch was reduced compared to amyloplasts. FITC-CF showed binding to some but not all amyloplasts. Fluorescence was absent from individual starch granules. Uncationised (native) ferritin controls exhibited no binding to amyloplasts.

Many of the central rootcap cells exhibited diffuse cytoplasmic CTC fluorescence (Fig. 1). Nuclei often had an intense halo of fluorescence and a less intense core. Fluorescence was lower on the cores of the vacuoles and amyloplasts. It was not possible to determine if the tonoplast or amyloplast envelope (in situ) fluoresced since CTC fluorescence was present in the immediately adjacent cytoplasm. However, isolated amyloplasts were surrounded by fluorescence which was absent on isolated starch granules (Fig. 2). The pattern of NPN fluorescence was similar to that of CTC. Rootcap walls but not protoplasts were lightly autofluorescent.

DISCUSSION

Electrophoretic measurements and labelling with CF show that the surfaces of amyloplasts isolated from corn coleoptiles are negatively charged. In this respect, amyloplasts resemble chloroplasts and the plant plasmalemma (1).

The decreased binding of CF to starch grains compared to amyloplasts may reflect an affinity of CF for membrane based charge. A negative zeta potential seems to be a general feature of starch granules from several species (3).

Wilkins (7) has hypothesized that if amyloplasts were electrically charged, their sedimentation could create a cell polarity; this might affect the permeability and transport

properties of nearby membranes. The demonstration here of a statolith surface charge supports that hypothesis but does not answer the question whether amyloplast charge is a component of graviperception.

CTC flouresces in the presence of calcium electrostatically bound to the Stern layer of membranes. It is not possible to distinguish between cytoplasmic and tonoplast and amyloplast envelope fluorescence in situ. However, CTC flourescence encircles amyloplasts isolated from coleoptiles in a medium without added calcium indicating that a calcium shell may exist on the amyloplast envelope. Punctate flourescence was found on amyloplasts in Haemanthus endosperm cells although the starch did not fluoresce (8). Antimonate precipitate containing calcium surrounds fixed Avena coleoptile amyloplasts in situ (6).

The reduced CTC flourescence from the amyloplast core could result from either a low concentration of membrane bound calcium or from a paucity of internal membranes. Amyloplasts do contain a system of thylakoids but most of the volume of the organelle is occupied by starch. NPN fluorescence is proportional to membrane density; the reduced fluorescence from the amyloplast core suggests that the internal membrane density of the amyloplast is lower than that of the cytoplasm (endoplasmic reticulum, dictyosomes etc.).

In the ion microscope, rootcap amyloplasts appear to contain more calcium than the cytoplasm (2). Since the ion microscope detects both free and membrane bound calcium, it is likely that much of the calcium in amyloplasts does not complex with CTC and is dissolved in the stroma and/or associated with the starch.

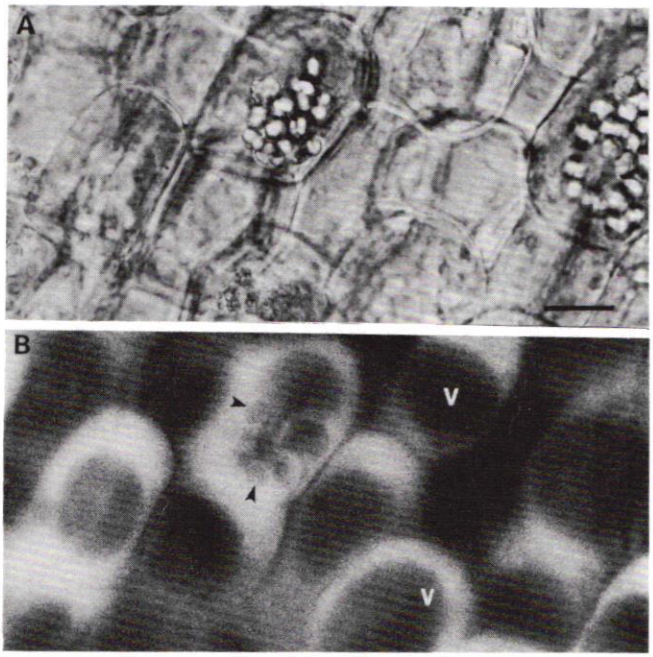

Fig. 1 and 2. Paired phase (A) and CTC fluorescence (B) micrographs. Bars = 15 µm. Fig. 1. Central cells of corn rootcap. Fluorescence from cytoplasm but not amyloplasts (darts) or vacuoles (v).

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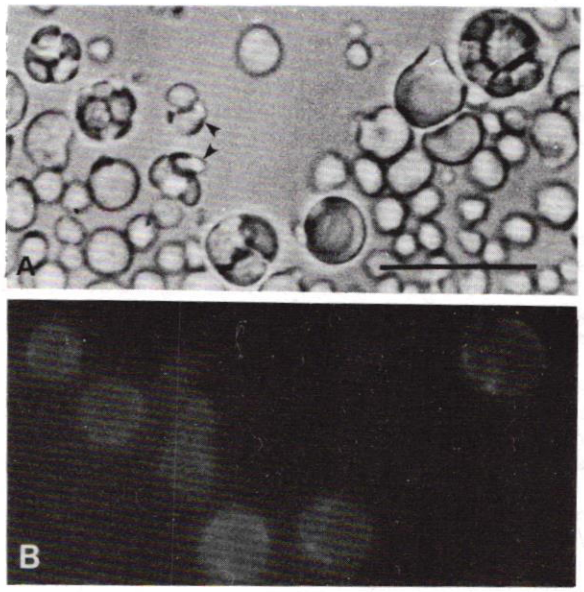

Fig. 2. Intact amyloplasts isolated from corn coleoptiles. CTC fluorescence from plastids but not starch grains. Disrupted amyloplast at dart.

THE MODE OF GRAVITY SENSING IN PLANT CELLS

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The role and the mode of action of gravity sensing cells were investigated in the *Asparagus officinalis* epicotyl and in the *Lens culinaris* root. In epicotyls, it has been shown that the ability of perceiving gravity is not only linked to the presence of statoliths, but also depends on the state of differentiation of the statocytes. In roots, the pressure of statoliths on the more viscous parietal cytoplasm could be responsible for the transmission of the geotropic stimulus.

INTRODUCTION

The majority of the plant organs which are able to respond to gravity possess particular cells (statocytes) containing voluminous amyloplasts (statoliths) (1, 2). These organelles sediment under the influence of gravity because their starch have a greater density than that of the surrounding cytoplasm (1, 2).

In roots, the statocytes are located in the cap (3 - 6). Removing the cap suppress their sensitivity to a geotropic stimulus (7). With the disappearence or the decrease in volume of plastid starch caused by the growth substances kinetin + gibberellic acid (GA_3) or GA_3 only, there occurs inhibition (8) or reduction (9) respectively of the geotropic response of roots. In the same way, a decrease in volume of the amyloplasts due to the etiolation of the seedlings of *Asparagus officinalis* induces a smaller geotropic reaction in their roots (10).

In *Asparagus* epicotyls, the statenchyma represents a monocellular sheath which comprises the outer layer of the pericycle (11, 12). The role of this tissue in the perception of gravity in these organs is not well established.

GRAVITY SENSING IN EPICOTYLS.

The geotropic curvature of the *Asparagus* epicotyl begins with a positive (downward) curvature and the negative (upward) response only occurs after 15 - 30 min of geotropic stimulation. Three phases of differentiation of the statocytes are distinguished by criteria of cell length, the state of vacuolisation and the sedimentation of amyloplasts and nuclei with respect to gravity. The statenchyma is located only in the first 10 mm of the epicotyls 1, 2 or 3 cm in length ; in effect the oldest statocytes differentiate into sclerenchyma beneath this level.

The upper limit of the bending is situated in the zone containing the mature statocytes, while the lower limit is much closer to the base of the epicotyl.

Experiments on decapitation of the epicotyls have indicated that the youngest and the mature statocytes do not play an important role in the perception of gravity. However it has been demonstrated that there is a statistical correlation between the initial rate of curvature of the epicotyls and the length of the sheath of the oldest statocytes.

The comparison of the ultrastructural features and the role of the different types of statocytes leads to the conclusion that the avility of perceiving gravity may be linked to the following sequence of events :
- 1. the decrease of the ribosome density and the decrease of the cytoplasmic viscosity,
- 2. the sedimentation of statoliths and their maturation,
- 3. the polarization of the endoplasmic reticulum along the distal wall,
- 4. a limited increase of the cytoplasmic viscosity and a lost of turgor of the statocytes.

GRAVITY SENSING IN ROOTS

The geotropic curvature of roots (20 mm long) stimulated horizontally occurs after a latent time of 30 min. The rate of curvature is maximal during 90 min and slows down progressively.

The central root cap cells contitute the statenchyma. When the root is in a vertical position, the statocytes show a polarization of their organelles. The amyloplasts are sedimented on large aggregations of endoplasmic reticulum lying along the distal wall whereas the nucleus is located against the proximal wall. When the root is placed horizontally the amyloplasts and the endoplasmic reticulum are subjected to a contrary movement.

A relationship between the geotropic response and the sedimentation of amyloplasts was studied.

The initial rate of curvature (v) was analysed as a function of the angle (α) of the root inclination relative to gravity. The number of contacts (c) between the amyloplasts and the parietal cytoplasm located along the longitudinal wall and their displacement (d) were also determined from roots inclined at various angles. The results obtained prove that the initial rate of curvature is a function of the product d c sin α. The geotropic stimulation is thus due to the pressure and the movement of statoliths on a sensitive surface located along the longitudinal wall.

The nature of the sensitive surface was analysed by examining the sedimentation of statoliths in roots stimulated for 20 min (Presentation time). It has been shown that the amyloplasts are able to provoke a geotropic stimulation though they are situated at a distance of 0.135 µm - 0,260 µm from the plasmalemma. The cytoplasmic structures which may play a role in the geotropic stimulation are :
- 1. the endoplasmic reticulum which is located at a distance of 0,185 µm from the plasmalemma ;
- 2. the microtubules or the plasmalemma if the pressure of statoliths can be transmitted by the more viscous cytoplasm.

From the numerous mechanisms which could be proposed it seems that the one involving a change of plasmalemma properties under the action of amyloplasts best explains the results obtained in roots and epicotyls.

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LINKAGE BETWEEN GRAVITY PERCEPTION AND RESPONSE IN THE GRASS LEAF-SHEATH PULVINUS

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Abstract. Cellulose synthesis inhibitors 2,6-dichlorobenzonitrile (DCBN) and coumarin inhibit the normal gravitropic response of the leaf-sheath pulvini in several grasses. These inhibitors induce thickenings that are mostly distributed in the radial walls. Their distribution patterns follow our previously published equation that describes the geometry of asymmetric growth in the leaf-sheath pulvinus (1). We propose that radial transport of growth promotors may account for the observed asymmetric growth rather than the classical concept of lateral downward transport in an horizontally placed shoot. Electron microscopic (EM) observation of an interaction between the tonoplast membrane and statoliths suggests that such an interaction might be a basis for gravity perception in grass leaf-sheath pulvini.

INTRODUCTION

The leaf-sheath pulvini of grasses are radially symmetric organs that do not grow in the normal vertical position. Upon gravistimulation, an asymmetric growth response occurs, leading to an upward (negative) curvature. The geometry of this response can be expressed by the equation: $S = 0.5 - r \cos \theta$, where S is the response of any cell in the organ, r is the radius of the organ and θ the angle of curvature in degrees (1). We now report on the use of inhibitors that further establishes the validity of this equation. Treatment with DCBN also reveals the presence of a cellular asymmetry which suggests a radial transport of hormones from statenchyma outwards; this may be a better explanantion for asymmetric growth in this organ. Ultrastructural proof is offered also for a possible mechanism of graviperception through a statolith-tonoplast interaction.

METHODS

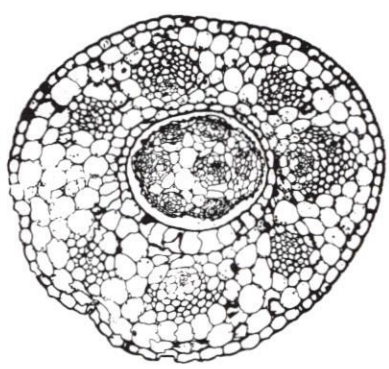
Leaf-sheath pulvini of barley (Hordeum vulgare), oats (Avena sativa), Agrostis nebulosa, and Lolium multiflorum were used in this study. Pulvini with 1 cm of shoot on either sides were held horizontally between glass plates. The following chemicals were supplied through filter paper in contact with the shoots: 0.1 M sucrose, varying concentrations of colchicine, coumarin, cytochalasin B, DCBN, isopropyl N-chlorophenyl carbamate (IPC), actinomycinD or cycloheximide, all in 0.1 M sucrose. At various intervals, the pulvini were excised, fixed in gluteraldehyde followed by osmium tetroxide, dehydrated and embedded in Spurr'd medium. Thick sections were stained in toluidine blue for light microscopy and thin sections with uranyl acetate and lead citrate for EM.

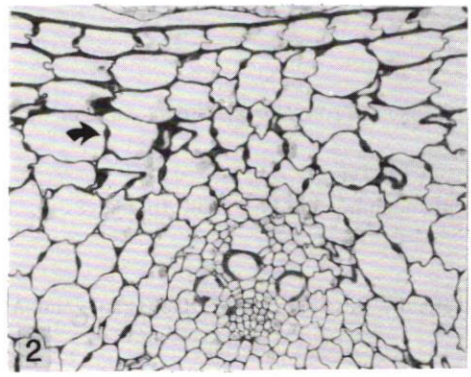
Free-hand sections of fresh pulvini were stained with a number of microchemical reagents to determine the chemical nature of the thickenings induced by DCBN and coumarin. Gravistimulated pulvini were also examined for esterase activity with naphthol AS-D acetate method and fluorescence microscopy method with fluoresein diacetate. Measurements of cell volumes were made with a Quantimet automatic image analyser.

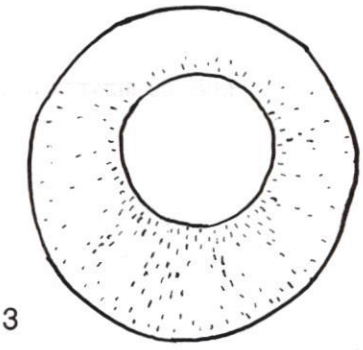
RESULTS

Actinomycin D and cycloheximide completely suppress the gravitropic responses, proving that RNA and protein synthesis are required for gravitropic bending. However, these two compounds do not cause any structural alterations observed at the light microscopic level. Cytochalasin B at 10^{-4}M causes only 15% reduction in curvature indicating a negative role for microfilaments in gravity perception or response. Millimolar concentrations of colchicine, IPC and coumarin, and 10^{-5}M of DCBN cause about 60% reduction in curvatures. The above four inhibitors also induce pronounced asymmetric swelling of the entire pulvinus. The swelling is an exaggeration of the normal cellular response that occurs during gravistimulation in control pulvini (Fig.1). In control pulvini, asymmetric cell elongation occurs throughout the pulvinus except at the uppermost region (1). As seen in Fig. 1, cell volume also increases in an asymmetric manner with no increase in volume at the top and maximum increase at the bottom. Colchicine, IPC, coumarin and DCBN further accentuate this volume increase at the expense of increase in length.

Colchicine and IPC are known inhibitors of the assembly of microtubules. Coumarin and DCBN are known to inhibit cellulose synthesis. While all four inhibitors cause asymmetric swelling, the cellulose synthesis inhibitors, in addition, induce characteristic thickenings on the radial walls. The arrow in Fig.2 shows such a thickening in the statenchyma region of a lower half of a barley pulvinus. These radial thickenings first appear in the statenchyma cells, and latter, may extend towards the outer epidermis. The distribution of these thickenings, as mapped from a typical section, is shown diagramatically in Fig. 3. The number of thickenings can be correlated with the maximum swelling response of cells. Microchemical analysis reveals that these thickenings have very little cellulose, but may possess other polysaccharides. The ultrastructure of these thickenings also confirms the absence of cellulose microfibrils but







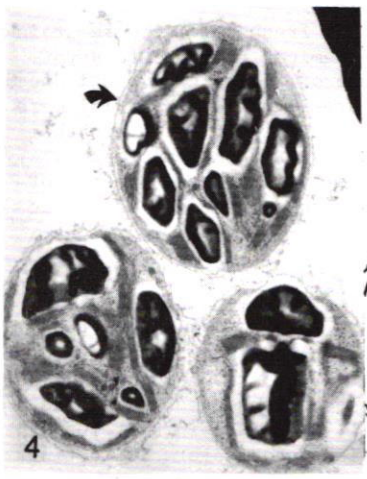


Fig. 1. Agrostis nebulosa x120. Fig. 2. Barley x200. Fig. 3. Barley. Fig. 4. Oats x7,500.

the presence of massive wall material, presumably hemicelluloses and polygalacturonans.

Statoliths sediment within about 10 min after gravistimulation. Fig. 4 is a cross section of a statocyte of oats obtained from the lower half of a pulvinus after 1 hr of gravistimulation. An interpretative diagram of the same is given in Fig. 5. The statoliths seem to be enveloped by the tonoplast membrane as they sediment towards the gravitational field (arrow). Higher magnification micrographs show that the tonoplast membrane is distinct from the amyloplast double membrane and never touches the latter.

In grass pulvini, the statoliths occur in small groups of statenchyma cells close to the inner epidermis (2). The number of such statenchyma groups varies from species to species. In barley, fluorescein diacetate staining shows intense esterase activity on the statoliths. On gravistimulation, increased activity is seen on the lower side. The naphthol As-D acetate staining method also confirms the increase in esterase activity on the lower side.

DISCUSSION

The asymmetric swelling induced by colchicine or DCBN is an indication that the gravistimulated pulvinus is continuosly sensing gravity and responding to this stimulus. However, the presence of these inhibitors interferes with cellulose microfibril orientation (colchicine) or prevents the supply of cellulose molecules (DCBN), thus altering the response phase by causing greater swelling to occur than the normal elongation. The distribution of the thickenings on the radial walls (Fig. 2,3) agree with our previous observations (1) that the response of cells is asymmetric and graded, with maximum growth occurring at the bottom and none at the top; other regions between show intermediate responses. The distribution of these thickenings also confirms our observation that all the cells, with the exception of the uppermost ones, are involved in response to gravity stimulation.

Radial Transport. Several features suggest that in the grass pulvinus a radial transport of growth promotors may be operating. First, the radial thickenings occur in the statenchyma before they occur elsewhere. Second, the thickenings occur preferentially on the radial walls, and not on the tangential walls. Third, the uppermost region does not develop any thickenings, while the lowermost region develops the most thickenings. The presence of increased esterase activity in the statenchyma might indicate that on gravistimulation, IAA might be released from conjugates in this region. Radial transport from the central circle of statenchyma towards the outside will depend on 1. The amount of hormone generated at each locus, and 2. The transport capacity of all the cells involved in such transport. A radial transport of IAA might account for observations, and those of others (3), that no lateral basipolar transport occurs in the grass leaf-sheath pulvinus. Only a radial transport mechanism can explain the fact that cell elongation occurs in regions well above the level of statoliths (2). Perhaps, a radial transport mechanism may be operative in other radial organs, such as dicot shoots, which have a central ring of statenchyma.

The interaction between sedimenting statoliths and the tonoplast membrane might be one mechanism for the perception of gravity. The stretching of the tonoplast membrane, or the proximity of the amyloplast and tonoplast membranes, might lead to biochemical changes, such as bringing an enzyme and its substrate together to release an active growth promotor from its inactive conjugate. Such amyloplast-tonoplast interaction may not be exclusive to grass leaf-sheath pulvinus, since micrographs that show such interaction have appeared for gravity-stimulated coleoptile (4).

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ROLE OF AUXIN AND PROTONS IN PLANT SHOOT GRAVITROPISM

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Abstract

Gravistimulation of sunflower hypocotyls results in an acceleration of cell extension on the lower surface and retarded growth on the upper surface. This pattern is evident along much of the hypocotyl axis. Auxin (10^{-5}M) and H^+ (pH 4.5) initiate greater extension rates over the same regions of vertical hypocotyls. Data are presented showing a redistribution of ^{14}C-IAA and ^{3}H-IAA occurs within 20-30 minutes of gravistimulation. The above data together with evidence that inhibitors of auxin transport or action prevent gravicurvature and asymmetric acid efflux indicate lateral auxin transport initiates asymmetric H^+ excretion which in turn causes the differential growth response associated with shoot gravitropism.

Recent work in our laboratory (4,5) and elsewhere (2) suggests that the negative gravitropic response of plant shoots may result from asymmetric acid efflux. Evidence for this notion derives from three pieces of data. First, it is well established that acid buffers enhance shoot cell elongation (3). Second, gravitational stimulation of shoots results in enhanced acid efflux from the lower surface while efflux from the upper surface is reduced (2,5). Lastly, shoot gravitropism is inhibited by neutral buffers (4).

In this paper we describe a line of experimentation designed to probe the relationship between asymmetric acid efflux and auxin redistribution during gravitropism. That is, since auxin is well known to stimulate H^+ excretion in straight growth systems (3), it is possible the lateral transport of auxin toward the lower side of a shoot (as postulated by the Cholondny/Went hypothesis) initiates asymmetric acid efflux. Alternately, graviperception could lead directly to differential proton excretion and thus auxin redistribution would not be required and/or be a secondary response to asymmetric acid efflux.

MATERIALS & METHODS

Seeds of sunflower (Helianthus annuus L. cv. Sungold) were germinated, grown and mounted in Plexiglas holders as previously described (4). In experiments localizing the magnitude of cell extension along the shoot axis, segments were first marked at intervals of approximately 1.5 mm with india ink. After mounting (usually 10 segments/holder), each Plexiglas platform was submerged in a 1-liter beaker containing 1,000 ml of test solution maintained at 32°C.

Prior to and after an appropriate period of straight growth (ie vertically oriented segments) or gravistimulation (horizontally mounted segments), the Plexiglas holders containing the segments were photographed with a 35 mm camera. The negative showing the straight growth or gravitational response was compared with negatives taken at zero time and the growth increment and/or degrees curvature determined. Patterns of H^+ efflux were determined by the method of Mulkey and Evans (2).

In experiments monitoring the lateral transport of isotopically labelled auxin, segments were prepared as described above. They then were allowed to take up either ^{14}C-IAA (50mCi/m mol) or ^{3}H-IAA (29 mCi/μ mol) while vertical in 1.0 mM phosphate buffer at pH 7.0. Uptake time was 60 min. After uptake segments were briefly raised and mounted in a horizontal position. During gravistimulation the segments and holders were placed in a chamber lined with moist paper towels and kept in darkness. At appropriate intervals the segments were removed and either bisected or stripped of their upper and lower epidermal layer. The harvested tissue was then counted for radioactivity using standard scintillation techniques. Data normalized for fresh weight are expressed as the % increase in cpm in lower portion relative to the upper portion.

RESULT & DISCUSSION

As shown in Figure 1 gravistimulation of sunflower hypocotyls results in the retardation of growth on the upper surface and the acceleration of growth on the lower surface relative to a vertically oriented control. When these growth responses are contrasted with the straight growth elicited by auxin (10^{-5}M, indole-3-acetic acid (IAA) at pH 6.0) and acid (5mM phosphate citrate buffer at pH 4.5), it is clear both agents elicit growth over a similarly broad region of the hypocotyl (data not shown). The correspondence between auxin, H^+, and gravisensitive tissues is consistent with the notion that auxin redistribution may initiate asymmetric acid efflux during gravistimulation. Further, these data are clearly inconsistent with the claim of Digby and Fern (1) that the magnitude of gravistimulated cell extension on the lower surface of shoots exceeds the capacity of shoot cells to respond to auxin. The smallest increment of growth produced by auxin over 4 hr was 14.7%. With acid the smallest increment was 16.6%.

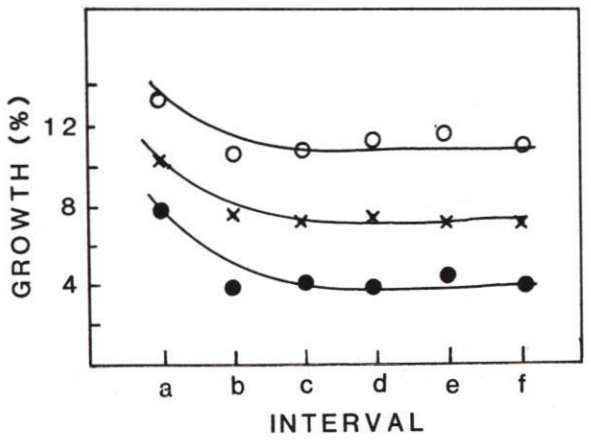

FIG. 1. Growth parameters. Increment a represents the apical-most portion of the segment, f the basal-most region. The % growth indicated represents changes from zero time values to 4 hr values. X=values for a vertical control. =the values for the upper surface of a gravistimulated segment and O=values for the lower surface.

In our hands there is a 25-30 min lag time before sunflower hypocotyls exhibit measurable curvature (data not shown). When segments are preloaded with either ^{14}C-IAA (Figure 2) or ^{3}H-IAA (data not shown) and then gravitropically stimulated there is a similar lag (about 20-30 min) before an isotope-asymmetry develops across the shoot.

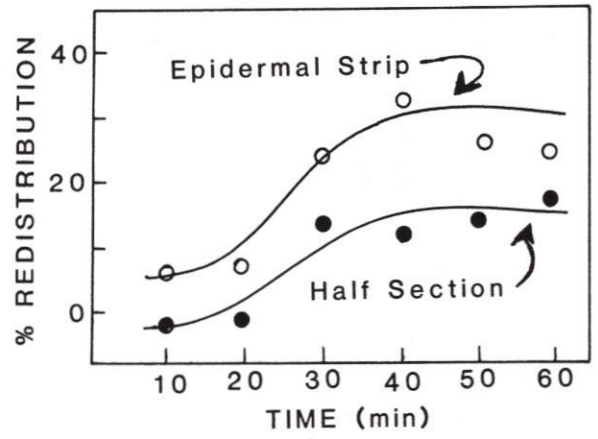

FIG. 2. Kinetics of ^{14}C-IAA Redistribution. Data are expressed as % increase in counts in the lower tissue relative to the upper, and are the average of 5 separate experiments.

The relatively rapid kinetics of isotope redistribution are consistent with the Cholodny-Went Model and the notion that auxin redistribution gives rise to asymmetric acid efflux. However, the magnitude of the isotope asymmetry is puzzling. That is, one might expect a larger asymmetry to develop across gravistimulated shoots. We are unsure how to rationalize this phenomenon but compartmentation and/or the formation of physiologically inactive analogs are possible explanations.

In order to further investigate the relationship between auxin and asymmetric proton efflux, we have examined the effect of antiauxins, auxin transport and/or action inhibitors, and vanadate (an inhibitor of auxin-induced H^+ excretion) on gravicurvature and H^+ efflux. Some of these data are summarized in Table I; a more detailed report has been published elsewhere (5). As shown in Table I all of the tested inhibitors of auxin transport and/or activity inhibited gravicurvature. Asymmetric acid efflux is also inhibited (data not shown).

Table 1. Effects of Selected Inhibitors on Shoot Gravitropism

Treatment	Degrees Curvature*
Control	80
PClB(10^{-4}M)	12
3,4,5-TIBA(10^{-3}M)	7
2,3,5-TIBA(10^{-3}M)	2
Vanadate (10^{-3}M)	30
Cycloheximide(5mg/l)	10

*Curvature determined after 4 hr of gravistimulation; segments were abraided to facilitate inhibitor penetration.

These data taken together with the sensitivity and location of gravisensitive cells and the kinetics of auxin redistribution, suggest that lateral transport of auxin initiates asymmetric acid efflux in gravitropically stimulated shoots.

This study was supported by NASA Grant NAGW-230. The technical assistance of Karen Ring is acknowledged.

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PROTEIN SYNTHESIS IN GEOSTIMULATED ROOT CAPS

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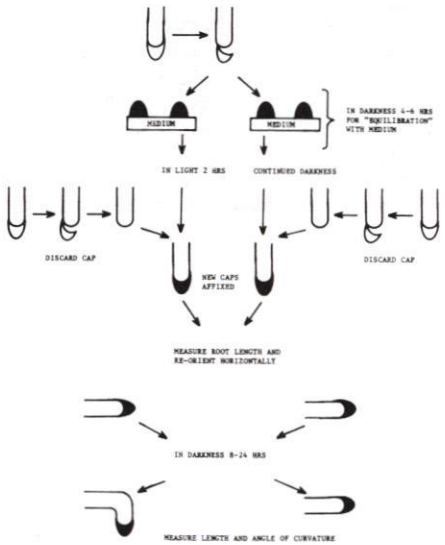
INTRODUCTION

The root cap is the site of perception of gravity (Juniper et al., 1966). As a result of gravitropic stimulation it is hypothesized that an inhibitory substance is produced in the cap (root cap inhibitor = RCI) and from there moves basipetally into the root where bending is initiated (Wilkins, 1978). While most roots will show a positive geotropic response when kept in total darkness, roots of several varieties of corn bend in response to gravity only after exposure of the root (specifically the root cap) to white light (Scott and Wilkins, 1969). Using light as a trigger it is possible to initiate production of the RCI. Recently it has been shown that the excised cap when placed in culture, illuminated, and subsequently explanted back to roots can induce downward bending in roots (Feldman, 1981). This downward bending presumably occurs because of the production of an inhibitor in the cultured, illuminated caps. The procedure used to assay for the RCI in cultured caps is shown in Fig. 1. With this technique we are able to include a variety of compounds in the culture medium and ask what their effect is on RCI production. This compound, auxin, or indole-3-acetic acid, must be included in the culture medium if RCI production is to occur in illuminated caps. Exactly how light and auxin interact to bring about downward (positive geotropic) root growth is not known. The objective of this paper is to identify processes occurring in the cap which are requisite for RCI formation and which can be triggered or modulated by both light and gravity.

Figure 1.



METHODS

Root caps are obtained from seedlings of Zea mays var. Merit. With dim green light (515-545 nm) as the only illumination and with the aid of a dissecting microscope root caps were excised and placed aseptically in culture on media designated S2M (Torrey and Fosket, 1970). For certain experiments the basic culture medium was supplemented either with cycloheximide (10^{-6} M), or 20 μCi ml^{-1} L-[4,5-^{3}H]leucine (Amersham, sp. act. 182 Ci $mmol^{-1}$), or indole-3-acetic acid (10^{-9} M), or with combinations of the above.

Caps were allowed to equilibrate in the dark in the various media, illuminated with white light for 10 min (1.3×10^{-2} E $cm^{-2}s^{-1}$) and then returned to the dark for varying intervals of time. To assess the incorporation of ^{3}H-leucine (that is, protein synthesis) caps (usually 30-50/treatment) were homogenized on ice in 0.1 M Tris buffer pH 7.4, spun at 10,000 g for 10 min and to the resulting supernatant an equal volume of 10% TCA added. Precipitation of proteins was allowed to proceed 1 hr on ice and the resulting precipitate collected on a millipore filter, washed and counted in a scintillation counter.

For other experiments cultured caps (either illuminated or maintained in darkness) were used as noted in Fig. 1 and the curvature of roots assessed from shadowgraphs made of the roots.

Protein levels were measured by the method of Lowry (1951).

RESULTS

Auxin must be included in the culture medium for bending to occur (Table 1) whereas inclusion of an inhibitor of protein synthesis (cycloheximide) retards bending (Table 2).

TABLE 1. Average Curvature in Degrees of Roots to Which Have Been Affixed Caps Cultured in the Presence or Absence of Auxin

	Amount of Auxin	
	0	10^{-9} M
Plus light	5	21
Continuous darkness	6	7

TABLE 2. Average Curvature in Degrees of Roots to Which Have Been Affixed Caps Cultured in the Presence of Cycloheximide

	Amount of Cycloheximide		
	0	10^{-7} M	10^{-6} M
Plus light	30	17	11
Continuous darkness	5	6	6

Light stimulate protein synthesis in caps compared to dark controls, with a peak in protein synthesis (μg/cap) occurring approximately 30 min after illumination. With increasing periods of time in darkness (subsequent to the 10 min illumination) protein levels drop, returning to levels observed in dark controls (Table 3).

TABLE 3. Protein as Measured by the Method of Lowry from Caps Illuminated 10 min and Then Returned to the Dark for Varying Intervals

Time in Dark After 10 min Illumination	µg Protein/Cap
0 (dark control)	1.30
30 min	1.73
60 min	1.50
120 min	1.35

The results of the Lowry are paralleled from experiments monitoring the incorporation of ^{3}H-leucine into protein (Table 4).

TABLE 4. Radioactivity in Protein from Root Caps Illuminated for 10 min and Then Returned to the Dark for Varying Intervals of Time

Time in Dark After 10 min Illumination	DPM (from 50 caps)
0 (dark control)	35,615
30 min	57,638
60 min	47,653
120 min	34,802

The highest incorporation of ^{3}H-leucine is observed 30 min after illumination with the amount of label decreasing with increased intervals of time in the dark.

Addition of cycloheximide (10^{-6} M) to media supplemented with ^{3}H-leucine shows incorporation of the label to be reduced by 90% (Table 5).

TABLE 5. Radioactivity in Protein from Root Caps Cultured in the Presence of Cycloheximide (10^{-6} M) in the Light or Dark

Treatment	DPM (from 50 Caps)
Light + cycloheximide	3,691
Dark + cycloheximide	4,160
Light, no cycloheximide	55,090
Dark, no cycloheximide	42,079

Pulse chase experiments show that auxin must be included in the culture medium if any light-induced protein synthesis is to occur (Table 6). Moreover, the rate of turnover of the label during the 4 hr interval following the light treatment is greater in caps cultured in auxin-supplemented media, compared to caps maintained in media lacking auxin.

TABLE 6. Radioactivity in Protein from Root Caps Labelled in Dark for 3 hr, Then Exposed to Light for 10 min, Chased Immediately After Exposure and Then Returned to Darkness for Varying Intervals of Time Prior to Assaying for Protein

Time in hrs After the Light Treatment	DPM x 10^{-3}	
	+ Auxin	No Auxin
0 (dark control)	39	33
1	51	29
2	41	26
4	31	23
8	16	17
20	11	12
25	10	8

DISCUSSION

The preliminary results of this work point to the importance of protein synthesis for light-induced gravitropic bending in roots. If protein synthesis is prevented, as when caps are cultured in media supplemented with cycloheximide, caps so treated are unable to induce downward bending when manipulated as shown in Fig. 1. These results suggest that, in part, light acts by stimulating proteins which are necessary for the translation of the gravitropic stimulus into a growth response (downward bending). Thus, since it appears that protein synthesis is necessary for RCI production, we examined the turnover of protein with time and in particular addressed the question of whether light acts by stimulating the synthesis of unique proteins required for downward growth. The pulse chase experiments (shown in Table 6) indicate that about 4 hrs after illumination that the amounts of ^{3}H-leucine remaining in the caps is approximately the same, irrespective of whether the caps were cultured in media supplemented with or without auxin. However, recall (Table 1) auxin is necessary for RCI production. We therefore interpret these results to mean that auxin in combination with light allows for the translation of the gravitropic stimulus into a growth response at least in part through the modification of protein synthesis. At this point, based on the pattern of protein turnover, we would infer that unique proteins are stimulated by light and are involved in promoting the downward growth in roots which are responding to gravity. (Supported by NASA grant NAGW-234).

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QUANTITATION OF CHLORPROMAZINE-BOUND CALMODULIN DURING CHLORPROMAZINE INHIBITION OF GRAVITROPISM

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ABSTRACT

The regulatory protein, calmodulin (CaM), controls the activity of a plasma membrane localized ATPase in plants which serves to pump calcium out of cells. Recent data are consistent with the hypothesis that activation of this pump is one of the early steps necessary for gravitropism. Chlorpromazine (CPZ), a CaM antagonist, reversibly inhibits gravitropism in oat coleoptiles at concentrations which permit normal growth rates. We used ^{14}C-labeled CPZ to photo-affinity label endogenous CaM *in vivo* to learn whether the drug is actually binding to some portion of endogenous CaM when it inhibits gravitropism. Under conditions in which CPZ inhibits gravitropism for over an hour, at least 11% of the CaM in gravitropically stimulated coleoptiles is bound to CPZ. In a given CPZ experiment the degree of inhibition of gravitropism correlates well with the amount of CaM bound to CPZ.

INTRODUCTION

An early response to gravitropic stimulation in oat coleoptiles, which precedes visible bending, is the biased accumulation of calcium on the prospective slower-growing side (1). Sub-cellular calcium localization studies indicate that the gravity stimulated redistribution of calcium results in an increased concentration of calcium in the walls of cells on the upper side of a horizontally positioned coleoptile (2). Because calcium can inhibit the extension growth of plant cell walls, the accumulation of calcium in walls may play a role in inducing the asymmetry of growth which characterizes gravitropism.

The transport of calcium from cells into walls is most likely carried out by a plasma-membrane localized calcium-dependent ATPase. The regulatory protein, calmodulin (CaM), controls the activity of this ATPase (3). Recent data from our laboratory indicate that chlorpromazine (CPZ), a CaM antagonist, can inhibit the gravitropism of oat coleoptiles at concentrations low enough to permit their normal growth rates (10^{-5}-10^{-7}M) (4). The same concentrations of CPZ inhibit the gravity-stimulated asymmetric accumulation of calcium on the prospective slower-growing side of coleoptiles.

Although these results were consistent with the idea that some CaM-regulated function (such as the CaATPase) is important for gravitropism, there were no data to show whether any of the endogenous CaM in the oat coleoptiles was associated with CPZ under conditions in which CPZ was inhibiting gravitropism. The experiments described below were designed to obtain information on this question.

MATERIALS AND METHODS

Four-day old etiolated oat seedlings were used for these experiments. Coleoptile sections (apical 27 mm) from these seedlings were perfused with 10^{-7}M CPZ at 23°C for 7 hrs., conditions in which about 50% of the coleoptiles show no gravitropic response after 3 hrs. of stimulus (Group A), and 50% show some response, ranging from about 10° to about 45° upward bending from the horizontal (Group B). Under these same conditions, more than 90% of the control (-CPZ) coleoptiles show ≥70° upward bending. Group A coleoptiles were compared with Group B coleoptiles for content of ^{14}C-labeled CPZ which had bound to CaM *in vivo*.

Group A and Group B coleoptiles were simultaneously irradiated with UV light following the method of Prozialeck *et al.* (5) to covalently link CPZ to endogenous CaM. Each group of coleoptiles was then processed separately by the following protocol. The coleoptiles were quick frozen in liquid N_2, then homogenized with a mortar and pestle in acetone at -15°C. The extract was centrifuged to separate precipitated material (including CaM) from the supernatant, which included free CPZ. The pellet was freeze-dried under vacuum, then solubilized in a borate buffer at pH 8.4. This solution was clarified by centrifugation, which pelleted less than 15% of the radioactivity. A 100 ul aliquot of the clarified solution was then reacted with enough antibody against CaM to precipitate 120 ng of CaM under the conditions used, as determined by previous radioimmunoassays. The radioactivity of the precipitate was measured to determine the quantity of ^{14}C-labeled CPZ present in it.

RESULTS AND DISCUSSION

Radioimmunoassay of CaM (kit from CAABCO, Inc.) in the acetone-extracted protein from etiolated oat coleoptiles indicates that about 1% of this protein can be precipitated by anti-CaM antisera. We will refer to this precipitated protein as CaM, even though in principle other CaM-like proteins which share antigenic sites in common with CaM could be precipitated by the anti-CaM antisera.

Prozialeck *et al.* (5) found that the binding stoichiometry of ^{14}C-labeled CPZ with CaM after UV irradiation was 1:1. We assumed this stoichiometry was correct when we estimated the amount of CaM

bound to ^{14}C-labeled CPZ in the results reported here.

When the acetone precipitate from Group A coleoptiles was assayed, 11% of the CaM in this precipitate was found to be associated with ^{14}C-labeled CPZ. In contrast, less than 4% of the CaM in the acetone precipitate from Group B coleoptiles was found to be associated with labeled drug. These data indicate that there is a correlation between the degree of inhibition imposed by CPZ on the gravitropism of treated plants and the level of CaM bound to CPZ in these plants. The results are consistent with the idea that CaM plays a role in the transduction of a gravity stimulus into gravitropism in plants, and they warrant the use of more specific inhibitors of CaM to test this hypothesis more rigorously.

ACKNOWLEDGEMENTS

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A REEVALUATION OF THE ROLE OF ABSCISIC ACID IN ROOT GRAVITROPISM

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Abstract. According to the root cap inhibitor theory of gravitropism, the downward curvature of roots in response to gravity is due to accumulation of a growth inhibitor on the lower side of horizontally placed roots. The inhibitor is thought to be produced in the root cap which is also the site of gravity detection. Since abscisic acid (AbA) has been suggested to be the root cap inhibitor, or at least a major component of the inhibitor, we examined the effects of AbA on root growth. Although we found that high concentrations of AbA do inhibit root growth in long term experiments, we noted that the initial effect of AbA over a wide concentration range is to promote root growth. This transient promotion lasts up to ten hours which is well beyond the time required for roots to complete gravitropism. In experiments involving asymmetric application of AbA to horizontally placed roots, we found that AbA applied to the top of the root accelerates gravitropism while AbA applied to the bottom retards gravitropism. Our results are inconsistent with the theory that AbA acts as a growth inhibitor in establishing the asymmetric growth causing root gravitropism.

INTRODUCTION

According to the root cap inhibitor model, positive gravitropism in roots results from gravity-induced displacement of a cap-produced growth inhibitor toward the lower side of gravistimulated roots. Since abscisic acid (AbA) has been suggested to be an important component of the root cap inhibitor (1), we have examined its potential involvement in root gravitropism by studying the kinetics of AbA effects on root growth and gravitropism.

METHODS

The experiments were performed using seedlings of three cultivars of maize (Zea mays L.): Bear hybrid WF 9 x 38 MS, LG 11 and Orla 231. The methods for raising the seedlings and performing the continuous high-resolution measurements of AbA effects on elongation of seedling roots were as previously described (2). Experiments involving unilateral application of AbA to the elongation zone of horizontally placed roots were done using intact seedlings mounted in a humidified chamber. The humidity was maintained at 100% using a distilled water mist aspirator with a chamber temperature of 22 ± 2 C. Abscisic acid (0.1 mM) was provided to one side of the elongation zone by applying an agar block containing the hormone. The time course of gravitropism was recorded by color cinematography. In addition to experiments on the action of AbA on growth and gravitropism, the effect of AbA on hydrogen ion efflux from apical segments of maize roots was measured. Sixty 1-cm segments were placed in 3.5 ml of 1 mM potassium phosphate buffer (initial pH 6.3). A semi-micro combination pH electrode was inserted into the medium which was constantly stirred and oxygenated. Output from the electrode was recorded on a recorder adjusted to give full scale displacement for 1 pH unit. Other conditions of the hydrogen ion secretion experiments were as described in reference 2.

RESULTS

The time course of AbA action on straight growth of primary roots of the Bear hybrid cultivar of maize is shown in Figure 1. With either high (0.1 mM) or low (0.1 μM) concentrations of AbA, the initial effect is to promote elongation. The promotive phase continues about 10 h. With low concentrations of AbA the elongation rate gradually returns to the initial rate, while with high concentrations, the rate declines more steeply reaching a value about 50% less than the initial rate by 20 h. After about 24 h the elongation rate begins to recover and eventually returns to within 50% of the control rate. Similar results were obtained with the other two cultivars of maize tested, though the timing varied somewhat.

The finding that AbA initially promotes root elongation is inconsistent with the proposed role of AbA as a growth inhibitor mediating gravitropism in roots. This is especially true in view of the fact that the stimulation of root elongation by AbA continues up to 10 h while gravitropism in these roots is complete within 2 h after placing them in a horizontal position.

Since AbA is commonly viewed as an inhibitor of root growth rather than a promotor (1), we have tested the ability of AbA to promote hydrogen ion efflux from roots. Enhanced hydrogen ion efflux has been shown to be associated with enhanced growth in roots. The rapidly growing cells of the elongation zone of roots vigorously secrete hydrogen ions and promotion of root growth by low concentrations of auxin is accompanied by enhanced hydrogen ion efflux from the cells of the elongation zone (2). Figure 2 shows that both low and high concentrations of AbA stimulate hydrogen ion efflux from maize roots with a latent period of 5 to 10 min. These results are consistent with

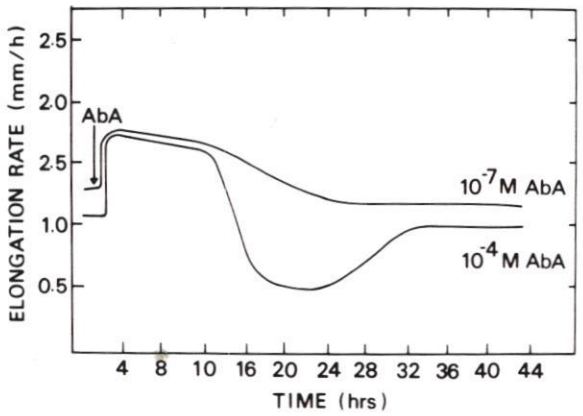

Fig. 1. Time course of AbA effects on root elongation in intact seedlings of the Bear hybrid cultivar of maize. The indicated concentrations of AbA were added at the arrow to the solution surrounding the primary root. Each curve is a representative curve for AbA treatment of a single intact root.

the finding that AbA initially promotes root growth since enhanced hydrogen ion efflux and enhanced growth appear to be coupled, perhaps in a cause-effect relationship (2).

In order to test the effect of AbA gradients on gravitropism in maize roots, we placed seedlings in humidified chambers in a horizontal position and applied agar blocks containing AbA to either the upper or lower side of the elongation zone. Figure 3 shows effects of such asymmetric AbA applications on gravitropism, with the photographs taken 1 h after placing the roots horizontally. In this figure, rows A, B and C represent maize cultivars Bear hybrid, Orla 231 and LG 11 respectively. Column 1 shows AbA applied to the top of the root (plain agar applied to bottom) column 2 shows AbA applied to the bottom of the root (plain agar applied to top) and column 3 represents control roots with plain agar blocks applied to both sides. With AbA applied to the top of the

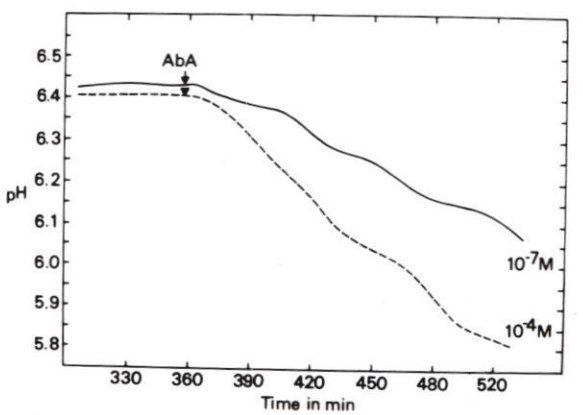

Fig. 2. Promotion of H^+ efflux from apical sections of the Bear hybrid cultivar of maize treated with AbA. Sixty 1-cm apical root sections per experiment.

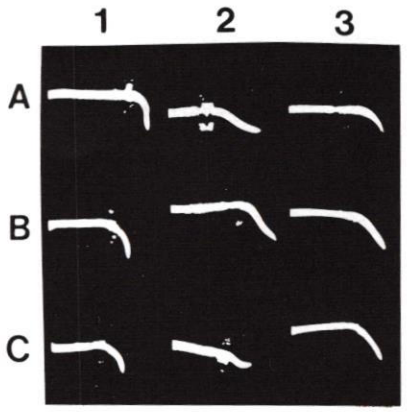

Fig. 3. Effect of unilateral application of AbA on gravitropism in roots. Rows A, B and C = maize cultivars WF 9 x 38, Orla and LG 11, respectively, column 1: AbA (0.1 mM) applied to the top (bottom block = agar only); column 2: AbA applied to the bottom (top block = agar only); column 3: control with agar blocks only. Photos taken 1 h after gravistimulation.

roots (column 1), gravitropic curvature is accelerated compared with the control (column 3). With AbA applied to the bottom of the roots (column 2), gravitropic curvature is retarded compared with the control. These findings are consistent with our results showing an initial stimulation of root growth by applied AbA. They are inconsistent with the hypothesis that AbA plays a role as an inhibitor mediating root gravitropism.

Since the early effect of AbA on root elongation is promotive, we may conclude that, if AbA plays a role in root gravitropism at all, it is more likely to act as a promotor of growth on the top side of the root than an inhibitor of growth on the bottom side. The evidence against AbA acting as a growth inhibitor mediating root gravitropism does not in itself weaken the root cap inhibitor model of gravitropism. There is at least one other inhibitor in the root cap (3) and this, or some other unidentified inhibitor may play a role in establishing the asymmetric growth which causes positive gravitropism in roots.

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THE MECHANICS OF GRAVITROPIC BENDING IN LEAFY DICOT STEMS

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INTRODUCTION

To understand the plant gravity receptors and biochemical transducers that account for gravitropic bending in plant organs, it is first essential to understand what is happening during bending: which cells are growing or not growing, in what ways, and exactly when. It would seem that such questions would have been answered many decades ago, and indeed, to an extent they were answered. Nevertheless, attempts to discover gravity receptors and transducers have often been based upon unfounded assumptions about the mechanics of bending. The durable Cholodny-Went theory, for example, proposes an auxin-induced promotion of growth on the bottom of a horizontal stem, a mechanism that is increasingly difficult to reconcile with observations.

DIMENSIONAL CHANGES DURING GRAVITROPIC BENDING

We confirm by photographic measurement the observations of Firn and Digby (1) that when a dicot stem is laid on its side, growth of the top surface ceases almost immediately. Growth on the bottom continues or may be slightly reduced or accelerated compared to growth of vertical stems.

That the cessation of growth on top is a fundamental feature of gravitropic bending is well illustrated by a procedure in which a horizontal stem between wire supports is wrapped with threads so gravitational bending is prevented. Upon release by cutting the threads some 48 hr later, bending occurs within seconds (3, 4). Bending is more extensive for plants held in the dark than for plants in the light and frequently overshoots the 90° bending of unrestrained controls, sometimes reaching 180° or more. Measurement by stereophotogrammetry shows that the bottom surface continues to elongate during restraint. The top surface does also but is apparently being stretched by growth of bottom tissues, since the top often shrinks upon release from restraint, sometimes almost to its initial dimensions when the stem was placed horizontally.

At the cellular level, cells near the top surface become shorter and thicker upon release from restraint, while bottom cells become longer and narrower. Within the range of experimental error caused by the difficulties of measuring comparable stem regions of different plants, it appears that cell volume is conserved during the rapid bending upon release (3).

THE FORCES OF GRAVITROPIC BENDING

A horizontal stem of *Xanthium strumarium* (cocklebur) or *Ricinus communis* (castor bean) was restrained by tying with a thread at the tip, the thread being attached to a horiziontal metal strip with strain gauges on top and bottom. Applying engineering techniques, it was possible to calculate the forces developed by the restrained stem and the stresses within the stem (3). As the stem attempts to bend upward against the restraint, compression develops on the bottom and tension on top. This is how bending energy is stored. Figure 1 shows degrees of bending as a function of time after release for several plants (each restrained for a period of time and then released) and for a single plant restrained, released, forced back to the horizontal position and restrained, this being repeated many times. Note that degrees of bending after release and stem pressure both stop increasing at the same time, and that the single stem that was repeatedly released and restrained gives essentially the same bending response as the various plants restrained for different times, each being released only once. Thus, the bending phenomenon (based on the above changes in cellular dimensions) is reversible, which strongly supports the concept that cell volumes do not change during the rapid bending after release. Note also that maximum calculated pressures on the bottom of a restrained stem are of the order of magnitude of cell turgor pressures that develop during growth--pressures that actually cause plant growth. We might expect that when turgor pressures (developed osmotically) equal external pressures caused by restraint of a bending stem, bending force can no longer continue to increase.

We have measured pressures directly with pressure-sensitive discs (ca. 3.5 mm diameter) inserted into stems. During restraint, pressures increased markedly on the bottom as expected, and pressures on top dropped compared to the reading when the transducer was inserted. Upon release, pressures on top suddenly increased; bottom pres-

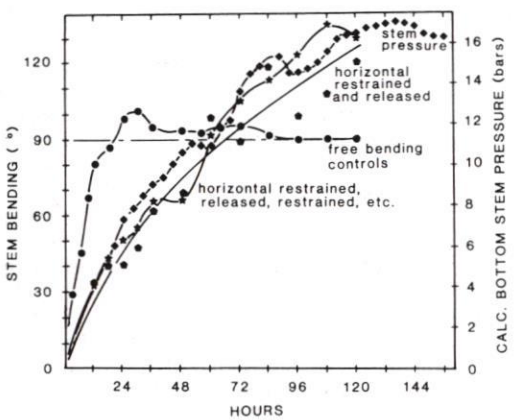

Figure 1. Stem bending (degrees) and calculated stem pressure (bars) with various treatments over time. Data are from reference (3).

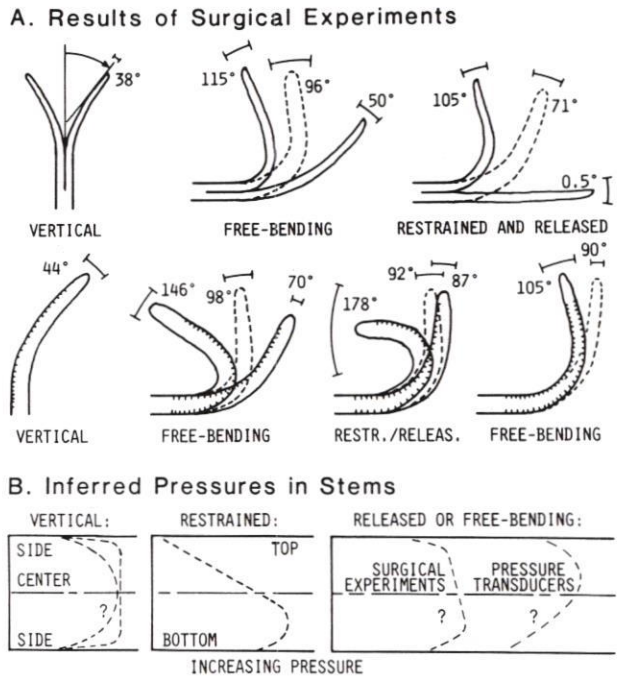

Figure 2. A. Results of cutting vertical or gravitroping stems as shown. Angles of bending are to scale, lengths are consistent, but thicknesses are arbitrary, and many stems zig-zag. Arcs above tips are ± standard errors. Dashed lines are before cutting; solid lines after. B. Hypothetical distribution of pressures in stems.

sures dropped sharply. Pressures on the bottom of free-bending stems increased at first (in response to stem weight), then decreased to the level of vertical control plants; pressures on top gradually increased during bending, indicating compression.

Since the top of a stem placed horizontally immediately stops growing, emphasis is shifted from a promotion of bottom growth as in most theories to an inhibition of top growth. Inhibition could be caused by increasing inhibitor(s) or a rapid removal of growth promoter(s). Of course, the real mystery has always been how cells on top "know" they are on top. All stem cells are initially equally horizontal, but response seems to be centered in top cells. Yet, the stretching of top cells in restrained horizontal stems clearly demonstrates the power of bottom cell growth.

PRELIMINARY SURGICAL EXPERIMENTS

To test our developing model, we have made longitudinal and shallow surface cuts in vertical stems and in gravitropically bending stems (Fig. 2). When vertical stems are bisected longitudinally, there is a marked outward bending as shown, clearly indicating that pressure is greater inside the stem than near the surface. This is true for several species we have tried, including the hollow-stemmed castor bean. Bending stems exhibit the same phenomenon. For free-bending stems the internal pressure gradients must be about the same as for vertical stems, with just enough asymmetry to account for bending. Higher pressures on the bottom probably develop as top tissues stop their growth. (Pressure lines in Fig. 2 are purely hypothetical.) The data of Figure 2 suggest that tensions on the top of gravitroping stems might exceed tensions on the bottom. When shallow cuts (about 1 mm deep) were made at intervals along the bottom of a horizontal restrained stem (cuts perpendicular to the longitudinal axis), stems sometimes bent over 180°. That there were pressure gradients beneath the surface layers was evident when tensions near the surface were relieved by cutting on both sides; such stems bent slightly more (15°) than before cutting. On the whole, our results strongly emphasize the resistance to bending of the bottom surface layers, a resistance apparently located in collenchyma and vascular tissues. These bottom outer layers, far from accounting for bending by their rapid growth (suggested by traditional models), actually *resist* bending.

As noted, our results emphasize the quick stopping of growth and the increased tensions on the upper layer of a horizontal stem. This suggests a model for gravitropic bending of stems. *Bending apparently occurs as the resistance of the upper surface layers is extended to the inner cells below.* This could be the key to the mystery posed above: the asymmetry of stem-cell response.

To summarize our developing model of gravitropic bending: Assume that the outer cell layers of stems always contain an inhibiting growth substance, so they always resist the elongation of the inner tissues. Assume further that movement of the inhibitor is strongly gravitropic, so whenever a stem is tipped from the vertical, the inhibitor moves from the outer layer downward and hence inward, increasing the amount of non-growing tissue on the top of the stem. Since bottom layers continue to grow, this accounts for upward bending. The inhibitor could be something as simple as a base that would neutralize the protons in the cell walls and thus limit plastic stretching (2). But how is its direction of movement controlled by gravity? What is the role of the amyloplasts in the starch sheath of cells just outside the vascular tissue? Is the actively growing tissue inside and around the vascular bundles? is the postulated inhibitor produced only close to the oxygen-rich surface? Such questions intrigue us.

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STRIATED ORGANELLES in HAIR CELLS of RAT INNER EAR MACULAS: DESCRIPTION and IMPLICATION for TRANSDUCTION

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Abstract: Use of unusual fixation procedures resulted in display of the several striated organelles that are present in hair cells of the inner ear. In the vestibular system these include the striated rootlets of the kinocilia, the striated cuticular plate and its attachments to the cell membrane at the zonula adherens, and a striated neck organelle (SNAP) that is present only in Type I hair cells. The possible roles of these organelles in vestibular hair cell transduction are considered here. It is suggested that the kinociliary apparatus serves as a pacemaker for hair cell activity, and that the cuticular plate functions in part to coordinate kinociliary and stereociliary interactions. The plate also could transmit signals from the apical to the lateral cell membrane and, if contractile, could amplify small signals and produce graded hair cell responses. SNAP is situated at the plasma membrane under the upper end of the calyx nerve ending where it could modulate hair cell electric conductance.

Neither the site of transduction of mechanical into electrochemical energy by hair cells nor the mechanisms involved are known with certainty for any inner ear receptor area. Hudspeth (1982) has suggested that initial transduction occurs at the tips of the stereocilia, and that current enters the cell's apex and exits through its basolateral surface. This concept treats the stereocilia as electrically conducting cables and the hair cells largely as dipoles. Earlier suggestions were that the transductive process involves some element at the base of the stereocilia or kinocilia (Engstrom et al., 1962) or occurs along the shafts or at the bases of the stereocilia (Flock et al., 1977). Recently, I modeled the mammalian gravity receptors on bioenergetic principles in which mechanical, chemical and electrical energies are interchangeable. This concept grew out of the observation that otoconia consist of highly ordered domains and might be piezoelectric; and out of results indicating the presence of striated organelles at or near sites in hair cells considered to be of importance in transduction (Ross, 1982). It was proposed that initial conversion of mechanical into electrical energy might occur at the level of the otoconial complexes, one step prior to the hair cells, to modulate the standing electric potential. Such modulation would then influence kinociliary activity and result in kinociliary-stereociliary interactions, with events coupled in part (at least) through the cuticular plate. The entire sequence of transductory events was not considered to be identical for the two kinds of hair cells below the level of the cuticular plate. A striated neck organelle (SNAP) and a microtubular array present in Type I hair cells was lacking in Type II vestibular hair cells, which showed instead much smooth endoplasmic reticulum apically. This paper deals further with the possible roles played by the striated organelles in vestibular hair cell transduction.

The striated organelles of vestibular hair cells include the rootlets of the kinociliary apparatus, the cuticular plate, and the attachments of the cuticular plate to electron-dense material lying at the zonula adherens, which are present in both kinds of hair cells; and SNAP, which is unique to Type I vestibular hair cells. These organelles are demonstrated optimally only under specific conditions that include glutaraldehyde as the primary fixing agent and a phosphate buffering medium (Ross and Bourne, 1982).

All of the striated organelles we have described are patterned upon the alternating electron-dense and more lucent bands present in the striated rootlets of the kinociliary apparatus. The more lucent bands are two or three times the width of the electron-dense stripes and are intersected by a fine, electron-dense line. The widths of the bands and the inter-stripe distances vary from one organelle to another. The electron-dense stripes are 13-28 nm wide in the striated rootlets and 45-70 nm wide in SNAP. The inter-stripe distance is 50-60 nm in the rootlets but 140-180 nm in SNAP. Differences in band width and periodicities occur within the same organelle, suggesting that the elements are contractile.

The role of striated rootlets is apparently unknown for any ciliated cell. The rootlets have been treated commonly as anchoring devices for cilia (and flagella), but evidence is accumulating that they might be contractile (see Sleigh, 1979). Human retinal rod ciliary rootlets contain ATP (Matsuka, 1967). Moreover, rhizoplasts (fibrous roots in algae) have a similar organization and have been shown to be contractile and under Ca^{++} regulation (Salisbury and Floyd, 1978). The hair cell kinociliary apparatus of the rat vestibular hair cells appears to have paired rootlets that are situated in the plane of hair cell polarization, i.e., in the direction of stereociliary motion resulting in hair cell activation or inhibition (Flock et al., 1977). This might be purely coincidental. Alternatively, the orientation of the rootlets and their possible contractility might indicate that they have a role in modulating

kinociliary activity, with the whole kinociliary apparatus serving as a pacemaker for hair cell responses (Ross, 1982). We have found the basal apparatus of the kinocilium to have robust attachments to the plasma membrane bordering the cuticular plate, and to electron-dense material at the edge of the plate below the surface membrane. Thus, an anatomical basis for kinociliary-cuticular plate interaction does exist, at least in the rat. It should be noted that some experimental results obtained under artificial in vitro conditions have been interpreted to indicate that the kinociliary complex is unimportant in hair cell transduction (Hudspeth, 1982). Further research is necessary to resolve this issue unambiguously. The hair cell kinocilium has the ultrastructure of a motile cilium. Moreover, the kinocilium has been observed to beat spontaneously and continuously, with the stereocilia following the kinocilium in integrated fashion (Flock et al, 1977; and others).

The kinocilium is constructed of a 9+2 system of microtubules and, therefore, has tubulin and dynein as its major protein constituents. The stereocilia and their rootlets, in contrast, have been shown to consist largely of actin in both vestibular and cochlear hair cells (Flock et al., 1981; Tilney et al., 1980; Slepecky and Chamberlain, 1982). The actin of the stereocilia has a paracrystalline order axially but liquid order transversely (DeRosier et al., 1980). This finding and other experimental evidence has led to the common assumption that stereociliary actin is stabalized in a rigid configuration. We do not yet know the nature of all the associated proteins in the stereocilia and their rootlets; thus assuming a rigid configuration to be the functional state of the actin is premature.

The stereociliary rootlets are not uniform in appearance along their extent in rat vestibular hair cells but show expanded and more narrow regions, granules, and even some cross-bands (Ross and Bourne, unpublished observations). Thus, proteins other than actin will likely prove to be present although localizing them to specific sites may be problematical. It is possible that proteins for dissassembly will be found, or perhaps myosin, which has already been localized to the upper part of the cuticular plate (and stereocilia) in mammalian cochlear hair cells (Macartney et al., 1980). Rat vestibular stereociliary rootlets are cross-linked to the striations in the cuticular plate. Whether contraction of the plate induced by kinociliary activity causes stereociliary "bending" through actin dissassembly or through actin-myosin interactions for contraction; or whether electric current invades the plate from the stereocilia as Hudspeth (1982) has proposed the net result would be the same. Signals would be transmitted from the apical cell membrane to the membrane below the occluding junction, through the striated cuticular plate. It is self-evident that, if the membrane at the zonula adherens is electrically excitable, hair cell electrical activity would be modulated. Intervention of a contractile (or tension-producing) cuticular plate would mean that small signals could be amplified and that graded hair cell responses could be initiated. In the Type I hair cells, both SNAP and the microtubular arrays would also be activated. SNAP is suspended from the same electron-dense material into which cuticular plate attachments insert, and the microtubules run in parallel from the plate into the cell interior.

The precise function of SNAP is unclear. It is remote from the sites of the ribbon synapses. It spirals along the hair cell plasma membrane under the upper end of the calyx nerve ending where it could modulate hair cell electric conductance. A major question is whether SNAP acts mechanically to "wring" the necks of the hair cells! A further question is whether the more phasic behavior of Type I receptors, and their greater sensitivity to natural stimuli (see Goldberg, 1981) might not have some basis in the presence of SNAP.

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TIMING OF NEURON DEVELOPMENT IN THE RODENT VESTIBULAR SYSTEM

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INTRODUCTION

The development of the mammalian central nervous system involves a sequence of precisely timed cell generations from the central neuraxis followed by the migration of the postmitotic neuron to a defined region/structure for terminal differentiation. The timing of the migratory and differentiation processes is highly regulated and failure to generate a sufficient cohort of precursor cells or for these cells to reach their proper terminal structure within the narrow time-span precludes the establishment of a functionally competent neuronal subsystem. During the process of subsystem differentiation, appreciable numbers (10-30%) of neurons become pyknotic and undergo degeneration (Glucksmann,1951;Prestige,1970; Rubel etal,1976). The potential for an adverse impact on elements of the mammalian CNS involved in vestibular and proprioceptive integration by spaceflight factors, either acting directly upon the developing embryo/fetus or indirectly thru the alterations in maternal physiology being expressed across the placenta, would vary with respect to the status of the timing sequence of neuronal development during the time of the insult to the system (Rodier,1976).

The only previous work in this area is a series of detailed studies in the rat brain stem by Altman and Bayer (1980a,b). The present report presents preliminary data concerning the timing of cell generation (onset and duration) in the developing rat vestibular and proprioceptive systems.

MATERIALS and METHODS

The material described in this report is derived from a larger series of pregnant Wistar=SPF rats that were treated with injections of tritiated thymidine (I.P., 0.5 mC, SA=20 c/mM) on embryonic days eight through birth. Each dam was given two injections with a three-hour separation. In our hands, such sequential injections label >95% of all neuroblasts during the eight to thirteen day embryo period and >90% of all neuroblasts in the remaining fetal periods. All dams were delivered and the litters reduced to six pups which were sacrificed on postnatal day 28. A second series of postnatal animals received similar dual injections on postnatal days 1, 7, 14 and 21 with sacrifice at 28 days. Fixation was in 10% neutral buffered formalin. Serial paraffin sections were cut at eight microns in either coronal or sagittal planes, mounted on slides and were dipped in dilute Kodak NTB2. The autoradiographic slides were stored with Drierite at 4°C for 60 days, developed in D19 and stained with H+E. The nuclear and sensory structures to be analyzed (see Table) were localized and every 10th section through the structures was surveyed with an ocular grid at 600x magnification. Heavily-labelled neurons were counted and their rostral-caudal/medial-lateral positions established. A minimum of 100 cells was counted in each of the structures.

RESULTS and DISCUSSION

Pooled data obtained from bilateral measurements on all structures are presented in Table 1. Each data-time point represents an averaging of the data from three non-littermates approximated to the nearest five per cent. Variation between individuals is appreciable although analysis of left-vs-right components within an individual reveals a close correlation.

The earliest staged embryo injections to result in heavily labelled neurons as components of any of the structures studied was on E10 with scattered large neurons of the LVN and smaller medial components of the trapezoid nucleus. With respect to the early generation of the LVN, it is significant that elements of the maculae of the utricles are also formed on E10. The generation of both maculae continues at a low level for at least one week postnatally.

The data clearly indicates a defined time-span for generation of all neurons in the CNS nuclei studied. This cytogenetic period in both vestibular and proprioceptive sensory nuclei occurs during and immediately after placentation, a potentially critical period for spaceflight exposure due to alterations in maternal physiology.

PER CENT NEURONAL POPULATION FORMED IN EACH STRUCTURE ON SELECTED EMBRYONIC DAYS
(Pooled bilateral data from three non-littermates/time point)

DAY	SVN	LVN	MVN	IVN	NMT	NGR	NCU	NCE	SSC	HSC	PSC	MUT	MSA	VGA
10		*			*							10	*	
11	5	10	*	*	15	*	*		*		*	30	15	*
12	20	45	10	20	20	10	10		15	10	10	25	40	10
13	50	30	20	45	10	60	40	5	35	25	10	15	25	35
14	15	10	40	25	*	10	25	15	20	30	40	10	15	25
15	10	*	30	10	45	15	15	30	15	20	25	5	5	10
16	*	*	*	*	10	5	10	40	10	10	10	5	*	10
17					*	*	*	10	5	*	*	*	5	5
18							*	*	*	*		*	*	*
19									*			*	*	
20									*			*	*	
P-1									*			*	*	
P-7												*	*	

(* - labelled cells comprise less then 3% of population)

HSC - horizontal canal crista
IVN - inferior vestibular nucleus
LVN - lateral vestibular nucleus
MSA - macula of the saccule
MUT - macula of the utricle
MVN - medial vestibular nucleus
NCE - external cuneate nucleus
NCU - medial cuneate nucleus
NGR - gracilis nucleus
NMT - Medial/lateral trapezoid nucleus
PSC - posterior canal crista
SSC - superior canal crista
SVN - superior vestibular nucleus
VGA - vestibular ganglion

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OCULOMOTORIC RESPONSE TO VOLUNTARY HEAD ROTATIONS DURING PARABOLIC FLIGHTS

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ABSTRACT

Eight voluntary subjects were exposed to zero-G environment during parabolic flight and to two-G conditions during the same flight. The oculomotoric response to voluntary horizontal head rotations at 0.4 Hz was measured and computed as the transfer function between the head rotations and the eye movements. The gain of the response varied proportionally to the G-load. This might be of importance for the development of space motion sickness because the variation results in unprecise eye movements in unusual G-environments.

INTRODUCTION

The free fall phase of parabolic flights is one of several possibilities for the creation of microgravity conditions for experimental purposes. It is well fit for physiologic experiments if short periods of microgravity are satisfactory for the experiments.

In our efforts to describe the influence of gravitation on the oculomotor response to horizontal head rotations, we have performed examinations of this reflex during parabolic flights. In order to be able to extrapolate from the zero-G and one-G results, the aircraft performed a two minute 60^{o} turn, obtaining a constant G-load of two G between two series of parabolic manoeuvres.

METHODS

Eight missions were flown with eight subjects. All subjects had normal vestibular pretests. None of them were professional pilots, but most of the subjects had some experience as passengers in small aircrafts. This qualification was prefered to avoid anxiety reactions during the experiments. During the flights, the subjects performed horizontal head rotations guided by a frequency modulated sound signal in order to examine all subjects at the same frequency, 0.4 Hz. The eyes were closed during the test. Eye movements were recorded by a standard electrooculographic measurement (EOG). The head rotations were recorded by a velocity sensitive device (Ratemeter) mounted on a firm head holder. An instrument tape recorder carried by the aircraft recorded the signals. A SAAB Supporter aircraft was supplied by the Royal Danish Air Force for the purpose. It is designated T-17 and is in daily service used for training and observation duties. It is a small two seated propeller driven aircraft well fit for aerobatic manoeuvres. In each mission two series of three consecutive parabolas were interupted by a two minute 60^{o} turn with a constant G-load of two G. Decent pauses were held between these manoeuvres. During the parabolic flights, a constant zero-G level was obtained in ten seconds in each parabola. Gain and phase deviation of the oculomotor response were computed by cross power spectral analysis. Because of unreliable calibrations of the eye movements in the air field, the gain results were normalized in relation to the one G values measured on ground.

RESULTS

One of the recordings was omitted because of a bad signal-noise ratio due to an unstable electrode.

Table I.

G	Mean gain $\pm$ SD	Mean phase $\pm$ SD
0	91.5 $\pm$ 21.6	$-5.6^{o} \pm 8.9$
1	100	$-8.0^{o} \pm 6.6$
2	117.0 $\pm$ 13.4	$-8.9^{o} \pm 6.6$

The results appear in fig. 1 and in table I. The difference between the gain results at zero G and two G is statistical significant ($p < 0.025$).

A tendency of an increasing phase lag with increasing G-load can not be confirmed by statistical analysis.

DISCUSSION

The head rotation test performed utilizes information two different afferent sources, the vestibular and the cervical afferent systems. The influence of the neck afferents on the vestibular afferents is negligible at 0.4 Hz, whereas the phase lag is expected to be about 100 msec at this fre-

quency (1). Lackner and Graybiel (2)have demonstrated that the gain of the oculomotor response to a pure vestibular stimulus varies proportionally to the G-load. Our study confirms this observation and proves, that the addition of the afferent neck system to the vestibular input does not compensate for this gravitational effect on the vestibulo-ocular response. Our type of stimulus can be compared with the spontaneous head movements appearing in daily life. These movements are unavoidable for the astronauts during orbital flights. The G-dependence of the compensatory eye movement response results in a sensory conflict between visual and vestibular information. This type of sensory conflict is sufficient for the development of motion sickness (3). Water immersion studies using the same vestibular test have demonstrated that the oculomotor response is unimpaired by the circulatory effects of microgravity, thus it seems probable that the vestibular disturbances are caused by the effects of gravitation on the otolith organs (4).

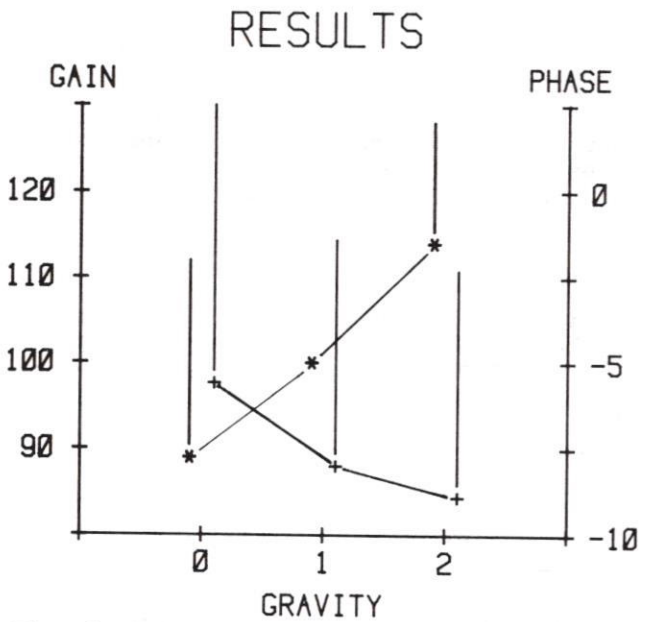

Fig. 1. Gain (*) and phase (+) variations with varying G-load.

CONCLUSIONS

The gain of the oculomotor response to head rotations varies proportionally to the G-load.

There is a tendency towards an increasing phase lag with increasing G-load.

This causes unprecise eye movements in unusual G-environments and might contibute to the development of space motion sickness.

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REVERSAL OF EARLY PATTERN FORMATION IN INVERTED AMPHIBIAN EGGS

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Xenopus eggs which were prevented from undergoing the classical rotation response to activation (PreFO eggs) displayed a reversal of pattern formation. Several aspects of the development of PreFO eggs is discussed.

Gravity orientation apparently plays an important and experimentally amenable role in "pattern formation" during early embryogenesis (1). Of the events which are potentially gravity dependent in early amphibian egg development none are more obvious and dramatic than the "rotation response" of the egg which accompanies activation. Unfertilized amphibian eggs display a clear asymmetrical animal/vegetal axis at many levels of observation (naked eye to ultrastructure). The most obvious animal/vegetal asymmetry is the darkly pigmented vegetal hemisphere. Likewise the location of yolk platelets according to size and density in the egg cytoplasm is an obvious example of animal/vegetal asymmetry (2).

Prior to fertilization eggs in the ovary as well as freshly spawned eggs display a random animal/vegetal orientation with respect to gravity. Fertilization and subsequent activation of eggs establishes, among other things, a peri-vitelline space which allows the egg to rotate so that the animal hemisphere opposes gravity (OpG) and the vegetal hemisphere faces gravity (G). This phenomenon, displayed by all amphibian species and all fertile eggs has historically been considered to be a prerequisite for normal embryogenesis.

In the present study Xenopus laevis eggs were immobilized shortly after fertilization under conditions which prevented the "rotation response". The observation was made that egg rotation is not a prerequisite for early development through oogenesis. The surprising observation that unrotated inverted eggs (animal hemisphere facing gravity) developed to the swimming stage (stage 46) establishes a model system for the investigation of the subcellular constituents of amphibian eggs that may adjust to gravity orientation. This experimental system should facilitate the identification of those cell components which either shift with gravity, against gravity, or do not respond to gravity orientation at all. Observations were made on cortical pigment shifts, first cleavage location, blastomere size, and the physical properties (density) of blastula stage blastomeres.

Freshly fertilized eggs which were prevented from undergoing rotation are termed prefertilization orientation (PreFO) eggs. They were prepared as follows: Eggs from hormone stimulated Xenopus laevis females were artificially inseminated with a minimal volume of concentrated sperm suspension. At 6 minutes after insemination they were flooded with a 20% ficoll (Type 400, Sigma, St. Louis) solution. Eggs treated in such a way did not rotate and were randomly oriented at virtually all angles (animal/vegetal axis was 0-180°). At the blastula stage embryos which developed from normal orientation (vegetal hemisphere facing gravity), 90° off-axis (animal/vegetal axis perpendicular to gravity) and inverted (animal hemisphere facing gravity) eggs were transferred to individual dishes at 15°C. The development of these blastula was scored at the neurula, tailbud, and swimming stages (stage 43-46).

Figure 1 shows that normal orientation eggs and 90° off-axis eggs displayed a similar frequency of survival. Inverted eggs, however, showed a somewhat lower survival frequency. Duplicate experiments at 22°C provided similar results except for a lower frequency of survival for inverted eggs (15°C-66%, 22°C-46%). These observations lead to the significant conclusion that the "rotation response" of Xenopus laevis eggs is not a prerequisite for survival to the swimming tadpole stage. Surviving embryos displayed axial structures and ranged in morphology from normal (similar to control embryos) to severely defective (abnormalities such as ascites, microcephaly, acephaly, and twisted axial structures). Inverted eggs exhibited the largest proportion of developmental abnormalities yet 26% of these eggs developed to normal swimming tadpoles that were indistinguishable from controls except for slightly lighter pigmentation of the head region. The conclusion from these experiments is that inverted amphibian eggs can successfully undergo reversal of their pattern formation.

Observations of living and inverted fixed PreFO eggs showed the following: 1) The cortical melanin pigment did not shift (it remained in the original hemisphere); 2) The location of the first cleavage furrow switched to the original vegetal hemisphere (OpG) of the egg; and 3) Blastomere size switched to the inverse of control animals. The original animal hemisphere blastomeres were large and the original vegetal hemisphere blastomeres were small. Yolk platelets are known to change position in inverted eggs. They clearly respond to novel gravity orientation. It is also known that isolated yolk platelets exist in at

least three different densities and that they are localized in the egg according to their density. It is, however, not known whether the egg cytoplasm density reflects the regional yolk platelet density.

Assuming that cleavage divides up the egg cytoplasm without any mixing of the major cytoplasmic components (e.g. yolk platelets), the density of the resultant blastomeres should reflect the density of the cyotplasm. A blastomere isolation and Percoll density gradient system were developed to answer the following question: Do blastomeres display uniform density? Dejellied (cysteine) morulas and early blastulas were dissociated in their vitelline membranes with 0.75% phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS) for one hour at 15°C. This procedure did not irreversably affect the blastomeres. Cell division continued during the actual dissociation process. Dissociated blastulas recovered and in several cases developed to swimming tadpoles when placed in 100% Steinberg's solution. The vitelline membrane was removed and the blastomeres gently layered on to a 45 to 95% continuous Percoll (Pharmacia) gradient at 15°C. Within one hour the blastomeres reached their isopycnic density. Figure 2(a) shows that the blastomeres were not of uniform density and that the smallest (pigmented) blastomeres were primarily located in the less dense region of the gradient while large non-pigmented blastomeres were found in the denser region of the gradient. Phase microscopic observations of disrupted isolated blastomeres showed that the denser blastomeres tended to possess large yolk platelets. It is clear the blastomeres are not of uniform density and their density differences reflect (at least in part) their yolk platelet composition.

In inverted eggs there is a mass shift of vegetal yolk mass toward the G (animal hemisphere) side (3). The following experiment was designed to determine whether this mass yolk shift could change the physical properties (density) of the blastomeres that inherit the vegetal yolk mass. PreFO normal orientation and inverted blastulas were dissociated and added to a Percoll density gradient. Figure 2(b) shows that the pigmented blastomeres of the normal orientation control blastula were found in the less dense region of the gradient. The pigmented blastomeres from the inverted blastula were located in the denser region of the gradient. Those large pigmented blastomeres contained large yolk platelets. It is clear that in the PreFO inverted blastulas the vegetal yolk mass had shifted towards the G hemisphere. The density of the G facing blastomeres reflect this shift. It appears, however, that inverted blastula PreFO animal hemisphere cells undergo a shift in response to gravity of yolk platelet distribution which depends upon the intrinsic buoyant density of individual platelets.

In addition to demonstrating that early pattern formation in Xenopus laevis does not require the classical "egg rotation" response for normal development, the PreFO experiments produced inverted amphibian eggs that were able to establish bilateral symmetry and develop through organogenesis. In addition, this study has shown that some cell components and developmental events respond to gravity in several of the following ways: (1) the vegetal yolk mass shifts with gravity; the blastomere size and density shifts may actually be a consequence of this yolk mass movement; (2) cleavage furrow formation shifted against gravity; and (3) the egg cortex and cortical pigment did not respond to gravity orientation.

This research was funded by NASA grant NAGW-60.

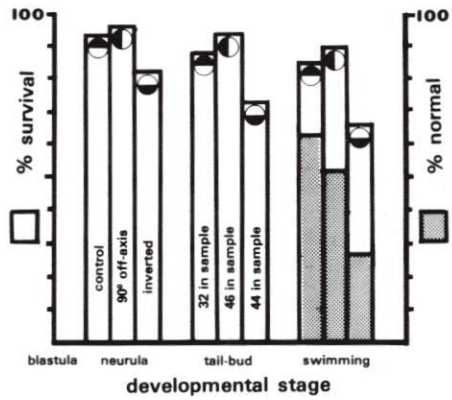

Fig. 1 A substantial proportion of both 90° off-axis and inverted PreFO eggs developed to the swimming stage. The frequency of normal development of inverted eggs was however diminished.

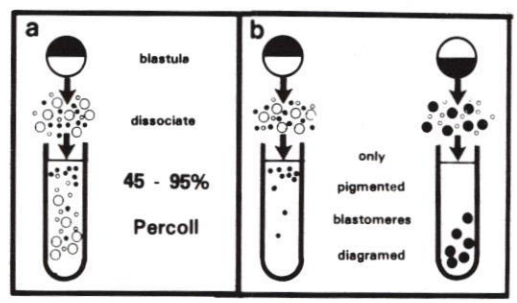

Fig. 2. (a) Dissociated blastula cells are not of uniform density. Small and pigmented blastomeres (less dense) and large non-pigmented blastomeres (more dense) were separated in the Percoll density gradient. (b) Large pigmented inverted PreFO blastula cells have a density characteristic of non-pigmented vegetal blastomeres from control blastula.

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GRAVITO-INERTIAL SENSITIVITY OF THE SPIDER: ARANEUS SERICATUS

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ABSTRACT

The gravito-inertial transfer function of the orb-weaving spider was evaluated by changes in the cardiac reflex. A non-intrusive method, using a laser system recorded the cardiac pulse. Between 1.001 and 1.5 Gz the data are 'best-fit' by a log function (r^2= 0.92). The response of the neurogenic heart is seen to be a good dependent variable for invetebrate research. The arachnid lyriform organ has those qualities which compliment the obtained gravity function. It is hypothesized that the cardiac pump maintains the spiders equilibrium in the gravito-inertial field.

INTRODUCTION

The importance of gravity(g) cues in spider behavior is evident from the geometry of the orb-web and the animals orientation. The receptor for g is unknown, nor has the issue been studied(4). An elaborate lyriform organ on the patella (Fig.1) presents characteristics which make it a good candidate for delivering g relevant information to the spider CNS. It is a known vibro/acoustic detector,has a spectral range from DC to + 10 KHz, and is strategically located near joints(1,2). The purposes of this research are to determine the operating characteristics of the spider with respect to g and to evaluate the role of the lyriform organ. This report describes the 'normal' g sensitivity curve in the unrestrained, unanesthetized orb-weaving spider.

METHODS

The response of the heart is the dependent variable for determining the g function. The spider heartbeat is neurogenic. The beat is initiated by a dorsal nerve arising from the CNS "pacemaker".The response of the spider heart to sensory stimuli and particularly to restraint of the animal has been previously observed(6,7). Thus, a method of recording action non-intrusively was developed. A low power laser transilluminated the heart and delivered an electrical representation of the pulse to a recording system and an instrument computer.

Adult, females of the species A. sericatus (70-115 mg) were exposed to Gz by centrifuge. The Gz intensities were given in an ascending series (1,2,5) between 1.001 and 1.5 Gz. The cardiac rate measurements were taken before and after each Gz stimulation. The presentation time and the interpolated post-rotatory intervals were 10 min each.

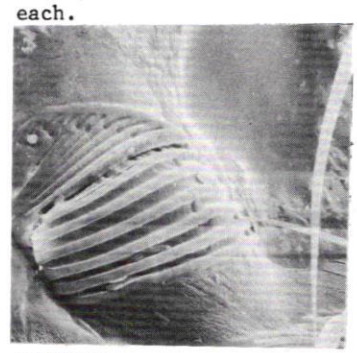

Fig.1. SEM, Lyriform Organ. 100 micron field.

RESULTS

Inter-beat-interval histograms (N=100) describe a bell-like curve not significantly different from a Gaussian distribution. The resting heartrates ranged between 15 and 60 BPM. Stimulation by 1.001 Gz usually produced a bradycardia (ca 5 to 20%). This rate reduction has been observed previously in restrained animals (ECG recordings). The spiders rarely moved during centrifugation, even during the higher Gz levels. They could move freely within the confines of a 5 cm diameter culture dish(the chamber on the centrifuge). Post-rotatory heartrates were slow adapting, perhaps on the order of 10 min for the 10 min presentation time.

Fig.2 is a plot of mean heartrate (± 1 standard deviation) as a function of log Gz. The "best fit" curve for these data is logarithmic with a goodness-of-fit accounting for 92% of the variance. The curves for individual animals were also logarithmic but showed substantial individual differences in sensitivity.

DISCUSSION AND CONCLUSIONS

Other than certain leg reflexes in response to pulsed vibro/acoustic stimuli the orb-weaver is quite sedentary(3). The cardiac reflex appears to be continually modulated by sensory inputs, including gravito-inertial stimuli, thus it serves a useful role as dependent variable. Perhaps the cardiac response can serve a similar purpose among other invetebrates having neurogenic hearts?

Bohnenberger (in 1) showed that the threshold force needed to just elicit an electrical discharge from a single lyriform slit was 40 microN.

In the present work, for the lowest Gz level employed (1.001), the force produced by the centripetal component (0.43 m/s/s) on a typical 100 mg spider would be 43 microN. Assuming that 1.001 Gz is supra-threshold for the spider an extrapolation of the obtained curve (Fig.2) estimates the absolute threshold intensity for the species at about 1.0008 Gz. This is not particularly surprising in view of reported displacement thresholds of the lyriform slits from 25 to 100 A (1,2,3). Thus the dynamic range of response may be on the order of 4 to 5 log units for the spider.

The spider does not present homologs of the utricular/semi-circular canal system of the vertebrates. Exoskeletal strain produced by compression (a consequence of g) is probably the " adequate stimulus". This would make arachnids and other invertebrates 'favored' subjects for the investigation of relatively "pure" gravitational effects. Consider that the salient manifestation of hyper-g is to produce a change in the weight of the spider,minus the disconcerting phenomenae associated with coriolus which obtains among the vertebrates.

Our "working hypothesis" that the cardiac reflex underlies postural homeostasis. The lyriform organ communicates compressive changes to a cardiac center. The heart then beats more rapidly and with an increased amplitude and in a syringe-like fashion ' pumps-up' the legs. Thus by means of a hydrostatic mechanism(5) the spider becomes supported when gravito-inertial fields are manipulated.

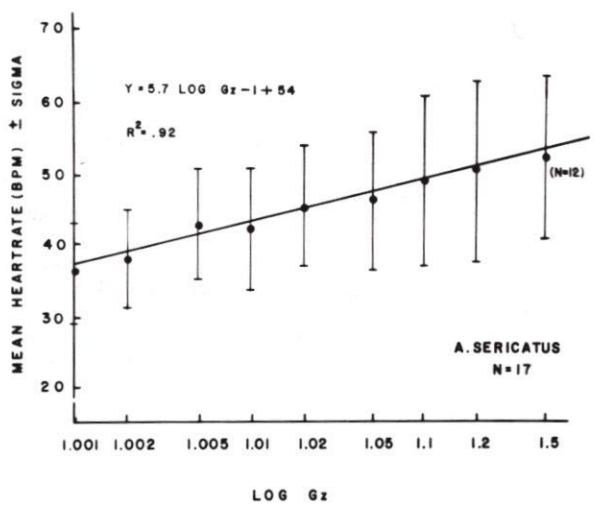

Fig.2. Gravity Sensitivity Curve for the Orb-Weaving Spider, Araneus sericatus.

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GRAVITY RECEPTORS IN A MICROCRUSTACEAN WATER FLEA: SENSITIVITY OF ANTENNAL-SOCKET SETAE IN DAPHNIA MAGNA

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ABSTRACT

Gravity receptors of Daphnia magna were discovered on the basal segment of the swimming antennae and were shown to respond to upward water currents that pass the animal as it sinks between swimming strokes. Sensitivity of the gravity perceiving mechanism was tested by subjecting daphnids to a series of five decreasingly dense aqueous solutions (neutral density to water) in darkness (to avoid visual cues). Three-dimensional, video analysis of body position (pitch, yaw and roll) and swimming path (hop and sink, vertical and horizontal patterns) revealed a gradual threshold that occurred near a density difference between the animal and its environment of less than 0.25%. Because daphnids do not sink but continue to glide after stroking in the increased density solutions, gravity perception appears to occur during a vertical swing of the longitudinal body axis to the vertical plane, about their center of gravity, and, thereby, implies a multidirectional sensitivity for the antennal-socket setae.

INTRODUCTION

Gravity (G) is believed to be an essential cue for continuously swimming members of the genus Daphnia to spatially orient at night (1). G-perception is assumed to be associated with setae on the swimming antennae (2) which are activated by the rush of water past the animal as it sinks between upward swimming strokes or hops (3). During the sink phase of this hop-and-sink swimming movement, the antennae are extended upward at 45° angles to slow descent. The upward currents generated during sink are thought to stimulate the antennal hairs, initiating stroke, thereby regulating stroke frequency and maintaining a constant horizontal swimming path through a feedback mechanism (4). The actual location of this proposed sensory apparatus has not been reported.

Daphnia magna Straus when experiencing neutral buoyancy in the dark exhibited disorientation that suggested a lack of internal g-sensing organs, e.g. statocysts (5). G-receptors were, therefore, postulated as being mechanoreceptive setae on the exoskeleton, as known in other zooplankters (6). Selective removal of paired setae on the basal socket of the swimming antennae (Fig. 1) resulted in daphnids that swam normally in water with overhead illumination and exhibited disorientation in the dark (5). These observations suggest that the antennal-socket setae are essential for indirect detection of gravity through changes in direction and velocity of water currents. As a continuation of these studies, the sensitivity of the g-receptor mechanism was investigated by exposing D. magna to a series of five decreasingly dense aqueous solutions from neutral buoyancy to water (Table 1). Quantitative and qualitative visual observations were used to discriminate the minimum density difference or threshold between the animal and its environment that elicited relatively normal spatially oriented body position and swimming behavior.

MATERIALS AND METHODS

Ten mature, parthenogenic female D. magna, 4 mm in length, were individually videotaped in a fluid filled plexiglass rectangular box, 3 x 3 x 5 cm. Pond water containing algae was increased in density with the addition of a non-toxic solution of colloidal silica particles (15-30 nm) coated with PVP. This admixture maximally effected density while minimally effecting viscosity and osmolarity. After an acclimation period of two hours, each animal was taped for two minutes at night in each of the five conditions. With 60 fields/second, this allowed the analysis of 7200 fields/condition or 36,000 fields/animal. By illuminating the vessel at 90° angles, reflecting the images off front surface mirrors and collecting them side-by-side in one infra-red (IR) sensitive (peak 890 nm) camera, two 90° split horizontally aligned views of the same animal are recorded. Darkness was attained by placing IR filter boxes (890 nm) over the vessel and light sources. With this system it is now possible to track the three dimensional x, y, and z coordinates of swimming path and body orientation.

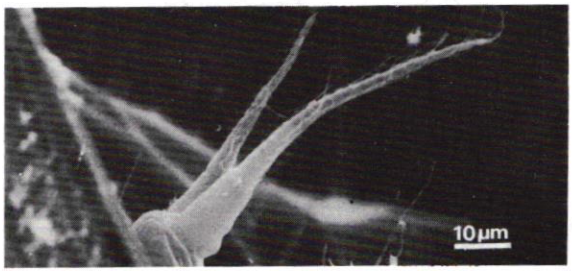

Figure 1. Gravity sensitive setae of D. magna

RESULTS

Pitch and yaw components of body orientation for a representative daphnid reveal a corresponding relationship between N3 and W (Fig. 2). The results for turns or rolls are not directly comparable. However, if the numbers of sequential turns are analyzed, the trend of similarity between the behavioral patterns in N3 and water is repeated (Table 2).

Swimming path reflects the same correspondence only in vertical hops, where direction and magnitude in N3 is nearly identical to that in water (Fig. 3). A downward vertical sink vector is exclusively representative of W, as are short horizontal movements.

Table 1. Mean densities (D) and standard errors (SE) of aqueous solutions used to compare the behavior of D. magna: in water (W); at decreasingly negative buoyancies (N3. N2. N1); and at neutral buoyancy (N). (25 vol-wts/density measurent).

	W	N3	N2	N1	N
D	1.0048	1.0375*	1.0381*	1.0384*	1.0400*
SE	±.0001	±.0001	±.0002	±.0001	±.0002

* significantly distinct from preceding density (t-test with Bonferroni multiple-comparison, inequality correction).

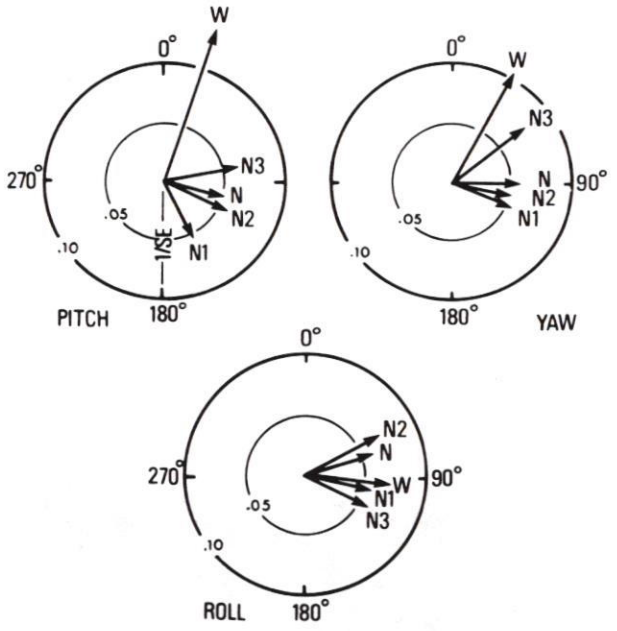

Figure 2. Body orientation as compared to body axes plotted on polar coordinates. (Each directional change was assumed to begin at 0° direction.) Arrows point in mean angular orientation; length is the inverse of angular deviation.

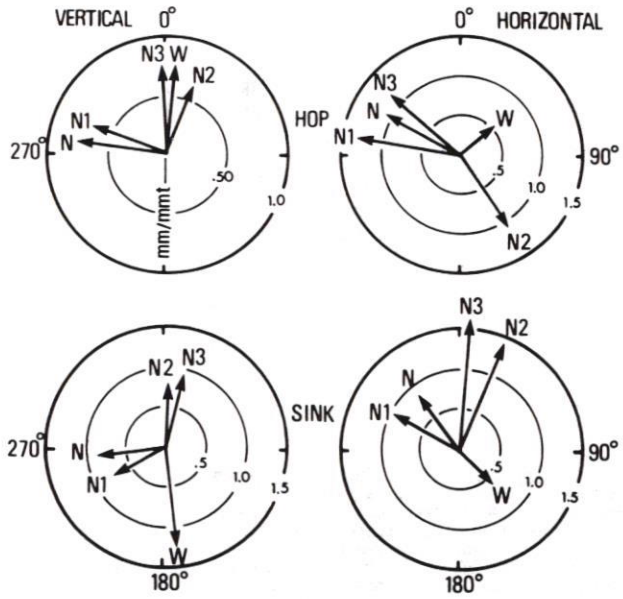

Figure 3. Vertical and horizontal swimming path vectors (in relation to x, y, and z coordinates of the experimental vessel). Arrows depict mean direction and magnitude (in mm) of movement (mmt).

DISCUSSION

Behavioral observations revealed that in the N1 condition the D. magna hugged the walls over two thirds of the time. Swimming paths free of the wall consisted of tight circular spinning with the animal continuously pitching forward or somersaulting, indicative of disruption and disorientation (1,5). N2 did not elicit wall hugging. Somersaulting occurred one third of the time and directional, dorsal-side-up movement alternately dominated, as depicted in the hop vertical vector (Fig. 3). No somersaults were observed in the N3 condition. Large, upward, directional spirals caused by yawing to the right were recorded one third of the time, and normally oriented swimming the rest. Body orientation, as measured by rotation about the lateral axis, appears to be g-sensitive only in N3 (Fig. 2, Table 2). Swimming path reveals a progressive sequence of directional orientation from N2 to N3 to W; vertical sink and horizontal movements do not appear to copy this trend.

Qualitative analysis of swimming movements shows that even though the animals are negatively buoyant in N1-N3, there is no sink phase. After hop, daphnids continue to move in the basic direction of hop. This hop-and-glide directional movement is reflected in the N1-N3 vertical sink vectors (Fig. 3). However, after a hop, while gliding, the center of gravity of the animal causes the posterio-ventral end to drop into alignment with the Y-axis. This vertical shift of longitudinal body axis is most evident in condition N3. Apparently this small but consistent change is enough to allow g-perception and a corresponding body orientation to the gravity vector.

Both body position and swimming path orientation indicate g-perception closest to the water condition in N3. The threshold appears abrupt from the pitch and yaw behavior but more gradual when combined with the vertical hop and swimming path analysis. Spatial orientation begins in N2 and closely reflects that in water in N3. This equates to a density difference of less than 0.25% between daphnids and their environment and indicates their extreme sensitivity to density changes. The replacement of a sink requirement with a consistent downward shift of longitudinal body axis prompts the hypothesis that the g-receptive, antennal-socket setae are multidirectional in sensitivity.

Table 2. Sequential body turns (rolls) in water compared to those in increased-density, aqueous solutions. Symbols: n, number of sequential rolls; X^2, chi square statistic used to compare patterns (%) of RR, RL, LR, and LL (right turns followed by right turns, right turns followed by left turns, etc.)

	W	N3	N2	N1	N
n	72	64	48	48	32
X^2		2.7	350*	42*	44*

* significance level below .01

ACKNOWLEDGEMENTS

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SOME KARYOLOGICAL OBSERVATIONS ON PLANTS GROWN IN SPACE

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Critical experimentation using plants under space flight conditions is in its infancy. As more opportunities arise for making observations on space grown material, it behooves investigators to learn as much as possible, however fragmentary, from available specimens. An opportunity arose to study root materials grown in the course of Heflex Bioengineering Test (HBT) made by Brown and Chapman Dept. of Biology, University of Pennsylvania, Philadelphia on STS-2/OSTA-1 in the late fall of 1981. Since this laboratory is interested in plant growth and development under space flight conditions (2), access to Shuttle material provided an opportunity to assess whether cell division in a root would be affected by prolonged exposure to micro-gravity.

Through the kindness and co-operation of Dr. Allan H. Brown and Mr. David K. Chapman, and NASA, arrangements were made to provide us with several roots of sunflower developed under space flight conditions. Because the results of STS-2 were interesting but by no means exhaustive or even really convincing, a further opportunity arose to attempt confirmation or extension of the results using sunflower material grown on STS-3 (again courtesy of Dr. Brown and Mr. Chapman and NASA Headquarters, especially Dr. Thora Halstead). Sunflower material (as part of HBT-II) was again made available; but we also had access to oat and mung bean material grown on STS-3 by Dr. Joe R. Cowles and Dr. H. William Scheld, Department of Biology, University of Houston.

PROCEDURE

Specimens were treated with prefixative as soon after recovery as possible prior to examination by squash. Procedures ensured intervention in the first cell division cycle undertaken on earth.

RESULTS

Six roots of STS-2 derived dwarf sunflower, cv. 'Teddy Bear', were examined after treatment with the cytostatic agent 1-bromonaphthalene for 4-1/2 hrs, fixing in alcohol-glacial acetic acid, staining in Feulgen and squashing in aceto-carmine (3). Relatively few cells were in division. The average percent was 2.29 (range 0 to 4.12). Laboratory controls were consistently 2 times greater. Roots of two plants were aneuploid, 2n-1=33 (Fig. 1). In each case the homologue of chromosome pair number 6 was missing. We have neither encountered in any of our many ground based studies of sunflower karyotypes nor heard of aneuploidy in sunflower. Other chromosomal aberrations were also encountered. Bridge formation was apparent in telophase and Feulgen-positive material which was assumed to have "diffused" from some of the nuclei was also encountered. Similar results were mentioned by Sparrow and his co-workers (5) in microspore cells of Tradescantia grown in Biosatellite II. Whereas there again was a reduced number of cells in division in roots of sunflower seedlings germinated aboard STS-3, no aneuploids were encountered out of a total of 16 roots examined.

"Flight" and "control" roots of oat seedlings exposed to 1-bromonaphthalene prefixative for 4-3/4 hrs were examined as well. There were about 1/10 as many divisions apparent as we had expected on the basis of ground studies. In flight material, out of a total of 80,000 cells examined from a total of 8 roots, only 0.04% were in division. The controls averaged some 2.43-2.84%. The most significant feature of the oat work was not only the paucity of cell divisions, but the presence of widespread aberrations in the flight samples. There was much evidence of fragmentation and breakage (Fig. 2). We have never encountered such abnormalities in control materials of oats but they resemble damage by alkylating agent mutagens. The basal growing zones of 2 oat plants were squashed as well. In one sample, there were 25 metaphases out of a total of 750 cells (or 3.3%); in the other 33 metaphases out of 1150 (2.8%) were found. Although based on only two plants, the number of divisions in the "shoot" growing region of oats was much greater than in the root tips of flight material (see above). This suggests that whereas the root cells had been adversely affected under flight conditions, the cells in the basal meristematic area of the shoot were not.

Observations on the root tips of mung bean (Vigna mungo) where cells had been pretreated with cycloheximide disclosed no unusual features. The overall number of division was about half that encountered in laboratory control experimentation. Even so, differences in percentage of cell divisions in the flight and ground controls could be established. (3.52% versus 8.90% respectively.) The full diploid complement of 2n=22 was encountered in all cells and the gross morphology of the chromosomes was generally comparable in flight and control material. It was equally true, however, that all flight samples--regardless of species--exhibited greater chromosome contraction and a poorer spread than those nuclei from the ground controls.

Fig. 1. Karyotype of 'Teddy Bear' from HBT. Inset shows a representative cell stopped in metaphase (scale bars, 5 μm).

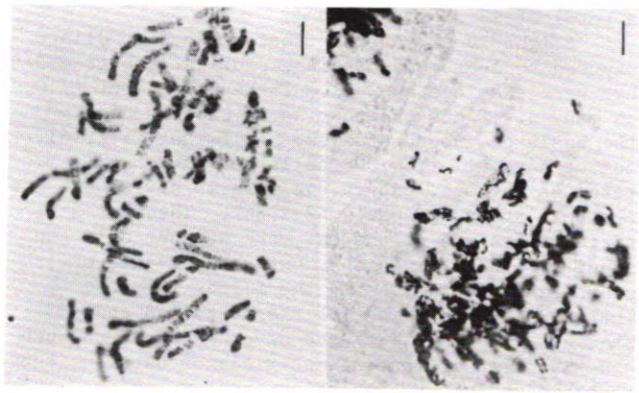

Fig. 2. Metaphases from oats showing multiple breaks and fragmentation (scale bar, 5 μm).

COMMENTARY

All oat, sunflower and mung seedlings showed a reduced number of cells in division as they went through their first cell division cycle on earth when compared to their ground controls. (1g controls aboard the spacecraft could not be made.) This implies that the scarcity of cells in division reflected the level of cell division activity in the spacecraft environment. It could equally well mean that upon return to earth, the root cells had been so affected by the conditions of re-entry etc. that cells were not in a state conducive to re-establishment of a normal cell division cycle. It is also possible that towards the end of the flight period (196 hrs in STS-3), cells which had hitherto been active were running "downhill" in terms of their capacity for cell division. While the cell division data can be variously interpreted, the severe cytological disturbances are difficult to explain away. Aneuploidy in H. annuus reflects spindle disturbances. These have been encountered before (6). Platonova et al. (4) attributed substantial increase in aberrations in microspores of Tradescantia to temperature problems. We see no such problems in our data. Moreover, potential problems due to seed age (1) or degree of hydration (7) do not seem to be responsible for the problems encountered. Therefore, space flight conditions affected the post-Flight cell division status of some of the roots of plants grown on STS-2 and 3. A significant number of oat, mung, and sunflower plantlets exhibited random root orientation and lack of strictly orthotropic growth of their shoot systems in flight samples. We made no attempts to correlate directional perturbations of roots and cytological disturbances. But, the observation that mung roots were apparently least affected in terms of their cytology despite the fact that their roots were often randomly oriented suggests at this time, at least, that this would not be a fruitful area for future study. All in all, it seems that the growing conditions were less than ideal, or, that the root environment provided was not well suited to the kinds of cytological investigation attempted. In any case, the cytological examination of the root tips raised more questions than it solved. Future flights will permit resolution of these problems. Research was supported by NASA Grant NSG-7270, Plant Cells, Embryos and Development in Space.

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EFFECT OF CULTURAL CONDITIONS ON THE SEED-TO-SEED GROWTH OF ARABIDOPSIS AND CARDAMINE: A STUDY OF GROWTH RATES AND REPRODUCTIVE DEVELOPMENT AS AFFECTED BY TEST TUBE SEALS

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ABSTRACT - A clinostat simulates weightlessness for plants by slowly rotating plants around a horizontal axis. Though not ideal, it is a practical device to study on Earth the effects of weightlessness on plants by simulation. About half of Arabidopsis thaliana (L.) Heynh. plants grown on clinostats die prior to flowering. The survivors do not differ in appearance from controls, but produce seeds less viable than controls. In a Soviet space experiment, only one A. thaliana plant was reported to survive. This plant was still in the vegetative stage on the 56th day of the experiment while ground controls were flowering and setting seeds. In this and other studies one or more stages of A. thaliana life cycle has been completed in space. However, the growth of plants through all stages of development in a single sequential episode has not been accomplished. It has been hypothesized that the difficulty of growing plants may be more from cultural than from gravitational conditions. Arabidopsis thaliana (L.) Heynh. and Cardamine oligosperma Nutt. were chosen for study for their short life cycle and small size. The initial results of test tube seals on the growth, flowering, and seed pod formation are discussed.

INTRODUCTION - This study is part of a task to grow plants in weightlessness from seed to seed. Planting of the seeds and culturing of plants to seed harvest are to be done in weightlessness.

A relatively simple task to perform on Earth, but apparently, as evidenced by the papers published [2,4,6,7,8,9] a rather elusive goal when attempted in space. At best, the Soviet workers were only able to grow the plants through one or two life-cycle stages. The plants were grown in sealed containers. As far as I know, mature seeds have not been obtained from plants that were started from seeds under weightlessness. Plants will grow up to a point and for reasons unknown, die. Thus, attempts to grow a plant through a complete life-cycle under weightlessness resulted in failure.

In other studies, but using clinostats to simulate weightlessness, Brown and co-workers [1] also cultivated Arabidopsis in sealed containers. They successfully grew Arabidopsis for over 21 days in test tubes sealed by a film of Saran. Since flowering occurred around the 26th day, the experiment was too short to complete a life cycle.

Attempts to grow plants from seed to seed in sealed containers are reported herein. The data are from experiments performed in the first four months of a 3-year task and are preliminary.

PLANT MATERIAL - Arabidopsis thaliana (L.), Heynh., mouse ear cress, flowers 3 to 4 weeks after planting and produces seed pods with mature seeds about 2 weeks after flowering. Cardamine oligosperma Nutt., flowers about 5 weeks after seed planting. Seeds mature 2 weeks later.

GROWTH MEDIA - The first medium was a 1:1 mixture of fine ground peat moss and fine vermiculite watered with 0.1 strength nutrient solution having an 18-20-16 formulation with chelated iron. The second was an agar medium made up according to Miksche and Brown [4].

SEALS - Seals used were Saran film (Dow Chemical), metal cap (Belco culture tube caps), and open-to-air (control). In the first two experiments, a single rubber band, size 12, was tightly wrapped over the Saran about 1cm below the test tube rim. In the third experiment, one treatment consisted of a single rubber band wrapped into 4 loops over the Saran about 1cm below the rim. The second treatment had a second rubber band similarly wrapped 2cm below the first band.

GROWING CONDITIONS - Fluorescent cool white lamps gave 340 ft.c. at plant level and temperature was maintained at 23° ± 1°. Seedlings were transplanted from a 10x10x10cm plastic pot containing vermiculite-peat mix into 25x200mm test tubes containing vermiculite-peat mix 2 to 4 weeks after germination and watered as required.

For the agar media experiments, sterilized seeds were planted directly on agar. No supplements were given after planting.

RESULTS - In the first experiment, growth (height) and flowering (date of bud appearance) were suppressed in mouse ear cress sealed with Saran (controls-300mm high, Saran-60mm high). Furthermore, seed pod formation, which occurred by day 45 in open-to-air controls, was still lacking in the sealed treatment even up to day 124. Thus, growth, flowering, and seed pod development in the mouse ear cress were strongly restricted by Saran. In the second experiment, growth and flowering of bitter cress were suppressed by the Saran seal. However, up to day 55, the Saran sealed plants were taller; but, by the end of the experiment, open-to-air control plants were more than twice as tall.

This early difference may be a response to higher humidity in the sealed tubes. Seed pod formation had not occurred in the Saran sealed treatment by the end of the 85-day experiment; Figure 1. Saran

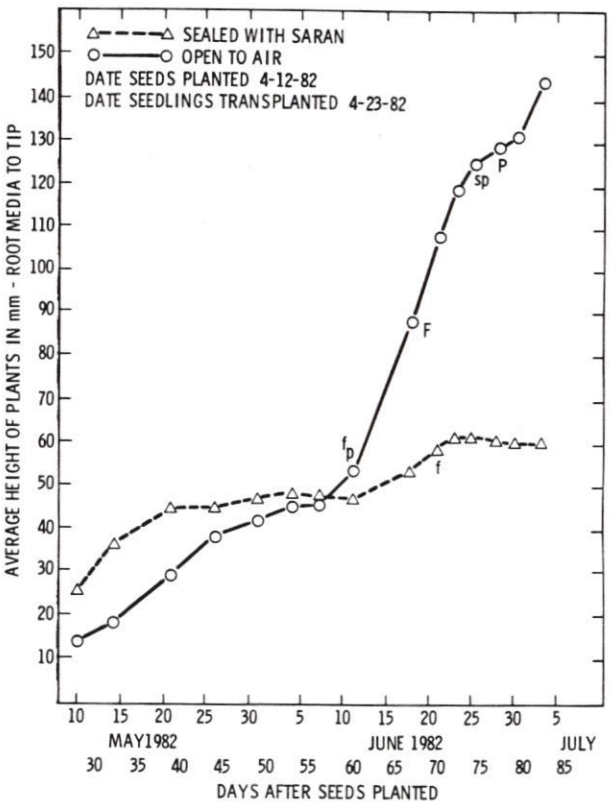

Figure 1. Growth - development of Cardamine oligosperma raised in 25x200mm test tubes on vermiculite-peat mix under 340ft.c. continuous fluorescent light. f-first flower, F-all plants flowering, p-first seed pod, P-all plants with pods, sp-first mature seed pod.

seal treatment again resulted in suppression. In the third experiment, Saran, metal cap, and open-to-air seals were tested. With the two types of Saran seals used, i.e., single and double rubber bands, a total of four treatments are presented in Figure 2. As in the previous two experiments, the growth, flowering, and seed pod development were suppressed by the Saran wrap. Formation of seed pods occurred 10 days after the Saran seals were deliberately broken open. Growth also resumed.

DISCUSSIONS AND CONCLUSIONS - What conclusions can be drawn from these results? What might be the cause or reasons for the observed differences in the height, delay in flowering, and inhibition in seed pod development?

Light quality and quantity are unlikely candidates. Saran, as far as I know, has very little effect on transmitted light, and since the differences in the plant responses are so great, light is set aside as a possible candidate for the present. Nutrients are also unlikely candidates since, in all treatments for a particular experiment, the levels were the same.

Atmospheric composition appears to be a likely candidate. The composition includes such physiological components as carbon dioxide, oxygen, and humidity. This choice is supported by the observation that the mouse ear cress renewed their growth and then set seed pods after the Saran seal was ruptured. This area is being investigated now.

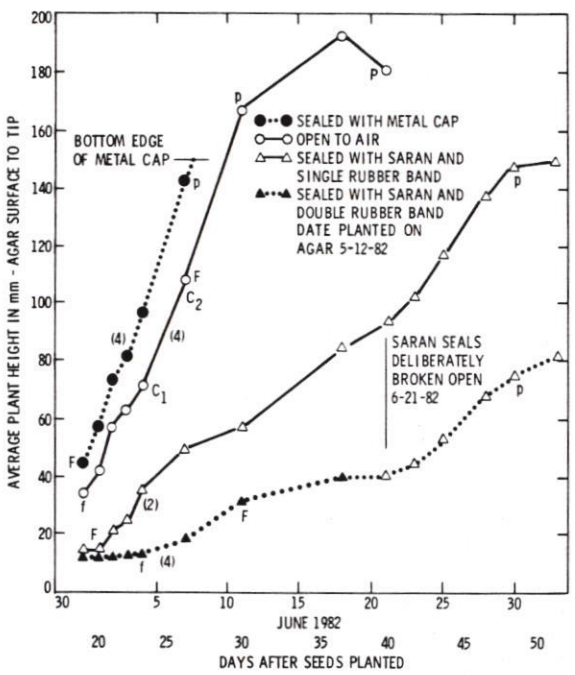

FIGURE 2. Growth - development of Arabidopsis thaliana raised in 25x200mm test tubes on agar medium under 340ft.c. continuous fluorescent light. C_1-one tube contaminated, C_2-all tubes contaminated, all other letters same as Figure 1.

Detrimental products of metabolism appear as unlikely candidates. However, gaseous effluent from algal cultures reduced growth and even killed higher plants.[3] Whether the gaseous effluent story can be applied here is not known at this time. The possibility of detrimental products as the cause or source is being left open.

There may be other factors to included as candidates; but for the moment, the two likely candidates are detrimental products of metabolism and atmospheric composition. The strongest candidate at the moment appears to be atmospheric composition. (Supported by NASA contract NAS7-918.)

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LIGHTIFICATION IN YOUNG PLANTS EXPOSED TO THE NEAR-ZERO GRAVITY OF SPACE FLIGHT

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Pre-germinated pine seedlings and oat and mung bean seeds were prepared for flight on STS-3. The experimental chambers were placed in two (flight and ground control) plant growth units (PGU) equipped with lights and capable of day/night cycling, data recording and some temperature regulation. Observations after the 8-day flight revealed that pine seedlings grew and developed similarly to ground controls. Oat and mung bean seeds germinated and also grew well in space. The most noticeable difference between flight and ground control seedlings was the number of oat and mung bean roots which grew upward out of the root support medium in the flight seedlings and certain orientation difficulties experienced by the mung bean. The lignin content in the whole stems of pine and mung beans was slightly lower in the flight vs. control tissues. Protein content and PAL and peroxidase activity in the upper hypocotyl sections of pine stems showed an increased amount of protein and a corresponding decrease in PAL and peroxidase activity.

INTRODUCTION

Plants, which constitute a major and critical component of the earth's biosphere, have been the subject of limited study in space. Experiments conducted primarily by Russian investigators have already demonstrated that near-weightlessness can disorient root and shoot growth, enhance plant sensitivity to substrate moisture conditions, and increase mortality rates. Our knowledge and understanding of plant growth and development in space, however, is still in its infancy.

The experiment described in this paper was flown on STS-3 in March 1982 and is one of the first experiments studying plant growth and development in the Space Shuttle era. The objectives of the experiment were: 1) to test the function and effectiveness of flight hardware developed to grow small seedlings in the Shuttle mid-deck; 2) to observe overall plant growth and development of space-flown seedlings; and 3) to establish whether synthesis of the structural polymer, lignin, was reduced in weightlessness.

HARDWARE DESCRIPTION

The Plant Growth Unit (PGU) was designed primarily by Lockheed Missles & Space Company, Inc., and was built by Lockheed and NASA-Ames. The hardware was designed to replace a Rockwell locker on the forward bulkhead of the orbiter mid-deck; overall dimensions are 51 x 36 x 27 cm. The PGU consists of two functional components, the Plant Growth Chambers (PGC) and the Plant Support Unit (PSU). The PGU contains six PGC's, each of which has a volume of approximately 2 liters. The PSU is the support component of the flight hardware. It is equipped with plant-growth lamps (Vita-Lite spectrum), timer for controlling day/night cycle, temperature sensors, electronically-controlled fans and heater strip for temperature modification, data recording system, and internal batteries. The PGU is nominally powered by 28-volt DC power.

EXPERIMENTAL DESIGN

The experiment was designed basically to study the influence of near-weightlessness on lignification in young plant tissues. Lignin, after cellulose, is the most abundant carbon compound in plants and provides much of their mechanical support. Since gravity is believed to be a primary controlling stimulus for lignification, the experiment was designed to evaluate how near-weightlessness affects the quantity and rate of lignin formation in different plant species during early stages of development. Three plant species, pine (seedlings), mung beans (seeds), and oats (seeds) were prepared for the STS-3 mission. The duration of the flight was 194 hours.

PRELIMINARY RESULTS AND CONCLUSIONS

Hardware Performance

The PGU functioned as designed and can be used to support growth of young plants during space flight. The PGU remained continuously powered during flight. The PGU modified PGC temperatures essentially as projected and provided day/night temperature differentials. The lamp status and temperature inside each PGC was recorded flawlessly at 15-minute intervals. The light cycle timer functioned properly to provide the designated day/night cycles. The back-up (ground control) PGU also functioned properly.

Observation of Plant Growth

GERMINATION -- The number of mung bean and oat seeds which germinated was about normal (87.5%) in both flight and 1g controls. Near-weightlessness, however, perturbed orientation of mung beans

during germination, an effect not seen in ground controls.

STEMS -- The aerial portions of the seedlings generally grew upward toward the light, especially in oats and pine. The majority of mung beans also grew upward, but some of the stems in the flight PGC's grew horizontally. This suggests that light was insufficient to correct an orientation problem during germination or is not a complete substitute for gravity in the upward orientation of stems in certain species. Stem heights of the flight seedlings were 10 to 30% less than the ground controls. Part of this difference is attributed to differences in rate of plant growth between flight and back-up PGU's.

ROOTS -- The roots were shorter in flight seedlings than in ground controls. Also, there were slightly more roots per seedling in the flight tissues. Neither light nor water source was adequate to orient all roots downward; approximately 26% and 38% of the oat and mung bean roots, respectively, were growing upward above the plant growth medium. Roots of flight pine seedlings (planted as seedlings), however, were all growing downward. The ability of roots to penetrate the agar also was reduced in the flight seedlings, especially in oats where no roots were seen in the agar; oat roots easily penetrate agar at 1g.

Plant Tissue Analysis

Seedlings from the flight and control chambers were sectioned and analyzed. In oats and mung beans, selected 12-mm sections were cut from designated stem regions. A 2-mm subsection was cut from the base of each section for histological analysis. In pines, the entire stem length was measured and subsequently sectioned into 6 equal sections. A 2-mm subsection also was cut from the base of each pine section for histological analysis. Spectrophotometric lignin determinations were made on 1.0-cm oat and mung bean sections and on 0.8- to 0.9-cm pine sections. Protein and enzyme analyses were carried out on proportionally cut pine stems of 1.0- to 1.1-cm lengths (seedlings not used in the lignin analysis).

LIGNIN -- Sixteen oat, mung bean and pine seedlings were analyzed for lignin. The spectrophotometric procedure involved sequential extraction of individual stem sections with acetone-HCl, NaOH and water, digestion of lignin with acetyl bromide, and quantitation at A_{280}. The total lignin content of the flight pine and mung bean stems was 3.6% and 25% less, respectively, than 1g controls.

PROTEIN -- Protein content was determined in proportionally-cut sections (average length 1.0 to 1.1 cm) from twelve pine seedlings. Six seedlings were sampled from each chamber and the respective sections were pooled for analysis. The sections were homogenized and subsequently centrifuged. Aliquots of the resulting supernatant were quantitated spectrophotometrically (A_{595}) after reaction with Coomassie Blue. There was slightly less (2.2%) overall protein in the flight seedlings than in the 1g controls. If the upper two-thirds of the pine seedlings are compared, however, the flight tissues contained about 5% more protein than the controls. It should be noted that this is the region of the pine stem which developed while in near-weightlessness.

ENZYME ACTIVITIES -- The activities of two enzymes, phenylalanine ammonia-lyase (PAL) and peroxidase, in the lignin biosynthetic pathway were determined in proportionally-cut sections from twelve pine stems (the same sections used for protein quantitation). Aliquots of the supernatant were assayed for PAL activity using ^{14}C-phenylalanine and for peroxidase activity using a spectrophotometric assay (A_{470}). PAL activity was 31% less in the flight pine stems than in the controls. Peroxidase activity in stems of the flight seedlings was 22% less than in the controls.

DISCUSSION AND CONCLUSIONS

Plants can germinate and grow in space. Most of the flight seedlings appeared healthy and did not experience any drowning problems. There was some difference in stem height and root length between flight and control seedlings, but part of this is attributable to growth differences between the two hardware units. Difficulty with the mechanics of seed germination and stem orientation was evident in mung beans. Light did not adequately compensate for gravity in this particular case. Mung bean and oat roots also experienced orientation problems.

The results of the chemical analysis are interesting but are complicated somewhat by the height differences between flight and control seedlings. Because of this, we have expressed the data on a total stem basis. The flight pine seedlings contained slightly less lignin than did the controls. This amount of difference neither supports nor negates the hypothesis that gravity reduces lignification. The approximately 25% reduction in lignin in the flight mung beans, on the one hand, supports the hypothesis. The pine seedlings flown in space contained more protein in the upper stems than did the controls and contained considerably less PAL and peroxidase activity. The reduction in PAL and peroxidase activity is more amplified than changes in lignin content. Since PAL activity in particular correlates with lignification, the reduced PAL activity in space-grown plants suggests that a larger difference in lignin content between flight and control seedlings would occur with an increased experimental period.

A COMPARATIVE STUDY OF MONOCOT AND DICOT ROOT DEVELOPMENT IN NORMAL (EARTH) AND HYPOGRAVITY (SPACE) ENVIRONMENTS.

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ABSTRACT

The anatomy and fine structure of roots from oat and mung bean seedlings grown under hypogravity conditions aboard NASA's space shuttle was examined and compared to that of roots from ground control plants grown under similar conditions. Oat roots from both sets of plants exhibited normal tissue organization and ultrastructural features, with the exception of cortex cell mitochondria, which characteristically showed a "swollen" morphology. Flight-grown mung bean roots differed significantly from the controls in that root cap cells were somewhat disorganized and degraded in appearance, especially at the cap periphery. At the EM level, these cells exhibited a loss of organelle integrity and a condensed cytoplasm. The potential significance of this finding for the putative gravity-sensing cap cells was noted.

INTRODUCTION

The growth and development of plants in hypogravity environments is poorly understood, largely because opportunities to examine this phenomenon have been limited. With the advent of the space shuttle and space flights of several days duration, seedlings can be germinated and grown to maturity entirely under hypogravity conditions prior to their return to earth.

Previous experimentation, conducted primarily with etiolated seedlings, has suggested that these plants experience various types of growth disorientation in the reduced gravity environment. In the few instances where fine structural studies have been conducted on these flight-grown plants (1), there are data which suggest that changes in basic cell architecture and organelle morphology may accompany growth in the hypogravity environment. The question as to whether larger-scale tissue organization differences also occur in space-grown plants remains unanswered. This question is especially relevant for tissues such as the root cap, the cells of which contain dense, multigranular amyloplasts (statoliths) which rapidly sediment in response to changes in the direction of mass acceleration, and thus are implicated in graviperception. Earlier Biosatellite II experiments (2) indicate that root tissue organization in space-grown plants is unaffected, although statolith orientation in the cap was random in seedlings fixed in flight. Recently, we gained the opportunity to re-examine root growth and development for seedlings grown in space during the 8-day STS-3 shuttle mission. Unlike previous investigators, we were able to examine plants which had been grown under more normal conditions of alternating L/D periods in controlled environment chambers. We report here the results of a comprehensive fine-structural study of morphologically well-preserved root tissues from the flight-grown plants.

MATERIALS AND METHODS

Oat (Avena sativa v. "Garry") and mung bean (Phaseolus aureus) seedlings were grown from seed entirely under hypogravity conditions aboard the shuttle (flight-grown) or in a 1-g (control) environment in a ground-based lab at the Kennedy Space Center. Growth parameters for the control plants were approximately the same as those experienced by the flight-grown seedlings. The seedlings were grown in completely enclosed plant growth chambers which were a part of the larger plant growth unit (PGU), the overall construction of which is illustrated in (3). Briefly, the metal base of the growth chamber contains two grooves into which 15 ml of agar in either distilled water (oats) or pH 6.8 Hoagland's solution (mung bean) was poured. Seeds were supported and germinated between two layers of Miracloth sandwiched between urethane foam. The foam was in contact with the underlying agar and served as a wick to pull moisture and nutrients up to the seedlings. Seedlings were grown under a 14/10 LD cycle beneath fluorescent light banks producing an average 400 ft-c. illumination at the center of the PGU. Temperatures in the chamber ranged from 23-24° C at night to 26-27° C during the day. Flight-grown seedlings remained under hypogravity conditions for 8 days. They did, however, experience various accelerating forces associated with landing and removal of the PGU

from the spacecraft. After landing, growth chambers were disassembled in the field and gross seedling morphology and growth orientation was photographically recorded. Plants were then removed from the chambers and prepared for anatomical and biochemical studies. Roots were carefully separated from the substrate, then excised and immediately immersed in fixative. Roots of control seedlings were handled in a similar manner, then both sets of samples were flown to the lab for further processing.

Roots were prepared for electron microscopy, employing standard fixation and embedding techniques for plant materials. Thin-sectioned samples were examined and photographed using a Phillips 300 TEM. Serial thick plastic sections (0.25 μm) of embedded roots were mounted on glass slides and stained with Azur I/II. These sections were used to correlate tissue structure at the light and electron microscopic levels.

RESULTS

The gross morphology of both control and flight-grown oat roots was normal. The meristem exhibited the usual monocot organization with three separate tiers of initials giving rise to a central vascular cylinder, cortex and rootcap, respectively. The peripheral cap cells were considerably more vacuolated in the flight-grown roots than in the control roots, but this was the only discernable difference between the two sets of plants. The central rootcap cells of both sets of roots exhibited numerous multigranular amyloplasts, which were randomly oriented. At the ultrastructural level, peripheral cap cells contained an extensive golgi apparatus. These cells produced copious amounts of "slime" sheath materials, presumably via golgi-mediated secretion. Cells of the meristem and central cylinder exhibited normal ultrastructural features, with the exception of mitochondria with a "swollen" morphology in the cortex cells. Cell-cell continuity in these tissues was maintained by plasmodesmatal connections and most cells exhibited prominent arrays of cortical microtubules. Various stages of cell division were observed in the meristem and cortex/cylinder tissues, but not in the rootcap. All stages of mitosis were seen in both sets of roots and EM characterization of the mitotic apparatus established the existence of typical spindle and kinetochore microtubules. Cytokinesis between daughter cells was completed by normal golgi/ER-mediated cell plate formation.

Flight-grown and control mung bean roots also exhibited normal tissue organization, with the meristem giving rise to a cortex, central cylinder and rootcap. In the flight-grown roots, however, the cap cells bordering the photodermis were degraded and compressed in appearance, as were the central cap cells. Overall, the cap was smaller in size than that of the control plants. Control cap cells were not collapsed, but were rounded with large, central vacuoles. The central cap cells were organized in longitudinal files, or a columella. The cap cells in both sets of plants exhibited numerous multigranular amyloplasts, similar to those seen in oat roots, but in flight-grown roots, there was a progressive degeneration of cell and organelle integrity peripheral to the meristem. Amyloplasts in these cells also were randomly oriented, as they were in the controls. The ultrastructural features throughout the remaining root tissues were normal and similar to those described for oat. No stages of cell division, however, were observed in either flight-grown or control mung bean roots, the interphase nuclei being characterized by small amounts of highly-condensed chromatin and prominent nucleoli.

Our preliminary observations suggest that hypogravity environments do not adversely affect gross organ differentiation, although in the case of the mung bean, certain tissues, such as the rootcap, may exhibit development irregularities. Whether such responses are species specific and the extent to which they might impair graviperception-response mechanisms remain intriguing and unanswered questions.

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GRAVITROPIC BASIS OF LEAF BLADE NASTIC CURVATURES

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The curvatures produced in leaf blades by auxin treatment have been described as nastic curvatures because the initial differential growth was always enhanced on the lower side regardless of the side of application. We now know, however, that blades can show differential growth of either the upper or the lower side depending upon conditions of treatment. Therefore, the dorsiventrality of the blade influences but does not limit the direction of curvature. Gravitational orientation is an important factor in determining the direction of curvature. The dorsiventral directionality of response to growth regulators and the response to changes in orientation to gravity suggest that blade curvatures are analogous to negative or positive gravitropism. Neither blade hyponasty or epinasty can be accounted for by ethylene alone. Petiole responses, however, are not directional, and the leaf angle changes produced by rotation or auxin treatment can be accounted for by ethylene production.

INTRODUCTION

Studies of the time course and quantity of ethylene production and the effects of growth regulators on the curvature of leaf blade, petiole, and pulvinus show that the nastic curvatures of the leaf have a gravitropic character.

MATERIALS AND METHODS

The primary leaves of seven-day old seedlings of Phaseolus vulgaris L. var. pinto were treated by spreading a solution of the indicated compound over the surface specified, adaxial (upper) or abaxial (lower). The compounds used in these experiments were: IAA (indole -3- acetic acid); TIBA (2,3,5-triiodobenzoic acid); NPA (Na-N-1-naphthylphthalamic acid); Ethrel (2-chloroethylphosphonic acid); CESA (2-chloroethylsulfonic acid); AVG (aminoethoxyvinylglycine, gift of Hoffman-LaRoche, Inc.); GA (gibberellic acid); K (Kinetin); ABA (abscisic acid); $AgNO_3$ (silver nitrate). Changes in blade form were measured by expressing the distance between laminar margins as a percentage of the full extended width. The leaf angle that subtends the laminar pulvinus was measured goniometrically. Curvature of the petiole was measured as the distance between the laminar pulvini. Ethylene production by excised seedlings or specified organs was measured by gas chromatography, allowing a two hour pretreatment time for wound ethylene to subside.

DISCUSSION OF THE RESULTS

While the amount of curvature produced is directly related to the concentration of auxin applied (9), the direction of curvature is determined by the conditions of application of auxin and other growth regulators. Auxin treatment can produce either epinasty or hyponasty depending upon temperature of incubation. (7) Studies of auxin transport through leaf discs show a preferential dorsi-ventral transport. In these experiments, auxin in lanolin paste was applied to the upper surface of leaf discs which were then placed on agar blocks. After two hours, the discs were discarded and the agar blocks were transferred to the upper side of fresh leaf blades and subsequent hyponasty was measured. These blocks produced 19% curvature, compared to 5% with agar control blocks and 47% with agar blocks containing 10^{-3} M.IAA. When the auxin was supplied to the lower surface of the disc and the receiver block was on the upper side, the block produced only 8% curvature. In future studies labelled IAA will be used and results will be quantitated directly instead of by the bioassay technique reported here. Studies of growth regulators applied in specified directions show that auxin transport inhibitors and ethylenegenic compounds are significantly more effective in inhibiting hyponasty when applied to the lower side opposite IAA upper than when the compounds are applied in the opposite direction. See Table I. The cytokinins inhibit hyponasty regardless of the side of application. (3) Gibberellic acid and abscisic acid do not produce, promote, or inhibit hyponasty regardless of the side of application. Only auxin transport inhibitors and ethylenegenic compounds have a consistent directional epinastic effect in the pathway of a preferred dorsiventral auxin response.

In its typical horizontal (diageotropic) position, the blade response to auxin application is hyponastic. When rotated on a clinostat or inverted, hyponasty of the blade is reduced, and the upper flank of the petiole is curved. (Table II). Rotation alone is sufficient to produce the

leaf angle response.

Table I. Effect of Direction of Application of Growth Regulators		
	% Inhibition of Hyponasty[a]	
Growth Regulator	Regulator Lower IAA Upper[b]	Regulator Upper IAA Lower[c]
TIBA	55	23
NPA	48	8
CESA	55	8
K	13	14
ABA	5	16
GA	7	8

a. maximum % hyponasty with IAA alone minus maximum % hyponasty with IAA plus regulator
b. mean % hyponasty with IAA upper side, 70%
c. mean % hyponasty with IAA lower side, 66%

Table II. Effect of Orientation to Gravity on Auxin Treated Leaves			
Treatment	% Blade Hyponasty at 6 hrs.	°Leaf Angle Change 24 hrs.	mm Petiole Distance Change 24 hrs.
Unrotated			
Control	4	-6	+3.5
IAA	49	-14	+6.7
Rotated (5 rpm)			
Control	12	-21	+3.0
IAA	26	-35	+6.7

The angle decrease is a well known gravitational response, (8) and can be shown to be associated with ethylene (1), (Table III). Auxin treatment of the blade results in promotion of ethylene synthesis, (4) which follows initiation of hyponasty but precedes the leaf angle change. Inhibition of ethylene synthesis by AVG or of ethylene action by silver nitrate inhibits the leaf angle decrease, and this is the only one of the three leaf responses to auxin that is inhibited by ethylene inhibitors.

Table III. Effect of Ethylene Inhibitors on Blade Hyponasty, Leaf Angle, and Ethylene Production			
Treatment	% Blade Hyponasty at 4 hrs.	°Leaf Angle Change at 24 hrs.	ppm Ethylene at 24 hrs.
Control	1	+2	0.2
$AgNO_3$	10	+5	-
AVG	10	+5	0.9
IAA	62	-30	2.4
IAA+AVG	75	-4	0.2
IAA+$AgNO_3$	68	-9	2.0

The pulvinus, which is the motor organ for leaf angle change, shows no directional response to the application of auxin. As in the petiole, application to the upper side of the pulvinus produces epinasty and application to the lower side produces hyponasty. (5) Immersion of excised petioles in auxin does not promote ethylene synthesis over that of sections immersed in buffer.

Table IV. C_2H_4 Production Promotion Over Controls by IAA μl/1/g. Fresh Weight		
Time	Blade Disc.	Excised Petiole
0 min.	0	0
30	2.4	-1
90	5.2	-1
180	10.8	-2

The blade appears to be the main source of the ethylene that produces the leaf angle decrease.

Since the potential exists for either dorso-concave or dorso-convex curvature of the blade in response to auxin treatment, these are not simply nastic curvatures. In the stem and the root, gravitropic responses can be easily demonstrated by a change in position from the vertical to the horizontal. The leaf blade is already in the horizontal position. Changes in its orientation will only reduce the gravitational influence on the cells. It is possible, though, to show through inversion and clinostat rotation the involvement of the gravitational vector in the response. Studies of auxin transport and transport inhibitors also show an upper to lower direction. Even though gravitropism cannot be directly demonstrated in untreated blades, the nastic curvatures of the leaf blade appear to be essentially gravitropic curvatures.

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Mechanical Stress Regulation of Growth and Photosynthetic Productivity of Glycine max (L.) Merr. cv. Wells II under Different Environmental Regimes.

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Brief seismo- or thigmo-treatments applied twice or three times daily to vegetative soybean plants as gyratory shaking or manual stem rubbing significantly retarded plant growth and photosynthetic productivity. Plants grown in a greenhouse in the summer under 1/3 to 1/2 of full sun showed a greater degree of sensitivity to mechanical stress than did plants grown under full sun. Growth dynamics analysis of plants grown in a controlled environment favoring mechanical responsivity indicated a decrease in relative growth rate (RGR) of shaken plants, indicating stress-induced inhibition of photosynthetic productivity. Changes in net assimilation rate (NAR) and leaf area ratio (LAR) are being assessed to determine which RGR components contribute to the observed changes in RGR. Short-term measurements of leaf gas exchange are being conducted to complement growth dynamics analysis.

Gravity is a physical force presumed important for the normal growth and development of higher plants. Mechanical disturbance is another type of physical force which has been found to affect plant development (4, 8, 13), although its influence is less vectorial and more dynamic than a static gravitational stimulus. On Earth, the natural physical force most important to plants is wind (5, 7, 10, 17). In our laboratory, we attempt to mimic aspects of wind action by agitating plants with a gyratory shaker. The usual response of herbaceous plants to periodic shaking is a general reduction in size, a response we have termed "seismomorphogenesis" (13). Typical growth responses to shaking include reduced internode length and diameter, reduced node number, smaller leaves, and lower shoot and root fresh and dry weight (1, 9, 12).

The importance of mechanical stress to the space program is not so much the reduction in plant size, but the reduction in photosynthetic productivity that accompanies it (12). Photosynthetic higher plants have been identified as key elements of human life support for future space-deployed, regenerative life support systems (3, 6, 18). In this role, plants may provide food, revitalize breathing air, purify waste water, and recycle nutrients in the closed system (16). It also is possible that mechanical forces may partially substitute for gravity in weightlessness. In this context, the acceleration and vibrations attending spacecraft operation may prove to be a cause for concern regarding photosynthetic productivity of "space crops." The present study characterizes and analyzes the effects of gyratory shaking on dry weight gain of young soybean (Glycine max (L.) Merr.) plants, a species with promising potential for supporting humans in a vegetarian diet.

Materials and Methods

Seeds of soybean (Glycine max (L.) Merr. cv. Wells II) were sorted for size and sown directly into 12.5 cm diam plastic pots containing a greenhouse soil mix. Seedlings were grown in a walk-in growth room having a photoperiod of 15.5 hr, a photosynthetic photon flux density (400-700 mm) of $310\mu Em^{-2}s^{-1}$, a diurnal temperature regime of 32.0°C day/28.5°C night, and a relative humidity of 43% day/70% night. Seedlings were selected for uniformity and thinned to one per pot six days after sowing. Plants were arranged in a completely randomized design before experimental treatments were initiated.

Plants to be shaken were carefully lifted by the pot and secured on a gyratory platform shaker, where they were agitated in a horizontal circular motion for 4 min three times daily (0830, 1430, and 2030 h) at 240 to 280 rpm. This treatment was termed "seismo-" stress. Other plants were manually rubbed three times daily with 20 up-and-down strokes along the upper stem and shoot tips, and side-to-side flexing of the stem. This treatment was termed "thigmo-" stress.

Upon termination of experimental treatment, plants were sectioned into various parts and measured for dimensions of length, diameter, area, and fresh and dry weight. Growth dynamics analysis was performed reporting parameters such as relative growth rate (RGR), leaf area ratio (LAR), and net assimilation rate (NAR). In one experiment, transpiration was monitored as water loss from the entire leaf canopy as a function of mechanical stress pretreatment. All data were subjected to analysis of variance and mean separation tested by LSDMOD at the 0.05 level of significance.

Results and Discussion

Preliminary greenhouse studies indicated that light level influences the extent of mechanical stress sensitivity exhibited by soybean. Photosynthetic photon flux densities ranging from 290 to $340\mu Em^{-2}s^{-1}$ from fluorescent and incandescent lamps in a growth room were found to result in good mechanical sensitivity and were used for all

subsequent experiments reported here.

Mechanical stress reduced the overall size of soybean relative to that of undisturbed controls, thigmo-treatments moreso than seismo-treatments. Although stem length was reduced by both mechanical treatments, leaf area was not reduced. Nevertheless, only shaking treatment reduced whole plant dry weight, and only stem rubbing increased stem density and leaf succulence. Thus, each mechanical treatment causes unique growth responses in soybean. Another difference between the two treatments included relative swelling of internodes of thigmo-treated plants, which was due mainly to the large decrease in internode length. This response did not occur with seismo-treatment.

Dry weight distribution among plant parts also was affected differentially by mechanical treatment. Rubbing the upper stem directed dry weight preferentially into lateral growth below the point of rubbing, suggesting that apical dominance had been broken. Enhanced lateral development occurred at the expense of stem and petiole development. The more general physical disturbance caused by shaking kept relatively more assimilated carbon in the main leaves while it reduced the proportion of dry weight partitioned into stem and roots.

Growth dynamics analysis revealed a reduction in RGR of seismo- and thigmo-treated plants caused by cumulative mechanical stress treatment. Relative growth rate of shaken plants averaged 4.7% lower than the RGR of controls, while that of rubbed plants was 8.6% lower. According to the relationship RGR = NAR X LAR (11), the overall decrease in RGR was due entirely to a reduction in NAR. Leaf area ratio actually increased in response to both treatments, but not enough to offset the decline in photosynthetic efficiency indicated by decreased NAR.

Transpiration rates were used to determine whether stress-induced stomatal closure was responsible for the observed decrease in NAR. Although transpiration rate of seismo-pretreatments averaged 9% lower than that of controls during the 45 min period immediately before a 4 min shake, this rate fell to 83% of the control rate from 0 to 45 min after the shake, and was 85% of control 45 to 90 min after the shake. Thus, the possibility of reduced carbon assimilation caused by shaking is inferred by these results, although this remains to be confirmed by direct measurement of CO_2 exchange rates as a function of mechanical pretreatment.

Comparison of soybeans under windbreak vs. exposed field conditions with undisturbed vs. mechanically-stressed plants reveals similarities in growth response, particularly between wind-exposed and shaken plants. Seismo-stress, like wind, represents general, diffuse, physical loading of shoots, whereas thigmo-stress may involve a more local, direct, physical contact. Wind has been shown to significantly limit the photosynthetic productivity and yield of soybeans in the field (2, 14, 15). Similarly, shaking or vibration may limit the photosynthetic productivity of soybeans growing in the space environment. The extent to which mechanical signals will impact on plant growth and development under hypogravity conditions remains to be determined by flight experiments.

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POLYAMINE FORMATION BY ARGININE DECARBOXYLASE AS A TRANSDUCER OF HORMONAL, ENVIRONMENTAL AND STRESS STIMULI IN HIGHER PLANTS.

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ABSTRACT

Recent evidence implicates polyamines including putrescine in the regulation of such diverse plant processes as cell division, embryogenesis and senescence. We find that the enzyme arginine decarboxylase, which controls the rate of putrescine formation in some plant systems, is activated by light acting through P_r phytochrome as a receptor, by the plant hormone gibberellic acid, by osmotic shock and by other stress stimuli. We therefore propose arginine decarboxylase as a possible transducer of the various initially received tropistic stimuli in plants. The putrescine formed could act by affecting cytoskeletal components.

INTRODUCTION

While polyamine biosynthesis in animal and microbial cells is initiated by ornithine decarboxylase, considerable evidence indicates a similar role in some higher plants for the enzyme arginine decarboxylase (ADC). In both the stem and leaves of pea seedlings and the young green leaves of oats, we have found a variety of physiological stimuli and stress reactions to affect the activity of ADC. Since the changes in ADC activity are often very rapid, require protein synthesis, and are reflected in changes in polyamine titer, ADC synthesis may be central to numerous rapid growth and adaptive reactions in plants. We shall describe here examples of control of ADC by light (absorbed by the pigment phytochrome) by a hormone (gibberellin), and by osmotic stress.

RESULTS

A. Control by Phytochrome. In plants, the photoreversible chromoprotein phytochrome controls many aspects of plant growth and development, including seed germination, leaf and stem growth, flowering and the onset of senescence. Red light (optimum near 660 nm) converts the inactive red-absorbing form of the pigment (P_r) to the active far-red-absorbing form (P_{fr}); far-red light (optimum near 730 nm) causes a reversion of P_{fr} to P_r and annuls the effect of red light. Since P_{fr} promotes leaf growth but inhibits stem elongation, it was of great interest to see whether the activity of ADC, which we had already shown to be influenced by light, would be affected in reverse fashion in the two organs.

Six day old etiolated pea plants were given a 5 min exposure to red light and the terminal buds (mainly leaf tissue) and subapical epicotyl (stem) tissue harvested and compared with dark controls. In buds, where the specific activity of ADC rose gradually in the controls over the eight hour test period, red light caused a highly significant doubling of ADC specific activity within two hours, and a continued rise until the end of the experiment. This effect was mediated by phytochrome, as shown by mutual red/far-red reversibility. In epicotyls, on the other hand, where the dark controls show a fairly steep rise in ADC activity over the eight hour test period, red light completely prevents this rise. This effect, too, is completely red/far-red reversible. Cycloheximide (5 µg/ml) strongly inhibits the increase of ADC activity in both rapidly-growing organs (darkened epicotyls and red-irradiated buds) but is without much effect on the already inhibited red-irradiated epicotyls and darkened buds. It thus appears that phytochrome controls the de novo synthesis of ADC in both organs, but in opposite ways.

Is the rise or fall of ADC activity merely a secondary consequence of the rise or fall of growth rate? We used a peculiarity of the etiolated pea system to show that changes in ADC are in fact independent of growth. If the terminal bud is excised together with a long piece of epicotyl consisting of several internodes, then red light promotes the growth of the terminal bud, although to a lesser extent than in the intact plant. If the bud is excised with only a short piece of attached epicotyl, red light cannot promote bud growth. Yet in both systems, red light promotes ADC activity in the buds. This shows that red light has its effect on ADC activity irrespective of its linkage to growth.

B. Control by Gibberellin. The isoprenoid plant hormone gibberellin, which promotes stem elongation, especially in dwarf and rosette plants, is also known to reverse

some effects of red light in etiolated plants and to mimic phytochrome effects in other systems. We therefore asked whether gibberellin could induce greater ADC activity in systems wherein cell division and growth are stimulated. We chose the light-grown Progress dwarf pea seedling as the experimental material. Gibberellic acid [GA; Sigma; 5-20 μg/ml in 0.03 M pH 6.4 phosphate buffer containing .05% pluronic L101 (Wyandotte) to aid penetration] was sprayed onto nine day old plants. Pluronic in buffer was sprayed as a control. GA treatment caused a great increase in internode elongation; thus two days after spraying, fourth internode length had increased from 9.78 mm in controls to 16.03 mm in treated plants and fifth internodes from 6.68 mm in controls to 21.50 mm in treated plants. ADC specific activity in the fourth internodes was increased threefold by 5 μg/ml gibberellic acid spray; at the same time ODC activity was decreased threefold. Putrescine content per internode was increased threefold in internode 5; spermidine and spermine increases were much lower.

A kinetic experiment showed that ADC activity was increased within 3 hours after application and peaked at about 9 hrs. ADC activity then declined and rose to a second (circadian?) peak at about 30 hours, accentuating a peak already apparent in the controls.

C. Control by Osmotic Stress. For several years, we have been concerned with the isolation, cultivation and attempted regeneration of protoplasts derived from the leaves of seedling oats. We noted that protoplasts, isolated from cells by a 2 hour cellulolytic digestion of walls in the presence of 0.6 M mannitol or sorbitol as an osmotic protectant, had a markedly altered polyamine profile. Putrescine increased 5-10 fold in 2 hrs and up to 60 fold in 6 hrs while spermidine and spermine were unaltered or slightly lower, and their oxidation product, 1,3-diaminopropane rose slightly. Control leaf segments floated on 1 mM phosphate buffer pH 5.8, showed no significant change in putrescine titer. Wild oat, barley, corn and wheat leaves showed similar changes; it is noteworthy that all were peeled except wheat, which showed distinct, albeit lower increases in putrescine without the complication of peeling injury.

The specific inhibitor of arginine decarboxylase DL-α-difluoromethyl arginine (DFMA) (0.1 to 1.0 mM) completely prevented the rise in putrescine level, while not affecting spermidine and spermine levels (Table I). DFMA also prevented the rise in ADC specific activity, but did not affect ODC. Conversely, DL-α-difluoromethylornithine (DFMO), a specific inhibitor of ODC in mammalian cells, had little effect on ODC activity, but significantly raised ADC activity and putrescine content. We conclude that the effect of sorbitol in increasing putrescine titer is mediated by an increase in ADC activity. Since other osmotica such as mannitol, proline, betaine and, to a lesser extent sucrose, produce the same effect, we conclude that water stress provided by the osmotica serves to induce the appearance of ADC.

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TABLE I. Polyamine titer and biosynthetic enzyme activity in oat leaf segments under osmotic stress. Numbers for polyamine levels represent mean ± S.E.M. *Significantly different from the 4 hr buffer control ($P<.05$). **Significantly different at $P<.01$. # Protein was determined by the Coomassie Blue G-250 assay, using gamma globulin as the standard.

Treatment	Polyamine (nmol/g.f.wt.) Putrescine	Spermidine	Spermine	Enzyme activity ($nmolCO_2$/hr/mg protein) # ADC	ODC
0 hr. Control	16±1	161±18	41±4	2.27	4.24
4 hr. Control	13±2	152±10	38±3	1.98	4.55
0.6 M Sorbitol	102±5**	120±13*	21±2**	3.81**	4.65
" + 1.0 mM DFMO	129±7**	186±10*	32±3	5.31**	4.68
" + 0.1 mM DFMA	20±2*	123±7*	36±2	1.24*	4.64
" + 1.0 mM DFMA	10±1	128±8	32±2	0.41**	4.55

THE INTERACTION OF CALLOSE AND ETHYLENE IN THIGMOMORPHOGENESIS.

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In the bean plant, thigmomorphogenesis is exhibited as a decrease in elongation coupled with an increase in thickening of the stem. We have observed several physiological and biochemical changes that occur and that seem to be part of the causal mechanism of this syndrome. Prominant among these is the production of a "pulse" burst of the phytohormone, ethylene, which begins 30 to 40 min after mechanical perturbation (MP) of the stem. This "pulse" of ethylene evolution usually peaks at 2 to 3 h and disappears by 5 to 6 h after MP. More recently, we have studied the deposition of the B-1,3-glucan, callose following MP. Callose deposition begins immediately after MP, increases up to 6 h, and the callose begins to become resorbed thereafter, decreasing steadily to the 0 h level by 3 days after MP. Furthermore, we have found that the glucose analog, 2-deoxy-D-glucose (DDG), specifically inhibits callose deposition, and that it also has a somewhat lesser inhibitory effect on the subsequent production of ethylene. In addition, DDG also inhibits thigmomorphogenesis, suggesting that callose deposition may play a causal role in thigmomorphogenesis.

We have studied these phenomena in a number of very different systems, and in each case, callose deposition (which is inhibited by DDG) precedes ethylene evolution, and DDG is always capable of inhibiting the physiological phenomenon, as well as callose deposition. For these reasons, it seems worthwhile to consider the possibility that callose deposition is the first link in the causal chain of MP-induced thigmomorphogenesis which is mediated by the "pulse" of endogenous ethylene. In other words, it is possible that in all of the systems which we have studied, callose deposition in some way causes the production of ethylene.

On the basis of the available evidence, 2 hypotheses have been formulated to attempt to explain such a causal relationship. The first is a "physico-chemical" one. according to this model, MP induces callose to be deposited on the inner face of the cell wall. As it accumulates, it presses against the plasma membrane, perturbing it. Since it is currently thought that the site of ethylene biosynthesis is the cell surface, the possibility exists that the perturbation of the plasma membrane may be part of the induction mechanism of ethylene biosynthesis during thigmomorphogenesis. We intend to test this hypothesis by doing electron microscopy of the regions of callose deposition to see if we can see any deformation of the plasma membrane.

The second hypothesis may be called the "elicitor" hypothesis. According to this model, elicitors, which may be oligosaccharide precursors of callose biosynthesis or oligosaccharide breakdown products of callose, induce the "pulse" of ethylene production by acting on some site of the biosynthetic pathway of ethylene biosynthesis. Such elicitors have been shown to cause the production of stress metabolites such as phytoallexins, and stress metabolites have been shown to be produced as a result of various types of environmental perturbations as well as by exogenous ethylene. If this model is correct, known elicitors should cause ethylene production, thigmomorphogenesis and the synthesis of stress metabolites.

We have tested the elicitor hypothesis using the bean first internode as assay material. Table 1 shows that two known oligosaccharide elicitors, one from pectin (P.E.) and one from soybean glucan (G.E.), as well as arachidonic acid, a fatty acid recently shown to have elicitor activity, all induce a decrease in elongation and

TABLE 1. The effect of known elicitors on bean 1st - internode growth and ethylene evolution.

Treatment	% of 0 h		C_2H_4
	Elongation	Thickness	µl/g/h
No MP, no additive	183	1.3	4.1
MP, no additive	98	4.4	5.5
No MP + 1µg/µl P.E.	183	4.8	4.3
No MP + 40µg/µl G.E.	170	4.9	3.2
No MP + 1mM Ar.A.	124	3.6	8.4

an increase in internode diameter comparable to the changes caused by MP. In addition, MP and al of the elicitors but G.E. induce an increase in ethylene production. The elicitors were also tested for ethylene and stress metabolite production in the soybean cotyledon bioassay. In all cases, the know elicitors caused both a significant increase in ethylene evolution, a well as an increase in stress metabolite. In addition, an elvant of bean first internodes, also had elicitor activity, in that it induced an increase in stress metabolite in the soybean cotyledon bioassay (Table 2).

Thus, it seems that elicitors may be causally involved in the thigmomorphogenetic syndrome. It remains to be seen if elvants from the internodes of MP plants contain more elicitor activity than do those of controls.

TABLE 2. The effects of known elicitors on ethylene evolution and stress metabolite production by the soybean cotyledon bioassay.

Treatment	C_2H_4 (µl/g/h)	Stress Metabolite (285 mm)
H_2O (control)	0.64 a	0.185 a
P.E.	1.10 b	0.450 c
G.E.	1.70 c	0.420 c
A.R.A.	1.80 b	0.430 c
BEAN ELUANT	——	0.279 b

CHANGES IN OSTEOBLASTIC ACTIVITY DUE TO SIMULATED WEIGHTLESS CONDITIONS.

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ABSTRACT

Using histochemistry and electron microscopy, we have studied the reduced bone formation which occurs in the hypokinetic, orthostatically treated adult rat. The two major changes noted occured in the osteoblast population, indicated by a reduced alkaline phosphatase activity and reduced numbers of gap junctions between cells. These results were most noticeable in the periosteum and endosteum of the long bones. Changes in osteoblasts lining the surface of trabecular bone were not as evident. These results indicate that the cells lining the surfaces of weight bearing bones are most affected by hypokinesia and this reduction in cellular activity may be a mechanically induced effect.

INTRODUCTION

The osteoblast lays down new collagen in an organized lamellar pattern, a pattern which may be influenced by the change in rate of collagen synthesis (1) or a change in mechanical stress placed upon the mineralized tissue (2). Subsequent disuse of bone results in its loss no matter if this occurs within normal gravity (3) or in the hypogravity environment (4). The model of Morey-Holton, whereby hypokinesis and ortho stasis are applied to animals, results in significant bone loss and reduced new bone formation (5).

The purpose of this study was to visualize by electron microscopy, the alkaline phosphatase activity of the osteoblast population and the gap junction connections between osteoblasts and the adjacent cells of the periosteum. It might be expected that in either case, a reduction in these parameters would be a good indication of an effect of hypokinesis and orthostasis on the ability of osteoblasts to form new bone.

METHODS

Adult rats were suspended in the hypokinetic/orthostatic model of Morey-Holton. After 14 days of treatment the animals were sacrificed and the distal end of the femur including portions of the diaphysis were preserved in 2% glutaraldehyde for electron microscopy and alkaline phosphatase localization (6).

RESULTS

Following 14 days of hypokinesia, the histochemical results indicated that alkaline phosphatase activity was reduced in the osteoblast population compared to controls (figure 1). This reduction was most obvious in those osteoblasts along the diaphyseal bone surface. We are presently trying to quantitate this activity to determine if osteoblasts in trabecular bone were affected to the same extent as osteoblasts in diaphyseal bone. It was noted that the alkaline phosphatase in the diaphyseal growth plates did not show any change in activity due to the hypokinesis.

Previous studies of normal bone (7) indicated that specialized "gap" junctions exist between all cells of bone and that perhaps this was a pathway whereby bone cells could communicate with each other. In the present study we located gap junctions by electron microscopy in those bone cells associated with periosteal surfaces (figure 2). Efforts to quantitate these results are in progress but it was initially determined that the number of gap junctions between cells of the suspended animals was greatly reduced compared to controls. There is some indication that the junctions present under hypokinetic conditions also occupied less surface area of the osteoblast compared to the junctional area on the control osteoblasts.

DISCUSSION

These results suggest that the reduced bone formation found in long bones of hypokinetic, orthostatic rats is caused by a reduced activity of the osteoblast population. A reduction in activity of differentiated osteoblasts is a critical observation because it suggests that these cellular responses are directly affected by the mechanical stress applied to bone and when this stress is relieved, the cells no longer receive "signals" to function in their normal capacity. Exactly how these signals are translated by the cell to modify their behavior is not presently known.

ACKNOWLEDGEMENTS

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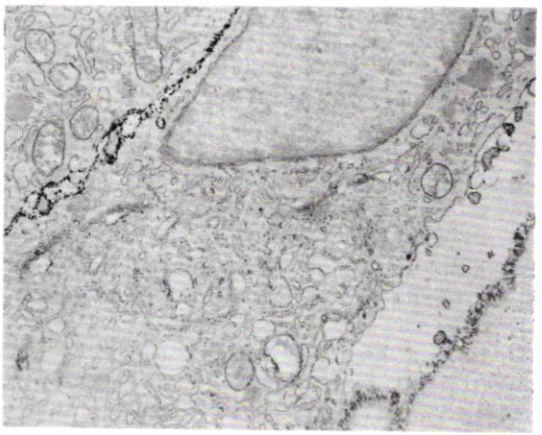

Figure 1.

Alkaline phosphatase activity along the osteoblast cell membrane demonstrated by electron microscopic histochemistry. The reaction product (the dense black deposits) is localized at sites of enzyme activity and was generally reduced in amount in those osteoblasts from the hypokinetically treated animals.

Magnification: 12,000X

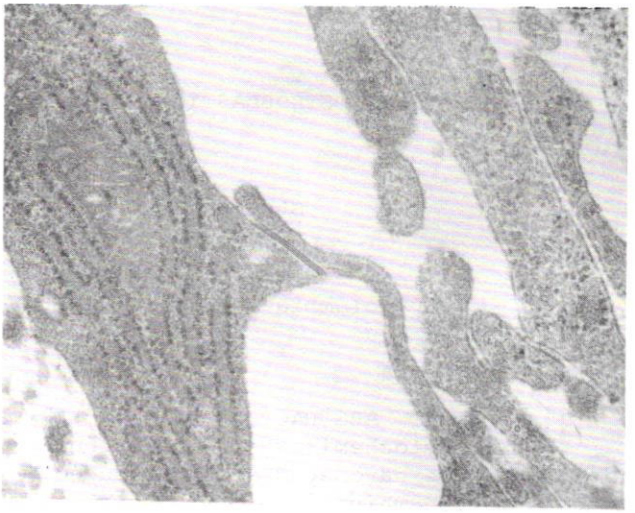

Figure 2.

Gap junctions occurred between adjacent osteoblasts and, as seen in this micrograph, between osteoblasts and the adjacent periosteal cells. These junctions occurred less often between cells when the animals had been subjected to hypokinesis.

Magnification: 40,000X

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CALCIUM TRANSPORT FROM THE INTESTINE AND INTO BONE IN A RAT MODEL SIMULATING WEIGHTLESSNESS

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ABSTRACT

The objective of this study was to determine whether a defect in transport of calcium in the duodenum was related to decreased bone formation in the suspended rat. Rats were suspended by the tail at a 40° angle for up to 15 days. ^{45}Ca was injected into the ligated duodenum in situ 15 minutes prior to sacrifice. Blood, tibia, vertebra and humerus were obtained for total calcium and ^{45}Ca analyses. Intestinal calcium transport did not appear to be significantly altered by suspension. However, by 5 days of suspension a significant decrease in accumulation of ^{45}Ca into tibia and vertebra was observed. A trend of decreasing bone mineral and mass was established in tibia and vertebra by the fifth day of suspension. The humerus failed to demonstrate a significant weight decrease or change in ^{45}Ca accumulation after 15 days of suspension. Results from this simulated weightlessness model suggest that transport of calcium from intestine into bone is decreased within 5 days of suspension. This deficiency appears to be associated with a progressive decrease in total mass of non-weightbearing bones.

INTRODUCTION

During space flight, animals including humans progressively lose bone mineral (1). This loss of bone could potentially limit extended space exploration. A similar, albeit slower, loss of bone occurs during prolonged immobilization (2). Suspension of rats to unweight their hindlimbs may provide a good model for simulating weightlessness. Our study was designed to determine whether suspension of the rat leads to a general loss of mass and mineral from both weighted and unweighted bones and to evaluate the role of intestinal calcium absorption in the negative calcium balance that ensues upon suspension. Our results indicate that local factors in bone, not systemic factors, are the principal mediators of bone loss in this model of simulated weightlessness.

METHODS

Male Sprague Dawley rats, 120-140 g, (Hilltop), were suspended at a 40° angle for up to 15 days by their tails such that their hindlimbs were unweighted but their forelimbs were normally weighted. They were otherwise free to move about the cage. Control rats of the same strain, sex and weight were pair fed to the suspended animals for the same period of time. At the appropriate times the rats were anesthetized with chloral hydrate (310 mg/kg/body wt), the proximal duodenum was surgically exposed and ligated to form an isolated sac with intact blood supply, and this sac was injected with 300 µl of a modified Trowells-HEPES buffer containing 2.5 mM calcium, 3 µCi ^{45}Ca and 4 µCi ^{3}H-polyethylene glycol (PEG) (3). After 15 minutes blood was collected for total calcium and ^{45}Ca determinations. The duodenal

TABLE 1. Effect of suspension on bone weight (wt), ash, and calcium content (Ca)

Days Suspended	N	Vertebra Wt[1]	Vertebra Ash[2]	Vertebra Ca[3]	Tibia Wt	Tibia Ash	Tibia Ca	Humerus Wt	Humerus Ash	Humerus Ca	Mandible Ash	Mandible Ca
Pair fed	9	3.61	1.99	.78	12.3	6.86	2.68	7.79	4.25	1.75	9.74	3.87
± SD		.44	.16	.05	.4	.45	.14	.30	.23	.25	.59	.43
5d	8	3.49	1.90	.75	12.4	6.89	2.62	8.06	4.43	1.79	9.82	3.92
± SD		.66	.26	.12	.9	.40	.19	.49	.20	.24	.56	.34
7d	8	3.13*	1.64*	.63*	13.1	6.75	2.51*	7.88	4.34	1.71	10.11	3.88
± SD		.39	.24	.09	2.3	.50	.06	.42	.23	.12	.81	.42
10d	7	3.01*	1.60*	.61*	11.4*	6.42*	2.52	7.88	4.22	1.73	10.51	4.13
± SD		.32	.19	.03	.7	.36	.16	.66	.30	.21	.67	.30
15d	8	2.90*	1.46*	.59*	11.0*	6.10*	2.31*	7.76	4.27	1.71	10.71	3.90
± SD		.34	.16	.07	1.4	.28	.19	.30	.24	.17	1.69	.21

[1] mg fat free weight/g weight x 10; [2] mg ash weight/g body weight x 10; [3]mg calcium/g body weight x 10

*P < 0.05 vs pair fed

sac was emptied, and the lumenal contents were analyzed for ^{45}Ca and ^{3}H-PEG. The duodenal tissue of the sac was solubilized in 2 ml 2 M NaOH, and then analyzed for protein, ^{45}Ca, ^{3}H-PEG, and total calcium. One tibia and humerus, the L1 vertebra, and the mandible (in some experiments) were removed, extracted sequentially in ethanol and ether, ashed in a muffle furnace, and analyzed for fat free weight, ash weight, total calcium, and ^{45}Ca (3).

RESULTS

The rats used in these experiments tolerated suspension by their tails remarkably well. Their weight was progressive and equivalent to pair fed unsuspended rats. Suspension results in a steady loss of mass from the unweighted tibia and vertebra but not from the normally weighted humerus and mandible (Table 1). The vertebra loses bone more quickly than the tibia. Calcium uptake by the tibia and vertebra is depressed compared to pair fed controls after 5 (but not 2) days of suspension (Table 2). However, this depressed calcium uptake is reversed by 10 days and is increased above pair fed controls after 15 days of suspension. The humerus does not show significant changes in calcium uptake during suspension. Duodenal calcium transport and serum calcium levels have not been consistently altered by suspension (Table 3). In the experiment contained in Table 3 an increase in serum calcium was observed during suspension which was associated with an initial fall in intestinal calcium transport. However, the differences are subtle and generally not significant.

TABLE 2. Effect of suspension on calcium-45 uptake by bone.

Days Susp.	N	Vertebra cpm[1]/bone	Vertebra bone[2]/serum	Tibia cpm[1]/bone	Tibia bone[2]/serum	Humerus cpm[1]/bone	Humerus bone[2]/serum
Expt 1							
2d	9	.89	1.01	.94	1.05	ND	ND
±SD		.23	.30	.22	.31		
5d	8	.72*	.72*	.59*	.60	ND	ND
±SD		.14	.10	.15	.12		
Expt 2							
2d	5	.88	.99	.96	.97	1.01	1.02
±SD		.25	.25	.29	.27	.26	.26
5d	4	.60*	.90	.46*	.70*	.68	.97
±SD		.17	.15	.13	.17	.38	.32
10d	5	.95	1.01	.78	.83	.99	1.05
±SD		.25	.26	.30	.33	.25	.25
15d	6	1.46*	1.35*	1.36*	1.26*	1.14	1.08
±SD		.27	.17	.21	.11	.19	.15

[1]Ratio of ^{45}Ca cpm/total bone in the suspended animals to that of the simultaneously studied pair fed controls.

[2]Ratio of specific activity (cpm ^{45}Ca/mg Ca) in bone/serum $\left(\frac{^{45}Ca \text{ bone} \times \text{mg Ca serum}}{\text{mg Ca bone} \times ^{45}Ca \text{ serum}}\right)$ of the suspended animals to that of the simultaneously studied pair fed controls.

*P < 0.05 compared to pair fed controls.

TABLE 3. Effect of suspension on duodenal calcium transport and serum calcium levels

Days Suspended	Control	5d	7d	10d	15d
Ca trans[1]	4.23	4.33	3.85	4.14	4.90
±SD	.42	.47	.69	.66	.83
Serum Ca[2]	9.29	9.83*	9.70	9.69*	9.68*
±SD	.39	.45	.64	.29	.33
N	9	8	8	7	8

[1]nmol Ca transported from the duodenal sac/mg prot

[2]mg Ca/dl serum

*P < 0.05 vs pair fed controls

DISCUSSION

The rat suspended by its tail provides a useful model for studies of the effect of gravity on the skeleton. In such a model only some of the normally weight bearing bones (e.g. lumbar vertebrae and tibiae) are unweighted, whereas other bones (e.g. humerus) remain weighted. This provides an internal control to determine whether changes in bone mass following the unweighting of selected bones are mediated by systemic factors such as parathyroid hormone and/or the vitamin D metabolites or by local factors such as changes in blood flow and/or alterations in piezoelectric ion fluxes. If systemic factors dominate, one would expect that all bones would be affected; if local factors dominate, one would expect that only the unweighted bones would be affected. Our data indicate that local factors dominate. Only the unweighted bones lose mass during suspension. Furthermore, duodenal calcium transport, a process regulated by 1,25-dihydroxyvitamin D (4) is not altered. The loss of bone could be due to an increased rate of resorption, a decreased rate of formation, or both. We measured calcium-45 uptake by bone as an index of bone formation. Our results indicate a decreased rate of calcium uptake by the tibia and vertebra after 5 d of suspension. This returns to control levels by 10 d and is greater than control levels by 15 d. The humerus does not show such changes. These results suggest an initial depression in bone formation. Bone formation may be stimulated subsequently if bone resorption is increased and is coupled to bone formation. Regardless, the continued loss of bone indicates that bone resorption exceeds bone formation at least for the 15 d of the study. In conclusion, we find a progressive loss of bone mass and mineral during suspension but only in the unweighted bones. The local factors mediating this selective bone loss need to be identified. The suspended rat appears to be a good model for such studies.

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IS SUPPRESSION OF BONE FORMATION DURING SIMULATED WEIGHTLESSNESS RELATED TO GLUCOCORTICOID LEVELS?

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ABSTRACT

To investigate the hypothesis that suppression of bone formation in the suspended rat model was the result of increased levels of corticosterone, experiments were performed on young, growing, male rats exposed either to 4°C or suspended for two weeks. Rats suspended on the model system, designed to simulate certain aspects of spaceflight, gained weight at a rate at least equal to control animals but still showed a significant suppression of bone formation within 7 days. Cold-exposed rats gained less weight than their corresponding control group and did not demonstrate any suppression of bone formation. These findings suggest: 1) tail suspension is less stressful than previously used harness systems, 2) suspension in young, rapidly growing rats causes a significant suppression of cortical bone formation, 3) cold exposure does not alter bone formation rate in rats of a similar age and strain to those suspended in this study, and 4) suppression of bone formation provoked by unloading the rear limbs is not due solely to sustained stimulation of the pituitary-adrenal system.

INTRODUCTION

Increased levels of glucocorticoids are known to influence bone cell activity (1). Skeletal alterations induced by simulated weightlessness (2) or spaceflight (3) in rats could be related to a continual stimulation of pituitary-adrenal function. To investigate the hypothesis that suppression of bone formation in the rat model designed to simulate certain aspects of spaceflight might be due to corticosterone, experiments were performed on young, growing rats exposed either to 4°C for 2 weeks or suspended for 2 weeks. Cold-exposure, a well-defined chronic "stressor" (4), should induce similar bone changes to suspended rats if, indeed, the bone perturbations are due solely to chronic stress.

METHODS

Male, Sprague-Dawley derived, Hilltop rats (Scottsdale, PA) were approximately 40 days of age at the beginning of the experimental period. Rats were randomized according to weight and were either suspended as previously described (5) or exposed to 4°C according to the procedure of Vernikos et al. (4) for two weeks. Control rats were fed the average amount of chow consumed by the suspended rats the preceding day or were fed ad libitum for the cold-stress portion of the experiment. Rats exposed to the cold tended to consume more food than did the control group while suspended rats were noted to eat about 20% less than the ad libitum controls. All animals were fed Wayne Rat Chow and water was always available. The light:dark cycle in the vivarium and in the cold chamber was 12:12 with lights on at 7AM. Rats were injected with demeclocycline, 10 mg/kg body mass, at the beginning and on the seventh day of the experiment. At the end of the second week of the experiment, rats were euthanized and the tibias removed. Cross-sections, approximately 50 μm thick, were sawed just distal to the tibiofibular junction and were photographed under UV light. The area between labels was measured using a digitizing tablet interfaced with a PDP 11/34, and bone formation rate was determined by dividing the area by the number of days between labels.

RESULTS

Tail suspension appears to be well tolerated by rats of this strain and age as indicated by the body mass data in Table 1. In fact, suspended rats gained significantly more weight during the first week as compared with the pair-fed controls. The rate of weight gain in the suspended animals was also significantly greater during the first week as compared to controls (8.6±1.18 g/day vs 6.6±0.79 g/day, $P<0.005$). However, the rate of weight gain over the two week period was very similar in both groups (6.6 ± 1.02 vs 6.5±0.49 g/day). The cold-exposed rats did not weigh significantly less than controls (Table 1), but the rate of gain was significantly less not only during the first week (5.1±0.38 vs 6.2±0.75 g/day, $P<0.005$) but also for the entire two week period (5.5±0.36 vs 6.5±0.81 g/day, $P<0.01$).

Table 1. BODY MASS DATA IN SUSPENDED AND COLD-EXPOSED ANIMALS

Group	N	Body Mass, g Initial	7 day	14 days
Suspended				
Control	12	141±7.1[1]	187±8.6	230±8.8
Experiment	7	138±7.2	198±9.3*	230±15.7
Cold-Stress				
Control	8	179±7.2	223±9.8	271±13.8
Experiment	8	180±10.9	216±11.0	257±12.4

[1] mean±1 S.D.
*$P<0.025$ compared to corresponding control

Table 2. PERIOSTEAL BONE FORMATION RATE AT THE TIBIOFIBULAR JUNCTION IN SUSPENDED AND COLD-STRESSED RATS

Group	N	Rate of Bone Formation at the Tibiofibular Junction, mm^3/day 0-7 days	N	0-14 days
Suspended				
Control	11	0.0605±0.00885[1]	12	0.0631±0.00753
Experimental	3	0.0481±0.00290	7	0.0478±0.01449
% change from control		-20		-24
P		<0.005		<0.025
Cold-Stress				
Control	8	0.0394±0.00549	8	0.0407±0.00540
Experimental	8	0.0406±0.00525	8	0.0397±0.00512
% change from control		+3		-2
P		>0.05		>0.05

[1]mean ± 1 S.D.

The rate of periosteal bone formation at the tibiofibular junction in suspended and cold-stressed rats as compared to control animals is noted in Table 2. Within the first week of suspension, a 20% reduction in bone formation was noted in the experimental animals as compared with the control group; the decrease was highly significant. No further reduction in bone formation was noted between 7 and 14 days of suspension. Animals housed at 4°C did not show any alteration in bone formation at this sampling site at either 7 or 14 days. (Table 2).

DISCUSSION

Young, rapidly growing rats appear to tolerate suspension by the base of the tail extremely well. The rate of weight gain is very comparable to control rats fed the same amount of food. No differences were noted in any adrenocortical tissue examined, e.g. adrenal weight, thymus weight, or serum corticosterone levels. Lack of change in such tissues may reflect adaptation as noted by Vernikos et al. (4). These authors noted that low A.M. levels of both plasma and adrenal corticosterone levels returned almost to control levels within 7 days of cold-exposure. However, the circadian peak rose significantly higher than the unstressed controls, and cold-exposed animals showed faster, greater, and more-sustained response to acute ip injection of saline. Our animals were always guillotined between 9 and 11AM, and great care was taken not to acutely stress any animal. Also, our control rats for the suspension experiments were not allowed to feed ad libitum which may have imposed some stress on the control group. However, unlike cold-stressed rats which gained weight at a significantly slower rate than controls, the suspended animals gained weight as rapidly as their control group. These data suggest that tail suspension is no more stressful than pair-feeding of control rats and produces a growth curve which nearly parallels that of ad-lib control animals.

Suspended, rapidly growing, male rats show significant suppression of bone formation at the periosteal surface of the tibiofibular junction during the first week of suspension as compared with control animals (Table 2). This suppression of bone formation rate continues during the second week of suspension. Although this suppression of formation is not as great as previously reported (5), animals used in previous experiments were from a different supplier and did not gain weight at a rate comparable to the animals used in these experiments. Interestingly, the cold-stressed rats which gained weight more slowly than their controls did not exhibit any alteration in periosteal bone formation rate (Table 2). Thus, suppression of bone formation provoked by unloading the rear limbs is not due solely to either an elevation of serum corticosterone or to sustained stimulation of the pituitary-adrenal system. However, changes in steroid receptor number or sensitivity might occur in unloaded limbs; such changes could not be ruled out in this study.

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BONE MINERAL ANALYSIS OF RAT VERTEBRA FOLLOWING SPACE FLIGHT: COSMOS 1129

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INTRODUCTION

Over the past ten years a vast amount of research pertaining to space flight induced osteopenia has been conducted. Attempts have been made to determine its etiology, the severity of its consequences and its reversibility upon return to earth's environment. These studies, incorporating biochemical, histological and biomechanical analysis techniques and various ground based experimental models to simulate the effects of space flight have centered mainly upon the cortical and cancellous regions of long bone to determine the bone dynamic change caused by weightlessness. Little, if any, of this work has concentrated on bone loss and skeletal readaptation within the vertebral column. Information pertaining to this skeletal region is important because of the association of reduced cancellous vertebral bone with decreased mechanical stress resistance within the vertebral column, the consequence of which are much more severe than fracture of appendages.

The mission of COSMOS 1129 was to study the adaptive abilities of organisms to microgravity followed by readaptation in earth's environment. One experiment of this project dealt with alteration in rat skeletal mineral dynamics during exposure to microgravity and earth's readaptation. This paper describes the methods and results of a comparative vertebral centrum bone mineral content analysis from ground based control and space flown rats.

MATERIALS AND METHODS

Experimental Animal. Male Wistar rats weighing approximately 270 to 320 gms. and about 85 days of age were subjected to orbital space flight aboard the COSMOS 1129 Biosatellite. A synchronous exposure experiment provided rats for controls. These animal groups were handled in the following manner.

Flight (F): The flight animals were exposed to 18.5 days of space flight in individual life support containers. After space flight recovery, these animals were either sacrificed immediately (R+0) or allowed to readapt to earth's environment for 6 (R+6) or 29 (R+29) days before sacrifice.

Synchronous (S): The ground based synchronous experiment simulated an environment as close as possible to that experienced by the flight rats without weightlessness exposure.

Specimen Preparation & Chemical Analysis. The vertebral centrums were prepared for analysis by disarticulation from the vertebral column via transection of the intervertebral disk and synovial articular capsules and by removal of posterior processes at the pedicle and all adjoining soft tissue. These specimens were subjected to compression tests then frozen in distilled water in preparation for mineral/element analysis.

Each sample was thawed, removed from its container and rinsed with distilled water (the rinse being saved in the original container). The rinsed specimen was dried, desiccated, and weighed to the nearest .01 mg. to obtain the total specimen weight. The weighed vertebral centrum and its accompanying distilled water were digested in 10% nitric acid. The prepared analytic was stored at 4°C.

Three basic chemical analysis techniques conducted in accordance with 40 CFR 136 were utilized for the bone mineral/element content analysis. The analysis technique employed and the mineral/elements analyzed included atomic absorption spectrophotometry for Ca, Mg, Na, K, Ba, Sr, Y, Pb and Mn, specific ion electrodes for F^- and Cl^-, and ascorbic acid colorimetric analysis method for PO_4^-. In certain cases for atomic absorption analysis technique, Lanthanum Chloride (Ca) and KCl(Ba) were used as additions for controlling chemical interference and ionization. For each technique, standardizing calibration procedures were accomplished daily to insure accuracy and reliability of the instrumentation. Because some of the minerals analyzed revealed low sample concentrations, the minimum detection limit capable for the analyses technique employed was determined. Also duplicate analysis were completed on a number of vertebra for each mineral analysis to determine the reproducibility of the data.

DATA ANALYSIS

To facilitate data analysis and comparison, the vertebral centra were grouped into six equal positions according to decending level. ($P_1=T_2-T_3-T_4$, $P_2=T_5-T_6-T_7 \ldots P_6=L_5-L_6-L_7$).

Means and standard deviations were determined for each exposure group (F and S), at each vertebral position (P_1 through P_6) and for each recovery period (R+0, R+6, and R+29). The students T statistical analysis method was used to determine significant differences between exposure groups for different recovery periods.

RESULTS

Seven of the total minerals/elements analyzed were found unacceptable for further consideration. The reasons for their rejection were either that the majority of data were at or below the detection limit (Cl^-, F^-, Mn, Pb and Y) or

contaminates from blood produced an unacceptable data error (Newman). Chemical content findings for Sr, Ba, Mg, PO_4^- and Ca follow.

Strontium. An analysis of Sr indicated levels ranging from 0. to .2% weight with the smallest concentration occurring in the upper thoracic and progressively increasing with decreasing vertebral position (P_1 to P_6). This trend remains constant over exposure and recovery period. There were no significant differences for F and S animals.

Barium. Barium ranged from .1 to 1% wt. The slope of all % wt. Ba vs. vertebral position curve was approximately 0. This trend remained the same for all exposure/recovery groups (F/R+0, F/R+6, F/R+29, S/R+0, S/R+6 and S/R+29). The S rat showed significantly decreasing amounts of Ba with increasing recovery period length. The F group, however, demonstrated no significant difference between recovery periods at a level corresponding to R+6 for the controls.

Magnesium. Magnesium indicated wt. % levels ranging from .3 to .5 with the slope of all % wt. mg vs. vertebral position curves approximately 0. The S animals showed no significant difference for Mg content between recovery groups. The F group wt. % Mg values showed increased levels at R+0 returning to S levels at R+6 and R+29.

Phosphate. PO_4^- ranged from 9 to 13% wt. All exposure/recovery groups indicated a decrease in wt. % value with decreasing vertebral level (P_1 to P_6). No significant difference was demonstrated statistically between F and S at any recovery period.

Calcium. Ca content ranged from 19 to 24% wt. (see Figure 1). These values showed decreasing mineral content with decreasing vertebral level for all exposure/recovery combinations excepting for F/R+6 and S/R+6. For these cases the mineral content increased with decreasing vertebral level. Statistically, there was no significant difference between recovery periods within either exposure, but there was a significant difference between F and S for each recovery period. The interrelationship between recovery period within F and S seem to have the same orientation graphically (see Figure 1).

DISCUSSION

The purpose of this paper is to present results from a selective mineral/element analysis of F and S rat vertebral centra, and no attempt will be made to explain these results with specific physiologic conclusions. A selective review of the literature was found to be useful in explaining some of the results in general. The observation that when comparing F and S rats, the wt. % of PO_4^- remains constant while the Ca values are decreased may be an indicator of incomplete osteoid mineralization. Neuman (1980) provides information suggesting that calcium to phosphorous ratio of newly forming bone is dependent upon the extent of mineralization in the osteoid. Further research is required to clarify this observation. The results of Ca content analysis seem to support the observations of Wronski, et al. (1981) pertaining to F rat bone formation and resorption. Their conclusions indicated that space flight exposure caused an arrest in bone formation with no change in resorption rate. This may be supported by the observation that the curves for wt. % Ca level for F and S are similarly oriented with the F set of curves at decreased wt. % values. These results may indicate that similar mineral dynamics are functioning with all gravity resisting skeletal structures in the rat. Lastly, these results do not correlate well with the results for higher order animals, as indicated by the results of Klein. Using Ca^{45} to study the steady-state relationship between bone and blood in dogs, chicks, and rats, he found that in the rats, bone resorption is dissociated early (2 weeks of life) from bone resorption. He concluded that the rat is not an appropriate model for studying active bone turnover. The results of this study seem to indicate a similar conclusion.

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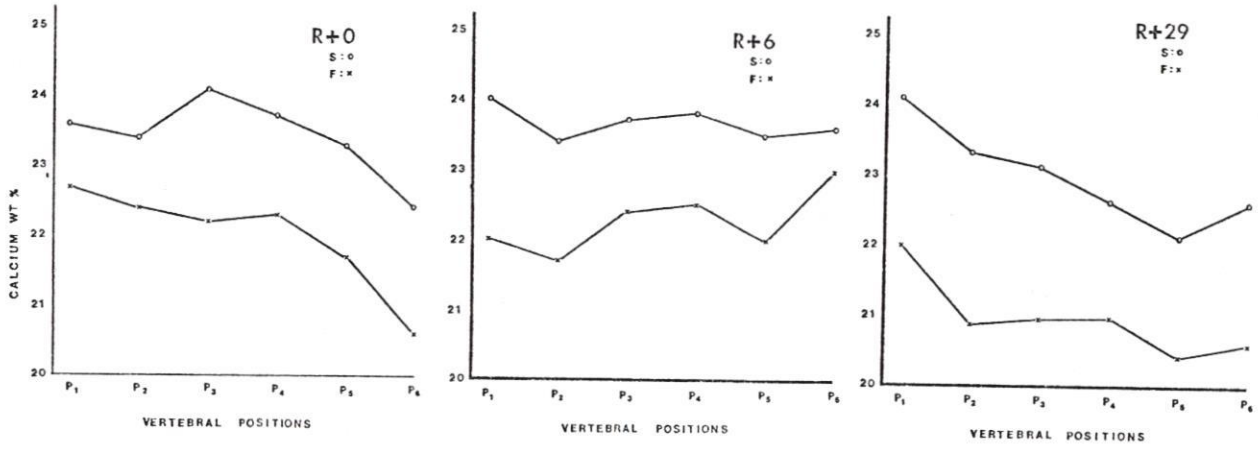

Figure 1. Rat vertebral centrum weight % Ca for Flight (F) and Synchronous (S) exposures at recovery periods 0 (R+0), 6 (R+6) and 29 (R+29) days

ALTERATIONS IN MITOCHONDRIA AND SARCOPLASMIC RETICULUM FROM HEART AND SKELETAL MUSCLE OF HORIZONTALLY CASTED PRIMATES

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INTRODUCTION

Weightlessness (zero G) produces a series of physiological changes collectively termed cardiovascular deconditioning. This syndrome is characterized by orthrostatic intolerance, decreased plasma volume, increased resting heart rate and fluid shifts to the upper body (6). These changes are also characteristic of bed-rested humans (6). Recent studies (1,2) have demonstrated that horizontally-casted primates exhibit the same physiologic changes seen in bed-rested humans and post-flight astronauts.

A common feature in all these conditions (1,2,6) is inactivity and loss of hydrostatic fluid volume. These changes may be due altered peripheral circulatory control, altered CNS influences or from primary myocardial involvement in cardiovascular deconditioning as seen in humans following bed rest and, to date, in all cosmonauts and astronauts after space flight. The horizontally body casted primate (Macaca mulatta) has been used as an animal model to simulate bed rest and space flight findings. The preliminary data clearly indicate depressed calcium transport activity in cardiac sarcoplasmic reticulum isolated from horizontally casted primates.

METHODS

Four male rhesus monkeys ranging in weight from 9 to 11 kg were used in these studies. Two animals were body casted for 30 days (1) and two uncasted animals served as controls. Cardiac mitochondria (9) and sarcoplasmic reticulum (3) were isolated by established techniques. Skeletal muscle mitochondria were isolated by modifications of a previously described method (4). Mitochondrial respiratory activity was measured by a polarographic technique (8). Mitochondrial and sarcoplasmic reticulum calcium transport were measured by dual-beam spectrophotometry as previously described (3,8).

RESULTS

No significant differences were found in heart mitochondrial respiratory functions between control

Table 1: Functional Activities of Mitochondria from Heart and Skeletal Muscle of 30-Day Casted Primates. (n = 2 for each group, respectively).

	Yield†	ADP:O*		Q_{O2}**		Cyt. Oxid.+	Ca^{2+} Uptake++
Heart		(G-M)	(Succ.)	(G-M)	(Succ.)		
Controls	24.5	2.8	1.7	322	281	0.902	360
30-Day	23.4	3.0	1.8	311	224	1.53	272
S. Muscle							
Controls	-	2.5	1.6	131	127	-	231
30-Day	-	2.9	1.5	84	70	-	103

†Yield = mgs mitochondria per gm weight tissue.
*ADP:O = Ratio of nmoles ADP phosphorylated to natoms oxygen consumed. g-m = glutamate-malate as substrate; Succ. = succinate as substrate.
**Q_{O2} = Rate of optimal phosphorylating respiration (state 3) expressed in: natoms oxygen consumed per min. mg^{-1} mitochondrial protein.
+Cytochrome oxidase specific activity expressed in: µmoles cytochrome c oxidized per min. mg^{-1} mitochondrial protein.
++Respiratory substrate-supported rates of calcium uptake expressed in: nmoles Ca^{2+} per min. mg^{-1} mitochondrial protein.

Table 2: Calcium Transport in Cardiac Sarcoplasmic Reticulum from Control and 30-Day Casted Primates.

	$R_B^{\dagger}$	Total B*	Uptake**
Controls	23.4	44.5	156
30-Day	11.1	29.8	61.3

$^{\dagger}R_B$ = initial rate of calcium binding in absence of oxalate following ATP addition expressed as: nmoles Ca^{2+} per mg SR protein 5 sec^{-1}.
*Total B = Total amount of calcium bound by SR in absence of oxalate expressed in: nmoles Ca^{2+} per mg sarcoplasmic reticulum protein.
**Uptake = Rate of calcium uptake by sarcoplasmic reticulum in presence of oxalate expressed in: nmoles Ca^{2+} accumulated per min. mg $protein^{-1}$.

and 30-day casted animals (Table 1: ADP:O and Q_{O2}). The efficiency of oxidative phosphorylation (Table 1; ADP:O) was within normal limits for both NADH-linked (G-M) and succinate supported (Succ) respiration in control and experimental animals. Rates of phosphorylating respiration (Table 1; Q_{O2}) were essentially the same for both control and 30-day casted animals. A marked increase in cytochrome oxidase activity (Table 1; Cyt. Oxid.) was observed in the casted primate heart mitochondria compared to controls. A 25% decrease in respiratory substrate-supported calcium uptake was found in casted primate heart mitochondria compared to controls (Table 1; Ca^{2+} uptake). Skeletal muscle mitochondria from casted animals exhibited marked decreases in both phosphorylating respiration and substrate-supported calcium uptake compared to controls (Table 1; S. muscle Q_{O2} and Ca^{2+} uptake).

Sarcoplasmic reticulum isolated from control and casted primate hearts revealed marked changes in calcium transport activities (Table 2). The casted primate had decreased rates of ATP-supported calcium binding (Table 2; R_B) and uptake. Total amounts of calcium bound by cardiac sarcoplasmic reticulum of casted primates was also depressed (Table 2; Total B).

DISCUSSION

It should be emphasized at the outset that these data are quite preliminary. However, the results are quite consistent even though the sample size is small. The lack of any differences in heart mitochondrial oxidative phosphorylation (Table 1) indicates that no apparent changes have occured in the primary energy-producing system of 30-day casted primate heart. The reasons for both the increased cytochrome oxidase activity and depressed calcium transport in the casted animal heart mitochondria are at present unclear. The decreased respiratory activity in skeletal muscle mitochondria from the casted animals (Table 1) is not too surprising. It has been repeatedly demonstrated that exercised-trained skeletal muscle mitochondria exhibit increased respiratory activity compared to sedentary controls (5). In the case of the casted primate, a "disuse atrophy" of skeletal muscle may very likely be the cause of decreased mitochondrial activity. The rates of calcium transport in these mitochondria can be explained by the decreased respiratory rates, since calcium transport is driven by respiratory substrate.

The changes in cardiac sarcoplasmic reticulum from casted primates are striking (Table 2), although any interpretation at this point must be approached with caution. Substantial evidence exists in the literature which correlates depressed sarcoplasmic reticulum function with altered states of myocardial contractile performance (7). Sarcoplasmic reticulum is considered to be a primary system in regulating beat-to-beat fluxes of calcium in the mammalian heart. The marked depression in cardiac sarcoplasmic reticulum functions suggest altered calcium homeostasis in the casted-primate heart which could be a factor in cardiovascular deconditioning.

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EFFECT OF SUSPENSION HYPOKINESIA/HYPODYNAMIA ON GLUCOCORTICOID RECEPTOR LEVELS IN RAT HINDLIMB MUSCLES

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ABSTRACT

Suspension hypokinesia/hypodynamia (H/H) results in differential atrophy of hindlimb muscles in rats. Data obtained from studies of humans and rats exposed to weightlessness suggests a potential role for glucocorticoids in disuse atrophy. Glucocorticoid receptor levels were assessed in muscles from control and 7 day suspended rats to evaluate a role for glucocorticoids. Specific binding of ^{3}H-dexamethasone in the gastrocnemius, plantaris, and EDL muscles of control rats was quantitatively similar while specific binding in the soleus was lower. Suspension resulted in a differential alteration of receptor levels which corresponded with the extent of muscle atrophy. These studies point to the involvement of glucocorticoids in muscle atrophy resulting from H/H.

INTRODUCTION

An examination of rats exposed to weightlessness during the COSMOS program revealed a marked atrophy of hindlimb muscles (1,2). A suspension model has been developed to simulate these weightlessness-induced alterations. In agreement with the COSMOS studies, suspension hypokinesia/hypodynamia (H/H) results in differential (soleus > gastrocnemius = plantaris > EDL) atrophy of hindlimb muscles in rats (3,4). Flight studies (5,6) and evidence from suspended rats (3,7,8) document alterations of adrenal morphology as well as plasma levels and urinary excretion of glucocorticoids. Recent studies (9,10) have demonstrated elevated glucocorticoid receptor numbers in the gastrocnemius muscle following immobilization and denervation. The present studies were undertaken: i) to evaluate the effect of suspension H/H on glucocorticoid receptor levels in rat hindlimb muscles, and ii) to ascertain whether altered receptor levels reflect the differential nature of hindlimb muscle atrophy during suspension H/H.

METHODS

Male Sprague-Dawley rats (180-200g) were suspended in a denim/velcro harness as previously described (3). After 7 days of suspension animals were sacrificed by cervical dislocation and the soleus, gastrocnemius, plantaris and EDL muscles from 3-4 animals were excised. Muscles from both age- and weight-matched animals were used to determine control parameters. Pooled samples of each muscle were minced, homogenized in 1-2 volumes of buffer (10mM Tris-HCl, 1.5mM EDTA, 10mM sodium molybdate, 10mM monothioglycerol and 10% glycerol, pH 7.6) and the 100,000 x g supernatants incubated for 20 hours with increasing concentrations of ^{3}H-dexamethasone (0.4-16nM) in the absence (total binding) and presence (nonspecific binding) of a 100-fold excess of unlabeled dexamethasone at 4°C. Incubations were terminated by adsorption of free steroids with dextran-coated charcoal and aliquots counted. Specific binding to receptor sites was determined as the difference between total and nonspecific binding.

RESULTS AND DISCUSSION

The time course of ^{3}H-dexamethasone binding to muscle cytosol was investigated and apparent equilibrium conditions were attained at incubation times of 20 hours at 4°C. The ligand specificity of the receptor site was typical of that anticipated for a glucocorticoid receptor. Receptor levels were similar in the gastrocnemius, plantaris and EDL muscles of control rats (Table 1). The soleus, composed primarily of slow twitch oxidative fibers, had a much lower receptor level. Following 7 days of suspension, receptor numbers were significantly elevated in all muscles but the EDL. The relative increase in receptor numbers thus reflects the differential nature of the muscle atrophy previously reported (3,4). Apparent k_d values were not altered by H/H, mean values for controls ranging from 3.0 to 6.0nM, while mean values for muscles from suspended

rats ranged from 3.5 to 5.5nM. Alterations in endogenous glucocorticoid levels cannot account for these observations as normal plasma corticosterone levels have been reported in the rat following 7 days of H/H (7,8). These findings suggest that differential muscle atrophy resulting from H/H may result from differential alterations of glucocorticoid receptor levels.

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Table 1. Maximum ^{3}H-dexamethasone binding capacity (fmol/mg cytosol protein) in hindlimb muscles from control and hypokinetic/hypodynamic(H/H) rats.

	Control	H/H
Soleus	25.0 ± 4.1 (3)	138.3 ± 17.7* (3)
Gastrocnemius	45.0 ± 4.1 (6)	59.0 ± 4.3* (8)
Plantaris	60.0 ± 5.7 (7)	82.7 ± 10.0* (8)
EDL	54.9 ± 7.9 (7)	53.9 ± 8.3 (7)

+ Mean ± SEM (number of observations)
* Significantly different from control by the t-test ($P < 0.05$)

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EVALUATION OF THE RESPONSE OF RAT SKELETAL MUSCLE TO A MODEL OF WEIGHTLESSNESS

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Suspension of rats in a head-down tilt position such that their hind limbs are non-load bearing has been proposed as a model for weightlessness. Changes observed in metabolism, bone formation (1), and muscle catabolism (2) support the validity of the model. To further document this model, we have investigated the effects of suspension on the mechanical, biochemical and histochemical characterisitics of two hind limb skeletal muscles, the gastrocnemius and the soleus.

Methods and Results

Our suspension technique is the same as that employed by Morey et al. (1) and Mussachia et al. (2), with the exception that we use a steel pin sutured to the rat's back to support the suspended rat. We have found this type of support to the spine to prolong the amount of time the rat can remain suspended. Our investigations have involved rats suspended either one, two or four weeks. Recovery from suspension has also been investigated in rats suspended for two weeks and subsequently allowed to recover for one week. In our studies experimental and control rats have been sex and weight matched, and when possible have been litter matched.

As indicated by Table I, rats suspended for either 1, 2 or 4 weeks gained weight. Although there was no difference in the body weights of suspended and control rats, the suspended rats were more stressed by their confinement as indicated by an increase in adrenal weights which was related to the length of the suspension period (Table I).

In agreement with a previously reported weight loss observed for the gastrocnemius after one week of suspension (2), we found the wet weights of both the gastrocnemius and soleus muscles to decrease during suspension (Table I) approaching asymptotic values by four weeks of suspension.

Corresponding to the muscle atrophy indicated by this weight loss, mechanical parameters of the soleus muscle declined with suspension. For this phase of the study, an *in situ* muscle preparation was used. Briefly, the rat was anesthetized and the soleus muscle and corresponding sciatic nerve were exposed. The muscle's distal tendon was severed and attached to a force transducer for isometric measurements. Muscle length was set at Lmax by clamping the knee and foot and by adjusting the muscle length by a micrometer attached to the force transducer. The blood supply to the muscle was intact and the muscle was electrically stimulated supermaximally through the sciatic nerve. The lower half of the rat with the exception of the nerve incision was immersed in Ringer's solution at 37°C and aerated with a gas mixture of 95% O_2 and 5% CO_2. For muscles obtained from suspended rats, developed tension, the maximal rate of tension development (dT/dt), contraction time and one-half relaxation time declined (Table I). In the rats suspended for two weeks and allowed to recover for one week before being studied, the contractile parameters of developed tension and one-half relaxation time recovered and were not significantly different from control values, whereas, dT/dt and contraction time remained significantly less than control values.

The distribution of Type I and II myosin in soleus muscles was determined in rats suspended two and four weeks and in rats allowed to recover for one week after a two week suspension period. The myosin content was determined by sodium pyrophosphate-solubilization and pyrophosphate-polyacrylamide gel electrophoresis. As shown in Table I, following suspension Type II myosin did not significantly change from control while the amount of Type I myosin decreased. The decline in Type I myosin agrees with the shortening of contraction and relaxation times observed for the soleus muscles receiving similar treatment.

A histochemical evaluation of the gastrocnemius and soleus muscles showed progressive atrophy in both muscles with suspension. Both also showed positive esterase and DPNH staining after two weeks of suspension. Whereas the gastrocnemius muscle maintained Type II predominance during suspension and recovery, the soleus muscles, which in the majority of cases showed Type I dominance, did in some muscles show fiber type reversal or Type II predominance. This greater atrophy of Type I fibers, like the change in myosin distribution, may partially explain our observed changes in the twitch time course for the soleus muscle.

Conclusions

A comparison of our data with those obtained from space-traveled rats of the Cosmos series

indicates the Morey technique of rat suspension is a valid model for weightlessness. A decrease in contractile strength and a decline of the contraction time duration of the soleus muscle was demonstrated in rats after both 20.5 and 22 days of space flight by Cosmos 690 and 605, respectively (3). Further, a possible explanation for the latter effect on the soleus muscles was provided by studies performed on space-traveled rats from Cosmos 1126 (4). These data showed that soleus muscles from space-traveled rats exhibited greater atrophy of Type I muscle fibers.

Acknowledgements

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TABLE I: Response of Rat Skeletal muscle to suspension-hypokinesia and recovery

	Control	Suspension duration 1 Week	2 Weeks	4 Weeks	Recovery[1]
Body weights (grams gained per day)		>1.0g	1.98g	1.43g	
Adrenal weights (mgms)					
Right	15.9±0.8		20.2±1.1	27.9±1.5	
Left	16.7±0.6		20.8±0.6	28.0±1.1	
Muscle wet weights					
Soleus (mgms)	154±8		80±2	62±2	
Gastroc (gms)	1.10±0.055		0.58±0.029	0.46±0.023	
Soleus Mechanical data:					
Developed tension (gms)	26.5±4	12±1.5	9.5±1.5		20±1
dT/dt (gms/sec)	3400±250	1550±160	1250±250		1850±100
Contraction Time (msec)	39±2	30±0.5	26.5±1.0		31.5±1.5
1/2 Relaxation time (msec)	73±4	54±3	38±2		60±3
Soleus Myosin (µg Myosin/mg tissue)					
Total Myosin	11.5±0.75	16±1.5	4.5±0.5	2.0±0.5	12±2
Slow twitch Myosin (Type I)	10.0±0.50	13.5±2	3.25±0.5	1.25±0.25	9±2
Fast twitch Myosin (Type II)	1.5±0.25	2.5±0.75	1.25±0.25	0.75±0.25	2±0.5
Histochemistry:					
Gastroc:					
Atrophy		↔	+	++	
Fiber type predominance		II	II	II	II
Small angular fibers:		↔		+	+
DPNH		↔		+	+
Soleus:					
Atrophy		+	++	+++	↔+
Fiber type predominance		I	I	Variable[2]	I
Small angular fibers:		↔	+	+	+
DPNH		↔	+	+	+
Esterase		↔	+	+	+

↔-no change from control
+-increase from control
I-slow twitch fiber predominance over fast twitch fiber type
II-fast twitch fiber predominance over slow twitch fiber type

1) Rats suspended two weeks and were allowed to recover 1 week.
2) of 13 rats, fiber Type I>II in 7 rats, equal distribution of I and II in 3 and type II>I in 3 rats.

SYNTHESIS OF AMINO ACIDS IN WEIGHT BEARING AND NON-WEIGHT BEARING LEG MUSCLES OF SUSPENDED RATS

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ABSTRACT

The effect of hypokinesia (HYP) for 6 days on the de novo synthesis of glutamine (GLN) and glutamate (GLU), and of alanine was tested in isolated leg muscles of intact, adrenalectomized (ADX) and ADX cortisol-treated rats. The net synthesis of GLN and GLU was lower in soleus muscles of HYP animals of these three groups of rats. The synthesis of alanine was lowered by HYP in ADX animals and apparently raised by HYP in ADX cortisol-treated rats. No HYP effect was seen in the extensor digitorum longus (EDL) muscles of these animals. Although ADX lowered the synthesis of GLN and GLU in soleus muscles of control rats, while cortisol treatment restored this process to near normal, neither ADX nor cortisol treatment produced any effect in the HYP animals. However, effects of ADX and cortisol treatment on synthesis of GLN and GLU in EDL muscles and of alanine in both muscles seemed normal in HYP animals.

INTRODUCTION

We have reported previously (1) that the tail suspension hypokinesia model described by Morey-Holton and Wronski (2) reduced the size of the soleus muscle to near maximum after 6 days, but had no effect on the size of the extensor digitorum longus muscles of these animals. Atrophy of the soleus muscle in hypokinesia must thereby result in the net liberation of amino acids from this tissue. In muscle, the de novo synthesis of alanine and glutamine provide a vehicle for removing nitrogenous waste from the tissue (3). Therefore, we tested whether hypokinesia might increase the de novo synthesis of these amino acids to facilitate removal of amino groups and free ammonia derived from the breakdown of excess amino acids in the tissue.

METHODS

Female albino rats (80-100 g) bred by the Division of Animal Resources at the University of Arizona were used in these experiments. Tail suspension hypokinesia was carried out for 6 days by the method of Morey-Holton and Wronski (2). Control animals had their tails suspended but their hindlimbs were allowed to remain weight bearing. Where indicated, bilateral adrenalectomy was performed 3 days prior to suspension and the animals were provided with 1% NaCl solution until sacrifice. Cortisol treatment was initiated on the day of suspension and animals were injected subcutaneously at mid-day with 2 mg cortisol acetate/100 g body wt. The cortisol acetate was suspended in 0.9% NaCl solution. Muscle incubations (4) and measurement of net synthesis of alanine, glutamine and glutamate (5) were carried out as described previously. Data were collected from 9 or 10 animals in each group and compared for significant differences by the unpaired Student's t test. The data are given as means $\pm$ SEM.

RESULTS

Caldwell et al (6) reported that production of glutamine and alanine by perfused hindquarters of adrenalectomized rats was lower than of intact rats. We found a similar result in isolated leg muscles of suspended weight bearing rats (Table 1). Although our data include both glutamine and glutamate, most of this combined analysis consistently reflects the synthesis of glutamine. Amino acid synthesis was lower by 29-53% in muscles of adrenalectomized, weight bearing rats than in intact controls. This effect was due to a lack of circulating glucocorticoids since administration of cortisol prevented the marked decrease in the synthesis of alanine and glutamine measured in the isolated muscles. Adrenalectomy of the hypokinetic animals resulted in a lower rate of alanine and glutamine synthesis in extensor digitorum longus muscles, and of alanine synthesis in soleus muscles. Cortisol administration prevented this lost capacity for amino acid synthesis.

Despite these normal responses of amino acid synthesis to adrenalectomy in muscles of hypokinetic rats, synthesis of glutamine was not significantly lower in soleus muscles of adrenalectomized hypokinetic rats. This apparent lack of response may be attributed to the already low basal rates of synthesis of glutamine seen in muscles of intact hypokinetic rats. Hypokinesia lowered this process in soleus muscles only, whether the animals were intact or adrenalectomized. Cortisol treatment had no effect on this synthesis of glutamine in the soleus muscles of hypokinetic rats.

Although the pattern of response of alanine synthesis to adrenalectomy and cortisol treatment seemed normal in hypokinetic soleus muscle, some apparent effects of hypokinesia on this process could be detected. In untreated adrenalectomized rats, hypokinesia lowered the synthesis of alanine but in cortisol-treated animals this process was apparently increased by hypokinesia.

DISCUSSION

The most sriking effect of hypokinesia on amino acid metabolism shown here is the diminution in the synthesis of glutamine by soleus muscles. The lack of any effect on this process in extensor digitorum longus muscles, which do not seem to atrophy in hypokinesia (2,7), suggest that decreased use of the soleus muscle may reduce its capacity for synthesizing glutamine. Since the results reported here are preliminary, we have no exact explanation for this observation. Several possibilities include: a) lower activity of glutamine synthetase, b) less free ammonia availability for glutamine synthesis, and/or c) decreased degradation of amino acids to provide α-amino groups for the synthesis of glutamine. This latter possibility may be unlikely since synthesis of alanine was unaffected in intact rats. However, this latter process is 79% slower than glutamine synthesis in control muscles and may not be as sensitive to decreased availability of amino groups under these conditions. These observations may reflect several metabolic changes in muscle occuring simultaneously and could involve alterations of both carbonous and nitrogenous precursors. Clearly extensive further studies are required to provide an explanation for these rather striking responses of the soleus muscle to hypokinesia.

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TABLE 1

Effect of Hypokinesia on Synthesis of Amino Acids by Leg Muscles of Intact and Adrenalectomized Rats

	Synthesis of Amino Acids (nmol/mg muscle/2h)					
	Intact		Adrenalectomized		Adrenalectomized plus cortisol	
	Glutamine + Glutamate	Alanine	Glutamine + Glutamate	Alanine	Glutamine + Glutamate	Alanine
	Soleus					
Suspended weight bearing	4.45±0.49	1.49±0.06	2.09±0.17[c]	0.95±0.08[d]	3.29±0.21[e]	1.25±0.09[c,f]
Suspended non-weight bearing	1.63±0.28	1.51±0.06	1.06±0.17	0.72±0.06[d]	1.08±0.19	1.61±0.09[e]
Difference (%)	-63[a]	ns	-49[a]	-24[b]	-67[a]	+29[b]
	Extensor Digitorum Longus					
Suspended weight bearing	3.69±0.36	0.94±0.06	1.78±0.15[d]	0.67±0.06[d]	3.96±0.24[e]	0.84±0.07[e]
Suspended non-weight bearing	3.60±0.33	0.97±0.05	1.76±0.15[d]	0.71±0.09[c]	3.54±0.27[e]	0.87±0.09[f]
Difference (%)	ns	ns	ns	ns	ns	ns

Significant difference from weight bearing: [a]$P<0.001$; [b]$P<0.050$.
Significant difference from intact: [c]$P<0.020$; [d]$P<0.001$.
Significant difference from adrenalectomized: [e]$P<0.001$; [f]$P<0.020$.
No significant difference from weight bearing: [ns]$P>0.100$

WEIGHTLESSNESS HYPOKINESIA: SIGNIFICANCE OF MOTOR UNIT STUDIES

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ABSTRACT

In preliminary experiments, we found that medial gastrocnemius (MG) motor units in cage-reared rats were unusually sensitive to fatigue, a test for which is required to distinguish between FF- and FR-unit types. This observation prompted an analysis of whole-muscle fatigue. It revealed an impairment of the electrochemical process of neuronal excitation-contraction coupling at one or several sites between motor axons and the contractile machinery. (Supported by NASA Grant NAGW-338).

INTRODUCTION

Following preliminary experiments (see Abstract), we undertook an analysis of whole-muscle fatigue in the MG of cage-reared rats, together with an estimation of the fatigability characteristics of MG in a theoretical "noncaged-reared" rat model, assuming the theoretical muscle would have unit properties like those found in hindlimb muscles of noncage-reared cats (3,5,6).

METHODS

We used 500 g male and female 100-day old Sprague-Dawley rats, who had spent their life in 46-49 x 25-28 x 20 cm cages (4-7/cage). Such confinement is typical for commercially bought rats. Under pentabarbitol sodium anesthesia (30-70 mg/Kg, I.P.), MG was prepared for force and EMG measurements (3,5,6).

RESULTS

Experiments. Fig. 1 shows force and EMG responses of MG (N = 7) . Note three points:

1. Before the fatigue test, the disparity between force output for direct and nerve stimulation was greater than considered normal (2,4).
2. The fatigue test produced more substantial reductions in force and EMG than anticipated (3,5). Unfortunately, there are no data comparable to Fig. 1 for noncage-reared rats. However, Table 1 (lines 8, 11) suggests that EMG (force) reduction would be 23% (62%) in the theoretical MG as compared to 66% (79%) in our test MG (Fig. 1).
3. Intermittent and abrupt changes in EMG amplitude were a feature of each stimulus train of the fatigue test, even before force declined.

Estimations. Three assumptions in the Table 1 estimations require explanation:

1. Values for the relative distribution of the different fiber types (Table 1, line 1) are only available for cage-reared rats. They should not differ markedly in noncage-reared rats (3).
2. The assumption that the amplitude of the EMG co-varies with muscle-fiber diameter$^{3/2}$ is based on measurements made on "in-continuity" mammalian nerve axons (8).
3. At first glance, the assumption might seem far-fetched that relative (inter-unit) features of motor-unit anatomy (Table 1, lines 1-4), force development (line 5) and fatigability (lines 7, 10) are similar for rat MG and cat tibialis posterior (TP; footnotes 3-4). However, the specific tension (force/cross-section) of muscle tissue is quite similar across species (6), as are presumably the EMG:force relationships.

DISCUSSION

Orderly motor-unit recruitment (3) should prevail in cage-reared rats with cumulative activation (recruitment) of progressively more forceful units for progressively stronger contractions. As a result, it is likely that the FF units of cage-reared rats are used to less extent than the less forceful FR and S units (3,6). On this basis, we propose that the impairment in neuronal excitation-contraction coupling observed in these experiments is more pronounced in FF than FR and S units. We also anticipate that the observed impairments would be less in the soleus (SOL) muscle because, for the motor activities possible in a confined environment, SOL is presumably used to a greater extent than MG (3,6). Conversely, the observed deficits should be similar in another commonly studied muscle, extensor digitorum longus (EDL), because, like MG, its usage is limited during less demanding contractions (unpublished observations).

To address these issues, we intend to examine the dependence of the observed impairments on the fatigability of muscle by comparing the fatigue profiles of MG, SOL and EDL in exercise-deprived, exercised-exposed and wild rats. The existance of such a relationship would underscore the desirability of assuring an appropriate level of functional integrity of test muscles prior to the application of a disuse protocol to simulate weightlessness.

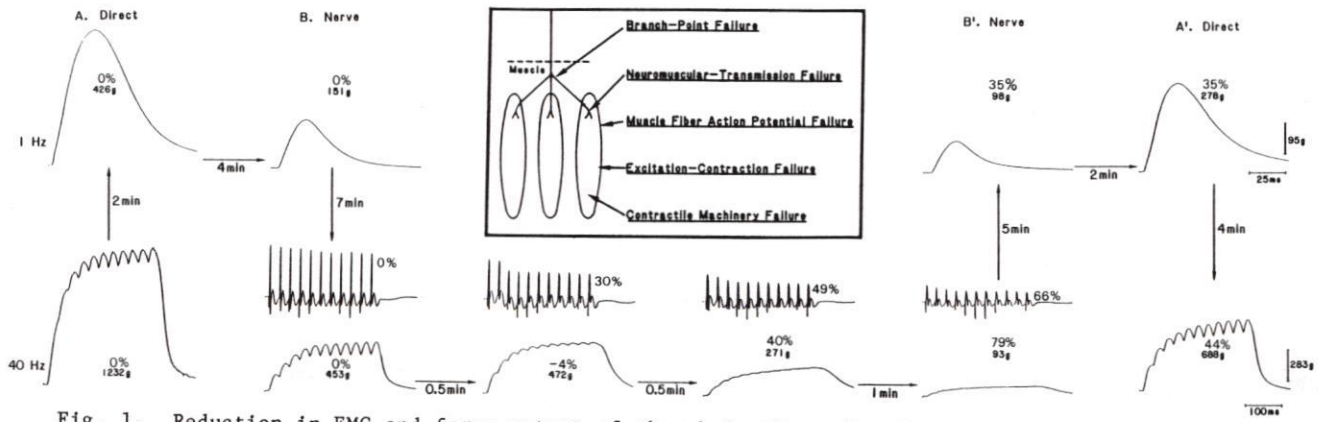

Fig. 1. Reduction in EMG and force output of the whole MG muscle of a cage-reared rat. Upper force profiles: averages of 8 supramaximal twitches (1 Hz). Lower force and EMG traces: single-sweep records of subfused tetani (40 Hz). Stimulus duration; 2 ms for direct intramuscular stimulation (A, A^1), 0.1 ms for muscle nerve (B, B^1). Arrows (mins): stimulus sequence (interstimulus intervals). Fatigue test: 330 ms-duration 40 Hz stimulus trains at 1 Hz for two min. Records indicate 1st (B), 31st, 61st and 121st (B^1) trains. Amplitudes of EMG (mean) and force (peak) expressed as reduction from "control" values (0%). Insert: sites at which fatigue might occur during muscle-nerve stimulation (7).

TABLE 1. Estimation of Mean Reductions in EMG and Force Output of MG Motor Units of Theoretical "Noncage-reared" Rats During a Fatigue Test[1]

		Unit (Fiber) Type		
		FF(FG)	FR(FOG)	S(SO)
(1)	Muscle fiber distribution (%)[2]	38	58	4
(2)	Relative muscle-fiber diameter[3,4]	1.26	0.95	0.79
(3)	Relative motor-unit innervation ratio[3,4,5]	1.26	0.84	0.90
(4)	Motor-unit distribution (%) $(100\cdot(1)\cdot(3)^{-1}\cdot\Sigma(1)^{-1}\cdot(3)^{-1})$	29	67	4
(5)	Relative force ratio[3,4,6]	2.20	0.61	0.19
(6)	Contribution to whole-muscle EMG[7] (%) $(100\cdot(1)\cdot(2)^{3/2}\cdot\Sigma(1)^{-1}\cdot(2)^{-3/2})$	49	49	2
(7)	EMG reduction after 2 min fatigue test (%)[3,8]	40	5	14
(8)	Contribution to % reduction[8] in whole-muscle EMG $((6)\cdot(7)\cdot100^{-1})$	20	3	0
(9)	Contribution to whole-muscle force (%)	60	39	1
(10)	Force reduction after 2 min fatigue test (%)[3,8]	96	11	1
(11)	Contribution to % reductions in whole-muscle force $((9)\cdot(10)\cdot100^{-1})$	58	4	0

1 Test described in Fig. 1.
2 Cage-reared rat MG data (1).
3 Noncage-reared cat TP data (4,5).
4 Ratio of mean value to mean value for all unit (fiber) types.
5 Mean number of muscle fibers in motor unit.
6 Based on fused (100-200 Hz) tetanus value.
7 Amplitude of extracellularly recorded EMG assumed to co-vary with fiber diameter$^{3/2}$ (7).
8 Reduction in mean value(s) for 121st stimulus train with respect to 1st train.

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$\dot{V}O_2$ KINETICS DURING SUBMAXIMAL EXERCISE FOLLOWING SIMULATED WEIGHTLESSNESS

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INTRODUCTION

The kinetics of oxygen uptake (VO_2) during performance of and recovery from constant-load exercise have been extensively studied. At the onset of muscular work, the VO_2 response exhibits the development of a deficit between oxygen supply and demand at the tissue which is incurred during the first minutes of exercise prior to reaching a steady-state VO_2 (6,8). Subsequently, the excess VO_2 measured during recovery from exercise constitutes the "payment" of the oxygen deficit (6).

Although there is no difference in steady-state VO_2 during constant-load ergometer exercise when comparing individuals with high and low maximal oxygen uptakes (VO_2 max), subjects with high VO_2 max tend to reach the steady-state at a faster rate (9). Likewise, exercise training significantly reduces oxygen deficit and recovery VO_2 induced by exercise (5). However, the effects of spaceflight deconditioning on VO_2 kinetics have not been determined. Bedrest has been employed as an effective method of examining the deconditioning effects of acute hypokinesia and weightlessness (1,2,3,4,7). In addition to the marked reductions in VO_2 max produced by continuous bedrest of 7 to 14 days (1,3,7), Convertino et al. (1,3) reported significant decreases in VO_2 at 3-min submaximal work intensities and suggested that this may be the result of a change in VO_2 kinetics rather than mechanical efficiency.

The purpose of this study was to determine the effects of deconditioning following 7 days of continuous head-down (-6°) bedrest on changes in steady-state VO_2, O_2 deficit, and recovery VO_2 during the performance of constant-load exercise.

PROCEDURES AND METHODS

Five healthy men, with a mean ± SE age of 38 ± 1 yr, a mean height of 174 ± 4 cm, and a mean weight of 69.7 ± 3.0 kg, gave written consent to participate as volunteers for this study.

During a 5-day orientation period, the subjects were familiarized with the mechanics of exercise using a cycle ergometer in the upright and supine positions and the procedures of the test protocol by performing two orientation exercise tests. Following the orientation tests, each subject performed a maximal exercise tolerance test in the supine position to obtain descriptive data for aerobic capacity. The mean maximal oxygen uptake (VO_2 max) of the subjects was 38.4 ± 2.4 $ml \cdot kg^{-1} \cdot min^{-1}$.

The overall experimental protocol consisted of a 14-day ambulatory control period followed by 7 days of continuous bedrest (BR) in the head-down (-6°) position and 10 days of recovery after BR. On days 2 and 13 of the control period (pre-BR), and again at the end of BR (post-BR), each subject performed a submaximal exercise test in the supine position on an electronic cycle ergometer. The exercise test consisted of a 5-min rest period followed by 5 min of exercise at 700 kgm/min (115 W) with 10 min of recovery from exercise. After the subject's heart rate returned to within 5 beats per min of the pre-exercise value, the submaximal test was repeated in the upright position.

The oxygen uptake was measured during each 30 s of all exercise tests using the analysis system previously described by Convertino et al. (4). Oxygen deficit was computed as the difference between the total VO_2 consumed during exercise and steady-state VO_2 multiplied by the duration of exercise. The steady-state VO_2 level was calculated by averaging the VO_2 values for the last 2 min of the 5-min workload. Recovery VO_2 was calculated as the total oxygen consumed during the 10-min recovery period. The halftime, $t_{\frac{1}{2}}$, of the VO_2 at the onset of exercise was determined for each subject as the time (sec) required for a 50% change in VO_2 from rest to steady-state exercise.

RESULTS

A constant-load exercise intensity of 700 kgm/min produced a steady-state VO_2 of 1.71 liters/min in pre-BR supine exercise and 1.70 liters/min in pre-BR upright exercise. These work levels were equal to approximately 65% of the subjects' supine VO_2 max. Following BR, steady-state VO_2 was unchanged in supine and upright exercise (Table 1).

In the supine position, BR did not change total exercise VO_2, O_2 deficit, or recovery VO_2 (Table 1). However, following BR, the VO_2 values during the initial 3 min of exercise were smaller ($P < .05$) and consistently larger ($P < .05$) during the first 3 min of recovery in the upright position. Consequently, total exercise VO_2 decreased ($P < .05$) from 7.41 ± .11 to 7.23 ± .17 liters, O_2 deficit increased ($P < .05$) from 1.10 ± .05 to 1.36 ± .07 liters, and recovery VO_2 increased ($P < .05$) from 5.17 ± .11 to 5.37 ± .17 liters during the post-BR upright test (Table 1). The elevation of O_2 deficit during post-BR upright exercise resulted from a significant increase in the VO_2 halftime from 52 ± 3 to 65 ± 10 sec ($P < .05$). The halftime for VO_2 during exercise in the supine position was not changed following BR.

TABLE 1. *Steady-state VO_2, O_2 Deficit, and Recovery VO_2 during supine and upright constant-load exercise at pre- and post-BR.*

SUPINE		UPRIGHT	
Pre-BR	Post-BR	Pre-BR	Post-BR
Steady-State VO_2, *liters/min*			
1.71 ±.05	1.72 ±.04	1.70 ±.02	1.72 ±.03
O_2 Deficit, *liters*			
1.68 ±.09	1.72 ±.15	1.10 ±.05	1.36 * ±.07
Recovery VO_2, *liters*			
5.35 ±.21	5.48 ±.38	5.17 ±.11	5.47 * ±.17

Values are mean ± SE.
* $P < .05$ from pre-BR value.

DISCUSSION

The deconditioning effects of bedrest on the physical working capacity were manifested in our subjects by significant changes in VO_2 kinetics during upright cycle exercise following 7 days of continuous -6° head-down tilt. During the 5-min constant-load exercise, a smaller portion of the total energy requirement was generated from systemic VO_2 mechanisms as suggested by a lower total VO_2 during exercise. Consequently, the significant bedrest-induced elevations of VO_2 halftime, O_2 deficit, and recovery VO_2 indicated that the body energy stores (i.e., oxygen stores, high energy phosphate stores and glycogen stores) made larger contributions to the energy pool required to perform the muscular work.

The deconditioning-induced change in VO_2 kinetics in this study might be attributed to (a) reduced physical activity during simulated weightlessness, and/or (b) a chronic reduction of orthostatic stress on the cardiovascular system which occurs during spaceflight. If reduced orthostatic exposure was an important factor in bedrest deconditioning, then the changes in the VO_2 kinetics during upright exercise following bedrest should be greater than those during supine exercise because of the added stress of orthostatic hypotension. Submaximal cycle ergometry performed in the upright position following bedrest produced significantly greater increases in VO_2 halftimes at the onset and cessation of exercise compared to the VO_2-time responses measured while the same subjects performed equal work rates in the supine position. In fact, there was no change in supine exercise and recovery VO_2 halftimes following bedrest suggesting that orthostatic stress was the major factor associated with altered VO_2 kinetics during constant-load exercise after deconditioning.

Despite the ability to attain similar steady-state VO_2, simulated weightlessness results in a reduction of total VO_2 capacity and an increase in the O_2 deficit and VO_2 halftime during submaximal constant-load exercise. This change in VO_2 kinetics was induced by re-exposure of the cardiovascular system to the $+1G_z$ (upright) environment.

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EFFECTS OF AGE AND SEX ON HORMONAL RESPONSES TO WEIGHTLESSNESS SIMULATION

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As the age of experienced astronauts increases and women become Shuttle crewmembers, the need to study effects of age and sex on physiologic responses to space flight is evident. We have examined effects of horizontal bedrest on excretion of catecholamines, aldosterone, and cortisol by human subjects grouped by age and sex. Responses were assessed by assays of 24-hr urine samples collected throughout the studies. In 36-45-yr-olds the excretion of epinephrine increased, while it decreased in the 46-55- and 56-65-yr-old groups. Norepinephrine excretion decreased (5-27%) in all groups during bedrest. Aldosterone excretion increased in the younger two groups of both males (19 and 6%) and females (47 and 9%). A slight decrease was seen in 56-65-yr-old males (6%) while excretion in females was unchanged. Cortisol excretion increased in the youngest groups of both men (12%) and women (13%) but decreased in the 56-65-yr-old groups (6 and 5%). In the two groups of intermediate age (46-55 yr) excretion in females decreased (15%) while in males it increased (19%). Hormone measurements may be of value in explaining variations in stress tolerance due to age and/or sex during space flight.

INTRODUCTION

As the age of experienced astronauts increases and as women become Shuttle crewmembers, the need to study the effects of age and sex on the physiologic responses to space flight becomes more apparent. In this series of studies bedrest was used to simulate weightlessness, and to study the extent of cardiovascular (CV) deconditioning by subject response to exercise, centrifugation and lower body negative pressure. We studied effects of bedrest on the excretion of aldosterone, cortisol and catecholamines on days not associated with CV testing.

METHODS

Groups of 7-10 male or female subjects in the age ranges 36-45, 46-55 and 56-65 years participated in the studies. Subjects resided in a controlled environment throughout the experiment. For 9 days prior to bedrest subjects remained ambulatory and active during the day and control data were collected. A period of 10 days of bedrest followed, during which subjects maintained a strictly horizontal position. Subjects remained in the study for 5 days after termination of bedrest for collection of recovery data.

Throughout the study, 24-hr urine samples were collected and measurements of aldosterone (ALDO) and cortisol (COR) were made by radioimmunoassay and epinephrine (EPI) and norepinephrine (NOR) by fluorimetric analysis. The amount of each hormone in the total volume of urine for each 24-hr period was calculated. Data were analyzed with the Student t-test for paired observations. Bedrest and recovery data are shown as percent change from control. Differences were considered significant if $p \leq .05$.

RESULTS

Bedrest affected the age groups and sexes differently, and it affected excretion of some hormones more than that of others (Tables 1 and 2). The most consistent change was a reduction in NOR excretion in all age groups of females and males (5-27% reduction), although the change was significantly different from the control period only for 46-65-year-old females. In males and the two older groups of females, EPI was decreased also (significantly in 46-55-year-old females and 56-65-year-old males). In 36-45-year-old males and females, the EPI/NOR ratio was considerably but not significantly greater (67 and 62% respectively) after bedrest than it had been during the control period. In older groups of males the ratio decreased, while in females it increased. In both males and females, excretion of ALDO increased with bedrest in 36-45-year-olds (19 and 47% respectively) and 46-55-year-olds (6 and 9% respectively). None of the changes in ALDO was significant, nor were the small increases and decreases in cortisol excretion.

Different groups varied in their ability to return to normal levels of hormone excretion after bedrest (Table 3). Most differences between the control and recovery periods were not significant, but apparently the four days for which urine was collected were not enough time for a return to normal in many cases. The oldest groups (56-65-year-olds) of both males and females showed a significant change in NOR excretion. In females there was an increase of 32% (after a decrease during bedrest) and in males a decrease of 32% from the control period.

DISCUSSION

NOR excretion has been reported to decrease as a result of bedrest (1) or space flight (2), and these results agree. In this study, EPI excretion generally decreased, although it has not been found to change with space flight (2). NOR

TABLE 1. HORMONE EXCRETION IN SUBJECTS BEFORE BEDREST
Mean ± SE

Parameter	Males 36-45 yr	Males 46-55 yr	Males 56-65 yr	Females 36-45 yr	Females 46-55 yr	Females 56-65 yr
ALDO, µg/24h	9.8±0.7	7.0±0.5	10.6±0.6	14.7±2.1	8.5±0.2	9.0±0.9
COR, µg/24h	44.1±4.8	43.8±2.5	52.5±3.3	55.6±5.3	46.8±1.9	37.2±4.2
EPI, µg/24h	35.0±3.6	21.0±2.6	22.2±3.6	22.2±2.9	27.2±4.0	20.9±2.0
NOR, µg/24h	53.5±4.2	76.6±7.1	89.6±5.6	56.3±4.6	58.8±2.6	58.8±4.1
EPI/NOR	0.80±.20	0.27±.03	0.26±.07	0.42±.10	0.54±.13	0.54±.19
Urine volume (ml/24h)	2817±178	1664±106	2395±181	2352±128	1686±7.4	2365±128
No. of Subjects	7	7	7	10	8	9

TABLE 2. PERCENT CHANGE IN HORMONE EXCRETION AS A RESULT OF BEDREST
Mean ± SE

Parameter	Males 36-45 yr	Males 46-55 yr	Males 56-65 yr	Females 36-45 yr	Females 46-55 yr	Females 56-65 yr
ALDO, µg/24h	19±12	6±13	-6±11	47±25	9±11	-0.3± 8
COR, µg/24h	12±15	19±17	-6± 5	13±13	-15± 9	-5± 5
EPI, µg/24h	3±19	-13±15	-26±12*	36±31	-20± 7*	-4±13
NOR, µg/24h	-27± 8	-5± 8	-13± 7	-27±12	-14± 9*	-15± 8*
EPI/NOR	67±52	-6±17	-16±12	62±28	11±14	20±19
Urine volume (ml/24h)	-20± 5*	-8± 6	-26± 6*	-9± 8	-13± 3*	-8± 3*

*p≤.05

TABLE 3. PERCENT CHANGE IN HORMONE EXCRETION DURING RECOVERY FROM BEDREST
Mean ± SE

Parameter	Males 36-45 yr	Males 46-55 yr	Males 56-65 yr	Females 36-45 yr	Females 46-55 yr	Females 56-65 yr
ALDO, µg/24h	17±14	9±21	3± 9	-16±18	13±16	33±14
COR, µg/24h	-6±13	12± 7	-9± 8	-7± 7	-6± 6	4± 4
EPI, µg/24h	8±30	19±27	7±29	38±24	13±12	25±16
NOR, µg/24h	-15±11	12±12	-32± 8*	38±20	-6± 8	32±10*
EPI/NOR	89±94	22±35	44±28	7±15	13±19	1±15
Urine volume (ml/24h)	-15± 5*	6±17	-26± 5*	-3± 6	-20±11*	-1± 9

*p≤.05

was the only hormone to show a significant change in excretion between the recovery period and the pre-bedrest period.

Changes in the ratio of EPI and NOR excretion have been considered to be indicative of the extent of cardiovascular deconditioning, a physiological result of bedrest (3). Significant CV deconditioning after bedrest has been found in both sexes of the two older groups in this study (4, 5). Our results indicate that the effects of bedrest on the EPI/NOR ratio vary with age and in the older groups with sex, but the changes were not significant and could not be correlated with deconditioning.

Urinary cortisol has been found to increase in astronauts after space flight (2) and in experimental subjects after bedrest (1), but there was little change in this hormone after bedrest in the present study.

CONCLUSIONS

In this study the ratio of epinephrine excretion to norepinephrine excretion was not found to be a a consistent index of cardiac decondition ing. Norepinephrine excretion was found to decrease in three age groups of each sex as a result of bedrest. Epinephrine excretion also decreased in the older groups (46-65 years old).

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SHORT TERM (1 AND 3 DAY) CARDIOVASCULAR ADJUSTMENTS TO SUSPENSION ANTIORTHOSTASIS IN RATS

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ABSTRACT

Antiorthostasis (AO) results in responses reflective of thoracic vessel loading. Initial findings included fluid and electrolyte shifts (diuresis and natriuresis) in AO but not in orthostatic (O) rats. This study aims at obtaining supportive evidence for cardiovascular responses, e.g., blood pressure and related parameters, in light of the original hypothesis. Tilting rats rapidly head-up from either horizontal (O) or head-down (AO) positions was used to assess cardiovascular sensitivities. O and AO rats were used after 1 or 3 days of suspension. Rats were controls (C), pre-tilted O and AO, tilted O and AO (rapid head-up 70-80°); post-tilted (to original postures). MAP in C rats was 108 +2 mmHg, in O, (117±1.02) and AO, (120±.58). MAP, diastolic pressure (DP) and pulse pressure were consistantly elevated in AO rats on day 3. With rapid head-up tilt, only MAP and DP showed significant increases. These changes were seen as cardiovascular responses to AO and support further use of this rat model for AO studies.

INTRODUCTION

The suspended rat has been proposed as an animal model for antiorthostasis (1,2). The term antiorthostasis (AO) and orthostasis (O) are borrowed from application to the human postures of standing (vertical) and lying down (horizontal). The development of a suitable animal model for studies aimed at understanding cardiovascular responses related to fluid and electrolyte balances was undertaken using a common laboratory animal, the rat. Previous investigations of functional responses to environmental modifications relating to animal antiorthostasis include those of Gauer et al (3). They expanded the thoracic blood volume in dogs by means of negative-pressure breathing and reported a diuresis. The interpretation was that cardiopulmonary stretch receptors responsed to apparent changes in blood volume. Neural and endocrine mechanisms affected an increase in the amount of filtered plasma voided as urine. More recently, Deavers et al (4) reported diuretic, natriuretic and kaliuretic responses in antiorthostatic suspended rats in a 15-20° head-down tilt posture. They also reported rapid, 1-2 day reversals after rats were returned to normal posture. Some cardiovascular parameters have been measured in attempts to assess the effects of head-up tilt and head-down tilt in anestetized rats (5) and in head-down tilted rats (6). Popovic (6) examined circulatory alterations in 30° head-down tilted rats.

In this initial study, measurements of routine circulatory parameters were made in an effort: to compare responses in O and AO suspended rats, to evaluate the effect of rapid head-up tilt in AO rats and to assess responses after return to the pre-tilt posture.

MATERIALS AND METHODS

Male Sprague-Dawley rats (180-200) gm were suspended in a harness (1,2) in either a head down tilted (15-20°) or a horizontal positon. Pre-suspension values were determined for each animal. A polyethylene cannula (PE 10) was introduced into the right carotid artery with the opening at the level of the aortic arch (confirmed at autopsy). Sodium pentobarbital (6 mg/100 gm) anesthesia was used. On the following day pre-suspension blood pressures were recorded (controls) and rats were suspended in AO or O positions; tilt tests were done on days 1 and 3. At each position a series of recordings were made for five (5) minute periods, from 3 to 7 animals in each group. Blood pressures were measured using a Statham pressure transducer (Model P23) and a Grass recording system (Model 79); heart rates were taken from blood pressure recordings.

RESULTS AND DISCUSSION

Data are summarized in Table 1. There was a significant increase in MAP on Day 1 comparing both O and AO suspension to presuspension values. In O rats, the MAP returns to control levels on Day 3 and, in contrast, MAP in AO animals remains elevated on day 3. This is seen as the result of the signficantly greater diastolic pressure and, to some extent, in the systolic pressure in the AO animals. We reasoned that the sustained elevated blood pressure at Day 3 is a reflection of the AO positioning, as well as previously reported fluid and electrolyte changes evidenced by diuresis and natriuresis (4). Pulse pressures were not altered significantly from presuspension control values in either O and AO subjects. In general, heart rates were slightly elevated following suspension.

Head-up tilt (70°) of both O and AO rats results in increased MAP and increased heart rate when compared with pre-tilted rats. However, the response is less pronounced in the AO subjects. On Day 3 lack of MAP response in AO rats suggests increased vasomotor tone in response to AO positioning and elevated fluid and electrolyte losses. There was a lack of pulse pressure change indicating minimal hypotensive response in these animals. In O and AO subjects returned to their original posture, following the head-up tilt, there is a generalized response of blood pressure return to pre-tilt levels.

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TABLE 1: Blood Pressures (mm Hg) in Antiorthostatic (AO) and Orthostatic (O) Rats. Mean ± SEM; Significant Differences: P Values of 0.05* or 0.01**

	PRE-SUSPENSION	SUSPENDED		
M A P		Pre Tilt	Tilt (70°)	Post Tilt
DAY 1 AO	108 ± 2	120 ± 0.58*	128 ± 2.75	119 ± 1.81
O		117 ± 1.02	135 ± 2.23	122 ± 2.63
DAY 3 AO	108 ± 2	142 ± 1.21**	137 ± 1.69	133 ± 0.86**
O		109 ± 1.87	125 ± 1.40	114 ± 1.33
SYSTOLIC				
DAY 1 AO	143 ± 4.5	158 ± 0.66**	164 ± 2.42	155 ± 1.78
O		150 ± 0.73	163 ± 4.41	158 ± 2.66
DAY 3 AO	143 ± 4.5	174 ± 1.63**	165 ± 1.85*	160 ± 1.44
O		160 ± 1.83	172 ± 1.75	161 ± 3.46
DIASTOLIC				
DAY 1 AO	90 ± 2	102 ± 0.37	109 ± 2.64	101 ± 1.74
O		101 ± 1.26	114 ± 5.23	105 ± 2.50
DAY 3 AO	90 ± 2	126 ± 1.16**	123 ± 2.12**	120 ± 0.93**
O		93 ± 4.11	103 ± 2.47	90 ± 1.03
PULSE PRESSURE				
DAY 1 AO	53 ± 4.26	56 ± 0.51**	55 ± 0.84**	54 ± 0.80
O		48 ± 1.29	45 ± 1.53	53 ± 0.75
DAY 3 AO	53 ± 4.26	47 ± 1.52**	41 ± 2.06**	42 ± 1.04**
O		72 ± 1.92	73 ± 0.90	72 ± 3.54
HEART RATE (B/M)				
DAY 1 AO	435 ± 22	479 ± 2.18**	518 ± 5.16**	498 ± 2.63**
O		518 ± 4.03	554 ± 4.56	538 ± 5.96
DAY 3 AO	435 ± 22	480 ± 0.75**	509 ± 4.13*	485 ± 3.61
O		492 ± 1.03	494 ± 6.27	487 ± 6.28

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DAILY RHYTHMS OF ACTIVITY AND TEMPERATURE OF Macaca nemestrina

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ABSTRACT

We have examined activity and temperature rhythms of pig-tailed macaques (Macaca nemestrina) maintained in LD 16:8 at 25°C in specially designed restraint chairs. Activity was monitored via a sensor that was attached to the restraint chair. Temperature was monitored at the axilla, ankle and ear. All variables showed prominent day-night variations, and except for ankle temperature, had highest values during the daytime. These results show that the regulation of the daily rhythm of body temperature involves anatomical sites that are utilized in a temporally distinct fashion.

INTRODUCTION

To investigate the effect of microgravity on body temperature rhythms, we plan to monitor activity along with core and skin temperatures on the macaques to be flown on the upcoming COSMOS primate Biosatellite. Aschoff and Heise (1972) have shown that in humans in the thermoneutral zone, the daily body temperature rhythm is primarily due to changes in heat loss, and not changes in metabolism. Heat loss from the extremities is relatively low during the day and elevated during the night. Skin temperature has often been used to indicate changes in vasomotor heat loss. High skin temperatures (approaching core body temperature) indicate dilation of subcutaneous blood vessels and consequent heat dissipation, while low skin temperatures (approaching ambient temperature) indicate vasoconstriction and consequent heat conservation.

Here we report the results of our preliminary studies to characterize activity and temperature rhythms. Macaques display prominent rhythms with the temporal patterns of the temperature rhythm depending on the site being monitored.

METHODS

Four young (4-7 kg) male pigtail macaques (Macaca nemestrina) were used in this study. To monitor activity and temperature patterns, the monkeys were conditioned to sit in a primate restraint chair. This chair is based on the design of Milhaud et al. (1980). Before any data were collected, the monkeys underwent a 2 month conditioning period. While in the chair, the animals continued to have food (Purina Monkey Chow and fruit) and water continuously available.

Activity was monitored via a sensor (L & M Electronics, Foster City, CA) attached to the neck restraint plate. The output of the sensor was filtered and amplified, and then fed into a digital data collection unit (Ambulatory Monitoring, Inc., Ardsley, NY) and totaled over 16 min intervals.

Temperature was measured by YSI thermisters (Yellow Springs Instruments, Yellow Springs, OH). The colonic probe was inserted about 10 cm above the anus, and taped to the base of the tail. Skin temperature sensors were attached by gluing the flat sensor to the skin with cyanoacrylate glue and then by placing tape over the sensor. The output of the YSI thermisters was recorded either on a strip chart recorder or on a digital data collection unit (Ambulatory Monitoring, Inc.). All analyses were done on an Apple II+ microcomputer. The data were plotted by a digital Hi-Plot plotter (Model DMP-4, Houston Instruments, Houston, TX).

RESULTS

Figure 1 shows the activity along with axillary, ankle and ear temperatures of a monkey measured for 5 days. Activity levels are highest during the day, as are axillary and ear temperatures. Ankle temperature shows a different pattern, with highest values during the night. Ear temperature has markedly episodic changes. It can go through an excursion from about 2°C above ambient temperature to about 2°C below axillary temperature and then back again within about 2 hr. To characterize the rhythms, average waveforms were constructed for the data of the 4 monkeys (Figure 2). Activity shows a large amplitude rhythm, with high counts during the day and low counts at night. It begins to increase before lights on, and begins to decline before lights off. Axillary temperature starts to increase about 30 min before lights on, continues to rise for the first 3 to 4 hr of light, and then remains relatively constant. Temperature begins to decline about 2 hr before lights off and reaches the lowest point in the middle of the night. The highest average daytime temperature is 37.8°C, and the lowest average nighttime temperature is 36.7°C. Ankle temperature is high in the night and low in the day, while the opposite is true for ear temperature.

DISCUSSION

The results of this study provide baseline data for the COSMOS primate Biosatellite experiment on circadian rhythms of activity and temperature. Our data show that there is not only differential

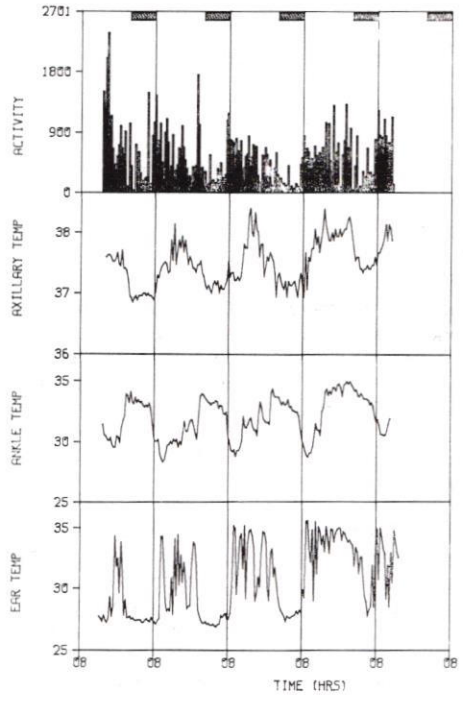

FIGURE 1. A plot of activity (counts / 16 min), axillary temperature (^{o}C), ankle temperature (^{o}C), and ear temperature.

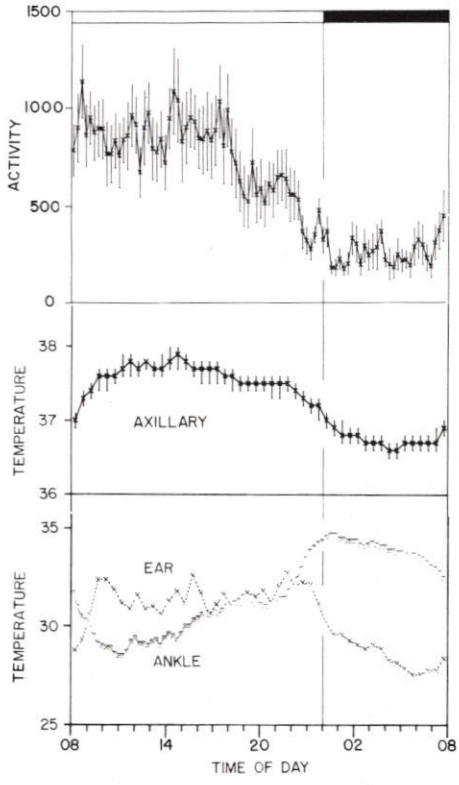

FIGURE 2. Average waveforms of the circadian rhythms of activity (counts / 16 min) (top), axillary temperature (^{o}C) (middle), and ankle (o) and ear (x) temperatures (^{o}C) (bottom). Plotted are the mean values of each variable

regulation of skin temperature at specific anatomical sites, but that this also varies with time of day. As was noted in the Introduction, the rhythm in body temperature of individuals in the thermoneutral zone is primarly due to changes in heat loss and not changes in metabolism (Aschoff and Heise, 1972). These results suggest that core body temperature is maintained at about 38^{o}C during the day by reduced cutaneous blood flow to the limbs. If excess heat is generated, it is lost through other sites, e.g., the ear. During the night, core body temperature drops by more than 1^{o}C, and this is controlled by increased cutaneous blood flow (heat loss) in the limbs.

The differential recruitment of anatomical sites for heat loss at specific times of the day and night underscores the importance of the circadian timing system in thermoregulatory function. Fuller et al. (1978) have shown that in the absence of adequate environmental temporal cues, thermoregulatory function can be impaired. In space, primates are exposed to an abnormal temporal environment and there are major changes in fluid distribution. It will be interesting to see how these affect the regulation of body temperature.

ACKNOWLEDGEMENTS

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INCREASED HEMATURIA FOLLOWING HYPERGRAVIC EXPOSURE IN MIDDLE-AGED WOMEN

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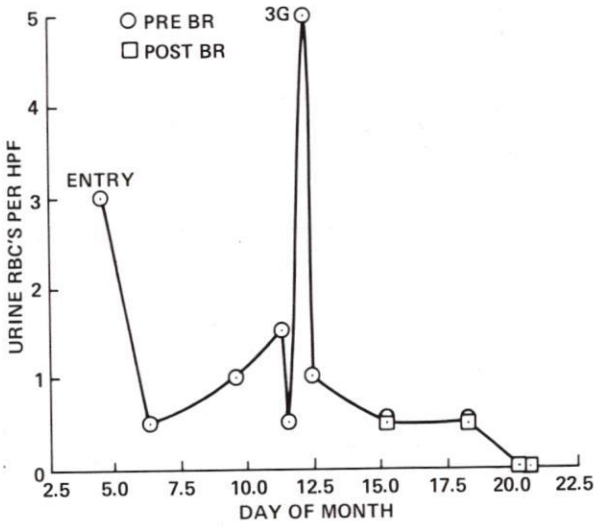
INTRODUCTION: To study the effects of simulated weightlessness on orthostatic tolerance of middle-aged women, 9 females (55 to 65 years old) underwent acceleration (+ G_z) and lower body negative pressure (LBNP) before and after 10 days of horizontal bed-rest (BR). This paper describes the first known association of microscopic hematuria with hypergravic and orthostatic stress suggesting similarities to the 'stress hematuria syndrome' previously seen with heavy exercise (2).

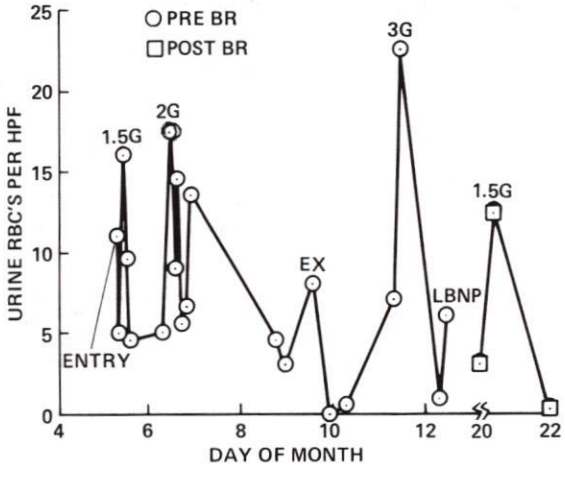
METHODS: All subjects underwent duplicate testing during a 9 day control period consisting of +1.5 G_z +2 G_z, and +3 G_z exposures, in addition to LBNP. The +1.5 and +2 G_z runs were modeled after projected Shuttle reentry profiles. The +3 G_z run was used as a physiological acceleration tolerance test. The +1.5 G_z exposure was repeated on BR Day 7, +2 G_z on BR Day 8, and +3 G_z on BR Day 9. All G levels were run with and without an anti-G suit inflated. Maximal bicycle ergometer tests (EX) were performed before and after BR. All subjects passed a screening exam including creatinine (Cr), BUN, and urinalysis (UA). All women gave a negative history for prior renal disease, hypertension or bleeding diathesis. Subjects were fed a diet containing high grade protein and maintained urine pH generally in the acid range preventing red blood cell (RBC) lysis. Mid-stream clean catch urines were tested using Labstix (Ames) for hemoglobinuria. Microscopic exam of well-mixed spun sediment was performed at a magnification of 45 X if the dipstick screen was positive and/or preBR before and after +3 G_z and LBNP, and during BR on Day 5 after a long period of inactivity.

RESULTS: Three women, subject #'s 167, 172, and 185 had hematuria from 1 to 5 RBC's/HPF on routine UA after entry into the study. The course of their hematuria during the study is shown on the accompanying figures. All women were past menopause. In two subjects, LBNP increased hematuria. Acceleration levels of +2 G_z or greater produced 3- to 5-fold increases in hematuria in these women. Within one day post +G_z or LBNP, hematuria subsided to basal levels. Horizontal BR per se did not affect the degree of hematuria. Individual serum Cr and BUN indicated no change in renal function. During and after the episodes of hematuria subjects reported no symptoms usually associated with traumatic injury such as flank, suprapubic, or perineal pain, dysuria, urinary tenesmus, passing clots or gross hematuria. Lab analyses revealed negative cultures and no pyuria, significant casts or other

HEMATURIA SUBJECT #167



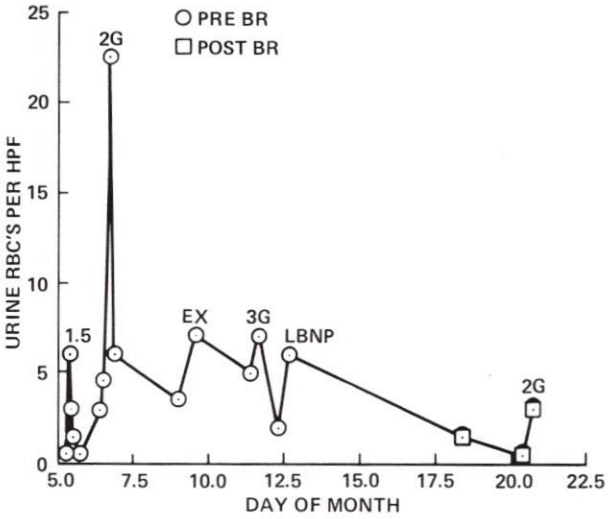
HEMATURIA SUBJECT #172



abnormalities. Associated proteinuria was absent by dipstick screening (detectable threshold >20 mg/dl). was absent. These three subjects had 24 hour urine collections preBR on the day following maximal exercise testing. All subjects had a low total urine Cr concentration from 20 mg/dL to 44 mg/dL (normal 80 to 170). Resulting Cr clearances

were in the low normal range when corrected for body surface area. Total 24 hour protein excretions were not elevated (range 20 to 48 mg). Total 24 hour urine volumes ranged from 2535 to 3750 ml.

HEMATURIA SUBJECT #185



DISCUSSION: In these subjects, microscopic hematuria was associated with exposure to orthostatic or acceleration stress. The limited data suggests but does not prove a causal relationship. The mechanisms underlying increased microscopic hematuria in these women may be similar to those implicated in the 'Stress Hematuria' or 'Athletic Pseudonephritis Syndrome' (2) which has been reported in male and female runners, crew racers, swimmers, hockey players, marine recruits, and bongo drummers (1,2,3,4,7,8). Some investigators (1) favor a traumatic mechanism of injury especially to the lower urinary tract as shown in Table I.

Table I STRESS HEMATURIA SYNDROME
LOWER URINARY TRACT ETIOLOGY

HEAVY EXERCISE ↓ MECHANICAL TRAUMA	LBNP, $+G_z$ ↓ MECHANICAL TRAUMA
– Repeated impact theory – male runners empty, flaccid posterior bladder impacted against trigone and prostate	– Sustained inferior displacement of bladder and compression against pelvic structures
– Repetitive contractions of underlying pelvic floor musculature during running	– Possible exacerbation with anti-G suit inflated compressing distended bladder
– Usually gross hematuria	– Urothelial petechiae and ecchymoses due to rupture of small vessels and increased capillary pressures
– Does not explain hematuria in female runners or exertional hematuria in swimmers and rowers	– Contribution of hemolysis and fibrinolysis unknown
– Traumatic hemolysis may → hemoglobinuria	
– ↑ fibrinolytic activity in athletes may ↑ hemorrhage	

Late stream dysuria and pain patterns also suggest involvement of the bladder neck and posterior urethra (4). These instances of gross hematuria in men do not explain microscopic hematuria seen in athletic women, or swimmers who do not experience lower body jarring like runners. Therefore, a renal - glomerular mechanism has also been postulated. However, in women exposed to $+G_z$ acceleration or LBNP, mechanical trauma to the kidney or bladder may account for transiently increased hematuria.

The contribution of traumatic rhabdomyolysis, traumatic hemolysis, and increased fibrinolysis, seen in runners, to a traumatic mechanism of hematuria during orthostatic stress or $+G_z$ is unknown, but probably not major. Myoglobinuria and hemolysis would give a positive dipstick reaction, but would not account for our microscopic results on which this report is based. Recent reports suggest that rhabdomyolysis is a distinct entity from the exercise or stress hematuria commonly seen during aerobic activities (2). In general microscopic RBC counts are more sensitive indicators of hematuria than dipstick readings, since the commonly used orthotolidine hydroperoxide stick test detects free hemoglobin better than intracellular hemoglobin with a threshold of at least 5 to 10 RBC's/HPF or an equivalent of 50,000 RBC's/ml necessary before a positive trace reaction results (8). This level is far above average physiological RBC loss for most men of <20,000/ml. Because dipsticks were used in some studies to initially screen runners (2), the incidence of microscopic hematuria of 17% to 22% in men and women respectively may have been underestimated.

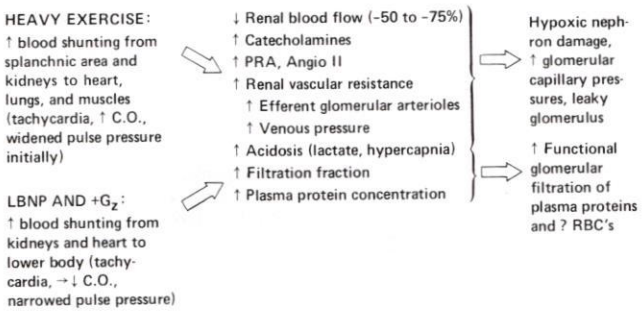
One factor favoring a traumatic origin of hematuria in our subjects over a glomerular or renal origin in the absence of associated proteinuria, or casts (7). However, proteinuria detectable on the standard urinalysis has not always been found in association with exercise-induced hematuria (3,4). In some subjects, total protein level may remain unchanged immediately after exercise or when measured 45 minutes post-stress (6); but protein electrophoresis and radial immunodiffusion may indicate a shift in the pattern of urinary proteins suggesting alterations in glomerular (predominant serum albumin excretion) and proximal renal tubular function (increased B2-microglobulin and urinary lysozyme bands) (3,6). Dipstick protein screens (threshold <20mg/DL) and total 24 hour quantitative protein determinations would not detect these subtler changes in function. Recent studies have rarely found RBC casts or nephritic sediment associated with microscopic hematuria without underlying pathology (2,3,7).

The glomerular origin of stress hematuria (Table II) is favored by some investigators (2,3) as a more cohesive way of explaining the phenomenon especially in women. In this theory which is compatible with $+G_z$ and LBNP, the critical triggering factor is a decrease in renal blood flow of up to 50 to 75% (2). Other factors which contribute to decreased RBF are the increase in catecholamines, plasma renin activity (PRA), and angiotensin II produced by $+G_z$, LBNP, and exercise which cause an increase in renovascular resistance and renal vein pressures leading to hypoxic nephron damage, increased glomerular capillary pressures and a "leaky" or more permeable glomerulus with pro-

teinuria and hematuria (2,3). In addition, the decrease in RBF shifts flow from the cortical to the deeper medullary nephrons increasing filtration fraction and increasing protein excretion. Hemoconcentration and acidosis during exercise or orthostatic stress contribute to the increase in filtration of plasma proteins and possibly RBC's (3).

STRESS HEMATURIA SYNDROME
RENAL ETIOLOGY*

Table II



*Excluding mechanical trauma, contusion, ecchymoses, petechiae

The overall effects of heavy exercise on renal function are shown in Table III.

EFFECTS OF HEAVY EXERCISE ON RENAL FUNCTION

Table III

Glomerular filtration rate ↓
Renal blood and plasma flow ↓↓
Filtration fraction ↑ (shift to deeper medullary nephrons)
Diuresis ↓
Free water clearance ↓
Osmolal clearance ↓
Na^+ excretion ↓
K^+ excretion ↓ or ↑
Protein excretion ↑ (plasma albumin predominates, some tubular secretion changes)
Plasma renin activity ↑

? Microscopic hematuria ↑

Adapted from Campanacci, L. et al., Contr. Nephrol. 26:31, 1981

Interestingly, mild exercise which increases cardiac output yet maintains RBF is not associated with stress hematuria, and proteinuria, or with the functional changes in renal function such as decreased GFR and free water clearance (2). In addition, aerobically trained people also maintain a higher RBF after maximal work and have less urinary protein abnormalities (albuminuria and hemoglobinuria) than untrained subjects (3). The effect of acceleration familiarization and training on stress hematuria is not known.

Serum creatinine and BUN measurements during this study and past studies, indicated no change in resting renal function due to weightlessness simulation or bedrest. During a previous study of 55 to 65 y.o. men, detailed renal function studies using inulin, para-aminohippurate and dextran showed no significant change in resting GFR, RBF, and filtration fraction following six days of horizontal BR. This is interesting since in another study, prolonged bedrest alone produced a significant increase in resting PRA levels (5) which might be expected to increase renovascular resistance through angiotensin II and decrease RBF during bedrest. This would trigger changes in glomerular function which increase microscopic hematuria. However, measurements on day 5 of bedrest indicated a return of hematuria levels to baseline in the three women we followed (first postBR point on Figures). This again is consistent with the resolution of stress hematuria within 48 to 72 hours following the acute stress (2,7,8). In general any hematuria which persists longer than this time period even if asymptomatic, requires further workup usually including cystoscopy and urography to rule out other common causes of hematuria including renal, bladder, or prostate carcinoma, glomerulonephritis, renal calculus, tuberculosis, sickle cell trait or disease, schistosomiasis, and other disorders (8). The degree of hematuria (from microscopic to gross) correlates poorly with pathological severity (8).

The sporadic and unpredictable occurrence of microscopic hematuria following LBNP and +G in our women is also consistent with the relatively low and unpredictable recurrence rate found in runners (2,4). A multifactorial etiology including stress intensity, duration, state of hydration, bladder volume, level of circulating catecholamines and PRA, and hemodynamic response (RPF) probably all contribute to the incidence of hematuria during a particular stress.

CONCLUSIONS:

1. Increased microscopic hematuria following orthostatic or acceleration stress may be similar to the 'Stress Hematuria Syndrome' occurring with heavy exertion.

2. The sporadic occurrence of this phenomenon suggests a multifactorial etiology in predisposed individuals. Mechanisms which contribute to this phenomenon probably include mechanical trauma (upper or lower urinary tract), and alterations in glomerular permeability due to renal hemodynamic changes and circulating neurohormonal factors.

3. Bedrest or weightlessness simulation per se does not appear to significantly alter renal function, but may decrease microscopic hematuria with an orthostatic component.

4. The use of a dipstick hematest may underestimate the frequency of microscopic hematuria.

5. Clinical evaluation (e.g., urography and cystoscopy) is recommended if hematuria persists longer than 72 hours following exertional or hypergravic stress.

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NASA Space Biology Program

The advent of the space age provided the first access to the "gravity-free" state and an opportunity to manipulate gravity from its norm of one down to zero. Therefore NASA has assumed the responsibility to investigate the biological significance of gravity and thereby expand biological knowledge.

Objectives

The objectives of NASA's Space Biology research program are *1*) to investigate the biological significance of gravity; *2*) to use gravity to solve relevant biological questions; and *3*) to enhance our capability to use and explore space.

Goals

The goals of the Program are *1*) to enhance our knowledge of normal physiological adaptive mechamisms in both plants and animals and thereby provide new insight into both normal and pathological mechanisms; *2*) to provide for the multiple generation survival of plants and animals in space through an understanding—and ultimately control—of the affects of gravity on development, adaptation, and evolution; and *3*) to enhance plant productivity through an understanding and control of gravitational and related environmental stimuli and the manipulation of response mechanisms.

The achievement of such goals depends on answers to basic scientific questions that include the following.

1) Does gravity influence fertilization and early development and can fertilization and early development proceed normally in a near 0-G environment? If gravity does affect fertilization and early development, what are the sensitive physiological systems and how are they affected? If early development is affected by gravity, is it a result of an affect on the parent or the direct affect on the embryo itself?

2) What is the role of gravity in the formation of structural elements, such as lignin, cellulose, chitin, and bone calcium, at the molecular as well as at the more complex organizational levels?

3) What role does gravity play in calcium-mediated physiological mechanisms and in calcium metabolism?

4) What is the gravity-sensing mechanism? How does it perceive information? How is the information transmitted to evoke a response?

5) How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology, and behavior of organisms? *Or* how do gravitational and other environmental stimuli interact in their control and direction of living forms? Can the action of gravity be replaced by different stimuli?

Strategy

The strategy so far has been to manipulate gravity on earth and develop weightless simulation models to develop and test gravitational hypotheses; to identify gravity-sensitive biological systems and interacting environmental response mechanisms; to address valid gravitational biological questions on earth when possible; and to plan and design future space experiments. As space-flight opportunities, either manned or unmanned, become more prevalent, increasing emphasis will be placed on flight experiments. Similarly, as longer flight missions become available, emphasis will be directed toward biological questions that require longer periods of microgravity for adequate experimentation.

Program Content

The program has been divided into the following three broad areas: *1*) the role of gravity in reproduction, development, maturation, and evolution; *2*) gravity receptor mechanisms (these include the identification of the organ or site of gravity reception and the biological systems and mechanisms that transmit the information to a responsive site); and *3*) the physiological effects of gravity (this includes the biological mechanisms by which living systems respond and adapt to altered gravity, particularly that of the space environment, as well as the interactive affects of gravity and other stimuli and stresses on the physiology, morphology, and behavior or organisms).

This NASA program in space biology is carried out intramurally by the NASA Research Centers and by a system of extramural grants. Qualified scientists interested in learning more about the program and the development of research proposals should contact:

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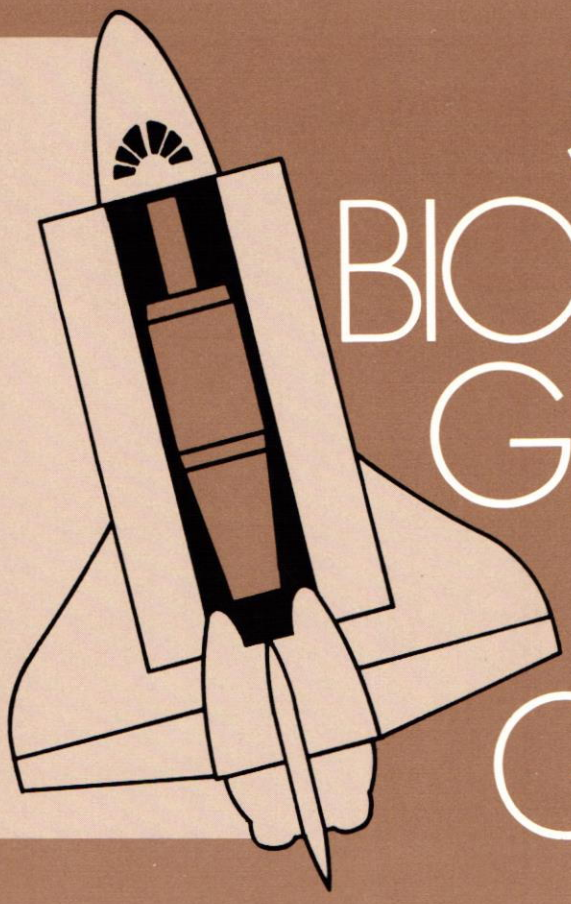

SPACE BIOLOGY GRANT PRO-GRAM