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

A Supplement to
The Physiologist
Volume 33, Number 1
February 1990

Published jointly by the
American Physiological Society and
The Galileo Foundation

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

September 25–27, 1989
Lyon, France

Orr E. Reynolds, Guest Editor

Auguste Salathé

The Proceedings of the 11th Annual Meeting of the IUPS Commission on Gravitational Physiology is dedicated to Dr. Auguste Salathé, a pioneer in the field. He was born 10 June 1849 in Mulhouse, France, to Judith and Charles Auguste Salathé, MD. After training in Natural History at the University of Strasbourg, he attended Medical School in Paris, receiving the MD degree in 1877 with a thesis entitled, "Movements of the Brain and Mechanisms of Circulation in the Central Nervous System."

Subsequently, Dr. Salathé worked in the Laboratory of M. Marey at the Collège de France where he had access to advanced physiological instrumentation developed by its director, Dr. E. Jules Marey. This included sensitive and accurate apparatus for the measurement and graphic recording of blood pressure and heart and respiratory frequencies (1).

Salathé studied the effect of the head-up ($+G_z$) orientation to gravity on hemodynamics in rabbits (2). He observed increases in femoral and decreases in carotid artery pressures, which were followed by unconsciousness, convulsions, and death within 45 minutes. All of these premortem changes could be reversed by returning the animal to a horizontal position. Salathé also observed that the head-downward position ($-G_z$) was well tolerated, not producing any adverse response for several hours. In some experiments, he used trephined animals with the opening covered by glass (3). In such animals head down ($-G_z$), cerebral congestion as well as a dark red color of buccal and nasal mucosae and injected eyes were apparent.

Experiments on the effect of orientation to gravity had been done by Piorry (1826) and Marey (1863). Salathé reviewed their reports and comparing them with this own observations concluded, "After a prolonged decubitus [recumbency] one can see the production of two phenomena when the subject is placed in an upright position, cerebral anemia and congestion of the legs because the deaccustomed vessels do not react against gravity." This is a clear statement of the deconditioning of the circulatory system and the resulting increased susceptibility of animals to orthostatic hypotension after chronic recumbency.

Salathé also developed a centrifuge to compare the effects of centrifugal force with those of gravitational orientation (2). He was aware of E. Darwin's proposal for the development of a human centrifuge (1803) and also Mach's hypothesis (1875) of an equivalence of the effects of gravity and centrifugal force on sensory systems.

In his experiments he centrifuged rabbits in a "head-out" ($-G_z$) or "head-in" ($+G_z$) position. The "head-in" position on the centrifuge essentially reproduced the "head-up" orientation in earth gravity, and the centrifuged animals exhibited similar response, dying within 6–15 minutes. Centrifugation in the "head-out" position was tolerated better, the animals surviving at least twice as long as with the "head-in" orientation. Salathé concluded that these observations with gravity orientation or with centrifugation experiments were the result of induced changes in hemodynamics and the distribution of blood. To test this, he devised an apparatus (a "hermetic vase") with which he could vary the air pressure around the lower body of rabbits, changing blood distribution (2). The effects of this treatment on circulation were the same as with a head-up orientation to gravity or with a "head-in position on a centrifuge.

Thus, over a century ago, Dr. Auguste Salathé anticipated the principle experimental techniques (excepting earth-orbital vehicles) of gravitational physiology. He greatly anticipated the use of the Tilt Board (1913) or the Lower Body Negative Pressure (LBNP) device (1964). His animal centrifuge studies were contemporary with those of Tsiolkovsky, to whom the Proceedings of the Fifth Annual Meeting of the Commission on Gravitational Physiology were dedicated. Salathé's pioneering achievements in gravitional physiology have not received the general recognition that they deserve.

References:

1. Marey, E. J. La Méthode Graphique. Travaux du Lobaortoire de M. Marey. *Physiol. Expér.* 1876, p. 251.
2. Salathé, A. De L'anémie et de la congestion cérébrale provoquée mécaniquement chez les animaux par l'attitude verticale ou par un movement giratoire. Travaux du laboratoire de M. Marey. *Physiol. Expér.* 3: 251–272, 1877.
3. Salathé, A. Mouvements du Cerveau. Travaux du laboratoire de M. Marey. *Physiol. Expér.* 1876, p. 365.

IMPORTANT
PRELIMINARY ANNOUNCEMENT

Twelfth Annual Meeting
IUPS Commission on Gravitational Physiology
October 14–18, 1990
Leningrad, USSR

The Twelfth Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences will be held in Leningrad, USSR, October 14–18, 1990.

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

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

Table of Contents

Gravitational Physiology IV

Hypergravic Conditions

Workshop on Head-Down Tilt

It gives me great pleasure, as Chairman of the International Commission of IUPS on Gravitational Physiology, to welcome you to the Commission's Eleventh Annual Meeting. It is the first time we have a Commission Meeting in France, and we are much honored to be here in Lyon as guests of the Claude Bernard University and the University of Saint Etienne.

The scientific program of this meeting includes over 90 presentations. In addition to the sessions in which the voluntary papers will be presented, there will be three symposia with invited papers. The first of these symposia, dealing with "Recent Space Flight Results in Gravitational Physiology", will be held this morning. The second symposium, that on "Current Concepts in Gravitational Physiology", will be held tomorrow morning, and the third, entitled "Gravity and the Cardiovascular System" is scheduled for Wednesday morning.

Current aereospace technology exposes human subjects to extreme variations in the gravitational forces that range from almost zero during space travel to as much as nine-times normal, or more, during operation of high performance military aircraft. When the Commission was established in 1974 by the IUPS Council, a driving force was the need for research in the new problem area associated with the advent of the space age. It is perhaps natural, then, that so far the scientific programs of our annual meetings have been mainly concerned with the biological effects of microgravity.

Many links are still missing in our understanding of the physiological chain of events that lead to adverse changes under the influence of microgravity. In prolonged space flight, we have so far attempted to counteract these changes by interventions that have necessarily been of a makeshift character because control experiments have been lacking. However, by regularly employing such countermeasures we have compromised the body's spontaneous adaptation to microgravity, and we therefore do not know, if with time, the body can truly adapt to this environment.

It is axiomatic that all problems connected with the physiological decay caused by microgravity can be solved by substituting centrifugal force for gravity during spaceflight. So, eventually, in order to facilitate long-duration residence in space, the engineers may have to step in and provide centrifugal force as the ultimate protective device. Even if this is bound to happen, we must continue to study the fascinating effects of the effective absence of normal gravity, perhaps the most familiar and the least understood of all environmental forces.

Physiologists have been successful in defining the environmental hazards encountered by man in high altitude flight; on the basis of such knowledge it has been possible for the engineers to create a safe microclimate that protects man in a hostile environment. On the other hand, man cannot be shielded from the action of high, sustained G forces as long as maximum aircraft maneuverability is a requirement. Here technology has little to offer in the way of protection. It is therefore the task of the physiologist to devise methods for minimizing the effects of the acting G force. The circulatory system is especially sensitive to the action of G forces. It is appropriate, therefore, that one of the symposia of this meeting has been devoted to cardiovascular adjustments to high, sustained G forces.

I would like to conclude these remarks by extending, on behalf of the Commission, my gratitude to the local organizing committe, Professors Claude Gharib, Ch. Alexandre and G. Gauquelin with coworkers and to supporting national organizations, which have so graciously contributed to making this meeting possible. I would also like to express the Commission's gratitude for the support given by the European Space Agency, the Soviet Academy of Sciences, the U.S. National Aeronautics and Space Administration, and the Galileo Foundation, a recently established organization for financial support of the Commission's activities.

Hilding Bjurstedt

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

RESULTS OF STUDIES OF MOTOR FUNCTIONS IN LONG-TERM SPACE FLIGHTS

I.B.Kozlovskaya, V.A.Barmin,
V.I.Stepantsov, N.M.Kharitonov

Institute of Biomedical Problems,
Moscow, USSR

Motor functions disturbances occupy an important place in the complex of effects caused by prolonged exposures to microgravity. Results of previous studies and observations performed on crew members of long-term space flights (SF) onboard of the "Salyut-6" orbital station have revealed a wide spectrum of changes in almost every component of motor system including muscles (hypo- or -atrophia, atonia), sensory inputs and spinal reflexes (hyperactivity of many if not all proprioceptive inputs, hyperreflexia of varying degrees), integrative motor control mechanisms (posture, locomotion, accuracy control). The intensity of disturbances in different components varying appreciably did not intercorrelate, thus pointing out to their independence and different genesis (I.B.Kozlovskaya et al., 1981).

Results of simulation studies (3-7-day dry water immersion, DI; antiorthostatic bed rest, ABR of 30-360-day of duration) have shown that depth and duration of motor effects caused by microgravity are defined at least by 3 factors, namely, duration of exposure, individual resistance and character of physical exercises used during exposure. The important role of the first factor has been obviously demonstrated in situations, where physical exercises were by some reason neglected. In this case the second factor - individual resistance become important especially in exposure of not very long duration defining the end results. But when physical exercises are used the role of above factors is diminished and the results of exposures are almost completely defined by PhE characteristics.

In accordance with these data it seemed extremely important to verify this conclusion in situation of real microgravity.

Material and Methods

The protocol of motor functions testing in flights onboard of "Salyut-7" and "Mir" stations was identical to that used previously (I.B.Kozlovskaya et al., 1981) and included tests and methods that allowed quantitative evaluation of changes in different parts of motor system. The state of support input was determined with respect to the vibrosensitivity threshold of the sole support areas (A.A.Otelin et al., 1976). The state of the muscle input and related mechanisms of spinal regulations was evaluated with the aid of the recruitment curve of the gastrocnemius muscles (T-reflex), showing the reflex amplitude as a function of stimulation strength. In order to evaluate the state of gastrocnemius (G) and tibialis anterior (TA) muscles the isokinetic dynamometry test was used in which the speed and strength of the muscle understudy were measured with respect to the force moments and the EMG when the subject was moving the foot with high (180°/s), moderate (120°/s) and low (60°/s) speed as well as during isometric contractions.

Motor control systems state was determined on the basis of stabilographic data and characteristics of postural synergies. The stabilographic recording were conducted according to the standard procedure for 3 min:

1 - comfortable posture with eyes opened;
2 - the same posture with eyes closed;
3 - Romberg's posture. The upright stability was also measured in relation to the characteristics of correction response in the test with perturbation of the body balance, generated by pushes of a known force against the subject's chest.

Observations were carried out before, and on days 2-4, 6, 11 and 45-72 after flights. The preflight data were used as controls.

The observations were performed on 25 crewmembers of long term space flights onboard of the "Salyut-6,7" and "Mir" stations. The durations of flights varied from 60 to 366 days. In 14 cases (of 25) cosmonauts visited space the first time; in 4 - the long duration flight was preceded by short duration (7-day) one; in 7 cases cosmonauts were already acquainted with long term space flights (Fig. 1).

Results and Conclusion

Results of studies revealed in all members of long term SF alterations of motor functions analogous to those, described earlier (I.B.Kozlovskaya et al., 1981, 1982, 1985). On return to the Earth the crewmembers showed certain changes in state of i) muscle properties demonstrated by an obvious decrease of the gastrocnemius muscle stiffness, lowering its strength - velocity abilities, subatrophy and even atrophy; ii) proprioceptive inputs and spinal reflex mechanisms, shown by a definite increase of sole support areas vibrosensitivity as well as muscle stimulation sensitivity

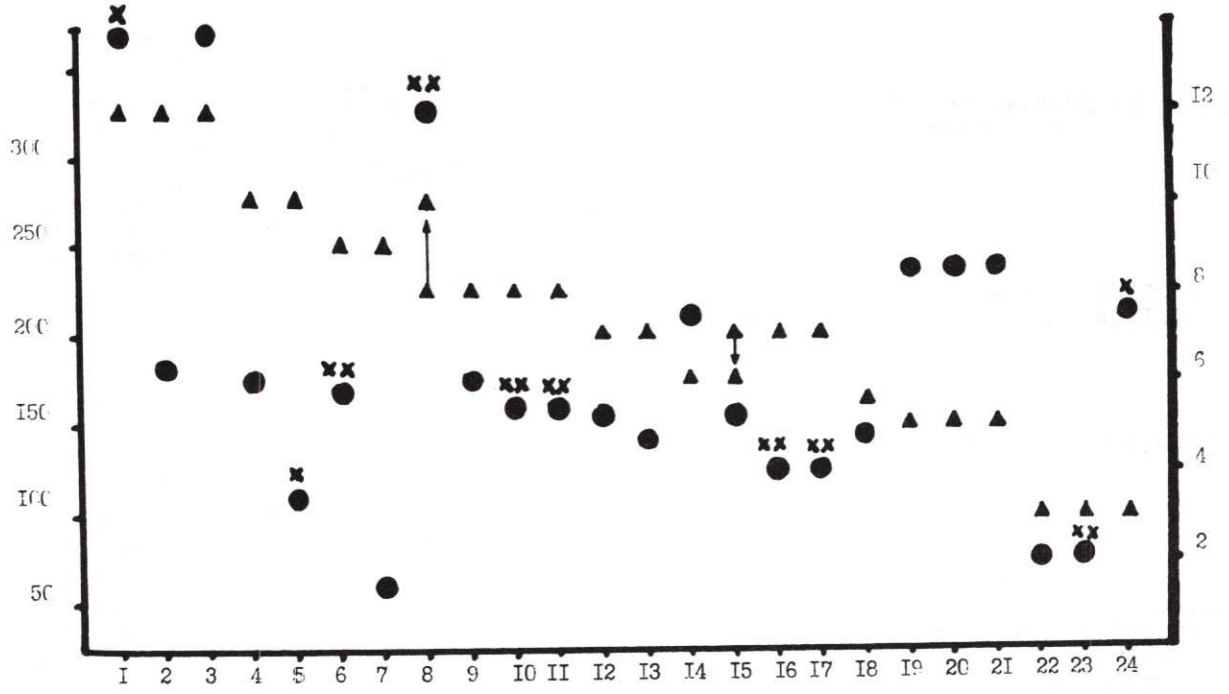


Fig. 1. Dependence of the intensity of microgravity effects on space flight durations (shown by circles) and on volume of physical exercizes (shown by triangles). The abscissae shows the rank number of the crew member according to the intensity of motor effects. The ordinate (on the left) shows flight duration, on the right - volume of physical exercises in marks.

(Achill reflex); decrease of the maximal EMG response and disturbances of interlimb synergy; iii) motor control systems, revealed by a decrease of upright posture stability, especially evident in perturbation test; locomotor disturbances; alterations of accuracy control (hypogravitational ataxia).

Intensity and duration of changes in different SF differed considerably. But again they did not show any correlation with SF duration, demonstrating at the same time quite strong correlation with the intensity and volume of PhE, used during flight (Fig. 1).

This conclusion is supported strongly by the results of studies, performed on members of crews of the 2nd and 3rd expedition onboard of "Mir" station, that are demonstrated in Figs. 2-6. As it is seen in Figs. 2,3, the decrease of the strength-velocity properties of gastrocnemius muscle was maximal in this group in one member of the 2nd crew with 160-day of SF - the value of loss reached in this case 60-70%; the minimal loss on the opposite was recorded in members of the 3rd expedition with 366-day flight duration and the 3rd member of the 2nd expedition with 330-day flight duration.

Analogous results were obtained when depth and duration of the upright posture alterations were compared. These results are shown in Figs. 5-6. It is seen that correction time in perturbation test increased after SF in one member of the 2nd crew 5 times (duration of SF in this case was 175-day); at the same time in 2 members of the 3rd expedition (360 day) and 1 of the 2nd - with 330-day of flight duration the increase reached hardly 1.5. The same was true also for the other characteristics of the correction responses.

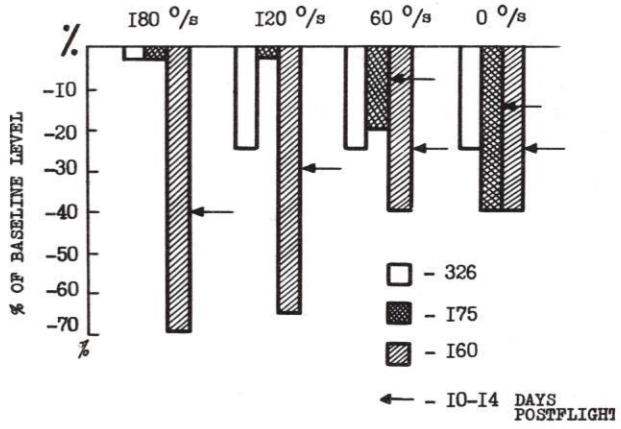


Fig. 2. Strength-velocity properties of m.gastrocnemius in crew members of the 2nd expeditions onboard "Mir" station.

Thus, in full accordance with the conclusions grew out of simulation studies the results of present studies demonstrated clearly the independence of the intensities of space flights motor effects on the flight duration and on the opposite their firm dependence on characteristics of physical exercise countermeasure used during flight.

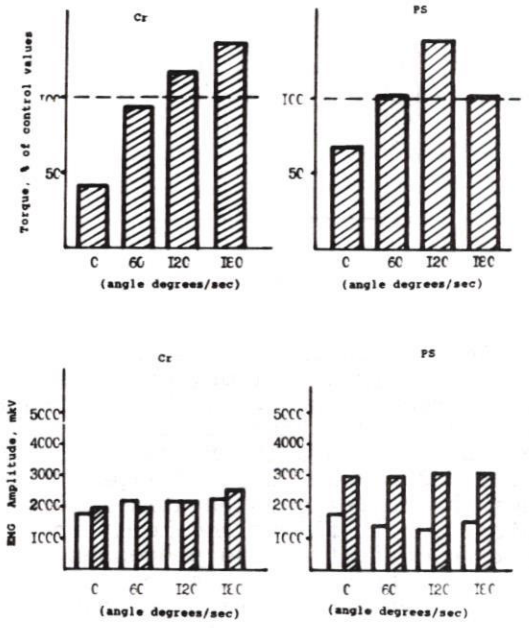


Fig. 3. Strength - velocity properties of m.gastrocnemius in crew members of the 3rd expeditions onboard "Mir" station.

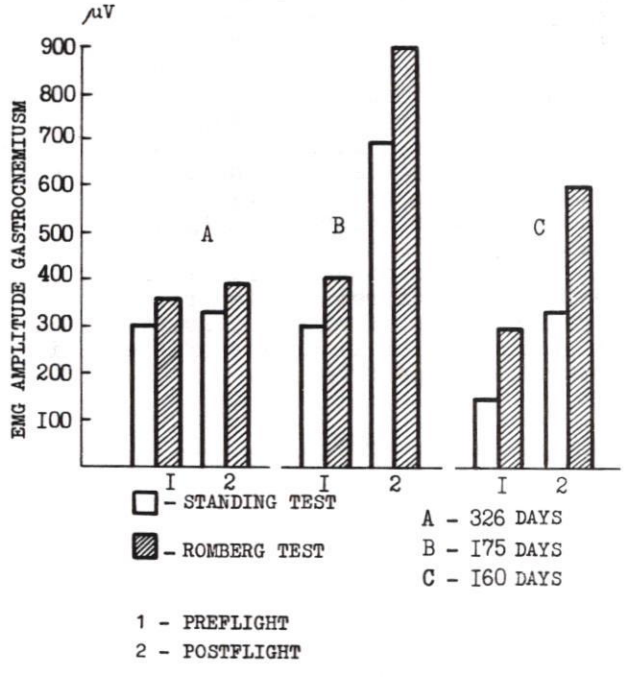


Fig. 4. Electromyographic cost of the upright posture in crew members of the 2nd expeditions onboard "Mir" station.

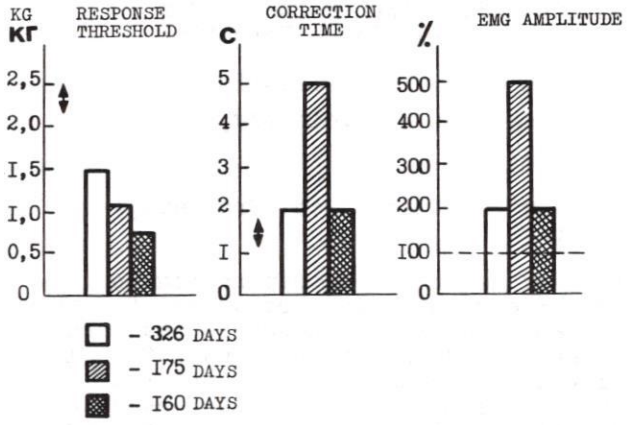


Fig. 5. Characteristics of corrective postural responses in crew members of the 2nd expeditions onboard "Mir" station.

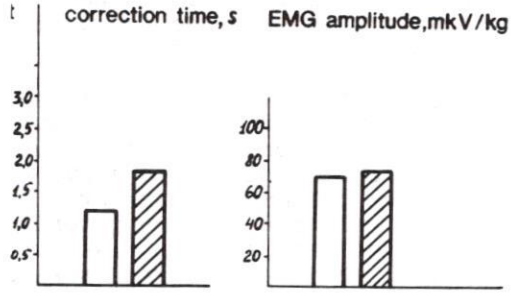


Fig. 6. Characteristics of corrective postural responses in crew members of the 3rd expeditions onboard "Mir" station.

References

1. Kozlovskaya I.B., Kreidich Yu.V., Rachmanov A.S. Mechanisms of the effects of weightlessness on the motor system of man. The Physiologist, 24, 59-63 (1984).

2. Otelin A.A., Mashansky V.F., Mirkin A.S. In: Fater-Pacini Corpusculae. Nauka, Leningrad, pp.403 (in Russian) (1976).

3. Kozlovskaya I.B., Aslanova I.F., Grigorieva L.S., Kreidich yYu.V. Experimental analysis of motor effects of weightlessness. The Physiologist, 25, 49-52 (1982).

4. Kozlovskaya I.B., Aslanova I.F., Kirenskaya A.V. The effect of support unloading an characteristics of motor control system activity. In: Motor Control, New York, Pergamon Press.

Destructive and Adaptive Processes in Human Vertebral Column Under Altered Gravitational Potential

G.P.Stupakov, Yu. V.Mazurin,
V.S.Kazeikin, Y.B.Moiseyev,
V.V.Kaliakin

Institute of Biomedical Problems,
Moscow,USSR

Laws of destruction, reparation and adaptive reformation processes of human vertebral column structures have been investigated in the paper as a function of gravitational exposure amplitude, duration and recurrence under aerospace flights by using the experimental dates and the mathematical model.

In the practice of aerospace flights human may be subjected to gravitational factors of various characteristics affecting state of muscular-skeletal apparatus. Moreover the most frequent and significant changes and after-effects have been observed in the vertebral column (5). The goal of this study is to establish general laws of destructive, reparative and adaptive processes in the human vertebral column related to gravitational potential changes during aerospace flight. Characteristics of this potential may be subdivided into several classes:

1. single high-intensity impact exposure related to an ejection process;
2. repeated impact exposures of average intensity related to parachute jumps and aircraft aerofinisher landings;
3. repeated in-flight accelerations;
4. weightlessness.

In the above mentioned classes gravitational potential has been shown to differ in magnitude (amplitude) and exposure time, i.e. "amplitude-temporal" characteristics of a stress exposure may determine differences in the vertebral column morphological changes under varions conditions.

Key processes which define these differences are:

- vertebra structure destruction;
- vertebra reparation;
- adaptive reformation.

Formalization of the mentioned processes is expected to require different approaches in investigations and theoretical generalizations.

Investigation of destruction processes for hypergravitational conditions (classes 1-3) has been conducted in the series of the human vertebra segment T_{II}-L_3 studies with various rates of loading corresponding to characteristics of impact exposures and in-flight accelerations. Processes of microdestructions of vertevra structures have been assessed by parameters of acoustic emission (8), i.e. by acoustic signals accompanying some crack formation recorded at a level of T_{12} (Fig. 1).

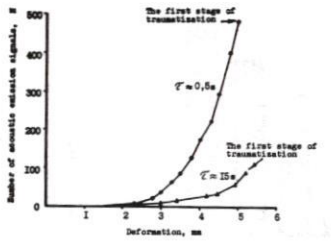


Fig. 1 Accumulation of acoustic emission signals as a function of an absolute deformation value of the vertebral column segments T_{II} - L_3 at a different duration of impulse loading (τ_s)

Fig. 1 illustrates that firstly in case of deformation arises, emission signals increase , secondly that the same magnitude of deformation is assumed to correspond to more intensive cracks formation under conditions of impact loadings than under quasi-static loadings typical for in-flight accelerations. In other words destructive processes have been shown to predominate in characteristics of vertebra deformation under impact, and conversely under quasi-static loading-elastic and plastic ones. This is particularly importante in a systemic investigation of different mechanical effects upon human vertebral column.

The most important aspect here is a rate of reparative processes which is difficult to assess in human experiments. Therefore an indirect method has been employed to express the early obtained relationship between clinical recovery time and the vertebral column destruction extent (5) and parameters of acoustic emission. The comparison of the acoustic signals number, corresponding to the first traumatization stage and a typical (for this stage) time of medical rehabilitation, permits to obtain a relationship reflecting time of reparative process for a certain number of microdestructions with an assumption of linearity of the indices interdependence (Fig. 2).

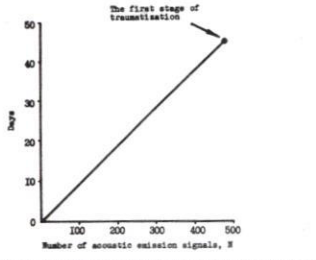


Fig. 2 Rate of vertebra microdestruction reparation according to the criterion AE following impact exposure.

The initial data obtained give an opportunity to study and explain after-effects of mechanical loading upon the vertebral column of the above mentioned three classes of hypergravitational potential.

Ejection Impact Acceleration

Operation of the most modern ejection systems results in vertebral column exposures amounting to about 60% of the value, which produces a traumatic damage of the first degree (stage) of severity. In ac-

cordance with the results of the experiments on the vertebra segments, the number of the vertebra microdestructions is estimated to average 30% of the overall number typical for macrotrauma. Taking into consideration the data of Fig. 2. the time of the reparative process of the developed microlesions accounts for about 14 days. The most probable result of ejection would be absence of any unfavourable remote after-effects.

Repeated impact accelerations of intermediate intensity

Results of acoustic emission recording under loading of the vertebra segments and measuring of accelerations affecting human body during simulated parachute landings demonstrate a possibility of some vertebra microfractures at each impact. Effects of these repeated impact exposures for remote periods are expected to be assessed by the ration of the characteristics "destruction-reparation". If a duration of a period between exposures is sufficient for reparation of the destructed structure integrity, then development of any pathological shifts in the vertebral column is unlikely. And on the contrary, in case of incomplete reparation, summation of effects of vertebra impact loading is possible along with variety of pathology. Such considerations are apparently relevant to pilots of naval aviation with aerofinisher landing.

Repeated in-flight accelerations

Practical absence of vertebra structure destructions at segment loadings due to exposure to in-flight accelerations of 6g allows to assume that even within a long-term flight practice there is no summation of the vertebral column destructive processes in pilots. Moreover taking into consideration existing theoretical ideas of bone structure reformation characteristics under their mechanical loading (3) it is possible to expect a development of adaptive vertebra processes manifesting by their density increase.

Validation of the suggested hypothesis has been performed by the results of the pilot examinations who have ejected from an aircraft without complications, sports-men-parachutists and sportmen-pilots.

In a year following the ejection there were no differences in the rate of roentgenographic symptoms of the vertebral column osteochondrosis in 20 pilots aged 25-30 in comparison to the pilots of the control group similar by composition and age. This fact allows to conclude that the vertebra microinjuries obtained at a single exposure to intensive impact acceleration heal practically without a trace.

Density of bone structure of vertebra bodies has been assessed in sportsmen-parachutists and sportsmen-pilots by a method of double photonic absorptiometry (jointly with V.S.Oganov and A.S.Rachmanov), besides roentgenodiagnostics of the vertebrae state has been conducted.

Monotonic, practically unchanged content of mineral substances in vertebral bodies has been observed in parachutists of different groups aged 20-39 years in a study of vertebra body density in comparison with its decrease in the average male population (in accordance with the literature data, 4).

The density of vertebral bodies has been increased in the pilots and by the age of 39 the differences reached about 16% in comparison with the control group. A significant direct correlation has been observed between the body mass and the overall bone vertebra mineral (correlation coefficient $r=0.520$ $p < 0.02$) in case of the similar correlation lack in the parachutists ($r=0.021$).

According to the data of our previous investigations (5), the correlation of the mentioned indices has not been practically shown in the average male population ($r=0.15$).

Pathological symptoms manifested in a form of marked osteochondrosis, multiple Schmorl's herniaes and deforming spondylosis have been revealed in parachutists at X - ray examination of the vertebral column 4–10 times more often than in pilots.

After-effects of gravitational exposures, different by magnitude and duration, may be reviewed under category of "dose-effect". The largest composite of a dose, i.e. of a load integral by time, depends on conditions of normal locomotor activity under vertical position and during sleep (horisontal position). The greatest amplitude of the gravitational exposure corresponds to impact accelerations in parachutists, and the lesser one -to in-flight accelerations in pilots. "Dose" characteristics of these exposures are estimated to be respectively 0.014% and 0.2% of the value related to normal locomotor activity in a vigilant state.

The comparison of dynamics of vertebrae bodies density with the hypergravity characteristics allows to conclude, that the structural changes of bone substance depend both on the magnitude and the duration of mechanical stress. The duration of the impact load on parachutists is evidently insufficient to induce the marked adaptive changes, directed at increasing the vertebrae resistance to mechanical load through increasing their structure density. For all that, one must take into account, that partially bone formation potential is spent on the reparation of damaged structures. The above pathological changes develop with time in case of incomplete reparation.

The effects of in-flight prolonged accelerations are characterized by the more marked adaptive changes of bone density, becoming greater with the increase of the force load, which is indicated by the magnitude of body mass. Meanwhile the increase of in-flight loads

also induces the activation of plastic processes in bone structures. The average increase of the mineral substance content by 4.6% in the vertebrae of four persons, exposed to centrifugation for 2 to 2.5 months at 9g, confirms this.

Thus, the direction and the markedness of reaction of the bone reparation in the spine in response to the hypergravity effects depend, on one side, on their amplitude-temporal characteristics and, on the other side, must apparently be determined by the speed of the reparative and adaptive processes, which varies individually. It is known, that this speed depends, for instance, on the initial bone density and organism metabolic speed, determined by oxygen consumption.

The amplitude-temporal characteristics of the force effect on the spine can be considered as a basis for the assessment of its bone tissue reaction to the conditions of the fourth class gravitational potential - e.g. weightlessness.

Earlier we proposed the hypothesis on the possibility of gradual increase of the interdisc pressure due to unloading of the spine, corresponding the osmotical level (6).

In other words, in spaceflight the level of the tense-deformed condition of vertebrae may correspond to its magnitude, characteristic of the vertical posture in the ground conditions. In this case the duration of the effect from such condition under weightlessness lengthens, because on the ground in the horizontal position during sleep the interdisk pressure decreases twofold. Hence, under weightlessness the time component of load integral might exceed the corresponding value typical for ground conditions. However this condition is possible only under sufficient hydratation capacity of the intervertebrae disks substances, but under insufficient hydratation the level of disks pressure and the level of the tense-deformed condition of vertebrae will decrease. If these considerations are true, under conditions of real and simulated weightlessness the options of vertebral bone mass gain or decrease are possible.

According to the literature data both options have observed (1,2), which substantiate further theoretical generalization.

Assuming the maximal recorded gain in the bone structure density as corresponding to the disks pressure in the vertical posture for the ground conditions 6.5 kgs/cm^2 and assuming that the theoretical speed of density decrease is of 7.3% within a month under absence of defensive mechanisms exhibited by intervertebrae disks (7), it is possible to establish the dependance of density changes from the temporal pressure integral (Fig.3).

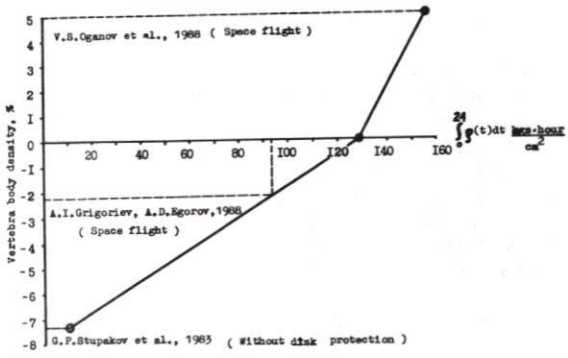


Fig. 3 Possible variant of vertebra body density as a function of integral of intradiscal pressure (ρ, kgs/cm^2) by time (t, hours) within twenty-four hours.

Using the dependance one can calculate, that in cosmonaut with speed of bone substance resorbtion in vertebrae of 2% within a month, the corresponding pressure in disks could be about 4 kgs/cm^2.

The destructive and adaptive processes in spine under 4 classes of the loads can be expressed (formalized) by the mathematical model. The model reflects the dynamics of changes of average number intact m_1 and destructed m_2 trabeculae against the time as a result of flow of events of resorption and synthesis with intensities $\lambda_{12}(R_i)$ and $\lambda_{21}(R_i)$, depending on the levels of spine deformation (induced by mechanical loads) and also on the metabolism speed.

A possibility of instant trabeculae destruction under the effects of the first and second class loads is taken into account. The diagram of the system state is shown on figure 4.

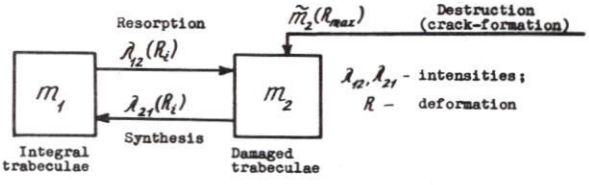


Fig.4 Diagram of system states.

The flow-graph model is described by the equations (1) - (3).

$$\ddot{R} + 2\xi\omega_n\dot{R} + \omega_n^2 R = a(t) \quad (1)$$

$$\dot{m}_1 = -\lambda_{12}(R_i)m_1 + \lambda_{21}(R_i)m_2 - \tilde{m}_2(R_{max})\mathbb{1}(\dot{R}-\dot{R}_i)\mathbb{1}(t-\tau_j) \quad (2)$$

$$\dot{m}_2 = \lambda_{12}(R_i)m_1 - \lambda_{21}(R_i)m_2 + \tilde{m}_2(R_{max})\mathbb{1}(\dot{R}-\dot{R}_i)\mathbb{1}(t-\tau_j) \quad (3)$$

Were $\mathbb{1}$ - the Haviside single function. The magnitude of the spine deformation as the dynamic reaction to the effect of accelerations $a(t)$ the right part (I) or forces computed with the help of the equation (I). The maximal magnitude of deformation is utilized to determine the number of the instantly destructed trabeculae $\tilde{m}_2(R_{max})$.

It is assumed that under the impact accelerations at the moment τ_j such destruction occurs only if the deformation speed $\dot{R}$ exceeds some threshold magnitude R_g. The instantly destructed trabeculae is described by the third summand of the equations (2) and (3). The first two summands of the equations (2) and (3) describe reparative and adaptive processes. Low level of mechanical loads induces small deformations R_i and the intensity of the resorption processes of bone tissue $\lambda_{12}(R_i)$ prevails the intensity of synthesis processes $\lambda_{21}(R_i)$.

With time the loss of bone mass will be increasing. Increase of mechanical load levels will result in opposite conditions.

Indentification of the model parameters requires a great deal of experimental studies with recording the histogram of the operating loads (amplitudes, durations, frequencies) and measuring the changes of bone structure density under different levels of physical activity.

The integration of the system equations (I) - (3) within one cycle of loading results in changes of the number of intact and resorbed trabeculae for one cycle, designated Δm_1 and Δm_2. In a particular case at fixed intensities of dissociation and reparation processes, when the sum of intact and resorbed trabeculae is constant and corresponds to the initial value $m_1(t) + m_2(t) = m_1(0) + m_2(0)$, then equations (2) and (3) permit the following analytical solution: (4).

$$\Delta m_1(t) = \frac{\lambda_{21}[m_1(0)+m_2(0)]}{\lambda_{12}+\lambda_{21}} + \frac{\lambda_{12}m_1(0) - \lambda_{21}m_2(0) - (\lambda_{12}+\lambda_{21})t}{\lambda_{12}+\lambda_{21}} \qquad (4)$$

Where $\Delta m_1(t)$ - the current change of gain in intact trabeculae number as a result one cycle of loading. The first summand is equal to the fixed value of the integral trabeculae number m_1, at t, tending to infinity. The second summand comprises the effect of the initial value $m_1(0)$ and $m_2(0)$ at start of the cycle, which is conditionally taken for t=0.

In the linear task setting the accumulated change of the intact trabeculae number within numerous loading cycles does not depend on the sequence of load applications and can be obtained by summation of increments for each loading cycle in accordance with the occurance rate an amplitude and the duration, which are determined by loads histogram.

The greater model generality would be achieved, if the limitations on the summary intensity of synthesis flow of resorbed trabeculae, related to metabolism intensity and bone structure density, are taken into account.

The limitations can be expressed by the equation (5):

$$\lambda_{21}(R_i)m_2 \leq L_\nu, \quad \nu = 1,2,\ldots n \qquad (5)$$

Where - constants, characterizing the limit values of the summary flow of reparation for different levels of physical activity with the number ν.

Correction for the limitation (5) makes the equations (I) and (2) essentially nonlinear. Meanwhile the hypothesis of the linear summation of changes within one loading cycle ceases to be applicable in calculation of the accumulated intact and resorbed trabeculae (m_1 and m_2), thus complicating the prediction, which requires integration of the equations systems (1) - (3) throughout the whole recorded history of load exposures.

It is known that bone structure density is related linearly to integral trabeculae number m_1, and the magnitude of effective destructed tensions is proportional to $\rho^{1/2}$. Therefore, the digital method of the density change prediction can be used for the estimation of the risk level of aircrew members during the ejection and the cosmonauts at landing after weightlessness exposures, prediction of repeated impact load effects and the prognosis of effective exercise complexes. The presented approach also allows to apply the methods of optimization (for example, the principle of the Bellman dynamic programming) to solve the tasks of the optimal planning specific physical activity patterns to prevent unfavourable bone changes.

References

1. Grigoriev A.I., Egorov A.D. Phenomenology and mechanisms of major physiological changes in weightlessness //Space Biology and Aerospace Medicine. - 1988. - N 6. - P. 4-17.

2. Oganov B.C., Rachmanov A.C., Morukov B.V., Janson Ch.A., Tatarinov A.M., Saichik B.E., Ternovoy S.K., Kann K. The investigations of the bone tissue state by noninvasive methods in the conditions of prologed hypokinesia //Space Biology and Aerospace Medicine. - 1988. - N 1. - P. 30-33.

3. Pugh J.W., Rose R.M., Radin E.L. Elastic and viscoelastic properties of trabeculae bone: dependence on structure //J.Biomech. - 1973. - V 6. - N 5. - P.475-485.

4. Riggs B.L., Wahner H.W., Dunn W.L. et al. Differential changes in bone mineral density of the appendicular and axial sceleton with aging //J. Clin Invest. - 1981. - V. 67. - P. 328-335.

5. Stupakov G.P., Kozlovsky A.P., Kazeikin V.S. Spine biomechanics in the condition of load impact effect in the practice of aerospace flights //The problems of Space Biology. - 1987. - V. 56. - P. 240.

6. Stupakov G.P. Biomechanical characteristics of bone structure changes folloving real and simulated weightlessness //The Physiologist. - 1988. - V. 31. - N 1. - Suppl. - P. 4-7.

7. Stupakov G.P., Volozhin A.I. Bone system and weightlessness//The problems of Space Biology. - 1989. - V. 63. - P. 185.

8. Stupakov G.P., Kazeikin V.S., Morukov B.V. Microgravity - induced changes in human bone strength //The Physiologist. - 1989. - V. 32. - N 1. - Suppl. - P. 41-44.

BONE CELLULAR EFFECTS AFTER WEIGHTLESSNESS EXPOSURE - AN HYPOTHESIS.

L. Vico, C. Alexandre.

Laboratoire Biologie Tissu Osseux LBTO - Faculté de Médecine - Saint Etienne - FRANCE

Our knowledge on the human bone adaptation to spaceflight has not been largely increased in the last few years. Data have been principally obtained from Skylab (1973-1979) astronauts (10, 28, 30) and Salyut-6 cosmonauts (23, 29). Perturbations of calcium homeostasis involve slightly increased plasma calcium, increased urinary calcium excretion and a negative calcium balance. Loss of mineral from the calcaneus was correlated with the calcium imbalance. On the contrary, no change was detected in the ulna and radius. The bone density in the vertebral column might slightly increase during a long-term spaceflight, but the reasons are unknown (4). Moreover, no dramatic bone loss was reported in Soviet cosmonauts after a year in space. Finally, measurements in one crewmember of the Soviet 28d. Aragatz mission using an x-rays scanner (15) was performed: an elevated bone density was observed in the distal radius whereas no change occured in the distal tibia. These data constitute preliminary results: the differential bone responses could be related to differences in weight-bearing function and/or in trabecular or cortical envelope nature. However, a redistribution of calcium ions could occur leading to the creation of a space skeleton different from the earth skeleton but with no great total bone loss. This is consonant with calciotropic hormonal status (principally serum PTH concentration) evaluated before and after the Skylab missions that showed no change that would account for the mobilization of bone mineral during spaceflight (2). Evaluation of vitamin D and PTH status have benefited from more reliable analysis in Spacelab-2 flight (13) and 51-G flight (1), of one week duration. No change was observed after the 51-G flight. In the Spacelab-2 flight, the vitamin D hormone was elevated in all astronauts at the end of the first inflight day but returned to normal by the seventh day. Thus, this hormone could have transiently activated the bone remodeling.

Flight	date	duration (day)	collaborative studies
Soviet Cosmos 605	1973	21.5	USSR-US
Soviet Cosmos 690	1974	20.5	
Soviet Cosmos 782	1975	19.5	USSR-US
Soviet Cosmos 936	1977	18.5	USSR-US
Soviet Cosmos 1129	1979	18.5	USSR-US
Soviet Cosmos 1514	1983	5	USSR-France
Soviet Cosmos 1667	1985	7	USSR-France
Soviet Cosmos 1887	1987	12.5	USSR-US-France
American Spacelab 3	1985	7	

Table 1 - Spaceflights with biological experiments

Bone investigations on rats from Soviet Cosmos biosatellites and American Spacelab-3 flight (Table 1) have contributed to our knowledge on the effects of spaceflight on the bone cells. However, we do not understand yet the dynamic processes according to flight duration: care must be taken when comparing different flights because rats are not of the same strain, sex and age and because differences in time of sacrifice after landing occur from an experiment to another. Futhermore aims and methodology of analysis are not homogenous in laboratories implicated in these studies (Table 2).

FLIGHT	RATS	SACRIFICE	EXPERIMENTS
Cosmos 605	male Wistar ~250g	1-2 d. after recovery	- histological investigations of long bones (33)
Cosmos 782 Cosmos 936	male Wistar ~215g male Wistar ~215g	immediate immediate	- long bone diaphyseal tetracycline labeling (11) - histomorphometric analyses of long bone metaphysis (24) - Ash content and strength testing analyses (18, 22)
Cosmos 1129	male Wistar ~290g	7-11h after recovery	- osteoblast differentiation - bone mineral content analysis - quantitative histochemistry (7) - evaluation of bone resorption by continuous tracer techniques (5) - tetracycline labeling and histomorphometric analyses of non-weight-bearing bones (19) and weight-bearing bones (9, 31)
Cosmos 1514	pregnant Wistar ~300g	no more than 6h after recovery	- histomorphometry and histoenzymology analyses of different skeletal sites (26)
Spacelab 3	male Sprague-Dawley large rats ~400g small rats ~250g		- density gradient analysis and X-rays diffraction (20) - chemical analysis (14) - osteoblast differentiation (16) - histomorphometric analyses of long bones and lumbar vertebrae (32) - morphological and mechanical properties of long bones (17)
Cosmos 1617	male Wistar ~300g	no more than 6h after recovery	- histomorphometry and histoenzymology analyses of different skeletal sites (27)
Cosmos 1887	male Wistar ~300g	2 d. after recovery	- histomorphometry and histoenzymology analyses of different skeletal sites

Table 2 - Experimental conditions of bone rats studies

Collaborative studies between the US and USSR on male rats flown onboard the first Cosmos biosatellites (18.5 to 21.5 d.) have been usefull in the investigations of some of the changes in bone occurring during spaceflight:

- Qualitative appreciation by microscopic examinations of long bones of Cosmos 605 rats primarily suggested an osteoporosis in methaphyses and, in some cases, an inhibition of bone longitudinal growth (33).
- The most striking effects of the Cosmos 782 flight was a cessation of bone growth in the tibial diaphysis as indicated by surimposed lines of two tetracycline labels (3 days before and 3 days after flight) nammed as arrest line (11).
- The mineralization defect appeared to be related to an abnormal organic matrix (21) that may be partially responsible for the decrease in torsional strength (22). Cessation of bone formation also occured after the Biocosmos 936 (24) and 1129 (31). In both Biocosmos 782 and 936, tibial, humeral and femoral longitudinal growth rate were not altered. In the Biocosmos 936 flight, some rats were maintained in a 1G centrifuge: the tibial shaft bone formation rate was reduced but not the femoral mechanical strength (22). As such, the decreased bone strength observed after spaceflight could only be attributed to null gravity (18).
- A histomorphometric study on metaphyseal tissue of proximal tibial and humeral metaphyses had been attempted in rats of the Cosmos 1129 experiments (9, 12). Measurements were done at various distances from the growth cartilage metaphyseal junction. A marked decreased mass of trabecular bone and an increased fat content of the bone marrow were observed. Bone cells were evaluated with morphological criteria. The osteoblast population appeared to decline immediatly adjacent to the growth plate metaphyseal junction, but osteoclast number was unchanged (Table 3).

		% from control
Trabecular bone volume	tibia (proximal)	-35
	humerus (proximal)	-30
Endosteal bone resorption	tibia (shaft)	0
	humerus (shaft)	0
Marrow fat	tibia (proximal)	+150
	humerus (proximal)	+270
Osteoblast population	humerus (proximal)	-40
Osteoclast population	humerus (proximal)	0

data from: Morey et Wronski (12)

Table 3 - Histomorphometric results obtained in long bones of rats flown onboard the Biocosmos 1129.

In addition to histologic methods, resorption was indirectly assessed as reflected by medullary area alterations (31, 12) and by calcium kinetic studies (5). All these studies indicated no elevation. Three week spaceflights did not only affect the weight-bearing skeleton. Quantitative histochemistry of rat lumbar vertebrae suggested slower bone turnover, resulting in accumulation of older bone (7). However, sections of rat mandible covered by masticatory muscles did not incur the decreased periosteal bone formation noted in weight-bearing bones, whereas impairment bone formation was evidenced in areas without muscle attachement (19). In tibial diaphyses, Spector et al.(21) concluded that cessation of bone formation did not occur where intrinsic muscle forces continue to act.

Collaborative studies between France and USSR gave us the opportunity to more specifically define the time course of inhibition of bone formation and differences in regional bone turnover at several sites in rats.

- The first of the series, the 5d. Biocosmos 1514 transported pregnant rats. Histomorphometric analyses in weight bearing (tibia) and non-weight-bearing (vertebrae) bones indicated no change in bone mass and inner structure in either type of bone. No alteration on bone formation occured but an increase of osteoclast number was evidenced at the vertebral level (26). This resorption stimulation could reflect an early and transient adaptation to microgravity or can be due to interferences between hormonal changes of pregnancy and microgravity effects.

- As early as the 7th day of microgravity exposure (Biocosmos 1667), marked alterations were detected in weight bearing bones (27): the tibial elongation rate was decreased (unpublished data); in proximal metaphysis, bone loss due to reduced number of trabeculae, the remaining ones being thinner, was observed. Bone cellular activities were evaluated at the level of the secondary spongiosae where remodeling occurs (3): formation activity, reflected by measurements of osteoid seams, was decreased. Resorption activity, estimated by count of osteoclast number and active resorption surfaces using a histoenzymologic method (6), remained unchanged. In normal mineral homeostasis, a decrease in bone formation will lead to a decrease in bone resorption as well, so that bone mass will be maintained. The uncoupling between cellular activities might be responsible for the bone loss.

In thoracic and lumbar vertebrae, bone mass, bone architecture and resorption activity were not changed.

In proximal femoral metaphysis, measurements were performed in an area where muscle inserted into bone via the Sharpeys' fibers. Unlike the tibia, no significant change was observed in bone mass and bone cellular activities. In view of these data, we hypothesized that the deleterious effects of mechanical unloading due to weightlessness can be partially avoid by muscle contraction strength, not totally inhibited during spaceflight. Thus bone mass, and not only bone formation (19, 21, 25) might be protected.

- In the 7d. Spacelab3 flight, histomorphometric analysis was performed in the long bones and lumbar vertebrae (32). Results showed strong trends of decreased tibial periosteal and longitudinal bone growth, reduced osteoblast size in the proximal tibia and decreased osteoblast surface and number in the lumbar vertebrae. Reduced accumulation of mineral and osteocalcin in humerus and vertebrae with no associated decrease in collagen suggested that both mineralization and collagen metabolism are impaired (14). For the most part, histological indexes of bone resorption were normal.

- Changes observed in the Spacelab3 flight are of minor extend compared to those of the Biocosmos 1667. Differences in strain and age of rats and in skeletal sites explored may explain some of these variations. Furthermore, the effects of spaceflight in the slower growing humerus should be retarded compared to those of tibia. This is consonant with Shaw et al.(17) results that indicated impeded maturation of bone strength and stiffness with the effects more pronounced in the tibia than the humerus.

Gradient density analyses provided interesting data about the matrix and mineral (20). The patterns of bone maturation indicated a decreased in bone growth / turnover and X-Ray diffraction of vertebrae indicated that spaceflight was associated with a decreased in apatite cristal size / perfection.

- The Biocosmos 1887 was of intermediary duration. Instead of the 14 days planned, it lasted 12,5 days and necropsy occured two days latter. We performed histomorphometric measurements identical to those of the Cosmos 1667 mission (Table 4). Same bone samples plus the proximal humerus were investigated. Compared with the 7d Cosmos flight we found no change in the tibial elongation rate. In the secondary spongiosa, bone loss was principally associated to trabeculae thinning. As in the 7d. flight, osteoid parameters were depressed whereas resorption activity remained unchanged. Femoral result confirmed those of the Cosmos 1667.

Quite unexplained was the stimulation of the resorption activity observed in the lumbar vertebrae. Possibly, because rats did not eat for nearly two days before sacrifice, the absence of a dietary source of calcium may have stimulated bone resorption.

In the proximal humerus, the primary spongiosa thickness was reduced suggesting a decreased rate of bone elongation. At the level of the secondary spongiosa, results were comparable to those of tibia but of minor extend.

In this Biocosmos the two days post-flight period could have mask microgravity effects. For example, surimposed stress and/or recuperation could have modify the primary spongiosa activity whose life span is about 4 days in 300g Wistar rats. A 14 day mission was realised in October 1989. Same investigations will be performed allowing a verification of Biocosmos 1887 results.

			7d. COSMOS 1667	12.5d. COSMOS 1887
TIBIA	1° Spongiosa	thickness	-53%	unchanged
	2° Spongiosa	bone volume	-47%	-31%
		trabecular number	-40%	unchanged
		trabecular thickness	-20%	-13%
		formation (osteoid)	-56%	-37%
		resorption	unchanged	unchanged
HUMERUS	1° Spongiosa	thickness	-	unchanged
	2° Spongiosa	bone volume	-	unchanged
		trabecular number	-	unchanged
		trabecular thickness	-	unchanged
		formation (osteoid)	-	-33%
		resorption	-	unchanged
FEMUR	bone mass	and architecture	no significant	no significant
		formation (osteoid)	change	change
		resorption		
VERTEBRAE	bone mass	and architecture	unchanged	unchanged
		resorption	unchanged	increased

Table 4 - Bone histomorphometric results (% change from control) obtained in Biocosmos 1667 and 1887 flights.

Pooling our data obtained in a very responsive weight-bearing skeletal site explored in most experiments, i.e. the tibial metaphysis, and taking into consideration preceeding qualifications, we proposed a kinetic of bone cellular variations (Fig. 1).

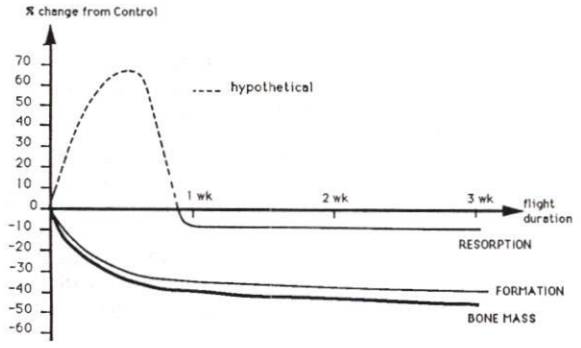


Fig 1 - Tibial secondary spongiosa - Evolution of bone mass and bone cell activities.

The first series of events occured during the first week and would include longitudinal growth failure (7d Biocomos 1667). A reduced primary spongiosa would constitute a poor substrate for the secondary spongiosa supply. It might explain why secondary spongiosa trabeculae appeared thin and less numerous. Futhermore, at the level of the secondary spongiosa, the reduced formation activity also contributed to trabeculae thinning. An earlier and transient increase in bone resorption cannot be ruled out (5d. Biocosmos 1514). This first phase may constitute an acute response of bone tissue to new environmental conditions.

In the 12.5d. Biocosmos 1887, events appeared less dramatic: a normalization of the primary spongiosa may explain the normal number of trabeculae found in the secondary spongiosa. But the trabeculae remained thin because formation activity continue to be low. The resorption activity appeared normal.

Three weeks spaceflights indicated that the bone elongation rate appeared normal. The formation activity continued to be depressed and the resorption activity was in the normal range with histological criteria or slightly reduced with data of calcium exchanges. The trabecular bone mass appeared similarly affected to those of rats of two weeks mission. Thus, after an acute response, a slow bone turnover principally characterized by net bone deficiency, takes place.

More details have been provided in osteoblast histogenesis in periodontal ligament (16). Proliferation and differentiation sequence of osteoblast histogenesis comprised steps with different cell types recognizable to their nuclear size. Briefly, there is two principal pools: the osteoprogenitors cells (nucleus inferior to 80 μm^3) and the preosteoblastic cell (nucleus 80 to more than 170 μm^3). The shift in last stage of osteoprogenitor to first stage of preosteoblast is thought to be mechanically induced (8). Microgravity conditions appeared to block this conversion (16).

Actually, our knowledge on the adaptation of the rat bone tissue to spaceflights would probably result from microgravity, launch and landing conditions, fluid shift, altered circadian rythmicity, physiological stress... Bone cell responses are probably a composite response to these multiple factors. Some of these artifacts could be minimized in *in vitro* studies. Osteoblast cultures, which are already available in various laboratories, need to be flown in space.

REFERENCES

1 - ALEXANDRE C., VICO L., PILONCHERY G., CHAPUY M.C., DELMAS P.D., CHAPPARD D. : Effets de l'apesanteur sur le métabolisme phosphocalcique et sa régulation hormonale chez l'homme au cours du vol spatial franco-americain 51G. Pathol. Biol., 36 (2), 144-148, 1988.

2 - ANDERSON S.A. et COHN S.H. : Bone demineralization during spaceflight. The Physiol., 28, 212-217, 1985.

3 - BARON R., TROSS R., VIGNERY A. : Evidence of sequential remodeling in rat trabecular bone: morphology, dynamic histomorphometry, and changes during skeletal maturation. Anat. Rec., 208, 137-145, 1984.

4 - BENSON A., BONDE-PETERSEN F., HINGHOFER-SZALKAY H., KIRSCH K., KLEIN K.E., MILHAUD C., STEGEMANN J. : Physiology and Medicine In: Life Sciences Research in Space. Ed. OSER H. and BATTRICK B., ESA SP-1105, pp14, 1989.

5 - CANN C.E., ADACHI R.R. : Bone resorption and mineral excretion in rats during space flight. Am. J. Physiol, 244, R327- R331, 1983.

6 - CHAPPARD D., ALEXANDRE C., RIFFAT G. : Histochemical identification of osteoclasts. Review of current methods and reappraisal of a simple procedure for routine diagnosis on undecalcified human iliac bone biopsies. Bas Appl. Histochem., 27, 75-85, 1983.

7 - EURELL J.A.et KAZARIAN L.E. : Quantitative histochemistry of rat lumbar vertebrae following space flight. Am. J. Physiol, 244, R315-R318, 1983.

8 - FIELDER P.J., MOREY E.R., ROBERTS W.E. : Osteoblast histogenesis in periodontal ligament and tibial metaphysis during simulated weightlessness. Aviat. Space Environ. Med., 57, 1125-1130, 1986.

9 - JEE W.S.S., WRONSKI T.J., MOREY E.R., KIMMEL D.B. : Effects of spaceflight on trabecular bone in rat. Am. J. Physiol, 244, R310-314, 1983.

10 - LEACH C.S. et RAMBAUT P.C. : Biochemical responses of the Skylab crewmen : an overview. In : Biomedical results from Skylab. Johnston R.S., Dietlein L.F. Eds NASA, Washington D.C., 204-216, 1977.

11 - MOREY E.R.et BAYLINK D.J. : Inhibition of bone formation during space flight. Science , 201, 1138-1141, 1978.

12 - MOREY-HOLTON E. et WRONSKI T.J. : Animal models for simulating weightlessness. The Physiol. 24(6), S45-46, 1981.

13 - MOREY-HOLTON E.R., SCHNOES H.K., De LUCA H.F., PHELPS M.E., KLEIN R.F., NISSENSON R.H., ARNAUD C.D. : Vitamin D metabolites and bioactive parathyroid hormone levels during Spacelab 2. Aviat. Space Environ. Med., 59, 1038-1041, 1988.

14 - PATTERSON-BUCKENDAHL P., ARNAUD S.B., MECHANIC G.L., MARTIN R.B., GRINDELAND R.E., CANN C.E. : Fragility and composition of growing rat bone after one week in spaceflight. Am. J. Physiol., 21, R240-R246, 1987.

15 - POUGET J.F., RUEGSEGGER P., ALEXANDRE C. : Aspects méthodologiques et résultats de l'étude de la minéralisation osseuse dans le cadre du Projet Aragatz. Résumé des présentations. Colloque Aragatz. CNES - GLAVKOSMOS. Villefranche Sur Mer. Oct. 1989.

16 - ROBERTS W.E, FIELDER P.J., ROSENOER L.M.L., MAESE A.C., GONSALVES M.R., MOREY E.R. : Nuclear morphometric analyses of osteoblast precursor cells in periodontal ligament, SL_3 rats. Am. J. Physiol., 252, R247-R251, 1987.

17 - SHAW S.R., VAILAS A.C., GRINDELAND R.E., ZERNICKE R.F. : Effects of a 1-wk spaceflight on morphological and mechanical properties of growing bone. Am. J. Physiol., 254, R78-R83, 1988.

18 - SIMMONS D.J. : Adaptation of the rat skeleton to weightlessness and its physiological mechanisms. Results of animal experiments aboard the Cosmos-1129 Biosatellite. The Physiol., 24, S65-S68, 1981.

19 - SIMMONS D.J., RUSSEL J.E., WINTER F., VAN TRAN P., VIGNERY A., BARON R., ROSENBERG G.D., WALKER W.V. : Effect of spaceflight on the non-weight bearing bones of rat skeleton. Am. J. Physiol., 244, R319-R326, 1983.

20 - SIMMONS D.J., RUSSELL J.E., GRYNPAS M.D. : Bone maturation and quality of bone material in rats flown on the space shuttle "Spacelab-3 Missions". Bone and Min., 1, 485-493, 1986.

21 - SPECTOR M., TURNER R.T., MOREY-HOLTON E., BAYLINK D.J., BELL N.H. : Arrested bone formation during spaceflight results in a hypomineralized skeletal defect. The Physiol., 26, S110-S111, 1983.

22 - SPENGLER D.M., MOREY E.R., CARTER D.R., TURNER R.T., BAYLINK D.J. : Effect of Spaceflight on bone strength. The Physiol., 22, S75-S76, 1979.

23 - STUPAKOV G.P., KAZEYKIN V.S., KOZLOVSKIY A.P., KOROLEV V.V. : Evaluation of changes in human axial skeletal bone structures during long-term spaceflights. Space Biol. Med., 18(2), 42-47, 1984.

24 - TURNER R.T., MOREY E.R., LIU C., BAYLINK D.J. : Altered bone turnover during spaceflight. The Physiol., 22, S573-S574, 1979.

25 - TURNER R.T., WAYLEY G.K., SZUKALSKI B.W. : Effects of gravitational and muscular loading on bone formation in growing rats. The Physiol, 28, S67-S68, 1985.

26 - VICO L., CHAPPARD D., ALEXANDRE C., PALLE S., MINAIRE P., RIFFAT G., NOVIKOV V.E., BAKULIN A.V. : Effects of weightlessness on bone mass and osteoclast number in pregnant rats after a five-day spaceflight (Cosmos 1514). Bone, 8, 95-103, 1987.

27 - VICO L., CHAPPARD D., PALLE S., BAKULIN A.V., NOVIKOV V.E., ALEXANDRE C. : Trabecular bone remodeling after seven days of weightlessness exposure (Biocosmos 1667). Am. J. Physiol., R243-R247, 1988.

28 - VOGEL J.M., WHITTLE M.W., SMITH M.C.Jr., RAMBAUT P.C. : Bone mineral measurements - experiment MO78. In : Johnston R.S., Dietlin L.F., eds Biomedical results from Skylab DC: NASA SD-377, 183-190, 1977.

29 - VOROBYOV E.I., GAZENKO O.G., GENIN A.M., EGOROV A.D. : Medical results of Salyut-6 manned spaceflights. Aviat. Space Environ. Med., 54, S31-S40, 1983.

30 - WHEDON G.D., LUTWAK L., RAMBAUT P.C., WHITTLE M.N., SMITH M.C., REID J., LEACH C., STADLER C.R., SANFORD D.D. : Mineral and nitrogen metabolic studies, experiment MO71. In : Johnston R.S., Dietlin L.F., eds Biomedical results from Skylab DC: NASA SP-377, 164-174, 1977.

31 - WRONSKI T.J. and MOREY E.R. : Effect of spaceflight on periosteal bone formation in rats. Am. J. Physiol., 244, R305-R309, 1983.

32 - WRONSKI T.J., MOREY-HOLTON E.R., DOTY S.B., MAESE A.C., WALSH C.C.: Histomorphometric analysis of rat skeleton following spaceflight. Am. J. Physiol., 252, R252-R255, 1987.

33 - YAGODOVSKY V.S., TRIFTANIDI L.A., GOROKHOVA G.P. : Spaceflight effects on skeletal bones in rats (light and electron microscopic examination). Aviat. Space Environ. Med., 47, 734-738, 1976.

EFFECTS OF SPACE FLIGHT ON DENDRITES OF THE NEURONS OF THE RAT'S BRAIN

P.V.Belichenko*, M.A.Machanov*, A.A.Fedorov*, I.B.Krasnov**, T.A.Leontovich*

*Brain Research Institute and
**Institute of Biomedical Problems,
Moscow, USSR

Introduction

Space exploration by a mankind needs thorough study of its influence on the brain structure and functions as well as determining the mechanisms of compensatory processes that accompany the flight. Some authors described structural changes of dendrites (D's) of neurons of adult mammalian brain under the influence of unusual environmental conditions or deafferentation (3,7,8). As it is known microgravity in space is accompanied by a fundamental changes of the character of sensory information entering the brain, first of all proprioceptive and vestibular ones (10). Although there are some studies of the morphology of the brain of animals subjected to a flight aboard the biosputniks, examination of their dendritic systems are lacking.

The aim of this study was to analyse the geometry of D's of the neurones of brain of rats that spent aboard biosputnik 7 days ("Cosmos-1667") or 13.5 ("Cosmos-1887) days and to find out whether there appear some changes in morphology of D's during flight. There were examined two classes of neurones: 1. related to functions known to be disturbed in space flight - the giant multipolar neurons (GMN's) of the reticular formation issuing reticulospinal pathways and related to automatic motor reactions (vestibulo-motor, trigemino-motor, etc.). GMN's are scattered along the brain stem being structurally alike everywhere. They long stright D's are adapted to integration of afferent impulses of quite different origin and from vast area; 2. cells that could probably take part in the compensatory processes - the pyramidal neurons (PN's) of the visual cortex (since physiological data point to an important compensatory role of vision during microgravity (9,12). Partly our results are published elsewhere (2).

Material and Method. Male Wistar-SPF adult albino rats (altogether 43) were used: 1. Flight group from biosputnik "Cosmos-1667" (7 days flight; controls: vivarium I and ground-based experiment with its vivarium control group). 2. Flight group from biosputnik "Cosmos-1887" (13.5 days flight; controls: vivarium and synchronous experiment). For analysis of GMN's all this material was used. Due to some peculiarities of research programs for biosputniks, the GMN's taken from biosputniks of 7 and 13.5 days flight belonged to different reticular nuclei (correspondingly, to n.gigantocellularis of medulla and to n.pontis oralis; 42 and 37 GMN's were analysed). For the visual cortex the small PN's of the II-III layers were examined, only in 13.5 days flight group with its two controls (29 neurons). Rats were decapitated and in 3-7 minutes the brain pieces were cut out and treated by Golgi method. Drawings of neurons were performed under the microscope by a drawing apparatus; certain parameters where measured and another calculated by our method (11). System ASM (Leitz) for measurings and computer PDP-11 for processing of figures were used. For GMN's the following parameters were obtained: number of D's, maximal radius of dendritic field, relative dendritic length, mean branching of a single D., branching of the whole neuron, number of foci of maximal branchings, (for a single D. and the whole neuron), volume of dendritic territory and the total length of D's in it, specific density of D's in the slide. By the small PN's of the visual cortex there were measured: the total length and the number of free ends of the apical and basal D's (separately), the length of the apical D. from the cell body till its first branch and till the most distal oblique D., the area of the dendritic field. The orientations of D's of both GMN's and PN's were also determined. For this purpose a transparent test-net with 15 sectors was placed on the drawing of the neuron and centered on the middle of the cell body. The total length of D's was measured in every sector and was calculated as a percentage total length of D's of the whole neuron (the polarogram of the neuron - Fig. 1). In order to determine the orientation of D's of every analysed GMN to either afferent input, slide was projected and the precise positions of this neuron and surrounding nuclei were marked. By combining the centre of the polarogram with the neuron's position one could disclose the orientation of it's D's. For determining the significant differences between the parametres of GMN's in the flight group and all the control groups Kolmogorov-Smirnov test was used. Since PN's possess a much more complicated and multiform structure than GMN's one could expect an unsynonymous reaction to the effect of the space flight within the same class of PN's. Therefore Q-factor analysis (4,18) was applied to a combined group of all PN's (flight group+control groups) to define whether there exist within the flight group some cell clusters differing from each other and from controls by measured parametres of their D's. Canonical analy-

sis followed (4,15) to define the significance of this parting (according to Hotteling's T test and Bartett's test) and the differing parametres by means of coefficients of discriminating function .

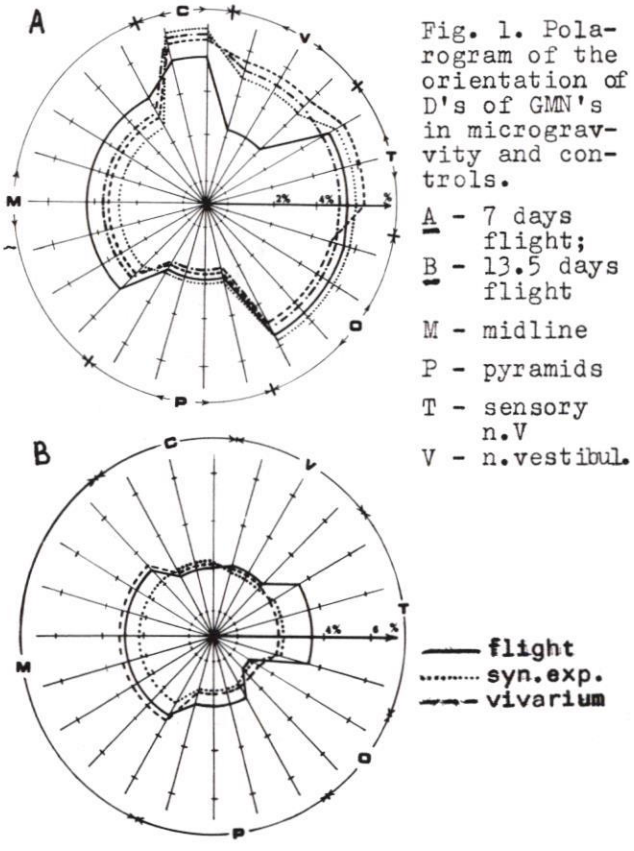


Fig. 1. Polarogram of the orientation of D's of GMN's in microgravity and controls.
A - 7 days flight;
B - 13.5 days flight
M - midline
P - pyramids
T - sensory n.V
V - n.vestibul.

Results and Discussion

Analysis of GMN's. 7 days space flight did not significantly change all the 11 measured parametres of D's, but did change however, their orientation. After 13.5 days flight both orientation and some structural parametres were altered. It deserves attention that the total length of D's of the whole neuron remained stable in both flight groups. Could it mean that in all revealed plastic changes of D's of GMN's in both flight groups the total mass of a neuron remain almost the same and presumably its redistribution occurs instead of real growth or atrophy?

Changes of Orientation of D's.(Fig. 1). The material of 7 days flight showed a significant decrease in the total length of D's oriented towards the vestibular nuclei, and conversely, an increase in orientation towards the midline. After 13.5 days flight, it was observed only a significant increase of the length of D's that stretched towards sensory trigeminal nuclei (p=0.05 for all cases). When interpreting these data one should remember that GMN analysed after 7 and 13.5 days flight belonged to different reticular nuclei and therefore their neighbourhood varied. GMN's of the 7 days flight (situated within the n.gigantocellularis of medulla) were close to the main volume of the vestibular sensory nuclei and to n.interpolaris n.V. In this case the decrease of the total length of D's in the sector oriented towards the vestibular nuclei may represent the deficit in vestibular information entering the brain during microgravity. GMN's of the 13.5 days flight (belonged to n.reticularis pontis oralis) were situated next to the most differentiated trigeminal sensory nucleus - its main nucleus; a region where the vestibular complex is only vaguely related. No decrease in the mass of D's of GMN's in the "vestibular" direction in these experiments could be explained by the latter fact.

An increase of the mass of D's in some sectors of dendritic fields of GMN's of both the 7 and 13.5 days flights may reflect compensatory search for additional sources of afferent input. Here again, the fact that the two groups of GMN's in question belong to different nuclei results in orientation of the mass of their D's towards different formations. Thus, the orientation of D's of GMN's of medulla (7 days flight) to the midline could supposedly be connected with a compensatory usage of proprioceptive information from medial lemniscus decussaiting fibres. Conversely, the orientation of D's of GMN's of the n.pontis oralis towards the main sensory trigeminal nucleus (13.5 days flight) could reflect compensatory usage of information from the vibrissae. It is known that inflow from the latter system is mainly processed by main sensory nucleus of n.V and that in their ordinary life on earth rats receive most information about their position through their vibrissae. So one can suppose that the change (diminishing?) of the vestibular information during microgravity which interferes with normal motor coordination, can be compensated for, at least partly, by the trigeminal vibrissae system. The lack of orientation of D's toward the trigeminal nuclei in the 7 days flight may supposedly depend on the location of these GMN's more caudally adjacent to n.interpolaris instead of the main nucleus n.V. N. interpolaris process mostly ordinary skin sensations (tactile, temperature and algesic) and to a much lesser extent information from the vibrissae. On the other hand, one cannot exclude the possibility that we were observing the successive involvement of different sensory systems in the compensatory process: the trigeminal system joining later than the proprioceptive(consider the increased total length of D's of GMN's towards the medial lemniscus decussation during 7 days flight).

We were surprized to observe in adult animals so an early (in 7 days) change in the orientation of D's. The constancy of all the 11 measured structural parametres of D's of GMN's including the total length of D's would suggest in this case a simple and rapid mobility of dendritic branches, may be of the nature of retraction and extrusion as

it was described for the neurons of invertebrates and of the autonomic nervous system of mammals after destruction of the synaptic connections of a neuron (16).

Structural Changes of D's (Fig. 2). Significant alterations of the structure of D's of GMN's after 13.5 days flight cover their increased total branching, total number of foci of maximal branchings and a greater volume of dendritic territory (p=0.05), all the other analysed parametres remained unaltered. These data would suggest there are superfluos structural elements of D's, similar to these described in kittens in the same GMN's during certain periods of their development (5). The authors consider these findings may be an attempt by the neuron to compensate for the deficit of information input that exist in early life. One can propose that the same mechanism might underlie the superfluous elements of D's of cells in question during microgravity in space. De novo foci of maximal dendritic branching probably arise at points where the most important information for this condition converges on the D's.

May be these "constructive" processes of alterations of D's deserve longer time and therefore were not evident by examination of GMN's after 7 days flight. Of course one could not exclude the possibility that these differences in reactions of GMN's of two flight groups depend upon their relations to different nuclei or upon some other peculiarities of these space flights.

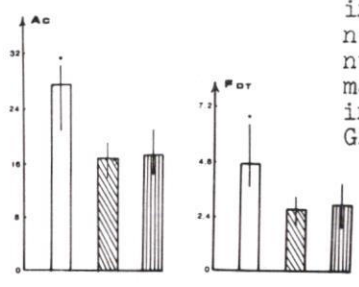


Fig. 2. Branchings of the whole neuron (Ac) and number of foci of maximal branchings (Fdt) of GMN's.
□- flight (13.5 days)
▨- vivarium
▥- synchronous experiment

Analysis of Small Pyramidal Nerons (PN's) of Layer II-III of the Visual Cortex

Multiparameter analysis revealed two clusters among these PN's of the 13.5 days flight material: one with significant changes of some of the parametres measured that could be attributed to the effects of the space flight; and a second one that lacked such changes (Fig. 3). The structural changes of the PN's in the first cluster involved both apical and basal D's. The apical D. did not change its branching, but the total length of its oblique D's grew, principally on account of branches that stretched towards I layer. As a consequence of this the dendritic field of these neurons was substantially widened. With the basal D's however one found a decrease in the total length of

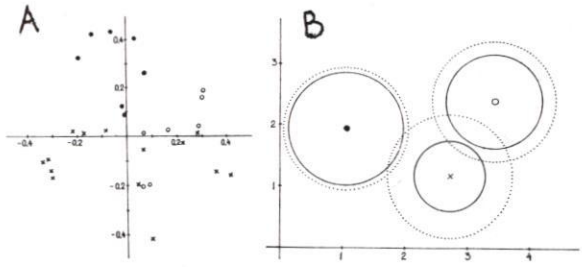


Fig. 3. Q-factor (A) and canonical (B) analysis of PN's of II-III layers of the visual cortex. **Two clusters of PN's:**
• first cluster(flight group changed);
∘ second cluster of flight group;
+ controls; —— confidence interval;
--- area, containing 68% of neurons.

D's and a change in their orientation away from the depths of the cortex (to layer IV) and towards layer I instead (Fig. 4). By the weight of parametres in the between group variance of discriminative function (4) one can come to a conclusion about a much greater significance of the changes in apical system of D's in comparison with basal. The total length of D's of the whole neuron, however, did not change significantly; there was only a slight tendency towards an increase. One can suggest that the structural changes of D's of PN's observed in visual cortex reflect the compensation by the visual sensory system during conditions of microgravity the failure of information from other sensory systems (first of all vestibular and proprioceptive) and related motor disturbances. Such interpretation of our data receives a support from other authors study (14), who reported an augmented number of dendritic spines on PN's of the auditory cortex of young rats after elimination of other sensory inputs (visual and from vibrissae) to the cortex that induced a spine loss in corresponding sensory cortices. It is pertinent that we revealed the lengthening of D's not in young, but in adult rats as has been similarly seen in adult rats after one month exposure to an enriched environment (8,17). Our data show the possibility of a more rapid growth of D's (in about two weeks) in an altered environment, at least in microgravity. It is noteworthy that exactly the oblique D's were found to grow in adult rats subjected to special long training (8), in contrast with young animals where mostly the basal system was involved. In our recent study of PN's in somatosensory cortex of adult rats that spent aboard biosputnik 7 days, there were again their oblique D's with an increased number of spines (1). Our data allow to propose that at least one of the compensatory structural changes of D's in the visual cortex of adult rats includes widening the extend their intrinsic connections (between different fields of the same hemisphere) and interhemispherical ones. This proposition is

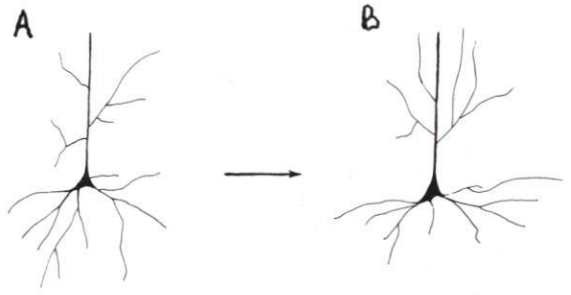


Fig. 4. Scheme of geometry of D's of PN's II-III layers of the visual cortex.

A - controls+stable flight cluster of cells; B - changed flight cluster of cells.

accordingly based on 1) the lengthening exactly of oblique D's of PN's - the region of termination of callosal fibres (6) and on the tendency of the basal D's of these neurons to change their ordinary orientation in parallel to the oblique D's; 2)the lengthening of the oblique D's mostly on account of those of their branches that stretch upwards to layer I, where fibers from neighbouring cortical fields in the visual cortex terminate (13). Since the total length of D's of PN's and their branching was constant, one may assume that new dendritic branches hardly appear, and that changes consisted in the first instance in lengthening of some branches and shortening of others, i.e. it would seem to be mostly a "reorientation" of the same dendritic mass. In this respect it resembles the forementioned change of D's of GMN's in reticular formation during 7 days flight. These results show a marked plasticity (reorientation and growth) of D's of adult mammalian brain during microgravity in space that could be the structural base of some of the compensatory processes that take place in these conditions.

References

1. Belichenko P.V. The quantitative analysis of dendritic spines of the pyramidal neurons of the layer V of somatosensory cortex of the rat that flew aboard biosputnik "Cosmos-1667". Bull. Exp.Biol. a. Med., 1988, 6, 736-738 (in Russian).

2. Belichenko P.V., Leontovich T.A. Morphometry of giant multipolar neurons of the brain stem of rats, that flew aboard biosputnik "Cosmos-1667". Bull. Exp.Biol. a.Med., 1989, 5, 618-620 (in Russian).

3. Černova V.L. Morpho-functional characteristic of the neurons of amygdala after section of its connections. Diss., M., 1983 (in Russian).

4. Derabin V.E. Multiparameter biometry for anthropologist. M., MGU, 1983.

5. Gladkovich N.G., Leontovich T.A., Shuleikina K.V. The quantitative morphological characteristic of the developing neurons of the brain stem reticular formation. Neurophysiology, 1980, 12, 53-60 (in Russian).

6. Globus A., Scheibel A.B. Synaptic loci on parietal cortical neurones: termination of corpus callosum fibers. Science, 1967, 156, 1127-1128.

7. Green E.J., Greenough W.F., Schlumpf B.E. Effects of complex or isolated environment on cortical dendrites of middle-aged rats. Brain Res., 1983, 264, 233-240.

8. Greenough W., Juraska T., Volknear Maze training effects on dendritic branching in occipital cortex of adult rats. Behav.Neurol.Biol., 1979, 26, 287-297.

9. Igarachi M. Vestibular compensation. An overview. Acta oto-laryngol., 1989, 97, Suppl.406, 78-82.

10. Kozlovskaya I.B., Kreidich Y.V., Rakhmanov A.S. Mechanism of the effects of weightlessness on the motor system of man. The Physiologist, 1981, 24, 6, Suppl., 59-64.

11. Leontovitsch. Methodik zur quantitativen Beschreibung subcorticaler Neurone. J.für Hirnfors., 1973, 14, 59-87.

12. Marchand A.R., Amblard B. Locomotion in adult cats with early vestibular deprivation. Visual cue substitution. Exp.Brain Res., 1984, 54, 395-405.

13. Ribak C.E. A note on the laminar organization of rat visual cortical projections. Exp.Brain Res., 1977, 27, 413-418.

14. Ryngo D., Ryngo R., Globus A., Killackey H.P. Increased spine density in auditory cortex following visual or somatic deafferentation. Brain Res. 1975, 90, 143-146.

15. Seal H.J. Multivariate statistical analysis for biologists. London, Methuen, 1964.

16. Sotnikov O.S. The structural dynamic of the living neuron. Leningrad, Nauka, 1985 (in Russian).

17. Uylings H.B., Kuypers R.,Diamond M.C., Veltman W.A. Effects of differential environment on plasticity of dendrites of cortical pyramidal neurons in adult rats. Exp.Neurol., 1978, 62, 658-677.

18. Überla K. Faktorenanalyse. Springer Verlag, 1977.

METABOLIC CONSEQUENCES OF FLUID SHIFTS INDUCED BY MICROGRAVITY

N. M. Cintron, H. W. Lane, and C. S. Leach

Biomedical Operations and Research
NASA/Johnson Space Center
Houston, TX 77058

Many systems in the body are affected by weightlessness. A change in the distribution of body fluid is thought to be one of the earliest responses (13) and to induce a number of further changes. This paper will review the status of knowledge about several areas that may be affected by fluid redistribution. These areas are fluid and electrolyte regulation; maintenance of optimum nutritional status; and absorption, distribution, and elimination of pharmacologic agents (pharmacokinetics).

Fluid and Electrolyte Regulation

When the pull of gravity is removed, fluid is lost from the legs and pools in the chest and head (22). This redistribution of body fluid is thought to be manifested temporarily as an increase in central blood volume and pressure, which activates baroreceptor reflexes and other mechanisms that bring about a reduction in body fluid. Recent studies on the Space Shuttle (8) are consistent with Skylab findings (11) that total body water is reduced during flight. Total body water was measured by the isotope dilution technique, using oxygen-18-labeled water as the tracer, for three crewmembers on one Shuttle flight. It was measured three times before flight, twice during flight, and for the last time several days after landing. A 3 percent reduction in total body water occurred within 2 days of launch, and several days later body water was still reduced.

At least part of the body water decrease is manifested as a reduction in blood volume, which amounted to 10.5% after the 10-day Spacelab 1 flight (9). As fluid is lost, blood electrolytes and other components should decrease also.

One of the consistent findings from previous space-flight programs is a decrease in blood sodium. On Earth, serum sodium is tightly controlled by several hormones. On three Spacelabs, serum sodium varied considerably but was usually lower than its preflight level (10). Plasma renin activity (PRA), or angiotensin I, is the precursor of angiotensin II. Angiotensin II stimulates secretion of the sodium-retaining hormone aldosterone. One might expect aldosterone to be secreted soon after a reduction in serum sodium. For the first 2 days after launch (10), levels of PRA during flight were lower than preflight values. After 3 days they were increased, however. Plasma aldosterone did not increase until almost 8 days after launch. The two hormones were not as closely coupled as would be expected (2).

The hormone atrial natriuretic factor or ANF is now thought to play an important role in regulation of body fluid volume (20). It has the effect of increasing the excretion of sodium. If ANF were increased, serum sodium might tend to be reduced. During space flight, ANF might be expected to increase because secretion of this hormone is stimulated by increased central blood volume (1). ANF was measured in blood samples obtained from four crewmembers on Spacelab 2 at two times during flight (Fig. 1). At the first sampling time, about 30 hours after launch, ANF was above preflight levels. The other in-flight time point was almost a week after launch, and for all crewmembers ANF at this time was lower than preflight levels. ANF was still reduced at landing but returned to preflight levels later (2).

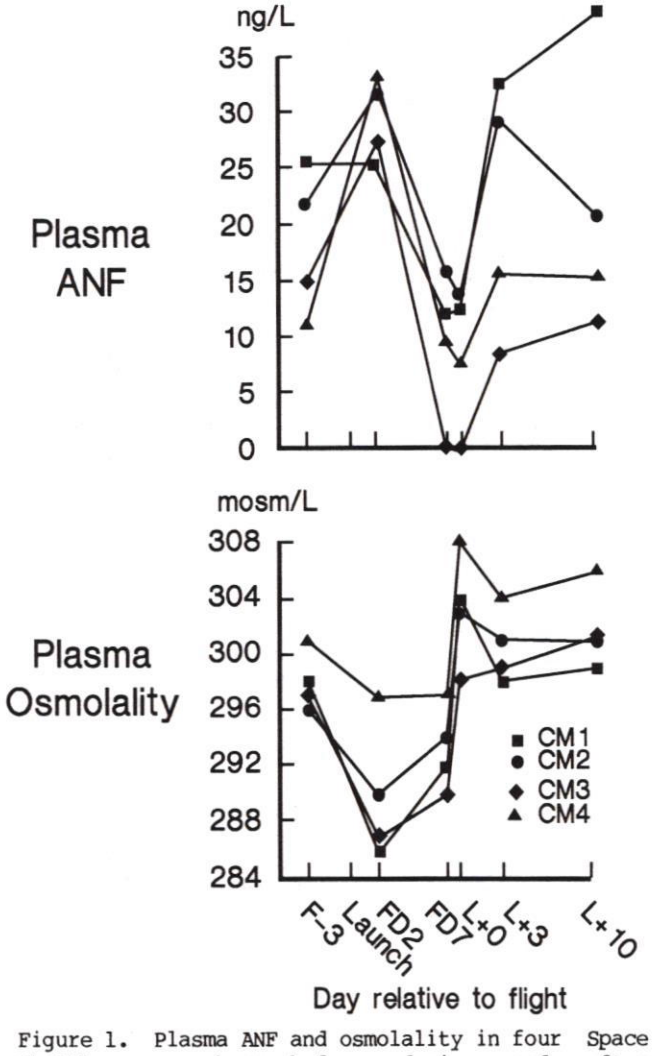


Figure 1. Plasma ANF and osmolality in four Space Shuttle crewmembers before, during, and after flight.

Changes in plasma osmolality during flight were in directions opposite to those of ANF (Fig. 1). Osmolality was reduced at first, when ANF was highest, but it increased later. At landing plasma osmolality was above preflight levels, while ANF was at its lowest point. Plasma osmolality did not necessarily reflect levels of serum sodium, which did not exceed preflight levels during flight. These results suggest that although ANF may promote a hyponatremic state at first, it is probably not

maintaining the low levels of serum sodium found at later time points.

Another hormone important in fluid and electrolyte regulation is antidiuretic hormone (ADH), which promotes fluid retention. Plasma ADH has been measured in samples from some of the Spacelab crewmembers. Every time plasma ADH has been measured during flight, it has been elevated (10). The increase in ADH, along with the other results mentioned above, is consistent with a tendency to prevent body fluid volume or the volume of one of its compartments from decreasing below a certain level, at the expense of Earth-normal levels of serum sodium.

Maintenance of Optimum Nutritional Status

Several body components in addition to sodium and water are reduced during space flight. Body weight has decreased after almost all missions (7,24). Analysis of data from all three Skylab missions indicated that more than half of the weight lost by crewmembers was lean body mass, including 1.1 kg of water and .3 kg of protein (12). Loss of protein contributed to the loss of muscle mass (5).

During long-term space flight, there was also continued loss of body nitrogen and potassium (23). Both were in negative balance on Skylab 2, even though calorie intake was considered adequate. The mineral loss was thought to come primarily from muscle because it paralleled reductions in muscle tissue in the legs. Because a loss of muscle mass affects exercise capacity during and after flight, dietary and exercise countermeasures are being studied to prevent muscle loss (6,14).

Several lines of evidence have indicated that energy utilization is elevated during space flight (19). The average energy utilization of Skylab crewmembers was 41.7 kcal/kg per day before the 3-month flight and 43.7 kcal/kg per day during the third month of flight. Plasma glucose and insulin were significantly decreased during Skylab flights but increased after landing.

Fasting blood glucose levels were determined for Shuttle crewmembers prior to flight, and blood glucose was measured again at landing (Fig. 2). The landing blood glucose was not fasting; in fact, most crewmembers consume some food after exiting the Shuttle but before the blood draw. However, even with food intake, the blood glucose levels at landing (102 mg/dl) were not statistically different from fasting preflight levels (94 mg/dl). Furthermore, of the 32 crewmembers studied, at least one had urinary ketosis. This suggests that in at least one individual glycogen stores were depleted at landing. For long-term missions, this could cause a decrement in psychomotor skills. Loss of muscle tissue and glycogen stores also represents loss of the fluid associated with these tissues. If loss of tissues is prevented, more fluid may be retained.

These data emphasize the importance of nutritional status of crews.

Pharmacokinetics

Pharmacokinetics is the mathematical description of the dynamic processes of drug absorption, distribution, and elimination and the interrelationships among them. Some of the effects of weightlessness, including altered fluid distribution, may affect one or more of these key processes and thereby change the overall kinetics of a drug. A change in the kinetics of a drug in turn alters the therapeutic benefit anticipated in terms of the rate of onset or of the magnitude or duration of the drug's effect.

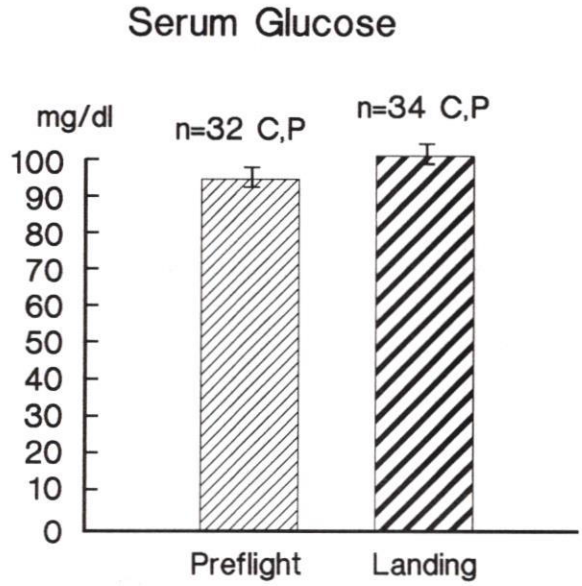


Figure 2. Serum glucose before and after space flight in Shuttle commanders (C) and pilots (P).

To evaluate the feasibility of assessing pharmacokinetic changes using ground-based models of weightlessness, scopolamine pharmacokinetics were studied in subjects placed on antiorthostatic bedrest (-6 degrees). A fluid shift toward the head and chest is one of the earliest consequences of antiorthostatic bedrest (15). The results of the study indicated that there were significant changes in absorption but not distribution and elimination of scopolamine after bedrest (17).

In preparation for performing experiments in space by noninvasive techniques, ground-based studies were conducted to verify the feasibility of using salivary drug levels of both acetaminophen and scopolamine for predicting blood concentrations following drug administration. Acetaminophen was used as a general "probe" of drug alterations. After oral administration, therapeutic concentrations of acetaminophen were detected in saliva. The saliva/plasma ratio of the drug was consistent and remained close to 1 over a wide range of plasma concentrations during the pre- and post-absorptive phases of drug dynamics. The correlation between plasma and saliva acetaminophen concentrations was 0.93 (18). Scopolamine, after intravenous or oral administration, readily distributed into saliva with consistent saliva/plasma ratios over the entire disposition profile (16). Pharmacokinetic evaluation and bioavailability estimations of both drugs using saliva and plasma concentration data were shown to be in agreement.

Salivary drug monitoring was used during flight to obtain the first preliminary information on the effects of space flight on the pharmacokinetics and dynamics of drugs. Salivary pharmacokinetics of acetaminophen and scopolamine/dextroamphetamine during space flight were evaluated following oral administration to crewmembers before and during missions. Inflight

saliva samples were collected using a system developed at the Johnson Space Center (4). Upon return, drug concentrations in the samples were determined by established methods.

Significant changes in concentration profiles of these two drugs were observed during space flight, with the alterations being more pronounced during the absorption phase than during the elimination phase. For acetaminophen, the rate of absorption and time to reach peak saliva concentration calculated from pre- and inflight data indicated that there was a significant decrease in the absorption rate of the drug during flight (4). A two-fold increase in the time to reach peak concentration resulted. Scopolamine analysis of a limited number of samples from inflight studies also suggested that detectable changes in saliva concentration-time profiles of scopolamine occur during missions (3). In one crewmember (Fig. 3, upper graph), a significant decrease in the peak concentration and an increase in the time to reach peak concentration were observed, while opposite results were obtained for another crewmember (Fig. 3, lower graph).

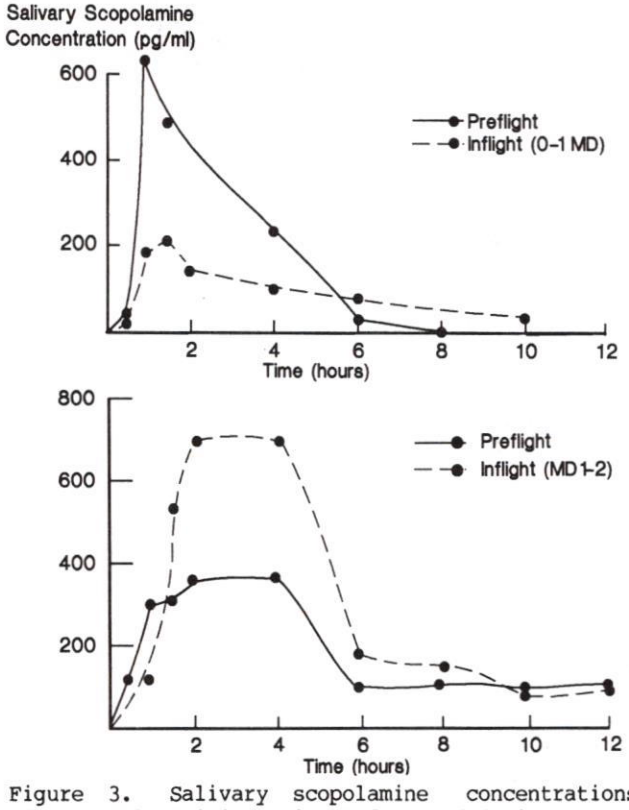


Figure 3. Salivary scopolamine concentrations after oral administration before and during space flight, for two Shuttle crewmembers.

For both drugs, there were large intersubject variations in peak concentration and time to reach the peak concentration during flight. It is important to note that the intersubject variability in these two variables was minimal during the preflight phase, suggesting that the physiologic response to space flight is likewise highly variable among individuals (4).

The results of our inflight investigations, although preliminary and limited, collectively support predictions that changes in drug dynamics may occur during flight as a result of the physiologic condition of crewmembers. Alterations in the absorption phase suggest that the gastrointestinal tract may be functioning differently in space, perhaps because of the loss or redistribution of body fluid. Such changes in the pharmacokinetics of drugs administered to crewmembers during a mission may result in ineffective therapeutic responses or unexpected side effects.

The limited inflight data accumulated thus far are inadequate to characterize the degree and magnitude of and the mechanisms underlying space-flight-induced pharmacokinetic changes, because a number of variables influence the disposition profiles and kinetic parameter estimates of drugs. While information about some of these variables (e.g., mission day) is available, information about such factors as ingestion of other medications during flight and the overall physiologic response of each participating crewmember to microgravity is unavailable. A comprehensive evaluation of inflight pharmacokinetics of drugs under controlled experimental conditions is planned for future Spacelab missions, where some of the factors contributing to the variability in data can be monitored if not controlled. The information thus obtained can be used not only to predict the therapeutic consequences of operationally critical drugs but also to describe physiologic alterations relevant to overall metabolic homeostasis in humans.

Conclusion

Space medicine research in the U.S. has focused on the 7- to 10-day Space Shuttle missions in recent years. As missions are lengthened, it is anticipated that the effects of space-flight-induced physiologic changes will be magnified and must be corrected to enable optimum health and performance during flight and prompt readaptation to gravity.

ACKNOWLEDGEMENTS

The authors thank Dr. Jane Krauhs and Mrs. Sharon Jackson for help in preparation of the manuscript.

REFERENCES

1. Anderson, J. V., D. N. Millar, J. P. O'Hare, J. C. MacKenzie, R. J. M. Corrall, and S. R. Bloom. Atrial natriuretic peptide: physiological release associated with natriuresis during water immersion in man. Clin. Sci. Lond. 71:319-322, 1986.

2. Cintrón, N. M., C. S. Leach, J. M. Krauhs, and J. B. Charles. ANP and other fluid-regulating hormones in spaceflight. In: American Society of Hypertension Symposium Series, Vol. 3, edited by B. M. Brenner and J. H. Laragh. New York: Raven Press, 1989.

3. Cintrón, N. M., L. Putcha, Y.-M. Chen, and J. M. Vanderploeg. Inflight salivary pharmacokinetics of scopolamine and dextroamphetamine. In: Results of the Life Sciences DSOs Conducted Aboard the Space Shuttle 1981-1986, edited by M. W. Bungo, T. Bagian, M. A. Bowman, and B. M. Levitan. Houston, Texas: Space Biomedical Research Institute, Johnson Space Center, 1987, p. 25-29.

4. Cintrón, N. M., L. Putcha, and J. M. Vanderploeg. Inflight pharmacokinetics of acetaminophen in saliva. In: Results of

the Life Sciences DSOs Conducted Aboard the Space Shuttle 1981-1986, edited by M. W. Bungo, T. Bagian, M. A. Bowman, and B. M. Levitan. Houston, Texas: Space Biomedical Research Institute, Johnson Space Center, 1987, p. 19-23.

5. Cintrón-Trevino, N. M., C. S. Leach, and P. C. Rambaut. Potential biochemical basis of muscle atrophy during prolonged weightlessness. In: Biochemistry of Exercise, edited by H. Knuttgen, J. A. Vogel, and J. Poortmans. Champaign, IL: Human Kinetics Publishers, 1983, p. 351-355.

6. Grigor'yev, A. I., V. U. Stepantsov, V. A. Tishler, V. M. Mikhaylov, Yu. D. Pometov, and V. R. Dorokhova. Means and methods for preventing the undesirable effects of weightlessness. In: Results of Medical Research Performed on Board the "Salyut-6"-"Soyuz" Orbital Scientific Research Complex, edited by N. N. Gurovskiy. Moscow: Nauka, 1986, p. 125-145.

7. Hoffler, G. W. Cardiovascular studies of U.S. space crews: an overview and perspective. In: Cardiovascular Flow Dynamics and Measurements, edited by N. H. C. Hwang and N. A. Normann. Baltimore, MD: University Park Press, 1977, p. 335-363.

8. Leach, C. S., L. D. Inners, and J. B. Charles. Changes in total body water during space flight. In: Results of the Life Sciences DSOs Conducted Aboard the Space Shuttle 1981-1986, edited by M. W. Bungo, T. Bagian, M. A. Bowman, and B. M. Levitan. Houston, Texas: Space Biomedical Research Institute, Johnson Space Center, 1987, p. 49-53.

9. Leach, C. S., and P. C. Johnson. Influence of spaceflight on erythrokinetics in man. Science 225:216-218, 1984.

10. Leach, C. S., P. C. Johnson, and N. M. Cintrón. The regulation of fluid and electrolyte metabolism in weightlessness. In: Proceedings of the 2nd International Conference on Space Physiology, Toulouse, France, 20-22 Nov., 1985 (ESA SP-237), edited by J. Hunt. Paris, France: European Space Agency, 1986, p. 31-36.

11. Leach, C. S., and P. C. Rambaut. Biochemical responses of the Skylab crewmen: an overview. In: Biomedical Results from Skylab, NASA SP-377, edited by R. S. Johnston and L. F. Dietlein. Washington, D.C.: National Aeronautics and Space Administration, 1977, p. 204-216.

12. Leonard, J. I., C. S. Leach, and P. C. Rambaut. Quantitation of tissue loss during prolonged space flight. Am. J. Clin. Nutr. 38:667-679, 1983.

13. Nicogossian, A. E. Overall physiological response to space flight. In: Space Physiology and Medicine, 2nd ed., edited by A. E. Nicogossian, C. L. Huntoon, and S. L. Pool. Philadelphia: Lea & Febiger, 1989, p. 139-153.

14. Nicogossian, A., F. Sulzman, M. Radtke, and M. Bungo. Assessment of the efficacy of medical countermeasures in space flight. Acta Astronautica 17:195-198, 1988.

15. Nixon, J. V., R. G. Murray, C. Bryant, R. L. Johnson, Jr., J. H. Mitchell, O. B. Holland, C. Gomez-Sanchez, P. Vergne-Marini, and C. G. Blomqvist. Early cardiovascular adaptation to simulated zero gravity. J. Applied Physiol. 46:541-548, 1979.

16. Putcha, L., N. M. Cintrón, and J. M. Vanderploeg. Salivary concentrations for clinical drug monitoring of scopolamine. Aviat. Space Environ. Med. 56:484 (Abstract), 1985.

17. Putcha, L., N. M. Cintrón, and J. M. Vanderploeg. Pharmacokinetics of scopolamine in normal subjects placed on antiorthostatic bedrest. Aviat. Space Environ. Med. 60:483 (Abstract), 1989.

18. Putcha, L., N. M. Cintrón, J. M. Vanderploeg, Y. Chen, and J. R. Dardano. Comparative concentration profiles of acetaminophen in plasma and saliva of normal subjects. World Conference Clinical Pharmacology and Therapeutics, Stockholm, Sweden, Aug. 1986.

19. Rambaut, P. C., C. S. Leach, and J. I. Leonard. Observations in energy balance in man during spaceflight. Am. J. Physiol. 233 (Regulatory Integrative Comp. Physiol. 2): R208-R212, 1977.

20. Richards, A. M., D. McDonald, M. A. Fitzpatrick, M. G. Nicholls, E. A. Espiner, H. Ikram, S. Jans, S. Grant, and T. Yandle. Atrial natriuretic hormone has biological effects in man at physiological plasma concentrations. J. Clin. Endocrinol. Metab. 67:1134-1139, 1988.

21. Rowe, J. W., R. L. Shelton, J. H. Helderman, R. E. Vestal, and G. L. Robertson. Influence of the emetic reflex on vasopressin release in man. Kidney Internat. 16:729-735, 1979.

22. Thornton, W. E., G. W. Hoffler, and J. A. Rummel. Anthropometric changes and fluid shifts. In: Biomedical Results from Skylab, NASA SP-377, edited by R. S. Johnston and L. F. Dietlein. Washington, D.C.: National Aeronautics and Space Administration, 1977, p. 330-338.

23. Whedon, G. D., L. Lutwak, P. C. Rambaut, M. W. Whittle, J. Reid, M. C. Smith, C. Leach, C. R. Stadler, and D. D. Sanford. Mineral and nitrogen balance study observations: the second manned Skylab mission. Aviat. Space Environ. Med. 47: 391-396, 1976.

24. Yegorov, A. D., I. I. Kas'yan, A. A. Zlatorunskiy, S. F. Khlopina, V. A. Talavrinov, I. A. Yevdokimova, Ye. M. Romanov, and V. I. Somov. Changes in body mass of cosmonauts in the course of a 140-day space flight. Kosm. Biol. Aviakosm. Med. 15(1):34-36, 1981.

HISTOMORPHOMETRIC ANALYSIS OF BONES OF COSMOS-1887 RATS

A.S. Kaplansky, G.N. Durnova, E.I. Ilyina-Kakueva,
Z.F. Sakharova, E.V. Vorotnikova
Institute of Biomedical Problems, 123007 Moscow, USSR

It was previously shown that exposure to microgravity may lead to bone mineral loss (1,4,5), osteoporosis (3,8,9, 12,15,16) and bone strength reduction (7,14). However the process has been inadequately studied and it is still unclear whether its development has time limits and reaches a plateau. It is however obvious that even a 7-day exposure to microgravity is a sufficiently long period for osteoporosis to occur and bone strength to change (3,11,15,16). In view of this, we carried out the present investigation the purpose of which was to identify structural changes in skeletal bones induced by a two-week space flight, to evaluate them quantitatively, and to clarify mechanisms underlying osteoporosis during the transient period of adaptation to microgravity.

Material and Methods

Tibia and lumbar vertebrae (L4-L6) of five male rats of the Wistar SPF strain were investigated. The rats weighing 318 g were sacrificed 2 days after the 13-day flight on Cosmos-1887. Also, tibia and vertebrae of vivarium rats and ground-based synchronous controls that were exposed to all physiologically significant flight factors were examined. The proximal part of the tibia was separated from the diaphysis, cut into two parts along the sagittal plane and fixed for 24 hrs in 4% paraformadelhyde (pH 7.0-7.2) at 4°C; then one portion of the proximal compartment was dehydrated in acetone replaced three times during 36 hrs at 4°C, embedded into the JB-4 medium (Polysciences, USA) in the cold (-15°C) (10); the second portion of the proxymal compartment and diaphysis were decalcified in 10% EDTA (pH 7.0), dehydrated in alcohols and embedded into Histoplast (Serva, FRG). Lumbar vertebrae were embedded in JB-4 or Histoplast.

Using the method of simultaneous coupling, the activity of lysosome acid phosphatase (the enzyme acting as an osteoclast marker) was measured in sections of undecalcified bones cut by a Polycuts microtome. For this purpose 50 mM sodium tartrate were added to the incubation medium (10). Sections of decalcified bones were stained using hematoxylin-eoxin, picrofuchsin, methyl green-pyronine and toluidine blue and section of the diaphyses were stained according to the method of Schmorle.

Histomorphometric analysis of the spongiosa of tibia and vertebrae was performed with the aid of an image analyzer MOP-Videoplan. The following parameters were measured in the bones: trabecular volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp.) (13); besides, the thickness of the cartilage growth plate and width of the primary spongiosa were determined. The bone formation rate was determined by counting the number of osteoblasts in 1 mm^3 of the primary spongiosa and determining the osteoblast activity index (2), because the amount of osteoid in bone trabecules was negligibly small and could not be measured by the image analyzer. The resorption activity was measured by counting the number of osteoclasts per 1 mm^3 of the spongiosa (N.Oc/TV) as well as per 1 mm^3 (N.Oc/BV) and 1 mm^2 of trabecular bone (N.Oc/BS) (16).

In cross-sections of the diaphyses the surface area of the cortical plate and bone marrow cavity were measured.

The results were statistically treated and differences were taken to be significant at $p < 0.05$.

Results and Discussion

Investigation of spongy bone of tibial metaphyses showed that a 13-day exposure to microgravity induced osteoporosis as indicated by a reduction of the bone amount in the primary and secondary spongiosa which occurred at the expense of a decrease in the number and thickness of bone trabecules (Table 1). The thickness of the epiphyseal cartilage growth plate and length of trabecules of the primary spongiosa also diminished. As a result, the area occupied by the primary spongiosa diminished. The reduction of the spongiosa of tibial metaphyses was combined with a decrease of the total number of osteoblasts in the primary spongiosa and their functional activity. At the same time the amount of osteoclasts in the primary spongiosa increased and in the secondary spongiosa remained unchanged. Acid phosphatase activity in osteoclasts diminished noticeably; this change can be viewed as a sign of lowered activity of osteoclasts as a result of adaptation to Earth's gravity that began after recovery.

Ground-based synchronous control rats showed only a reduction of the volu-

the Life Sciences DSOs Conducted Aboard the Space Shuttle 1981-1986, edited by M. W. Bungo, T. Bagian, M. A. Bowman, and B. M. Levitan. Houston, Texas: Space Biomedical Research Institute, Johnson Space Center, 1987, p. 19-23.

5. Cintrón-Trevino, N. M., C. S. Leach, and P. C. Rambaut. Potential biochemical basis of muscle atrophy during prolonged weightlessness. In: Biochemistry of Exercise, edited by H. Knuttgen, J. A. Vogel, and J. Poortmans. Champaign, IL: Human Kinetics Publishers, 1983, p. 351-355.

6. Grigor'yev, A. I., V. U. Stepantsov, V. A. Tishler, V. M. Mikhaylov, Yu. D. Pometov, and V. R. Dorokhova. Means and methods for preventing the undesirable effects of weightlessness. In: Results of Medical Research Performed on Board the "Salyut-6"-"Soyuz" Orbital Scientific Research Complex, edited by N. N. Gurovskiy. Moscow: Nauka, 1986, p. 125-145.

7. Hoffler, G. W. Cardiovascular studies of U.S. space crews: an overview and perspective. In: Cardiovascular Flow Dynamics and Measurements, edited by N. H. C. Hwang and N. A. Normann. Baltimore, MD: University Park Press, 1977, p. 335-363.

8. Leach, C. S., L. D. Inners, and J. B. Charles. Changes in total body water during space flight. In: Results of the Life Sciences DSOs Conducted Aboard the Space Shuttle 1981-1986, edited by M. W. Bungo, T. Bagian, M. A. Bowman, and B. M. Levitan. Houston, Texas: Space Biomedical Research Institute, Johnson Space Center, 1987, p. 49-53.

9. Leach, C. S., and P. C. Johnson. Influence of spaceflight on erythrokinetics in man. Science 225:216-218, 1984.

10. Leach, C. S., P. C. Johnson, and N. M. Cintrón. The regulation of fluid and electrolyte metabolism in weightlessness. In: Proceedings of the 2nd International Conference on Space Physiology, Toulouse, France, 20-22 Nov., 1985 (ESA SP-237), edited by J. Hunt. Paris, France: European Space Agency, 1986, p. 31-36.

11. Leach, C. S., and P. C. Rambaut. Biochemical responses of the Skylab crewmen: an overview. In: Biomedical Results from Skylab, NASA SP-377, edited by R. S. Johnston and L. F. Dietlein. Washington, D.C.: National Aeronautics and Space Administration, 1977, p. 204-216.

12. Leonard, J. I., C. S. Leach, and P. C. Rambaut. Quantitation of tissue loss during prolonged space flight. Am. J. Clin. Nutr. 38:667-679, 1983.

13. Nicogossian, A. E. Overall physiological response to space flight. In: Space Physiology and Medicine, 2nd ed., edited by A. E. Nicogossian, C. L. Huntoon, and S. L. Pool. Philadelphia: Lea & Febiger, 1989, p. 139-153.

14. Nicogossian, A., F. Sulzman, M. Radtke, and M. Bungo. Assessment of the efficacy of medical countermeasures in space flight. Acta Astronautica 17:195-198, 1988.

15. Nixon, J. V., R. G. Murray, C. Bryant, R. L. Johnson, Jr., J. H. Mitchell, O. B. Holland, C. Gomez-Sanchez, P. Vergne-Marini, and C. G. Blomqvist. Early cardiovascular adaptation to simulated zero gravity. J. Applied Physiol. 46:541-548, 1979.

16. Putcha, L., N. M. Cintrón, and J. M. Vanderploeg. Salivary concentrations for clinical drug monitoring of scopolamine. Aviat. Space Environ. Med. 56:484 (Abstract), 1985.

17. Putcha, L., N. M. Cintrón, and J. M. Vanderploeg. Pharmacokinetics of scopolamine in normal subjects placed on antiorthostatic bedrest. Aviat. Space Environ. Med. 60:483 (Abstract), 1989.

18. Putcha, L., N. M. Cintrón, J. M. Vanderploeg, Y. Chen, and J. R. Dardano. Comparative concentration profiles of acetaminophen in plasma and saliva of normal subjects. World Conference Clinical Pharmacology and Therapeutics, Stockholm, Sweden, Aug. 1986.

19. Rambaut, P. C., C. S. Leach, and J. I. Leonard. Observations in energy balance in man during spaceflight. Am. J. Physiol. 233 (Regulatory Integrative Comp. Physiol. 2): R208-R212, 1977.

20. Richards, A. M., D. McDonald, M. A. Fitzpatrick, M. G. Nicholls, E. A. Espiner, H. Ikram, S. Jans, S. Grant, and T. Yandle. Atrial natriuretic hormone has biological effects in man at physiological plasma concentrations. J. Clin. Endocrinol. Metab. 67:1134-1139, 1988.

21. Rowe, J. W., R. L. Shelton, J. H. Helderman, R. E. Vestal, and G. L. Robertson. Influence of the emetic reflex on vasopressin release in man. Kidney Internat. 16:729-735, 1979.

22. Thornton, W. E., G. W. Hoffler, and J. A. Rummel. Anthropometric changes and fluid shifts. In: Biomedical Results from Skylab, NASA SP-377, edited by R. S. Johnston and L. F. Dietlein. Washington, D.C.: National Aeronautics and Space Administration, 1977, p. 330-338.

23. Whedon, G. D., L. Lutwak, P. C. Rambaut, M. W. Whittle, J. Reid, M. C. Smith, C. Leach, C. R. Stadler, and D. D. Sanford. Mineral and nitrogen balance study observations: the second manned Skylab mission. Aviat. Space Environ. Med. 47: 391-396, 1976.

24. Yegorov, A. D., I. I. Kas'yan, A. A. Zlatorunskiy, S. F. Khlopina, V. A. Talavrinov, I. A. Yevdokimova, Ye. M. Romanov, and V. I. Somov. Changes in body mass of cosmonauts in the course of a 140-day space flight. Kosm. Biol. Aviakosm. Med. 15(1):34-36, 1981.

HISTOMORPHOMETRIC ANALYSIS OF BONES OF COSMOS-1887 RATS

A.S. Kaplansky, G.N. Durnova, E.I. Ilyina-Kakueva,
Z.F. Sakharova, E.V. Vorotnikova
Institute of Biomedical Problems, 123007 Moscow, USSR

It was previously shown that exposure to microgravity may lead to bone mineral loss (1,4,5), osteoporosis (3,8,9, 12,15,16) and bone strength reduction (7,14). However the process has been inadequately studied and it is still unclear whether its development has time limits and reaches a plateau. It is however obvious that even a 7-day exposure to microgravity is a sufficiently long period for osteoporosis to occur and bone strength to change (3,11,15,16). In view of this, we carried out the present investigation the purpose of which was to identify structural changes in skeletal bones induced by a two-week space flight, to evaluate them quantitatively, and to clarify mechanisms underlying osteoporosis during the transient period of adaptation to microgravity.

Material and Methods

Tibia and lumbar vertebrae (L4-L6) of five male rats of the Wistar SPF strain were investigated. The rats weighing 318 g were sacrificed 2 days after the 13-day flight on Cosmos-1887. Also, tibia and vertebrae of vivarium rats and ground-based synchronous controls that were exposed to all physiologically significant flight factors were examined. The proximal part of the tibia was separated from the diaphysis, cut into two parts along the sagittal plane and fixed for 24 hrs in 4% paraformadelhyde (pH 7.0-7.2) at 4°C; then one portion of the proximal compartment was dehydrated in acetone replaced three times during 36 hrs at 4°C, embedded into the JB-4 medium (Polysciences, USA) in the cold (-15°C) (10); the second portion of the proxymal compartment and diaphysis were decalcified in 10% EDTA (pH 7.0), dehydrated in alcohols and embedded into Histoplast (Serva, FRG). Lumbar vertebrae were embedded in JB-4 or Histoplast.

Using the method of simultaneous coupling, the activity of lysosome acid phosphatase (the enzyme acting as an osteoclast marker) was measured in sections of undecalcified bones cut by a Polycuts microtome. For this purpose 50 mM sodium tartrate were added to the incubation medium (10). Sections of decalcified bones were stained using hematoxylin-eoxin, picrofuchsin, methyl green-pyronine and toluidine blue and section of the diaphyses were stained according to the method of Schmorle.

Histomorphometric analysis of the spongiosa of tibia and vertebrae was performed with the aid of an image analyzer MOP-Videoplan. The following parameters were measured in the bones: trabecular volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp.) (13); besides, the thickness of the cartilage growth plate and width of the primary spongiosa were determined. The bone formation rate was determined by counting the number of osteoblasts in 1 mm^3 of the primary spongiosa and determining the osteoblast activity index (2), because the amount of osteoid in bone trabecules was negligibly small and could not be measured by the image analyzer. The resorption activity was measured by counting the number of osteoclasts per 1 mm^3 of the spongiosa (N.Oc/TV) as well as per 1 mm^3 (N.Oc/BV) and 1 mm^2 of trabecular bone (N.Oc/BS) (16).

In cross-sections of the diaphyses the surface area of the cortical plate and bone marrow cavity were measured.

The results were statistically treated and differences were taken to be significant at $p < 0.05$.

Results and Discussion

Investigation of spongy bone of tibial metaphyses showed that a 13-day exposure to microgravity induced osteoporosis as indicated by a reduction of the bone amount in the primary and secondary spongiosa which occurred at the expense of a decrease in the number and thickness of bone trabecules (Table 1). The thickness of the epiphyseal cartilage growth plate and length of trabecules of the primary spongiosa also diminished. As a result, the area occupied by the primary spongiosa diminished. The reduction of the spongiosa of tibial metaphyses was combined with a decrease of the total number of osteoblasts in the primary spongiosa and their functional activity. At the same time the amount of osteoclasts in the primary spongiosa increased and in the secondary spongiosa remained unchanged. Acid phosphatase activity in osteoclasts diminished noticeably; this change can be viewed as a sign of lowered activity of osteoclasts as a result of adaptation to Earth's gravity that began after recovery.

Ground-based synchronous control rats showed only a reduction of the volu-

me of the primary spongiosa whereas the volume of the secondary spongiosa did not differ from that in the vivarium controls. The reduction in the primary spongiosa volume was accompanied by a decrease in the total number of osteoblasts and their functional activity. The number of osteoclasts in the primary and secondary spongiosa was similar to that in vivarium controls and acid phosphatase activity was slightly lower.

The thickness of the cortical plate of tibial diaphyses and the area of the bone marrow cavity of the flight rats remained unaltered when compared to those of the controls.

In contrast to the tibia, lumbar vertebrae of flight rats displayed only early signs of osteoporosis, viz. thinning of bone trabecules and increase of space between them (Table 2). Osteoporosis developing in both lumbar vertebrae and tibia is primarily caused by a decrease in the rate of bone neoformation which is indicated by a decline in the number of osteoblasts. No signs of enhanced resorption were found in lumbar vertebrae as suggested by the number of osteoclasts.

Histomorphometric analysis of lumbar vertebrae of ground-based synchronous rats did not reveal any changes as compared to the vivarium rats.

Our results give evidence that a 13-day exposure to microgravity causes spongy bone osteoporosis developing primarily due to inhibition of bone neoformation and, in a lesser degree, stimulation of bone resorption. Osteoporosis of the spongiosa of tibial metaphyses developed in parallel with inhibition of bone growth in length which manifested morphologically as a reduction of the thickness of the cartilage growth plate and primary spongiosa.

Unlike spongy bone that responds rapidly to the effects of microgravity, compact bone of the diaphyses proved more stable; after the 13-day flight its amount was close to normal.

Comparative evaluation of the severity of osteoporosis in tibia and vertebrae demonstrated that the spongiosa reduction in tibia was more significant which lends support to the previous findings (3,11,16).

In summary, among the changes seen in bones of rats flown on Cosmos-1887 variations of two types can be identified: some changes emerged during flight in response to the effects of microgravity, i.e. osteoporosis, and other changes occured immediately after flight, i.e. decrease of acid phosphatase in osteoclasts, indicating the onset of readaptation to Earth's gravity.

Table 1 Histomorphometric analysis of tibial metaphyses

Parameter	Rats					
	V	S	F	V	S	F
	Primary spongiosa			Secondary spongiosa		
Thickness of growth plate, μm	177.0±4.0	190.0±9.0	146.0±9.2^{x,xxx}			
Width of primary spongiosa zone, μm	411.0±21.0	399.0±8.0	269.0±23.0^{x,xxx}			
BV/TV,%	35.4±0.8	30.0±1.0^{xx}	19.5±0.6^{x,xxx}	27.7±0.7	28.7±1.3	18.7±1.0^{x,xxx}
Tb.N,mm	8.5±0.1	7.5±0.2^{xx}	6.1±0.1^{x,xxx}	4.7±0.1	5.0±0.1	3.8±0.2^{x,xxx}
Tb.Th.,μm	41.0±0.7	39.0±0.8	31.0±0.7^{x,xxx}	59.0±1.2	56.0±1.6	47.0±1.1^{x,xxx}
Tb.Sp.,μm	76.0±1.7	96.0±3.0^{xx}	136.0±3.5^{x,xxx}	157.0±4.0	150.0±6.0	260.0±18.0^{x,xxx}
Osteoblasts per mm^3	1134.0±42.0	866.0±42.0^{xx}	925.0±25.0^{x}			
Osteoblasts index	1.5±0.05	1.4±0.04	1.3±0.02^{x}			
N.Oc./TV per mm^3	65.0±2.0	70.0±3.5	75.5±3.8^{x}	32.5±2.1	27.4±1.5	22.0±1.4^{x,xxx}
N.Oc./BV per mm^3	185.0±12.7	241.5±24.0	402.5±53.2^{x,xxx}	118.7±18.0	99.3±16.6	135.9±29.1
N.Oc./BS per mm^2	5.0±0.3	5.7±0.4	7.5±0.7^{x,xxx}	4.2±0.4	3.3±0.3	3.8±0.7

Designations: V = vivarium rats; S = synchronous rats; F = flight rats; x indicates significant differences between V and F rats; xx, between V and S rats; xxx, between F and S rats.

Table 2 Histomorphometric analysis of lumbar vertebrae

Parameter	Animals		
	V	S	F
Thickness of growth plate, μm	140,0±8.6	141,0±9.9	143,0±7.0
Osteoblasts per mm^3	461.0±35.0	375.0±42.0	$268.0\pm28.0^{x,xxx}$
Osteoblasts index	1.3±0.02	1.2 ± 0.03^{xx}	1.2 ± 0.01^{x}
BV/TV, %	27.5±1.0	27.6±0.7	$22.8\pm0.9^{x,xxx}$
Tb.N, mm	4.8±0.1	5.2±0.1	4.7 ± 0.1^{xxx}
Tb.Th., μm	56.0±1.2	54.0±1.1	$48.0\pm1.0^{x,xxx}$
Tb.Sp., μm	162.0±7.0	151.0±5.2	182.0 ± 8.0^{xxx}
N.Oc/TV per mm^3	21.0±1.5	15.6 ± 1.9^{xx}	24.9 ± 3.7^{xxx}
N.Oc/BV per mm^3	79.5±9.6	57.2±6.4	111.9 ± 21.0^{xxx}
N.Oc/BS per mm^2	2.9±0.2	2.2 ± 0.2^{xx}	3.5 ± 0.4^{xxx}

References

1. Volozhin A.I. Pathol.Physiol.Exper. Therapy 18, 1, 19-27, 1984 (in Russian).
2. Durnova G.N., Vorotnikova E.V., Sakharova Z.F. et al. Space Biol. 23, 2, 22-26, 1989 (in Russian).
3. Kaplansky A.S., Durnova G.N., Sakharova Z.F. Space Biol. 21, 5, 25-31, 1987 (in Russian).
4. Prokhonchukov A.A., Kolesnik A.G., Komissarova N.A. et al. In: The Effect of Space Flight Dynamic Factors on the Animal Body. Moscow, Nauka, 1979, 146-148 (in Russian).
5. Prokhonchukov A.A., Desyatnichenko K.S., Tigranyan R.A. et al. Space Biol. 16, 2, 62-64, 1982 (in Russian).
6. Rogacheva I.V. et al. Space Biol. 18, 5, 39-44, 1984 (in Russian).
7. Stupakov G.P., Korolev V.V. In: The Effect of Space Flight Dynamic Factors on the Animal Body. Moscow, Nauka, 1979, 174-179 (in Russian).
8. Esling S.W. In: Ibid, 157-165.
9. Yagodovsky V.S., Gorokhova G.P. In: Ibid, 165-174.
10. Cole A.A., Walters L.M. J.Histochem. Cytochem. 35, 2, 203-206, 1987.
11. Gazenko O.G. et al. The Physiologist, Suppl., 30, 1, 53-55, 1987.
12. Jee W.S.S., Wronski T.J., Morey E.R. et al. Am.J.Physiol. 244, 310-314, 1983.
13. Parfitt A.M., Drezner M.K., Gloriex F.H. et al. J.Bone and Mineral Research. 2, 595-610, 1987.
14. Simmons D.J. The Physiologist, Suppl., 24, 6, 65-68, 1981.
15. Vico L., Chappard D., Alexandre C. et al. Proc. of the 2nd Intern.Conf. on Space Physiology. Toulouse, France, 20-22 Nov., 1985, ESA SP-237, 1986, pp.67-78.
16. Vico L., Chappard D., Bakulin A.W. et al. The Physiologist, Suppl., 30, 1, 45-46, 1987.

THE PATTERNS OF SPONTANEOUS OCULOMOTOR ACTIVITY DURING WEIGHTLESSNESS AND RE-ADAPTATION TO GRAVITY

L.N. Kornilova, A.M. Goncharenko, V.Grigorova*, A. Manev**

The Institute of medical and biological problems, the USSR Health Ministry
* The Institute of Physiology, the Bulgarian Academy of Sciences, People's Republic of Bulgaria.
**The Institute of Space Research, the Bulgarian Academy of Sciences, People's Republic of Bulgaria

Introduction

During the period of adaptation to microgravity and the following readaptation to the Earth conditions cosmonauts may have specific sensory-motor reactions, in particular in the oculomotor sphere. The extent and pattern of the oculomotor activity are governed on the one hand by the intensity and pattern of afferentation of different sensory modalities (visual, vestibular, proprioceptive), and on the other hand by the state of central mechanisms, which control the oculomotor reactions.

This problem is of particular interest because the reactivity of the oculomotor system characterizes the functional state of the vestibular system and its interacting sensory systems and topology of changes. Besides it is rectly related to the state of an operator's watching capabilities which are among other things a part of cosmonauts' professional activities.

The preflight and postflight investigations of the vestibular function and its interaction with sensory systems, which are being conducted since 1974, showed that most cosmonauts had a spontaneous positional nystagmus (7). One of the first space investigation of spontaneous oculomotor reactions was carried out aboard the Vostok spacecraft (1). However, no goal-oriented or systematic investigations had been carried out before 1984. During the period of 1984-1987 the Soviet and the joint American and European programs of investigations of the vestibular function under weightlessness have been realized. Individual reactions and some general laws of the vestibular and oculomotor systems of Primates (Kozlovskaya I.B.(8)) and of a man (the "Optokinez" experiment, Kornilova L.N.(6)) have been studied. The logical continuation of the "Optokinez" experiment is the Soviet-Bulgarian experiment, "Labirint"; the results of one fragment of this experiment (an investigation on "spontaneous oculomotor activity") are given in this report.

Methods

Investigations were carried out prior to (before 40 and 30 days) and after (on the 2nd, 3rd and 75th days) the space flight with five cosmonauts being examined. The flight duration of four cosmonauts (Nos.1-4) was 10 days and one cosmonaut (No.5) had the space flight duration of 241 days.

Two cosmonauts were examined in the course of the space flight: one of them (No.4) was examined twice - on the 3rd and 5th days of the space flight, and the other (No.5) was examined 4 times - on the 3rd day of the space flight before and after (4 hours later) taking of the diuretic "Furosemid" (0.04 g), then on the 5th and 164th days of the space flight.

During the "Labirint" experiment the automated data acquisition and processing system (ADAPS) "Zora" was used, which system was developed by Bulgarian specialists based on personal computers. Using analog-to-digital converter discretization of physiological systems was fulfilled and digital information was stored on diskettes to be handled later.

Recording of oculomotor activity was carried out by electrooculography (EOG). Investigations on "spontaneous oculomotor activity" were carried out with the stationary position of the cosmonaut's head. Spontaneous activity was recorded with the eyes closed and open and with the eyes open and goggled. EOG was recorded with the eyes in the following positions: direct and the extreme left, right, up and down. It took 10 seconds for each position.

While handling the oculograms, the amplitude, frequency and velocity of the slow phase of a spontaneous nystagmus were analyzed along with the amplitude of the eyes' saccadic and smooth movements, amplitude and velocity of the horizontal and vertical eye movements in response to a command and the duration of fixing reactions. The analysis of EOG was performed from diskettes on a computer using special programs.

Result and Discussion

Before the spaceflight all those examined, except cosmonaut No.2, had no vestibular disfunction in the form of a spontaneous nystagmus.

One cosmonaut examined (No.2) had an upward horizontal spontaneous nystagmus that was first recorded after his 75-days' space flight in 1983. The results of a dynamic clinical physiological investigation showed that the nature,

direction and intensity of a spontaneous nystagmus of that cosmonaut was not changed in time. The said nystagmus was not subjected to adaptation, did not fade away and was never followed by dizziness when changing the head and body position.

The background examination of other cosmonauts (No.1, No.3, No.4, No.5) showed the stable position of the eyeballs with irregular involuntary micro movements (micro saccades) in the form of quadricular (30%) or nystagmus (70%) jumps with an amplitude up to 2° and frequency of 0.8-1°/sec.

We considered such involuntary symmetric eyes' micro-movements of the central nature, generated on the automatic principle as a functional physiological oculomotor activity. The occurrence of the eyes' jumps and smooth movements with an amplitude of more than 2° was considered as a spontaneous activity and destabilization of the eyeball's position, and involuntary rhythmical eyes' jerks as a spontaneous nystagmus.

The background investigations showed that all those examined when moving the eyes in a horizontal and vertical directions (Fig. 1.A.1; Fig.2.A.1) had eye movements in responses with an amplitude of 8°±1° and, as a rule, followed by a short increase of a fixing nystagmus-like microsaccadic activity (the duration of a microsaccadic reaction was 2÷3 sec). Sometimes at the moment of moving the eyes there occurred single correcting: saccadic movements of the eyes within 2 to 3°.

In the course of the background investigations of cosmonauts with the eyes closed there occurred a spontaneous smooth eye movements activity with the amplitude of 3 to 4°, or the amplitude of saccadic eye movements in response to a command was increased when moving the eyes along a horizontal (19±2°) or a vertical line (14±2°) (Fig. 1.A.2; Fig. 2.A.2).

In the case of the eyes open and dark-goggled the amplitude of horizontal and vertical eye movements in response to a command increased still further, while the amplitude of smooth movements was reduced in comparison with that with the eyes closed (Fig. 1.A.3; Fig.2.A.3).

The distinctive features of oculomotor activity with the eyes open and closed and with the eyes open and dark-goggled, which are observed during the background investigations are characteristic for normal healthy people under similar conditions.

When examined on the 3rd day of the space flight both cosmonauts revealed the increase of a number of involuntary micro saccades of the eyes and change of the proportion of their forms, i.e. micro saccadic quadricular motions 50% and nystagmus - 50%. During the space flight the cosmonauts had the smooth eyes movements even with the eyes open (A_{mpl}=3-4°). On Earth such reactions occur as a rule with the eyes closed.

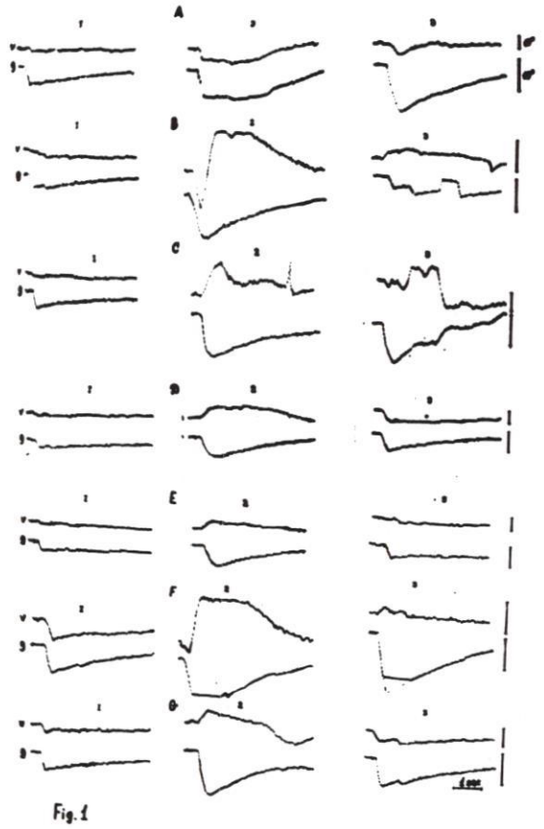


Fig. 1. Horizontal eye movements in test subject 5.

A - before flight;
B - flight day 3 before a diuretic is administered;
C - flight day 3 after a diuretic is administered;
D - flight day 5;
E - flight day 164;
F - postflight day 2;
G - postflight day 75;
EOG vertical lead;
EOG horizontal lead;
← direction of eye movements ;
1 - eyes open; 2 - eyes closed; 3 - eyes open and dark-goggled.

The amplitude of eyes movements in in response to a command when moving the eyes into the extreme horizontal or vertical positions with the eyes open did not practically differ from that of preflight.

However the increase of a number of correcting saccadic movements of the eyes was noted practically each time when moving the eyes. The duration of fixing reactions has increased more than twice (t=7÷9 sec.).

The vertical (EOG) for the eyes closed and "straight" showed for both

cosmonauts a spontaneous up-oriented and basically clonus-like vertical nystagmus of an average amplitude (A=5-7°, RSP (rate of slow phase) = 32±3.2), and changing periodically from tone to undular.

The eyes' movement into the extreme horizontal positions caused decrease of the vertical nystagmus, while the vertical gaze moving resulted in an increase of the vertical nystagmus (Fig.3B).

The vertical spontaneous nystagmus was isolated and it was recorded both at the straight gaze and the extreme vertical eyes moving, i.e. in the direction of the fast and slow nystagmus phases. Such a nystagmus can be related so the Ist, IInd and IIIrd grades, which points to the participation of the vestibular system both in peripheral and central formations. Besides the eyes' vertical moving caused a fixation nystagmus (A=3--4°), which passed into a fixation reaction (t=10 sec) and then into a spontaneous nystagmus (A=6-8°).

The amplitude of eyes movements in response to a command with the eyes closed was markedly increasing, particularly in a vertical direction, and its magnitude (A=22±2°) was greater than in the background before the flight (Fig.1.B.2; Fig.2.B.2).

Also increasing was the amplitude of smooth (6-7°) and saccadic movements (3-5°). By the end of the accomplishment of test exercises spontaneous oculomotor activity went more expressive.

With the eyes open and dark-goggled as compared with the eyes closed both cosmonauts revealed increasing of spontaneous oculomotor activity of the smooth and saccadic pattern. At the eyes' horizontal removing the amplitude of smooth eye movements recorded was 6±2° and saccadic movements - 4±2°, while at the vertical removing against the background of smooth eye movements there was recorded a spontaneous up-oriented nystagmus of the Ist, IInd and IIIrd grade basically clonus--like and periodically undular or tone (Fig. 3B).

The amplitudes of horizontal and vertical eye movements and correcting saccadic movements with the eyes open and goggled were identical to those recorded with the eyes closed.

After horizontal and vertical eye movements prolonged (up to 10-11 sec) micro saccadic reactions were retained as a high-frequency and low-amplitude nystagmus. At the vertical eye movements a spontaneous vertical nystagmus (A=3-4°, RSP - 30.2±2.70).

Cosmonaut No.5, had more pronounced features of oculomotor reactions, than cosmonaut No.4.

Retesting of cosmonaut No.5 according to the "Labirint" program the same day (i.e. the 3rd day) but in 4 hours after taking diuretic (Furosemid - 0.04) revealed no essential changes in the pattern of oculomotor activity.

In the same way as before taking diuretic only functional, physiological or micro saccadic activity was recorded with the cosmonaut's eyes open. The amplitude of horizontal and vertical eye movements in response to a command was similar to that before taking diuretic and the gaze movement was exact without any correction. (Fig. 1.C.1; Fig.2.C.1.).

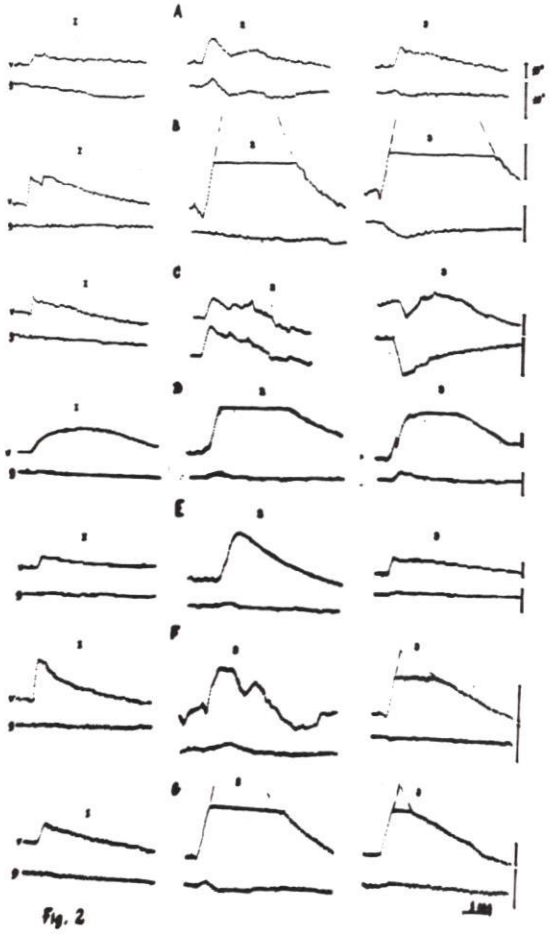


Fig. 2. Vertical eye movements in test subject 5.

For designations see Fig. 1.

↓ direction of eye movements

In the case with the eyes closed there were recorded as in the previously examination, the eyes smooth movements on the background of which in a vertical canal there was as before a spontaneous up-oriented clonus-like and periodically changing into undular or tone nystagmus of the Ist, IInd or IIIrd grade (Fig.3.C).

In the same way as before a spontaneous nystagmus was recorded with the ga-

ze straight-oriented. It increased when moving the eyes moving to the right or to the left.

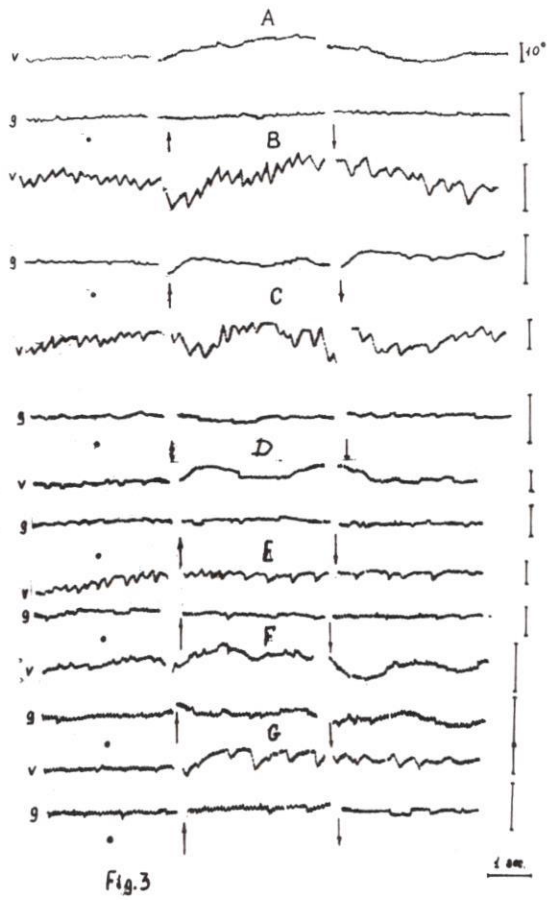


Fig.3

Fig. 3. Spontaneous oculomotor activity in test subject 5 before, during and after flight (with eyes closed).

A - before flight;
B - flight day 3 before a diuretic is administered;
C - flight day 3 after a diuretic is administered;
D - flight day 5;
E - flight day 164;
F - postflight day 2;
G - postflight day 75;
center;
direction of eye movements.

Higher saccadic activity was observed in a horizontal EOG. The amplitude of eye movements to a command was larger tha than with the eyes closed but of the same magnitude as before taking diuretic.

Because of the presence of a vertical nystagmus and higher horizontal saccadic activity the eyes movements to a command were performed with difficulty and were accompanied by correcting saccades ($A_{cor.sac}$=3-4°) and prolonged fixation reactions (t=10-11 sec).

With the cosmonauts' eyes open and dark-goggled there was observed an increase of smooth eye movements. In this case in a vertical EOG there was recorded a spontaneous nystagmus as before, and in a horizontal EOG it was saccadic activity.

The amplitudes of eye movements to a command were the same with correcting saccadic movements and prolonged fixation reactions.

On the 5th day of the space flight both cosmonauts (No.4 and No.5) revealed no spontaneous smooth or saccadic movements with the eye open. The eyeball's positions were stable. Moving the eyes in any directions were exact without correction. (Fig.1.D; Fig.2.D).

With the cosmonauts' eyes closed neither spontaneous vertical nystagmus nor horizontal saccadic activity were observed (Fig. 3.D).

When moving the eyes in a vertical direction the cosmonauts had the larger amplitude of eyes movements to a command than with the eyes open or than on the 3rd day of the flight, and in a horizontal direction it was less than on the 3rd day.

Both cosmonauts revealed the change of the velocity of command eye movements. Velocities of the eye movements are decreased from 70-60°/sec to 25-40°/sec (Fig. 4B). The time of the eye movements was greatly increased from 41±2.2 to 72±4 sec. EOG represented not a saccadic movement, but a smooth sine curve (Fig. 1.D; Fig.2.D).

The character of oculomotor activity with the eyes open and dark-goggled was similar to that with the eyes closed, the difference being in that the amplitude of vertical eye movements in response to a command was larger than that of horizontal (A_{hor}=21÷24°, A_{vert}=40÷50°). Vertical eye movements were dissymetrical ($A_{up} > A_{down}$ by 5°).

It should be noted that on the 3rd and 5th days of the space flight both cosmonauts revealed the eyes voluntary movements from the left to the right and upward not in a horizontal or vertical line but diagonally, since the eyes movement was registered simultaneously in a vertical and in a horizontal canal of EOG (Fig.1.C; 1.D. Fig.2.C;2.D).

On the 164th day of the space flight spontaneous activity was practically not recorded with the eyes open. Moving the eyes into the extreme horizontal and vertical positions occurred with a small amplitude without correcting saccades.

The eyes closing resulted in the eyeballs' destabilization and occurrence of smooth movements (A=4-6°), in the EOG vertical or horizontal canal. On the background of smooth movements there were recorded a spontaneous up-oriented vertical low-amplitude clonus-like nystagmus (of Ist-IInd grade)(Fig. 3.E).

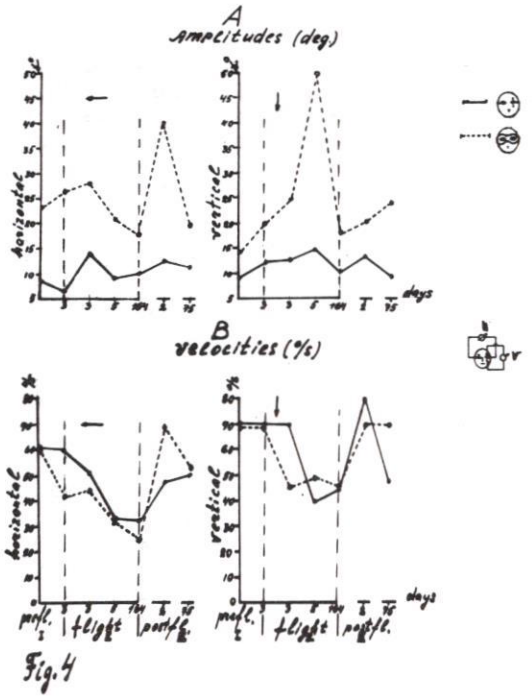


Fig. 4. Oculomotor responses to eye horizontal and vertical movements toward extreme points.

A - amplitude of eye movements;
B - velocity of eye movements;
Abscissa: examination days;
Ordinate: parameters measured;
Arrows indicate eye movement direction.

I - before flight; II - during flight; III - after flight.

The eyeballs' voluntary movements into the extreme positions took place not in a horizontal or vertical direction, but diagonally and their amplitude was less than on the 5th day of the space flight (Fig. 4.A). The velocity of the eyes movements into the extreme positions was lower (Fig. 4.B) so the eyes movements represented not saccadic but smooth movements with a prolonged fixation reaction (t=12÷14 sec). With the eyes open and dark-goggled we observed the decrease of the level of spontaneous oculomotor activity, but spontaneous vertical nystagmus was recorded (Fig.3, E). Other characteristics of voluntary movements were identical to those with the eyes closed.

According to the data of the onboard "Form" and "Questionnaire", filled in by the cosmonauts after the flight, there was only one cosmonaut (No.1) at the initial period of adaptation to weightlessness who felt vestibular discomfort (illusory reactions and sickness). The rest asserted that during the space flight felt themselves well without illusory or vegetative reactions.

After the flight (on the 2nd day) the cosmonauts' spontaneous activity was not not pronounced except No.2 and the eyeballs' position was stable. The eyeballs' voluntary movements into the extreme positions were of a small amplitude without correction, and the duration of fixation reactions corresponded to that of normal type (Fig.1.F; Fig.2.F).

With the eyes closed or open and dark-goggles spontaneous activity of the saccadic and smooth pattern increased especially at the eyes' vertical removing. There was noted an increase of the amplitude at the extreme eyeballs' removing as compared with the eyes open and also when compared with the results of the preflight examination with the eyes closed; the amplitude increase of the eyes movements at the gaze extreme removing (more pronounced with the eyes open) is comparable to the results obtained during the flight. In most cases as also during the flight at the extreme horizontal and vertical removing of the eyes there were recorded the eyes correcting saccades followed by a prolonged (10÷11 sec) tail of fixation reactions.

Cosmonaut No.2 retained a spontaneous nystagmus recorded before the space flight also after the flight. While with the eyes open a nystagmus occurred periodically, it was regular, mainly up-oriented and of an average amplitude (4÷5°) with the eyes closed or open and dark-goggled.

Because of a spontaneous nystagmus the cosmonaut's eye movements in response to a command were broken both with the eyes open and closed (the eyes had nystagmus and floatation). Cosmonaut No.5 being examined on the 75th day after the 241-days space flight did not showed the characteristics and intensity of oculomotor activity identical to the pre-flight data: the vertical EOG for the eyes closed spontaneous nystagmus.

Correlation between the results of investigations of spontaneous oculomotor reactions before, during and after the space flight made it possible to reveal in those examined along with their individual features a number of regular changes of the character and intensity of oculomotor activity. Thus, with the eyes open during the space flight and after it only some cosmonauts had the eyes destabilization and spontaneous activity in the form of smooth and saccadic movements. While all the cosmonauts had such features with the eyes closed or open and dark-goggled especially at the eyes' removing reflecting the eyeballs' vertical movements.

Moreover, one cosmonaut (No.5) revealed when being first examined during the space flight a spontaneous nystagmus with the eyes' vertical removing, which was not recorded on the 5th day but occurred again on the 164th day.

That cosmonaut had no spontaneous nystagmus after the 241-days space flight

and returning to Earth but had it again. on the 75 day.

During the space flight and after it all the cosmonauts with the eyes closed or open and dark-goggled showed an essential increase of the movements' amplitude when removing the eyes into the extreme positions especially in a vertical direction, occurrence of correcting saccadic movements (or nystagmus), an increase in time of fixing reactions.

Eye movements in response to a command of some cosmonauts after the flight were somewhat laboured, i.e. time of eyes' removing into the extreme positions increased and the eyeball floated.

The intensity and character of spontaneous oculomotor reactions observed the space flight and after were very much similar to those previously recorded in the "Optokinez" experiment (6).

The occurrence of higher spontaneous activity of a smooth or saccadic character or even the occurrence of a spontaneous nystagmus, a sharp increase of an amplitude when removing the eyes into the extreme positions (especially up and down), followed by the eyes correcting saccadic movements (or by nystagmus), an increase in duration of fixing reactions - all this is indicative of relieving vestibular and oculomotor reactions. The changes of parameters of spontaneous oculomotor reactions may be due to the chang changes of afferentation at the vestibular input (an increase in activity of the vestibular nerve), or to the changes of neuron activity on the level of relay structures - flocculus and vestibular nuclei of the cerebrum (Kozlovskaya I.B. et al. (8)).

The dependence of the eyeball and nystagmus stability on the visual input attests to the leading part of the peripheral branches of the vestibular system. With the eyes open, reference points being present, the eyeballs' position is stable and no nystagmus is recorded. The occurrence of a spontaneous nystagmus with the eyes closed and retaining it with the eyes open and goggled is testimony to the participation of peripheral formations in its genesis. According to Igarashi M. (5) the changes in the vertical oculomotor system observed in space (a spontaneous vertical up-oriented nystagmus, dissymetry of vertical fixing reactions - up down) may be due to distorted afferent impulses from the otolith sacculus. Besides a spontaneous nystagmus of the Ist, IInd and IIIrd grade and the eyes up- and downward smooth movements are indicative of the participation of central structures and the vestibular system's sections. The increase of a number of quadricular micro saccadic movements, their dissymmetry and the eyes' incidental oscillations (an undular nystagmus) - all this is probably the result of the interaction of the cerebellum and vestibular system under weightlessness. It should be taken into consideration that under weightlessness "supporting unloading" also takes place the so-called (proprioceptive deprivation), which fact also to a certain extent causes modulation of oculomotor reactions (3).

The eyes smooth movements recorded under weightlessness perhaps reflect changes of functioning levels of the brain "controlling" central formations including reticular formation of cerebrum and the cortex of the cerebral hemispheres (2,4). We may suggest that under weightlessness the back corticifugal flow is reduced and the cortex holding influence on sub-cortical structures is also decreased.

When sensory functions are free of the central influence, their automation leads to an increase of the sensory systems' functional activity, in particular of vestibular and related oculomotor activity.

We may also expect that the redistribution of the liquid media in man's constitution in a cranial direction results in the character of sensory-motor reactions under weightlessness. However, there was no such an effect in cosmonaut No.5 examined after taking diuretic. Perhaps some violation in the sensory motor sphere of that cosmonaut was not due to the hemodynamic factor.

References

1. Akulinichev I.T., Emelyanov M.D., Maksimov D.G. et al. Biomedical Investigations in Weightlessness. Moscow, Medizina, 1968, pp.367-370 (in Russian).

2. Durinyan R.A. Cortical Control of Sensory Reactions. Moscow, Medizina, 1975 (in Russian).

3. Kozlovskaya I.B., Kreidich Yu.V., Barmin V.A. et al. Space Biol., 1982, No.5, pp.41-45 (in Russian).

4. Khilov K.L. Brain Cortex in the Vestibular Function. Moscow-Leningrad, 1952 (in Russian).

5. Igarashi M. Vestibular-related neuroscience and manned space flight. IAF/IAA-88 - 495.

6. Kornilova L.N., Bodo G., Kaspransky R.R. Physiologist, 1987, v.30, N 1, pp.85-89.

7. Kornilova L.N., Jakovleva J.Ja., Tarasov J.K. Physiologist, 1983, v.26, N 6, pp.34-36.

8. Kozlovskaya I.B., Sirota M.G., Babaev B.M. Physiologist, 1984, v.27, pp. 111-114.

THE EFFECT OF SPACE FLIGHT ON THE ULTRASTRUCTURE OF THE RAT CEREBELLAR AND HEMISPHERE CORTEX

I.B. Krasnov* and L.N. Dyachkova**

* Institute of Biomedical Problems and
** Institute of Evolutionary Morphology and Ecology of Animals, Moscow, USSR

Introduction

Electron microscopy of the brain of animals flown in space is one of the methods to examine its function and structure in microgravity. Of interest is the study of the cerebellar nodulus cortex as well as the somatosensory and visual cortex which receive graviceptive, proprioceptive and photoperceptive impulses providing information about the gravitational level and spatial body position. Electron microscopic examination of the nodulus cortex of rats flown for 18.5 days on Cosmos-936 (3) revealed ultrastructural changes in terminals of mossy fibers (TMF), i.e. axonal terminals (AT) of neurons of the vestibular ganglion. These changes were indicative of TMF superexcitation and reflected superexcitation of the "receptor cell of the utriculus - neuron of the verstibular ganglion" system that developed postflight. The purpose of the present study was to investigate the ultrastructure of the nodulus cortex of pregnant rats after a 5-day flight on Cosmos-1514 and of the nodulus cortex as well as the somatosensory and visual cortex of the brain of male rats after a 7-day flight on Cosmos-1667.

Methods

Brains of rats from flight, synchronous and vivarium control groups, each consisting of 5 (Cosmos-1514) or 7 (Cosmos-1667) animals, were investigated. The rats were decapitated 6-8 hrs after recovery of Cosmos-1514 or 4-8 hrs after recovery of Cosmos-1667. Two-three minutes after decapitation strips of the nodulus, somatosensory and visual cortex were fixed by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) at 4°C. Then the biosamples were postfixed in 2% OsO_4 in the same buffer and embedded in araldite. Frontal ultrathin section were examined electron microscopically. The III layer of the somatosensory cortex, at x6000, was used to count the following parameters in sequential 160 $/\mu m^2$ pieces: normal 1.5-2.5 um thick ATs; "light" and "dark" degenerated ATs; axonal growth cones and axo-dendritic synapses. The data were treated using the Student's t-test.

Results and Discussion

Having in view the concept that structural and functional changes in nerve synapses are correlated (6), it can be postulated on the ground of ultrastructure analysis that 6-8 hrs after recovery (Cosmos-1514) TMFs in the granular layer of the rat nodulus were in the state of superexcitation (Fig. 1) (3,4). This is indicated by: 1) densely packed synaptic vesicles (SV) that fill in the terminal; 2) unusual SV clustering near presynaptic membranes of axo-dendritic synapses; 3) increased electron density of pre- and post-synaptic membranes and post-synaptic thickening; 4) enlargement of the synaptic gap. One third of the axo-dendritic synapses showed synaptic transmission blockade; this was suggested by the fact that there was a 10-20 nm distance between SVs and the presynaptic membrane, the post-synaptic thickening was well delineated, and the structure of mitochondria and cisternae in post-synaptic elements was well preserved (4).

In rats flown for 7 days on Cosmos-1667, TMFs in the nodulus cortex were in the state of excitation 4-8 hrs after recovery (2). This is indicated by: 1) greater area of distribution and amount of SVs; 2) noticeable SV clustering near presynaptic membranes; 3) increased electron density of pre- and post-synaptic membranes and post-synaptic thickening; 4) enlargement of the synaptic gap. Blockade of synaptic transmission occurred in approximately every tenth axo-dendritic synapse of TMFs.

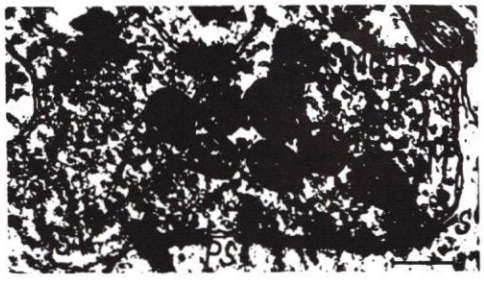


Fig. 1. Ultrastructure of the mossy fiber terminal in the granular layer of the nodulus cortex of a Cosmos-1514 rat. State of TMF superexcitation. Magnification x42,000. Bar indicates 0.4 um. TMF - terminal of a mossy fiber, PS - postsynaptic segment of a granular cells dendrite; SV - synaptic vesicle; M - mitochondria; S - synapse.

Thus, the ultrastructure of TMFs in the granular layer of the nodulus cortex of rats exposed to 0 G for 5 or 7 days and to 1 G for 4-8 hrs after recovery points to their excitation. This excitation of TMFs, which are axonal terminals of

neurons of the vestibular ganglion, reflects, in one's turn, excitation of the "receptor cell of the utriculus - neuron of the vestibular ganglion" system. The latter should be viewed as a result of enhanced sensitivity of the otolith organ in microgravity: after recovery the rats were for 4-8 hrs exposed only to Earth's gravity which may stimulate the otolith function only in case of increase of its sensitivity. The stimulating effect of Earth's gravity on the otolith organ after recovery is supported by ultrastructural studies of TMFs in the ground-based experiment in which postflight exposure to it was simulated by rat centrifugation at 2 G for 6 hrs. The results of TMF ultrastructural studies serve as morphological evidence in favor of a higher sensitivity of the otolith organ in microgravity. They give support to the observation we reported previously on the basis of our electron microscopic examination of the rat nodulus cortex after a 18.5-day flight on Cosmos-936 (3).

In the somatosensory cortex of the 7-day Cosmos-1667 rats we revealed ultrastructural changes (most expressed in the III layer) which suggest a microgravity-induced reduction of afferent impulsation. This is evidenced by: 1) drastic decrease of the number of axo-dendritic synapses in the III layer, where fibers from the ventral nuclei of the thalamus end, which receive impulses from proprio- and exteromechanoreceptors (Fig. 2); 2) drastic increase of the number of "light" degeneration ATs which normally emerge after prolonged decline of neuronal activity (6) and are characterized by a significant decrease of the number of synaptic vesicles and mitochondria, decline of electron density of the matrix, aggregation of a small number of synaptic vesicles near the presynaptic membrane with integrity of the membrane of the outline of TMFs; 3) fragmentation of structures in post-synaptic segments of dendrite spines pointing to extended reduction of afferent impulsation (5). At the same time the ultrastructure of the somatosensory cortex showed signs of its excitation similar to those found in TMFs of the nodulus cortex of Cosmos-1667 rats as well as signs of its superexcitation represented by a greater number of "dark" degeneration ATs (Fig. 2) which are a solid osmiophilic condensation produced by an active stimulation of neurons (1). This state of excitation of somatosensory structures reflects an enhanced flow of proprio- and exteroceptive impulses entering the cortex during 6-8 hr exposure to Earth's gravity after recovery and indicates a higher sensitivity of proprio- and exteromechanoreceptors of the rat body and limbs in microgravity. At 0 G disorders of synaptic links that exist on the Earth develop in parallel with an active formation of new links in the somatosensory cortex. The latter is evidenced by a significant increase in the amount of axonal growth cones (Fig. 2). The II-IV layers of the somatosensory cortex showed a noticeable increase of the area occupied by processes of glial cells.

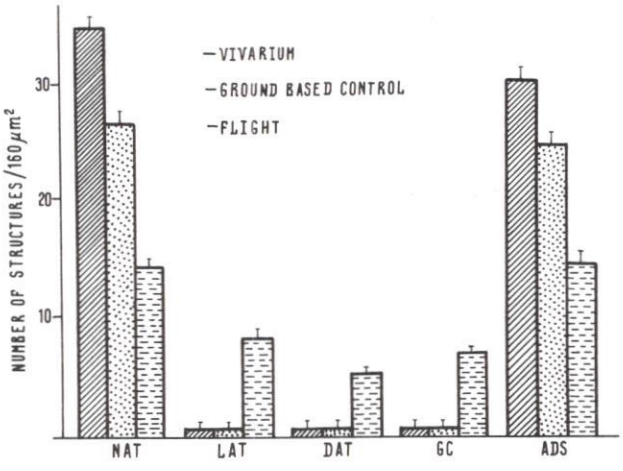


Fig. 2. Distribution of structures in the III layer of the somatosensory cortex of Cosmos-1667 rats. NAT - normal axonal terminals; LAT - "light" degeneration of axonal terminals; DAT - "dark" degeneration of axonal terminals; GC - axonal growth cones; ADS - axo-dendritic synapses.

The visual cortex of the 7-day Cosmos-1667 rats displayed ultrastructural signs of the processes described above in the somatosensory cortex; however most changed ATs developed "light" degeneration and morphological signs of excitation were less expressed than those seen in the somatosensory cortex. In the visual cortex growth cones were mostly concentrated in the II-IV layers.

References

1. Iontov, A.S. Electron microscopic examination of synapses of spinal cord anterior horn cells after electric stimulation. Arch.Anat.Histol.Embryol. 57:4-18, 1974 (in Russian).

2. Krasnov, I.B. Increase in sensitivity of the otolith apparatus in microgravity. Morphological evidence. Abstr. Papers, XX Meeting of Intercosmos Space Biol.Med.Group, Berlin, GDR, 1987, 127 (in Russian).

3. Krasnov, I.B., Dyachkova, L.N., Anders, V.N. Abstr. Papers, XVIII Meeting of Intercosmos Space Biol.Med.Group, Gagra, USSR, 1985, 143-144 (in Russian).

4. Krasnov, I.B., Dyachkova, L.N. Kosm. Biol., 20:45-48, 1986 (in Russian).

5. Taxi,J. Ann.Sci.Natur.Zool. et Biol. Anim. 11:413-674, 1965.

6. Walberg, F., Mognani, E. Brain Res. 14:67-75, 1969.

CARDIOVASCULAR DECONDITIONNING DURING WEIGHTLESSNESS SIMULATION AND THE USE OF LOWER BODY NEGATIVE PRESSURE AS A COUNTERMEASURE TO ORTHOSTATIC INTOLERANCE

Antonio Güell, MD*, Laurent Braak, Ph.D*, Claude Gharib, MD **

* Centre National d'Etudes Spatiales, 18 Avenue Edouard Belin, 31 055 TOULOUSE CEDEX, FRANCE

*** Laboratoire de Physiologie, Faculté de Médecine Granges Blanches, 8 Avenue Rockfeller, 69 373 LYON CEDEX, FRANCE

When man is subjected to weightlessness, the body fluids in the lower part of the body move to the thoraco-cephalic regions in the absence of hydrostatic pressure : this fluid, shift estimated at approximatively 1.500 to 2.000 cc, is the main cause of the Cardiovascular Deconditionning Syndrom (C.D.S.) (4) ; the main symptoms of which appear on returning at earth after space missions. This syndrom is characterized by :

- a higher heart rate at rest,
- an orthostatic intolerance (drop in systolic and diastolic pressures with an increase in heart rate) possibly accompanied by a presyncopal state or a syncope,
- a higher submaximal oxygene consumption for an equivalent work load.

Orthostatic intolerance with its accompagying risks of syncope, is a particularly topical problem with the coming for Space Transportation System, it's being known that astronauts would suffer from the effects of reentry acceleration along the + gz axis ; the risk of syncop is increased as this new stress is added to C.D.S (5).

Many different methods were suggested to counteract the orthostatic intolerance including anti-g suits, exercices, electrical muscle stimulation saline loading and various pharmacologic agents ; if some countermeasures had been evaluated in controlled experiments, their efficacy is uncertain and their mechaninsm of action unknown.

The main purpose of this study was to assess the specific effects of periodical Lower Body Negative Pressure (L.B.N.P.) sessions on the C.D.S. during a bedrest experiment and more specifically on the orthostatic intolerance postural hypotension is invaraibly observed after prolonged bedrest such as when astronauts following return to Earth from missions lasting at least several days.

MATERIALS AND METHODS

General procedures : two bedrest studies were performed at an interval of one year, with the participation of the same volunters (except one subject) after the agreement of the French National Ethics Commitee : (1) the first one, in November-December 1987 ; (2) the second one, one year later in September-October 1988. The all duration of 42 days each experiment was of, including :

- ambulatory period : 7 days,
- antiorthostatic bedrest at 6 head down : 30 days,
- recovery period : 5 days.

The subjects : 5 healty volunteers took part in this study : in the first part of the experiment (November-December 1987) 3 subjects (A, B, C) were submitted to periodical sessions of L.B.N.P. and the 2 others (D, E) served as controls.

In 1988 (September-October) the 3 subjects (A, B, C) having undergone L.B.N.P. in 1987, made up the control group : the 1987 control group (D, E) became, in 1988, the L.B.N.P. group.

L.B.N.P. procedures : three 20 minutes sessions per day -35 mb for the first three weeks of the antiorthostatic bedrest were followed by 4 sessions a day for the first 4 days of the last week, culminating in 6 daily sessions over the last 3 days. The subjects were monitored throughout these L.B.N.P. sessions : continuous electrocardiogram, systolic, diastolic and mean arterial pressures each minute.

During les L.B.N.P. sessions, the soles subjects' feet were against a foot-rest.

Orthostatic exploration : 2 orthostatic investigations using the tilt-test were performed, the soles of the subjects' feet against a foot-rest ; (1) the first, on day-5 (i.e. 5 days before the beginning of the bedrest during the ambulatory period) ; (2) the second immediately after the end of the bedrest ; each of these two tilt tests was performed at the same time during the day.

Tilt-test protocol consisted in tilting the subject to + 60° in 2 seconds after 30 minutes in a horizontal position. The + 60° position was maintained for 60 minutes or until a presyncopal state or syncope ensued. During the tests, the subject's heart rate was monitored and electrocardiogram taken continuously, and systolic, diastolic and mean arterial blood pressures monitored semi continuously (each minute).

Blood samples were taken before the tilt test (5 minutes before to tilt the subjects) and when subject were in + 60° Head up position (3 minute after tilting) to measure Plasma Renin Activity, vasopressine and catecholamines.

Plasma volume was not measured by classical techniques (Evan's blue, radio-isotipic method) for ethical reasons ; an index of plasma volume was however obtained by considering the hematocrit values and the weight of each subject (12)

Statistical analysis

The subject's data were analysed using a paired test. The orthostatic tolerance data were analysed with a one-way repeated measures Anova design. Post hoc testing was accomplished with Duncan's multiple range test.

All statistical analyses employed an p level of 0.05 to denote significance.

Results

The results are summarized in table 2, and in figures 1 and 2. They show that :

1. - Orthostatic tolerance (table 2 and (figures 1 and 2)

- When the subjects were in the control groups (i.e. absence of countermeasure) : (1) during pre-bedrest there were no significant variations in blood pressure at the end of the tilt test with respect to the supine position ; the heart rate was slightly increased ; (2) during post-bedrest a significant drop in systolic and diastolic blood pressures along with a very sharp increase in heart rate which led, in 3 of the 5 subjects to a true syncope with loss of consciousness and, in one subject, to a presyncopal state (sweating, paleness, thirst).
- When the subjects were subjected to periodic L.B.N.P. : (1) there were no significant variations in the systolic or diastolic blood pressures at the end of the tilt test compared to the supine position whether

before or after bedrest ; (2) a significant increase was observed in the heart rate at the end of the post-bedrest tilt test compared to the value obtained during the ambulatory period. It is interesting to note that this increase was less marked than that observed when the subjects had not been subjected to the countermeasure.

2. - Plasma volume

The variations observed in the plasma volume index show differences according to whether the subjects were L.B.N.P. or controls ; for the ambulatory period no variation was seen in the index when the subjects were placed under L.B.N.P. ; in control subjects however, a significant decrease was seen in the index from the first week of bedrest up to the end of the bedrest period.

3. - Vasopressine, Plasma Renin Activity and Catecho-lamines

The variation of these parameters, during the tilt tests carried out before and after bedrest, showed a tendancy towards a greater increase during tilt tests performed at the end of bedrest in both groups. Considering the small number of samples and the large individual variations the results cannot be considered as significant but the general trend remains.

4.- Finally, no side-effects, such as varicose veins appearing in the lower half of the body, were observed. Moreover, signs of orthostatic intolerance were noted during any of the various L.B.N.P. sessions.

DISCUSSION

The preliminary results (1) concerning the prevention of post-bedrest orthostatic intolerance were confirmed ; repeated application of L.B.N.P. prevented the drop in blood pressure observed after 30 days bedrest in an antiorthostatic position.

Several mechanisms are involved in the physiopathogeny of the orthostatic intolerance observed during cardio-vascular deconditioning syndrome : decrease of the plasma volume, role of the baroreflex arc, role of the low pressure venous system of the lower limbs.

The redistribution of the body fluids towards the thoracocephalic region is interpreted by the Central Nervous System as a state of relative hypervolemia and stimulates a certain number of pressure and volume receptors ; a number of hormonal parameters are consequently modified in the aim of reaching a new balance point with a decrease of the central blood volume (8). The short term (< 24 h) modifications are :

- a hypothetical decrease in the synthesis and secretion of anti-diuretic hormone causing a rise in diuresis (8) ; this reflex path, which starts at the right auricle, has never been proven to exist in man during space flights,
- a drop of sympathetic tonus with a decrease of the plasma renin activity and of aldosterone secretion bringing about sodium leakage (10),
- finally an increase in the synthesis and secretion of the atrial natriuretic factor (9).

In the longer term, during the first days of bed-rest there is a fluid loss from interstitial fluid and plasma volume resulting from an increase in diuresis and a decline in fluid intake. The result is a decrease in central venous pressure (1, 3, 6), and the subsequent stimulation of the renin aldosterone system. Such a decrease was found by Kirsch (14) during the Spacelab-1 Mission. The renin-aldosterone system is also sensitive to sodium and potassium balance. Now it is well known that during the first day of hypogravic studies there is a sodium and potassium loss which can stimulate the renin aldosterone system. So the observed long-term responses are probably linked mainly to sodium loss and mediated by the automatic nervous system (beta adrenergic stimulation) and also to kaliemia disturbances.

It is argued that the relative increase in the central blood volume should give rise to an attenuation of the activity of the baroréflex arc (2). Recent studies (13) have shown modifications of this activity in man during postural changes (orthostatism, antiorthostatism).

Baroreception responsiveness is a first phase probably heightened by the new redistribution of body fluids to the thoracocephalic regions. When the astronauts return to earth, the baroreceptor responsivness can be attenuated, contributing to the orthostatic intolerance observed after spaceflights, Baroréflex function needs to be evaluated under conditions of prolonged weightlessness or simulated weightlessness (13).

Finally, about 85 % of the venous volume of the leg is stored in the deep vein network, they are muscle veins with a poorly innervated wall. It appears that the skeletal musculature of the lower limbs plays an important part in the phenomenon of compliance and thus in capacitance. However, muscular atrophy occurs during space flights and simulations : Thorton et al (16, 17) suggest that decreased muscle tone contributes to an increased degree of blood pooling after bedrest or spaceflight ; so, the drop in muscle tone could partly explain the increase in compliance.

In normotensive (15) subjects in head-down position, a decrease in venous tone was observed, similar findings were reported during hypervolemia induced by head-out water immersion by Echt and Gauer (7). Changes in venous capacity can explain the modifications in venous tone : these modifications of tone could be related to :

- changes in hydrostatic forces,
- decreased activity of smooth muscle cells,
- possibility of an action of humoral factors on venous tone.

But, findings observed in hypertensive subjects in head-down tilt are in favour of changes in the viscoelastic properties of venous walls which are responsible for the passive behaviour of veins.

In fact, the combined data, although incomplete and inconclusive, suggest that orthostatic intolerance after bedrest and related conditions is a multifactorial disorder. Changes in effective venous compliance and perhaps also subtle autonomic dysfunction appear to amplify the effects of a moderate absolute hypovolemia.

The results obtained during these two experiments support the large, but often underestimated, role of the low-pressure venous system of the lower limbs in the origin of post-space-flight and post-bedrest orthostatic intolerance since both groups presented the same muscular atrophy.

Future basic research must consider : (a) the possible links between the distribution of blood through the veins, orthostatic intolerance and the functioning of skeletal muscle ; (b) a detailled study of the distensibility and compliance of the venous territory of the lower limbs ; (c) the role of the capillaries and the trans-membrane exchanges of the venous territory ; (d) the role of the vegetative nervous system in vein distendability ; (e) the neurochemical mechanisms involved.

Future applied research should consist of new experiments on the ground to define the use of L.B.N.P. in prophylaxis and, in particular, to investigate the actual pressures and durations to be used so as to interfere as little as possible with the work activities

of the future crews. Moreover, it would be apt to immediately start studying the efficiency of L.B.N.P. as a countermeasure on a large number of astronauts during short-term missions of the STS type missions.

Finally, on a technical level, considering use in the future orbital infrastructures with limited living space available, L.B.N.P. devices should be designed for maximum ergonomy and, of course, minimum bulk. The idea of developing a depressurised sleeping bag type of system would save a considerable amount of time as L.B.N.P. sessions could be conducted while the astronaut is asleep ; however, a wide range of physiological studies will have to be conducted and validated beforehand.

This is why we aim to produce a prototype of this sleeping bag. The system will comprise an airtight cloth held in place by rings around the astronaut's legs and a flexible airthight seal attached to the upper part at waist level. The padded rings will be inflatable, so this system has the advantage of taking up little space when folded away, compared to other, bulkier, types of structure.

In conclusion, the beneficial effect of L.B.N.P., as a prophylactic, has been proven. The two experiments reported open new perspectives into the concept on in-flight cardiovascular prophylaxis suggesting further research on fundamental, applied and technological levels.

REFERENCES

1.- ATKOV O., BEDNENKO V., FOMINA G. - Ultrasound technique in space medicine. Aviat. Space and Environ. Med., 1987, 58,9,69-73.

2.- BILLMAN G.E., DICKEY D.T., TEOH K., STONE H.L. - Effects of central venous blood volume shift on arterial baroreflex of heart rate - am. J. Physiol. 1981, 241, 571-575.

3.- BLOMQUIST, C.G., J.V. NIXON, R.L. JHONSON, JR. and JH. MITCHELL -Early cardiovascular adaptation to zero gravity simulated by head down tilt. Acta Astronautica 1980 7 : 543-553.

4.- BUNGO M.W., CHARLES J.B., JHOHNSON Ph.C. - Cardiovascular deconditionning during space flight and the use of saline as a countermeasure to orthostatic intolerance. Aviat. Space and Environ. Med., 1985, 56, 985-90.

5.- CHARLES J., BUNGO M. - Cardiovascular research in space : consideration for the design of the human research facility of the U.S. Space Station. Aviat. Space and Environ. Med., 1986, 46, 1000-1005.

6.- CHARLES J.B., BUNGO M. - Non invasive estimation of Central Venous Pressure using a compact Doppler ultrasound system 1987 - In : NASA - TM - 5820, p.p. 69-74.

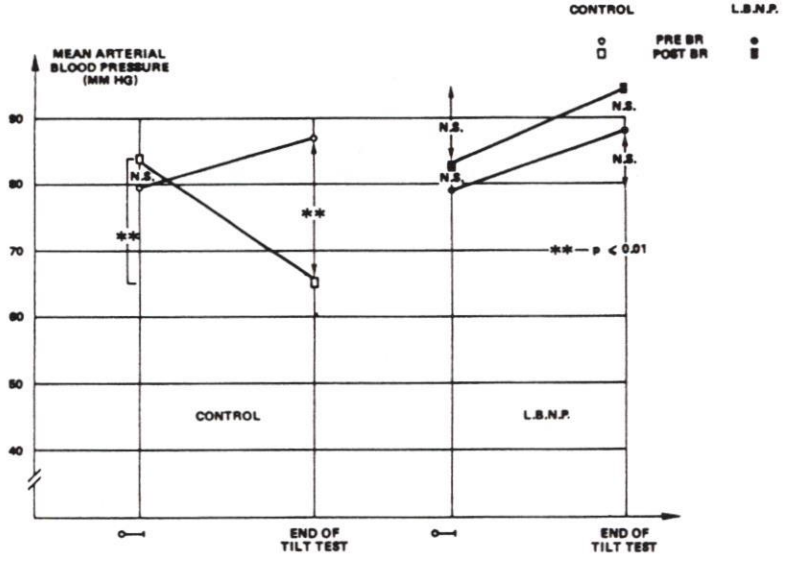


FIGURE 1 -

RESPONSES OF MEAN ARTERIAL BLOOD PRESSURE TO TILT TEST
PRE AND POST BEDREST WITH AND WITHOUT L.B.N.P.

7.- ECHT M. and GAUER O. - Changes in peripheral venous tone and central transmural venous pressure during immersion in thermo neutral bath - 1974, Pfluegers Arch. 352, 211-217.

8.- GAUER O. - Circulatory basis of fluid volume control.Physiol. Rev. 1963, 43, 423-481.

9.- GHARIB Cl., GUAQUELIN G., GEELEN Gh., CANTIN M., GUTKOWSKIA J., MAUROUX J.L., GUELL A. - Volume regulating hormones in weightlessness, 1985, The Physiologist 28, 530-534.

10.- GUELL A., GHARIB Cl., BES A., GERAUD G. - Physiological effects of bedrest 1982. The Lancet, 6/02, 328

11.- GUELL A., BRAAK L., MAUROUX J.L., PAVY A. - The preventive effects of L.B.N.P. (Lower Body Negative Pressure) on orthostatic intolerance after a 30 day bedrest, 1988, Proceedings of the 39th congress of the International Astronautical Federation, October 8-15 Bangalore, India

12.- GUYTON A.C. 1981. Partition of the body fluids : osmotic equilibria between extra and intracellular fluids. In Textbook of Medical Physiology, Ed. W.B. Saunders Company, Philadelphia, 391-402.

13.- HARISSON M.H., RITTENHOUS D., GREENLEAF J.E. - Effect of posture on arterial baroreflex control of heart rate in humans - Europ. J. Appl. Physiol. 1986, 55, 367-373.

14.- KIRSCH K., ROCKER L., GAUER O., KRAUSE R., LEACH C., WICKE M.J., LANDRY R. - Venous pressure in man during weightlessness - Science, 1984, 225, 218-219.

15.- LONDON, G.M., LEVENSON J.A., SAFAR M.E., SIMON A.C., GUERIN A.P., PAYEN D. - Hemodynamic effects of head down tilt in normal subjects and sustained hypertensive patients. Am. J. Physiol, 1983, 245, 194-202.

16.- MORE Th., THORTON W. - Inflight and postflight fluid shifts measured by leg volume changes. In : NASA TM-5820, p.p. 59-65, Johnson Space Center, Houston, 1987. TALAVRINOV V., ANACHKIN O., BRAGAMOV K., VOLGUINE V., aviakosm. med. 1988, 22, 22-26.

17.- THORTON W., HOEFFLER G. - Hemodynamic studies of the legs under weightlessness. In NASA SP-377, p.p. 37-44, Johnson Space Center, Houston, 1977

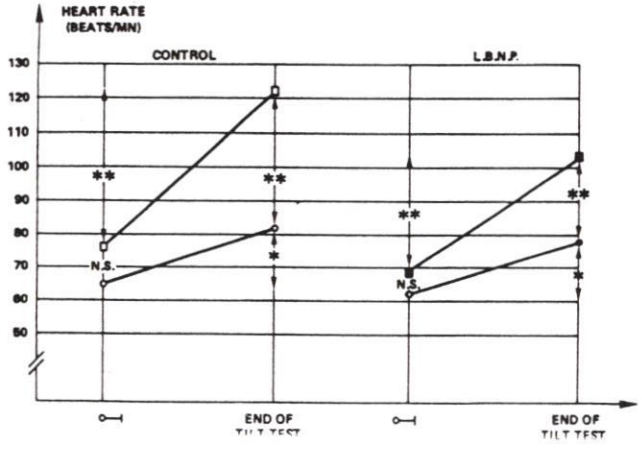


FIGURE 2 -

RESPONSES OF HEART RATE TO TILT TEST
PRE AND POST BEDREST WITH AND WITHOUT L.B.N.P.

PHYSICAL FITNESS BEFORE AND AFTER ONE MONTH HEAD-DOWN BEDREST, WITH AND WITHOUT LOWER BODY NEGATIVE PRESSURE

D.RIVIERE *, A.PERE *, F.CRAMPES *, M.BEAUVILLE **, A.GUELL * and M.GARRIGUES *

* Service d'Exploration de la Fonction Respiratoire et de Médecine du Sport - HOPITAL PURPAN - 31059 TOULOUSE CEDEX FRANCE

** C.N.E.S. - 31055 TOULOUSE CEDEX FRANCE

It is now a well-documented fact that long duration exposure to actual and/or simulated weightlessness leads to a decrease in physical fitness (1,8). This is usually assessed in terms of the decrease in maximal oxygen uptake (4,6). Exercise during spaceflight has been suggested as a countermeasure (10). The aim of this paper, is to study the effect of another countermeasure, lower body negative pressure (LBNP) on the physical fitness of men subjected to weightlessness simulated by 30 days of anti-orthostatic bedrest.

MATERIAL AND METHOD

Two bedrest studies were performed with the same subjects, the first in November-December 1987 and the second in September-October 1988.
After a 7-day ambulatory period, subjects were submitted to 30 days of anti-orthostatic bedrest at -6° head-down. This was followed by a 5-day recovery period.
- Subjects : 5 healthy male volunteers took part in this experiment, with the National Ethics Committee's agreement. They served alternately as controls and then were subjected to LBNP sessions or vice versa. Subjects' characteristics are given in table 1.

Table 1 : Descriptive statistics for subjects

	AGE	HEIGHT	WEIGHT	VO2 MAX.	VO2 MAX.
SUBJECTS	yr	cm	kg	l.min.-1	ml.min.-1.kg.
CONTROL	33.0 +/- 3.1	180 +/- 6	71.2 +/- 2.5	3.3 +/- 0.2	46 +/- 3
LBNP	32.8 +/- 3.0	180 +/- 6	71.7 +/- 2.4	3.4 +/- 0.2	47 +/- 4

Values are means +/- SE ; no significant differences.

- LBNP procedures : three 20-minute sessions per day at - 35 mb for the first three weeks, followed by 4 sessions a day for the first 3 days of the last week and by 6 daily sessions over the last 4 days.
- Physical fitness assessment : On day -6, the maximal oxygen uptake (VO2 max.) was determined for each subject by the direct method (EOS Sprint Jaeger) during a cyclo-ergometer exercise test called codification test. Then, in view of studying the effects of bedrest with and without LBNP on physical fitness, 2 other tests were performed at 11 a.m., the first 1 day before the bedrest period (D-1) and the second 3 days after the bedrest period (R+3). During these two tests, the following parameters, oxygen uptake (VO2) (by the direct method), heart rate, blood lactates and hemoglobin concentrations, were determined at rest and after 10 minutes cycling at 60 % VO2 max., 2 min. cycling at 70, 80 and then 90 % VO2 max. and finally during a recovery period after 3 minutes cycling at 30 % VO2 max. These different levels of work load were determined from the results of the codification test performed on day -6. We insist on the fact that the two exercices were submaximal and that for each test, the same absolute intensities were used.
Blood samples for lactates and hemoglobin were obtained from a small catheter placed in a forearm vein.
- Statistical procedures : Since, after a 1-year interval, the physical fitness of the subjects had inevitably changed, we compared the results obtained during the two bedrest studies for the 5 subjects before bedrest, i.e 10 results before bedrest (group 1) to the results obtained after bedrest without LBNP (5 results : group 2) and to the results obtained after bedrest with LBNP (5 results : group 3). We also compared group 2 and group 3. The results are expressed as means +/- SE. Variance analysis was used to evaluate the significance of differences. We used the Student's T test on the differences.

RESULTS

- Oxygen uptake (VO2) (Fig.1)
VO2 was not different after the bedrest period from the oxygen uptake before, either with or without LBNP except during the recovery period after the test where VO2 was significantly higher without LBNP after bedrest than before ($p < 0.05$). VO2 was not different after the bedrest period between groups 2 and 3.
- Heart rate (Fig.1) :
The heart rate was significantly higher after the bedrest period than before, at all the levels of exercise, both without LBNP ($p < 0.05$) and with LBNP ($p < 0.01$). But, again there was no difference after bedrest between group 2 and group 3.
- Blood lactates (Fig.2)
The lactate production was increased after the bedrest period. With LBNP, the difference was not significant while without LBNP, the difference was significant ($p < 0.05$) for 80 % VO2 max. and during the recovery period at 30% VO2 max. There were no differences after bedrest between groups 2 and 3.
- Hemoglobin concentration (Fig.2)
At rest, there was a significant decrease in hemoglobin concentration ($p < 0.05$) after bedrest in both groups. During exercise, the hemoglobin concentration was not different after bedrest from the hemoglobin concentration before. During the recovery period after the test, the hemoglobin concentration was significantly lower after bedrest than before in the group subjected to LBNP ($p < 0.05$). There were no differences after bedrest between groups 2 and 3.

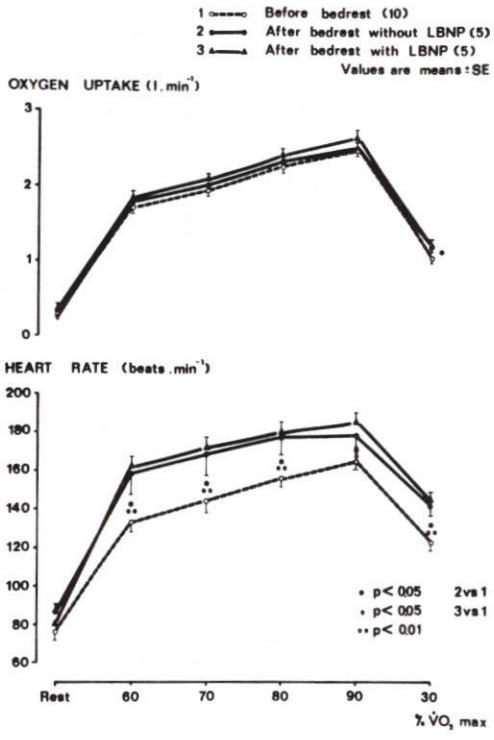


Fig.1

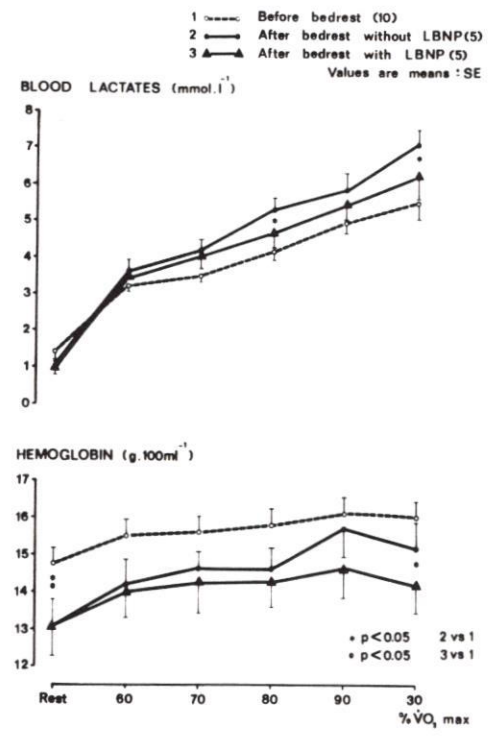


Fig.2

DISCUSSION

First, we can say from the results of the codification test that these subjects were not athletes ; so, they were not concerned by the present controversy about the greater sensitivity to cardiovascular deconditioning in well-trained subjects (2,5).

Second, we found that for the same absolute intensity of exercise, the oxygen uptake of the subjects was not decreased after bedrest. This has already been shown : "the efficiency of oxygen transport, transfer and utilization in the muscle tissue was not reduced in men" (9). But, the increase of heart rate, which was nearer the maximal heart rate, indicates that in fact, after bedrest, each work load represented a higher percentage of VO2 max., i.e a higher relative intensity. This must be regarded as a decrease in physical fitness. This statement is confirmed by the increase in blood lactate concentrations after bedrest.

This decrease of physical fitness may be due partly to one month of physical inactivity and partly to the head-down position i.e simulated weightlessness. It seems that the physical inactivity played the predominant part for two reasons. First, we found no difference during exercise after bedrest with and without LBNP. Second, our results were in agreement with those of authors who only studied the effects of detraining on responses to submaximal exercise (3). However, it seems probable that the head-down position also played a part in the decrease in physical fitness since it appears that without LBNP the recovery of the subjects following the test was less efficient after bedrest than before. This difference did not appear with LBNP.

The last point we shall discuss is the hemoglobin concentration. At rest, the decrease observed after bedrest has already been described and attributed to a diminution of erythropoiesis during bedrest (7). During exercise, the hemoconcentration was approximately the same before and after bedrest in both groups. However, during the recovery period after the test, the return to basal values seems faster with LBNP, may be due to a difference in volemia between groups 2 and 3.

In conclusion, from the results obtained during the recovery period after exercise, we think that it is possible that LBNP has a favourable effect on the decrease in physical fitness but that this influence is probably masked by the magnitude of the specific effect of one month of inactivity.

REFERENCES

1- BLOMQVIST CG, STONE HL. In : Handbook of Physiology - Section 2. The cardiovascular system. Vol.3. Peripheral circulation and organ blood flow. SHEPHERD JT, ABBOUD FM eds, Bethesda, MD : The American Physiological Society, 1983, 1025-1063

2- CONVERTINO VA, SATHER TM, GOLDWATER DJ, ALFORD WR. Med. Sci. Sports Exerc. 1986 ; 18 : 551-556

3- COYLE EF, MARTIN III WH, BLOOMFIELD SA, LOWRY OH, HOLLOSZY JO. J. Appl. Physiol. 1985 ; 59 : 853-859

4- FREY MAB. Aviat. Space Environ. Med. 1987 ; 58 1014-1023

5- HARRISON MH. Sports Medicine, 1986 ; 3 : 428-435

6- HUNG J, GOLDWATER D, CONVERTINO VA, Mc KILLOP JH, GORIS ML, De BUSK RF. Am. J. Cardiol., 1983 ; 51 : 344-348

7- JOHNSON PC, DRISCOLL RB, LEBLANCE AD. In : Biomedical Results from Skylab, JOHNSTON RS, DIETLEIN LF eds, Washington, DC ; Natl Aeronaut. Spce Adm., 1977, SP-377, 235-241

8- LEVY ML, TALBOT JM. Final report, Phase I, Washington, DC : NASA, 1983

9-SANDLER H. In : Zero-g Simulation for Ground Based Studies in Human Physiology, with Emphasis on the Cardiovascular and Body Fluid Systems, Paris : ESA SP-180, 1982, 107-146

10-TIPTON CM. Med. Sci. Sports Exerc., 1983 ; 15 441-444

EFFECTS OF LOWER BODY NEGATIVE PRESSURE ON CARDIOVASCULAR ADAPTATION TO ONE MONTH HEAD-DOWN BEDREST

D.RIVIERE *, F.CRAMPES *, A.PERE *, M.BEAUVILLE **, A.GUELL * and M.GARRIGUES

* Service d'Exploration de la Fonction Respiratoire et de Médecine du Sport - HOPITAL PURPAN - 31059 TOULOUSE CEDEX FRANCE

** C.N.E.S. - 31055 TOULOUSE CEDEX

In the aim of suppressing or minimizing the cardio-vascular disadaptation observed when subjects return to normal gravity after exposure to weightlessness, some countermeasures have been applied during spaceflights (3,5). Among these, lower body negative pressure (LBNP), which leads to a distribution of blood volume similar to that observed in normal gravity, has been suggested (1). In this paper, we study the effects of daily LBNP sessions on the cardiovascular system during weightlessness simulated by 30 days of anti-orthostatic bedrest.

MATERIAL AND METHODS

Two bedrest studies were performed with the same subjects, the first in November-December 1987 and the second in September-October 1988.
After a 7-day ambulatory period, subjects were submitted to 30 days of anti-orthostatic bedrest at -6° head-down. This was followed by a 5-day recovery period.
- Subjects: 5 healthy male volunteers took part in this experiment, with the National Ethics Committe's agreement. They were subjected to LBNP sessions (LBNP subjects) and then they served as controls (control subjects) or vice versa. The subjects' characteristics are given in table 1.

Table 1 : Descriptive statistics for subjects

SUBJECTS	AGE	HEIGHT	WEIGHT
	yr	cm	kg
CONTROL.....	33.0	180	71.2
	+/-	+/-	+/-
	3.1	6	2.5
LBNP.....	32.8	180	71.7
	+/-	+/-	+/-
	3.0	6	2.4

Values are means +/- SE ; no significant differences

- LBNP procedures : three 20-minute sessions per day at -35 mb for the first three weeks, followed by 4 sessions a day for the first 3 days of the last week and by 6 daily sessions over the last 4 days.

- Cardiovascular adaptation measurements :
To investigate cardiovascular adaptation, we measured cardiac output and heart rate. The cardiac output was estimated by carbon dioxide (CO_2) rebreathing (8) (Infrared absorption analyser Jaeger). For each measurement, the result was the average of 4 successive measurements. The heart rate was continuously mesured (Servomed SMS 182 Hellige). Then, the mean stroke volume was calculated by dividing cardiac output by heart rate.
These 3 parameters were determined before bedrest (Day -5) during bedrest (Days 1, 7, 21 and 28) and after bedrest at recovery + 5. Before and after bedrest, cardiac output was measured in the supine horizontal position. During the bedrest period, the measurements were made between 2.5 and 3.5 hours after the last LBNP session.
- Statistical procedures : results are expressed as means +/- SE. Variance analysis was used to evaluate the significance of differences. We used a one-factor model for comparisons between the two groups of people at a given day and a block method without repetition (2-factor model) for testing the evolution in each group. We used the Student T test upon differences.

RESULTS

- Cardiac output (Fig.1) :
In controls, there was a decrease in cardiac output, which was maximum on day 21 (-19 %). Then cardiac output increased, but five days after the subjects had got up, it remained lower than the basal value. However, these modifications were not statistically significant. In LBNP subjects, we observed, on the first day, a significant increase ($p < 0.05$) in cardiac output (+29 %). Then, cardiac output decreased, but during the whole bedrest period it remained above the basal value, but not significantly. Five days after the subjects had got up, cardiac output was the same as that observed before the bedrest period.
Before the bedrest period, cardiac output was identical in control and LBNP subjects. On day 1, there was a significant difference ($p < 0.05$) between the two groups, but for all the following days, there were no significant differences.
- Heart rate (Fig. 1) :
The evolution of the heart rate during bedrest was quite similar in control and LBNP subjects, with a decrease in the first seven days (significant in controls, $p < 0.05$), and then an increase and a return to the basal value at the end of bedrest period. Five days after the subjects had got up, the heart rate was above that observed before bedrest and the difference was significant in control subjects ($p < 0.01$). There was no significant difference between the two groups for any given day.
- Stroke volume (Fig.2) :
In both groups, the changes which occurred in mean stroke volume were quite similar to changes in cardiac output ; however, in controls, at day 21 and at recovery + 5, mean stroke volume was significantly lower than the basal value ($p < 0.05$ and $p < 0.01$, respectively). There was no significant difference between the two groups for any given day.

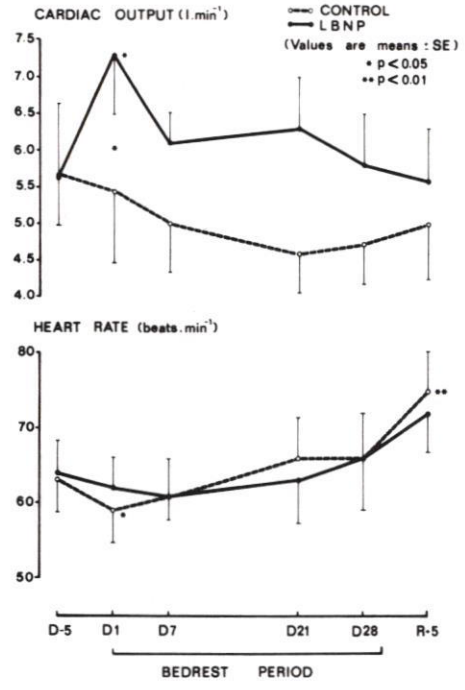


Fig.1

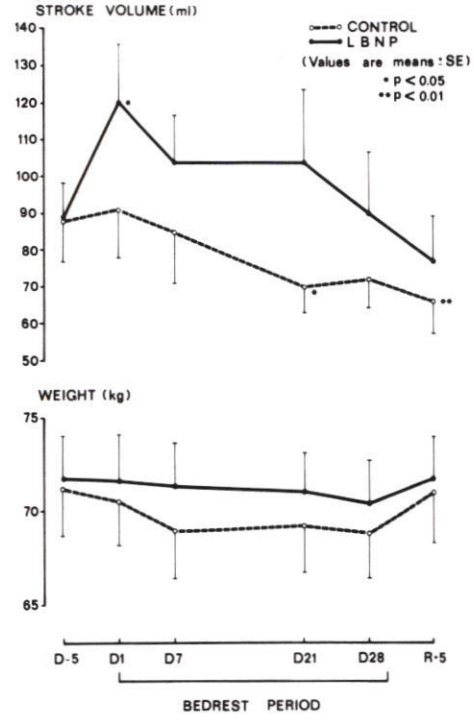


Fig.2

DISCUSSION

From our results, we can say that without LBNP there was a tendency for cardiac output to decrease while with LBNP cardiac output was increased or at least maintained.

Without LBNP, a similar evolution of cardiac output during head-down bedrest or during weightlessness has already been described in previous papers (2,9). Exposure to actual and/or simulated weightlessness causes fluid shifts from lower to upper portions of the body. This triggers a complex set of cardiovascular and systemic adaptations which lead mainly to hypovolemia (6). This is responsable for the orthostatic intolerance observed after returning to normal gravity. From our results, we can hypothesize that LBNP sessions during bedrest, which periodically cause fluid shifts from upper to lower portions of the body, could prevent or counteract some of these adaptations. This statement is in agreement with results obtained during 8-day bedrest (7) and 4-week bedrest (10), which showed a higher blood volume and a better orthostatic tolerance when LBNP was applied. This last result was also observed by other authors in the present experiment (4).

In our study of these subjects, the differences observed in cardiac output changes between the two groups actually appear to be related to a difference in volemia ; in fact, there was no difference in the evolution of heart rate of the two groups of subjects and consequently, the calculated mean stroke volume showed the same changes as cardiac output. This difference in volemia was indirectly confirmed by the changes in the subjects weight (Fig.2). The control subjects lost more weight than LBNP subjects.

In conclusion, although the interpretation of our results is limited by the small number of subjects, differences seem to exist in the cardiovascular adaptation to one month head-down bedrest, with and without LBNP. They appear to be related to a difference in volemia changes and to be favourable to LBNP subjects who had a better orthostatic tolerance after returning to normal conditions.

REFERENCES

1-BAISCH F, BEIER J, GEBHARDT U, HORDINSKY JR, MORSBACH H. In :Zero-g Simulation for Ground-Based Studies in Human Physiology with Emphasis on the Cardiovascular and Body Fluid System, Paris : ESA SP-180, 1982, 43-75

2-BLOMQVIST CG, STONE HL. In : Handbook of Physiology - Section 2.The cardiovascular system. Vol. 3. Peripheral circulation and organ blood flow.SHEPHERD JT, ABBOUD FM eds, Bethesda, MD : The American Physiological Society, 1983, 1025-1063

3-GRIGORIEV AI. Aviat. Space Environ. Med. 1983 54 : 318-323

4-GUELL A, BRAAK L, MAUROUX JL, PAVY A. Communication au 39th Congress of the International Astronautical Federation, Bangalore, INDIA, October 8-15, 1988

5-HYATT KH, WEST DA. Aviat.Space Environ.Med. 1977 ; 48 : 120-124

6- JOHNSON PC, DRISCOLL RB, LEBLANCE AD. In Biomedical Results from Skylab, JOHNSTON RS, DIETLEIN LF eds, Washington, DC ; Natl Aeronaut. Spce Adm., 1977, SP-377, 235-241

7- LAMB LE, STEVENS PM. Aerospace Med. 1965 ; 36 : 1145-1151

8-MARKS C, KATCH V, ROCCHINI A, BEEKMAN R, ROSENTHAL A. Sports Medicine 1985 ; 2 : 432-446

9-SANDLER H. In : Zero-g Simulation for Ground-Based Studies in Human Physiology, with Emphasis on the Cardiovascular and Body Fluid Systems, Paris : ESA SP-180, 1982, 107-146

10-STEVENS PM, MILLER PB, GILBERT CA. Aerospace Med. 1966 ; 37 : 357-367

CARDIORESPIRATORY RESPONSES TO MAXIMAL AND SUBMAXIMAL EXERCISE IN SUPINE AND UPRIGHT POSITIONS

R. L. HUGHSON, H. XING, C. BORKHOFF AND G. C. BUTLER

Department of Kinesiology,
University of Waterloo,
Waterloo, Canada

An immediate effect of space travel is the removal of the normal head-to-foot gravitational force on the cardiovascular system. The supine posture on earth also removes the head-to-foot gravity vector and affects the cardiovascular response to exercise (1,4). The purpose of this study was to examine the cardiorespiratory responses to maximal and submaximal exercise in both the upright and supine postures. Maximal exercise testing has been a standard employed on earth to characterize the function of the cardiorespiratory system (2). However, these are not always reliable for repeated testing of fitness (14). Submaximal testing has been observed to be sensitive to acute alterations in function with beta-blockade (8) or hypoxia (13). The most common approach to submaximal testing has been with step transitions in work rate; but several repetitions are needed to extract the signal from the noise (12). A new approach to submaximal testing is with the pseudorandom binary sequence (PRBS) (11,15). Within a single test of 30 min or less duration several repetitions of identical sequences can be completed and the signal can be extracted from the noise.

Methods:

Nine healthy male volunteers (age = 22±1, mean ±S.E) were tested in three exercise protocols on an electrically braked cycle ergometer in both upright and supine positions: (i) incremental exercise with 4 min at 25W, then work rate (WR) increased as a ramp at 20W/min until exhaustion; (ii) following a 4 min baseline period at 25W, WR increased as a step function to 105 W for 6 min, 6 repetitions were made; (iii) a PRBS test with each sequence having 63 units, each of 5 s duration, for a total period of 315s with WR set at either 25 or 105W by a subroutine of the breath-by-breath program. A total of 6 identical sequences were performed as a continuous test; the first was treated as warm up and the remaining 5 were analyzed.

Oxygen uptake ($\dot{V}O_2$), ventilation and heart rate (HR) were determined breath-by-breath with a computer program (First Breath Inc. St.Agatha, Ontario, Canada) as described previously (10). In both step and PRBS tests, each breath's value for VO_2 was linearly interpolated to the next to obtain time series data at 1s intervals. Step data from 6 repetitions and PRBS data were ensemble averaged to yield a single data set. Step data were fit by a two component exponential model (10) to estimate the time taken to achieve 63% of the final response; this was called the total lag time (TLT) (16). PRBS test data were subjected to autocorrelation of the WR and cross-correlation of $\dot{V}O_2$ and WR (15). These data were fit by a model adapted from the step test to account for the shape of the autocorrelation function (9) to yield a TLT estimate. Comparisons between TLT estimates were by repeated measures analysis of variance. Simple upright vs. supine comparisons were by paired t-tests.

Results:

Each of maximal WR, $\dot{V}O_2$ and HR were reduced in the supine compared to the upright posture (317±17 vs 278±16W; 3676±201 vs 3295±194 ml · min^{-1};198±2 vs 185±3 beats · min^{-1}, $p<0.01$). The TLT was greater during step transitions in supine (42.0±3.1s) than upright exercise (31.7± 1.3s, $p<0.05$). Oxygen deficit was greater (531±120 vs 403±56 ml O_2; $p<0.05$). Estimates of TLT from PRBS testing also showed slower responses in supine than upright positions (40.1±2.7 vs 32.0±1.2 s, $p<0.05$). The TLT estimates from step and PRBS tests were highly correlated ($r = 0.99$).

Discussion:

Acute changes in posture from upright to supine positions resulted in impairment of the cardiorespiratory responses to both maximal and submaximal exercise. The new findings of the present study were the ability of the PRBS exercise test to detect this impairment and also the high correlation between kinetics determined by submaximal step and PRBS exercise tests.

Previously several investigators had provided evidence of slower adaptation of $\dot{V}O_2$ following step increases in work rate in the supine position (3,4). The findings of this study confirm these results. The 30% longer estimate of TLT in supine than in upright exercise translated into a 128 ml greater oxygen deficit. Because the energy to perform work during this deficit period did not come from atmospheric O_2, it had to come from some or all of O_2 stores, high energy phosphocreatine stores or anaerobic glycolysis with lactate production (5,8). When the lactate production rate is increased, it can result in metabolic acidosis and is potentially an inefficient use of intramuscular glycogen stores. The implication for repeated bouts of work in a space environment might be a more rapid onset of fatigue.

The PRBS exercise tests have the advantage shown by the present study to be able to extract information about $\dot{V}O_2$ kinetics from a single test session, as opposed to the 6 or more repeats often used for the step test (10,12). We have reported only time domain analysis, although frequency domain analysis can also be performed (11,15). Because the estimate of TLT obtained by PRBS testing was slowed in the supine posture by the same amount as it was during step changes in work rate, it can be assumed that the oxygen deficit was also greater. The same mechanisms described above can be assumed to operate to supply energy to account for the deficit.

Maximal exercise testing has been widely used to assess cardiorespiratory function. Clearly in the present study, maximal performance was impaired by the supine posture. Although $\dot{V}O_2$ max is often reported to have a high test-retest reproducibility, it should be noted that when 3 tests were obtained over a one month period, the second test results were lower than the first and third (14). This suggested that the subjects might have been hesitant to provide maximal efforts on all occasions. Therefore, we would not recommend maximal testing on a repeated basis to follow changes in fitness with space travel.

The major mechanism responsible for impaired cardiorespiratory function in the supine posture appears to be the loss of hydrostatic contribution to arterial perfusion pressure. Recently, Eiken (6) has shown that application of negative pressure to the lower body during supine exercise restored leg blood flow and improved exercise performance to near upright levels. In the O-g environment, a similar loss of driving pressure can be expected. This might be confounded by the reduction in blood volume normally associated with prolonged O-g exposure (7).

In summary, the PRBS exercise test provides a sensitive method to quickly detect altered cardiorespiratory function that occurs with loss of the normal head-to-foot gravity vector. Because this is a submaximal test, it can be applied repeatedly with minimal imposition to the subjects and it is not influenced by the effort of the subject. It is possible to conceive of a scheme to monitor fitness and prescribe exercise training based on these test results.

Acknowledgements: This work was supported by the Natural Science and Engineering Research Council of Canada.

References

1. BLOMQVIST, C.G. Cardiovascular adaptation to weightlessness. *Med. Sci. Sports Exerc.* 15: 428-431, 1983.

2. BRUCE, R.A., T.A. DEROUEN, and K.F. HOSSACK. Value of maximal exercise tests in risk assessment of primary coronary heart disease events in healthy men. *Am. J. Cardiol.* 46: 371-379, 1980.

3. CERRETELLI, P., D. PENDERGAST, W.C. PAGANELLI, and D.W. RENNIE. Effects of specific muscle training on VO_2 on-reponse and early blood lactate. *J. Appl. Physiol.* 47: 761-769, 1979.

4. CONVERTINO, V.A., D.J. GOLDWATER, and H. SANDLER. Oxygen uptake kinetics of constant-load work: upright vs. supine exercise. *Aviat. Space Environ. Med.* 55: 501-506, 1984.

5. DI PRAMPERO, P.E. Energetics of muscular exercise. *Rev. Physiol. Biochem. Pharmacol.* 89: 143-222, 1981.

6. EIKEN, O. Effects of increased muscle perfusion pressure on responses to dynamic leg exercise in man. *Eur. J. Appl. Physiol.* 57: 772-776, 1988.

7. GHARIB, C., M. MAURICE, G. GEELEN, G. GAUQUELIN, M. VINCENT, J.M. POTTIER, H. MEHIER, B. ROUSSEL, A. GUELL, and A. BES. Volume regulating hormones during weightlessness and simulated weightlessness. In: *Angiologie*, (edited by BOCCALON, H.) Paris:John Libby Eurotext, 1988, p. 657-664.

8. HUGHSON, R.L. Alterations in the oxygen deficit-oxygen debt relationships with beta-adrenergic receptor blockade in man. *J. Physiol. (London)* 349: 375-387, 1984.

9. HUGHSON, R.L. Exploring cardiorespiratory control mechanisms through gas exchange dynamics. *Med. Sci. Sports Exerc.* 21: in press, 1989.

10. HUGHSON, R.L., D.L. SHERRILL, and G.D. SWANSON. Kinetics of $\dot{V}O_2$ with impulse and step exercise in man. *J. Appl. Physiol.* 64: 451-459, 1988.

11. HUGHSON, R.L., D.A. WINTER, A.E. PATLA, G.D. SWANSON, and L.A. CUERVO. Investigation of VO2 kinetics in man with psuedorandom binary sequence work rate change. *J. Appl. Physiol.* In press: 1989.

12. LAMARRA, N., B.J. WHIPP, S.A. WARD, and K. WASSERMAN. Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *J. Appl. Physiol.* 62: 2003-2012, 1987.

13. MURPHY, P.C., L.A. CUERVO, and R.L. HUGHSON. Comparison of ramp and step exercise protocols during hypoxic exercise in man. *Cardiovascular Research* submitted: 1989.

14. SMYTH, G.A., R.L. HUGHSON, D.J. WALTERS, and D.A. RANNEY. Short- versus long-term effects of oral beta blockers on exercise performance. *J. Cardiac Rehab.* 4: 227-237, 1984.

15. STEGEMANN, J., D. ESSFELD, and U. HOFFMAN. Effects of a 7-day head-down tilt (-6°) on the dynamics of oxygen uptake and heart rate adjustment in upright exercise. *Aviat. Space Environ. Med.* 56: 410-414, 1985.

16. SWANSON, G.D., and R.L. HUGHSON. On the modeling and interpretation of oxygen uptake kinetics from ramp work rate tests. *J. Appl. Physiol.* 65: 2453-2458, 1988.

BAROREFLEX RESPONSES AND LBNP TOLERANCE FOLLOWING EXERCISE TRAINING

V.A. Convertino, C.A.Thompson, D.L. Eckberg, J.M. Fritsch, G.W. Mack, and E.R. Nadel

National Aeronautics and Space Administration, Biomedical Operations and Research Office, and The Bionetics Corporation, Kennedy Space Center, FL 32899; VA Medical Center and Medical College of Virginia, Richmond, VA 23249; J.B. Pierce Foundation Laboratory, New Haven, CT 06519

INTRODUCTION

About 15 years ago, Stegemann et al. [5] and Luft et al. [3] made independent reports of lower orthostatic tolerance in athletes trained for endurance events compared to sedentary subjects. The results from these studies and a review written by Klein and co-workers [2] raised concern about the issue of whether astronauts and high-performance aircraft pilots could compromise the function of their blood pressure control by engaging in regular exercise which enhances aerobic capacity. During the next 12 years, some investigators have suggested that lower responsiveness of high- or low-pressure baroreflexes, greater limb compliance, and greater vagal tone reported in endurance-trained athletes may contribute to the reduced effectiveness of their blood pressure control system [1]. However, other investigators have failed to find evidence to support this hypothesis [1]. Most of these studies have been cross-sectional comparisons of athletes and sedentary subjects, and were designed to examine only one or two mechanisms of the blood pressure control system. It was our purpose, therefore, to conduct a longitudinal exercise training study in which tolerance to orthostasis and several primary mechanisms associated with blood pressure control could be measured before and after the increase in aerobic capacity.

METHODS

Twenty-four healthy nonsmoking normotensive men, with a mean (±SE) age of 36 ± 1 years, a mean height of 177 ± 1 cm, and a mean weight of 80.5 ± 3.0 Kg, gave written informed consent to participate in this study, which was approved by the Kennedy Space Center Human Research Review Board. All subjects were relatively inactive at the time of the study as indicated by a mean maximal oxygen uptake (VO_2max) of 40.8 ± 0.9 ml/(kg·min) and never had participated in any formal endurance training program. Following selection, subjects were randomly assigned to either an exercise group (N = 16) or a control group (N = 8); the groups were matched for age, height, weight and VO_2max.

Prior to initiation of the experimental protocol, all subjects underwent a 2-week orientation period in which they were familiarized with test protocols and sequences. Tests were conducted at the same time of day and in the same sequence pre- and post-training. All subjects stated that they abstained from autonomic stimulants such as tobacco and caffeine and did not exercise for 24 hours prior to each testing period.

Following pre-training testing, control subjects were asked to maintain normal activities and refrain from participation in formal exercise programs for 10 weeks. The exercise subjects, however, underwent a 10-week endurance exercise training program consisting of cycle ergometry in the upright posture for 30 mins/day, four days/week at a work intensity of approximately 70-80% VO_2max. Heart rates (HR) and blood pressures (BP) were taken every 10 mins of each training period to assure attainment of the targeted work intensity. Every second week of training, the same absolute workrate was performed for 10 min and VO_2, as well as HR and BP, were measured as indices of training effects. Training workloads were then adjusted to maintain a work intensity of 70-80% for subsequent training sessions.

Pre- and post-training measurements included VO_2max, blood volume, the stimulus-response relation of the carotid-cardiac and cardiopulmonary baroreflexes, calf compliance, hemodynamic responses to lower body negative pressure (LBNP), and LBNP tolerance. VO_2max was measured using a graded workrate protocol on a Quinton electronically-braked cycle ergometer. The protocol allowed for the determination of VO_2max by demonstrating no change in VO_2 despite an increase in workrate. Plasma volume was measured by an Evans blue dye technique and blood volume was calculated from plasma volume and hematocrit. The response relation of the carotid-cardiac baroreceptor was determined with the Eckberg technique using a complex sequence of rapid pressure changes delivered to the carotid area by a neck chamber device and measuring beat-to-beat R-R interval changes. The stimulus-response relation for reflex control of vascular resistance was measured by selective unloading of cardiopulmonary baroreceptors using LBNP levels of -5 to -20 mmHg. The slope of the linear relationship between peripheral venous pressure (PVP) and forearm vascular resistance (FVR) was used to characterize the gain of the baroreflex control of FVR.

The protocol to determine LBNP tolerance began with a 5-min resting period followed by a 3-min exposure to -20 mmHg and then continuous decompression of 10-mmHg increments each 3 min until test termination. LBNP tolerance was determined by any one or combination of the following criteria: (a) completion of 3 min at -100 mmHg; (b) onset of presyncopal symptoms such as a precipitous fall in systolic blood pressure (SBP) greater than 15 mmHg and/or a sudden bradycardia greater than 15 bpm between adjacent 1-min measurements; (c) progressive diminution in SBP below 80 mmHg; and (d) voluntary subject termination due to discomfort such as nausea or dizziness. Stroke volume (SV, impedance plethysmography), HR, arterial BP (sphygmomanometry), and leg circumference (strain gauge plethysmography) were measured during the LBNP tolerance test.

RESULTS

VO_2max increased by 20% ($P < 0.05$) from 2.97 ± .11 to 3.55 ± .11 L/min, resting HR decreased ($P < 0.05$) from 63 ± 3 to 57 ± 2 bpm, standard deviation of the R-R intervals was increased ($P < 0.05$) from 59.4 ± 8.2 to 74.0 ± 8.2 msec, and blood volume increased ($P < 0.05$) from 63.6 ± 2.1 to 69.3 ± 2.8 ml/kg in the exercise group after 10 weeks of endurance training. VO_2max, blood volume, resting HR, and R-R interval standard deviation were unaltered over the 10-week period of normal activity for the control group.

The response relation of the carotid-cardiac baroreflex shifted significantly on the R-R interval axis, but did not shift on the pressure axis in the exercise subjects (Fig. 1). There were no changes in maximum slope, carotid distending pressure (CDP) at minimum R-R interval and maximum R-R interval, and range of R-R interval response. There were no changes in the carotid-cardiac response relation in the control subjects.

The gain of the baroreflex control of FVR was significantly reduced in the exercise group from -5.72 ± 0.71 to -4.15 ± 0.51 PRU/mmHg ($P < 0.05$) in response to endurance training. The mean (±SE) responses of PVP/FVR stimulus-response relations before and after training in the exercise group are illustrated in Figure 2. The change in baroreflex gain following exercise training was linearly related to the change in total blood volume ($r = 0.65$, $P < 0.05$), but not VO_2max. The gain

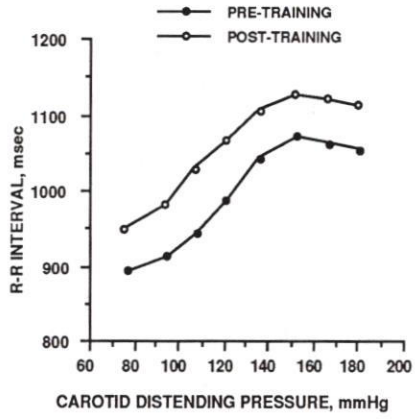


FIG. 1: Mean carotid-cardiac baroreflex response relations of exercise subjects before and after training.

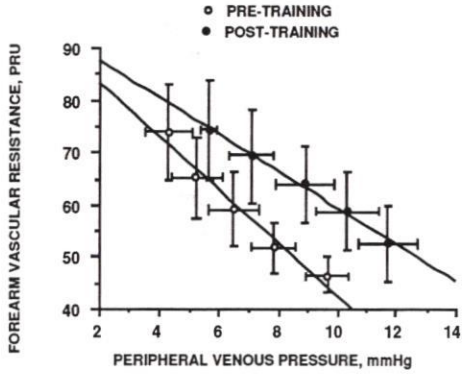


FIG. 2: Mean (±SE) stimulus-response relations of the cardiopulmonary baroreflex of exercise subjects before and after training.

of this baroreflex in the control group at pre-training (-6.10 ± 0.57) was unchanged after 10 weeks of normal activity (-6.22 ± 0.94).

Calf compliance was reduced by 20% ($P < 0.05$) in the exercise group from 4.27 ± 0.24 to 3.41 ± 0.20 ml/mmHg following endurance training. Calf compliance was unchanged from pre-training (4.41 ± 0.39) to post-training (4.41 ± 0.49) in the control subjects.

LBNP tolerance was significantly increased from 747 ± 61 to 958 ± 88 torr-min following 10 weeks of exercise training ($P < 0.05$). No change in LBNP tolerance was observed in the control group and averaged 838 ± 70 and 785 ± 84 torr-min before and after 10 weeks of normal activity, respectively. Changes in calf circumference, HR, SV, BP, cardiac output and systemic resistance at the point of LBNP termination were similar before and after training in both exercise and control groups. However, HR and SPR were lower and SV, CO, and BP were higher at subtolerance levels of LBNP after training.

DISCUSSION

In this study, we re-examined the hypothesis that endurance exercise training designed to significantly increase aerobic capacity results in alterations of blood pressure control mechanisms associated with reduced orthostatic tolerance. We specifically selected a population of subjects whose age and fitness relate to those of astronauts. Using our specific exercise training, our results provide evidence that VO2max can be significantly elevated in populations of average fit individuals without reducing LBNP tolerance.

Our exercise training regimen caused a resting bradycardia, which may have been mediated by increased cardiac vagal tone as indicated by increased HR variability at rest. However, these changes had no effect on the cardiac vagal reflex response as indicated by unaltered gain (maximum slope) and minimum to maximum R-R interval difference of the carotid-cardiac baroreflex relation. Finally, exercise training in this study resulted in attenuated gain of baroreflex control of FVR. No change in these variables was observed in the control subjects, indicating that changes in autonomic function observed in this study were the result of training per se. Our results regarding these autonomic responses after exercise training are nearly identical the those reported by Seals and Chase [4].

We are unaware of any previous data similar to ours that demonstrate a significant increase in LBNP tolerance with endurance training. Our results may result from the specific mode of exercise since reduced episodes of fainting are associated with training programs using cycling rather than running [1]. Further, increased LBNP tolerance is related to expanded blood volume and lower limb compliance [1,3]. Therefore, the 9% increase in blood volume and 20% reduction in calf compliance observed in our exercise subjects may represent the primary adaptations associated with the increase in LBNP tolerance following our training protocol.

Our results cannot exclude the possibility that endurance exercise training designed to increase maximal oxygen uptake to athletic levels, i.e., greater than 60 ml/(kg·min), may reduce orthostatic responses. We have however identified an exercise training regimen which can increase aerobic capacity and LBNP tolerance without compromising baroreflex mechanisms associated with blood pressure control. Operationally, our data suggest that astronauts and pilots with average maximal oxygen uptakes can undertake regular exercise which can increase their aerobic capacity to moderate levels without significant concern about reducing their orthostatic tolerance.

REFERENCES

1. Convertino, V.A. Aerobic fitness, endurance training, and orthostatic intolerance. *Exer. Sports Sci. Rev.* 15:223-259, 1987.

2. Klein, K.E., H.M. Wegmann, and P. Kuklinski. Athletic endurance training - advantage for spaceflight? The significance of physical fitness for selection and training of Spacelab crews. *Aviat. Space Environ. Med.* 48:215-222, 1977.

3. Luft, U.C., L.G. Myhre, J.A. Loeppky, and M.D. Venters. A study of factors affecting tolerance of gravitational stress simulated by lower body negative pressure. *Research Report on Specialized Physiology Studies in Support of Manned Space Flight.* NASA Contract NAS 9-14472. Albuquerque, NM:Lovelace Foundation, 1976, pp.1-60.

4. Seals, D.R., and P.B. Chase. Influence of physical training on heart rate variability and baroreflex circulatory control. *J. Appl. Physiol.* 66:1886-1895, 1989.

5. Stegemann, J., U. Meier, W. Skipka, W. Hartlieb, B. Hemmer, and U. Tibes. Effects of multi-hour immersion with intermittent exercise on urinary excretion and tilt table tolerance in athletes and nonathletes. *Aviat. Space Environ. Med.* 46:26-29, 1975.

ORTHOSYMPATHETIC ACTIVITY DURING HEAD-OUT WATER IMMERSION AND DURING FLOATING

J. Lecomte, G.V. Lecomte and D. Lagneaux

Institut Léon Fredericq, Department of Physiology, University of Liège, 17 Place Delcour, B - 4020 - Liège

During vertical immersion in thermoneutral water, the orthosympathetic activity is lowered as soon as the human subject is immersed. A reduction in the urinary excretion of noradrenaline (NA) is one of the proofs of this hypoactivity, while adrenaline (A) excretion is slightly increased (1). Urinary excretion of A depends mostly on the suprarenal medullary secretion and is specially in relation with mental stress. NA excretion is related to the overflow of the neuromediator at the heart and blood vessels postganglionic junctions. So, during immersion, it can be inferred that sympathetic efferences to the cardiovascular system are diminished. In fact, total peripheral resistances are reduced by about 30% and the heart rate is slowed down (2).

Depending on the density of the bathing medium, the human body can be immersed (low density medium) or left floating on the surface of the bath (high hypertonic medium). We have compared the orthosympathetic activity level in both conditions: floating on a hypertonic medium versus head-out immersion, in thermoneutral tap-water.

METHODS

Ten normal subjects (19-21 years old), in good physical conditions, previously trained to relaxation, to immersion and to floating, were submitted, in random order, two different days at 10-11 am to one of the following procedures.

After a first miction whose urine was discarded, an equivalent volume of water was ingested. The subjects were left in a near-horizontal position during one hour, relaxed, head and chest supported (30°). After this control period, the urine was collected and stored for further analysis. Again, an equivalent water volume was ingested. Then, head-out immersion or floating were performed.

In the first case, the subjects were introduced up to the neck into a tap-water bath, at 34.2 °C, and maintained during 30 min in the same near-horizontal position as in air with the right arm positioned rest at the surface of the water for arterial blood pressure and heart rate measurements.

In the second one, they were laid down during a same time, on the surface of a hypertonic aqueous solution of magnesium sulphate (300 gr/l).

In both cases, at the end of the test, urines were collected and stored. Catecholamines were measured in triplicate by a fluorimetric method (3). Their elimination has been expressed in relation with creatinine excretion taken as an index of the glomerular filtration rate. Heart rate and brachial systemic pressure were measured during all the procedures by non invasive methods.

RESULTS

Concerning diuresis, natriuresis and creatinuria, no consistent differences were found between figures obtained during near-horizontal position in air and floating on the hypertonic solution. Moreover, there were no changes in the cardiovascular data. On the contrary, during immersion, diuresis, natriuresis and creatinuria were statistically increased (respectively 169±11%, 51±13% and 27±5%). Systemic blood pressure was significantly increased (12±3 mmHg); heart rate was slowed (-3±1 b/min).

As can be seen in Fig.1, NA excretion was significantly reduced during immersion but not during floating. Urinary excretion of A remained unchanged in both procedures.

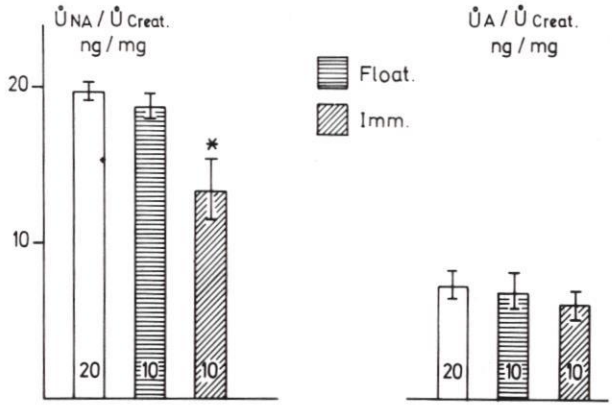


Fig.1.- Urinary excretion of free noradrenaline (NA) and adrenaline (A) during the 30 min immersion (Imm.) and floating (Float.) periods, compared to the control one. Values in ordinate are expressed in ng NA or A/mg creatinine excreted in the same period. Differences between immersion on one side, floating or control on the other side, are significant for NA (*). No changes were found during floating compared to control.

During floating as well as during immersion, a sensation of relaxation has been described by the subjects.

DISCUSSION

In order to appreciate the orthosympathetic activity level during the whole period of the test, we have decided to measure the urinary excretion of catecholamines rather than to do punctual plasmatic determinations. In such a way, we got a better approach of the mean global orthosympathetic control during a long time. In preliminary assays conducted with horizontal or vertical head-out immersion, we have established that NA excretion was reduced since the first 15 min from the start of immersion for all that thermal neutrality was strictly observed (4) and that the subject has been accustomed to balneation avoiding all mental stress.

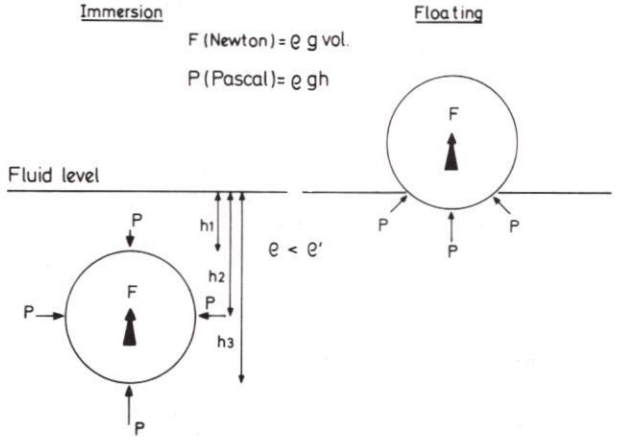


Fig.2.- Schematic illustration comparing forces (F) and hydrostatic pressures (P) acting on the body mass during immersion and during floating. h (1,2 and 3) are the distances between the fluid free level and the P application points on the body during immersion. P application points are reduced during floating on a hypertonic medium ($\varrho' > \rho$). For explanations, see text.

Floating differs thus from immersion by the absence of any modification in the orthosympathetic control of cardiovascular system. In both situations (Fig.2), the hydrostatic pressure (P) is operating, but in different ways. Due to the high density of the medium, during floating, this pressure is acting only on a limited area of the body. Contact with the hypertonic medium is restricted, Archimedes'forces (F) being sufficient to maintain most of the body mass above the free level of the medium. Deep immersion is avoided. On the contrary, in the water bath, the body is immersed and the hydrostatic pressure operates on the whole body surface. This external compression produces the well-known central shift of fluids, with a redistribution of the blood volume into the thoracic reservoirs (5). The repletion of the cardiac chambers increases the ventricular filling pressures, setting up a Frank-Starling mechanism. Systemic blood pressure increases with a larger solicitation of the carotid baroreflexes conducting to a decrease of the sympathetic efferents to the vessels and to a reduction of the heart rate. Bradycardia may also be facilitated by the stretching of the diffuse veno-atrial receptors (6).

In conclusion, during immersion, orthosympathetic control of the systemic circulation is diminished, due to the action of hydrostatic pressure. The crucial modification acting as a stimulus is the importance of the blood volume shift to the cardiac chambers. When immersion is avoided as during floating on a hypertonic medium, such a variation is absent and sympathetic activity remains the same as in clinostatism.

REFERENCES

1. Goodall, McC., McCally, M. & Graveline, D.E.,1964, Am.J.Physiol., 206, 431-436.

2. Arborelius, M.Jr., Ballidin, U.I., Lilja, B & Lundgren, C.E.G., 1972, Aerosp.Med., 43, 592-598.

3. Anton, A.H. & Sayre, D.F., 1962, J.Pharm.exp.Therap., 138, 360-375.

4. Johnson, D.G., Hayward, J.S., Jacobs, T.P., Collis, M.L., Eckerson, J.D.& Williams, R.H., 1977, J.Appl.Physiol., 43, 216-220.

5. Gauer, O., 1975, Acta Astron., 2, 31-39.

6. Blomqvist, C.G. & Stone, H.L., 1983, Handbook of Physiology, Sect.2: Circulation, Vol III, Part 2: 1025-1063. Am.Physiol.Soc., Bethesda, Maryland.

CARDIOVASCULAR RESPONSES TO MODERATE EXERCISE DURING WATER IMMERSION IN WOMAN

J. NAGANO+, S. TORIKOSHI++, K. YOKOZAWA++, K. ITOH+++, Y. FUKASE++++, andY. SUZUKI+++++

+; Lab. of Physical Education, Bunka Woman's Univ., 3-22-1, Yoyogi,Shibuya-ku, Tokyo, Japan 151, ++; Tokyo Woman's Christian Univ., +++; Turumi Univ., ++++; Rikkyo St. Margurite Woman's College, +++++; Lab.of Health Adoministration, Faculty of Medicine, Univ.of Tokyo,Hongo,Bunkyo-ku,Tokyo,Japan113

When a moderate upright exercise was perfo-rmed for 20 minutes in the two conditions of axilla level water immersion (WI) and air, despite of the same mechanical work load, oxygsn uptake during exercise was significa-ntly lower in the WI than in air ($P<0.01$). The lowered oxygen uptake during exrcise in the water should be due to the reduction of pedalling resistance while rotating water according to the pedalling movement. Also, keeping posture and lifting legs weight should make to decrease recluiting active muscle mass. Cardiovascular responses to the exercise were significsntly corresponded to oxygen uptake and exercise intensity identified as $\%VO_2max$ except systolic arte-rial pressuer. Under the weightlessness simulated by water immersion, the activating muscle mass should be decreased in a moder-ate upright dynamic exercise. So that oxygen uptake in WI is decreased. Cardiovascular responses in WI are at least decreased like-ly to corresponded to the decreased oxygen uptake and thus to the lowered exercise intensity.

As immersed in water (water immertion:WI) to level of diaphragm or axilla, central venous pressure and blood volume distribution stimulate these in supine posture, which is almost the same as weightlessness situation. Further cardiovascular responses to conbined stimulaion of exercise and weightlessness. In the present study, using a bicycle ergo-meter settled in water bath, cardiovascular responses to moderate dynamic exercise in upright posture were compared in two conditions of axilla level WI and air under atmospheric pressure to dynamic exercise under weightlessness.

Subjects and Methods

Five normative female students of average 21 years old (19-25yrs.) participated as the subjects whose averaged body weight and hig-ht were 55.2(±7.7)kg and 160.4(±9.4)cm, respectivery. The risks of the atudy were descrived and informed content obtained. Before the study the maximum oxygfn uptake (VO_2max) in each subjects was examined by exahaustive treadmill running of 6min. to 12 min. and the average VO_2max of the subjects was 42.0(±5.4)ml/kg/min.

In WI experiment, the bath settled an ergo-meter was filled up by 33℃ water until the axilla level of each subjects. Following one hour sitting on the ergometer in the water, a 320kpm/min load pedalling was performed for 20 minutes. Through the the experimrnt, heart rate (HR) was counted depending on the EKG records by computer, arterial pressure (AP) was measured on the left upper arm by means of automatic blood pressure meter, forearm blood flow (FBF) was measured on the right arm by rubber strainguage pletysmogra-phy method. To determine oxygeu uptake (VO_2) expired gas collected by mean of Douglus bag method and % of CO_2 and O_2 were analyzed by mass-spectrometer. Cardiac out put (CO) was measured by acetylene gas rebreathing techn-ique. In the air experiment, following 15min sitting rest on the saddle in the bath without water, the same load exercise was ailso performed for 20min. in 27℃ room tem-perature, which should make about 33℃ of mean skin temperature during exercise as de-pending on the results given in the pre-experiment. All of the measurements were the same as these in WI experiment. Each of mea-surements was carried out around every ten minutes during one hour rest in WI, and from 10th min. to 15th min. during 15min. rest in air. During exercise in the both experiments HR, AP, and FBF were measured every minutes, and CO was done at 10th min. and 20th. Expired gas for VO_2 determination was collec-ted for two minuts period from 4th min, 6th min, 14th min, and 16th min during exercise.

Results

Following sharply increase for inicial 3 minutes, HR responses during exercise shift-ed to gradually increasing tendency in the both conditions of experiments. However, HR averaged in each experiment was significant-ly lower at every measured point except it at the first minute in WI than in air ($P<0.05$ or <0.01). VO_2 at 60th min during WI rest was significantly lower than it at 15th min during rest in air ($P<0.05$). VO_2 reached steady state during exercise was about 58% in WI of it in air ($P<0.01$). Systolic arter-ial pressure (SAP) at 60th min during WI rest was littlc bit higher but not signific-ant as compared with it at 15th min during rest in air. During exercise, SAP was not different between the both experiments. Rate pressure products(RPP=SAP×HR) which suggests the cardiac work significantly lower during exercise in WI than in air ($P<0.01$).

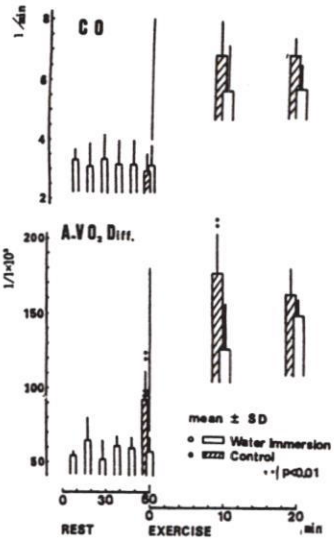


Fig.1 The time couses of CO and A-VO_2Diff. averaged in all subjects.

Fig.1 shows the averaged time courses of CO and systemic arterio-venous oxygen difference(A-VO_2 diff.). In the upper part of the figure, CO during exercise in WI as about 15% lower of it in air but not significant. In the down part of the figure, A-VO_2 diff. given at 60th min during WI rest was significantly lower than it at 15th min during rest in air. During exercise, A-VO_2 diff. steped up in WI but steped down in air from the level given at 10th min in WI than in air ($P<0.05$) but not different at 20th min.

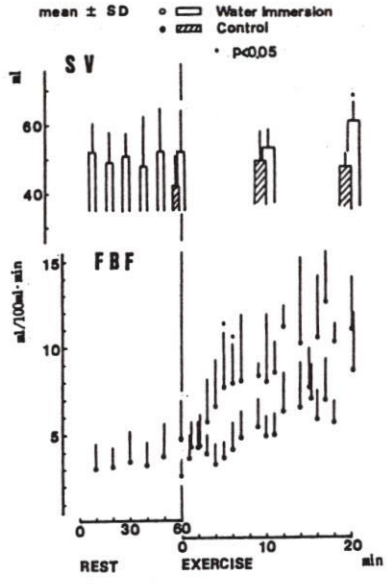


Fig.2 The time courses of SV and FBF averaged in all subjects

As shown in upper part of Fig.2 averaged stroke volume (SV) given 60th min during WI rest was about 25% higher than it at 15th min of rest in air, but there was not significantly different. During exercise, SV was almost same at 10th min between the two experiments. Then, it increased at 20th min between the two conditions ($P<0.05$).
In down part of the figure, FBF given at 60th min during WI rest was about 45% higher than it at 15th min during rest in air, but period of exercise, there were significantly different between the two conditions of WI and air. However, during later period of exercise, the significance was not shown.

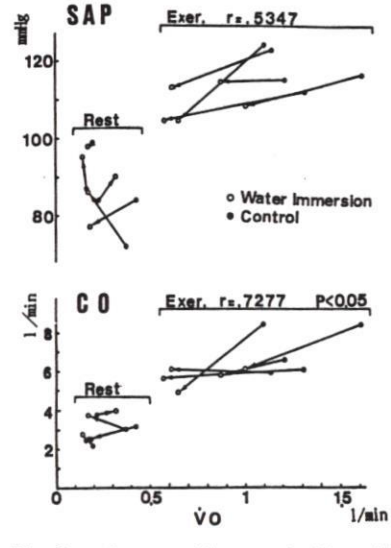


Fig.3 The relationships VO_2 and SAP and also VO_2 and CO during rest and exercise.

Fig.3 shows the relationships between VO_2 and during exercise. Allow marks in the figure indicate the changing situation from the values given in WI to these in air. During exercise, VO_2 was correlated to CO ($P<0.05$) but not to SAP.

Fig.4 shows the relationships between work intensity (%VO_2max) and RPP and HR using all of individual mean values likely to these in Fig.3. During exercise HR and RPP were significantly correlated to %VO_2max ($P<0.05$).

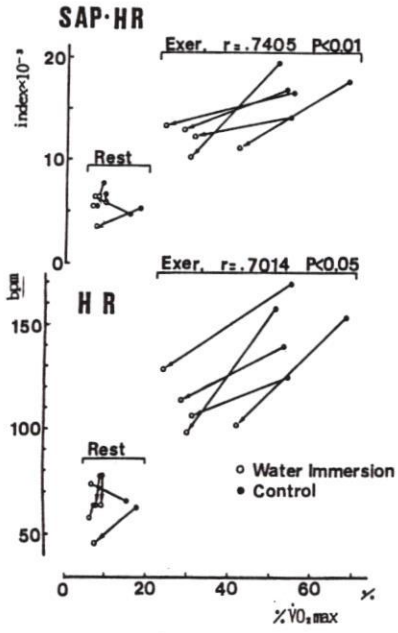


Fig.4 The relationships between %VO_2-max and SAP× HR and also %VO_2max and HR.

Discussion

In the present study, when a moderate upright pedalling exercise (55%VO_2max) was performed for 20 minutes in axilla level WI and in air in female students, despite of the same mechanical work lord (320kpm/min) VO_2 during exercise was significantly lower in WI than in air ($P<0.01$). HR, CO, and RPP during exercise were also significantly lower in WI than in air, while SAP was almost same in the two conditions. The reason why VO_2 during exrecise in WI was decreased from the level given in air should be due to the reduction of pedalling resistance while rotating water according to the pedalling movement. Also, keeping exercise posture and lifting legs weight should make to decrease recluiting active muscle mass.
The decreases in cardiovascular responses except SAP to exercise in WI should be due to the decrease in VO_2 and %VO_2max, so there were significant correlations of HR, CO and RPP to VO_2 and %VO_2max respectively, That is corresponding to work intensity should be depend on cardiac work and cardiac output.
In the other hand, the reason why SAP during exercise in WI was not lowered in spite of the decrease in not only VO_2 and/or %VO_2-max but also CO HR, and RPP cuold not explain from the presented results in this study. Probably, one of the reason is due to the functions of mechano-receptors in exercising muscles and body parts. The another is that WI to the diaphragm level shoud induce increases in central blood volume and central venous pressure, even if during exercise, suggested by the increase in SV as compared with it in air in the present study and then the increases should consequently make to increase intrathoracic blood volume expantion likely to elevate SAP.
In conclusion, because the activating muscle mass in a moderate upright dynamic exercise in WI should be decreased, VO_2 is decreased as compared with it in air. Cardiovascular responses to exercise in WI are therefore decreased like that corresponded ajustedly to the decreased oxygen transport.

FLUID/ELECTROLYTE BALANCE AND CARDIOVASCULAR RESPONSES: HEAD-DOWN TILTED RATS.

X.J. Musacchia,[1] D.R. Deavers[2] and G.A. Meininger[3]

[1]Department of Physiology and Biophysics, University of Louisville, Kentucky 40292, [2]Department of Physiology and Pharmacology, University of Osteopathic Medicine, Iowa 50312, and [3]Department of Medical Physiology, College of Medicine, Texas 77843.

INTRODUCTION

The head-down tilted (HDT) rat is widely accepted as an experimental subject to study physiological and biochemical responses characteristic of those experienced during exposure to microgravity. Cardiovascular responses and body fluid shifts, generally measured in terms of blood pressures and renal responses, respectively, are better understood in man and in much less detail in the rat. This is understandable, since measurements in man are often routinely made during flight and post flight but only under post flight conditions in the rat.

The HDT rat has been used to simulate pathophysiological changes and to predict events that may occur during flight. To date, much information concerning cardiovascular changes and fluid and electrolyte modification in rats comes from several COSMOS biosatellite experiments. However, it must be noted that the information is obtained from rats during post flight conditions and not much information is yet available from in-flight animals.

The current presentation deals with two experimental areas of interest: cardiac responses to a week of whole body suspension (WBS) with about a 20° HDT and post tilting recovery, and fluid and electrolyte balance studies during one week of HDT and one week of recovery. In both cardiovascular, and fluid and electrolyte experiments, horizontally positioned non-head-down tilted (N-HDT) rats were used for comparison. In both HDT and N-HDT the WBS system is used and the hind limbs are unloaded; this is a common and widely used approach to skeletal muscle studies in earth side laboratories.

Some years ago we showed that the HDT rat, for periods of one week, responded with diuresis, natriuresis and kaliuresis (1); in addition, we also reported that renal distribution of electrolytes and urea was heavily concentrated in the renal papilla (3). In the latter study, the kidney slice method, perfected earlier with kidney slices from the hamster, was used (7). Deavers et. al. (1) also reported that following WBS with HDT, urine, sodium and potassium excretion rapidly returned to control levels during a few days of recovery.

In 1984, Gazenko et. al. (2) reported that during post flight periods of from 2 to 22 days, in rats from COSMOS 605 and 782, the percentage of renal excretion of water was lower than that in synchronous controls. From their ground based experiments they suggest the occurrence of natriuresis in rats during flight, this was ascertained during water loading following the second day of recovery. Thus, it may be reasoned that there is a similarity between responses to weightlessness and HDT in rats.

One interpretation suggested by Deavers et. al. (1) was that the water and salts depleted during HDT were rapidly reversed with the termination of HDT. This indication of a relatively rapid compensation, i.e. within a couple of days led us to the current hypothesis that the responses during recovery constituted a retention of water and salts.

The objective of our research was to make a quantitative assessment of the balance of water, sodium and potassium during HDT and recovery, and to determine if there were any relationships to be made with cardiovascular responses in similarly treated rats.

MATERIALS AND METHODS

The experimental features are summarized:

Rat:	Sprague-Dawley	170-190 gm	males
	HDT 20°	7 days	WBS
	Recovery from HDT	7 days	---
	N-HDT	7 days	WBS

Animal Room:

Light:Dark	12:12 hr
Temperature	23-24°C

Measurements taken daily:

Body weights, urine and fecal output, evaporative water (2/day), food and water intake.

RESULTS AND DISCUSSION

In control rats, the ingested water (both from drinking and food), and metabolic water were consistently higher (20 to 25%) than the water excreted in urine and feces and lost from evaporation. The intake total for 22 animals ranged from 32 to 40 ml/day and the total water loss ranged from 22 to 25 ml/day. In the HDT rats, negative water balance was particularly severe during the first day; this is attributed to a highly reduced intake, about 12 to 14 ml/day compared with 20 to 22 ml/day output. Although during days two and three there is significant increase in water intake, and there continued to be a consistent increase in urine output throughout the seven day period. Upon removal from the WBS, in HDT rats the urine output and total water output fell drastically, from 50 to 30%. The N-HDT rats also responded with a marked drop in water intake during the first day of WBS but they did not show the diuresis seen in the HDT rats. We calculated that the water retention during the first day of recovery is about 12% of the body water. During recovery the greatest change occurred in increased drinking with a decreased urine output. During the seven days of recovery there is a progressive increase in urine output and on the sixth and seventh days of recovery the animals are comparable to metabolic cage controls. For the most part the preformed water and metabolic water remain relatively unchanged in experimental and control subjects (except for the first day of WBS). Thus, in future experiments concerned with water balance both of these features may not be required.

Sodium intake was significantly reduced during the first three days of HDT and urinary and fecal sodium loss exceeded the sodium intake during days two through three. This early net sodium loss, particularly evident on the first day, was markedly elevated during days five and six (2.6 to 2.8 mEq/day). This increased sodium loss was highly evident when compared with the control rats (1.8 to 2.2 mEq/day) and the N-HDT rats (2.2 to 2.5 mEq/day). Thus, natriuresis is a feature of HDT. During the first day of recovery there was a marked retention of sodium down to 1.5 from 2.7 mEq/day and, at the same time, intake remained high. The changes during the following days of recovery showed significant adjustments and a re-establishment of sodium balance.

The metabolic cage controls were always in balance. The N-HDT were in negative balance

during the first three days, a slight change toward balance during days four and five and a change into balanced condition during days six and seven.

Urinary potassium losses increased progressively during the seven days of HDT. With the exception of days one through three when potassium intake was significantly lower, the rats remain in potassium balance. There was a marked potassium retention during the first day of recovery from HDT. This retention was not relatable to potassium intake, since it remained relatively constant during the recovery period and the rats remained in potassium balance.

In the N-HDT rats, although the potassium intake and excretion showed parallel progressive increase, there were no significant imbalances during days one through four; and in day four through seven there was a balanced condition.

There are parallel changes in cardiac responses and fluid and electrolyte balance in seven day HDT rats. The blood pressure changes include elevations in mean arterial pressure (MAP), diastolic pressure (DP) and systolic pressure (SP). Typical changes in mmHg are: MAP controls 101±2.1; and in HDT one day 111±2.9, three day 118±2.9 and seven day 121±4.4; also, within one day after HDT, 104±1.5. All N-HDT rats responded with slight elevations and none were significant until seven days. A comparable pattern was seen in the recordings of DP and SP.

At this time we do not conclude that there is a cause and effect relationship between the fluid and electrolyte modification and the blood pressure changes, however it is notable that within a short time after removal from HDT the blood pressures return to control levels. It appears that the cardiac responses are transitory.

There continue to be points of controversy concerning the blood pressure responses in HDT rats. In 1981, Popovic (5) reported that in head-down tilted rats during the first two days there is an increase central venous pressure (CVP), and a decrease in MAP and from the third to the seventh day the blood pressures return to control levels. His animals were not anesthetized nor were ours. In 1982 Musacchia et. al. (4) reported that during three days of HDT there are elevations in MAP, SP and DP. In the same year, Tucker et. al. (9) reported that MAP was significantly decreased with prolonged head-down tilt (tail suspended 20-25°). However, these rats were anesthetized (Inactin 100 mg/kg i.p.). Shellock et. al. (6) reported increases in CVP in HDT rats and that the degree of tilt was directly related to the increase. More recently Tipton et. al. (8) reported rapid increases in CVP with a decrease to baseline values after 90 minutes and also that the blood flow to the hind limb and the mesenteric vascular beds tended to decrease with several hours of HDT suspension. One thing is certain, HDT produces changes in the dynamics of cardiovascular blood flow. The question remains are these changes in blood pressure a reflection of cephalad fluid shifts and how are they related to the increased capillary density of hind limb skeletal muscle such as the soleus in HDT and weightlessness?

Lastly, because these reports deal with simulations of weightlessness it is essential that flight experiments be done with rats. Chronic cannulae placed in the aorta and right atria, such as those used in the laboratories of Musacchia and Popovic, are suggested for in-flight rats. Also, the same rats can be used for blood withdrawal during and following flight.

Supported by NASA Grants: NSG-2191, NSG-2325 and AMES Grant #2-386.

REFERENCES

1. Deavers, D.R., X.J. Musacchia, and G.A. Meininger. Model for antiorthostatic hypokinesia: head-down tilt effects on water and salt excretion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49 (4):576-582, 1980.
2. Gazenko, O.G., Yu. V. Natochin, Ye. A. Ilyin, N.A. Ilyusko, Yu. I. Kondratiev, Ye. A. Lavrova, and Ye. I. Shakhmatova. Fluid-electrolyte metabolism and renal function of white rats in experiments aboard COSMOS biosatellites. Aviat. Space Environ. Med. 55: 685-691, 1984.
3. Musacchia, X.J., D.R. Deavers, and G.A. Meininger. Water balance and renal cortico-medullary electrolyte distribution in hypokinesia. The Physiologist 20:67, 1977.
4. Musacchia, X.J. and J.M. Steffen. Short term (1 and 3 day) cardiovascular adjustments to suspension antiorthostasis in rats. The Physiologist 25 (6) Suppl:S163-164, 1982.
5. Popovic, V. Antiorthostatic hypokinesia and circulation in the rat. The Physiologist 24 (6) Suppl:S15-16, 1981.
6. Shellock, F.G., S.A. Rubin, D. Michele, G. Nevitt, and H.F.C. Swan. Early right atrial pressure changes during simulated weightlessness in rats. The Physiologist 27:261, 1984.
7. Tempel, G.E. and X.J. Musacchia. Renal function in the hibernating, and hypothermic hamster Mesocricetus auratus. Am. J. Physiol. 228 (2):602-607, 1975.
8. Tipton, C.M., J.M. Overton, M.J. Joyner, and A.R. Hargens. Local fluids shifts in humans and rats: comparison of simulation models with actual weightlessness. The Physiologist 30 (1) Suppl:S117-120, 1987.
9. Tucker, B.J., A.R. Hargens, O.W. Peterson, and R.C. Blantz. Alterations in glomerular and tubular dynamics during simulated weightlessness. The Physiologist 25 (6):S67-68, 1982.

HEMODYNAMIC RESPONSES TO -6 DEGREES HEAD-DOWN REST IN DOGS: A MODEL OF WEIGHTLESSNESS

W. G. Squires, M. K. Smith, S. Nunneley, and P. B. Raven

Department of Biology, Texas Lutheran College
School of Aerospace Medicine, Brooks AFB, and
Department of Physiology, Texas College of Osteopathic Medicine

INTRODUCTION: Humans have been placed in a 5°-6° head-down rest (HDR) position ranging in time from 1 hour to 24 hours(1). This experimental protocol has been used to produce intravascular fluid volume shifts towards the head and subsequently to resemble the intravascular fluid shifts observed during exposure to microgravity similar to that encountered during space flight(1). Alternatively, small animals have been used in hind-limb or tail suspension models to allow a more invasive investigation of the effects of microgravity(6). In the present experiment, we have carefully described the hemodynamic changes in the dog associated with time during the 6° HDR condition of simulated microgravity, as a comparison to the human HDR model.

METHODS: In this study all animals were treated according to the guiding principles of animal care of the American Physiological Society. Sixteen male dogs, 15-25 kg, were separated into three experimental groups. Group One (G1). Six animals were anesthetized and ventilated. Electrocardiogram (ECG) was monitored. A No. 7 catheter, connected to a pressure transducer was inserted into the jugular vein and advanced into the right atrium under fluoroscopy to measure right atrium pressure (RAP). Systolic, diastolic and mean arterial (MAP) pressures were obtained from a catheter in the left iliac artery to the descending aorta. Thru a mid-line thoracotomy, the heart was suspended and a pressure transducer was inserted for left ventricular pressure (LVP) and LV dp/dt. The animal was placed in the supine position for HDR. Group Two (G2). Six animals were intubated and placed on an anesthesia machine. HR, RAP and MAP were monitored as in G1, with the exception that pressures were obtained with the chest closed. A ultrasonic probe was placed around the right iliac artery for measurement of blood flow (BF). Cardiac output (Q) was measured by thermodilution. The animals were placed in the lateral decubitus position for HDR. Group Three (G3). Four animals were studied in the conscious state. Each dog was anesthetized as described for G2. A 7 French vascular access port (VAP) in the left iliac artery was advanced into the aorta for measurement of MAP. A second VAP was placed fluoroscopically in the right atrium to measure RAP. Animals accepted HDR in a conscious state, standing upright in a sling. Data were obtained before HDR and at 15, 30, 45 and 60 min. of HDR. The animal was returned to the horizontal position and rested for an additional 15 min. during which recovery data were obtained. Univariate analysis of variance (ANOVA) was used to analyze the data with an alpha level set at 0.05. Two analyses were performed: first, common variables of all groups (MAP, RAP and HR) were pooled for each of the experimental groups 1, 2, and 3 and tested for the experimental condition (HDR) effect. Second, LVP and LV dp/dt in G1 and iliac BF and cardiac flow in G2 were analyzed.

RESULTS: A. HDR Responses: Figure 1 summarizes the response of HR, right atrial mean pressure and mean systemic arterial pressure of all 16 animals to HDR. The data of G1, G2, and G3 were combined to describe the response to HDR as there were no differences due to surgical conditions of the groups. (P>0.05). RAP significantly increased during HDR reaching a peak response at 15 min. and remaining elevated through 60 min. of HDR. MAP and HR were unchanged throughout HDR, P>0.05. Figures 2A & B, summarize the Q and BF, G2, LVP and LV dp/dt during HDR of G1. There were no differences between control, HDR or recovery measure for any of the variables, P>0.05.

Figure 1

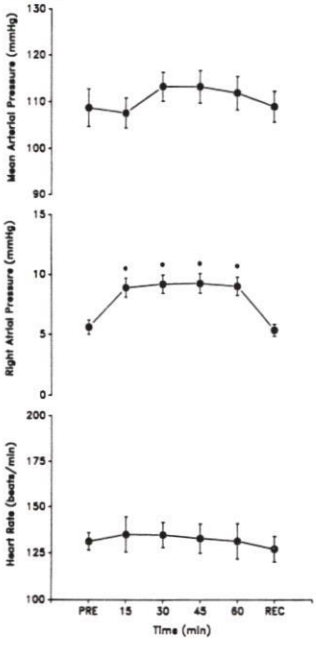
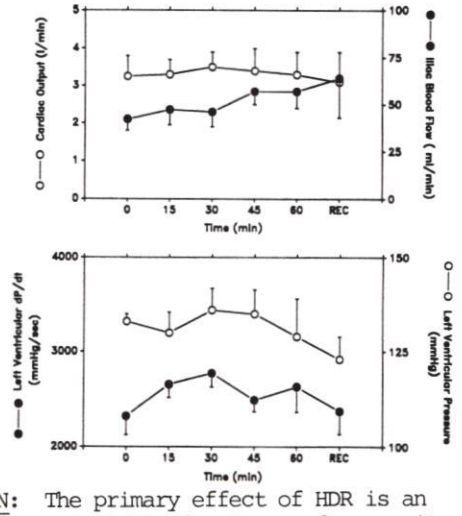


Figure 2



DISCUSSION: The primary effect of HDR is an increase in central blood volume and concomitant increase in central venous pressure(CVP)(2). In the present investigation RAP, an index of CVP, increased rapidly with HDR and plateaued early; both the rate of change and the plateau were comparable to values reported in human experiments (3,4,8). In their review, Blomqvist and Stone (2) described a bell-shaped CVP response to the first 6 hours of HDR of humans with a minimal plateau

over time at the peak response. The data from present experiments in the dog suggest that CVP rises quickly over the first 15 min., then remains stable for at least 60 min. This observation is consistent with the findings of Lollgen et al, (4) who demonstrated a pronounced increase in RAP at minute 15, with a subsequent plateau through minute 60 of -6° HDR in humans. Nixon et al (8) observed that CVP was increased significantly at minute 30 of HDR and returned to near baseline at minute 120 of HDR. Gaffney et al (3) observed a similar return of CVP during the first hour of -5° HDR. Therefore, it appears that CVP does plateau during the first hour of moderate (5°-6°) HDR and returns to baseline during the first hour of -6° HDR in both anesthetized and conscious dogs. In man, MAP typically does not change during HDR of -5° to -10° (3,5,8), although small increases and decreases (<10 mmHg) have been documented after one hour of HDR (4). We observed similar small (5-10 mmHg), but insignificant, increases in mean and systolic arterial pressure in both anesthetized and conscious dogs. However, the averaged responses of all dogs demonstrated a lack of effect of HDR on MAP. The HR and Q varied widely among individual dogs within experimental protocols. In general HR and Q did not change significantly during HDR. Previous investigations in humans suggest that HR remains unchanged or decreases slightly during -5° to -10° HDR (3,4,8, 10). The available data regarding Q and stroke volume do not change significantly during HDR in humans (4,8,10). However, Gaffney et al (3) observed a significant increase in Q and stroke volume at 15 min. of HDR with subsequent return to baseline at 60 min. of HDR. London et al (5) observed a significant increase in Q after 30 min. of -10° HDR compared to -6° HDR in humans. The HDR results in an immediate increase in central blood volume. Several studies have shown that central blood volume increases dramatically and remains elevated during prolonged HDR (5,7). In addition, a large proportion of the translocated blood appears to be sequestered rapidly in the pulmonary circulation (4,5,7). Much of this volume is probably filtered into extravascular spaces, since increasing perfusion pressure augments the filtration process. Echocardiography measurements of end-diastolic volume suggest that the heart volume returns to normal within 30 min. (8). Therefore, the increased central blood volume associated with the onset of HDR is rapidly redestributed away from the heart. Consequently, the initial effect of HDR on Q is transient. Different peripheral vascular beds may show differing vasomotor responses to HDR. In our experiments BF increased and remained elevated throughout the one hour of HDR. These data are consistent with the observations of London et al (5) who found increased forearm blood flow and decreased forearm venous tone and vascular resistance after 30 min. of -10° HDR in man. This vasodilation was probably mediated by reflex inhibition of vasoconstrictor tone by stimulation of the volume-sensitive cardiopulmonary mechanoreceptors. Stimulation of these mechanoreceptors produces sympathoinhibition and vasodilation, whereas, unloading the mechanoreceptors produces vasoconstriction (9,11). Assuming that -6° HDR produces a similar effect on these mechanoreceptors as -10° in humans, our data demonstrate a similar effect on vascular resistance in selected peripherals beds served by the iliac artery of dogs. This increase in flow with Q being constant has a differential effect on vascular resistance indicating a decreased resistance, therefore, the sustained vasodilation in the iliac artery was not observed globally throughout the vasculature, suggesting heterogeneous control of the circulation during HDR. Gaffney et al (3) found similar transient changes in total peripheral resistance, with early decreases followed by a return to baseline levels in humans. Other studies have reported no significant resistance changes during HDR (4,5,10). Vascular resistance changes that do occur during HDR in humans appear to be transient and our data demonstrate a similar response in this dog model. In summary, it would appear that the dog when placed in the HDR position has similar cardiovascular responses to that of the humans and could be used as an animal model for more invasive investigations of the micro 'g' environment.

ACKNOWLEDGEMENTS

This research was funded by U.S. Air Force contract numbers F49620-82-C-0035, F33615-84-R-0514, and F33615-83-D-0602-0018.

REFERENCES

1. Blomqvist, C.G., J.V. Nixon, R.L. Johnson Jr., and J.H. Mitchell. Early cardiovascular adaptation to zero gravity simulated head-down tilt. Acta. Astronaut. 7:543-53, 1980.
2. Blomqvist, C.G., and H.L. Stone. Response to stress: gravity. In: Handbook of Physiology: Peripheral Circulation and Organ Blood Flow. Vol. III, Chap. J.T. Shepherd and F.M. Abbounds (Eds.). Bethesda, MD: American Physiological Society, 28, pp. 1025-1063, 1983.
3. Gaffney, F.A., J.V. Nixon, E.S. Karlsson, W. Campbell, A.B.C. Downey, and C.G. Blomqvist. Cardiovascular deconditioning produced by 20 hours of bedrest with head-down tilt (-5°) in middle-aged healthy men. Am. J. Cardiol. 56:634-638, 1985.
4. Lollgen, H., U. Gebhardt, J. Beier, et al. Central hemodynamics during zero gravity simulated head-down bedrest. Aviat. Space Environ. Med. 55:887-892, 1984.
5. London, G.M., J.A. Levenson, M.E. Safer, A.C. Simon, A.P. Guerin, and D. Payen. Hemodynamic effects of head-down tilt in normal and sustained hypertensive patients. Am. J. Physiol. 245 (Heart Circ. Physiol. 14):H194-H202, 1983.
6. Mussacchia, X.J., Steffen, R.D. Fell, and J. Dombrowski. Physiological comparison of rat muscle in body suspension and weightlessness. Physiologist. February 1987, 30(1 Suppl): S102-S105.
7. Nikolayenko, E.M., V.Ye. Katkov, S.V. Gvozkev, et al. Human lung fluid content during 7-day head-down tilt. Space Biol. Aerosp. Med. 18(6):55-60, 1984.
8. Nixon, J.V., R.G. Murray, C. Bryant, et al. Early cardiovascular adaptation to simulated zero gravity. J. Appl. Physio. 46:541-548, 1979.
9. Roddie, I.C., J.T. Shepherd, and R.F. Wheland. Reflexes changes in vasoconstriction tone in human skeletal muscle in response to stimulation of receptors in a low-pressure area of the intrathoracic vascular bed. J. Physiol. London. 139:369-376, 1957.
10. Tomaselli, C.M., R.A. Kennedy, M.A.B. Frey, G.W. Hoffler. Cardiovascular dynamics during the initial period of head-down tilt. Aviat. Space Environ. Med. 58:3-8, 1987.
11. Zoller, R.P., A.L. Mark, F.M. Abbound, P.G. Schmid, and D.D. Heistad. The role of low pressure baroreceptors in reflex vasoconstrictor responses in man. J. Clin. Invest. 51:2967, 1972.

COMPARATIVE STUDY OF CARDIOVASCULAR RESPONSES IN PRIMATES EXPOSED TO TILT TEST AND LBPP.

**TRAN C.C., *PAILLARD F., **LANGERON O., ***LABORDE F., **PAYEN D., ***GALLIX P., *LISCIA P., ****LIEBAERT P., *QUANDIEU P.*

* **Laboratoire Central de Biologie Aérospatiale**
** **Hopital Lariboisière**
*** **Centre d'Etudes et de Recherches Animale**
******Direction des Recherches Etudes et Techniques**
(PARIS-FRANCE)

INTRODUCTION

Tilt test and LBPP (Lower Body Positive Pressure) are two distinct methods to simulate on the ground the shift of biological fluids toward the upper part of the body such as it is observed in space. In microgravity, hydrostatic pressure disappears and low peripheral resistances do not increase. The tilt test inverses hydrostatic pressure and does not modify peripheral resistances. LBPP increases low peripheral resistances and does not suppress hydrostatic pressure. Cardiovascular responses to hemodynamic changes created by the tilt test and LBPP must a priori be different from those which exist in microgravity. The purpose of this study was to compare both simulation methods.

MATERIALS AND METHODS

The use of an animal model (baboon) permits the direct implantation of transducers in the studied artery. This animal was chosen because the anatomic configuration of its cardiovascular system is similar to man's.

Cardiovascular responses were studied through the pulsed ultra-sound doppler velocimetic signal. Two systems were used : Echovar F8C of Alvar Electronic operating at an emitting frequency of 8 MHz to measure carotid blood velocities and AEM 248 operating at an emitting frequency of 4 MHz to measure aortic blood velocities.

For tilt test studies, an easy method is to use a support allowing a rotation of 360° of the animal around a horizontal axis. Doppler measurements can thus be made for various animal positions : orthostatic, head-down recumbency.

To place the animal under LBPP conditions a hyperbaric chamber was used. It was a rigid plexiglass enclosure with a circular opening on the top to allow passage of a baboon in a restraining chair down to the animal's waist. The opening was made leakproof by a latex skirt around the animal's abdomen. A pumping system increased overpressure levels by plateaus of 10 mbar up to 50 mbar.

. Doppler 8 MHz and 4 MHz probes were calibrated on a hydraulic bench according to a method directly derived from Peronneau's (4). Two baboons were chronically bioinstrumented. During the same operation the surgeon implanted a 4 MHz doppler sensor on the ascending aorta and an 8 MHz sensor on the primitive carotid; wires were passed under the skin.

Experiments only started after animals had recovered from surgery. Animals were anesthetized with 10 mg/kg ketamine i.m. followed by a half dose every half hour. Sensors were connected to data loggers through a small skin incision. Subjects were exposed to ten successive rotations on the support. Carotid and aortic doppler measurements were successively made for each stabilized position of the animal. LBPP experiments required a special preparation : shaving of the chest, applying vaseline, positioning of the latex skirt held in place by surgical adhesive tape. The animal was then placed in the system and pressure rose in the lower part of the body by increments of 10 mbar up to 40 or 50 mbar (1,2). A complete rise in pressure lasted approximatively 45 minutes. On the support and in LBPP, measurements were made at some distance from ketamine injections (after ten minutes and when heart rate was stabilized). When the carotid sensor was connected, velocity profiles were recorded. Carotid blood flows were calculated by digital integration of velocity profiles. When the aortic sensor was connected, the instantaneous velocity was recorded on the aortic axis. The stroke volume (SV) was obtained by planimetry.

RESULTS

An initial experimental protocol consisted in picking up doppler carotid and aortic signals in the same animal. Because of certain problems in signal acquisition, results are presented for two pairs of animals, one for the aorta, the other for the carotid. Pratical investigations show that the change from the sitting to the head-down position increased carotid blood flow by 11% in one pair (2.34 ± 0.19 ml vs 2.61 ± 0.21 ml) and by 33% in the other pair (2.98 ± 0.15 ml vs 3.97 ± 0.77 ml). Doppler signals in the aorta are unfortunately unusable.

In LBPP, regression calculations did not evidence any relationship between changes in carotid blood flow and external overpressure in the two animals (fig.1, baboon N : r= -0.2878 ; baboon O : r= -0.0338). The same was true for the stroke volume (fig. 2, baboon M2 : r= 0.43; baboon O8: r= 0.30) .

DISCUSSION

Results expressed in terms of carotid blood flow were obtained from a velocity measurement. If the diameter of the blood vessels increases, bloodflow can be stable or increase even though velocity is reduced. However, our method of calculation takes into account any possible change in the arterial diameter since velocity profiles on this diameter are resed for annular integration. However the hypothesis of an axisymmetric flow must be accepted. But for the calculation of the stroke volume a constant value must be given at the aortic section, supposed to be circular. True, these results were obtained on only two pairs of animals. However, data reported here shows two opposite tendencies : an increase in carotid blood flow in the tilt test, which is classically observed, but no change in carotid blood flow in response to changing levels of overpressure. The cardiovascular response of primates should therefore a priori also be different in tilt test and under overpressure.

Our results show that carotid blood flow remains stable in LBPP, that heart rate tends to decrease and that SV remains stable. Why does carotid blood flow remain stable while cardiac out put decreases ? In LBPP carotid blood flow must, in fact, decrease but since low peripheral resistances are increased, distribution of the aortic blood flow favors carotid arteries, which tends to compensate for the decrease in blood flow in these aeras. This explains observed experimentally the absence of

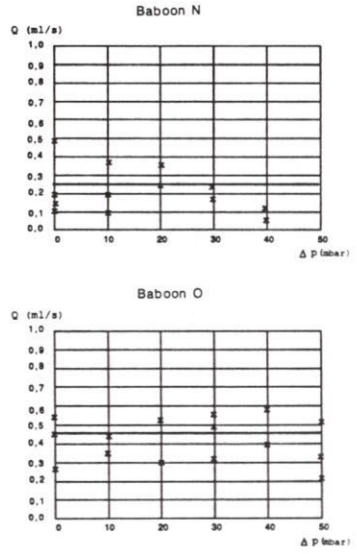


Fig. 1- Carotid blood flow vs over pressure

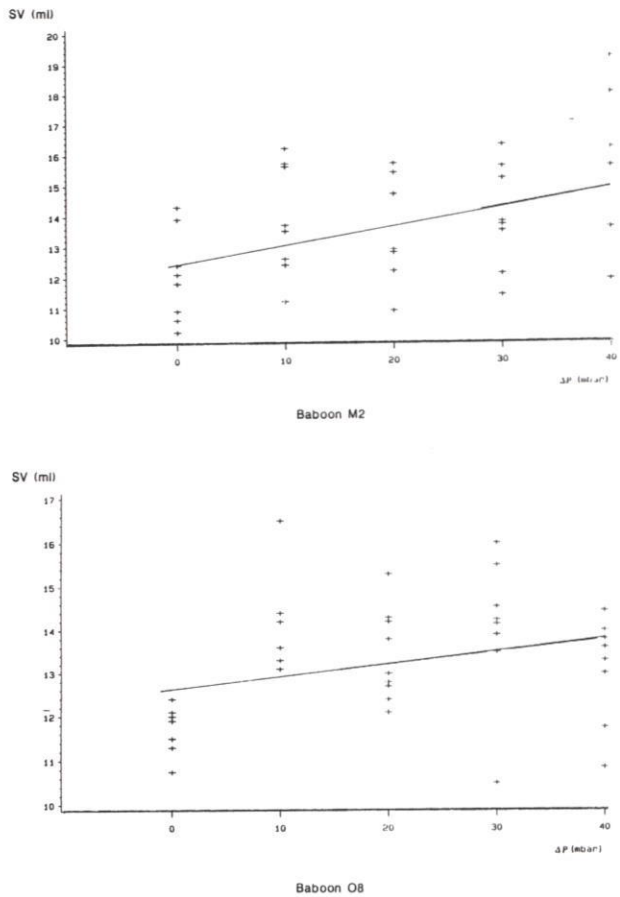


Fig. 2- Stroke volume vs overpressure

changes in carotid blood flow. The positive pressure applied to the lower part of the body enhances the venous return, but experimental results do not evidence any increase in SV. In this context, it is legitimate to think that the blood mass is shifted under the diaphragm under LBPP but with lung storage, or still, that blood is stored in digestive tract veins considering the increase in low peripheral resistances.

This interpretation, based on our experimental results is in contradiction with results obtained in man wearing the anti-G suit inflated at similar or higher overpressure levels. Seaworth et al. (5), using a two-dimensional echocardiographic technique, evidenced an increased in SV and end- diastolic volume in standing subjects wearing anti-G suits. However, SV and end-diastolic volume decreased one minute after inflation. This could be due to increased venous compliance for example in the lungs or digestive sphere. Kravic et al. (3) studied changes in plasma vasopressine, renine activity and arterial pressure, and confirmed the cephalic bloodshift. This shows that the experimental model influences results by its own response to stress. Which explains the sometimes contradictory results obtained by different teams.

Choosing primates as animal model our results show that it is better to use the tilt test than LBPP for short-term ground simulation of microgravity on cardiovascular biodynamics.

REFERENCES

1 - FERRARIO C.M., NAZDAM G., FERNANDEZ L.A. and GARDNER W.J.

Effects of pneumatic compression on the cardiovascular dynamics in the dog after hemorrhage. Aerospace Med. 41 (4) : 411-415, 1970.

2 - KASS D., SULZMAN F., FULLER C. MOORE-EDE M.

Renal responses to central vascular expansion are suppressed at night in conscious primates. Am. J. Physiol. 239 (Renal Fluid Electrolyte Physiol. 8) F 343 - F 351 1980.

3 - KRAVIK S.E., KEIL L.C., GRELEN G., WADE C.E., BARNES P.R., SPAUL W.A., ELDER C.A. and GREENLEAF J.E.

Effect of antigravity suit inflation on cardiovascular, PRA, and PVP responses in humans. J. Appl. Physiol 61 (2) : 766-774. 1986.

4 - PERONNEAU P.

Analyse de l'écoulement sanguin dans les gros vaisseaux par méthode ultrasonore.

Thèse de Doctorat d'Etat en Sciences Naturelles. Université Paris Sud. 1987. 159 p.

5 - SEAWORTH J.F., JENNINGS T.J., HOWELL L.L., FRAGIER J.W., GOODYEAR C.D., and GRASSMAN E.D.

Hemodynamic effects of anti-G suit inflation in a 1-G environment.

J. Appl. Physiol. 59 (4) : 1145-1151, 1985.

EFFECTS OF LOWER BODY POSITIVE PRESSURE ON FOREARM VASCULATURE

LEFTHERIOTIS G. (1), GEELEN G. (2)
SAUMET J.L. (1), HEMERY N. (1)

1: Lab. Physiol. et Explo. Fonct. Vasc., CHRU ANGERS CEDEX 49033, FRANCE.
2: Lab. Physiologie, UFR Grange-Blanche, LYON CEDEX 08, FRANCE.

INTRODUCTION :

The main physiological effects of lower body positive pressure induced by antigravity suit (Anti-G suit) inflation are a headward shift of body and an increase in blood pressure (1, 2) resulting from increased cardiac output and/or peripheral resistance. However the underlying mechanisms remain unclear. A central redistribution of fluids with an increase in central venous pressure has been shown to occur in head down tilt (HDT), water immersion (WI) as well as Anti-G suit inflation (3, 4, 5, 6). In addition, a decrease in forearm venous tone has been shown both in HDT (7) and WI (8). From our knowledge, no data are presently available on the effects of Anti-G suit inflation on the peripheral circulation.

MATERIAL AND METHODS :

Eight healthy euhydrated subjects volunteered for the study (age : 23 ± 5 (SD) yr). All the experiments were performed at the same time of day. The subject seated at rest for 30 min (Tdb = 25.6 ± 0.2°C). At 0830 the subject donned the Anti-G suit (MAST III A, David Clark) and stood on a tilt table with a footboard at +70° (70 HUT) for 2 hours. The first hour consisted in a quiet standing and the Anti-G suit was inflated (legs : 60 Torr and abdomen : 30 Torr) during the second hour.

Arterial blood pressure (SBP, DBP and MAP) as well as heart rate were measured every 10 min on the left arm (DYNAMAP SXP, Critikon). Forearm cutaneous temperature was measured with a Cu-Const thermocouple.

Forearm venous compliance (FVC) and arterial blood flow (FBF) were mesured with the venous occlusion plethysmography technique (Perivein - Janssen Instrument) on the right forearm. The forearm was held by an adjustable arm rest at the heart level to insure the free emptying of the veins. The changes in forearm venous compliance were determined by inflating the cuff at 4 occlusion pressures : 10, 20, 30, 40 Torr. The FVC was measured at the end of the 1 st hour of + 70° HUT (pre-inflation period, T0), just after the Anti-G suit was inflated (T1), 1 hour after Anti-G suit inflation (T2) and just after the Anti-G suit deflation (T3). FVC was determined by plotting dV% against the cuff occlusion pressure. The slope of the volume-pressure curve (venous elasticity coefficient-VEC) was also calculated and used as an index of venous tone. It was expressed as dV% per torr. after each FVC measurement, 3 determinations of FBF were made by the occlusion technique with the cuff inflated at 50 Torr.

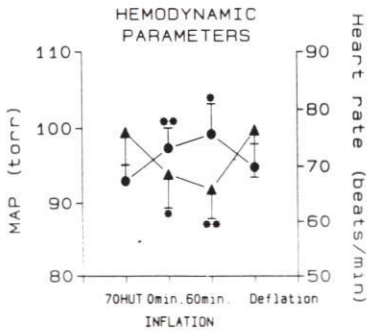
Values are mean ± SD. The data at T1, T2 and T3 were compared to T0 with the non-parametric Wilcoxon matched-pairs signed rank test. The null hypothesis was rejected for $p < .05$.

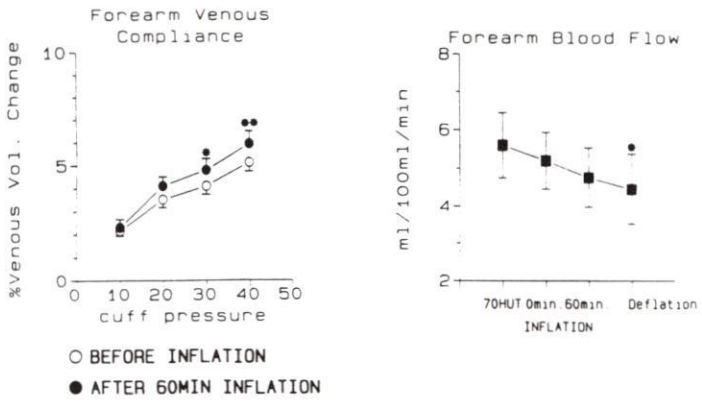
RESULTS : (Figure 1)

During the initial period of Anti-G suit inflation, MBP increased by 4% ($p < .02$), SBP by 9% ($p < .05$) and DBP by 7% ($p < .05$). A concomitant decrease in heart rate of 15% ($p < .01$) was also observed. These changes in arterial pressure and heart rate persisted until the end of the Anti-G suit inflation period. After Anti-G suit deflation. All parameters returned to their initial control values.

The initial period of Anti-G suit inflation (T1) had no significant effect on FVC. After 1 hour of Anti-G suit inflation, there were no significant changes at venous pressure of 10 and 20 mmHg, while FVC increased by 16% at 30 mmHg ($p < .05$) and 40 mmHg ($p < .02$). After Anti-G suit deflation, FVC returned to its control values. After 1 hour of Anti-G suit inflation, the VEC increased significantly by 42% ($p < .05$). The initial period of Anti-G suit inflation as well as Anti-G suit deflation had no significant effects on this coefficient.

Forearm blood flow remained unchanged during the Anti-G suit inflation period but decreased by 21% ($p < 0.05$) after Anti-G suit deflation.





MEAN ± SEM. ✱p<.05, ✱✱p<.02

DISCUSSION :

The results suggest that peripheral venous tone is decreased after one hour of Anti-G suit inflation as indicated by the increase in venous compliance and VEC. In our study, the change in venous tone is delayed compared to that previously reported during -10° HDT or WI (5, 6). The lack of significant change in the early period of inflation (T1) does not support the view of a venous response mediated through a reflex activation of cardiopulmonary and/or arterial baroceptors. However, an activation of such receptors does occur since inflation induced an immediate, marked and prolonged reduction in heart rate. The change in venous tone was neither dependent upon arterial inflow since there were no consistant changes in FBF (likely related to the small increase in MAP). Furthermore, the changes in VEC are independant of the arterial inflow (5, 6). The change in venous tone is thus neither hydrostatic nor reflex in origin. This, in addition ot the fact that it occurs as a delayed phenomenon suggest that it could be hormone-mediated. Indeed, it has been shown that inflation induces a significant decrease in both PRA (1, 2) and norepinephrine (personal communication) which is already maximal after one hour of inflation.

In conclusion, our results would support the view that the hormonal-mediated changes in the tone of the capacitance vessels plays a major role, and the reflexly-mediated only a minor role in the compensatory responses induced by Anti-G suit inflation. In addition, the role of other capacitance vascular beds such as pulmonary veins, which study was beyond the scope of the present work, should also be considered and investigated.

REFERENCES :

1) KRAVIK S.E., KEIL L.C., GEELEN G., WADE C.E., BARNES C.E., SPAUL W.A., ELDER C.A., GREENLEAF J.E., Effects of antigravity suit inflation on cardiovascular, PRA and PVP responses in humans. J. Appl. Physiol., 1986, 61 : 766-74.

2) GEELEN G., KRAVIK S.E., HADJ-AISSA A., LETHERIOSTIS G., VINCENT M., BIZOLLON C.A., SEM-JACOBSEN C.W., GREENLEAF J.E., GHARIB C., Antigravity suit inflation : kidney function and cardiovascular and hormonal responses in men. J. Appl. Physiol., 1989, 66 (2) : 792-9.

3) PELLIGRA R., SANDBERG E.C., Control of intractable abdominal bleeding by external counterpressure. J. Am. Med. Assoc., 1979, 241 : 708-13.

4) GREENLEAF J.E., Physiological responses to prolonged bedrest and fluid immersion in humans. J. Appl. Physiol., 1984, 57 : 619-33.

5) TOMASELLI C.M., KENNEY R.A., FREY M.A.B., HOFFLER G.W., Cardiovascular dynamics during the initial period of head down tilt. Aviat. Space Environ. Med., 1987, 58 : 3-8.

6) EPSTEIN M., Renal effects of head out water immersion in man : implication for an understanding of volume homeostasis. Physiol. Rev., 1978, 58 : 529-81.

7) LONDON G.M., LEVENSON J.A., SAFAR M.E., SIMON A.C., GUERIN A.., PAYEN D., Hemodynamic effects of head-down tilt in normal subjects and sustained hypertensive patients. Am. J. Physiol., 1983, 245 (Heart Circ. Physiol. 14) : H194-202.

8) ECHT M., LANGE L., GAUER O.H., Changes of peripheral venous tone and central transmural venous pressure during immersion in thermoneutral bath. Pflügers Arch., 1974, 352 : 211-17.

EFFECT OF SIMULATED WEIGHTLESSNESS ON THE POSTURAL RESPONSE OF MICROVASCULAR CUTANEOUS BLOOD FLOW

M. Aratow, A.R. Hargens, S.B. Arnaud, and J.-U. Meyer

Life Science Division (239-17)
NASA/Ames Research Center
Moffett Field, CA 94035-1000

Introduction

Astronauts frequently complain of facial puffiness and nasal congestion while in microgravity (10); similar complaints have been documented in the bedrest model of weightlessness (4). It is thought that these symptoms are caused by the cephalad shift of fluids resulting from absence of a hydrostatic gradient in the weightless environment. The cutaneous circulation serves mainly a thermoregulatory function, and can in extreme conditions account for as much as 30% of the cardiac output or 5-10% of blood volume (3). Regulation of the cutaneous microcirculation in response to intravascular pressure alteration remains controversial. The fluid shifts that occur as a result of postural changes or a microgravity environment can cause altered transmural pressures. We hypothesized that cutaneous hemodynamic regulation, regardless of the mechanism, would be altered with simulated weightlessness. This study evaluates the response of cutaneous microcirculatory blood flow to arterial pressure changes using the stimulus of postural change before and after one week of bedrest.

Methods

Eight healthy males (age 32-44 years) volunteered to participate in this study after giving informed, written consent. Cutaneous blood flow was measured in the central forehead and the dorsum of the left foot (proximal to the 4th web space) with a laser Doppler flowmeter (TSI, Inc.). This instrument expresses flow in relative millivolt units, so absolute values of flow cannot be determined. Probe location was marked by ink and measured from easily identifiable landmarks to ensure reproducible placement. Flow values were collected every 7 seconds, digitized, and stored by a microcomputer data acquisition system. Room temperature was 25.7 $\pm$ 1.9°C. An electric tilt table with a footboard produced changes in posture known to alter arterial pressure (7). Tilt speed was approximately 2°/sec. The Doppler probe was placed on the dorsal foot for 3 minutes while the subjects were at 6° head-down tilt (6° HDT). With the probe still on the foot, the subject was moved to 60° head-up tilt (60° HUT), and microvascular flow was again measured for 3 minutes. The probe was then switched to the forehead, and the subject remained at 60° HUT for another 3 minutes. Finally, with no movement of the probe, the subject was brought back to 6° HDT and flows were recorded for the last 3 minutes. Vascular regulation in the forehead was examined by movement from 60° HUT to 6° HDT, which increases arterial pressure to this region. Sixty degrees was chosen because it represented a hydrostatic compromise for foot blood pressure between sitting and standing. In the dorsal foot, the regulatory response was examined by movement from 6° HDT to 60° HUT, which increases arterial pressure to cutaneous vessels in the lower extremities. Subjects were evaluated 4 days before a one week period of bedrest at 6° HDT (simulated microgravity), and at 1 and 2 days after bedrest.

Data Analysis

Baseline flow was determined by averaging the flow values during the last minute of the 3 minute recording period before a tilt maneuver began. The flow response to tilting stimuli (resultant flow) was the average of the values in the last minute of the 3 minute recording period after a tilt maneuver had been completed. The responses to tilting were analyzed by paired t tests. The baseline flow of each subject was used to normalize resultant flows. These resultant flows were then used to evaluate the differences of the magnitude of responses to tilting between the study period before bedrest and the 2 study periods following bedrest by repeated measures ANOVA.

Results

Compared to baseline, forehead cutaneous blood flow **increased** 26 $\pm$ 7% (mean $\pm$ S.E.) ($p<0.05$) when subjects were moved from 60° HUT to 6° HDT before the bedrest period, as illustrated in Figure 1.

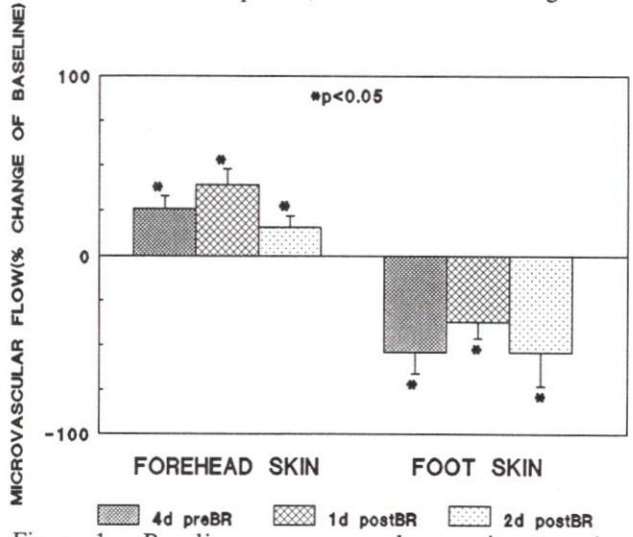


Figure 1. Baseline versus resultant microvascular flows at 4 days before, 1 day after, and 2 days after one week of bedrest (BR). Forehead cutaneous flow **increases** due to arterial pressure increase (HUT to HDT), whereas that in the dorsum of the foot **decreases** before and after bedrest with an arterial pressure increase (HDT to HUT). All blood flow responses are significantly different ($p<0.05$) from baseline flow.

This response of increased flow in the head was the same on the first ($p<0.05$) and second days ($p<0.05$) after bedrest (Figure 1). Dorsal foot cutaneous blood flow **decreased** 46 $\pm$ 12% in response to tilting from 6° HDT to 60° HUT before bedrest (Figure 1). This was not significantly different in magnitude or direction from responses 1 and 2 days after bedrest (Figure 1). An example of dorsal foot flow response to tilt from 6° HDT to 60° HUT is shown in Figure 2.

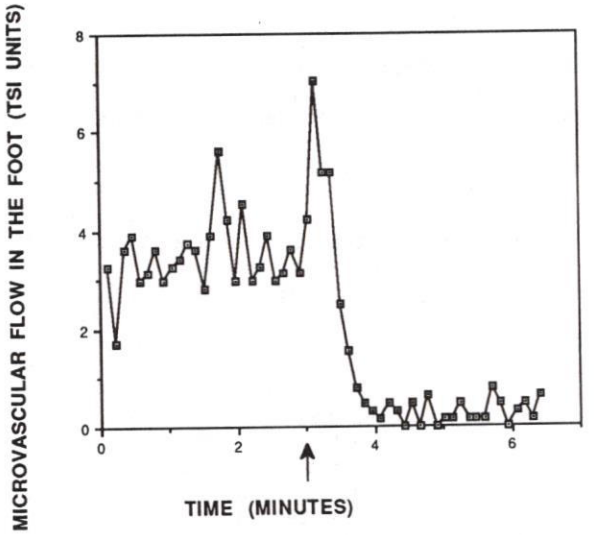


Figure 2. Example of decreased cutaneous blood flow in the foot when subject is moved from 6° HDT to 60° HUT. A persistent decrease of flow to near zero follows the tilt maneuver (arrow).

Discussion

The literature describes mechanisms of cutaneous vascular control such as a local, cutaneous veno-arteriolar reflex that causes vessel constriction in response to an increased transmural pressure in the extremities (6), and possible additional sympathetic control (1,2,8). Our data from dorsal foot skin agree with the results of Henriksen et al. (5) who found a 40% decrease of cutaneous blood flow upon passive displacement of the hand 50cm below the jugular notch. From this and additional studies, the same investigators postulated that edema of the lower extremities in the upright position is prevented by a local veno-arteriolar reflex (6). Our study documents that the vasoconstrictive response to increased arterial pressure in the lower extremities is preserved and unchanged after one week of bedrest. Previous studies of sympathectomized patients (6) and tetraplegic patients (9) demonstrate a persistence of this response in the cutaneous and subcutaneous vasculature of the legs, implying significant local reflexive control. If the local veno-arteriolar reflex is the major determinant of cutaneous blood flow reduction due to increased arterial pressure in the legs, longer periods of weightlessness, whether simulated or actual, may not affect this response. In contrast, cutaneous flow increased in the forehead with elevated local blood pressure in the head. This opposite response suggests an absence of this reflex in the forehead. A lack of repetitive exposure of the head to elevated intravascular pressures over man's evolution may be the reason for the lack of development of this reflex. In addition, no formation of such a reflex is seen to occur in the forehead after 7 days of simulated weightlessness.

We suggest that in microgravity, the cutaneous blood flow in the face increases due to absence of blood pressure gradients. Because there are no regulatory mechanisms to respond to this blood pressure increase, facial edema and its associated symptomatology occur. Lack of adaptation of this reflex in the face is supported by reports of persistent facial edema on longer duration space missions (10). Further studies should be performed in actual microgravity and for longer periods to characterize cutaneous microvascular control further. In addition, nerve blocks can be used to eliminate sympathetic or local veno-arteriolar reflex control selectively and to delineate cutaneous vasculature control mechanisms more completely.

Acknowledgement

This research was supported by NASA. We thank our volunteers, Bionetics, and the Human Research Facility staff.

References

1. Delius, W., K. E. Hagbarth, A. Hongell, and B. G. Wallin. Manoeuvres affecting sympathetic outflow in human skin nerves. *Acta Physiol. Scand.* 84:177-186, 1972.
2. DiSalvo, J., P. E. Parker, J. B. Scott, and F. J. Haddy. Carotid baroreceptor influence on total and segmental resistances in skin and muscle vasculatures. *Am. J. Physiol.* 220:1970-1978, 1971.
3. Guyton, A. C. *Textbook of Medical Physiology.* Philadelphia, PA: W. B. Saunders, 1986, chapter 29, pp. 343-345.
4. Hargens, A. R. Fluid shifts in vascular and extravascular spaces during and after simulated weightlessness. *Med. Sci. Sports Exerc.* 15:442-447, 1983.
5. Henriksen, O., S. L. Nielsen, W. P. Paaske, and P. Sejrsen. Autoregulation of blood flow in human cutaneous tissue. *Acta Physiol. Scand.* 89:538-543, 1973.
6. Henriksen, O. and P. Sejrsen. Local reflex in microcirculation in human cutaneous tissue. *Acta Physiol. Scand.* 98:227-231, 1976.
7. Katkov, V. E. and V. V. Chestukhin. Blood pressure and oxygenation in different cardiovascular compartments of a normal man during postural exposures. *Aviat. Space Environ. Med.* 5: 1234-1242, 1980.
8. Rowell, L. B., C. R. Wyss, and G. L. Brengelmann. Sustained human skin and muscle vasoconstriction with reduced baroreceptor activity. *J. Appl. Physiol.* 34: 639-643, 1973.
9. Skagen, K., K. Jensen, O. Henriksen, and L. Knudsen. Sympathetic reflex control of subcutaneous blood flow in tetraplegic man during postural changes. *Clin. Sci.* 62: 605-609, 1982.
10. Thornton, W. E., G. W. Hoffler, and J. A. Rummel. *Biomedical Results from Skylab.* Washington, D.C.: NASA, 1977, chapter 32, pp. 330-338.

ENDURANCE EXERCISE TRAINING REDUCES ORTHOSTATIC TOLERANCE IN HUMANS!

PETER B. RAVEN AND GLEN H. STEVENS

TEXAS COLLEGE OF OSTEOPATHIC MEDICINE
DEPARTMENT OF PHYSIOLOGY
3500 CAMP BOWIE BOULEVARD
FORT WORTH, TEXAS 76107-2690

Introduction

A continuing debate exists as to whether endurance exercise training incurs a predisposition to orthostatic intolerance (3, 7, 10, 12, 16, 18, 22, 23). Investigations that suggest no change or an increase in orthostatic tolerance with endurance exercise training (4, 6, 7, 12) suggest individual genetic susceptibility as a ready explanation for the converse results obtained in cross-sectional designed studies (7, 12). In addition, other factors involved in the debate were poorly defined criteria for orthostatic intolerance, factors of adaptability, and a diverse number of protocols of orthostatic challenge (7, 10, 22, 23). Therefore, the purpose of this study was to specifically determine the effect of an eight month endurance training protocol designed to increase VO_{2max} 25-30%, on the individuals susceptibility to orthostatic intolerance (OIT) as determined by direct arterial pressure measures during graded lower body negative pressure (LBNP).

Materials and Methods

Seven male subjects (28.43±3.6 yrs; x±SD) (see Table 1 for descriptive data) were studied before and after an 8 month aerobic training program, during which time the individuals OIT and physiologic responses to perturbations of the arterial blood pressure system by LBNP were monitored. All subjects were informed in writing as to the nature of the experiment and completed a subject consent form as approved by the Institutional Review Board for use of human subjects at the Texas College of Osteopathic Medicine.

A treadmill ramp protocol was used to determine parameters of metabolism during the ramped exercise stress test (GXT) and the determination of maximal aerobic capacity (VO_{2max}) and performance. The speed and grade were ramped by 0.15 mph and 1.5% grade every minute until volitional fatigue. Subjective termination of the test and objective criteria of Taylor, Buskirk and Henschel (24) were used to qualitatively and quantitatively determine if the subject had made a maximal effort. A determination of VO_{2max} was accepted if a plateau of oxygen consumption which changed less than 100 mls/min for 1 minute was demonstrated. Oxygen uptake was determined using a dedicated breath-by-breath analysis system incorporating a mass spectrometer (Perkin-Elmer M6A1100B) to determine gas concentrations (O_2 and CO_2) in the inspired and expired air, and a turbine flowmeter (Alpha Technologies VMM-2) to measure tidal volume. All variables were collected on-line using a dedicated micro computer (Digital Equipment Corporation MINC-23) and a customized software package to account for differences in delay and response time. Oxygen uptake kinetics were determined during the testing by determining the time lag between start of a steady increase in mechanical work (beginning of the ramp) and the detection of an increase in oxygen uptake (8). The ventilatory threshold (O_{an}) was determined as the work rate at which ventilation increased disproportionally from a linear increase in the mechanical work rate (8). These two measures were used to determine and monitor exercise training.

EXERCISE TRAINING PROTOCOL:

Each subject performed an aerobic training program that consisted of a walk, and jogging protocol 4 x's/week. Subjects exercised at a heart rate calculated at 1% below the individual ventilation threshold as determined by their VO_{2max} test. Most of the training sessions were monitored, and training heart rates adjusted as submaximal heart rates decreased with training. Subjects exercised approximately 45 minutes per session.

LOWER BODY NEGATIVE PRESSURE: (TOLERANCE TEST):

LBNP was used to induce a central hypovolemia by having the subjects lie in the supine position within a box (LBNP chamber), with an airtight seal engaged around the subjects iliac crests. Variable autotransformers that regulated voltage to two vacuum motors connected to the LBNP chamber were used to control the degree of suction applied to the subjects. A digital pressure read-out was supplied by a pressure meter (Biatek Instruments) that was connected directly to the LBNP chamber. Subjects were exposed to progressively increasing levels of LBNP through stages of control (0), -5, -15, -25, -35, -45 torr for 5 minutes per stage except -15 torr (2 minutes) after which time pressure was increased by -5 torr for 2 minutes to a maximum of -100 torr or until pre-syncopal conditions occurred,

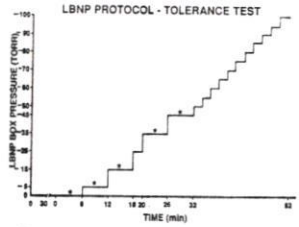


Figure 1.
LBNP Protocol - Tolerance Test: Subjects were sealed in a LBNP box and pressure was lowered in the box until (pre)syncopal symptoms arose. The stairs denote times when heart rate (HR), blood pressure (BP), forearm blood flow (FBF), cardiac output (Qc), and leg volume (LV) were measured. At the other time points only HR, BP and LV were recorded.

All LBNP procedures were performed at the same ambient room temperature. LBNP was performed at a time when subjects were at least 2 hours post prandial and free from physical activity or any stimulants from the previous 12 hours. Since extraneous muscle tension has been shown to augment blood pressure and offset the decrease in blood pressure during LBNP (20) electromyograms were measured in the legs and abdomen using surface electrodes and the subject was requested to maintain a relaxed state. In addition, as Lightfoot et al (11) has suggested that learning effects can be involved with a standard LBNP tolerance testing, all subjects underwent a LBNP pre-syncopal exposure on a separate day prior to any experimental exposures.

Direct invasive measures of arterial pressure recorded from a radial catheter and test termination was based on the following criteria, a precipitous decrease in arterial blood pressure, once pressure had fallen to 90/50; symptoms of lipthymia, such as general weakness, nausea, sweating and pale skin color. The catheter was

inserted into the radial artery under local anesthesia (1% lidocaine). A 1.1mm ID 3.2 cm long teflon catheter was inserted into the radial artery at the wrist of the right arm. The catheter was connected to a sterile tubing set and transducer (Hewlett Packard 1280, Waltham, MA). The catheter was kept patent with a continuous heparinized (2U/ml) saline flush connected to a pressurized system.

Cumulative stress index (CSI, torr x min) was used as a measure of tolerance. The CSI was first used by Luft et al (13) and was derived by summing the products of the duration and pressure magnitude of each complete or partial LBNP stage. During the tolerance testing the EKG was obtained by a lead II recording and along with the arterial pressure measurements, were recorded on a strip chart recorder (Narco Physiograph, Model Six B), see Figure 2, and on-line utilizing a DEC (MINC-23) minicomputer.

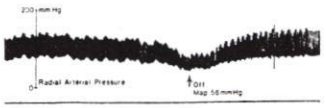


Figure 2.
A sample recording of one subject at termination of the LBNP tolerance test. Note rapid fall in radial artery blood pressure which was accompanied by lipthymia.

BLOOD VOLUME DETERMINATION:

The total blood volume and plasma volume of each subject was obtained using the carbon monoxide (CO) dilution technique of Myhre et al (14). Administration and equilibration of the CO was performed by having the subject lie supine and breathe into a closed system containing a carbon dioxide (CO_2) scrubbing unit.

During the first minute of breathing, 50 mls of pure (100%) CO were injected into the breathing system. The subject then continued to breathe into the system for an additional 10 minutes to allow for equilibration of the CO bound on the subject's circulating hemoglobin (Hb). 5 mls of blood were collect from the antecubital vein before and after the breathing procedure. The blood samples were analyzed for hematocrit (corrected) for trapped plasma), Hb and carboxyhemoglobin (COHb) using a co-oximeter (Instrument Laboratories, Model 282) within 1 hour of collection.

DATA ANALYSIS AND STATISTICS:

The data presented in this paper examines pre-and post-training effects and has been analyzed using the student 'T' test from the Statistical Analysis System (SAS). Level of significance was set at p 0.05.

RESULTS

Subject characteristics: Table 1 illustrates the results for the pre-versus post-8 month aerobic training program. VO_{2max} significantly increased from 43.89 ml/kg/min to 56.91 ml/kg/min without a significant change in weight, lean body mass or body surface area. Maximal performance time also increased from 15.50 minutes to 18.13 minutes, p 0.001.

TABLE I
UNCONDITIONED vs CONDITIONED TRAILS
(Before and after 8 months of aerobic training)
n=7*

Variable	Mean	±	SD	Mean	±	SD	Probability
HEIGHT (cm)	183.34		5.24	183.34		5.24	1.000
WEIGHT (kg)	88.63		15.57	84.19		15.69	0.605
% FAT	21.07		5.14	18.49		5.76	0.395
LBM (kg)	69.35		9.31	68.24		9.31	0.827
BSA (m^2)	2.11		0.16	2.06		0.17	0.615
RHR (bpm)	67.71		9.83	58.00		11.59	0.117
VO_{2max} (l/min)	3.85		0.52	4.72		0.62	0.015
VO_{2max} (ml/kg/min)	43.89		5.25	56.91		8.31	0.0043
TIME to VO_{2max} (min)	15.50		1.69	18.13		1.57	0.011
HR_{max} (bpm)	202.00		11.42	191.86		9.70	0.099

*Mean age of subjects for both pre-and post-training trials was averaged and was 28.43 ± 3.60 years. This variance accounted for changes in age due to the 8 month training period.

Figure 3 depicts the significant increase in absolute blood volume (mls), that occurred due to training. As there was no significant decrease in total body weight or lean body mass these differences were significantly different whether expressed in absolute levels mls, ml/kg of body weight or ml/kg of lean body mass.

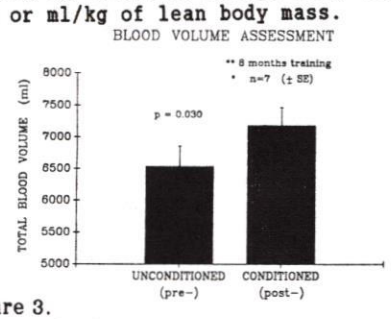


Figure 3.
Changes in absolute blood volumes (ml) of seven subjects pre-and post-conditioning.

A significant reduction in orthostatic tolerance was seen post-training and is evidenced by the change in CSI defined as the point at which symptoms of lipthymia and rapid falls in blood pressure occurred for each individual and is summarized in Figure 4 below.

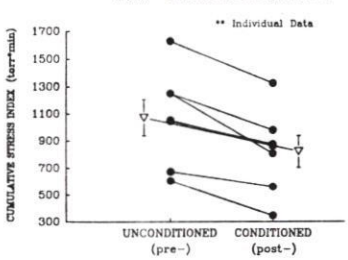


Figure 4.
Changes in individual (.) and mean ( ) orthostatic tolerance prior to and following the conditioning program.

Discussion

The data obtained in these experiments clearly challenges the notion that aerobic exercise training has no effect on orthostatic tolerance (4, 6, 7, 12). Furthermore, although based upon a limited number of subjects the idea of a genetic susceptibility to orthostatic intolerance that is not linked to a specific disease entity must be questioned. Clearly the confusion present in the literature today is a result of differing approaches to answering the basic question. Training modes and length of training are markedly different between the present investigation and that reported by others (6, 12). Specific physiologic adaptations may have occurred within the blood pressure regulating system over the 8 months of training, which were not manifest in the 8 day (6), or 10 week (12) training studies reported previously.

In attempting to explain these divergent results we propose two possible mechanisms which are firmly based upon the ideas of Blomqvist (1, 2). In 1986 Blomqvist (1) identified two principal mechanisms

of clinical orthostatic hypotension these being:
1)abnormally low central hypovolemia; and
2)inadequate cardiovascular regulatory responses (or hypoadrenergic responses).
Based upon the blood volumes of each individual the present study and those observed in our previous work (18-21), we suggest that the low fit subjects (<40 ml O_2/kg/min), were intravascularly hypovolemic and resembles persons exposed to micro 'g' environments. Subsequently, during 1-2g stresses the central hypovolemic person manifests as having poor orthostatic tolerance. In contrast, the high fit subjects (>55ml O_2/kg/min) are centrally hypervolemic and have attenuated regulatory responses during 1-2 g stresses because of either (a) decreased afferent information; (b) baroreceptor resetting; or (c) end-organ adaptations. Recently, we (18) and others (15, 17, 23) have identified significant differences in baroreceptor function in high fit compared to low fit subjects.
In summary, we propose that the divergent data concerning the role of aerobic exercise training on orthostatic tolerance are indeed component parts of the blood pressure regulatory system as a whole, which has a bimodal distribution of susceptibility to orthostatic hypotension probably related to central hypovolemia and central hypervolemic individuals.

References

1)Blomqvist, C. G. "Orthostatic Hypotension". Hypertension 8:722-731, 1986
2)Blomqvist, C. G. "Orthostatic Hypotension". Medical Grand Rounds. Southwestern Medical School, Dallas. July, 1988.
3)Burton, R. R., and J. E. Whinnery. "Operational G - induced loss of conscious: something old, something new". Aviat. Space Environ. Med. 56:812-817, 1985.
4)Convertino, V. A., T. M. Sather, D. J. Goldwater, and W. R. Alford. "Aerobic fitness does not contribute to prediction of orthostatic intolerance". Med Sci Sports Exerc. 85:167-173, 1975.
5)Convertino, V. A., P. J. Brock, L. C. Keil, E. M. Bernauer, and J. E. Greenleaf. "Exercise training-induced hypovolemia: role of plasma albumin, renin, and vasopressin". J. Appl. Physiol. 48:665-669, 1980.
6)Convertino, V. A., L. D. Montgomery, and J. E. Greenleaf. Cardiovascular responses during orthostasis: effect of an increase in VO_{2max}". Aviat. Space Environ. Med. 55:702-708, 1984.
7)Convertino, V. A., "Aerobic fitness, endurance training and orthostatic intolerance". Exercise Sports Sci. Rev. 15:223-260, 1987.
8)Davis, J. A., B. J. Whipp, N. Lamarra, D. J. Huntsman, M. F. Frank, and K. Wasserman. "Effect of ramp slope on measurement of aerobic parameters from the ramp exercise test". Med. Sci. Sports Exerc. 14:339-343, 1982.
9)Greenleaf, J. E., P. J. Brock, R. F. Haines, S. A. Roitanp, L. D. Montgomery and L. C. Keil. "Effects of hypovolemia, infusion, and oral rehydration on plasma electrolytes, ADH renin activity, and + Gz tolerance". Aviat. Space Environ. Med. 48:693-700, 1977.
10)Harrison, M. H. "Athletes, astronauts and orthostatic tolerance". Sports Med. 3:428-435, 1986.
11)Lightfoot, J. T., F. Hilton, and S. Fortney. "The effect of stage duration on lower body negative pressure (LBNP) tolerance". Med. Sci. Sports Exerc. 19 (supp):S91, 1987 abstract.
12)Lightfoot, J. T., R. P. Clayton, D. J. Torok, T. W. Jorunell and S. M. Fortney. "Ten weeks of aerobic training do not affect lower body negative pressure responses". J. Appl. Physiol. 67:894-901, 1989.
13)Luft, U. C., L. G. Myrhe, J. A. Leopsky, and M. D. Veters. "A study of factors affecting tolerance of gravitational stress simulated by lower body negative pressure. In: specialized physiological studies in support of manned space flight". Contract NAS9-14472. Albuquerque: Lovelace Foundation, 2-60, 1976.
14)Myhre, L. G., D. K. Brown, F. G. Hall and D. B. Dill. "The use of carbon monoxide and T-1824 for determining blood volume". Clin. Chem. 14:1197-1205, 1968.
15)Mack, G. W., X. Shi, N. Nose, A. Tripathi, and E. R. Nadel. "Diminished baroreflex control of forearm vascular resistance in physically fit humans. J. Appl. Physiol. 63:105-110, 1987.
16)Nicogossian, A. E., and J. F. Parker, Jr. Space Physiology and Medicine NASA, SP227, 1982.
17)Pawelczyk, J. A., W. L. Kenney, and D. Kenney. "Cardiovascular responses to head-up tilt after an endurance exercise program". Aviat. Space Environ. Med. 59:107-112, 1988.
18)Pawelczyk, J. A., S. Stern, and P. B. Raven. "Baroreflexes are less effective in endurance athletes". Med. Sci. Sports and Exerc. 21:S42 (Abstract #249), 1989.
19)Raven, P. B., B. Rohm-Young, and C. G. Blomqvist. "Physical fitness and cardiovascular responses to lower body negative pressure". J. Appl. Physiol. 56:138-144, 1984.
20)Smith, M. L., D. L. Hudson, and P. B. Raven. "Effect of muscle tension on the cardiovascular responses to lower body negative pressure in man". Med. Sci. Sports Exerc. 19:436-442, 1987.
21)Smith, M. L., and P. B. Raven. "Cardiovascular responses to lower body negative pressure in endurance and static exercise trained men". Med Sci Sports Exerc 18:545-550, 1986.
22)Stegemann, J., A. Busert, and D. Brock. "Influences of fitness on the blood pressure control system in man". Aerospace Med. 45:45-48, 1974.
23)Stegemann, J., H. D. Framing and M. Schieteling. "Der ein flub einer 6-studigen immersion in thermo-indifferentem wasser aut die regulation des kreilaufes und der leistungsfahigkeit bei traineirten und untrainierten". Pfluger Arch. 312:129-138, 1969.
24)Taylor, H. L., E. Buskirk, and A. Henschel. "Maximal oxygen uptake as an objective measure of cardiorespiratory performance". J. Appl. Physiol. 8:73-80, 1955.

MICROCOMPUTER-BASED MONITORING OF CARDIOVASCULAR RESPONSES TO MICRO-GRAVITY SIMULATION (-7 DEGREES HDT)

E.Länsimies, K.Tahvanainen, M.Mäntysaari, J.Hartikainen, T.Kärki and P.Tikkanen

Department of Clinical Physiology, University Central Hospital of Kuopio, P.O.Box 21, SF-70211 Kuopio, Finland

INTRODUCTION

Simple and reliable methods for the evaluation of cardiac autonomic function were sparse until the 1970's, when a new generation of simple bed-side tests were introduced. These tests are based on cardiovascular reflexes to different simple loading situations measured as changes of heart beat intervals (R-R intervals) and blood pressure. The most widely used tests are the Valsalva manoeuvre (Levin 1966), the deep breathing test (Wheeler and Watkins 1977), the orthostatic test (Drischel et al. 1963) and the isometric hand grip test (Ewing et al. 1974).

The aim of this study was to develop and test an integrated microcomputer-based system for the evaluation of cardiovascular responses to micro-gravity simulation and classical cardiovascular autonomic function tests.

METHODS

ECG and continuous blood pressure signals (non-invasive and/or invasive) were simultaneously obtained with a sampling frequency of 200Hz using an IBM PC/AT compatible microcomputer (MEDIKRO AT, Medikro Oy, Finland) equipped with an A/D converter (DT2801-A, Data Translation Inc., USA). Non-invasive arterial blood pressure signal was measured from a finger by vascular unloading method (FINAPRES, Ohmeda, Inc., U.S.A.). Pressure calibration, data acquisition and analysis were performed with a dedicated, interactive software package (Medikro CAFTS, Medikro Oy, Finland).

The software package (**CAFTS** - Cardiovascular Autonomic Function Test System) includes procedures with on-line help for the following cardiovascular autonomic function tests:

- active orthostatic test
- passive orthostatic test on a tilt table (from supine to 70 deg head-up tilting)
- microgravity simulation tests on a tilt table (from 0-G to 1-G and from 1-G to 0-G)
- study at rest in different body positions
- deep breathing test
- Valsalva manoeuvre
- sustained handgrip test
- pharmacological interventions (phenylephrine, atropine and nitroglycerine tests)
- mental arithmetics test

In each test a total acquisition time of 60 seconds is used and the subject is holding the finger with pressure monitoring at the level of the heart.

Heart beat interval (R-R interval, RRI) evaluation is based on a software QRS detector (matched filter technique). Beat-to-beat arterial systolic (SAP), mean (MAP), diastolic (DAP) and pulsearterial pressure (PP) are defined thereafter in each R-R interval.

(A) POSTURAL CHANGES

To monitor the beat-to-beat interval (RRI) changes or fluid shifts (blood pressure changes) between the upper and lower body parts in postural tests the subject moves from supine to standing posture (active orthostatic test) or the subject is moved from supine to 70 degrees standing posture (passive orthostatic test) using a tilt table. To simulate acute hemodynamic responses to weightlessness, the subject is moved on the tilt table from 70 degrees (1-G) to -7 degrees (0-G) (HDT) or vice versa (HUT).

(B) STUDY AT REST IN DIFFERENT BODY POSITIONS

To evaluate heart rate, RRI and blood pressure variability at rest or stationary conditions the subject either is breathing spontaneously or controlled with a constant frequency (0.2Hz cycle) and tidal volume (Medikro 101 spirometer, Medikro Oy, Finland).

In the analysis of a stationary study, mean heart rate, RRI, SAP, MAP, DAP and PP are defined. In addition, range and standard deviation of R-R intervals and blood pressure values are determined. To evaluate the frequency content of RRI signal and both systolic and diastolic finger blood pressure, a **power spectral estimation** is performed using AR modeling. Frequency spectrum is divided into three frequency bands: low-frequency (LF) band at DC-0.07Hz, medium-frequency (MF) band at 0.07Hz- 0.15Hz and high-frequency (HF) band at 0.15Hz-0.50Hz.

Total power (variance) of RRI's, SAP and DAP are calculated and variance is divided into the three spectral bands. In addition, MF/HF ratio for RRI, SAP and DAP spectral estimate are defined expressing sympathetic/ parasympathetic balance.

(C) VALSALVA MANOEUVRE

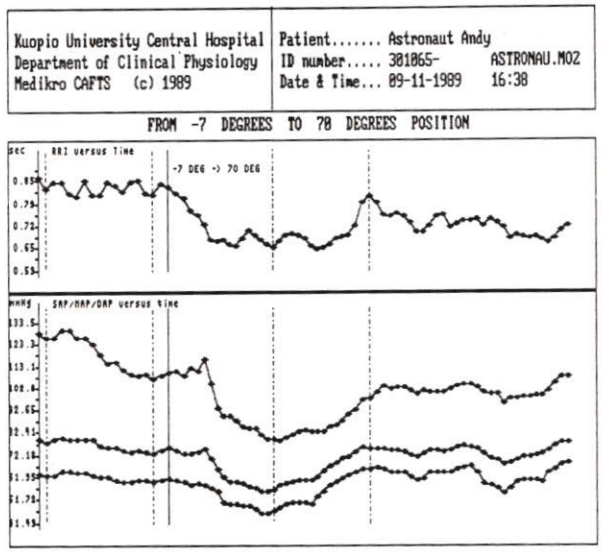
To perform a standardized Valsalva manoeuvre in sitting, supine, -7 degrees, 70 degrees or standing position, the subject blows against the pressure of 40mmHg (5.3kPa) for 15 seconds while continuous ECG and finger blood pressure are recorded with the microcomputer. Care is taken that the glottis is open and that the subject does not blow with the cheeks thus ensuring transmission of the expiratory pressure to the chest. The test is usually carried out three times and between each manoeuvre the subject rests at least for one minute.

In the analysis of Valsalva manoeuvre the RRI's and arterial blood pressure values during the test are defined and the Valsalva ratio is calculated. The Valsalva ratio is defined as the ratio of the longest RRI shortly after the strain to the shortest RRI during the strain. In selecting the maximal and minimal RRI's the intervals associated with premature beats are excluded. In addition, the longest RRI is selected within four beats from the maximal blood pressure value detected after the strain. Baroreflex sensitivity is defined as a ratio of the maximal RRI change to the maximal change in SAP after the expiratory strain.

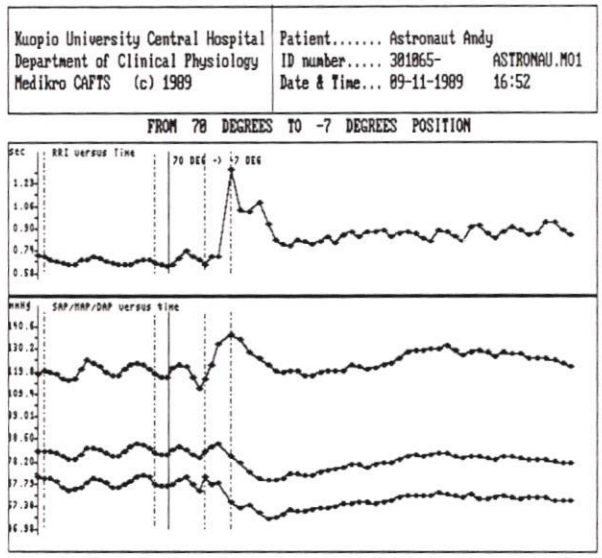
RESULTS

An example of acute changes in RRI and finger blood pressure in a tilt table experiment, where the subject was moved from -7 degrees (0-G) to 70 degrees (1-G) position is described in Fig.1a. In Fig.1b. the subject was tilted 14 minutes later from 1-G to 0-G. A markedly greater change of RRI and blood pressure can be observed in Fig.1b. Maximal RRI change was 685ms in 1-G to 0-G postural change when compared to 156ms in 0-G to 1-G.

Power spectral estimates of RRI, SAP and DAP signals are described in Fig.2a. in 0-G (-7 degrees) and Fig.2a. in 1-G (70 degrees) position. The subject was breathing with 0.2Hz frequency and a clearly dominant vagal tone is seen in 0-G, whereas in 1-G 0.1Hz oscillations become clearly more apparent as a sign of increased sympathetic tone.

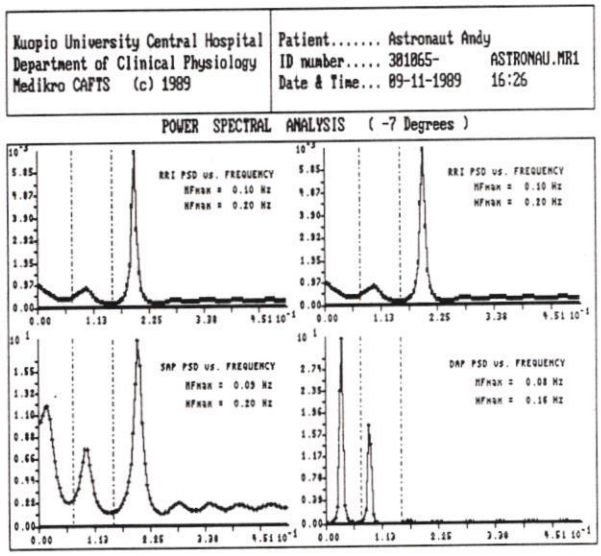


1a

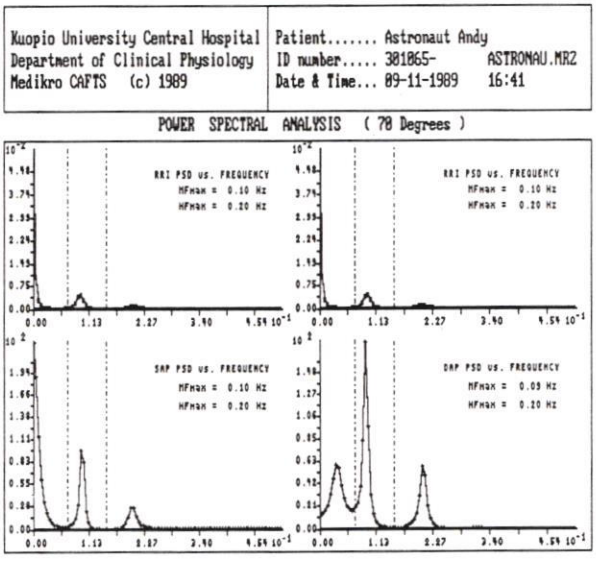


1b

Fig.1.: Acute RRI and blood pressure response to 0-G->1-G (a) and 1-G-> 0-G transition (b) in a healthy subject. Postural change at 15 sec from the start (even vertical line).

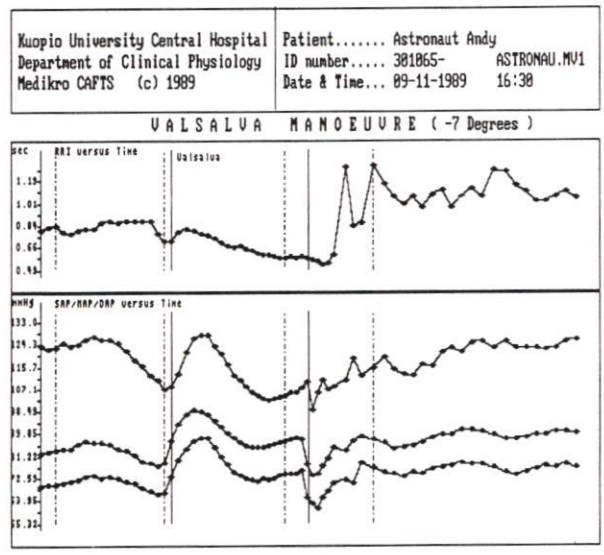


2a

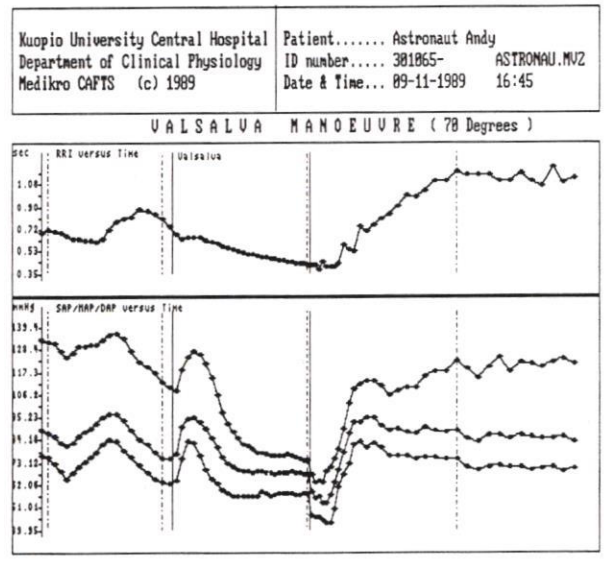


2b

Fig.2.: Power spectral estimation of RRI (upper panels) and SAP (lower left) and DAP (lower right) in 0-G (a) and in 1-G (b).



3a



3b

Fig.3.: RRI and blood pressure response to Valsalva manoeuvre in 0-G (a) and in 1-G (b).

R-R interval and finger blood pressure to a standardized Valsalva manoeuvre is described in Fig.3a. in 0-G (-7 degrees) and in Fig.3b. in 1-G (70 degrees) body position. A markedly greater decrease of finger pulse pressure is observed in Fig.3b. in 1-G position.

DISCUSSION

The role of autonomic nervous regulation in circulatory adaptation to gravitational changes is an essential part of space research.

The non-invasive system described (Medikro CAFTS) is designed for bed-side evaluation of autonomic control of heart rate and arterial blood pressure. It is a menu-driven IBM PC/AT based comprehensive evaluation system with on-line help for clinical and research problems involved to cardio-vascular autonomic function and

dysfunction. The system includes evaluation of heart rate and blood pressure variability both in time domain and frequency domain (power spectral analysis).

Some of Medikro CAFTS applications include:

- evaluation of heart rate and blood pressure response to simulated microgravity
- beat-to-beat evaluation of orthostatic hypotension
- evaluation of sympathetic/parasympathetic balance of R-R interval and blood pressure using power spectral analysis
- evaluation of autonomic neuropathy (e.g. in diabetes and Parkinson disease)
- evaluation of baroreflex sensitivity in hypertension and after myocardial infarction using intravenous phenylephrine test
- drug interventions
- evaluation of heart rate and blood pressure response to mental loading

The preliminary data obtained by our group indicates that vagal and sympathetic changes occurring during tilting from 1-G to 0-G are not just reversal when compared to the changes in tilting from 0-G to 1-G. The more drastical changes of RRI and blood pressure may be partly explained by the passive manoeuvre of tilting without muscular effort from 1-G to 0-G.

Power spectral approach to the stationary signals in different body positions gives a deeper insight to the basic regulatory mechanisms. Increase of variability in the HF (parasympathetic) band indicate an increase of vagal tone in 0-G.

To assess adaptation to gravitational stress, a battery of cardiovascular tests included in the system described may provide new information of the problem.

REFERENCES

Levin A.B.: A simple test of cardiac function based upon the heart rate changes induced by the Valsalva maneuver. Am J Cardiol 1966; 18:90-99.

Wheeler T. and Watkins P.J.: Cardiac denervation in diabetes. Br Med J 1973; 4: 584-586.

von Drischel H., Fanter H., Gurtler H., Labitzke H. and Priegnitz F.: Das Verhalten der Herzfrequenz gesuner Menschen beim Ubergang vom Liegen zum Stehen. Arc Kreislaufforsch 1963; 20, 190-194.

Ewing D.J., Irwing J.B., Kerr F., Wildsmith A.W. and Clarke B.F.: Cardiovascular responses to sustained handgrip in normal subjects and in patients with diabetes mellitus: a test of autonomic function. Clin Sci Mol Med 1974; 46: 295-306.

THE EFFECT OF LBNP (LOWER BODY NEGATIVE PRESSURE) ON LOWER LIMB CAPACITANCE VESSELS DURING A 30 DAY -6° HEAD- DOWN BEDREST.

F. Louisy, C. Gaudin, J.M. Oppert, A. Güell and C.Y. Guezennec.

Centre d'Etudes et de Recherches de Médecine Aérospatiale
5 bis, avenue de la Porte de Sèvres
75731 Paris Cedex 15 - France.

INTRODUCTION

The response of lower limb capacitance vessels to microgravity is thought to be one of the determining factors of the orthostatic intolerance syndrome observed in astronauts when they return to earth (4, 8). We studied the leg venous distensibility of six subjects exposed to -6° head-down bedrest with two objectives : 1) to test the validity of -6° head-down bedrest as a ground model to simulate the effects of weightlessness on lower limb capacitance vessels. Indeed, increases in leg venous distensibility have been reported during spaceflight (8) but it is not known wether a long-term bedrest acts as long-term spaceflight 2) to test the effect of LBNP on venous distensibility changes. In fact, during spaceflight, LBNP is used as a countermeasure against cardiovascular deconditioning (5). The question is : does the preventive action of LBNP depends on a specific action on the lower limb capacitance vessels ? This hypothesis would be verified if lower limb venous tone is controlled by neuro-adrenergic mechanisms. Thus, this experiment would provide a better understanding of venous tone regulation in the legs.

MATERIAL AND METHODS

Leg venous distensibility was assessed by strain gauge plethysmography with venous occlusion (3, 9). This technique consists in measuring leg venous volume changes at different levels of counterpressure applied at the root of the lower limbs.

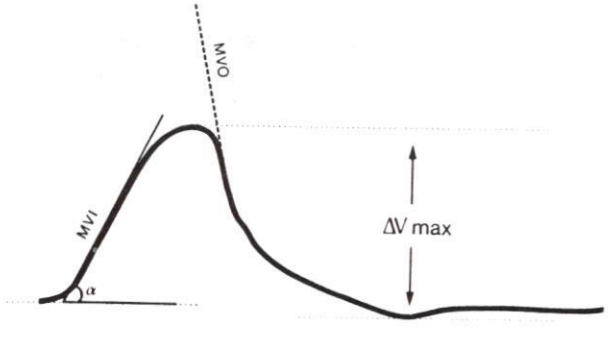


Figure 1. Variations in signal in leg volume obtained by mercury strain gauge plethysmography. ΔVmax = volume of venous emptying.

The nature of the plethysmographic signal is the following (figure 1) : when a given counterpressure is applied on the thigh, calf volume increases rapidly because of the cessation of venous outflow and the maintenance of arterial inflow. After a few minutes, calf volume reaches a plateau as venous pressure equals cuff pressure. By deflating the cuff, calf volume decreases towards the initial value and the total change in calf volume represents the volume of emptying. It is expressed as ΔVmax (in milliliters per 100 milliliter of tissu). It has the value of a venous capacity i.e the maximum quantity of blood which can be contained in the leg venous network at the considered counterpressure. It therefore measures the distensibility of the venous network at a given counterpressure. If cuff pressure is successively increased to 25, 30, 35, 40, 45 and 50 mmHg, six different ΔVmax are obtained. So, under these conditions lower limb venous distensibility is measured between 25 and 50 mmHg.

The six subjects underwent twice the bedrest experiment with a one-year interval, once with LBNP used as a countermeasure (LBNP group), once without LBNP (control group).Thus throughout the entire experiment we had six subjects in each group (LBNP and control), each one being his own control.

When LBNP sessions were programmed, the protocol for its application was the following : three daily exposures to - 35 mb for 20 minutes during the first three weeks of bedrest; during the fourth week, two protocols were applied i.e four daily LBNP exposures at - 35 mb for 20 minutes for the first four days and six daily LBNP exposures at - 35 mb for 20 minutes for the last three days. Heart rate, systolic and diastolic pressures were monitored during each LBNP exposure.

For each subject, leg volume changes ΔVmax were measured four times during a bedrest session. The first measurement (control = D_c) was made four, six or seven days before the start of bedrest. Other measurements were made on the 6th day (D_6) and on the 20th day (D_{20}) of bedrest, and on the 5th day of recovery (D_{+5}).

RESULTS

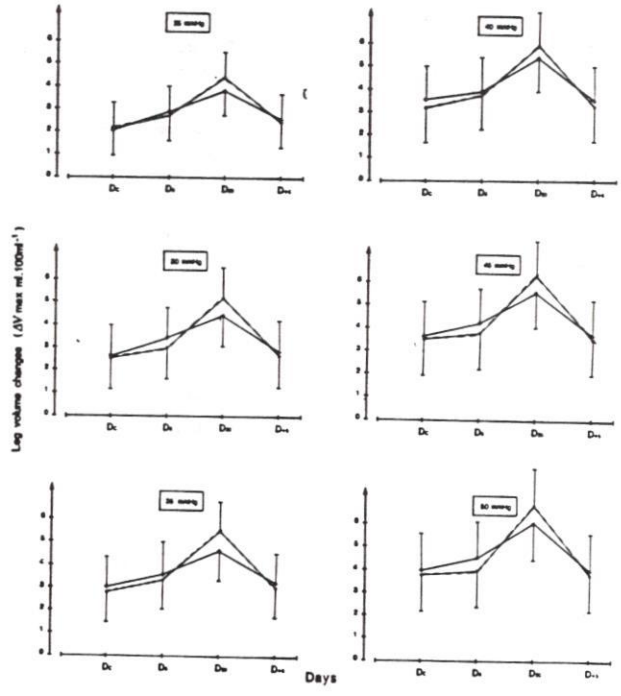


Figure 2. Evolution of ΔVmax during bedrest (Dc, D6, D20, D+5) at different levels of occlusion counterpressure (25, 30, 35, 40, 45 and 50 mmHg).

Mean results of venous capacity measurements are presented in figure 2 that shows the changes in Vmax before (D_c), during (D_6 and D_{20}) and after (D_{+5}) bedrest for each counterpressure applied to the root of the leg (25, 30, 35, 40, 45 and 50 mmHg). Dotted lines connect mean values of ΔVmax in the control group, solid lines connect mean values of ΔVmax in the LBNP group. Both in control and LBNP groups, there is a significant difference in venous capacity values depending on time and this is true for all counterpressures (the p of ANOVA for comparison between days is lower than 0.001 at all level of counterpressure). Compared to the control value, a gradual increase in ΔVmax is observed during bedrest. For instance, in the LBNP group, at 25 mmHg, for ΔVmax (ml.100 ml^{-1}) = 2.07 ± 0.71 on D_c, ΔVmax = 2.85 ± 1.19 (+37.7%) on D_6, 3.75 ± 1.74 (+81.2%; $p < 0.05$) on D_{20}. In the control group, ΔVmav = 2.11 ± 0.54 on D_c, 2.69 ± 0.29 (+27.5%) on D_6 and 4.39 ± 2.08 (+108.1%; $p < 0.05$) on D_{20}. On the 5th day of recovery, venous capacity values had practically returned to baseline : ΔVmax = 2.43 ± 0.94 on D_{+5} for the LBNP group and 2.39 ± 0.69 on D_{+5} for the control group ($p < 0.05$). No significant LBNP effect was observed : the p value for the ANOVA test in comparing LBNP and control groups is higher than 0.05.

DISCUSSION.

The first finding in this experiment is that head-down bedrest at -6° increases the distensibility of capacitance vessels in the lower limbs. Thornton and Hoeffler (8) reported increased venous capacities at 30 mmHg couterpressure in Skylab 4 astronauts. These increases were respectively 2.1, 5.3 and 7 ml.100 ml^{-1} for the commander, the pilot and the scientist on the 15th day of flight. They were not very different from mean results obtained in our subjects on the 20th day of bedrest (4.27 ± 2.5 ml.100 ml^{-1} for LBNP subjects, 5.05 ± 2.19 ml.100 ml^{-1} for control subjects) at the same counterpressure of 30 mmHg. Bedrest can therefore be considered as a good model to simulate the effects of microgravity on the venous hemodynamics of lower limbs. The second finding is that during exposure to head-down bedrest, leg venous capacity changes are not statistically different when LBNP and control groups are compared. This means that LBNP seems not to be a good procedure to counteract the adverse effects of weightlessness on venous distensibility. Such a finding could be explained by the following hypothesis : at the level of counterpressure used, transmural pressure across the vein must result in stretching of venous wall. As was stated by Buckey and Coll. (2), the veins involved in this response are predominantly the deep veins that comprise the majority of the venous volume of the legs (85%). These veins are thin-wall conduits with little intrisic smooth muscles and sympathetic innervation, and the response of such vessels to plethysmography or to any orthostatic stimulus should be more dependent upon the surrounding somatic muscles than upon cardiovascular reflexes involving neuro-adrenergic mechanisms (6, 7). Thus, our interpretation is that the increase in venous distensibility during exposure to real or simulated weightlessness is determined by surrounding muscles that are known to atrophy and lose their tone under these conditions. Probably the lack of significant effect of LBNP on venous dilation could be explained by its inability to act on muscle tone considering that one of its protective actions is to preserve the sympathetic nerve activity in smooth muscles of the vascular wall (1, 10).

In conclusion 1) long term head-down bedrest is a good model to study the adverse effects of weightlessness on the capacitance vessels of the lower limbs 2) LBNP seems not to be an efficient mean of preventing these effects because of the predominant role of skeletal muscles in controlling capacitance of the deep leg veins. As it has been showed elsewhere that LBNP is indeed a good way to prevent postflight orthostatic intolerance we have to address the problem of the contribution of leg venous hemodynamics to this syndrome.

REFERENCES.

1. **Baisch F, Beier J, Gebhardt U, Hordinsky JR and Morsbach H.** Lower Body Negative Pressure (LBNP). A method of controlled circulation disorder. In : Zero-G simulation for ground based studies in human physiology, with emphasis on the cardiovascular and body fluid systems. ESA SP - 180, 43 - 75, 1982.

2. **Buckey JC, Peshock RM and Blomquist CG.** Limb compliance by NMR : deep venous contribution to volume changes (abstract). JACC, 5 (2), 537, 1987

3. **Kenney LW and Armstrong GC.** The effect of aerobic conditionning on venous pooling in the foot. Med Sci Sports Exerc., 19 (5), 474 - 479, 1987.

4. **Panferova NY.** Vascular tone in different parts of the body during prolonged restriction of muscular activity. Kosmicheskaya Biol i Med., 6, 74 - 79, 1972.

5. **Pestov ID and Asyamolov BF.** Negative pressure on the lower part of the body as a method for preventing shifts associated with changes in hydrostatic pressure. Kosmicheskaya Biol i Med., 6, 59 - 64, 1972.

6. **Pollack AA and Wood EH.** Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. J Appl Physiol, 1, 649 - 662, 1949.

7. **Shepherd JT.** Role of venoconstriction for circulatory adjustements to orthostatic stress. In : The sympathoadrenal system, Alfred Benzon Symposium 23, Eds : Christiensen NJ, Henriksen O and Lassen NA, Munksgaard, Copenhagen, 103 - 105, 1986.

8. **Thornton WE and Hoeffler GW.** Hemodynamic studies of the leg under weightlessness. In : RS Johnson and LF Dietlein (Eds), Biomedical results from Skylab (NASA SP - 377), US Government Printing Office, Edit, Washington DC, 1977.

9. **Whitney RT.** The measurements of volume changes in human limbs. J Physiol, 121, 1 - 27, 1953.

10. **Wolthuis RA, Bergman SA and Nicogossian AE.** Physiological effects of locally applied reduced pressure in man. Physiol Rev., 54 (3), 566 - 595, 1974.

GRAVITY, CALCIUM, AND BONE: UPDATE, 1989

Sara B. Arnaud and Emily Morey-Holton
Life Science Division, NASA Ames Research Center
Moffett Field, CA 94035-1000

This update highlights some of the results of recent short-term flight and ground-based experiments that have contributed new insight into skeletal adaptation, calcium metabolism, and growth processes in 0 g. After 6 months in space, bone demineralization, invariably involving the os calcis (20), was found not to extend to the lumbar spine in 4 exercising cosmonauts (3). A flight experiment in the Space Shuttle crew has documented the early events in the calcium endocrine system during spaceflight (12).

On the ground, brief (<35 days) and long-term (>4 months) bed rest studies of healthy volunteers in the head-down tilt (HDT) model of weightlessness have been completed. The skeleton of the adult male responds more rapidly to unloading than previously recognized (2). Regional changes in bone density can be quantified in only 30 days, are highly individual, and follow the direction of gravitational forces in the HDT model during inactivity (1). Bone biopsy results in healthy volunteers after bed rest (11) differ from results in paraplegics from the same sampling site (21).

Flight experiments in growing rats reveal changes in the composition of bone mineral and matrix in the femur postflight that were found to be highly regional and suggestive of an effect of gravity on mineral distribution (10). These observations may be relevant to the results from an earlier Cosmos flight where artificial gravity in space was found to maintain bone strength, but not to correct the radial growth deficit (19).

Mineral in the Lumbar Spine

Ever since Krolner and Toft reported a reduction (–3.8%) in the average density of the 2nd to 4th lumbar vertebrae following therapeutic bed rest in 28 patients suffering from prolapsed intervertebral discs, there has been some concern that vertebrae in bed rest subjects and space travelers may demineralize (7). Fortunately, no significant changes were observed by Drs. Cann and Oganov, who used quantitative computer tomography to quantify the mineral content of the body of the 2nd lumbar vertebra of 4 cosmonauts before and after 6 months in space (3). These data have not completely erased the concern of osteoporosis in the lumbar spine because the Cosmonauts exercised daily.

Nevertheless, nonexercising bed rest subjects have also failed to show reduced bone density in the lumbar spine. LeBlanc et al. found no change in the density of the 2nd through 4th lumbar vertebrae in 5 of 6 subjects after 5 weeks of bed rest (horizontal); one showed a 3% decrease (8). Oganov et al. reported average increases to 12.6% in density of the spongiosa of the lumbar vertebrae of 3 bed rest subjects after 120 days in a –5° head-down tilt position (HDT) (14).

We used dual photon absorptiometry to measure the density of the 2nd through 4th lumbar vertebrae before and after 30 days HDT (–6°). Subjects were participating in a study designed to test the effect of isokinetic and isotonic exercise on orthostatic tolerance (5). Our results, shown as percent change in the histogram in Fig. 1, revealed no differences in 17 subjects, irrespective of the exercise group. Two subjects showed changes in opposite directions (–7 and +10%), well outside the error of the test.

Calcium Endocrine System

To identify factors that could have contributed to the change in lumbar spine mineral in 2 of 19 subjects, we examined the diet and a variety of parameters known to influence bone metabolism. Table I enumerates the values in these two subjects before and after bed rest. Figures 2a and 2b illustrate changes in circulating hormone concentrations that may be related to the alteration in lumbar spine density. None of the values except for serum parathyroid hormone (PTH) reached values outside the normal range. Combined increases in serum PTH and weight loss (4) favored decrease in lumbar spine mineral; the opposite changes were associated with an increase. These data are consistent with the known effects of PTH to enhance bone resorption. The precise role of serum 1, 25-dihydroxyvitamin D in the change in bone mineral content is not clear.

The early response of the calcium endocrine system in 4 astronauts was documented in serum obtained before, during, and after 7 days in space on the SL2 mission (12). The published data are summarized in Fig. 3. An increase in the vitamin D hormone, 1, 25-D, within the first 36 hours of launch was the only significant change, although trends toward increases in total serum calcium and phosphorus and decreases in bioactive PTH were present in 3 astronauts. Possible explanations for the early increase in 1, 25-D include perturbations during launch, transient lack of dietary calcium associated with space motion sickness, a nonspecific stimulation of renal 1-alpha hydroxylase connected with fluid shifts, or a specific

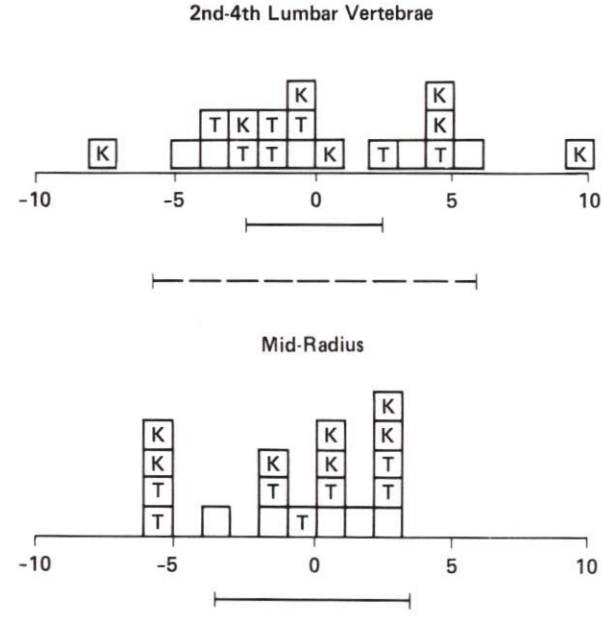


Figure 1. Percent change in density of the lumbar spine and mid-radius of 19 men referenced to each subjects basal level (0). Distribution was not affected by exercise (isotonic = T, isokinetic = K, no exercise = blank). The error of the tests are indicated by the bars, ⊢⊣ and $2 \times \sqrt{2} \times$ coeff. of variation, by (⊢------).

Table I. Comparison of clinical data in two exercising subjects who showed opposite changes in lumbar spine density after 30 days head-down tilt bed rest.

	A			B		
Study day, from 1st bed rest day	–5	4	27	–5	4	27
Age, years	42			37		
Weight, kg	68		68.6	83.8		82
Height, cm	171			183		
Plasma volume,[a] ml/kg	46.3		40.5	48.2		39.9
Body fat,[a] %	7.7			8.0		
Serum						
Total calcium, mg/dl	9.1		8.8	9.9		9.8
Ionized calcium, mg/dl	4.20		5.00	4.88		4.96
Total protein, g/dl	7.2		7.0	7.2		7.2
Phosphorus, mg/dl	2.0		3.2	2.3		2.3
Parathyroid hormone,[b] pg/ml	20	24	17	24	59*	44
1, 25-dihydroxyvitamin D, pg/ml	16	17	37	33	28	30
Cortisol,[c] ug/dl	16.9		15.6	9.5		11.3
Testosterone,[c] total, ng/ml	882			871		
Testosterone,[c] free, ng/dl	25			22.2		
Urine						
Creatinine clearance, ml/min/1.73m^2	128		123	108		118
Calcium, mg/24 hr	229		165	182		301
Hydroxyproline, mg/24 hr	21		15	36		42
Diet during study[d]						
Calories, kCal/kg	42		45	34		36.8
Calcium, mg	1281		1398	1274		1431
Phosphorus, mg	1816		1959	1883		2020
Sodium, mg	5756		5941	5615		5976
Protein, g	117		119	114		119
Bone density[e]						
Radius, gm/cm	1.304		1.337	1.422		1.434
Lumbar spine, L2-4, gm/cm^2	1.175		1.293	1.875		1.725

Analysis of [a] by J. Greenleaf, [b] by R. Marcus, [c] by C. Wade, [d] by R. Williams, and [e] by M. Powell.
*Above the normal range.

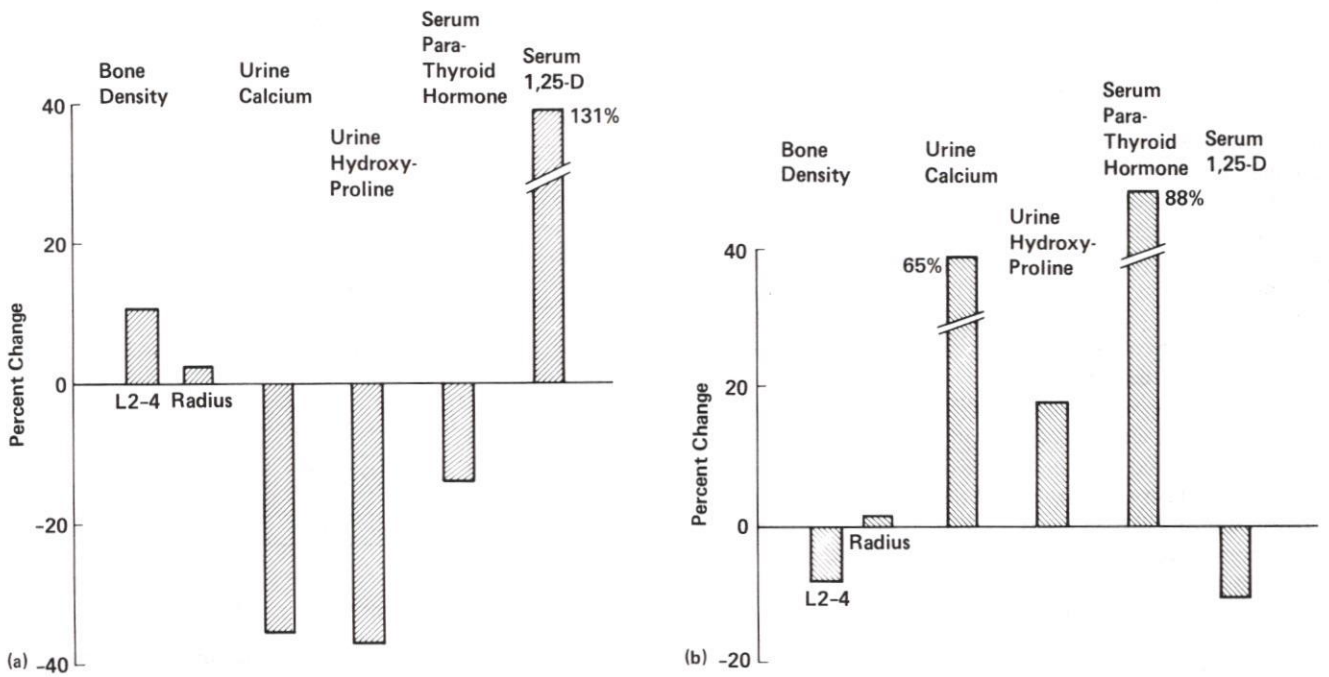


Figure 2. Changes in some parameters of calcium homeostasis, referenced to pre-bed rest levels, in subject A (a) who showed an increase, and subject B (b) who showed a decrease in lumbar spine density after 30 days bed rest. Of interest, both subjects performed isokinetic exercise for 30 min twice daily (5).

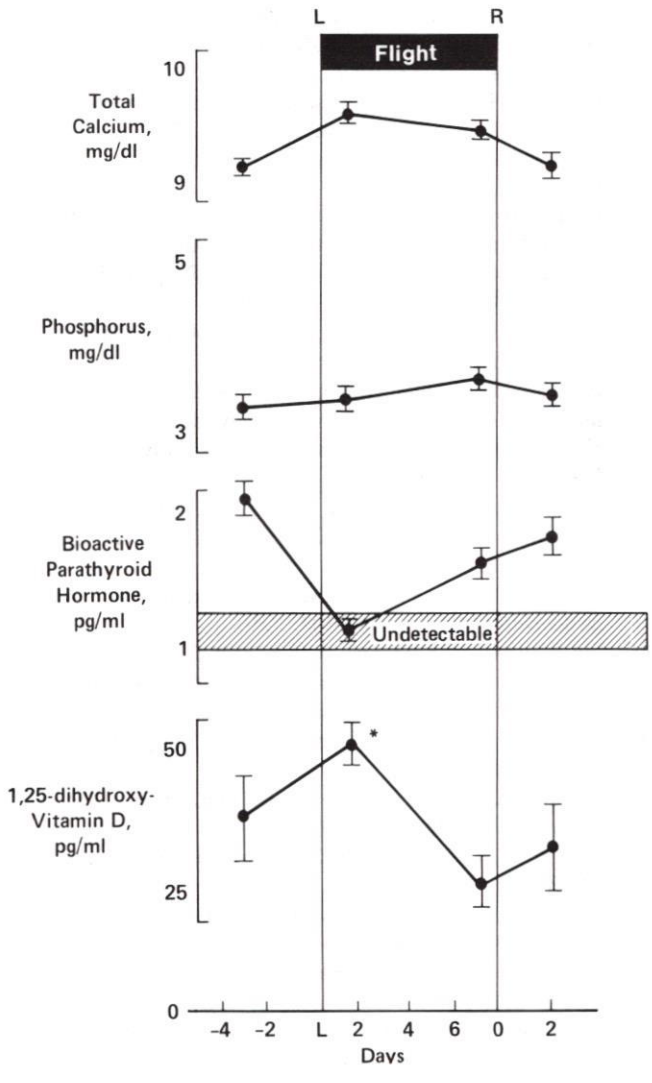


Figure 3. Mean values (±SE) in the serum of four astronauts obtained 1 week before, during, and the first week after launch (L) of a 7-day shuttle spaceflight (SL2) (data replotted from reference 13).

response in vitamin D metabolism to a change in a biomechanical stimulus originating in bone or muscle. Differences in the values during the first 24 hours did not seem to affect 7-day values, which are in the direction of being lower, but are not different from preflight values. The important contribution of these few samples taken during a flight is the preliminary knowledge that biologically active PTH, undetectable in serum after 36 hours in space, was not increased after 7 days, nor was 1, 25-D. While excesses of serum PTH cannot be responsible for the early mobilization of bone calcium, transient increase in 1, 25-D may be.

The above short-term data in flight differs from the results of a 7-day HDT study conducted at Ames Research Center, in which no changes were found during the first 36 hours. However, after 7 days, the trends to lower serum PTH and 1, 25-D in flight and on the ground were similar (11). The long-term Soviet bed rest study shows changes compatible with parathyroid hyperplasia with increases in serum calcium and PTH (especially after 49 days), suggestive of differences in early and late responses in the calcium endocrine system (13).

Of interest, in the Soviet bed rest study, were early increases in serum levels of calcitonin, an inhibitor of bone resorption, that gradually decreased to lower than basal levels after 3 months. Given the variations in both assay methods and bed rest protocols, the status of the calcium endocrine system, at least, after the first week in space or bed rest in healthy individuals, remains uncertain.

Bone Morphology

If newer concepts in the role of PTH and 1, 25-D in the processes of bone remodeling are correct, i.e., that PTH governs the differentiation and number of bone cells, and 1, 25-D, cell activity (9), the pattern of circulating hormone levels from the SL2 mission suggests the following early sequence of events: enhanced mobilization of calcium from bone related to the increase in 1, 25-D followed by suppressed mineralization in unloaded bones after a few days, with no increase in the number of osteoclasts or osteoblasts. Standard post mortem examination of some of the bones of 3 Cosmonauts after 28 days in space showed normal histology, fewer vascular channels than a control sample, and some increase in the porosity of the femoral epiphysis and diaphysis, but not in the rib, vertebrae, or calcaneus (16). Jowsey's analysis of the iliac crest of patients after 4-17 days horizontal bed rest for conditions unrelated to the skeleton, demonstrated reduced bone formation and no difference in the extent of resorption surfaces from normal in 11 of 14 patients. Cell counts are not in the report (6).

Following a 4-month period of bed rest in 3 healthy Soviet volunteers, Vico et al. found a two-fold increase in resorption surfaces, no increase in cell number, and reduced bone formation rate in specimens from the iliac crest (21). A puzzling observation was no measurable change in the volume of bone in healthy bed rest subjects, unlike patients with paraplegia (11). That the normal subject shows changes in surface morphology indicative of bone loss with no apparent diminution in volume at the two-dimensional level, suggests some form of compensation in microarchitecture. Either standard measurements may not be sensitive enough to detect losses in volume or other measurements involving the three-dimensional structure of bone, not usually done, may be needed to show how normal subjects maintain bone volume.

Gravity-Dependent Gradients of Mineralization

Comparison of the increments in whole-body calcium of rats exposed to 0, 1, and 2 g reveals accumulation of bone mineral directly related to the gravitational force (15). The mechanism of this acquisition of skeletal mineral must involve systemic as well as local processes. The cardiovascular system, whose general structure is oriented in the direction of gravity and where blood vessels, flow, and volume are known to differ at the local bone level in active and inactive individuals, is the most obvious candidate to influence bone mass.

Until recently, however, there were no data that suggested that there was a generalized cardiovascular effect on bone or that a shift of the hydrostatic column of pressure with changes in position, was associated with changes in bone mineral. In the tail-suspended rat, Roer and Dillaman found the expected decrease in ash in the bones of the unloaded lower extremities, no change in the humerus and ulna, and

importantly, an increase in bone ash in the skull (17). By dual photon absorptiometry, the density of the head region of adult bed rest subjects was found to be increased an average of 10% after a 30-day HDT study (1). These two studies suggest a gravity-dependent distribution of mineral in the whole skeleton, which may be a function of changing pressures, fluid flow, or volume in the cardiovascular system in response to change in position.

During the Cosmos 936 mission, centrifugation in orbit permitted comparison of the effects of gravity on the strength and growth of the femur of young rats in space (19). Rats treated with artificial gravity showed the same increases in density and strength during the 18.5-day flight as ground controls; however, the growth defect was not improved. Spengler et al. attributed the growth deficit to poor adaptation of the rats to the short-arm radius centrifuge and concluded that centrifugation normalized material properties, i.e., quality, but not the quantity of the femur. These paradoxical findings following artificial gravity could be explained by the recently observed linear gradients of mineralization in the diaphysis of the femur (10). At 1 g bone mineral concentration was lower in the distal than in the proximal diaphysis of the femur of the 14-week old rat, a disparity that persists in flight, but tends to disappear by 16 weeks on Earth. Because of the logistical problems connected with the 1887 flight where these diaphyseal gradients of mineralization were observed, and because the results differ from our expectation that mineral deposition proceeds from the center of a growing bone proximally and distally, confirmation of this observation is needed. Collectively, all of the above studies reveal an important connecting link between gravity, per se, and bone mineral distribution and deposition, most likely related to the cardiovascular system. The interaction of what appear to be gravity-dependent gradients visible at the whole-body and organ level, with the highly regulated processes that change bone structure at the local tissue level in response to biomechanical forces is not now apparent.

In summary: Advances in recent years have enabled us to recognize that two principal components of calcium metabolism, the calcium endocrine system and bone, respond promptly (within days), to changes in body position and weightlessness. The vitamin D hormone may be the best candidate for mobilizing bone mineral early, and newly identified gravity-dependent gradients, probably involving the cardiovascular system, may have a significant role in its distribution at the whole-body level. These observations have given us a new perspective on the results of balance studies in healthy subjects and astronauts (18,22). During inactivity or weightlessness, negative balances in bone minerals may be more directly a reflection of diets, and alterations in the function of the gastrointestinal tract and kidney that parallel, but do not necessarily derive from the highly localized activities concerned with the restructuring of bone and redistribution of bone mineral to meet new functional requirements. These studies imply that bone biomechanics are more severely affected by spaceflight than bone mass.

Acknowledgment: This work was supported by NASA programs 199-26-12, 199-26-22, and 199-18-12.

References

1. Arnaud SB, MR Powell, J Vernikos-Danellis, et al. *J. Bone Mineral Res.* 3:S119, 1988.
2. Arnaud SB, DJ Sherrard, N Maloney, et al. *J. Bone Mineral Res.* 4:S233, 1989.
3. Cann CE. Technology assessment: Calcium homeostasis and bone demineralization research. *In: USRA Proceedings: Calcium Science Working Group.* Sept. 1987, p. 97.
4. Greenleaf JE, EM Bernauer, LT Juhos, et al. Effects of exercise on fluid exchange and body composition in man during 14-day bed rest. *J. Appl. Physiol*: Respirat. Environ. Exercise Physiol. 43(1):126-1432, 1977.
5. Greenleaf JE, CE Wade, G Leftheriotis. *Aviat. Space Environ. Med.* 60: 537-42, 1989.
6. Jowsey J. Bone at the cellular level: the effects of inactivity. *In: Hypogravic and Hypodynamic Environments*, ed. RH Murray and M McCally. NASA SP-269, 1971, p. 111-119.
7. Krolner B, B Toft. *Clin. Sci.* 64:537-540, 1983.
8. LeBlanc A, V Schneider, J Krebs, et al. *Calcif. Tissue Int.* 41:259-261, 1987.
9. Malluche HH, C Matthews, M Faugere, et al. *Endocrinology* 119:1298-1304, 1986.
10. Mechanic GL, SB Arnaud, A Boyde, et al., Regional distribution of mineral and matrix in the femurs of rats flown on Cosmos 1887 biosatellite. *Faseb J.*, in press.
11. Minaire P, P Meunier, C Edouard, et al. *Calcif. Tissue Res.* 17:57-73, 1974.
12. Morey-Holton ER, HK Schnoes, HF DeLuca, et al. *Aviat. Space Environ. Med.* 59:1038-41, 1988.
13. Morukov BV, OI Orlov, AI Grigoriev. *The Physiologist* 32:S37-S40, 1989.
14 Oganov VS, AS Rakhmanov, BV Morukov, et al. *Moscow Kosm. Biol. Aviakosm. Med.* 22:30-33, 1988.
15. Pace N. AH Smith, DF Rahlman. *The Physiologist* 28:S17-S20, 1985.
16. Prokhonchukov AA, NA Zhizhina, RA Tigranyan. Homeostasis of bone tissue under normal and at extremal action. *In: Problems in Space Biology*, ed., PD Gorizontov, Nauka Press, Moscow, 1984, p. 152-165.
17. Roer R, R. Dillaman. Bone growth and calcium balance during simulated weightlessness in the rat. *J. Appl. Physiol.*, in press.
18. Schneider VS, J McDonald. *Calcif. Tissue Int.* 36:S151-S154, 1984.
19. Spengler DM, ER Morey, DR Carter, et al. *Proc. Soc. Exp. Biol. Med.* 174:224-228, 1983.
20. Stupakov GP, VS Kazeykin, AP Kozlovskiy, et al. *Space Biol. Med.* 18:42-47, 1984.
21. Vico L, D Chappard, C Alexandre, et al. *Bone Mineral* 2:383-394, 1987.
22. Whedon GD, L Lutwak, PC Rambaut, et al. Mineral and nitrogen metabolic studies, experiment MO71. *In: Biomedical Results from Skylab*, ed. R Johnston and L Dietlein. NASA SP-377, 1977, p. 164-174.

CATECHOLAMINES DURING SHORT- AND LONG-TERM HEAD-DOWN BEDREST.

JM. COTTET-EMARD*, JM. PEQUIGNOT*, A. GUELL**, L. PEYRIN* and C. GHARIB*

*Lab. Physiol., Fac. Méd. Lyon Grange-Blanche 69373 LYON Cédex 08, France
**CNES, 31055 TOULOUSE, France

On earth the standing humans are exposed to a hydrostatic pressure due to gravity which causes a fluid shift towards the lower part of the body. When changing from the standing to the supine position, the body fluid distribution is modified and the adaptation to the new position involves neurohormonal mechanisms.

Similarly, weightlessness causes a marked shift of fluids towards the upper part of the body and thus elicits strong physiological alterations which have been regularly described during the American or Soviet space flights. These alterations include cardiovascular deconditioning, bone demineralisation, orthostatic intolerance, reduced muscle capacity. Furthermore, some of these effects are amplified after landing.

Several simulations of microgravity have been used in order to study these alterations and to find countermeasures.

The horizontal or head-down bedrest is a simple method wich allows long investigations (one month or more) and is able to induce cardiovascular effects similar to those produced by microgravity. This is currently the most used model.

The sympathetic nervous system is involved in the cardiovascular regulatory processes and in particular is stimulated when changing from the horizontal to the vertical position. It is therefore to be expected that the sympathetic nerve activity may be altered during bedrest.

The influence of bedrest on catecholamines has been studied only recently after sensitive assays for plasma levels had been developed. For this reason there are few studies and the results vary according to the control position used. Only the data relating to humans or to monkeys will be discussed here and we will examine successively the short-term and long-term effects of bedrest.

SHORT-TERM EFFECT OF BEDREST -

London et al (1983) found decreased levels of plasma catecholamines after half an hour of head-down tilt at -10° but the control values in supine subjects are about 2-3 times higher than those regularly reported in the literature. The high level of catecholamines may reflect a stressful situation probably due to the sophisticated environment in this complex experiment including a measurement of central venous pressure. In contrast, Goldsmith et al (1985) failed to show any changes in plasma norepinephrine (NE) after one hour of head-down tilt at -30° as compared to supine controls.

Also during a head-down bedrest (HDBR) lasting up to 4 days performed in our laboratory there was no change in plasma catecholamines compared to supine controls (Pequignot et al, 1985). After this study, we wondered whether if the supine position was a good control because the main shift of the body fluids has already been achieved during the change from the standing to the horizontal position. It is thus possible that we overlocked the expected catecholamine response.

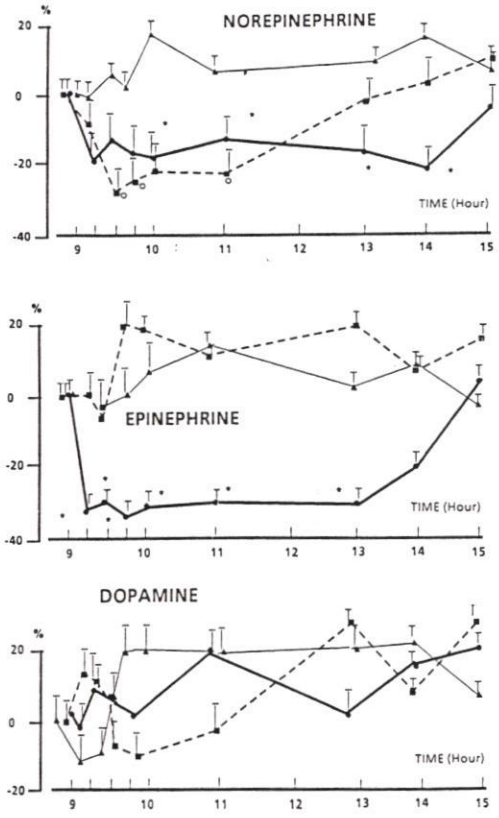


Fig 1. Time course of plasma catecholamines during 5 hours of: ◆——◆ sitting position; ■- - -■ supine position; ●—● head-down tilt (adapted from Gharib et al., 1988)

Therefore, in order to study the effects of the control position, Gharib et al (1988) studied the changes in plasma catecholamines induced by a horizontal bedrest and by a HDBR (-10°). Both conditions lasted for five hours and the control subjects were sitting. As expected, there was a rapid decrease in plasma NE (about -20%) during the horizontal bedrest but interestingly the NE fall was similar when the subjects were head-down tilted (Fig. 1). Epinephrine (E) was diminished only during the HDBR and dopamine (DA) was not altered.

LONG-TERM EFFECT OF BEDREST -

All studies have been performed with ambulatory control. Chobanian et al (1974) reported that the horizontal bedrest (28 days) elicited a decrease in plasma NE which was not significant (Fig. 2). Sandler et al (1978) showed in women a significant decrease in urinary NE after 17 days of horizontal bedrest (-48±7%). It is worth noting that during the post bedrest recovery the urinary NE output was higher than in controls (+71±26%).

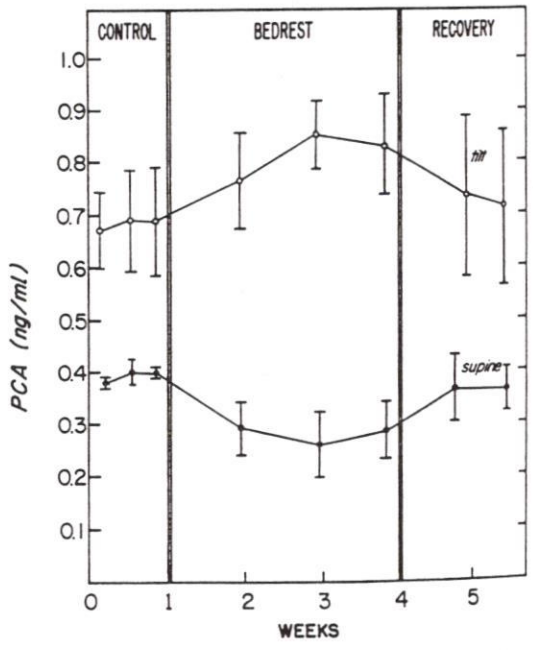


Fig 2. Plasma catecholamines (PCA) during a 28 days horizontal bedrest:●—●recumbency; O—O passive tilt test.(adapted from Chobanian et al.,1974)

In our laboratory Cottet-Emard et al (personal data,. 1989) have studied the effects of the HDBR (-6°, 28 days). They found a decrease in plasma NE which did not reach the of level significance (Fig. 3). The decrease was of the same magnitude as that reported by Chobanian in horizontal bedrest. In contrast to NE, E was increased up to 15 days but the variations between subjects were very high. DA did not change.

Quite recently, Karemaker et al (1989) reported a decrease in urinary output of NE during HDBR (-6°, 10 days). The laboratory data are in agreement with the few data available from space flights. Indeed, Leach et al (1981) showed a sustained decrease in urinary NE during the Skylab flights lasting at least two months. Kvetnansky et al (1989) also found a decrease in urinary metabolites of catecholamines during a Saliout flight.

DISCUSSION -

On the whole the data suggest a rapid and prolonged decrease in NE release during horizontal bedrest as well as during HDBR. The primary effect of the change from the standing to the supine position is the suppression

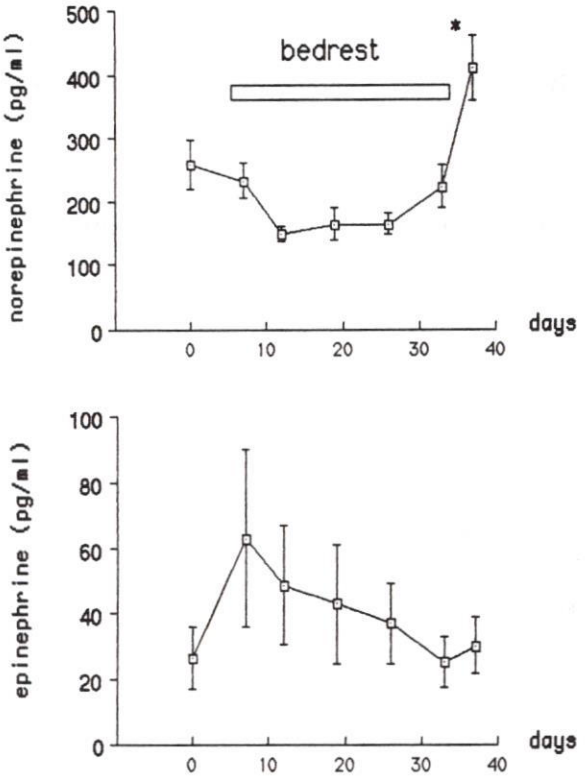


Fig 3. Time course of plasma catecholamines during a 28 days head-down bedrest (-6°)s .(Cottet-emard et al.,personal data, 1989)

of hydrostatic pressure exerted from the head downwards. The new fluid distribution after lying induces an immediate increase in plasma volume which is maximal after one hour. The fall in plasma level does not result from this hemodilution because the three catecholamines follow different patterns.

Also after lying there is an immediate increase in central venous pressure. Subsequently, the cardiopulmonary receptors are stimulated leading to a decrease in renal sympathetic nerve activity. After two hours of HDBR, Nixon et al (1979) found that the central venous pressure returns to baseline and even more diminishs after 24 hours. The initial central hypervolemia is rapidly corrected by an increase in diuresis and natriuresis. Thereafter, a progressive hypovolemia develops when prolonging the bedrest as shown by Greenleaf et al (1983). Thereafter, the influence of the stimulation of cardio- pulmonary receptors on sympathetic activity should be of short duration.

Now, the question is how to explain the sustained decrease in sympathetic nerve activity during long-term HDBR ? Of course, in this situation there is a strong decrease in physical activity which leads to a reduction in the energy output and cardiovascular adaptations occurring in ambulatory subjects. It is thus reasonable to assume that the recuced energy output and cardiovascular adjustments, which are primarily controlled by the sympathetic nervous system, induce a prolonged decrease in NE release. This assumption is supported by the early work of Schmid et al (1971) who found a reduced in release of vascular NE after 12 days of

bedrest. Actual decrease of NE release might not be reflected by the plasma level. Indeed, Linares et al (1988) have shown that the reduced volume of distribution of NE induced by a sodium restricted diet can increase the plasma NE level without any enhanced NE release.

In striking contrast, the sympathetic nerve activity is strongly enhanced during the post bedrest recovery. The plasma NE levels or urinary output is higher after bedrest than in controls. The cardiovascular responses to a tilt test are also enhanced compared to the controls and the NE release is exaggerated (Chobanian et al, horizontal bedrest). Cottet-Emard et al. have confirmed these data in HDBR. Similarly, Kvetnansky et al (1988) found also an activation of the sympathetic nervous system after landing (Saliout 7 flight, 237 days).

The sympathetic alterations following bedrest are not only caused by a persistent hypovolemia because restoring a normal volemia either by saline infusion or by regular muscle work does not abolish the orthostatic intolerance. The sensitivity of adrenoceptors is not altered by bedrest. In fact, the exaggerated sympathetic response might be explained not by a dysfunction of the adrenergic system but perhaps by a diminution of the arterial barosensitivity according to Billman et al (1982). Another possible cause is the decreased cardiac output which provokes a higher stimulation of the heart rate in response to tilt test. Finally, the venous tone might be also modified during the bedrest (Buckey et al, 1989, this symposium) but there is to date no clear evidence for any role of this factor in orthostatic intolerance.

CONCLUSIONS -

1/ During HDBR there is a decrease in sympathetic nerve activity. The early diminution in sympathetic tone might result from the stimulation of cardiopulmonary receptors due to the fluid shift to the upper part of the body. Thereafter the lack of physical activity, the decreased energy output, the reduction in cardiovascular adjustments might contribute to the diminution of sympathetic tone.

2/ HDBR elicits an exaggerated sympa- sympathetic response to the tilt test. This hetic response to the tilt test. This alteration appears to be multifactorial including hypovolemia, decreased cardiac output, modification of the barosensitivity and/or venous tone.

3/ It is difficult to compare the effects of HDBR and the few data obtained (particularly in plasma) from space flights because the astronauts are submitted to an environment able to modify quickly the environment able to modify quickly sympatho-adrenal activity.

Acknowledgments : This work was supported by grants of CNES, DRET, Fondation pour la Recherche Médicale.

REFERENCES -

1 - G.E. Billman, D.T. Dickey, H. Sandler and H.L. Stone. Effects of horizontal body casting on the baroreceptor reflex control of heart rate.
J. Appl. Physiol., **52** : 1552-1556, 1982

2 - J.C. Buckey, L.D. Lane, G. Plath, F.A. Gaffney, F. Baisch and G.G. Blomqvist. Effects of a 10 days of head-down tilt on the compliance of the lower limb.
11è Annual Meeting IUPS Commission on Gravitational Physiology, Lyon September 1989, 44

3 - A.V. Chobanian, R.D. Lille, A.D. Tercyak and P. Blevins. The metabolic and hemodynamic effects of prolonged bedrest in normal subjects.
Circul., **49** : 551-559, 1974

4 - C. Gharib, G. Gauquelin, J.M. Pequignot, G. Geelen, C.A. Bizollon and A. Guell. Early hormonal effects of head-down tilt (-10°) in humans. Aviat. Space Environ. Med., **59** : 624-629, 1988

5 - S.R. Goldsmith, G.S. Francis and J.N. Cohn Effect of head-down tilt on basal plasma norepinephrine and renin activity in humans. J. Appl. Physiol., **59** : 1068-1071, 1985

6 - J.E. Greenleaf. Bedrest studies : fluid and electrolyte responses. In "Space Physiology", 1983, CNES Ed., Cepadues Editions, Toulouse (France)

7 - J.M. Karemaker, A.D.J. Ten Harkel, D.L. Eckberg, J.M. Fritsch, H. Maass and L. Beck. Autonomic cardiovascular control during 10 days head-down tilt (HDT).
XXXI Int. Congr. Physiol. Sci., July 1989, Helsinki (Finland), p 1565

8 - R. Kvetnansky, N.A. Davydova, V.B. Noskov, M. Vigas, I.A. Popova, A.C. Usakov, L. Macho and A.I. Grigoriev. Plasma and urine catecholamine levels in cosmonauts during long-term stay on sapce station Salyut-7.
Acta Astronautica, **17** : 181-186, 1988

9 - C.S. Leach. An overview of the endocrine and metabolic changes in manned space flight. Acta Astronautica, **8** : 977-986, 1981

10 - O.A. Linares, L.A. Zech, J.A. Jacquez, S.G. Rosen, J.A. Sanfield, L.A. Morrow, M.A. Supiano and J.B. Halter. Effect of sodium-restricted diet and posture on norepinephrine kinetics in humans.
Am. J. Physiol., **254** : E222-E230, 1988

11 - G.M. London, J.A. Levenson, M.E. Safar, A.C. Simon, A.P. Guerin, D. Payen. Hemodynamic effects of head-down tilt in normal subjects and sustained hypertensive patients.
Am. J. Physiol., **245** : H194-H202, 1983

12 - J.V. Nixon, R.G. Murray, C. Bryant, R.L. Johnson, J.H. Mitchell, O.B. Holland, C. Gomez-Sanchez, P. Vergne-Marini and C.G. Blomqvist. Early cardiovascular adaptation to simulated zero gravity.
J. Appl. Physiol., **46** : 541-548, 1979

13 - J.M. Pequignot, A. Guell, G. Gauquelin, E. Jarsaillon, G. Annat, A. Bes, L. Peyrin and C. Gharib. Epinephrine, norepinephrine and dopamine during a 4-day head-down bed rest.
J. Appl. Physiol., **58** : 157-163, 1985

14 - H. Sandler and D.L. Winter. Physiological responses of women to simulated weightlessness. NASA SP-430, Washington 1978, 1-87

15 - P.G. Schmid, M. Mc Cally, T.E. Piemme and J.A. Shaver. Effects of bedrest on forearm vascular responses to tyramine and norepinephrine.
In "Hypogravic and Hypodynamic Environments", NASA SP-269, R.H. Murray and M. McCally Eds, Washington 1971, 211-223

Toward an univocous interpretation of cardiovascular biomechanics in hyper and microgravity

*Quandieu P., *Tran C.C., **Liebaert Ph., ***Gaffié D.

*Laboratoire Central de Biologie Aérospatiale (LCBA)
5 bis Ave de la Pte de Sèvres 75015 Paris France
**Direction des Recherches Etudes et technique (DRET-G9)
26 Bvd Victor 75996 Paris Armées France
***Office National d'Etudes et de Recherches Aérospatiales
29 Ave de la Division Leclercq 92320 Chatillon France

Mister President, Ladies and Gentlemen,

Originally, I was supposed to talk about cardiovascular effects of short duration hypergravity: I mean +Gz accelerations. Rather than imposing upon you a nth repetition of an already well documented, but not really well known question, I suggested to the organizing committee to tackle this exciting question not from its physiological standpoint but from its biomechanical standpoint which still has many unknowns.

Thank you very much for giving us the opportunity to explain how starting from the study of the loss of consciousness of fighter pilots under +Gz acceleration (GLOC) we propose the use of the physical laws of the deformable continuous media to explain it.

Using these laws:

- we can propose to complete the brain hypoxia theory with an intracranial hypertension syndrome to explain the GLOC
- we assume, the rapid inflation of the anti G suit may be potentially dangerous
- we think the cardiovascular biomechanics has to be studied with the same laws in hyper and microgravity.

As an invited speaker I will confine myself to generalities and I leave it to Mr. Liebaert Ph. and Mr. Gaffié D. to be more precise during this session.

Those of our colleagues who are not familiar with hypergravitation phenomena will easily understand that the behavior of biological fluids is opposite in two situations:

- a fluid shift toward the upper part of the body in microgravity
- a blood shift toward the lower part of the body in hypergravity, if:

*first: the hypergravity field is parallel to the axis of major blood vessels i.e. carotido-aorto-femoral arteries axis

*second: direction is headed from feet to head (+Gz).

The effects of the +Gz acceleration were modelized by Alice Stoll, years ago. This model of fighter pilot's tolerance of +Gz acceleration, shows three areas according to Gz amplitude as a function of time.

- a lower one, in which nothing occurs
- an intermediate area in which subject experiences a vision grey out followed by a black out
- an upper area possibly associated with loss of consciousness (GLOC) (See intra p.2).

Therefore the target of this mechanical aggression is a biophysical target, I mean -hρg- the hydrostatic component of blood arterial and venous pressures, which increases linearly with hypergravity. Consequently the heart has to struggle against this load factor. Finally, because of the heart capability limits and because the vascular system is compliant, LOC is due to a decrease in brain blood perfusion and the brain hypoxia theory is now admitted when the onset rate of the hypergravity field is gradual (GOR in Gz per second).

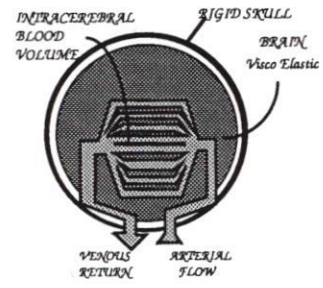
However, in the early 80's technical advances in aeronautics increased dynamics capabilities of aircraft . Not only they became capable of accepting accelerations of higher amplitude and longer duration but, above all, of more rapid onset rate (ROR). In the same time, numerous scientific publications on GLOC reported syndrome changes frequently characterized by the lack of black out and often associated with lacunar amnesia.

In spite of that, hypoxic etiology keeps all its credit for the pathophysiological explanation of the new syndrome.

But, conceptually, a mechanical excitation of the same nature (hypergravity), of the same degrée (amplitude) and of a different order (value of first derivative of acceleration) would generate the same syndrome, the only change being a more rapid onset of the syndrom. If this is not the case, we have to consider that another biophysical target exists, different from the hydrostatic component of arterial and venous blood pressure.

According to this conclusion, we can propose a slightly more general interpretation of the effects of short duration hypergravity. In a first approach of the biomechanics of the brain it will be sufficient to consider it, as a deformable spherical mass enclosed in a totally rigid envelope (the skull), the brain being separated from this envelope by a liquid type film (cerebrospinal fluid). The vascular system is tree-shaped according to the most classical description of the vascular systems. The geometry of large vascular trunks is elbow-shaped to account for difficult flow motions.

Fig1 *BRAIN/SKULL MODEL*



The brain is a deformable spherical mass enclosed in a totally rigid envelope (the skull), the brain being separated from this envelope by a liquid type film (cerebro-spinal fluid).

The brain itself is composed of two phases: a solid phase (nerve tissue) and a liquid phase : the blood volume is contained in a complex vascular system inside the solid phase.

Mechanical behaviors of these various structures are:

- purely rigid for the skull
- purely viscous for the CSF
- viscoelastic for the brain

Two parameters are to be studied:

*Mechanical stresses whose amplitude variations are represented by shades of grey

*general deformation (therefore mechanical stress) related to the decrease in blood mass under the effect of accelerations, namely a shift toward the lower part of the body.

CONDITIONS OF EQUILIBRIUM

To study a small brain volume unit of invariable volumic mass in equilibrium in a field of forces, we must consider the condition of stresses and the state of deformations .So dynamics of material particle is obviously insufficient and we have to use the deformable continuous media equilibrium equation i.e.

$$\vec{div}\,\bar{\bar{\sigma}} + \vec{F} - \rho\vec{\gamma} = 0$$

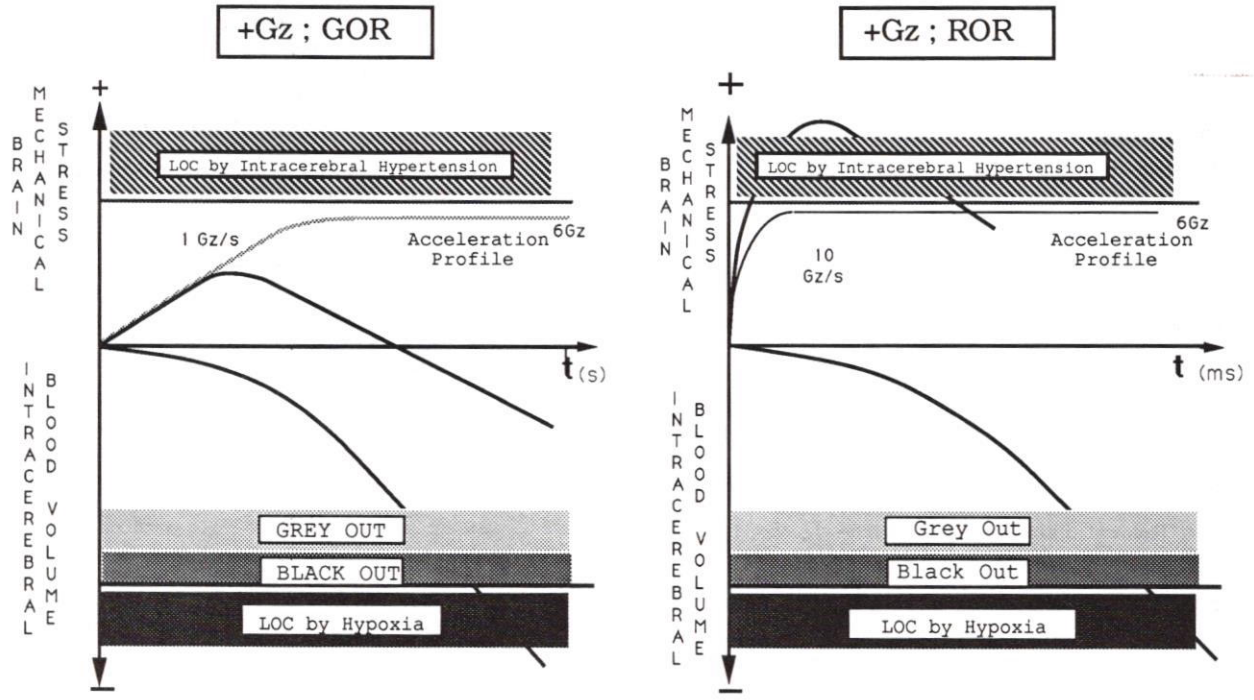


Fig 2 **Integration of the intra cerebral hypertension (ICH) in the set of LOC. Hypoxia explains grey and black out, ICH explains lack of black out and possibly lacunar amnésia post LOC.**

EFFECTS OF Hs+Gz GOR (Figure 2)

According to this model, what might be the effects of hypergravitation slowly established:

*Gz accelerations act mostly through amplitude and time. The target is the hydrostatic component of the blood pressure. The effect is a blood mass migration toward the lower part of the body. The consequence is brain hypoxia with grey and black out followed by LOC, as previously described.

*Intrabain mechanical stresses that will eventually appear with the increase of Gz levels may even "benefit" from the shift of the blood and remain equal or slightly increase and are in no case adverse.

EFFECTS OF Hs+Gz ROR (Figure 2)

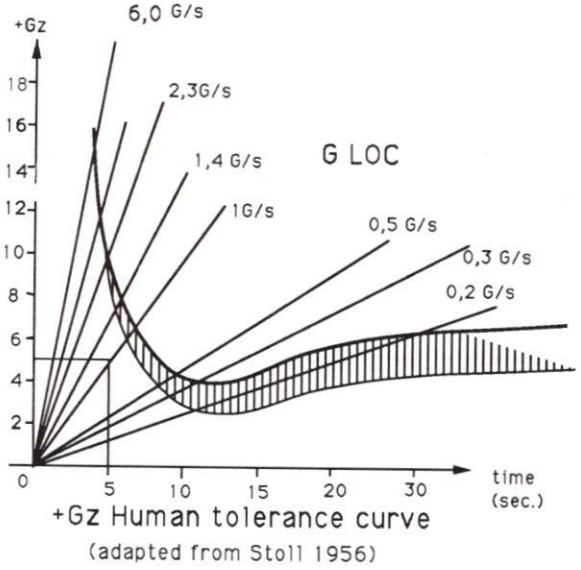
According to this model, what might be the effects of rapidly established +Gz accelerations ?

In this case, the interpretation which would be prevalent to account for LOC would be purely mechanical. The target would no longer primarily be the hydrostatic component of blood pressure: inertia, viscosity and probably the geometry of the vascular system would oppose blood migration toward the lower part of the body. No brain hypoxia would occur due to lack of time. LOC would then result from a sudden increase in forces amplitude inducing a no less sudden increase in mechanical stress. Since mechanical stress is, physically speaking, homogeneous with pressure dimension LOC is most exactly due to sudden intracranial hypertension.

Presented hypotheses (lack of hypoxia) are enough to explain the absence of grey and black out before LOC under Gz, ROR. In other word, if the "physical time" of application of the load factor is extremely short, blood does not have time to be expelled toward the lower part of the body, $\rho\gamma$ increases, and consequently stresses (in the sense of a compression) also increase. New conditions of the mechanical equilibrium of brain extend beyond normal possibilities of the physiological function of cells in the central nervous system and LOC occurs without preliminary signs.

I want to make it very clear, at this stage of my expose, that this interpretation has not been experimentally verified yet and that I would, in no way, present it as such !

My colleagues and myself are well aware of the fact that it will probably take many years of works before the concept we propose can be accepted or rejected. Nevertheless, during this session Mr. Gaffié, an Mr. Liebaert will have the opportunity to demonstrate that cautions optimism is possible.



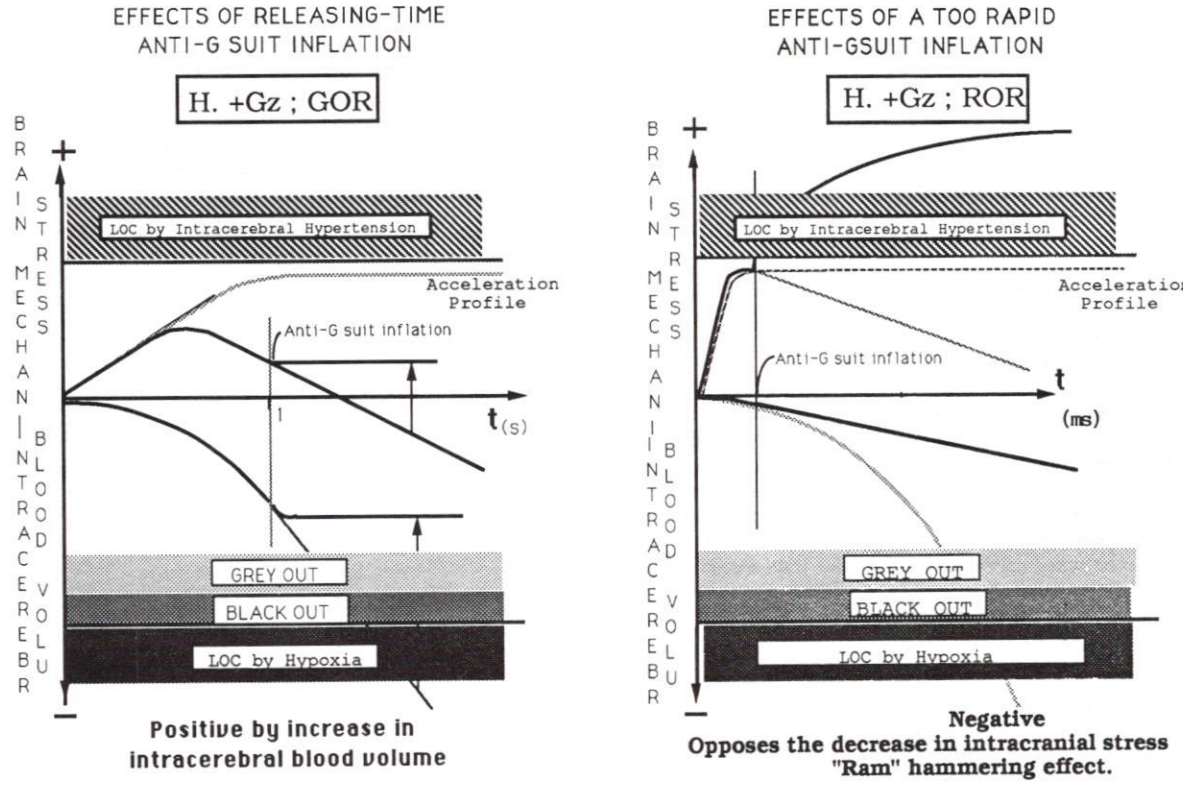


Fig 3 : Explanation of the positive effect of anti G suit inflationin case of Hs+ Gz;GOR and possibly reverse effects in case of Hs+ Gz;ROR

GSUIT INFLATION TIDE (Figure 3)

However, if as it is anticipated, at least part of these hypotheses are consistent, they would find an immediate application for the pressurizing rate of the anti G-suit. In fact the use of G-suit (which increases the useful time before LOC and offers good protection in +Gz, Gor) would have negative effects on pilot's consciousness... which could even be disastrous under certain flight configurations ! The hypothesis of these effects are schematically shown in figure 3. It is necessary to realize that a time scale factor exists which can be extremely important (sixty, for example, when acceleration rate increases from 0.1 Gz.s-1 to 6 Gz.s-1). The axis of ordinates shows positive changes in tissue stresses (above) and negative changes in brain blood mass (below).

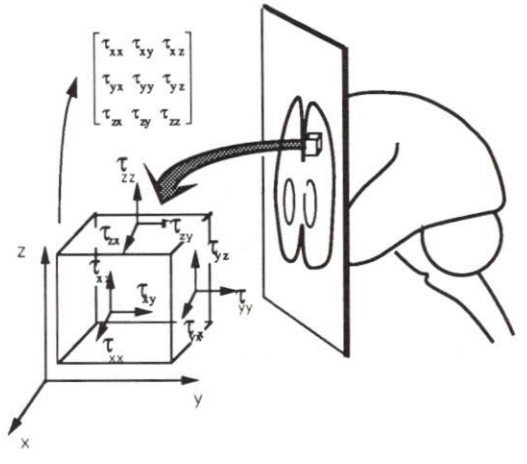
In the case **H+Gz, GOR,** considering what was said, the increase in peripheral vascular resistance of the abdomen and lower limbs tends to increase brain circulation and therefore, has a beneficial effect on the oxygenation of brain cells. The effect on mechanical stress is small when compression of the lower part of the body is sufficiently delayed, the brain blood volume being already reduced.

In the case of **H+Gz, ROR,** also coherent with the proposed biomechanical interpretation, increased low peripheral vascular resistances tend to enhance brain blood volume if the compression is rapid. However, even if this increase is small, the "battering ram" effect associated with the compression of the lower part of the body can amplify brain stress, increasing even more LOC hazard by intracranial hypertension. No one would consider denying the use of the G-suit to increase tolerance to positive Gz. However, its beneficial effect by rapid, even advanced pressurizing is not at all verified in the case of **H+Gz-ROR.**

"Toward a generalized interpretation of biomechanical volume forces"

The reason why we chose this slightly provocative title is also very simple. Saying that a subject is experiencing a +Gz acceleration is equivalent to saying that he is diving in a field of forces. It means that these forces are not surface forces (like the punch of a boxer or inflation of anti-G suit) but "volume forces" applied at distance, to the mass of this individual. From this angle hypergravity and hypogravity become a unified problem.

Indeed from a mechanical standpoint an organ (brain, heart...) is a continuous medium characterized by fiels n(r, t); p (r, t); v (r, t)... mass, pressure velocity function of position r, at every moment t. To study a small organ volume unit of invariable volume mass in equilibrium in a field of forces, we must consider the condition of stresses and the state of deformation.



Mechanical stress tensor.

The behavior $(\vec{div})$ of this tensor $\bar{\bar{\sigma}}$ gives the stress status inside the brain.

Therefore, we have to take into account the equation of equilibrium of the deformable continuous media that is

$$\text{div}\ \sigma + F - \rho\gamma = 0$$

where

*$\rho\gamma$ is the density of inertial force per unit of volume
*F is the density of volume force per unit of volume
*Div σ accounts for the behavior of stress tensor when one of the other two parameters changes. The previously described hypotheses stem from simple considerations. Nevertheless, only digital computation could now verify these hypotheses.

Nevertheless, we arrive at the most possible generalized interpretation of the biological effects of hypergravity or microgravity, for it is restricted to the only variations of force's field intensity:

- constant on the planet
- increases in hypergravity
- tends toward zero in astronautics.

Since it is a continuous parameter, we can easily prove that physiological phenomena of biomechanical nature observed in astronauts (cephalad fluid shift, especially) in weightlessness can be perfectly well explained using the mathematical model which was just presented, where G tends to zero.

Of course an other parameter has to take into account: the time:

- extremely short in the case of hypergravity
- extremely long in microgravity

Using this model it is easy to understand that organ volumes and mechanical stress of the tissue tend toward other equilibria. µGravityis biomechanics of other equilibria whereas hypergravity is biomechanics of transient phenomena. It is the reason why experiments developped on the ground which are supposed to simulate the adverse effects of microgravity will never be capable of reproducing them, whatever biological system is considered ! For example, in space the weight of one ventricular end diastolic blood volume is zero newton, on the earth it weights fourteen hundred newtons even though the subject is placed in a minus six degrees tilt position. So initial mechanical tensions of the myocardium are indisputably different in two cases even though cardiac muscle has to reacts on the same mass of blood at the very first beginning of the systolic time.

All this is not new, everyone knows that the re-establishment of a nearly normal physiology therefore requires calculation of a minimum field of force. The way of achieving this field is naturaly in theory the use of a rotary space laboratory, and this has been considered from the very first days of the space age (although Coriolis acceleration must not be negligeable !).

It is clear, therefore, that we have, at low cost, no other mean that math modelling to predict the value of this threshold. It will be an extrapolation from a predictive model describing the long term effects on biological structures, systems or organs (the heart hold the foremost place) in the hypergravity domain. Although, the mechanical behaviours are non linear and the biological structures have, as they will be called in another meeting "a sensitive dependance on initial conditions" the task is enormous but not impossible.

Unfortunately, today, we don't have any true information on the long term effect of+Gz acceleration (I mean repeated hypergravity load factor) on cardiovascular system.

Talking recently, with Pr. Hickmann chief of the clinical sciences division at the USAF aerospace medical school about the need to determined these long term effects of hypergravity on cardiovascular system and asking his opinion about this subject he wrote almost in one shot a few lines which will now be my conclusion with his kind authorization.

"Whether reapeted exposure to hypergravity has any long term, deletorious or otherwise effects, on any of the human subsystems is unknown.

Whether any of the ancillary problems hypergravity (G-induced loss of consciousness) or whether appliances and anti-G devices (e.g; positive pressure breathing) have any persistant effects is unknown. There is a need to formulate a plan of basic laboratory and clinical investigational studies to detect such long term effects, if indeed, any such effects exist.

As an Aerospace Medecine imperative, persons undertaking high performance flying as our occupation should be aware of any occupational risks to which they many be exposed. At present no clear picture has emerged of the presence of such risks for the musculoskeletal, cardiovascular or central nervous systems. However, the absence of a coherent structure inquiry lowers the confidence with which the Aerospace Medical Services may state that such effects do or do not exist".

The set of references about 199 papers are collected in a thesis:
LESTRADE : Analyse en temps réel de la fréquence cardiaque enregistrée en vol chez le pilote de chasse soumis à des accélérations +Gz.
Paris 1989 : Fac. Méd. Cochin, Port Royal.

AN APPROACH TO COUNTERACTING LONG-TERM MICROGRAVITY-INDUCED MUSCLE ATROPHY

P.A. Tesch[1], P. Buchanan[2], and G.A. Dudley[3]

Department of Environmental Medicine, Karolinska Institutet, S-104 01 Stockholm, Sweden[1]. Biomedical Operations and Research Office, National Aeronautics and Space Administration[2] and Biomedical and Environmental Laboratories, The Bionetics Corporation[3], Kennedy Space Center, FL 32899, USA

Introduction

Numerous animal and human studies suggest that space flight or simulated microgravity results in marked muscular atrophy and dysfunction (1, 2, 4-6, 12, 14, 15, 17). Thus, substantial decreases in muscle strength of the knee extensor muscle group, attributed mainly to a decrease in muscle fiber size and thus over-all muscle cross-sectional area, have been reported following 30 days of bedrest (4, 6, 9). It appears that the magnitude of these responses are greater for the lower limb than for upper body muscle groups (7, 13, 17) and greater for the knee extensors than for the knee flexors of the calf muscle group (4, 6, 7, 9).

Heavy-resistance training, as typically performed by athletes like bodybuilders or power lifters, is known to increase muscle strength and mass (15). Endurance training, in contrast, aiming at improving aerobic capacity does not produce these responses. Because heavy-resistance training obviously is the best model to induce muscle hypertrophy this training regimen should be employed to combat muscle atrophy produced by long-term space flight. Typically, resistance training consists of both concentric (shortening) and eccentric (lengthening) muscle actions with the use of free weights or weight-stack machines. For a given speed of muscle action numerous previous studies have shown maximum voluntary force production to be greater for eccentric than concentric muscle actions (3, 6, 11). If high tension production is the ultimate stimuli for increases in muscle strength and mass the performance of eccentric muscle actions should then be more effective than concentric muscle actions. Results from studies comparing responses to eccentric and concentric resistance training, however, are equivocal (10, 11). Intrigued by this fact a study was designed to examine whether the performance of ecccentric muscle actions in addition to concentric muscle actions is necessary to induce optimal increases in muscle strength and mass. Exercise tools, such as barbells or dumb-bells, traditionally used in weight training, cannot be employed in space because of the lack of gravity. Devices that produce high resistance in this environment therefore must be developed. For this purpose an important issue to address is whether concentric, eccentric or combinations of both types of muscle actions should be performed. Eccentric exercise is associated with markedly lower energy cost than concentric exercise when performed at the same power output. This greater efficiency, as evidenced by lower oxygen consumption during eccentric than concentric exercise (8) suggests that the energy intake provided as an exercise fuel would be lower for eccentric than concentric exercise. Because there is a desire to limit food intake for space crews the benefit of performing eccentric exercise is obvious.

This study therefore examined two groups of male subjects, who performed heavy-resistance training for 19 wks using either concentric muscle actions only or concentric and eccentric muscle actions. The purpose of the study was to compare changes in concentric or concentric/eccentric muscle strength between the two groups. A second aim was to compare the energy cost for concentric and concentric/eccentric heavy-resistance exercise.

Methods

Subjects: Eighteen healthy men were randomly assigned to two different training regimens. Age, height and weight for Grps CON and CONECC, respectively averaged (mean ±SE) 33.4±2.8 and 31.2±2.4 yrs, 176.2±2.4 and 178.5±1.9 cm and 85.1±3.5 and 80.8±3.7 kg.

Training: Both groups went through a familiarization program of progressive training before entering the actual program. Grp CON, performed concentric training only and Grp CONECC did both concentric and eccentric muscle actions. They performed heavy-resistance training using two exercises; supine leg press and seated knee extensions, respectively twice weekly for 19 wks. Grp CON performed five sets of 6-12 repetitions of concentric muscle actions. Grp CONECC performed five sets of 6-12 repetitions of concentric and eccentric muscle actions. Both groups performed each set until muscle failure. Bilateral leg presses and unilateral extensions, respectively were performed on Universal® and Nautilus® machines. These machines were modified using pneumatic devices for passive return of the weights or weight stack to "unload" the quadriceps muscle in Grp CON. This principle was not used during exercises performed by Grp CONECC. Thus Grp CONECC, not Grp CON, resisted the load during the descending phase.

Strength measurements: Three repetition maximum (3RM; i.e. the greatest load that can be performed in three consecutive muscle actions) was assessed during leg press pre-and post training. It was measured when performing CON (CON 3RM) actions only or when performing CON and ECC (CONECC 3RM) muscle actions.

Energy cost: Work performed during CON and CONECC exercise was calculated as number of repetitions times the vertical load acting during the concentric phase (i.e. the "negative work" performed during the descending phase was dismissed). Oxygen uptake was measured continuously five min before and during leg press exercise sessions and 15 min into recovery following exercise in Grps CON and CONECC using a semi-automatic system (Beckman® MMC Horizon System).

Results

Leg press CON 3RM and CONECC 3RM, respectively were similar in Grp CON (169±15 and 166±13 kg) and Grp CONECC (159±9 and 155±9 kg). Leg press CON 3RM increased ($p<0.05$) by 12 and 22%, respectively in Grp CON and Grp CONECC in response to training (fig. 1). The increase was greater ($p<0.05$) for Grp CONECC than for Grp CON. Leg press CONECC 3RM (fig. 2) increased in Grp CONECC (26%; $p<0.05$) but not in Grp CON (8%; $p>0.05$). The calculated work during

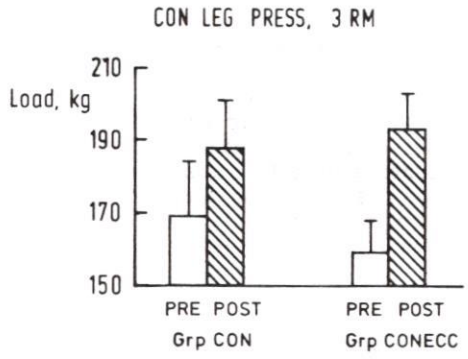


Fig. 1

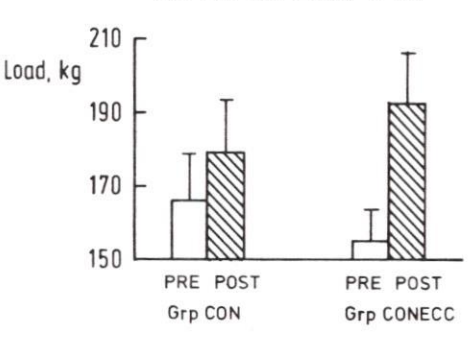


Fig. 2

CON and CONECC exercise sessions were 2614±136 and 2292± 109 kgxm, respectively. Work, expressed in absolute terms but not relative to body weight ($p>0.05$), was greater ($p<0.05$) for CON than CONECC exercise. The net total oxygen uptake (fig. 3) was similar ($p>0.05$) for CON (upper panel) and CONECC (lower panel) exercise (15.8±1.2 vs. 15.8±1.5 l).

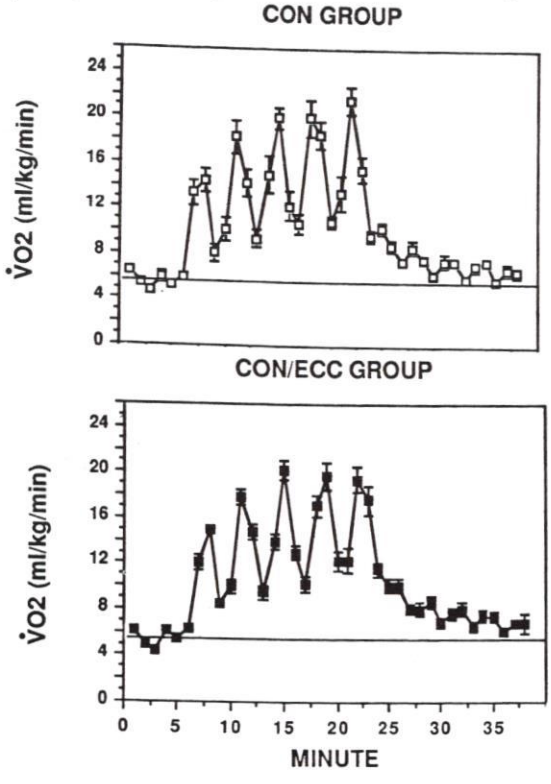


Fig. 3

Discussion

The results of this study show that a concentric-eccentric heavy-resistance training program produces greater increases in concentric or concentric-eccentric strength than a regimen comprising concentric muscle actions only. The time required to complete these different exercise regimens was identical despite the apparently greater total stress during concentric-eccentric training. The present findings accord with those of Komi and his associates (11) in that the performance of eccentric muscle actions appears essential to induce optimal increases in muscular strength. Although this may suggest the importance of performing eccentric muscle actions as a counter-measure for the physiological changes occurring during long-term microgravity it does not necessarily imply that muscle mass increases more in response to CONECC than CON training.

Previously, it has been shown that CON exercise is associated with markedly greater energy cost than ECC exercise carried out at an equivalent power output (8). The present data suggest that this difference is similar or even potentiated if CON and combined CON and ECC heavy-resistance exercise is compared. Hence, the eccentric or descending phase during leg press exercise was performed at a very low additional energy cost.

In summary, the results of this study indicated a substantially greater increase in maximal strength for Grp CONECC than Grp CON when the 3 repetition maximum was performed with concentric only or with concentric and eccentric muscle actions. The time required to perform a training session was similar for each group. Moreover, the apparent energy cost was only slightly greater for Grp CONECC. Accordingly, we suggest that simulated heavy-resistance training with both concentric and eccentric muscle actions must be seriously evaluated for use by space crews to counteract muscle atrophy that may occur during long-term space missions.

Acknowledgement

This work was suported by NASA Contract NASIO 10285.

References

1. Appell H-J. Skeletal muscle atrophy during immobilization. Int. J. Sports Med. 1986; 7:1-5.
2. Buchanan P, Convertino VA. A study of the effects of prolonged simulated microgravity on the musculature of the lower extremities in man: An introduction. Aviat. Space Environ. Med. 1989; 60:649-52.
3. Colliander EB, Tesch PA. Bilateral eccentric and concentric torque of quadriceps and hamstring in females and males. Eur. J. Appl. Physiol. 1989; 59:227-232.
4. Convertino VA, Doerr DF, Mathes KL, Stein SL, Buchanan P. Changes in volume, muscle compartment, and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity. Aviat. Space Environ. Med. 1989; 60:653-658.
5. Dietlein LF. Summary and conclusions. In: Johnston RS, Dietlein LF, Berry CA, eds. Biomedical results of Apollo. Washington, DC: National Aeronautics and Space Administration, 1975; 573-9: SP-368.

6. Dudley GA, Duvoisin MR, Convertino VA, Buchanan P. Alterations of the in vivo torque-velocity relationship of human skeletal muscle following 30 days exposure to simulated microgravity. Aviat. Space Environ. Med. 1989; 60:659-663.
7. Gogia PP, Schneider VS, LeBlanc AD, Krebs J, Kasson C, Pientok C. Bed rest effect of extremity muscle torque in healthy men. Arch. Phys. Med. Rehabil. 1988; 69:1030-1032.
8. Hesser CM. Energy cost of alternating positive and negative work. Acta Physiol. Scand. 1965; 63:84-93.
9. Hikida RS, Gollnick PD, Dudley GA, Convertino VA, Buchanan P. Structural and metabolic characteristics of human skeletal muscle following 30 days of simulated microgravity. Aviat. Space Environ. Med. 1989; 60:664-670.
10. Jones DA, Rutherford OM. Human muscle strength training: The effects of three different regimens and the nature of the resultant changes. J. Physiol. 1987; 391:1-11.
11. Komi PV, Buskirk ER. Effect of eccentric and concentric muscle conditioning on tension and electrical activity of human muscle. Ergonomics, 1972; 15:417-434.
12. Kozlovskaya IB, Kreidich YuU, Rakhmanov AS. Mechanisms of effects of weightlessness on the motor system of man. Physiologist 1981; 24 (Suppl.):S59-S64.
13. LeBlanc A, Gogia P, Schneider V, Krebs J, Schonfeld E, Evans H. Calf muscle area and strength changes after five weeks of horizontal bed rest. Am. J. Sports Med. 1988; 16: 624-629.
14. Nicogossian AE, Parker JF. Space physiology and medicine. Washington, DC: National Aeronautics and Space Administration, 1982;1-324: SP-447.
15. Panov AG, Lobzin VS, Belyankin VA. Changes in the function of the nervous and muscular systems under the influence of prolonged hypodynamia. In: Genin AM, Sorokin RA, eds. Problems of space biology. Washington, DC: National Aeronautics and Space Administration, 1969;133-145.
16. Tesch PA. Acute and long-term metabolic changes consequent to heavy-resistance exercise. Medicine Sport Sci. 1987; 26:67-89.
17. Thornton WE, Rummel JA. Muscular deconditioning and its prevention in space flight. Biomedical results from skylab. In: RS Johnston and LF Dietlein: NASA Special Publication SP-377, Washington, DC 1977: 191-197.

COMPARED EFFECTS OF A 13 DAY SPACEFLIGHT ON THE CONTRACTILE PROTEINS OF SOLEUS AND PLANTARIS RAT MUSCLES

X. Holy, L. Stevens, Y. Mounier

Laboratoire de Physiologie des Structures Contractiles, Université des Sciences et Techniques de Lille Flandres Artois, 59655 Villeneuve d'Ascq Cedex. FRANCE.

It is now well known that the musculo-skeletal system is particularly disturbed after spaceflight. A muscle mass decrease, a reduction in fiber diameter (2), a decline in strength (6) were described. Moreover the slow twitch muscles were more affected by microgravity than the fast ones.

The main aims of our work were first to determine and compare the modifications of the contraction development in slow and fast isolated skinned muscle fibers after a 13 day spaceflight, secondly to correlate these physiological modifications with the structural changes of the contractile apparatus. To achieve these objectives the maximal tensions developed by single skinned fibers were analysed. Then we studied the amplitudes and the kinetics of the fiber contractions when solutions of various calcium concentrations were applied. This permitted to determine the calcium sensitivity of the contractile proteins. Finally, the content of the isoforms of the myosin light chains and of the troponin C were analysed.

METHODS

The experiments were performed on two muscles from male Wistar rats. The muscles used were the slow postural antigravitational soleus and the fast plantaris. The animals were divided into two groups. The flight rats (F) were the animals placed in orbit for 13 days in the Biocosmos 1887. This group was compared to a synchronous control group (S) which received a simulation of the flight conditions in a spacecraft mock-up.

Animals were killed by decapitation two days after landing or after the end of the simulation. The muscles were removed and biopsies were immediately taken off and exposed to a skinning solution. The force measurements and recording were made as previously described (4).

For the SDS-PAGE analysis, single skinned were solubilized overnight (5) in 15 µl of a SDS solution (2.3 % SDS, 5 % β mercaptoethanol, 0.625 M Tris HCl, 10 % glycerol). The electrophoresis was performed in a 18 % Acrylamide gel in the Laemmli conditions (3). The gels were silver stained after the gel run.

Statistical significance of the difference between means was determined using the student's t test. All the values were reported as mean +/- SEM.

RESULTS AND DISCUSSION

Fiber diameter was measured before each experiment. On soleus, the diameter significantly decreased by 41 % (from 78.33 +/- 6.50 to 46.07 +/- 1.92 µm for S and F animals respectively). On the contrary, no modification in fiber size was found on the plantaris (66.07 +/- 2.30 and 64.08 +/- 2.85 µm for S and F animals respectively).

Maximal tensions (P_o) were induced by a solution of high calcium concentration (pCa 4.8) large enough to saturate all the calcium sites of the contractile proteins. The results observed on the soleus decreased significantly by 60 % from 41.39 +/- 5.27 mg for S animals to 16.89 +/- 1.45 mg for F animals. However, when the tensions were related to the cross-sectional area no difference appeared (0.96 +/- 0.10 $kg.cm^{-2}$ and 1.08 +/- 0.15 $kg.cm^{-2}$ for S and F respectively). Therefore, the decline of the fiber size appeared as the main cause of the tension decrease observed after spaceflight. On the plantaris, we did not find any difference in maximal tension neither in absolute value nor in values related to the cross-sectional area (1.30 +/- 0.25 $kg.cm^{-2}$ and 1.39 +/- 0.22 $kg.cm^{-2}$). So, as generally reported, the atrophy was more pronounced on the slow soleus muscle, more implied in an antigravitational function, than on the plantaris muscle. The atrophy was usually correlated to a decrease in myofibrillar protein content and the main explanation was a reduction in the rate of protein synthesis in conjunction with an elevation in the rate of protein degradation (1). Fig. 1 showed typical records of contraction obtained on single skinned fibers of soleus and plantaris muscles for both groups of rats.

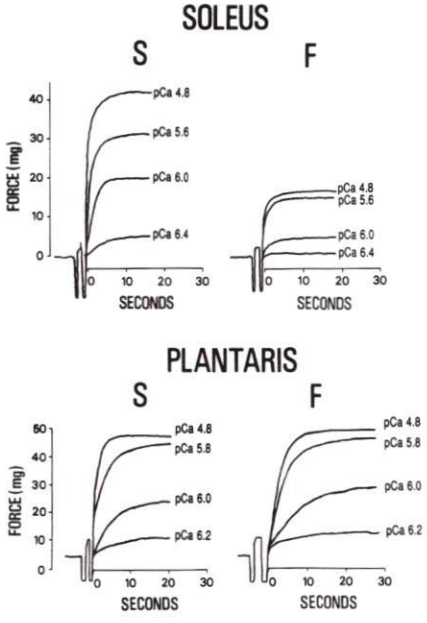


Fig. 1. Records of tension induced by various calcium concentration solutions for S and F animals.

On the soleus, the calcium threshold for the protein activation became higher after the flight. Moreover, the amplitudes of tension elicited by a given pCa solution decreased after the flight while the rate of force development appeared faster. On the plantaris similar threshold and amplitudes were obtained for S and F animals. However, the rate of force development appeared clearly slower after the flight.

To control the calcium sensitivity of the contractile system, Tension/pCa relationships (Fig. 2) were established. The tensions values (P) were normalized to the maximal tension (P_o).

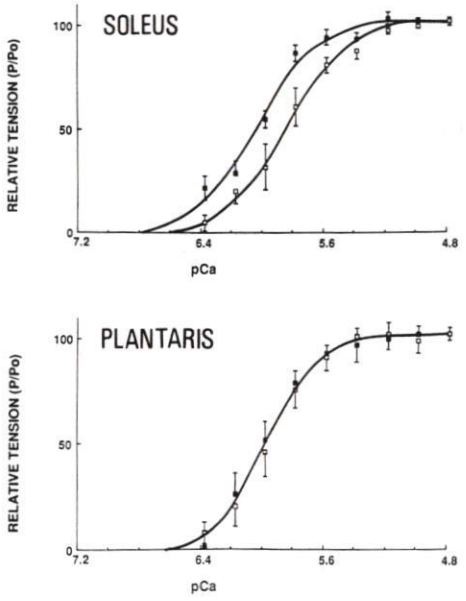


Fig. 2. Tension/pCa relationships of soleus and plantaris fibers from S (filled symbols) and F (open symbols) animals.

After the flight, the T/pCa relationship for the soleus was shifted towards lower pCa values, i.e towards higher calcium concentrations. The values of the calcium threshold (pCa 6.8 and 6.6 for S and F animals respectively) and of the pCa_{50} (the value of pCa which induced half maximal tension, pCa 6.06 and 5.85 for S and F animals respectively) declined by 0.20 pCa unit after the flight. Therefore, the apparent affinity of the contractile proteins for calcium decreased after the flight. Moreover, the slope of the relationship was slightly higher especially in the range of low calcium concentrations. Indeed, the linearization of the curve could be treated as two straight lines, described by the n_1 (for $P/P_0 > 0.5$) and n_2 (for $P/P_0 < 0.5$) parameters or Hill coefficients. After the flight n_1 remained unchanged (2.00 for S and 2.03 for F lines) while n_2 increased from 2.50 for S to 2.85 for F experiments.

For the plantaris, similar threshold (pCa 6.6), pCa_{50} value (6.00) and Hill coefficients (n_1 = 2.50; n_2 = 3.50) were obtained after the flight.

Therefore, after spaceflight, the calcium affinity of the contractile proteins appeared modified only for the slow twitch muscle.

In order to examine the contractile proteins content after spaceflight, a SDS-PAGE was performed. The isoform content of the myosin light chain and of the troponin C were compared for synchronous and flight animals (Fig. 3). The isoforms were identified in agreement with Salviati's results (5). On the soleus, for S animals, the light chain isoforms can be spotted as the LC_{1sa}, LC_{1sb} and LC_{2s} slow isoforms usually described. The isoform of the TnC of the slow fibers, TnC_s, appeared. After the flight two other myosin light chain isoforms appeared. They had a faster migration than the slow ones and were identified as the LC_{1f} and LC_{2f} fast isoforms. Moreover, a fast isoform of the TnC appeared. The results confirmed the transformation of the fibers from a slow type towards that of a fast one. The emergence of the TnC_f might explain the modification of the calcium binding properties observed in the soleus fiber after spaceflight while the changes in the light chain isoforms might explain the change of the rate of force development. The appearance of a LC_3 isoform was more rarely observed after the flight.

On the plantaris, for S animals, three light chain isoforms can be spotted and identified as the LC_{1f}, LC_{2f} and LC_3 isoforms. After the flight, the LC_3 content decreased. Since this light chain was more characteristic of a fast muscle, its decreased content could explain the slowing down of the force development previously described.

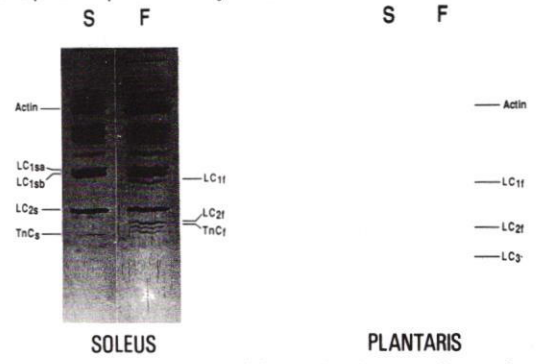


Fig. 3. SDS polyacrylamide gel electrophoresis of soleus (left part) and plantaris (right part) single fibers for both groups of rats.

To conclude, all these results suggested that the changes in calcium sensitivity as well as those of kinetic parameters observed after spaceflight could be correlated to changes in the type and/or in the proportion of the myosin light chain and TnC. Moreover, the modifications were more important for the antigravitational soleus muscle than for the plantaris.

REFERENCES

1. Booth F.W., Seider M.J. 1979. Early change in skeletal muscle protein synthesis after limb immobilization of rats. J. Appl. Physiol., 47, 974-977.
2. Ilyina-Kakueva E.I., Portugalov V.A. and Krikenkova N.P. 1976. Spaceflight effects on the skeletal muscles of rats. Aviat. Space Environ. Med., 47, 700-703.
3. Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227, 680-685.
4. Mounier Y., Holy X. and Stevens L. 1989. Compared properties of the contractile system of skinned slow and fast rat muscle fibres. Pflügers Arch., in press.
5. Salviati G., Betto R. and Danieli-Betto D. 1982. Polymorphism of myofibrillar proteins of rabbit skeletal-muscle fibres. Biochim. J. 207, 261-272.
6. Oganov V.S., Potapov A.N., Skuratova S.A. and Shirvinskaya M.A. 1980. Variability of physiological properties of rat skeletal muscles at different gravity levels. The Physiologist, 23, S71-S75.

The authors thank the institute of Biomedical Problems (Moscow) for providing the biological material from the biocosmos 1887 and they are grateful to Drs Oganov, Skuratova and Murashko for their scientific co-operation.

This work was supported by grants from the "Centre National d'Etudes Spatiales".

WATER INTAKE AT POST-EXERCISE COMPARING BETWEEN HEAD-UP AND -DOWN TILTING IN WOMAN

Kikuko YOKOZAWA , Shigeyo TORIKOSHI
Junko NAGANO and Yoji SUZUKI

Lab.of Human Physiol., Tokyo Wowan's Christian Univ.,Suginami-ku,Tokyo,JAPAN 167, Lab.Health Administration, Faculty of Medicine, Univ. of Tokyo,Bunkyo-ku Tokyo,JAPAN 113

As changed the body position to head-up and -down tiling immediately after a mild prolonged supine exercise, water intake at the post-exercise was investigated with measurements of cardiovascular responses ,body temperature and body weight to know if the water intake was depressed by the head down tilting(HDT). 5 female students have performed a 300 kpm/min. exercise for 50 minutes in high enviromental temperature of 35°C .Immediately after the exercise the body position was changed to 10° HUT or HDT and then maintained for 15 minutes of recovery. At the 15th minute of recovery, the subjects intaked cold water of 7±1°C at will. Through the experiments , VO2, HR, BP, CO, FBF, Tsk and TEAM were measured. Water intake at the 15 minutes of the post-exercise was significantly smaller in 10°HDT than in 10°HUT($p<0.01$). The water intake was not significantly correlated to the decrement in the body weight ,and also to the change in body temperature. Cardio-vascular responses during recovery of the exercise were not significantly different between the two tilting positions,except diastolic arterial pressure which was significantly lower in the HDT($P<0.05$). The presented results suggest that as changed body position immediately after a mild prolonged supine exercise in high temperature condition, water intake at the post-exercise should be depressed by the HDT as well as shown during exercise.

In the some previous studies,water intake in man should be affected by the change in central blood volume with gravitational stimulus. In our previous study presented in IUPS meeting of 1988, we have reported that the increased central blood volume in HDT depressed the center of water intake during prolong mild exercise under the two enviromental conditions of 26°C and 37°C. In this stusy,as changed the body to the HUT and the HDT immediately after a mild prolonged supine exercise, water intake at the post-exercise was investigated with measurements of cardiovascular responses,body temperature,and body weight to know whether the water intake was depressed by the HDT.

(Methods)

Five female students participated as the subjects in the study. Their averaged body weight(Wt.),body height(Ht.) maximum oxygen uptake(VO2max) were 60.2kg,163.6cm and 39.7ml/kg/min. respectively. The averaged values were little higher than the values of Japanese same aged women. Before the experiment,the subjects were informed details of the study.They could not intake any water and food for about six hours before the start of experiments. In Figure 1,protocol of experiment was illustrated. That is,after 15 minutes rest ,a supine pedaling exercise of a 300/kpm/min.was performed for 50 minutes in high enviromemtal temperature of 35°C,then,immediately after exercise,the body position was changed to 10 HUT or HDT and maintained for 15 minutes of recovery,the subjects intaked cold water of 7±1°C at will.The amount of water drunk determined as the volume wanted in each subjects and was identified as water intake.Through all experiments,oxygen uptake (VO2),heart rate(HR), arterial blood pressure(BP),cardiac output (CO),fore-arm blood flow(FBF),mean skin temperature(Tsk) and external audiomutus temperature were measured at each point along the experimental time table.In measuring each VO2 with breath by breath was averaged for every 30 second and determined as VO2 at the point by a metabolic analying system which was constructed with mass-spectrometer,gas flow-meter and computer.HR was counted on the record of EKG by computer system.Arterial blood pressure was measured by means of auscultation method in the right upper arm. CO was measured by a method of acetylen+argon gases rebreathing technique,of which the gases were analysed by a mass spectrometer

FBF was measured in the left fore-arm by using rubber straingauge plethymography. Each of body temperatures was measured by a thermister system. We used the student t-TEST for statistical comparisons between each other of all averaged values given in this study.

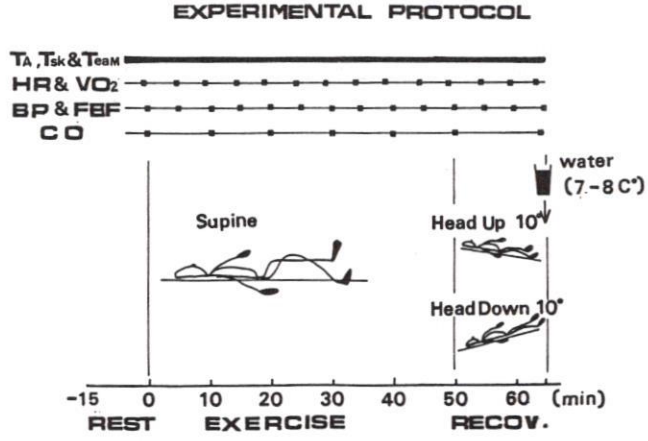


Fig 1. Protocol and Time Table in Experiment

(Results and discussion)

In Figure 2,the upper part of the figure shows the averaged water intake in the sub-jects given at 15th minute of recovery in each experiment.The down part of it shows the averaged decrement in Wt.of the sub-jects between before and after experiments There was no significant difference in the decrement of Wt. between the two body positions. The water intake in HDT was significantly greater than it in HUT.VO2 during exercise was about 1l/min. in the body conditions and corresponded almost 40% of VO2max in the subjects. Comparing between the two positions made during recovery of the exercise,VO2 was not different with each other,while Tsk and Team were little bit lower than in the HUT but not significant different.All of HR ,CO and systolic arterial pressure(SAP) at every point during exercise and recovery were no significant difference between the two body positions,while diastolic arte-rial blood pressure(DAP) was significantly lower in HDT than HUT.Even if the DAP was lowered by HDT,mean arterial pressure (MAP) was not different between the two positions.Comparing between the two tilt-ing positions at recovery, the both of total perpherial resistance (TPR) and stroke volume(SV) presented almost the same levels,although fore-arm volume con-ductance (FVC) in HUT was little bit lower than in HDT, but not significant.Figure 3 shows the relationships between water intake and stroke volume (SV).It was shown that an inverse significant correlation in the relationship was given during exer-cise in the previous study,but in spite of the different tilting posi- tions,such a significant correlation was not present-d at the post-exercise in this study.The fact is due to that SV was not influenced by alternating body position during recov-ery after mild prolong supine exercise.

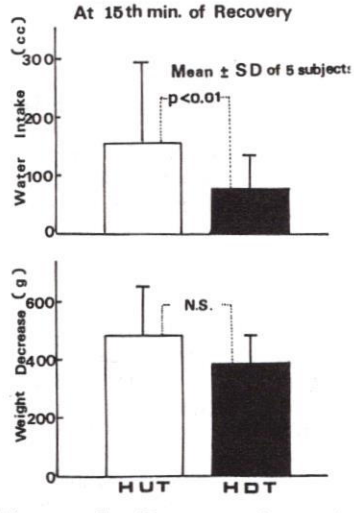


Figure 2. Water volume intaken at 15th minute of recovery and the difference body weight bet-ween before and after exercise

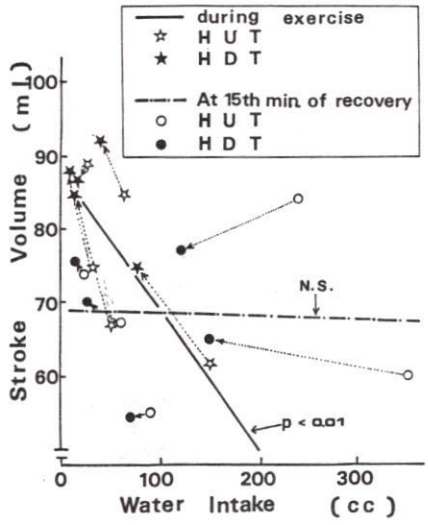


Figure 3. The relationship bet-ween water intake and stroke volume

(Conclusion)

As changed body position immediately after a mild prolonged supine exercise in high temperature condition(36°C),water intake at the 15th minute of the post-exercise was significantly smaller in 10° HDT than in 10° HUT (P<0.01).The water intake was not significantly correlated to the decrement in Wt.,and also to the change in body temperature. Both Tsk and Team during recovery of for the exercise were rather little bit higher in HDT than in HUT .Cardio-vascular responses during recovery of the exercise were not signifi-cantly different between the two tilting of HU and HD,except DAP which was signifi-cantly lower in HDT (P<0.05).In conclusion ,as changed body position immediately after a mild prolonged supine exercise in high temperature condition,water intake at the post-exercise should be depressed by the HDT as well as shown during exercise. Because the central blood volume and brain blood flow are filled up by the HDT,the center of water intake was made to lower the activity.

(Reference)

1.Kikuko YOKOZAWA,etal.,10°head-down and -up tilting on the water intake and cadiovascular responses during mild exercise in woman.,Proceedings of the eighth annual meeting of the IUPS commission on gravitational The Physiologist-30 S-66-67,1986

2.Kikiko YOKOZAWA.,et al.,Increasing cent-ral blood volume with head-down til -ting would inhibit water intake during mild pedaling at25°C and 35°C room temperature in woman.,Proced-ing of tenth annual meeting of IUPS comission in gravitational. Physiologist,Vol.32 S90-91 1989

EFFECT OF SIMULATED MICROGRAVITY ON THE ULTRASTRUCTURAL COMPOSITION OF RAT SOLEUS MUSCLE.

D. DESPLANCHES[1], S.R. KAYAR[2], B. SEMPORE[1], R. FLANDROIS[1] and H. HOPPELER[2]

1 URA 1341 CNRS, Laboratoire de Physiologie, Faculté de Médecine 69373 LYON Cédex 08 France
2 Institut of Anatomy, University of Bern, CH 3000 BERN 9

INTRODUCTION -

Previous reports have demonstrated that slow-twitch muscles such as soleus, atrophy to a greater extent than fast-twitch muscles, in response to a simulated or real microgravity (2, 3, 7, 8, 9). In addition to the loss of muscle mass, numerous investigators have studied changes in muscle structure and function. However, little is known on the effects of microgravity on the components of the respiratory system, namely on capillaries and mitochondria (4, 5). The present study was designed to examine the effects of a 5 wk-tail suspension period (6) on the ultrastructural composition of soleus muscle. Stereologic techniques (10) were used to estimate muscle capillary and the volume density of mitochondria, myofibrils and satellite cells. This morphometric approach allowed us to quantify the topological relationships among the various sites of cellular activity (4, 5).

MATERIAL and METHODS -

The experiments were performed on ten female pathogen free Wistar rats (mean body weight : 150 g). The rats were housed in a temperature controlled room (22-24°C) with a 12:12 light-dark cycle. They were provided with water and Purina laboratory chow ad libitum. One week later, half of them were suspended in individual cages using Morey's tail suspension model (6). Five weeks later, all the rats were anesthetized by an inhalation of halothane. The soleus was processed for morphometric analyses by fixation in a 6.25% solution of glutaraldehyde as previously described (4). Ultrathin sections (60-90 nm) were cut with an LKB ultramicrotome from two randomly chosen tissue blocks per each soleus muscle. Twelve micrographs per muscle were taken on 35 mm film with a Philips 300 electron microscop. Capillary number, fiber number and fiber area were estimated at a final magnification of x 1500, analysing 150 muscle fibers profiles in each muscle. The number of fibers and capillaries were counted directly. For mean fiber cross-sectional area, sampling was done by point-counting with a grid A 100 (100 test points), as previously described (10). A final magnification of 24000 (40 micrographs) was used to estimate the volume density of interfibrillar mitochondria Vv (mi,f), subsarcolemmal mitochondria Vv (ms,f), total mitochondria Vv (mt,f), myofibrils Vv (fi,f) and satellite cells Vv (sat cell,f). Point counting was performed with a grid C 16 (144 test points). Absolute values were obtained by multiplying the volume densities of these components with the muscle volume. Statistical analyses were performed using the Mann-Withney Sum-Rank test.

RESULTS -

After suspension, the soleus atrophy was great (-60%). The mean fiber cross-sectional area was significantly reduced from 1876±180 to 478±75 μ m2. Capillaries per fiber were decreased (2.24±0.14 vs 1.4±0.13) while capillary density was higher (1231±98 vs 3054±277 cap.mm-2).

Mean myofibril volume density was lower (-6%) after 5 wk of hypokinesia (Table 1). There was a tendency to an increase for total mitochondrial volume density (17%) that did not reach statistical significance on the bulk estimate of the whole fiber. This because a shift from the subsarcolemmal to the interfibrillar mitochondria occurred (Table 1). The satellite cell volume density was increased 6-fold (Table 1).

	Control	H/H 5 wk
V_V (fi,f)	78.2 ± 1.0	73.0 ± 1.0*
V_V (mt,f)	13.8 ± 1.1	16.1 ± 0.3
V_V (mi,f)	9.9 ± 0.9	13.5 ± 0.3
V_V (ms,f)	3.9 ± 0.5	2.5 ± 0.2
V_V (sat cell,f)	0.05± 0.04	0.32± 0.10*

Table 1 - Volume density of myofibrils (Vv (fi,f), total mitochondria Vv (mt,f), interfibrillar mitochondria Vv (mi,f), subsarcolemmal mitochondria, Vv (ms,f) and satellite Vv (sat cell, f), from soleus of control and 5-wk hypodynamic-hypokinetic rats (H/H 5 wk). Values are means; units, cm3/cm3; *significant $p<0.05$.

Absolute values of mitochondrial and myofibrillar volumes were obtained by multiplying the volume densities of these components with the muscle volume. The absolute volume of myofibrils (0.083±0.005 cm3), interfibrillar (0.010±0.001 cm3), subsarcolemmal (0.004±0.001 cm3) and total (0.015±0.001 cm3) were dramatically decreased by 63, 45, 73 and 53% respectively.

DISCUSSION -

A marked decline (60%) in muscle mass occurred after hypodynamia-hypokinesia. The fiber mean cross-sectional area decreased to a greater extent (75%) than the capillaries per fiber (37%) leading to a higher capillary density (148%). The large drop in muscle volume seemed to affect the composition of the muscle cells only a little. The volume density of myofibrils was slightly but significantly reduced (-6%) while the total mitochondrial volume density remained unchanged, on the bulk estimate of the whole fiber. These findings are in agreement with the results of Steffen

and Musacchia (8). They reported that sarcoplasmic protein accounted for 37.5% of the noncollagen protein in soleus rats exposed to 7 days of weightlessness (Spacelab-3 shuttle flight) but only 32.5% in ground controls. On the other hand, Tsika et al (9) noted a marked loss in soleus myofibril protein concentration (45%) in 6-wk suspended rats.

A shift from the subsarcolemmal to interfibrillar mitochondria occurred, according to the findings of Riley et al (7) who described fewer subsarcolemmal mitochondria in flight muscle fibers. The increase in volume density of satellite cells suggested muscle regenerative events (1) but it is currently open to question wether satellite cells are activated in hypokinesia-hypodynamia.

ACKNOWLEDGEMENTS - This research was supported by a grant from Fondation pour la Recherche Médicale.

REFERENCES -

1 - ALLBROOK D.B. Skeletal muscle regeneration Muscle and Nerve, **4** : 234-245, 1981

2 - DESPLANCHES D., M.H. MAYET, B. SEMPORE and R. FLANDROIS. Structural and functional responses to prolonged hindlimb suspension in rat muscle. J. Appl. Physiol., **63** : 558-563, 1987a)

3 - DESPLANCHES D., M.H. MAYET, B. SEMPORE, J. FRUTOSO and R. FLANDROIS. Effect of spontaneous recovery or retraining after hindlimb suspension on aerobic capacity. J. Appl. Physiol., **63** : 1739-1743, 1987b

4 - HOPPELER H., O. MATHIEU, R. KRAUER, H. CLAASSEN, R.B. ARMSTRONG and E.R.WEIBEL. Design of the mammalian respiratory system. VI- Distribution of mitochondria and capillaries in various muscles. Respir. Physiol., **44** : 87-111, 1981

5 - KAYAR S.R., H. CLAASSEN, H. HOPPELER and E.R. WEIBEL. Mitochondrial distribution in relation to changes in muscle metabolism in rat soleus. Respir. Physiol., **64** : 1-11, 1986

6 - MOREY E.R. Spaceflight and bone turnover : correlation with a new rat model of weightlessness. Bioscience, **29** : 168-172, 1979

7 - RILEY D.A., S. ELLIS, G.R. SLOCUM, T. SATYANARAYANA, J.L.W. BAIN and F.R. SEDLAK. Hypogravity-induced atrophy of rat soleus and extensor digitorum longus muscles. Muscle and nerve, **10** : 560-568, 1987

8 - STEFFEN J.M. and X.J. MUSACCHIA. Space flight effects on adult muscle protein, nuclei acids and amino acids. Am. J. Physiol., **251** : R1059-1063, 1986

9 - TSIKA R.W., R.E. HERRICK and K.M. BALDWIN. Effect of anabolic steroids on skeletal muscle mass during hindlimb suspension. J. Appl. Physiol., **63** : 2122-2127, 1987

10 - WEIBEL E.R. Stereological methods. Volume I. Practical methods for biological morphometry. London, Academic Press, 415 p, 1979

BIOMECHANICAL CHARACTERISTICS OF M.SOLEUS AND M.GASTROCNEMIUS OF RATS IN MUSACCHIA'S MODEL

J.Hideg, F.Guba[+], A.Pozsgai, I.Ocsovszki[+], F.Kékes-Szabó[++], P.Remes, M.Rapcsák[++]

Hungarian People's Army Medical Corps
[+]Institute of Biochemistry, Szent-Györgyi University Medical School, Szeged
[++]Institute of Pathophysiology, University Medical School, Debrecen

The experiences of space flights prove that under the influence of weightlessness, several such functional- and morphologic changes come into being in different organ systems - among others in the muscular system - which influence the physical work ability. The long term weightlessness leads to deterioration of muscular functions.

Several methods have been elaborated in the field of animal experiments for modelling weightlessness in the earth. From these the whole body hypokinesis, brought about by a special cage, stood the test. During these experiments it was stated that the muscular atrophy emergence is the most rapid and its degree is the biggest in the so called slow type muscles.

Recently Musacchia worked out a new kind of model for examining short term hypokinesis: in this model the rat is so hanged up that its weight is set to its upper limbs and the hind limbs are eased of burden. Musacchia and his co-workers stated that the weight of m.gastrocnemius explicitly decreased during the 7 days term of hanging up while the weight of m.EDL did not change. The decrease in the number of myofibrils can be regarded as one of the causes of the increased potassium evacuation. In connection with the m.soleus they stated that the number of its slow fibres notably decreased while these changes can not be noticed in the fast fibres. In the plasma the corticosterone level while in the muscles first of all in m.soleus the number of the glucocorticoid-receptors significantly increased. According to Musacchia, his method makes possible a good comparison - in the respect of muscle-morphometric changes - with the changes produced by space flights.

In our present work we looked for an answer that how can be characterized the changes, caused by whole body hypokinesis and Musacchia's immobilization method, by biomechanical parameters.

Material and method

In our experiments we used CFY male rats with mean weight 23o g. One group of the animals were exposed to hypokinesis, by means of special cages, in the case of the other group we used Musacchia's immobilization method. The control animals could move without restriction. 20-20 animals were in all groups. We used 14-28 days of exposition and at the end of it m.soleus and m.gastrocnemius were prepared in Nembutal anaesthesia. Following muscle weight measuring the convulsions were produced by indirect electric stimuli under isometric conditions. After digitalization we analised the convulsive diagram by computer. We determined the following parameters: contraction time /T_c/, convulsive half-time /$T_{1/2}$/, maximum tension /Te_m/, maximum speed of contraction /V_c/ and relaxation /V_r/. From these, for comparison of the two different method, we used the values of Te_m.

Results

Examining the body weight of the animals we noticed that weight growth of hypokinetic animals considerably falls behind, compared to those of controls, while in the animals immobilized according to Musacchia - after a short stagnancy - the weight growth began and it nearly coincided with the weight growth of controls.

We illustrate the muscle weight changes in fig. 1.

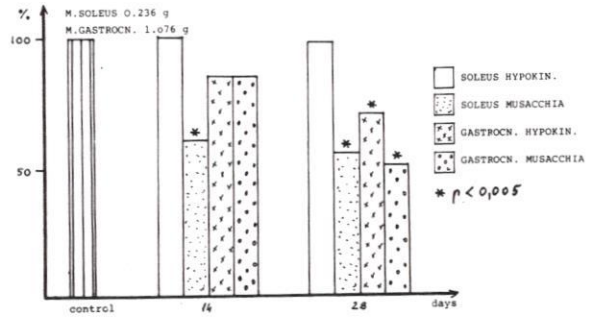


Figure 1. Muscle weight changes

As it can be seen the 14-28 days of hypokinesis period does not influence the weight of m.soleus but the Musacchia's immobilization has greatly decreased it already on the 14th day. The weight of m. gastrocnemius similarly decreased after 14 days in both weightlessness models, this decrease is more significant in Musacchia's model after 28 days.

Comparing the values of Te_m of hypokinetic and Musacchia's model we noticed the following. The hypokinesis does not cause tension decrease in m.soleus and it is little /not significant/ even in m. gastrocnemius on the 28th day. At the same time the Musacchia's immobilization produces an explicit tension decrease in m.soleus already on the 14th day and in m.gastrocnemius on the 28th day.

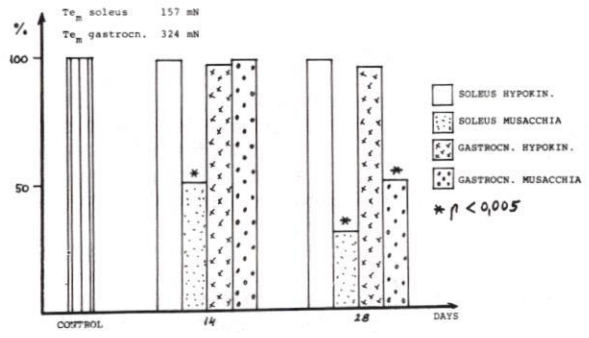


Figure 2. Tendency of Te_m values in the two different models

In the field of the other measured biomechanical parameters the two different muscles showed the following changes: the T_c and $T_{1/2}$ significantly decreased after 14 days in m.soleus. These values increased in m.gastrocnemius. After a 28 days period the above values decrease became more expressed in m.soleus and we tested a minimal decrease of T_c, and $T_{1/2}$ in m.gastrocnemius.

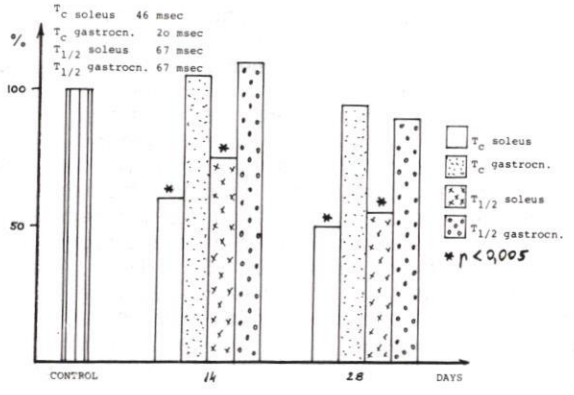


Figure 3. Tendency of T_c and $T_{1/2}$ in the two different muscles

V_c increased while V_r remained unchanged in m.soleus. In m.gastrocnemius V_r significantly increased by an unchanged V_c.

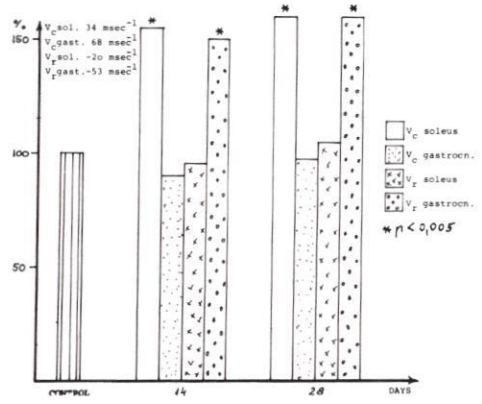


Figure 4. Tendency of V_c and V_r in the two different muscles

Conclusion

In the animals exposed to whole body hypokinesis - the applied exposition time- did not cause neither muscle weight decrease nor tension decrease in m.soleus. Muscle weight and tension decrease was rather slight in gastrocnemius.

In the animals -immobilized according to Musacchia- the atrophy of slow fibres could be seen as a prime factor. The slow type m.soleus showed changes, characterisctic to fast muscles. M.gastrocnemius is less sensitive to such kind of immobilization, in regard to biomechanical characteristics.

In the muscles, examined by us, the changes in biomechanical parameters - resulting from Musacchia's immobilization - correspond to so far studied earthy, furthermore even to bio-sputnik experimental biochemical and histochemical changes. The Musacchia's model simulates well the biomechanical changes, resulting in weightlessness.

TIME-COURSE OF SOLEUS MUSCLE-CHANGE IN AND-RECOVERY FROM DISUSE ATROPHY

M. Falempin, T. Leclercq, D. Leterme and Y. Mounier

Laboratoire de Physiologie des Structures Contractiles, Université des Sciences et Techniques de Lille, 59655 Villeneuve d'Ascq Cedex, FRANCE.

INTRODUCTION

Numerous observations on animals and human beings flown into space demonstrated the development of skeletal muscle atrophy particularly at the level of the antigravity muscles. These atrophic responses observed in hindlimb muscles after spaceflight were faithfully reproduced in ground based models of weightlessness such as hindlimb suspension used on rats (3,4). With these models, many authors described muscular atrophy and changes in the mechanical properties on different fast and slow muscles after various hindlimb suspension durations. However, few papers focused on the time-course of muscle changes and recovery for a very same muscle.

In the present investigation, an attempt was made, first, to demonstrate that the degree of atrophy and the changes of the mechanical parameters were dependent on the duration of the disuse and, secondly, to examine the kinetic of recovery after the development of a maximal atrophy.

METHODS

Male Wistar rats weighing 200-300 g were randomly distributed into three series : control rats, hindlimb suspended (HS) rats and rats submitted to hindlimb suspension followed by a recovery period (Rec). (HS) and (Rec) animals called "experimental" in the following description had their hindlimbs suspended in an apparatus as previously described (3). Periods of suspension were 2, 5, 8, 15 and 30 days. Recovery was tested on rats suspended for 15 days and allowed afterwards to walk normally on the floor of individual cages during periods of 2, 5, 8 and 15 days. For each period of suspension or recovery, groups of 5 animals (10 muscles) were studied. Simultaneous experiments were performed on age-matched control groups of 3 animals (6 muscles).

For experimental procedures, animals were anaesthetized using sodium pentobarbital (35 mg/kg, ip). The contractile properties of the soleus were recorded in situ after stimulation of the severed tibial nerve with square wave pulses (100 µs duration, supramaximal voltage). Measurements were made at the muscle length L_o for which maximal twitch tension was attained. Experiments were performed at 35°C.

The results were analysed using a two-way analysis of variance (ANOVA) and appropriate post hoc tests. A level of $P < 0.05$ was chosen as significant. Data are presented as means ± SE.

RESULTS

Table 1 shows the morphometric characteristics and the maximal peak tetanic tensions P_o on HS and Rec animals. In order to ease the reading of the table, absolute values for age-matched control groups were omitted and we chose to report the differences between each experimental group and its corresponding age-matched control group.

After 2 days of suspension, the soleus wet weight remained similar to that of the control group. Later on, the longer the suspension period, the larger the soleus atrophy, that reached a maximum effect after 15 days of suspension. No significant change was observed between 15 and 30 days. So, it appeared clearly that a structural atrophy occured on the soleus as early as after 5 days of disuse and that this atrophy happened progressively depending on the duration of the disuse until it reached a maximal state. When atrophy was achieved, the recovery was controlled after various periods of time. The soleus muscle weight showed a significant and progressive increase. After 15 days, the recovery remained incomplete compared to age-matched rats but the MW value reached that of the control group tested just before suspension (S, 0 days). The peak tetanic tension (P_o) either expressed in g or per cross-sectional area unit (g/mm^2) decreased continuously simultaneously with the development of atrophy until it reached a reduced level after 15

Table 1. Morphometric characteristics and peak tetanic tensions after different suspension and recovery periods.

		MW		CSA	P_o		
		mg	Diff	mm^2		g/mm^2	Diff
Suspension (days)	0	137.4 ± 8.6	-	7.8 ± 0.6	146.5 ± 7.7	18.7 ± 2.1	-
	2	133.4 ± 6.2	- 2.7	7.5 ± 1.8	123.2 ± 6.1	16.4 ± 2.4	- 14.6
	5	117.8 ± 7.4 ; * C,2	- 18.1	6.7 ± 1.2	81.0 ± 4.2	12.0 ± 1.2 ; * C,2	- 37.9
	8	112.1 ± 9.1 ; * C,2	- 24.0	6.4 ± 0.9	53.2 ± 3.5	8.3 ± 0.9 ; * C,5	- 57.5
	15	87.6 ± 4.7 ; * C,8	- 46.4	5.0 ± 1.0	30.7 ± 0.7	6.1 ± 0.7 ; * C,8	- 68.9
	30	88.1 ± 11.6; * C,8	- 50.8	5.1 ± 0.7	27.0 ± 2.4	5.2 ± 1.0 ; * C,8	- 70.0
Recovery after 15 days suspension (days)	2	92.5 ± 4.8 ; * C	- 45.6	5.4 ± 0.7	32.4 ± 2.2	6.0 ± 0.6 ; * C	- 70
	5	114.2 ± 7.2 ; * C,2	- 34.4	6.6 ± 1.0	57.4 ± 2.8	8.7 ± 0.8 ; * C,2	- 56.5
	8	138.4 ± 9.4 ; * C,5	- 21.4	7.7 ± 1.8	92.4 ± 3.1	12.1 ± 1.1 ; * C,5	- 38.6
	15	134.4 ± 12.0; * C,5	- 19.8	7.6 ± 2.2	136.8 ± 18.0	18.0 ± 2.6 ; * C,8	- 9.1

*MW : muscle wet weight ; CSA : cross-sectional area estimated by dividing MW by L_o ; P_o : peak tetanic tension recorded at 40 Hz ; Diff : differences as expressed in per cent of each age-matched control group. * C, day : significant differences, first, between control and experimental group and, secondly, between the considered experimental group and the nearest significantly different group of the same serie of animals ($P < 0.05$).*

days of HS. Then it remained unchanged. After reaching the maximal decrease, recovery started after 5 days and returned progressively to a value similar to that of the control group before suspension. However a fortnight is not enough for complete recovery compared the age-matched group. The difference remained equal to 9.1 per cent.

Different parameters can be used to characterize a muscle type. In our experiments, three were measured (Fig. 1) : the contraction time (CT) or time to peak tension, the (P_{20}/P_0) ratio which corresponded to the subtetanic tension at 20 Hz relative to P_0 and finally the fatigue index (FI). This latter parameter was determined by 330 ms trains of impulses delivered at 40 Hz once a second for a 2 min period. FI was calculated as the ratio between the relative tension at 2 min and the maximum tension obtained at the beginning of the stimulation.

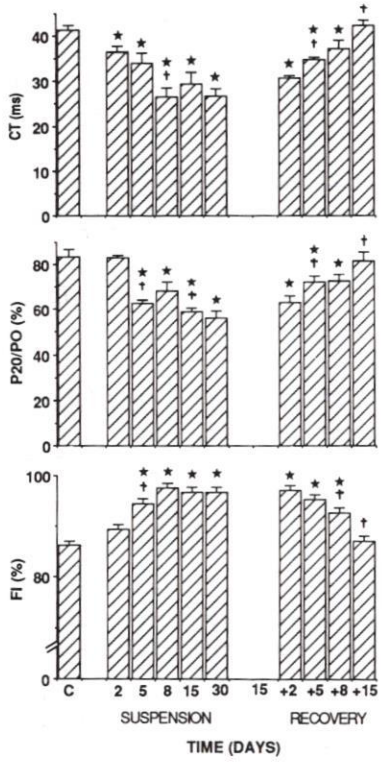


*Figure 1. Influence of suspension and recovery durations on the contraction time (CT), the P_{20}/P_0 ratio and the fatigue index (FI) of soleus muscle. C : control values ; * : significant difference between each experimental group and its age-matched control group ; † significant difference between the considered group and that just preceeding.*

Since no significant changes were found between the different control groups, control values for each parameter were considered as a single group for the graph and were reported on the extreme left. However all our statistical analyses were made between each experimental group and its corresponding age-matched control group.

CT progressively decreased in HS conditions from a mean value of 42 ms to 27 ms after 8 days of HS. No other change was observed for longer suspension. So the slow soleus became faster after HS.

The mean P_{20}/P_0 ratio equalled 83.5 % for the control groups, a value similar to those generally reported for a slow muscle. It significantly decreased after 5 days of HS to about 60 % after 15 days of HS. This lower value indicated a pattern more typical of a faster muscle since this ratio was equal to about 30 % in a fast muscle such as the EDL.

Finally, the fatigue index FI increased during suspension indicating that the soleus became more resistant.

For the three parameters, recovery appeared as a progressive process and was maximal after 15 days.

DISCUSSION

The present study dealt with the rates at which muscular atrophy and recovery of a slow postural muscle, the soleus, occured after different periods of HS. Our results showed that the muscular atrophy was a process which developped progressively until it reached a maxima after 15 days of HS. A "plateau" in the development of the muscular atrophy was suggested so far as the values of the MW and P_0 were not significantly different between 15 and 30 days of HS. The muscular atrophy could be correlated to the decrease in the cross-sectional area of the muscular fibers observed after HS (1) or after spaceflight (5) and partly explained by a decrease in the protein content (2). Our data showed that after HS, the slow soleus muscle reacted as a faster muscle. The CT and the P_{20}/P_0 ratio decreased significantly comparatively to control values indicating a transformation of the muscular phenotype towards that of a faster muscle. The FI was also modified : the soleus muscle became more resistant. This latter result might appear in contradiction with the changes of the two other parameters. However, all these modifications could be explained by an increase of the percentage of type II_A fast, fatigue resistant muscular fibers, observed after different periods of HS (1).

Our results also showed that a period of 15 days of spontaneous recovery was sufficient since most of the studied parameters recovered values comparable to control values. A significant recovery was observed after 5 days and was achieved progressively up to 15 days. A similar progressive readaptation of the soleus muscle was also found after spaceflight cosmos 1129 (6). These two data suggested that the readaptation period required for normalization of the soleus muscle after disuse seemed to be positively associated with the length of the disuse period itself.

REFERENCES

1. Desplanches D., Mayet M.H., Sempore B. and Flandrois R. 1987. Structural and functional responses to prolonged hindlimb suspension in rat muscle. J. Appl. Physiol., 63, 558-563.
2. Loughna P.I., Goldspink D.F. and Goldspink G. 1987. Effects of hypokinesia and hypodynamia upon protein turnover in hindlimb muscles of the rat. Aviat. Space Environ. Med., 58, 133-138.
3. Morey E.R. 1979. Spaceflight and bone turnover : correlation with a new rat model of weightlessness. Bioscience, 29, 168-172.
4. Musacchia X.J., Deavers D.R., Meininger G.A. and Davis T.P. 1980. A model for hypokinesia : effects on muscle atrophy in the rat. J. Appl. Physiol. 48, 479-486.
5. Oganov V.S., Skuratova S.A., Potapov A.N. and Shirvinskaya M.S. 1980. Physiological mechanisms of adaptation of rat skeletal muscles to weightlessness and similar functional requirements. The Physiologist, 23, 16-21.
6. Szilaqyit T., Rapcsak M., Szöor A., Oganov V.S. and Skuratova S.A. 1984. Readaptation of rat's muscles following space flight. The Physiologist 27, 79-80.

This work was supported in part by the Centre National d'Etudes Spatiales.

EVIDENCES FOR SLOW TO FAST CHANGES IN THE CONTRACTILE PROTEINS OF RAT SOLEUS MUSCLE AFTER HINDLIMB SUSPENSION : STUDIES ON SKINNED FIBERS.

L. Stevens, Y. Mounier

Laboratoire de Physiologie des Structures Contractiles, Université des Sciences et Techniques de Lille, 59655 Villeneuve d'Ascq Cedex. FRANCE.

INTRODUCTION

Muscular functions are well known to be altered by an exposure to weightless environments, i.e. after spaceflights (6). The effects were found to be greatest on muscles which were responsible for postural and loading functions. To assess physiological and/or biochemical responses of muscles exposed to weightlessness without any spaceflight experimental limitations, a variety of animal models were developed (4). In this study we used a hindlimb suspension model which consisted in a suspended rat whose hindlimbs were non-load-bearing for a certain period. Previous works on rats reported that hindlimb suspension (HS) provoked muscle atrophy, alterations in muscle contractile properties (8) and in fiber type distribution (2).

To approach these problems we chose the soleus muscle since it provided good representation of load-bearing functions. Our study of the contractile properties focused on the contractile protein comportment towards the calcium ions (tension/pCa, V_{max}). Moreover, the fiber sensitivity towards the strontium ions was controlled since it is known that slow or fast muscle fibers can be distinguished by their strontium sensitivity.

METHODS

Adult Wistar rats (250 g) were suspended for fifteen days as previously described in Wronski and Morey-Holton tail suspension model (4). This form of hindlimb suspension (HS) allowed the rats to walk freely on their forelimbs and gain continuous access to food and water. A group of non-suspended or control rats was used for comparison.

On the 15th day of HS, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg body wt) and the muscles were rapidly excised from the hindlimbs of the rats. A chemical skinning procedure was performed by exposure of the muscular biopsies to "a skinning solution" containing EGTA (5). The use of skinned fibers permitted a direct investigation of the contractile apparatus. The experiments consisted in recording isometric contractions developed by a single skinned fiber bathed in pCa or pSr activating solutions ($pX = - \log[X]$). These solutions were calculated using a computer program (5).

Statistical analysis (significance at 95 % confidence level) was performed using the Student's t test comparing suspended (n = 12) and control rats (n = 10). Data were presented as mean ± S.E.M.

RESULTS AND DISCUSSION

After two weeks of HS, the soleus muscle fibers were first characterized by a pronounced atrophy. Indeed, our results showed that fiber diameter significantly decreased from 76.07 ± 2.57 µm to 53.57 ± 1.30 µm. Moreover, the maximal isometric tension $\bar{P}_o$, expressed in mg and elicited by a high calcium solution (here pCa 4.6) significantly dropped from 32.00 ± 3.62 mg to 16.00 ± 0.64 mg. However, this maximal force P_o when expressed per cross-sectional area remained unchanged : 0.91 ± 0.12 $kg.cm^{-2}$ for control and 0.84 ± 0.13 $kg.cm^{-2}$ for suspended rats. This indicated that the force loss in terms of absolute tension could directly be correlated with the decrease in fiber diameter and thus with the muscle atrophy. This muscle atrophy was in complete agreement with the one generally described after similar periods of muscle disuse and was reported to be primarily due to a loss in muscle myofibrillar proteins, resulting from decreased protein synthesis and/or increased protein catabolism (1).

One way to examine the properties of the contractile proteins of muscle fibers was to establish the relation between calcium concentration (expressed in pCa units) and developed tension. Fig. 1 showed, on control and suspended rats, typical records of tension developed by a single fiber after the application of various pCa solutions. These records illustrated the loss of muscle strength after atrophy since for a given pCa value, the tension (in mg) decreased on suspended rat fibers.

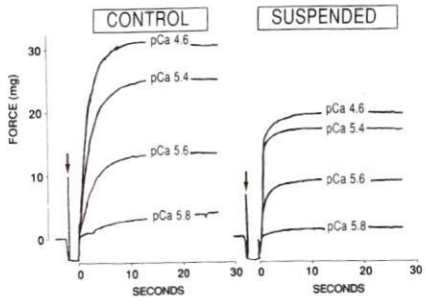


Fig. 1. Representative tension records for a control and a suspended rat fiber.

According to these records, the relationship between relative tension and pCa (T/pCa) could be established : the tension P at each pCa value was normalized to P_o and related to the calcium concentration, for control and atrophied animals (Fig. 2, A). Various parameters derived from these curves described the characteristics of the contractile proteins : the first observation was that after HS the T/pCa curve was not shifted along the pCa axis. So, the value of the pCa_{50}, corresponding to the value of the pCa necessary to obtain half maximal tension, was not changed. Since this value indicated the apparent calcium affinity of the contractile apparatus, no change in such affinity could be considered here.

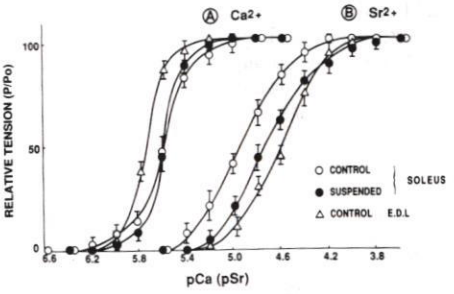


Fig. 2. T/pCa and T/pSr relationships of soleus and EDL muscle fibers.

Another parameter describing the T/pCa relationship was the value of the calcium threshold of activation. The value of this parameter appeared higher after suspension since it was pCa 6.4 for control animals and pCa 6.2 for suspended ones. This meant that after HS, fibers started to contract with a higher calcium concentration when compared to control fibers. By establishing the T/pCa curve for a typical fast muscle, the EDL, we showed that the value of the Ca^{2+} threshold of activation for soleus after HS was comparable to the one found for EDL (pCa 6.2). Finally another result could be derived from the T/pCa curve : the Hill coefficient which described the steepness of the curve.

A convenient way to examine the changes in the steepness was to linearize the T/pCa experimental curve (Fig. 3). The transformation resulted in plots that could be treated as two straight lines, one for P/P_0 above 50 % and another one for P/P_0 under 50 %.

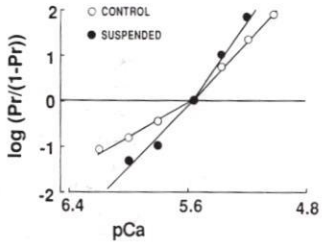


Fig. 3. Hill plot transformation of the T/pCa experimental data.

It appeared that the Hill coefficients n_1 (for $P/P_0 > 50$ %) and n_2 (for $P/P_0 < 50$ %) were equal to 2.9 and 2.4 under control conditions. After HS, both slopes of the relation were higher, n_1 and n_2 becoming respectively equal to 3.4 and 3.0. In most current models of muscle activation, the value of the Hill coefficient reflected the cooperativity among Ca^{2+} binding sites along the thin filament. So, the observed difference in steepness after HS might be explained by a greater cooperativity in the soleus of the suspended animals, which seemed to behave more like a fast muscle. Indeed, a much steeper T/pCa relationship was also found for the EDL muscle and was generally described for fast skeletal muscle (5, 7).

To get more information about this assumed evolution, we established the tension response to strontium ions (T/pSr) since it is used to classify the different fiber types of slow and fast skeletal muscles (3). As generally described all skeletal muscles presented less affinity to Sr^{2+} than to Ca^{2+} ions. However, slow skeletal muscle fibers are known to be more sensitive to Sr^{2+} ions than fast skeletal muscle fibers. Here, the T/pSr relationship was established for control and atrophied soleus fibers, and also for fast EDL control fibers. Fig. 2B showed that for control soleus the T/pSr was clearly shifted to the right by 0.7 unit when compared with the T/pCa curve. After HS, although the calcium affinity was unchanged, we found a difference of 0.9 unit between the T/pCa and the T/pSr curve. So, after HS, the typical slow soleus fibers got less Sr^{2+} affinity. Moreover, the T/pSr position of HS animals was close to the one of the fast EDL muscle, which was shifted by 1.0 unit when compared with the EDL T/pCa curve.

To bring more evidences of slow-to-fast changes in the soleus contractile properties after disuse, fiber contraction kinetics were studied. The contraction speed for control and HS rat fibers was estimated by measuring electronically the first derivative of the force development. That derivative corresponded to the maximal value of the ratio dP/dt also called V_{max}. We can see on the records of Fig. 1 that after HS the force developed faster. V_{max} was related to the corresponding pCa value (Fig. 4) : for a same pCa value, V_{max} was higher after HS.

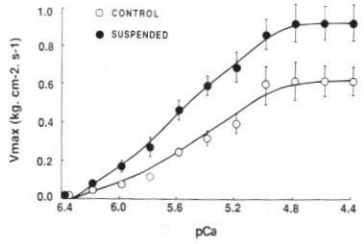


Fig. 4. V_{max} of control and HS rat muscle fibers.

This increase in contraction speed was consistent with the transformation of type I slow fibers into type II fast fibers described after HS (2).

To conclude, after a period of disuse induced by fifteen days of HS, the soleus muscle showed very important changes in its contractile properties. All the data provided additional evidences of a transformation of its usually slow characteristics into those of a faster muscle.

REFERENCES.

1. Booth F.W., Seider M.J. 1979. Early change in skeletal muscle protein synthesis after limb immobilization of rats. J. Appl. Physiol., 47, 974-977.
2. Desplanches D., Mayet M.H., Sempore B., Flandrois R. 1987. Structural and functional responses to prolonged hindlimb suspension in rat muscle. J. Appl. Physiol., 63, 558-563.
3. Fink R.H.A., Stephenson D.G., Williams D.A. 1986. Calcium and strontium activation of single skinned muscle fibres of normal and dystrophic mice. J. Physiol., 373, 513-525.
4. Morey-Holton E.R., Wronski T.J. 1981. Animal models for simulating weightlessness. The Physiologist, 24, S45-S48.
5. Mounier Y., Holy X., Stevens L. 1989. Compared properties of the contractile system of skinned slow and fast rat muscle fibres. Pflügers Arch. In press.
6. Oganov V.S., Potapov A.N., Skuratova S.A., Shirvinskaya M.A. 1980. Variability of physiological properties of rat skeletal muscles at different gravity levels. The Physiologist, 23, S71-75.
7. Stephenson D.G., Williams D.A. 1981. Calcium-activated force responses in fast- and slow-twitch skinned muscle fibers of the rat at different temperatures. J. Physiol., 317, 281-302.
8. Templeton G.H., Paladino M., Manton J., Glasberg M., Silver C.J., Silver P., Demartino G., Leconey T., Klug G., Hagler H., Sutko J.L. 1984. Influence of suspension hypokinesia on rat soleus muscle. J. Appl. Physiol., 56, 278-286.

This work was in part supported by the "Centre National d'Etudes Spatiales".

THE EFFECT OF THE ANGULAR VELOCITY ON THE MUSCLE COMPOSITION IN JAPANESE QUAIL

JANKELA J., BOĎA K., BARANOVSKÁ M.[x]

Institute of Animal Physiology
Slovak Academy od Sciences, 900 28 Ivanka pri Dunaji, Czechoslovakia
[x]Institute of Zoohygiene and Veterinary Technique, 918 21 Trnava, Czechoslovakia

Experimentation in gravitational biology is aimed at the study of the effect of acceleration on biological subjects. Acceleration fields can be produced by increasing the rate of motion (linear accelera tion) or changing the direction of motion (centrifugation). For long-term experiments only centrifugation can be employed. However rotation, a property of centrifugation, may have separate biological effects. Rather slow processes, such as growth or feed intake are probably not affected (5), however, in our previous ex periment on Japanese quail (2) a great stress effect of the rotation was shown followed by the changes in muscle composition. The objective of the present work was to study the effect of chronic rotation at stabile hypergravitation (2 G) on the composition of two muscle systems in Japanese quail.

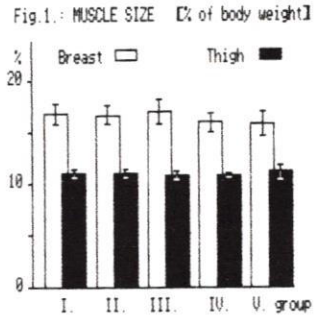
MATERIALS AND METHODS

30 hens of Japanese quail aged 68 days maintained on a commercial mash "ad libitum" were divided into 5 groups. The control group of 6 birds was reared under stationary conditions and mechanically loaded with an additional load of 2 G value for 96 hours continually. The other four groups (6 birds each) were continually exposed to a different angular velocity by centrifugating animals at 2 G hypergravitation for the same time period. The different angular velocity was reached by the alternation of the diameter and frequency of rotation the parameters of which are in table I.

TABLE I.	PARAMETERS OF THE EXPERIMENT		
Group	Angular Velocity [$\Pi.s^{-1}$]	Frequency [RPS]	Radius [cm]
I.	-	-	-
II.	0.8	23.6	320
III.	0.9	27.0	245
IV.	1.0	30.0	198
V.	1.1	33.0	164

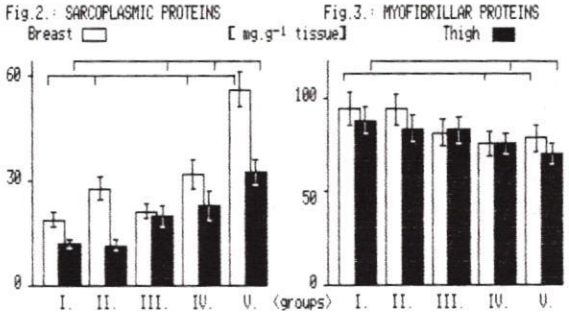
After 96 hours the animal were exsanguinated by decapitation and samples of breast and thigh muscles were taken. The sarcoplasmic and myofibrillar protein fractions were separated by the method of Hellander (3); protein contents were determined by the method of Lowry et al. (4) and nucleic acids, expressed as phosphorus, were determined by the method of Canev and Markov (1). The analysis of variance and multiple comparisons among treatment means were made using the Duncan's test. The statistical differences at the level of $P < 0.05$ were indicated in the figures.

RESULTS



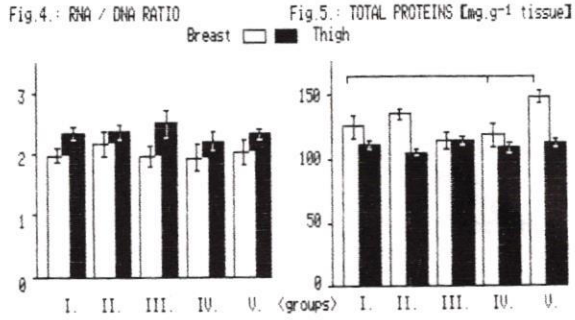
No significant differences in muscle size expressed as percentage of body weight (Fig.1) were found between experimental groups. Howewer the rotation lasting four days tends to the slightly lower size of the breast muscle without any effect on the size of thigh muscle.

The concentration of sarcoplasmic proteins (Fig.2) was significantly enhanced namely in breast muscle due to an increased angular velocity. The highest value was obtained in the animals rotated at the highest frequency (group V). The sarcoplasmic fraction in the thigh muscle was affected in aproximately the same manner.

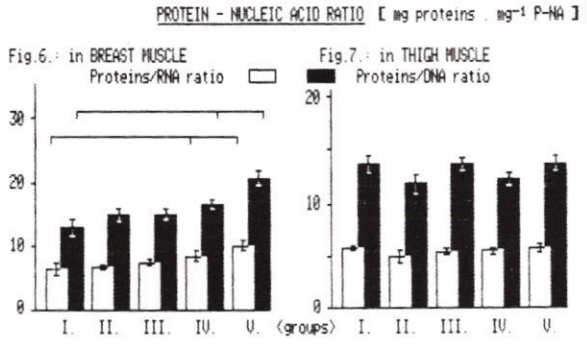


The content of myofibrillar proteins (Fig. 3) was reduced in the rotated animals. Significant differences, in breast as well as in thigh muscles, were obtained only in animals kept under high angular velocity (group IV, V).

The contents of ribonucleic (RNA) and desoxyribonucleic (DNA) acids in the muscles



were not significantly affected by employed treatments. Rotated animals showed only the tendency to diminution of the nucleic acid contents as a function of angular velocity. However, the ratios of RNA/DNA remained unchanged (Fig. 4). The values of total proteins in breast and thigh muscles did not show any, observed dependence (Fig. 5).
The expression of the amount of total proteins per unit size of nucleic acids changed these relations. The rotation evoked a significant increase of protein-RNA or



protein-DNA ratios (Fig. 6) in breast muscle of animals kept under high angular velocity (groups IV,V). Values of protein - nucleic acids ratios in thigh muscles had no significant differences between groups of animals (Fig. 7).

DISCUSSION AND CONCLUSION

Chronic rotation influenced some parameters of protein metabolism in muscles of Japanese quail. The comparison with animals loaded mechanically to the same extent of hypergravitation as at rotation enabled to eliminate the effect of hypergravitation alone at least in distinct muscles. It was shown that effects of a 4-day exposure of animals to rotation seems to be in dependence on the value of angular velocity of that motion. It was surprising that examined parameters were influenced predominantly in breast muscles. Muscle size, though nonsignificantly, was diminished in dependence on the increased angular velocity. The most detectable changes were obtained in the proportion of the main muscle protein fractions. The ratio of sarcoplasmic to myofibrillar proteins was gradually brought down first of all due to an expressively significant increase of sarcoplasmic fraction. It could testify to the modifications in protein equipment of the muscle cell. The relations between total proteins and nucleic acids confirm it. Though the diminution of nucleic acids such as changes in total protein contents were not significant, the increase of protein-DNA ratio was statistically important and enlarged in dependence on the growth of angular velocity. The course of protein-RNA ratio was in the same manner. These processes may signify that the increase of angular velocity influenced the growth of muscle cell size, probably on the level of protein synthesis. Observed tendencies to the decrease of total muscle size accompanied with the decrease of nucleic acid concentration in muscle is then achieved by the decline of the cell number.

REFERENCES

1. Canev,R.G.,Markov,G.G.:Biochimija,25, 151-159 /1960/
2. Gažo,M.,Boda,K.,Jankela,J.,Výboh,P., Juráni,M.,Baranovská,M.,Sabo,V., Strážnická,H.:The physiologist,31, 138-139 /1988/
3. Hellander,E.:Acta physiol.Scand.,41, 141 /1957/
4. Lowry,O.H.,Rosenbrough,N.J.,Farr,D.L., Randall,J.:J.biol.chem.,193,265-275 /1951/
5. Wunder,C.C.,Milojevic,B.,Eberly,L.: Nature,210,177-179 /1966/

BONE EFFECTS OF 13 DAYS OF WEIGHTLESSNESS ON RAT AND MONKEY SOME RESULTS OF BIOCOSMOS 1887 AND GROUND SIMULATIONS

ZÉRATH E., NOGUES C., BORNE M., SOURDAINE P.

Centre d'Études et de Recherches
de Médecine Aérospatiale
75731 PARIS Cedex 15 - FRANCE

The loss of bone mass is one of the main effects of microgravity. It has been observed in man using external methods (2), but in animals special techniques provide direct access to skeletal structures. This study was carried out during the Biocosmos 1887 program. It is a continuation of research already done in rats and primates bone tissue, both inflight and using experimental ground methods.

METHODS

Two different experiments are described : the 1887 Biocosmos spaceflight, and a rat-tail suspension experiment.

1- Biocosmos 1887

- Monkeys

Two male Rhesus monkeys approximately 4kg, Drioma and Erocha, flew in a 13-day spaceflight, and were exposed to a "synchronous" experiment reproducing flight conditions 43 days after landing. Their bones were labelled with calcein (20mg/kg) two days prior to and one day after flight as well as for the synchronous experiment. Drioma was used for two iliac crest biopsies, 16 days postflight on the right side, and 16 days after the synchronous experiment on the left side. Only one biopsy was performed on Erocha, after the synchronous experiment. Indeed, upon return to earth this animal received no calcein and was not subjected to a biopsy due to a degradation in its general condition resulting from a decreased amount of ingested food inflight.

- Rats

The left humerus and the 9th thoracic vertebra of twenty 3-month old rats were studied. Five flight animals were compared with 3 control groups of 5 rats: the synchronous group was exposed on Earth to the same experimental conditions as the flight batch, the basic control and vivarium batches were maintained on Earth in a vivarium and respectively sacrificed at the beginning and at the end of the experiment. After landing, the flight animals remained 1 day in the biosatellite, and 2 days at Earth gravity before they were sacrificed.

2- The rat-tail suspension experiment :

Ten 180g-male Wistar rats were randomly distributed into two groups of five : a vivarium group, and a 14 day-tail suspension group, according to Morey's technique (5). The study of weight curves (fig 1), which reflects the general health of the animals, shows that suspended animals continued growing during suspension but less than controls. After suspension animals were returned to their natural horizontal position for two days. They were then sacrificed with a high dose of Nesdonal(R) and their humeri were removed.

For all undecalcified bone samples, histomorphometric measurements were made using image analysis systems (Videoplan Zeiss-Kontron and NS-1500 Nachet) on 5-7µm thick slides stained using a Goldner-Masson trichromic technique. The following parameters were investigated :

- trabecular bone volume,
- relative osteoblast apposition surface,
- relative osteoclast resorption surface,
- calcification rate, in monkey iliac bone biopsies.

All these measurements were performed at a 250 enlargement factor. Results for rats were submitted to a statistical analysis of variance. For primates, each monkey was considered as its own control.

RESULTS

1- Monkeys

- Drioma : in the endocortical area, it appeared that the calcification rate was the lowest during spaceflight (1.25±0.11 µm/day) and gradually increased during the recovery period (1.40±0.11 µm/day) and during the synchronous experiment (1.65±0.13 µm/day).

- Erocha : inflight calcification rate could not be measured because of the absence of the second labelling, but three good quality labellings made it possible to compare the synchronous experiment (1.77±0.46 µm/day) with the preceding flight and post-landing recovery period (1.22±0.18 µm/day).

2- Rats

- Vertebrae

- The smallest trabecular bone volume was observed in "Flight" animals (21.08±1.16 %), and showed a significant difference (P<5%) with the "Vivarium" animals (29.58 ± 2.69 %).
- The relative osteoblastic apposition surface was the smallest in "Flight" animals (5.0±1.1 %), with a significant difference (P<5%) with the "Vivarium" animals (13.9±2.8 %).
- The osteoclastic resorption showed no significant difference between "Flight" and control animals

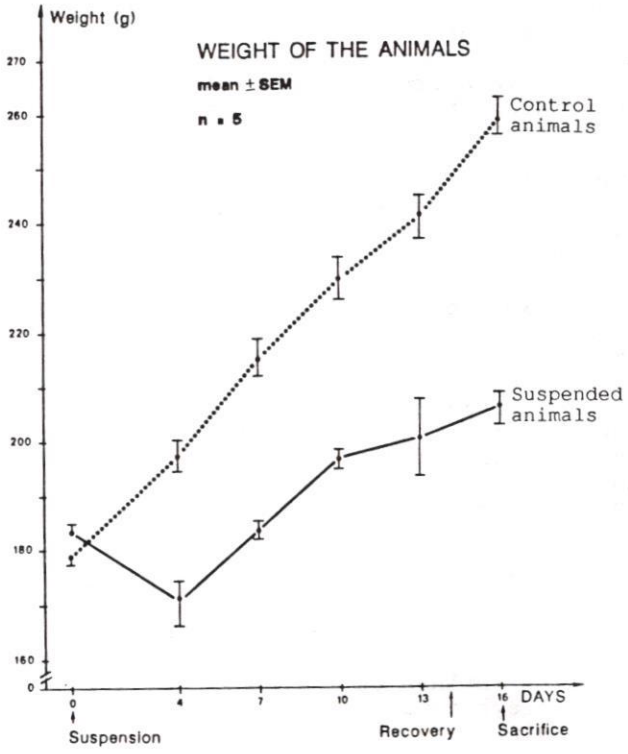


fig 1

- Humeral metaphyses

- Flight animals had the smallest trabecular bone volume (10.8±3.2 %). The greatest trabecular bone volume was observed for the vivarium animals (17.7±1.4 %), and there was a significant difference (P<5%) between "Flight" and ground animals.

- but osteoblastic apposition surface was larger in the "Flight" batch (33.1±8.1 %) than in vivarium animals (24.8±2.3 %).

- and no significant difference was observed between means of osteoclast resorption surfaces.

These results, and especially the measurements of the osteoblastic apposition surface in the humeral metaphysis can be compared to results provided by our experiment with rats suspended for 14 days and returned to the vivarium 48 hrs before sacrifice (fig 2). We actually observed not only a non significant decrease in trabecular bone volume of humeral metaphyses in the suspended batch, but also that same relative increase in osteoblastic populations as was observed in flight animals (34.5±4.9 % ; 17.0±3.6%).

HUMERAL METAPHYSIS

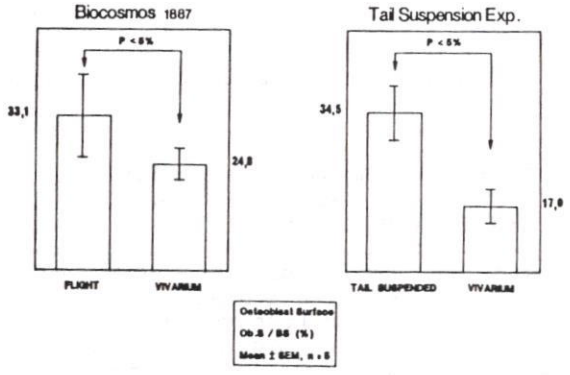


fig 2

DISCUSSION

1- Monkeys

Results should first have to take into account the fact :

- that animals were young, still growing, and had not reached full skeletal maturity,

- that with no preflight double labelling, it is not possible to assert how flight has really reduced the calcification rate.

- and that it is also difficult to describe postflight the dynamics of cell activities since there were 16 days between landing and biopsy.

2- Rats

Results must be discussed with the circumspection required when using an animal model whose bone growth characteristics are different from man's, especially the bone growth activity which is permanent.

Flight animals mass parameters of both proximal humeral metaphyses and vertebrae show a 30 % decrease, whereas they were not affected either by 5 days (3) or by 7 days (4) of flight. However, 18 days of spaceflight (COSMOS 1129) (1) already induced a decrease in humeral trabecular bone volume in rats.

But the most important question which we have to discuss concerns postlanding time and events before animal sacrifice. This is a very important point because the animals had nearly 48 hours on Earth, under difficult survival conditions :

Two questions should be reflected upon :

- First, the stress associated with these events : difficult to evaluate, but probably significant,

- Second, the effect of 48 hours spent at earth gravity on bone cell populations. Although it may not be enough to alter bone mass parameters, it could have affected cell activity as we know that preosteoblasts, in particular, have a cycle of less than 2 days to transform into active osteoblats (6). Results of our experiment with suspended rats could support this hypothesis.

We must therefore discuss the bearing property of the humerus in a suspension experiment. We have seen that bone alterations in mass parameters and cell activity follow the same trend as those induced by flight, where the humerus was exposed to a real microgravity. This suggests a possible systemic factor involvement in suspension effects.

Finally, the attention should be drawn to the small number of animals in the various experimental batches of rats (n = 5) which often makes result interpretation difficult.

CONCLUSION

During this 13-day spaceflight, we observed :

- In monkeys : a lower calcification rate inflight than during the following ground periods.

- In flight rats : lower bone mass values in the humeral metaphyseal and vertebral areas.

However, for this flight, the two days of postlanding recovery most probably induced measurable changes in cell osteoblast activity.

Acknowledgement : This work was realized in collaboration with Moscow IMBP (Dr Bakulin and Dr Novikov) and supported by the CNES. The authors are grateful to Danielle Freund for her very helpful translation work.

REFERENCES

1- JEE W.S.S., WRONSKI T.J., MOREY E.R., and KIMMEL D.B.: Effects of spaceflight on trabecular bone in rats. Am J Physiol 244 (Regulatory Integrative Comp Physiol 13):R310-R314, 1983.

2- RAMBAUT P.C., JOHNSTON R.S. : Prolonged weightlessness and calcium loss in man. Acta Astron 6: 1113-1122, 1979.

3- VICO L., CHAPPARD D., ALEXANDRE C., PALLE S., MINAIRE P., RIFFAT G., NOVIKOV V.E., and BAKULIN A.V.: Effets de l'apesanteur sur le tissu osseux de rat après un vol spatial de cinq jours (COSMOS 1514). J Physiol Paris, 82,1-11, 1987.

4- WRONSKI T.J., MOREY-HOLTON E.R., DOTY S.B., MAEJE A.C., and WALSH C.C. : Histomorphometric analysis of rat skeleton following Spaceflight. Am J Physiol 252 (Regulatory Integrative Comp Physiol 21) R252-R255, 1987.

5- WRONSKI T.J. and MOREY-HOLTON E.R. : Skeletal responses to simulated weightlessness : a comparison of suspension techniques. Aviat Space Environ Med, 58:63-68, 1987.

6- YOUNG R.W. : Regional differences in cell generation time in growing rat tibiae. Exp Cell Res 26 : 562-567, 1962.

EFFECTS OF A 40 DAY TAIL-SUSPENSION ON RAT WEIGHT-BEARING BONES

L. Vico[1], V.E. Novikov[2], J.M. Very[1], D. Chappard[1], C. Alexandre[1].

1- Laboratoire Biologie Tissu Osseux LBTO - Faculté de Médecine - Saint-Etienne - France
2- Institut des Problèmes Biologiques et Médicaux IPBM - Khoroshevskoye Shosse 76 A - Moscou - URSS

INTRODUCTION

Relatively short duration suspension experiments (few days to few weeks) were done since spaceflights of Biocosmos series and Spacelab-3 mission were not longer than 3 weeks. The adaptation of bone tissue until two weeks of skeletal unloading by tail suspension is well documented (4, 14). We performed 7-day (12) and 14-day (13) tail-suspension experiments: in the first experiment, a severe imbalance in bone cell activities (increased resorption and decrease formation) results in bone loss; in the second experiment, bone cellular activities come back to equilibrium and bone tissue loss is no longer observed.

A 40-day tail-suspension was undertaken in order to test whether the normalization seen in the two week experiment constitute a stable phase despite continued unloading.

MATERIALS AND METHODS

Male Wistar rats (140 day old) weighed about 400g at the beginning of the experimental period. These rats were in their lower growing phase. Tail suspension was performed according to Wronski and Morey-Holton (14) recommendations. The tail bandage covered less than one-half the tail surface, allowing adequate thermoregulation through the tail. Five rats suspended (group S) in this manner supported as well as possible the experimental period. Five control rats (group C) were housed in individual cages and five basal control rats (group BC) were sacrificed at the beginning of the experimental period. All rats were fed and watered *ad libitum* with a standart rat chow. Room conditions were 23° ± 1°C and light cycle LD 12:12. Body weights of S and C groups were recorded at the beginning, in the middle and at the end of experiment. At time of sacrifice all rats were guillotined and selected bones were removed for analysis.

Proximal tibiae were analysed in S, C and BC groups. Thoracic and lumbar vertebrae were analysed in S and C groups.

Longitudinal sections were made in long bones. Transversal sections in the midpart of the body were made in vertebrae at the level of the secondary spongiosa. Height sections per sample, 7 µm thick, were sawed for trichrome Goldner staining, allowing evaluation of bone mass and osteoid parameters. Six others 7 µm sections were used for tartrate resistant acid phosphatase evidencing osteoclastic cells (2).

Measurements similar to those of previous suspensions were done. Briefly, in tibiae, primary and secondary spongiosae were considered as two distinct areas. The thickness of the primary spongiosa in µm was used to evaluate possible alteration of longitudinal elongation of the bone. In the secondary spongiosa, bone mass parameters measured were: the trabecular bone volume per tissue volume (BV / TV in %), trabecular thickness (Tb.Th in µm), trabecular number (Tb.N in /mm). The resorption activity was reflected by the number of osteoclast per bone perimeter (N.Oc+ / B.Pm in c/mm) and the active resorption surfaces osteoclast surfaces per bone surfaces (Oc+. S/BS in %). The osteoid surfaces per bone surfaces were determined at cancellous (Cn OS/BS in %) and endocortical (Ec OS/BS in %) levels.

Statistical analysis was performed with the non parametric Mann and Whitney test. P values less than 0.05 are considered to be significant.

RESULTS

Mean body weights were exposed in Table 1. No statistical difference is observed between S and C at the end of the experiment.

Body weights (g)	beginning	middle	end
Basal control rats	398 ±49	–	–
control rats	407 ± 32	444 ± 32	450 ± 32
suspended rats	426 ± 32.5	404 ±57	430 ± 43

Table 1 - Body weights in different groups at the beginning, the middle and the end of the experimental period (means ± SD).

Tibial bone histomorphometric data are listed in Table 2. No difference was seen between B and BC parameters except for the mean thickness of the primary spongiosa which is decreased in older rats. In S rats, the primary spongiosa was inexistant. Bone mass parameters were also affected in the secondary spongiosa where trabeculae were thin and less numerous. No statistical difference was seen in bone cell activities, however osteoclastic parameters showed higher values and osteoid parameters lower values in S group.

	suspended rats (n=5)	control rats (n=5)	basal control rats (n=5)	basal control vs control	suspended vs control
1° Spongiosa mean thickness	0	94.8 ± 9.88	120 ± 5	P = 0.08	
2° Spongiosa BV/TV (%)	7.09 ± 3.81	12.45 ± 4.6	14.67 ± 6.6	NS	P = 0.056
2° Spongiosa Tb.Th (µm)	36.8 ± 4.21	43.4 ± 2.30	49.2 ± 5.39	NS	P = 0.056
2° Spongiosa Tb.N (/mm)	1.86 ± 0.8	2.86 ± 1.06	2.92 ± 0.50	NS	P = 0.056
2° Spongiosa N.Oc+/B.Pm (c/mm)	4.64 ± 0.93	3. 59 ± 1.09	2.63 ± 0.68	NS	NS
2° Spongiosa Oc+.S/BS (%)	7.45 ± 1.00	5.89 ± 1.77	4.45 ± 1.19	NS	NS
2° Spongiosa Cn OS/BS (%)	4.14 ± 2.38	5.02 ± 1.44	5.36 ± 0.82	NS	NS
2° Spongiosa Ec OS/BS (%)	64.1 ± 14.5	77.3 ± 10.3	77.38 ± 9.33	NS	NS

Table 2 - Quantitative bone histomorphometry in the proximal tibial metaphysis of suspended, control and basal control rats. (means ± SD, NS = non significant).

Vertebral bone mass and osteoclastic parameters and listed in Table 3. No change was observed in the thoracic vertebrae whereas important increase in osteoclastic parameters occurs in S group at the level of the lumbar vertebra.

	Suspended Rats n = 5	Control Rats n = 5
THORACIC VERTEBRA		
BV/TV (%)	18.7 ± 1.26	19.67 ± 4.5
Tb.Th (µm)	40.06 ± 5.58	45.16 ± 6.32
Tb.N (/mm)	4.78 ± 0.87	4.39 ± 0.94
N.Oc+/B.Pm (c/mm)	1.93 ± 0.78	1.9 ± 0.54
Oc+.S/BS (%)	3.43 ± 1.05	3.02 ± 1.17
LUMBAR VERTEBRA		
BV/TV (%)	13.45 ± 2.39	16.46 ± 4.27
Tb.Th (µm)	37.30 ± 2.79	42.86 ± 5.8
Tb.N (/mm)	3.64 ± 0.85	3.82 ± 0.79
N.Oc+/B.Pm (c/mm)	2.89 ± 0.89[a]	1.43 ± 0.49
Oc+.S/BS (%)	5.05 ± 1.74[a]	2.30 ± 0.85

Table 3 - Quantitative bone histomorphometry in vertebral bodies of suspended and control rats (means ± SD - a : p = 0.03)

DISCUSSION

The body weight of S rats decreased within the first period of suspension followed by a progressive weight gain leading to a total positive balance (+1%) which was however inferior to that of C rats (+9.5%). Some studies have reported a decreased growth rate based on body weight (5, 8, 15) whereas others have not, but in this case, control rats are often pair-fed with suspended animals (4, 6 14). The reasons of these discrepancies are unclear but could be related to a stressfull situation due to method of suspension. This study as others (9) suggested that after stressfull first days, rats adapted to their new posture.

The epiphyseal plates in rat bone do not close over the whole life span; this means that the rat skeleton continues to grow. However, it has been found that the remodeling rate and the apposition rate decrease with increasing age (10). In this study, the decreased primary spongiosa thickness found in C compared to BC is indicative of mature animals. Baron et al. (1), demonstrated that, in 320 g male Wistar rats, the trabecular sequential bone remodeling activity at the level of the 2° spongiosa of tail vertebra is similar to that of adult human iliac crest. However, this might not be true in 2° spongiosa of long bone metaphysis. Kimmel and Jee (7) have shown a tibial growth rate of about 10 times higher than the growth rate of the tail vertebra in Baron et al. (1) study. Thus, when long bones are studied, it would be more interesting to use older rats (of 400 g rather than of 300 g) in spaceflight and simulation programs in order to extrapolate cell behaviour from rat to man.

The most striking effect of a 40-day tail suspension was the disappearance of the tibial 1° spongiosa in suspended animals. An important decrease of this area was found in a 7d. tail suspension (12) with no more alteration in a 14d. tail suspension (13). In a two week period, back harness suspension induced a depressed tibial longitudinal bone growth whereas tail suspension did not (14). In the last case, a decrease trabecular bone mass was observed possibly resulting from an earlier inhibition of bone growth. All these results might be indicative of an acute response of bone within the first week of mechanical unloading followed by a normalization. Our long term experiment suggested that the normalization is not permanent. In tibial 2° spongiosa, alterations in trabecular arrangement could lead to weaker mechanic competence.

As far as bone formation and resorption activities are concerned, a normalization was also observed during second week of suspension. Using radioisotope incorporation (with ^{45}Ca and [^{3}H]proline) and assuming that bone weight in the growing rat is primarily determined by bone formation, Globus et al. (4) showed that bone formation was inhibited by the fifth day and returned toward normal by the 10th to 12th day. Results of this study were confirmed by triple tetracycline labeling at the level of cortical bone - tibiofibular junction (4) and trabecular bone - proximal tibial metaphysis (13). In the tibial 2° spongiosa, osteoid surfaces and resorption activity also reached normal or subnormal values within two weeks (13).

In this study, a non significant desequilibrium between osteoid apposition and osteoclastic activity was observed and could be related to bone loss. Thus, after an initial bone loss followed by a reversal phase, we observed an other phase characterized by a slower but more sustained trabecular bone loss. This last stage probably permits bone to adapt to decreased mechanical environment. With a different time course, our results could be related to those of Uhthoff and Jaworski (11) obtained with dogs. The right forelimb was encased in plaster and the left served as control. An initial and rapid bone loss followed by recuperation was evidenced. After that, a slower but longer lasting bone loss was observed (the periosteal envelope being the main contributor). Finally, a stabilization at some 30 to 50 per cent below original values occured. It would be now important in rat suspension model, to better control the timing of observations in long term experiments in order to know the exact pattern on bone changes.

As in previous suspension experiments (13) parameters measured in thoracic vertebrae were not affected by the hindlimb unloading. Alterations were observed in lumbar vertebral bodies: the bone volume was non significantly decreased and the osteoclastic activity was importantly increased. In lumbar spine, support reaction was suppressed but tail traction could transmit tension stress leading to mechanical perturbations.

REFERENCES

1 - BARON R, TROSS R, VIGNERY A. Anat. Record, 208, 137-145, 1984.
2 - CHAPPARD D, ALEXANDRE C, RIFFAT G. Basic Appl. Histochem., 27, 75-85, 1983.
3 - GLOBUS RK, BIKLE DD, MOREY-HOLTON E. Endocrinol., 114, 2264-2270, 1984.
4 - GLOBUS RK, BIKLE DD, MOREY-HOLTON ER. Endocrinol., 118, 733-742, 1986.
5 - GRAHAM SC, ROY RR, WEST SP, THOMASON D, BALDWIN KM. Aviat. Space Environ. Med., 60, 226-234, 1989.
6 - HALLORAN BP, BIKLE DD, WRONSKI TJ, GLOBUS RK, LEVENS MJ, MOREY-HOLTON E. Endocrinol., 118, 948-954, 1986.
7 - KIMMEL DB and JEE WSS. Calcif. Tissue Int., 32, 113-122, 1980.
8 - MUSACCHIA XJ, STEFFEN JM, DEAVERS DR. Aviat. Space Environ. Med., 54, 1015-1020, 1983.
9 - SIMMONS DJ and KIDDER L. ASGSB Bulletin, 1, pp 34, ISSN 0897-4697, 1988.
10 - SONTAG W. Bone, 7, 63-70, 1986.
11 - UHTHOFF HK and JAWORSKI ZFG. J. Bone Joint Surg., 60, 420-429, 1978.
12 - VICO L, NOVIKOV VE, VERY JM, ALEXANDRE C. Aviat. Space Environ. Med., 1989. (in press).
13 - VICO L and ALEXANDRE C. The Physiol., 1989. (in press).
14 - WRONSKI TJ and MOREY-HOLTON ER. Aviat. Space Environ. Med., 8, 58-63, 1987.
15 - YAMAGUCHI M, OZARIK K, HOSHI T. Res. Exp. Med., 189, 9-14, 1989.

Acknowlegement: This research was partially supported by "Direction Recherches, Etudes et Techniques"(DRET), N° 87/040.

COMPETING ANTAGONISTS TO ENERGY PRODUCTION IN SPACE AND THEIR EFFECT ON CALCIUM METABOLISM

Harvey A. Tanner

Retired
1357 N.E. Ocean Blvd. Apt. 420
Stuart, FL 34996

In microgravity, reduced energy demand and its production results in negative calcium balance and osteoporosis. Recent observations have been made linking calcium as a mediator in a diversity of physiological functions; included are its regulatory effect on endocrine and exocrine secretions, the development of the neuromuscular potential in muscle contraction, its effect on fluid effusions and on membranous electrolyte transport (1). Factoral in disorders of this calcium messenger system is negative calcium balance resulting in a multiplicity of diseases, some of which are exacerbated in microgravity.

When reduced energy requirements are met, increased adenosine triphosphates, ATP, will inhibit energy production resulting in inhibited decarboxylation. Increased citrates will activate the citrate cleavage enzyme and acetyl CoA carboxylase which eventuates in the conversion of the tricarboxylate to fat (2). Pertinent to calcium homeostasis is that the ubiquitous tricarboxylate is essential for the accretion, resorption and transport of soluble mono and dicalcium phosphate which is precipitated as tricalcium phosphate to form the bone hydroxyapatite (3).

This relationship of citrate to calcium balance is exemplified in vitamin D intoxication and in D avitaminosis. Administered vitamin D to rats resulted in decreased metabolic respiration in tissue homoginates and increased citrates (4). Concomitant with the hypercitricemia is a hypercalcemia (5). Conversely, in vitamin D deficiency, a hypocitricemia and a hypocalcemia occur. Investigators postulated that in D avitaminosis, citrates could be substituted for vitamin D. Their prediction was correct. Citrate administered to vitamin D deficient experimental animals and to rachitic infants resulted in the transport of the calcium phosphate salts and the resumption of mineralization of the repaired organic matrix (6).

In experimentally induced hyperparathyroidism, oxidative glycolysis in metaphyseal bone was considerably decreased 24 to 48 hours after animals were administered parathormone. Decreased oxygen consumption was followed by an increase of citrate which complexed and mobilized calcium in the growth zone of the bone (7).

Metabolic respiration is inhibited in diabetes. In man a variable increase of blood citrates occurs with the hyperglycemia. When blood sugar increases to levels that induce diabetic coma, a profound hypercitricemia is observed (8). With the progression of the endocrinopathy, osteolytic bone becomes demonstrable.

Inhibited biosynthesis will also incur a serious threat to astronauts when exposed to the influence of microgravity. Biosynthesis, expressed thusly; AMINO ACIDS + ENERGY $\longrightarrow$ PROTEIN, is impaired in microgravity by diminished energy production. This is manifested by chronic exhaustion of astronauts. The clinical consequences of inhibited biosynthesis is myopathy and impaired immunity.

Inhibited biosynthesis in microgravity is chemically unrelated to reduced biosynthesis observed with administered corticosteroids at 1G. In space, reduced energy requirements significantly inhibit energy production. On the other hand, administered corticosteroids at 1G results in a hypocitricemia (9). The reduced endogenous citrates will inhibit decarboxylation and energy production. The steroid related energy loss decreases biosynthesis which is evident by a hyperaminoacidemia and a hyperaminoaciduria (10). Increased renal clearance of amino acids also occurs in vitamin D deficiency. The aminoaciduria disappears with the administration of vitamin D and biosynthesis of the organic matrix of the bone hydroxyapatite is resumed (11).

In view of the fragility of life in space, innovative measures designed to maintain optimal energy production must be considered. Alterations in dietary regimens programmed to compensate for depressed energy requirements would have to be instituted. Pharmaceuticals whose action would enhance metabolic respiration, such as selected calcium channel blockers, may be productive; however, the pharmacodynamics of the prescribed drugs should be evaluated. The drug must be effective in the milieu of space where fluid shifts and electrolyte loss occur. The following is a resume of catabolic factors that are competing antagonists to energy production in microgravity:

1. All metabolic depressants, including preflight, inflight and postflight sedatives, muscle relaxants, antiadrenergic agents, alcohol, and cigarette smoking can severely reduce cellular respiration in space. The antagonistic effect can endure long after the precipitating factor is eliminated.

2. Contraindicated are a select number of calcium channel blockers that inhibit myocardial oxygen consumption. This would result in reduced energy production, increased calcium mobilization, and impaired biosynthesis. The pharmacodynamics of calcium channel blockers must be considered because an investigator designing a procedure to suppress calcium mobilization in space may indeed opt for a calcium blocker that does not adequately inhibit calcium withdrawal from tissues.

3. Vitamin D supplements in physiological doses may be especially toxic to astronauts in space by enhancing calcium mobilization.

4. Exogenous citrates have been observed to complex calcium resulting in muscle spasm and tetany. With the use of citrated transfused blood, membranous calcium which maintains the integrity and contiguity of capillaries will be mobilized resulting in hemorrhage which will be additionally enhanced by the loss of blood calcium required for clotting (12).

5. Investigators have administered citrates to immature rats resulting in a hypercalcemia, a hypercitricemia, osteoporosis and myelofibrosis (13). Clinical observers have attributed gingival bleeding to the indiscriminate consumption of citrates. Citric acid, aside from being a formidable component of citrus fruits, is widely incorporated in foods as preservatives and flavorants. The consumption of citrates, while tolerated at 1G, may become a major factor in disorders associated with negative calcium balance and compromised biosynthesis in space.

The following are anabolic factors that may enhance metabolic respiration in space.

1. Nifedipine, a calcium blocker, chemically and pharmacologically unrelated to verapamil and diltiazem, acts by increasing myocardial oxygen consumption. The withdrawal of tissue calcium is thus inhibited. The retained in situ myocardial ca++, acting like glycocide digitalis, positively effects myocardial inotropism. This is in contrast to verapamil and diltiazem which inhibit the quality of myocardial contraction and reduce the heart rate. Nifedipine also reduced cardiac arrhythmias. This is briefly explained by the lowering of the myocardial cell membrane permeability in the presence of calcium which inhibits fluxing of sodium complexed with water, and enhances the fluxing of potassium. The electrolyte control is essential for the development of the neuromuscular potential required for myocardial rhythmic polorization and contraction(14).

2. It is conceivable that in situ calcium may act to inhibit fluid effusions through capillary membranes therefore limiting the undesirable fluid shifts occurring in space.

3. In cellular respiration coenzyme Q, ubiquinone, observed to play an important role in electron transport, dramatically reduced the hypercalcemia and hypercitricemia as well as the histopathology observed in citrated animals (13).

4. Fluoride, in physiological doses, has been found to reduce bone hydroxyapatite solubility.

5. Vitamin C, as a dietary supplement, enhances metabolic respiration. In scorbutic animals a hypercalcemia and hypercitricemia occur which are reversible with the administration of the vitamin (15). Like citric acid it could be used as a flavorant and preservative without the undesirable side effects of the citrate additive.

Conclusion

In the biohostile environment of space, man's ingenuity will be taxed to the utmost in quest for compensatory adjustments and alternative life support systems that would enable him to survive in the environment of inhibited energy demand and reduced energy production. Success will be determined by his ability to maintain calcium homeostasis and biosynthesis. It is very likely that new parameters will have to be developed to determine biological requisites for optimal accommodation of man to microgravity.

References

1. Rasmussen, H. The calcium messenger system. N. Engl. J. Med. 314:1094. 1986.
2. Lowenstein, J.W. Citrate and the conversion of carbohydrate to fat. p. 71, Biochemical Society No. 27. Goodwin, T.W. ed. Academic Press, London, 1968.
3. Siefter, E., Lavine L.S. Aspects of citric acid chemistry related to bone. Bull. N.Y. Acad. Med. 37:157-59, 1960.
4. Carlsson, A., Hollunger, G. The Effect of vitamin D on citric acid metabolism. Acta Physiol. Scand. 31:317, 1953.
5. Harrison, H.E. The interrelationship of citrate and calcium matabolism. Amer. J. Med. 20:1-3, 1950.
6. Harrison, H.E. Harrison H.C. Vitamin D and citrate metabolism. Studies on rachitic infants. Yale J. Biol. Med. 24:273-83, 1952.
7. Lekan, E.C., Laskin, D.M., Engel, M.B. Effect of parathyroid extract on citrate metabolism of bone. Amer. J. Physio. 199:856-8. 1960.
8. Devillers, D.C., Dixit, P.K., Lazarrow, A. Citric acid metabolism in diabetes. 1. Plasma in alloxan rats and in clinical diabetes. Metabolism. 15:548-65. 1966.
9. Agress, I.G., Tindell, S.E., Westerling, H. The influence of adrenal cortex on citric acid concentration of the Guina pig's blood serum. Acta Physiol. Scand. 34:135, 1955.
10. Smith, E.L., Hill, E.L., Lehman, I.R., et al. Principles of Biochemistry. 7th ed. p. 964. McGraw Hill, New York 1983.
11. Jonix, T.H.P., Huisman, T.H. Aminoaciduiria in rachitic children. Lancet. 2:428:31, 1953.
12. Peach, M.J. Cations: calcium, magnesium, barium, lithium, ammonium p. 784. In The Pharmacological Basis of Therapeutics. 5th ed. Goodman, L.S., Gilman, A. eds. MacMillan, New York, 1975.
13. Matsumura, M., Kimata, A., Akkiyoshi, M. Effect of coenzyme Q on the periodontium of citrated rates. J. Dent. Res. 48:1264-67, 1967.
14. Needleman, P., Corr, P.B., Johnson, E.M. Drugs used for the treatment of angina. p. 818. In The pharmacological Basis of Therapeutics. 7th ed. Gilman, A.G., Goodman, L.S., et al., eds. MacMillan, New York, 1985.
15. Bannergee S., Kawiskawar, W.J. Studies on the operation of the tricarboxylic cycle in scurvy. J. Biol. Chem. 234:1347-49, 1959.

PLANTS AND MEN IN SPACE

A NEW FIELD IN PLANT PHYSIOLOGY

M. ANDRE (1)

R.D. MACELROY (2)

(1) CEA CEN CADARACHE - S.R.A.
13108 ST PAUL LEZ DURANCE - FRANCE

(2) NASA Ames Res. Center - Moffet Field
CALIFORNIA 94035

New developments in manned space flight are of interest not only to human physiologists, but to plant physiologists as well. The reason is that the growth of plants in space can solve many of the problems of life support. This is because as space crew members and mission durations increase, the cumulative mass of life support materials required to be lauched also increases, and becomes a significant logistics burden. Plants reduce this mass by recycling CO_2 and water, and by generating food and O_2 from wastes.The initial launch, with recycling, is costly (or heavy) because of the apparatus able to produce food and to recycle wastes. But the following resupplies are light. There is a breakeven time for which the cumulative cost with recycling crosses over the cost with resupply. The breakeven depends on the type of the mission. Several cases were studied by Gustan and Vinopal (1). In the case of a lunar base with 12 persons, the breakeven is for 6 to 7 years for a realistic recycling of 50% of the food. The over cost without recycling would be 34% after 15 years of operating. This last time is common for any great scientific plant.

Food requirement

If we consider the production of food by photosynthesis as the best well-tried process, the criteria of choice for green plants cultivation are :

- Yield : quantity (per surface, volume, mass) and ratio edible/total weight
- Digestibility spectra of amino acids, fibers, etc...)
  . Spectra of vitamins and salts
- Palatability (taste, etc...)
- Cost of food processing
- Psychological and cultural problems (variety of menus, nature, aspect, etc...)

The first solution studied was the algae culture. The algae solution, which interests engineers, presents several difficulties and risks :

Advantages :

- continuous culture (automatisable in principle)
- possibility to fix nitrogen in cyanophycees
- high level in proteins ≈ 50 % biomass
- high productivity per unit of volume of reactor

Disadvantages :

- costly harvesting
- low productivity per unit of mass (including water of culture)
- genetic stability not sure
- often bad digestability (cell wall)
- indesirable elements (pigments, metals...)
- too many nucleic acids ; tolerance 2g/day :
  . maximum 50 g per day in a human diet, or
  . complex food processing
- taste - color - texture
- problems of maintainance of system, adherence of algae, cleaning, washing, sterilization
- problems of contamination by bacteria, other algaes (safety).

This solution was actively studied in the Soviet Union 15 years ago, with interesting essays of diet with volunteers. Higher plants have now the priority and the role of algae in the far futur will only be possible as a source of proteins with automated apparatus able to isolate proteins and to produce equivalents of milk, cheese, meat. Concerning the higher plants, we can consider :

As advantages

- good ratio edible/total weight (> 50 %)
- low cost of food processings
- genetical stability
- very good adaptation to human feeding
- great variety of menus

As disadvantages :

- lower yield per unit of volume than algae
- sophisticated automatization :
  . change in density of cultivation,
  . harvesting...
- needs of the crew-work very probable (can be turned in advantage for cultural and psychological reasons)

The role of diet is essential in isolated long term mission. Remember the role of the food in the Navy. In addition, the problem of the crew's free time is also crucial, for example in strategic submarines or in polar stations. Farming activities could be a serious advantage in totally isolated long term missions.

To sum up, the algae scenario is not so advantageous as expected in the first analysis. People are fascinated by their great relative growth rate but if the mass of water containing the micro-organisms is included in the growth analysis, to compare with higher plants where the mass of all organs and the intercellular water are also included, the advantage of growth rate disappears. Hence in pratice, the photosynthetic quantum yield of the daily production of biomass by algae or by higher plants is similar and tends to the theoretical maximum in the best experiments of CELSS program (2-6). The higher plants scenario has the priority in USA and USSR for dietetics and, above all, psychological reasons.

Examples of researches

In the U.S.A., studies, started in seventies, have two important aspects : science and technology. Until now the first aspect has priority because of the lack or the uncertainty of the knowledges, especially in plant physiology.

For example, the uncertainty about the tolerance of ammonia by plants was not acceptable for space engineers because the ammonia is more easily obtainable than nitrates from wastes processing. It was well admitted in a recent past, after classical experiments in field and in lab, that the ammonia nutrition strongly depressed the growth of most crop plants.
After a reexamination by Raper et al (2) in controlled roots environment, it was demonstrated that the toxicity of ammonia was an artefact of the pH shift.

With a careful control of nutrient conditions and pH, there was no difference of growth in Soybean with either pure ammonia supply or pure nitrate or a mixture of both (3).

Another typical example of study supported by NASA (T.W. Tibbitts, Madison Univ.) concerns the genetical adaptation to the terrestrial photoperiod (2-5-7). In space the photoperiod is different. It can be suppressed by a continuous lighting. In potatoes the same amount of light was distributed either with classic photoperiod 12 - 12 hours or with half of lighting maintained continuously at the bottom. Five varieties of potatoes were tested. The growth was inhibited for two varieties; root growth was stimulated for two other ones. The development of tubers was possible in only one variety. That introduces an interesting possibility of study of the genetic and molecular bases of the adaptation to photoperiodism.

In wheat plants, similar experiments were made by Buggbee and Salisbury (2-6-8), but, the most interesting results concern the improvement of yield in grain in artificial conditions. The studies are performed in Utah University in closed chamber or in green house. The selection of wheat was restarted to have plants adapted to new conditions. For example the selection to the tolerance to continuous lighting permitted to have plants being mature after 60 days instead of 120 days.

In USSR researches are more empirical and are probably considered as operationnal. No scientific publication was availlable after the results presented in 1980 (4) showing the possibility to improve by eight the yield of wheat per unit of surface and per year in comparison with field records. The last Cospar revealed a continous work from this date and interesting results (8). That concerns a ground based station Bios 3. It has about 100 square meters including cabins for the crew and the workshop of gardening surfaces of plant cultivation. The station was air tight and totally locked during experiments. The system has been working for more than ten years. The autonomy in food production was 95% (the remaining 5 percent were sugar, pepper, salt, tea, etc.... The record was a total closure of 6 months for 4 men without negative sign for their health.

In Japan (2-7) studies concern mainly algae cultivation with high yield per unit of mass of reactor and experiments in lab with a closed cycle with plant cultivation associated with a little animal.

In Europe, studies in CELSS program are in progress but the only experimental results related to CELSS program are issued from the Laboratory of CADARACHE (2-7-8). It concerns the response of wheat crop to high $C0_2$, but also the cultivation in depressurised chambers.

Hence we made experiments in very low total pressure with ray grass (2) : two experiments was made at the atmospheric pressure, in standard atmosphere and with only five percent of O_2. Two other experiments was made at the same partial pressure of CO_2 and O_2 but without nitrogen. The total pressure was reduced respectively by four and by fourteen. Similar experiments were performed with Barley and Wheat. They show the plants are sensitive only to partial pressure of useful gases. That is an advantage for space cultivation where the containers must face vacuum or very low atmospheric pressures.

Problem of wastes recycling

Problems of waste recycling the treatment and the storage of wastes, which pose serious technical problems and risks for human health in space can be positively solved in association with plant cultivation. An high degree of autonomy of food production can be obtained with simple methods of recycling.

The explanation is in the fact, that the amount of minerals is very small in food (1 to 2 %). To produce food in space it is sufficient to store and to launch a small mass of fertilizers able to produce plants and food by photosynthesis with CO_2, light, and water. The carbon being recycled by combustion, the factor of autonomy will be around 85 percent or more if some minerals are reprocessed from ashes. That is the solution of Bio 3 of the Soviet Union. The cycle of nitrogen is improved by the use of urea from human wastes in plant nutrition. That explains the high rate of autonomy.

With an oxydation in humid and supercritical phase (at four hundred degrees Celsius) it is possible to reprocess between fifty to eighty percent of fertilizers including nitrogen in form of ammonia (8). In such a case the input of fertilizer is very small and the autonomy higher than 91 percent.

Conclusion

Based on comparisons of nutritional capabilities, human psychological responses and technlogical complexity, the use of higher plants appears to be more appropriate than algae. Empirical results suggest that a high level of material recycling can be obtained wiht plants, but scientific studies of plant physiology must continue if the bioregenerative system is to be adequately controlled. The issue of space gravity, which is of central importance in human physiology, may not be a major problem for plants because of the possibility that phototropism can provide an alternative sense of direction. If ingeneers are implicated in this research, physiologists must be concerned in the field open by the change of parameters of terrestrial environments : low gravity but also CO_2 and 0_2 concentrations, light period and intensity, atmospheric pressure, radiations, effect of traces.

Références :

1) GUSTAN E. and VINOPAL T. 1982 Controlled Ecological Life Support System. Transportation analysis NASA CR 16.64.20

2) MACELROY R.D., MARTELLO N.V., SMERNOFF D.T. 1986. Controlled Ecological Life Support Systems : CELSS 85 workshop NASA TM 88215

3) RUFTY T.W., RAPER C.D., JACKSSON W.1983. Growth and nitrogen assimilation of soybean in response to ammonium and nitrate nutrition. Bot.gaz.144 :466-470

4) POLONSKII V.T. and LISOVSKII G.M. 1980. Net production of wheat crop under high Ph AR irradiance with artificial light. Photosynthética 14 : 177-187

5) WHEELER R.M. and TIBBITTS T.W. 1986. Growth and tuberization of Potato (solanum tuberosum L) under continuous light.Plant physiol. 80 : 801-804

6) BUGBEE B.G. and SALISBURY F.B. 1988. Exploring the limits of crop productivity. Plant Physiol. 88 : 869-878

7) MACELROY R.D. and SMERNOFF D.T. (eds) 1987. Controlled Ecological Life Support Systems, Adv. Space Res. 7(4) 153 p. Pergamon Press, Oxford.

8) MACELROY R.D., TIBBITTS T. and THOMSON B (eds) 1988. Controlled Ecological Life Support Systems (2). Adv. Space Res. (in press).

OPPORTUNITY AND CHALLENGE IN LIFE SCIENCES RESEARCH ON SPACE STATION FREEDOM

Milton R. Heinrich

Zerog Corporation
27200 Deer Springs Way
Los Altos Hills, CA 94022, U.S.A.

In the 1990s the United States will place a space station in permanent orbit. One of the functions of Space Station Freedom is the provision of a manned laboratory for the study of the interaction of gravity and living organisms.

The absence of gravity during spaceflight results in many physiological changes in crew members, as well as in other animals and plants that have been flown in orbit. Some of these changes are uncomfortable to the crew and possibly deleterious. The new laboratory in space will provide facilities to study crew problems and possible countermeasures. In addition, there will be facilities to study animals and plants in microgravity, and to compare them with similar specimens held at the normal 1g on a centrifuge in the same spacecraft. This paper will discuss primarily the areas of animal and plant research on Space Station Freedom, and will point out both advantages and limitations of the facilities.

The Freedom Station will consist of four pressurized modules mounted on a truss structure approximately 500 feet long. The Truss also carries panels of solar cells, radiators, and external instruments for the study of sky and Earth. S. S. Freedom is an international effort, with two modules (Habitation and Laboratory) supplied by the United States, a Columbus module by the European Space Agency, and a Japanese Experimental Module. When the station is permanently manned, it will at first carry a crew of four, and this will later be increased to eight. Crew members will stay in orbit for 90 days in early missions, and the mission length will probably be increased later. Space Shuttle flights will provide crew rotation, resupply, and return of samples to ground. It should be pointed out that the design of the Freedom Station, and its operation plans, have not been finalized.

In the U. S. Laboratory Module, the total volume will be shared by Life Sciences and Material Sciences approximately equally. The planned outfitting of the Life Sciences area is the result of approximately 10 years of NASA meetings and workshops involving life scientists from all disciplines.

There are several major facilities which will be used in all of the life sciences activities. A Habitat Holding System will maintain animal and plant specimens in microgravity. There is a S. S. Freedom requirement for complete bioisolation of biological specimens, to prevent cross-contamination between specimens and crew. The Habitats which fit into the Holding System are being designed to hold rats and squirrel monkeys at first, and capability for other species will be provided in the future. (Mice may be housed in rat habitats with minor modifications.) NASA and CNES are jointly developing a Rhesus monkey Habitat.

A Centrifuge will maintain specimens at 1g, to serve as controls for those in microgravity, in the same environment. A Centrifuge 1.8 meters in diameter, to be mounted in the Laboratory Module, would accommodate ca. 24 rats. It may be possible to mount a larger Centrifuge in another location. The animal and plant habitats for the Centrifuge would be identical to those in the Habitat Holding System. The Centrifuge may also provide gravity levels both lower and higher than 1g. Fractional gravity levels would allow study of gravity thresholds at which changes in particular physiological systems begin to appear, or levels at which changes are prevented. Hypergravity studies (up to 3g planned) would indicate whether exposure to periods of increased gravity would prevent or reverse the changes observed in microgravity.
Another important use for the Centrifuge will be in studies of the rapid changes upon exposure to microgravity. The rapid changes in physiology are of immediate importance to the health of crew members. They are also of interest to those studying the mechanisms of adaptation. The opportunity to study this phase in crew members is limited by the number of instrumented subjects available at that time, and by the type of instrumentation which can be used in humans. Animals which are held at 1g on the Centrifuge, however, can be removed to microgravity at any time, and both their rapid and slower responses studied. Conversely, the processes of readaptation to 1g or higher can be studied by transferring animals from microgravity to the Centrifuge.

Life Sciences Glovebox--The bioisolation requirements of S. S. Freedom also dictate that all handling of biological material be done inside a closed glovebox. This includes all procedures with plants and animals, their tissues, and wastes. Habitats must be designed to fit in the Glovebox, or be attached to it by a system which allows the Habitat to be opened only to the inside of the Glovebox.

Equipment Washer/Sanitizer (or Service Unit)--a machine whose major use will be to wash and sanitize Habitats. It will also clean other laboratory equipment.

In addition to the major facilities above, the Freedom Station will provide specific pieces of equipment used in Life Sciences and Materials Sciences research. They were selected as those required by the majority of likely experiments. These items are called Laboratory Support Equipment; those of most interest to Life Sciences are listed in Table 1. The Laboratory Support Equipment is generic, and obviously many experiments will require additional equipment. NASA has studied those requirements and has prepared a list of supplementary items which could be supplied for particular missions. If more specialized equipment is needed for certain experiments the Principal Investigator may be required to supply it. All equipment is designed for rapid changeout during a Shuttle resupply visit.

Table 1

LABORATORY SUPPORT EQUIPMENT

Surgery/Dissection Instruments
-70°C Freezer
Snap Cryogenic Freezer
Storage Cryogenic Freezer
Freeze Dryer
Incubator
Small Mass Measurement Unit (1 gm to 10 kg)
Micro Mass Measurement Unit (1 mg to 10 gm)
Passive Dosimeter
Specimen Labeling Device (Bar codes)
Microscope System
Autoclave
pH Meter
Fluid Handling Tools
Digital Thermometer
Cameras

Life Sciences research in space will be greatly facilitated by Space Station Freedom. Some of the advantages of the new facility over unmanned missions or accommodations on the Shuttle are:

1. Increased laboratory volume, larger crew, more specimens and equipment.
2. Longer missions, allowing long-term studies on the adaptations to microgravity.
3. If longer times are needed for some studies (e.g., reproduction and development over multiple generations), specimens may be maintained in space for as many missions as necessary.
4. The capability to examine or dissect specimens in space without exposing them to the g-forces of reentry, or the readaptation to lg after landing.
5. Facilities for freezing and fixing large numbers of tissue samples, and returning them to ground for study.
6. Availability of a Centrifuge to provide lg control specimens, and to study effects of fractional gravity and hypergravity.
7. Sufficient numbers of specimens so that groups of specimens may be sampled at intervals during a mission to determine the time-course of changes.
8. Provides a permanent microgravity laboratory which is available continuously.

Although the Freedom Station will provide facilities and accommodations never available before, it should not be assumed that laboratory procedures used on the ground can easily be adapted for use in space. Some of the limitations in this space laboratory include:

1. Bioisolation must be maintained for all biological material. This requires the use of the Life Sciences Glovebox for all specimen handling, including examination, testing, mass determination, Habitat servicing, transfer to clean Habitats, injections, blood sampling, dissection, tissue preservation (freezing or fixation), tissue cultures, bacterial cultures, microscopy, and analysis. The glove box must be cleaned after each use. Glove box procedures can be tedious and demanding, especially when volume in the box is limited. In any case, procedures will be slower than on the ground.
2. Fluid handling is difficult in microgravity. Sampling or volume measurement require a coherent volume, which can be obtained, e.g., by use of a syringe or elastic bladder. All liquids must be confined.
3. Water supplies are limited, including those for cleaning. Water is recycled.
4. In the closed environment, toxic or hazardous materials must be avoided or strictly contained.
5. All loose items must be restrained.
6. Crew time will be limited.
7. Special procedures will require extensive crew training.

These are some of the factors to be considered in planning experiments in space.

RELATIONSHIP BETWEEN HORMONES AND BRAIN WATER CONTENT MEASURED BY 1H MAGNETIC RESONANCE SPECTROSCOPY DURING SIMULATED WEIGHTLESSNESS IN MAN.

N.MAURICE * ,B. ROUSSEL *, H. MEHIER *
G. GAUQUELIN**, C. GHARIB **

* LAB RMN BP 88 74402 CHAMONIX
** LAB PHYSIO FAC MED 69373 LYON CEDEX 8.

INTRODUCTION

Space flight conquest is submitted to our understanding of men physiological behaviour in long time space flight. Effect of space flight on crewmen is essentially due to weightlessness. It is generally admitted that initial changes induced by space flight as well as ground base experiments simulating weightlessness, are characterized by a reduction of hydrostatic gradient which conduct to a baroreflex response (6). This response modify both heart rate and peripheral resistance of the vascular bed (5), as well as hormonal levels. In this work we were interested in short term effect of simulated weightlessness on hormones levels, namely Plasma Renin Activity (PRA) and Atrial Natriuretic Factor (ANF), which are involved in liquid volumes regulation (3,4). Concurrently, water volumes in head were measured by proton NMR Spectroscopy to appreciate the well known redistribution of the fluid volumes in the upper part of the body and the relationship with the former hormones.

Materials and methods

Subjects:

Physiological values were measured on four female healthy volunteers, aged 20 to 30.

Weightlessness simulation:

We used head down tilt at -6° (HDT).

Water volume measurement :

Relative liquid volumes variations were measured by NMR proton spectroscopy (7). The spectrometer is a DRUSCH SRM200 . It is based on a 12 tons 1.2 Tesla resistive magnet with 20 cm by 120 cm gap. The measurement volume is a sphere

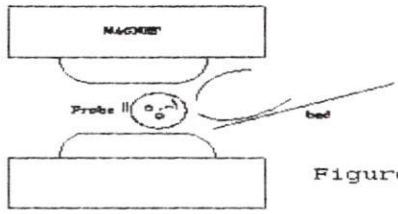


Figure 1

of 10 cm diameter. The center of this volume is located in the center of the gap and at only 25 cm deep from the edge of the magnet, this give a very large accessibility and allow a wide range of body position for measurement. In this work we could perform NMR acquisition during head down tilt at -6 °(fig 1). The radio antenna used was a surface coil of 65 mm diameter. It's a one turn Hertzian loop tuned to 20,7 MHz. The volume of interest explored is near a half of a sphere wich is 0.6 time the diameter of the coil(in our case about 40 mm). A particularity of this spectrometer is that it does not need to be shimmed on each patient as other superconducting magnet. Shimming on an homogenous ghost is sufficient. So if different compartments in the body have different magnetic susceptibility, the local magnetic field is different and the water resonances in these compartment are different. this allow separate measurement of their water volumes. The surface coil was placed on the vertex of subjects. As the radio frequency coupling between the antenna and the body may vary from one subject to another, we can only give relative volumes variations.
It is shown in figure 2 a typical NMR head spectrum acquired in one second. There are three important peaks: at left the cerebrospinal fluid (CSF) resonance, in the middle the tissue water (TW) resonance and at right the broad small peak of subcutaneous fat. We used this subcutaneous fat as an internal standard, assuming that it does not vary during the four hour experiment.

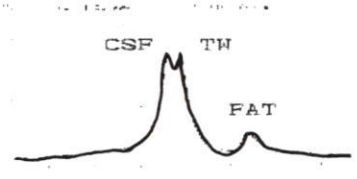


Figure 2

Hormones level determination

Venous blood sample were collected each half an hour. A teflon type catheter was used to avoid the presence of metallic parts near the magnet. Hormones levels were determined using standard radioimmuno assay methods. Blood samples were freezed for transportation before assaying.

Experiment protocol:

The experiment was realised in the morning, beginning with a rest of the subject in sitting position during 30 mn with a first blood sample after 15 mn. Thereafter the subject was head down tilted at -6° during four hours. Blood samples and NMR spectras were collected each 30 mn. At the end of the four hours, the subject sit for 30 mn and then the last values are collected

RESULTS

NMR values of water volume are expressed in ratio from height of the subcutaneous fat peak.
All values are referenced to control values measured in sitting position.

We found a significantly increase in total water content (TW+CSF) in head during HDT ($p<0.05$, fig 3). CSF increases but not significantly. TW too. Water volumes do not come back to their control values at the end of the experiment. A decrease in PRA and an increase in ANF (fig 4) is observed. Both hormones did not return to their control level at the end of the experiment.

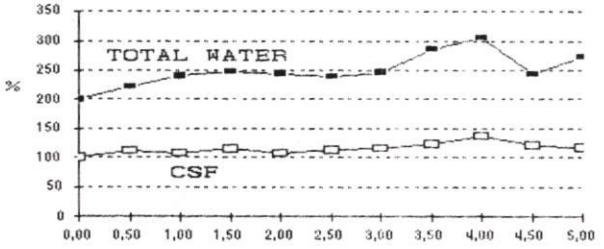


Figure 3

DISCUSSION

NMR results: . This experiment clearly demonstrate that a 4 hour HDT induce an increase in total head water. This increase may be due to at least three compartment: subcutaneous tissue, CSF and brain tissue. We think that the increase in CSF could push away a part of the brain seen by the probe (as could do an oedema or an hematom) and so, artificially decrease TW. Subcutaneous and brain tissue water are summed in the tissue water peak. The increase in CSF water peak observed is not sufficient to explain the total water increase (Fig 3) so the HDT induce an increase in water both in the CSF and subcutaneous+brain tissue peak. It could be possible to determine exactly the involvment of the subcutaneous tissue water in the increase of the peak by realising NMR measurement with a little surface coil which measures the water only in the subcutaneous tissue.

Hormonal results: the recorded values agree with many other experiments in the same conditions. This pattern of variation are now well known.

The most interesting of these results (fig 4) is the same pattern in ANF and water volume variation. Since it has been shown in the litterature (1,2) the effect of ANF on CSF secretion and the presence of ANF receptors in the blood brain barrier, further investigations should be undertaken to study the role played by ANF in the brain water regulation.

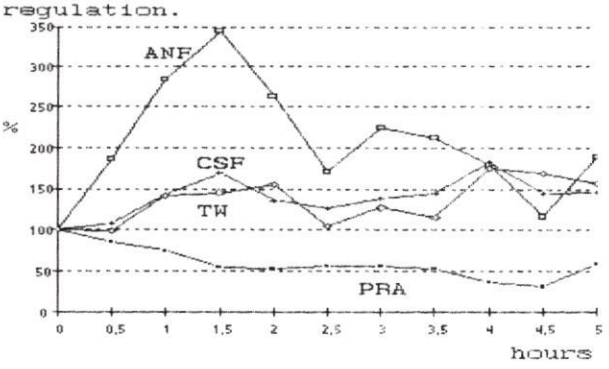


Figure 4: Values from 1 subject

REFERENCES

(1) Chabrier P.E., Roubert P., Plas P. and Braquet P.: Blood brain barrier and atrial natriuretic factor. Can. J. Physiol. Pharmacol. 66: 276-279 (1987)

(2) Doczi T., Ferenc J.,Szerdahelyi P. and Bodosi M.: Regulation of brain water and electrolyte contents: The possible involvment of central atrial natriuretic factor. Neurosurgery 21 (4) 454-458 (1987)

(3) Gharib C.,Gauquelin G., Pequignot J.M., Geelen G.,Bizollon C.A., Güell A.,: Early hormonal effect of head down tilt (-10°) in humans. (1988) Aviat. Space Environ. Med. 59, 624-629 (1987)

(4) Gharib C.,Gauquelin G., Geelen G., Cantin M., Gutkowska J., Mauroux J.L., Güell A. : Volume regulating hormones (renin, aldosterone, vasopressin and natriuretic factor) during simulated weightlessness. The Physiologist 28,S.30-S.33 (1985)

(5) Güell A., Rous de Feneyrols A., Comet B., Dupui Ph., Geraud B., and Bes A.: Neurocirculatory modifications caused by prolonged Anti-orthostatic position at -4°. in Space Physiology, Eds cepadues 439-445 (1983)

(6) Hinghofer-Szalkay H. : Fluid volume changes in astronauts- How they fit to our understanding of physiology. (1987)Proceed of the 3rd European Symposium on Life Science research in Space, Graz, Austria 14-18 Sept. 1987 (ESA SP 271, Dec 1987)

(7) Mehier H., Maurice M., Bonche J.P., Gaillard E., Foray J., Roussel B.: Spectrometrie RMN in vivo avec un aimant resistif à 1,2 Tesla. (1988) C.R. Acad Sci. Paris, 306, 313-316

MECHANISMS OF LOWER BODY POSITIVE PRESSURE-INDUCED NATRIURESIS.

GEELEN G., A. HADJ-AISSA*, G. GAUQUELIN, P. PASI, N. POZET* and C. GHARIB

Laboratoire de Physiologie UFR Grange-Blanche, Université Claude Bernard, 69373 LYON Cédex 08, and *Service d'Exploration Fonctionnelle rénale, Hôpital E. Herriot, 69003 LYON France.

INTRODUCTION -

The use of lower body positive pressure (LBPP) has been proposed by our group as a valuable alternative technique to bedrest (BR) and water immersion (WI) for studying blood volume regulation and for examining early cardiovascular, hormonal and renal responses to simulated weightlessness (6, 3). A headward redistribution of blood is indeed the common feature of BR, WI, and antigravity suit inflation (2, 3, 4, 6). While determining the effects of LBPP on kidney function in normal men during head-up tilting, we showed previously that the changes in kidney excretory patterns brought about by antigravity (anti-G) suit inflation (mainly a marked diuresis and natriuresis) were similar in nature and magnitude to those observed during WI or the early phase of bedrest (3). The goal of the present study was thus to investigate the mechanisms of LBPP-induced natriuresis.

METHODS -

For this purpose, after an overnight fast (no food, no drink) 6 male subjects (34±2 (SE) yr) underwent 30 min of sitting during which they received a light breakfast, followed by 3 hr of 70° head-up tilting. An anti-G suit (MAST III A, David Clark) was applied (60 Torr legs; 30 Torr abdomen) during the last 2 hr of tilt. To provide adequate urine flow, the subjects were given 500 ml of water at breakfast, then 200 ml at the start of each hour. Urine collection was made hourly. Urine flow, osmolar and free water clearances, total and fractional Na+ excretion were measured. In addition, glomerular filtration rate (GFR) and proximal Na+ reabsorption were measured by means of creatinine and lithium clearances (8) respectively. Pulse rate (PR) was monitored, and blood pressure was measured using a Dinamap.

Hematocrit, plasma osmolality and electrolytes were determined. Plasma renin activity (PRA) and plasma atrial natriuretic factor (ANF) were measured at 30 min. intervals throughout the experiment (9, 5). Plasma ANF was also measured 15 min. after inflation. Data are expressed as mean ± SE. They were analyzed with the Wilcoxon matched-pairs signed-ranks test. The null hypothesis was rejected when $p<.05$.

RESULTS -

Inflation of the anti-G suit induced the usual immediate and sustained increase in mean arterial pressure (+11±3 Torr, $p<.05$) and decrease in PR (-15±3 beats/min, $p<.05$).

Hematocrit was decreased ($p<.05$) throughout inflation, while plasma Na+ and osmolality remained essentially unchanged. PRA was significantly decreased by 30 min of inflation, and maximally suppressed ($p<.05$) by 55±4.4% after 2 hr of inflation, while plasma ANF remained unchanged. Creatinine clearance (GFR, corrected to 1.73 m2 body surface area) was unchanged, while inflation induced marked increases ($p<.05$) in urine flow (V), osmolar (Cosm) and free water (C_{H2O}) clearances, Na+ excretion rate ($U_{Na}.V$) and decreases in fractional total and proximal sodium reabsorption (Table 1). Potassium excretion ($U_K V$) increased after 2 hr of inflation.

	Hour 1	Hour 2	Hour 3
GFR (ml/min)	106.8±6.3	111.7±6.3	108.3±2.8
V (ml/min)	1.55±.43	3.34±.79*	5.85±.85§
C_{H2O} (ml/min)	-0.54±0.46	0.98±0.82	2.98±0.97*§
$U_{Na}V$ mole/min	62±10	77.2±10.1*	120.3±25.8*§
%Na réabsorption			
Total	99.64± .04	99.57± .05*	99.26± .20*
Proximal	79.04± 1.82	75.72± 2.02*	73.93± 1.62*
Distal	20.63± 1.97	23.87± 2.20*	25.32± 1.97*
$U_K V$ mole/min	34.01± 3.7	48.04± 10	70.37± 13*

*$p<.05$ vs hour 1
§$p<.05$ vs hour 2

Table 1 : Changes in kidney excretory patterns induced by inflation of an anti-G suit on six male standing subjects.

DISCUSSION -

Together with increased MAP and decreased PR and PRA, natriuresis appeared again in this experiment to be a characteristic feature of anti-G suit application (3). Natriuresis occured as early as the first hour of inflation and increased markedly thereafter. The question arises as to its mechanisms. Since creatinine clearance (GFR) and plasma Na did not change significantly with inflation, a role for increased Na load, if any, can only be modest. An increase in plasma ANF does not appear to be a causal factor either since, in opposition with the findings of WILKINS et al (10), ANF in our study was not increased by inflation. A possible explanation may be found in the fact that our model realizes the unusual combination of a central hypervolemia together with a prompt, marked and sustained bradycar-

dia, so that the latter would oppose the former stimulus, the result being an absence of change in ANF secretion.

The increase in urinary potassium makes it unlikely for the suppression of aldosterone secretion to play a role in the natriuresis of the first two hours of inflation. Lithium clearance was increased and an increase in distal reabsorption occured, which only compensated partially for the decreased proximal reabsorption. On the other hand, we have previously shown inflation to result in an increase in effective renal plasma flow (ERBF, 3). The increase in Na excretion was accompanied by an increase in K excretion, which suggests a decreased K proximal reabsorption and/or an increased K distal secretion in relation to both the increase in V and in distal Na delivery. Thus, the natriuresis of anti-G suit inflation appears to involve a decrease in Na reabsorption proximal to the distal tubule, which could be ascribed to either/or one of the two following mechanisms : i) a change in peritubular Starling forces, due to the pressure-natriuresis mechanism (7), ii) a decrease in sympathetic renal nerve activity (1).

ACKNOWLEDGEMENTS - We wish to thank UFR Grange-Blanche, UFR de Biologie Humaine and Université Claude Bernard (Physiologie de l'Environnement) for their financial support.

REFERENCES -

1. CAMPESE V.M. and W. HSUEH. Recent advances in the role of the renal nervous system and renin in hypertension. Contemporary Nephrology IV Ed. by S. KLAHR and S.G. MASBRY Plenum Medical Book Co New York & London, 1987, p. 283-322
2. EPSTEIN M. Renal effects of head-out water immersion in man : implications for an understanding of volume homeostasis. Physiol. Rev. **58** : 529-581, 1978
3. GEELEN G., KRAVIK S.E., HADJ-AISSA A., LEFTHERIOTIS G, VINCENT M., BIZOLLON C.A., SEM-JACOBSEN C.W., GREENLEAF J.E. and C. GHARIB. Anti-gravity suit inflation : kidney function and cardiovascular and hormonal responses in men. J. Appl. Physiol., **66** : 792-799, 1989.
4. GREENLEAF J.E. Physiological responses to prolonged bedrest and fluid immersion in humans. J. Appl. Physiol., **57** : 619-633, 1984
5. GUTKOWSKA J., M. BOURASSA, D. ROY, G. THIBAULT, R. GARCIA, M. CANTIN and J. GENEST. Immunoreactive atrial natriuretic factor (IR-ANF) in human plasma. Biochem. Biophys. Res. Commun., **128** : 1350-1357, 1985
6. KRAVIK S.E., L.C. KEIL, G. GEELEN, C.E. WADE, P.R. BARNES, W.A. SPAUL, C.A. ELDER and J.E. GREENLEAF. Effect of anti-gravity suit inflation on cardiovascular, PRA and PVP responses in humans. J. Appl. Physiol., **61** : 766-774, 986
7. ROMAN R.J. Pressure diuresis mechanism in the control of renal function and arterial pressure. Fed. Proc. **45** : 2878-2884, 1986
8. THOMSEN K. Lithium clearance : a new method for determining proximal and distal tubular reabsorption of sodium and water. Nephron, **37** : 217-223, 1984
9. VINCENT M., J. SASSARD and J.F. CIER. Méthode rapide de détection radioimmunochimique de l'activité rénine du plasma. Rev. Europ. Et. Clin. Biol., **17** : 1001-1006, 1972
10. WILKINS M.R., M.D. GAMMAGE, L.B. TRAN, M.J. KENDALL, C.J. LOTE, P.L. WEISSBERG and W.A. LITTLER. Effect of lower body positive pressure on atrial dimension and plasma atrial natriuretic peptide concentratrion. J. Hypertension, **4**, suppl. 6 : S500-S502, 1986

HEMODYNAMIC, PLASMA RENIN ACTIVITY AND NOREPINEPHRINE CHANGES INDUCED BY ANTI-G SUIT INFLATION IN MAN.

GEELEN G., J.L. SAUMET*, P. ARBEILLE**, F. PATAT**, J. GUILLUT** and C. GHARIB

Lab. Physiol. UFR Grange-Blanche 69373 LYON Cédex 08 - France
*Lab. Physiol. Fac. Médecine 49045 ANGERS - France
**Lab. Biophysique Fac. Médecine 37032 TOURS France

INTRODUCTION -

Antigravity suits have been used increasingly as life-saving devices in emergency medicine in the treatment of hemorrhagic shock and postural hypotension (6) and proposed as an alternative to bedrest and water immersion for studying the early cardiovascular, hormonal and renal effects of weightlessness (3, 5), since their application results in an increase in arterial blood pressure and a headward redistribution of fluid. However, some degree of controversy still exists as to the mechanism whereby the antigravity suit (anti-G suit) augments systemic arterial pressure (increased preload and/or mainly increased afterload) (1, 2, 4, 6, 7). On the other hand, attention has mostly been paid to the hemodynamic changes that occur immediately after inflation, and not to the longer term (more than 30 min.) effects of inflation, i.e., to the adaptive mechanisms to the long lasting inflation-induced increase in arterial pressure. This study was thus designed to assess the effects of lasting anti-G suit inflation on cardiac volumes and cardiac output (CO) and clarify the participation of increased CO in the LBPP-induced increase in blood pressure, while controlling certain neurohormonal responses.

METHODS -

For this purpose, after an overnight fast (no food, no drink) 6 male subjects (36±2.5 (se) yr) underwent a 30 min control period sitting, at the beginning of which they received a light breakfast, then a 3 hr head-up tilting to 70° (70° HUT). An anti-G suit (MAST III, David Clark Co) was applied (60 Torr legs, 30 Torr abdomen) during the last two hours of tilt. The subjects received 500 ml of water with breakfast, then 200 ml at the start of each hour. Two dimensional echocardiography was used to measure the inflation-induced changes in cardiac volumes. The ultrasound device was an echocardiograph (Aloka SSD 118) including two working modes : a 3 Hz real time B mode imaging (Sector Scan) and a time motion mode; such a system allows the visualization of the cardiac chambers as well as the display of the ventricular and auricular wall movement. Measurements were made -30 min before, then +30 and +90 min after inflation.

Chilled heparinized blood samples were taken every 30 min throughout the study and also 15 min after inflation for plasma renin activity (PRA) and norepinephrine (NE) measurements. Pulse rate (PR) was recorded, and mean arterial pressure (MAP) was calculated from systolic and diastolic blood pressure measurements made using a sphygmomanometer (MAP = DBP+1/3(SBP-DBP). Data are expressed as means ± SE. They were analyzed using the Wilcoxon matched-paired signed ranks test. The null hypothesis was rejected when $p<.05$.

RESULTS -

Immediately upon inflation, MAP increased by 7±3 Torr ($p<.05$) and PR decreased by 20±4 beats/min ($p<.05$). Plasma NE and PRA were significantly decreased as early as 15 min after inflation, and maximally suppressed ($p<.05$) after 15 and 60 min of inflation, respectively. Compared to preinflation values, left ventricular end diastolic volume (LVEDV), left ventricular end systolic volume (LVESV) and systolic volume (SV) were increased +30 and +90 min after inflation (table 1). Cardiac output (calculated as SV × PR) rose transiently after 30 min of inflation, despite the important bradycardia, but the increase was at the limit of significance (most likely on account of the small number of subjects), and was back to preinflation values at +90 min.

	-30 min	+30 min	+90min
LVEDV (ml)	93±5	122±13*	110±6.5*
LVESV (ml)	46±3	55± 7	57±5.5*
SV (ml)	46.5±4.5	67± 8*	53.5±5.5*
PR (b/min)	75± 1	64± 3*	62±2*
CO (ml/min)	3501±346	4382±595	3342±353

*$p<.05$ vs -30 min

Table 1 - Echocardiographic measurements of cardiac changes (mean ±SE) induced by anti-G suit inflation in six 70° head-up tilted subjects.

DISCUSSION -

When CO has been measured in previous work under anti-G suit inflation in man as in animals, measurements have led to conflicting results. However, two main points can be derived from those studies : firstly, the pre-existing level of blood volume appears to be an important determinant of the direction in which CO changes (1, 2, 4, 6, 7). Secondly, an anti-G suit induced increased preload is susceptible to occur as a first step of the suit action : i) when the starting central venous pressure is very low (as in patients in shock), ii) when the pressure applied with the suit is in the low range, iii) when venous pooling of blood has occurred, as in our study where the subjects have been head-up tilted before inflation (2, 6, 7). Thus, our data show that in the standing condition, i.e. with mild unloading of the left ventricle, anti-G suit inflation induces a significant increase in LVEDV, indicating an increase in preload. This is in agreement with SEAWORTH et al's data (7). However, the increase in CO occurs early and is short-lasting, since it has disappeared after 90 min of inflation, so that

it certainly contributes but only transiently to the LBPP-induced MAP increase. In addition, an inflation-induced baroreceptor-mediated decrease in sympathetic activity does occur, as reflected by the early, marked and sustained decrease in PRA and plasma NE.

ACKNOWLEDGEMENTS - We wish to thank Dr. M. VINCENT and Dr. J.M. COTTET-EMARD for the PRA and NE measurements, UFR Grange-Blanche, UFR de Biologie Humaine and Université Claude Bernard (Physiologie de l'Environnement) for their financial support.

REFERENCES -

1. BELLAMY R.F., L.R. DEGUZMAN and D.C. PEDERSEN. Immediate hemodynamic consequences of MAST inflation in normo- and hypovolemic anesthetized swine. J. Trauma. **24** : 889-895, 1984
2. GAFFNEY F.A., E.R. THAL, W.F. TAYLOR, B.C. BASTIAN, J.A. WEIGELT, J.M. ATKINS and G.G. BLOMQVIST. Hemodynamic effects of medical antishock trousers (MAST garment). J. Trauma. **21** : 931-937, 1981
3. GEELEN G., S.E. KRAVIK, A. HADJ-AISSA, G. LEFTHERIOTIS, M. VINCENT, C.A. BIZOLLON, C.W. SEM-JACOBSEN, J.E. GREENLEAF and C. GHARIB. Antigravity suit inflation : kidney function and cardiovascular and hormonal responses in men. J. Appl. Physiol., **66** : 792-799, 1989
4. GRAY S.III, J.A. SHAVER, F.W. KROETZ and J.J. LEONARD. Acute and prolonged effects of G suit inflation on cardiovascular hemodynamics. Aerosp. Med. **40** : 40-43, 1969
5. KRAVIK S.E., L.C. KEIL, G. GEELEN, C.E. WADE, P.R. BARNES, W.A. SPAUL, C.A. ELDER and J.E. GREENLEAF. Effect of antigravity suit inflation on cardiovascular, PRA and PVP responses in humans. J. Appl. Physiol., **61** : 766-774, 1986
6. PELLIGRA R. and E.C. SANDBERG. Control of intractable abdominal bleeding by external counterpressure. J. Am. Med. Assoc., **241** : 708-713, 1979
7. SEAWORTH J.F., T.J. JENNINGS, L.L. HOWELL, J.W. FRAZIER, C.D. GOODYEAR and E.D. GRASSMAN. Hemodynamic effects of anti-G suit inflation in a 1 G environment. J. Appl. Physiol., **59** : 1145-1151, 1985

SEX DIFFERENCES IN BLOOD CONSTITUENTS OF RATS FOLLOWING TAIL SUSPENSION

Masayuki Nakaya, Sachio Ikawa, Kazuo Kosugi and Shuji Takeuchi

Space Medicine Laboratory and First Department of Anatomy, The Jikei University School of Medicine, Tokyo 105, Japan

ABSTRACT

Tail suspension was utilized to study sex differences in the effects of hypokinesia on blood constituents of rats. Twelve male and twelve female rats were used. After four weeks of exposure to tail suspension or non-treatment, the rats were laparotomized under ether anesthesia and blood samples were obtained. Biochemical tests on the blood samples were then performed immediately. Hematocrit and serum iron were also estimated. The results were as follows: 1) The same tendencies were noted in blood constituents between male and female rats in the control group and the tail suspension group. 2) The average levels of blood albumin, creatinine, uric acid, glucose, triglyceride, serum iron and hemoglobin in the tail suspension group were lower than those in the control group, especially in male rats.

INTRODUCTION

Muscle dystrophy and bone demineralization have been widely reported during simulated weightlessness produced by hind-limb suspension in animals. We have been utilizing the suspension method for studying the metabolic and hormonal mechanisms of mineral metabolic adaptation to induced hypokinesia in rats. Recently, tail suspension has been utilized for simulation of weightlessness. We therefore used tail suspension to study the influence of hypokinesia on blood constituents in rats.

MATERIALS AND METHODS

Twelve male and twelve female rats (Wistar strain, nine weeks old) were used, and divided into two groups, a control group (C) and a tail suspension group (TS).

The period of tail suspension loading was four weeks. Rats were kept at a room temperature of 23±2°C and 50-80% relative humidity and allowed food and water freely. TS rats were suspended by a stainless steel wire (0.7 mm) passed through the root of the tail, taking care to insert the wire through the bone correctly. Rats subjected to tail suspension were raised in separate cages. After four weeks of exposure to tail suspension or non-treatment, the rats were laparotomized under ether anesthesia and blood samples were obtained from the abdominal aorta with an intravenous infusion set (winged needle). Biochemical tests on the blood samples were then performed immediately using a VISION-TM system (Dynabot Co.). The tested parameters included the levels of total protein, albumin, urea nitrogen, creatinine, uric acid, glucose, cholesterol, triglyceride, alkaline phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and hemoglobin. Hematocrit and serum iron were also estimated.

RESULTS AND DISCUSSION

A comparison of albumin values is shown in Fig. 1. A significant difference in albumin was found between the C group and the TS group. As also found in the Skylab crew, albumin decreased on the third day and 14th day after recovery. This decrease in serum albumin was due to suppression of synthesis, reduced absorption and leakage. This is also well known to occur in cases of hepatic injury, malnutrition and starvation.

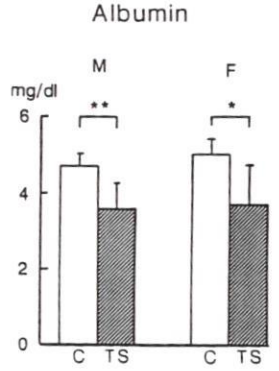


Fig. 1. Albumin after four weeks of tail suspension. Value is means±SE. Significantly different from control at $*p < 0.05$ and $**p < 0.01$. C:control, TS:tail suspension, M:male, F:female rats

As for creatinine and uric acid, the TS group showed lower mean values, the difference being significant in male rats (Fig. 2). Slight increases were observed in plasma creatinine, which were presumably indicative of slightly decreased creatinine clearance. The excretion of uric acid was decreased throughout the mission in most of the Skylab crew. It is well known in a clinical setting that a disease involving a decreasing level of uric acid occurs due to a decline of uric acid synthesis and its accelerated excretion from the kidney.

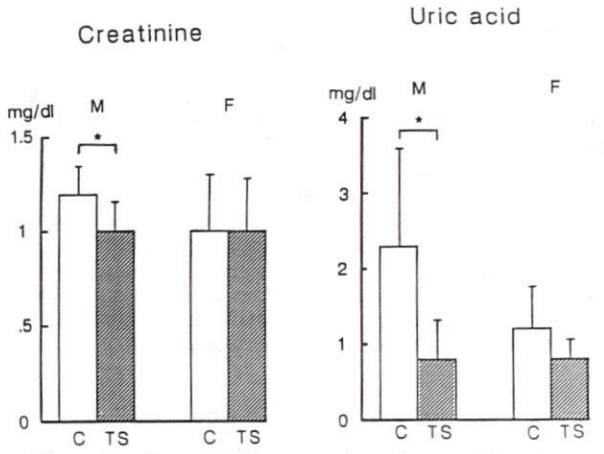


Fig. 2. Creatinine and uric acid after four weeks of tail suspension. Values are means±SE. Significantly different from control at *$p < 0.05$.
C:control, TS:tail suspension,
M:male, F:female rats

A comparison of blood glucose and triglyceride values is shown in Fig. 3. A significant difference in glucose was found between the C group and the TS group in male rats. In the Skylab crew, insulin and glucose were significantly decreased during the flight and increased after recovery. The level of glucose changes according to the quantity of nourishment absorbed. A decrease in the quantity of glucose seems to be due to its absorption from the blood by skeletal muscle, because glucose in serum is utilized for skeletal muscle metabolism. Triglyceride was significantly decreased in the TS group in male rats. Triglyceride is obtained almost totally from dietary fat, and fat stored in the body. The change in fat metabolism due to tail suspension was correlated with body weight. Loss of body energy stores throughout the long mission may have accounted for the mobilization of triglyceride observed after recovery in the Skylab crew.

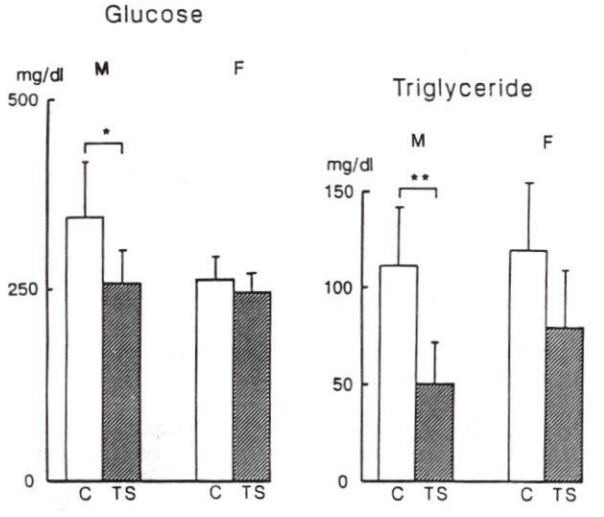


Fig. 3. Glucose and triglyceride after four weeks of tail suspension. Values are means±SE. Significantly different from control at *$p < 0.05$ and **$p < 0.01$.
C:control, TS:tail suspension,
M:male, F:female rats

A comparison of hemoglobin and serum iron is shown in Fig. 4. No significant differences in hematocrit were found. As for hemoglobin and serum iron, lower mean values were found in the TS group, the differences being significant.
These results imply that TS-group rats were in an anemic state. It is interesting that this condition was induced by tail suspension. In the case of space flight, astronaut anemia has been reported, the main phenomenon involved being a decrease of red blood cell mass. This is thus clearly different from the anemia observed in TS rats. Therefore in this study, no significant difference was found in other blood biochemical parameters.

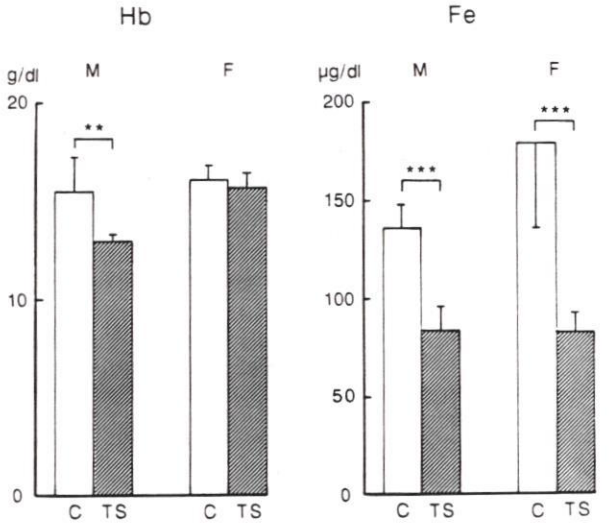


Fig. 4. Hemoglobin(Hb) and serum iron (Fe) after four weeks of tail suspension. Values are means±SE. Significantly different from control at **$p < 0.01$ and ***$p < 0.001$.
C:control, TS:tail suspension,
M:male, F:female rats

Our results indicate that decreases of nitrogen, fat and iron metabolism occurred in the tail suspension group, and it was evident that these changes were more marked in male than in female rats.

REFERENCES

1) Kimzy, S. L. Hematology and immunology studies. In: Biomedical Results from Skylab, edited by R. S. Johnston, and L. F. Dietline. NASA Document SP-377: Washington, DC. 1977, p.249-282.
2) Leach, C. S. and P. C. Rambaut. Biochemical responses of the Skylab crewmen. In: Biomedical Results from Skylab, edited by R. S. Johnston, and L. F. Dietline. NASA Document SP-377 : Washington, DC. 1977, p.204-216.

DEVELOPMENT OF AN ARTIFICIAL GRAVITY SLEEPER (AGS)

David Cardus*, Peter Diamandis**
Wesley G. McTaggart* and Scott Campbell***

*Baylor College of Medicine, Department of Rehabilitation, Houston, Texas 77030
**International Space University, Boston, MA
***Institute for Circadian Physiology, Boston, MA

Extended periods of exposure to microgravity lead to significant pathophysiologic changes in various systems of the body. Most of these changes, while not detrimental during space flight, present a major problem on return to earth's gravity and, in the future, in the descent to any planetary surface. Current efforts toward maintaining earth-normal physiological condition involve physical exercise and lower body negative pressure. Though these efforts are partially successful, they utilize a significant portion of the crew's potential time. In addition, these countermeasures do not address the full spectrum of physiologic conditioning needs.

The objectives of our project are: 1) To design and build a 2-meter radius "human compatible" centrifuge (termed the Artificial Gravity Sleeper or AGS) as an advanced version of an original radial centrifuge developed at MIT. The advanced version will be able to accomodate up to 4 subjects at a time, operate at a broader range of speeds, and have greater safety features. 2) To expand on a previous pilot study to investigate the quality of sleep during 100% gradient centrifugation on the AGS by studying a larger group of subjects. 3) To conduct a microgravity simulation study using bed rest to assess the ability of 100% gradient centrifugation to function as a countermeasure to cardiovascular deconditioning.

At the time of preparing this paper the following steps had been accomplished:

1. Design of the centrifuge.

The design phase has been completed. The new AGS (Fig. 1) will be a circular platform on a 2 m. radius, 76 cm. high and with a 80 cm. high circular enclosure. The platform will rotate on a central support structure and on a 3.6 m. diameter track supported by six air inflated wheels.(Fig.2) A magnetic breaking system will be able to stop the platform in approximately 10-15 seconds. During operation, it will be possible to spin the centrifuge to a maximum of 37 rpm, which will cause approximately 2 g at the feet. Within the central support structure there will be a slip-ring assembly through which power and experimental data will flow. The surface of the platform will have areas for housing monitoring equipment and a track system to which up to four beds can be mounted. When two or more beds are mounted on the AGS, they will be stationary. This will locate the subject's heads just off the center of rotation. The beds will have the ability to tilt at least 6 degrees in order to raise the level of the feet above the head. The beds will be fully enclosed under clear canopies in order to prevent the sleeping subjects from feeling the breeze when rotating, while allowing the researchers to keep visual contact at all times. The subjects will also be monitored by video cameras which will be mounted on the head of each canopy. An intercom system will be installed to allow researchers and subjects to be in constant communication. The subjects will sleep with eye masks on and the canopies will be closed to eliminate all physical and visual clues indicating that the platform is spinning. An additional canopy will be built opaque and with lights to be able to observe unmasked subjects while they are awake and active (i.e. reading, exercising, etc.)

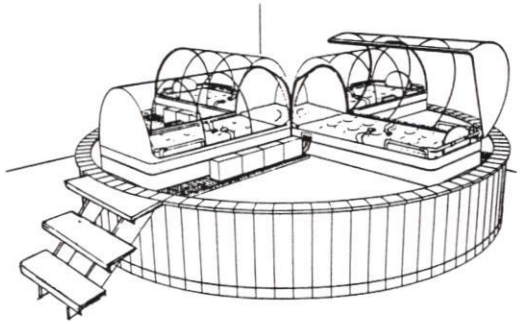


Fig. 1. Centrifuge platform with four beds and canopies.

2. Construction of the centrifuge.

Construction of the centrifuge was started in early August of this year. The platform is anticipated to be built by the middle of October. At that time instrumental testing will be done. This will include performance of the centrifuge platform, motors, power train and controls, equipment attachment systems, modular patient beds with mattresses, and shrouds for each bed.

3. Planning of human experimentation.

Human experimentation will consist of five types of experiments.

The purpose of the first type of experiment is to study the physiological response to various rotation speeds, and to position of the head in relation to the axis of rotation. The principal objective is to determine human tolerance to centrifugation in terms of sleep, alertness and performance and to study the cardiovascular responses.

The purpose of the second type of experiment is to characterize the physiological response to bed rest. The period of bed rest will last up to two or three weeks. Testing of the cardiovascular system will be done before and at the end of one week and again at the end of two or three weeks to ascertain the minimal stay in bed necessary to elicit substantial cardiovascular deconditioning. The head-down tilt (HDT) position will also be tried as a procedure for accelerating the deconditioning effects of simulated microgravity.

The purpose of the third type of experiment is to study the reconditioning effect of centrifuge rotation on previously deconditioned subjects. We will determine the cardiovascular reconditioning effects, if any, of intermittent 100% gradient rotation at 1 g. Rotation on the centrifuge will be up to 7 continuous hours each day for two or three weeks. While not on the centrifuge the subjects will remain in the horizontal position in bed rest.

The purpose of the fourth type of experiment is to study the preventive effect of centrifuge rotation on normally conditioned subjects. We will determine the effects of 100% gradient centrifugation at 1 g in maintaining the cardiovascular condition. The subjects will be submitted to continuous bed rest for up to two or three weeks with daily rotation on the centrifuge for 7 continuous hours each day.

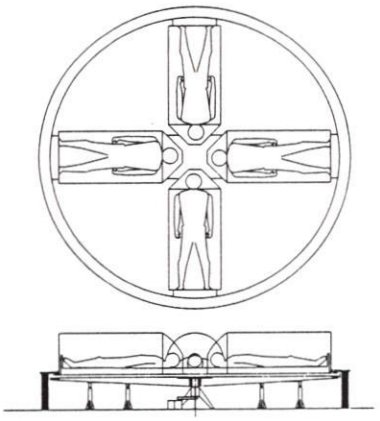


Fig. 2. Overhead view and lateral view of the centrifuge with pneumatic support.

The purpose of the fifth type of experiment is to study the physiological response to a combination of bed rest, centrifuge rotation and physical exercise. We will determine if physical exercise added to rotation has an additive effect in preventing the cardiovascular deconditioning that results from simulated microgravity (bed rest).

4. Measurements and techniques.

The effects caused by the experimental situations described above will be assessed by conducting psychological studies on sleep, alertness and performance, and on physiological studies focusing but not limited to cardiovascular conditioning.

Sleep, alertness and performance studies. Major sleep episodes will be polygraphically (EEG, EOG, EMG) recorded during rotation on the centrifuge. Data will be stored on magnetic tape, printed out on paper and subsequently analyzed using standard scoring procedures and computer assisted power and frequency analysis. Particular interest will be placed on measures of sleep efficiency, sleep continuity and "depth of sleep." The latter being assessed by delta (0.5 - 3Hz) power frequency across the sleep period.

Post sleep alertness will be objectively evaluated by a Maintenance of Wakefulness Test (MWT) administered at two-hour intervals through the subjects wake periods. During a 20 minute trial, the subject will be instructed to relax but remain awake. EEG will be continuously monitored. If sleep is detected within the 20 minutes, the subject will be awakened and the test terminated. Performance capabilities will be assessed prior to and following each period of rotation on the centrifuge. A brief testing battery, requiring approximately 15 minutes to complete, will be administered. These tests comprise a subset of instruments contained in the Walter Reed Performance Assessment Battery. These instruments will evaluate levels of vigilance, decision making ability, psychomotor skills, and cognitive performance.

Physiological studies. Testing will be done before and after each experiment to evaluate the subject's cardiovascular condition. Two levels of testing will be done.

Orthostatic tolerance. The following variables will be monitored before, during and again after head-up tilt: a) ECG pattern, b) heart rate, c) blood pressure, d) stroke volume and cardiac output, e) bodily fluid distribution and f) baroreceptor-cardiac reflex response.

Exercise tolerance. Response to exercise will be studied by leg or arm exercise in the supine position. Variables to be monitored are: a) ECG pattern, b) heart rate, c) blood pressure, d) stroke volume and cardiac output, e) blood oxygen saturation, and f) oxygen consumption. During rotation on the AGS these cardiovascular parameters will be also monitored continuously for safety and research purposes, excepting oxygen consumption. The methods to be employed are: ECG by modified V5 lead to assess ischemia, rhythmicity and heart rate. Blood pressure (BP) and pressure-rate product (RPP) by an automatic system, and stroke volume (SV), cardiac output (CO) and thoracic fluid index (TFI), by an electrical impedance technique, to assess the function of the heart as a pump. Oxygen consumption will be determined by collecting expired gas in Douglas bags, measuring respiratory gas concentrations by mass spectrometry and gas volumes with a wet-gas meter. Blood oxygen saturation will be recorded by infrared oxymetry in the ear lobe to detect cerebral ischemia. Bodily fluid distribution will be estimated by total body and leg electrical impedance using two energy levels to assess changes in distribution of total body and extracellular water as the major components. Photoplethysmography will be done to assess peripheral arteriolar activity during the head-up tilt. Creatinine clearance and free water excretion studies will be done to assess renal function.

The planned experiments should allow an evaluation of the centrifuge in terms of human tolerance to rotation and of its use as a countermeasure to the physiological effects of microgravity. As a result of this project, the AGS may be found to have potential usefulness as a tool for studying the mechanisms of some of the physiological alterations which occur as a consequence of microgravity or certain hypokinetic conditions.
Supported by NASA Grant No. NAGW-1691.

References:

1. Diamandis PH: The Artificial Gravity Sleeper: A deconditioning countermeasure for long duration space habitation. Master of Science Thesis in Aeronautics and Astronautics, M.I.T., 1988.
2. Piemme TE, et al: Renal response to +Gz 100 gradient acceleration in man. Aerospace Medicine 37:1253-1256, 1966.
3. Piemme TE, et al: Human tolerance to Gz 100 per cent gradient spin. Aerospace Medicine 37: 16-21, 1966.
4. Thorne D, Ginser S, Sing H, Higge F: The Walter Reed Performance Assessment Battery. Neurobehav. Toxycol. and Teratol. 7: 415-418, 1985.
5. White WJ: A History of the Centrifuge in Aerospace Medicine. Biotechnology Branch. Missile and Space Systems Division, Douglas Aircraft Co., Inc. 1966.

INFLUENCE OF SHORT AND LONG DURATION EXPOSURE TO HYPERGRAVITY IN A SINGLE-CELL ORGANISM, PARAMECIUM TETRAURELIA.

G. RICHOILLEY, G. GASSET, R. TIXADOR and H. PLANEL

Laboratoire de Biologie et Radiobiologie spatiales
Faculté de Médecine - Toulouse - France.

In previous experiments, we have shown that the growth rate of a single-cell organism, Paramecium tetraurelia, was stimulated when cultures were exposed to the space flight factors (1-2). This effect can be ascribed to microgravity, as it was demonstrated aboard Challenger (D1 mission) using a 1g centrifuge: the stimulatory effect, observed in static cultures exposed to microgravity, disappeared in the centrifuged cultures (3-4).

Based on these data, effects of hypergravity were investigated on earth. Paramecia were exposed to various gravitational levels, in short term or long duration experiments. Secondary effects and the cell movement were also studied. Results of our experiments are in good agreement with those of space experiments and confirm the negative geotaxis of Paramecium.

Material and technics

Paramecium tetraurelia (wild strain 82b) was cultivated in a straw infusion bacterized with Enterobacter aerogenes. Sixteen divisions after autogamy, cells in division were isolated. After division, one sister cell was used for the experiment, the other one for the control experiment.

Each culture tube (1 cm diameter) included one cell and 4 ml of medium. The culture tubes, hermetically closed, were placed, in a vertical position, on the rotating platform of the centrifuge. Five experiments were carried out for each g level. Cells were centrifuged at 130 rpm (2 to 10 g) or 190 rpm (20 g). Fifteen cultures were used at each fixation. Cell counts were performed using a haematocytometer and repeated three times. Generation times were measured after cloning and transferring the paramecia in depression slides.

Results

1) As shown in Fig. 1, the cell growth rate decreased in 3 day-centrifuged cultures, the greatest inhibition occuring at the highest gravitational levels. Differences between controls and centrifuged cultures are significant ($p < 0.05$) at every g level.

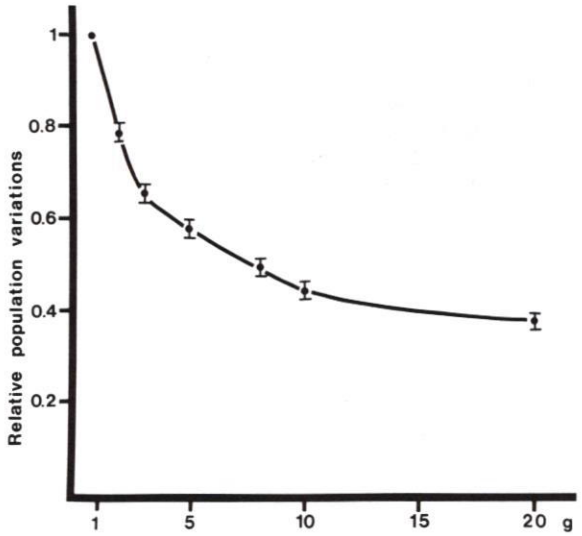


Figure 1. Effects of 3 day-exposure to hypergravity on relative variations of cell populations (Error bars show SEM).

The inhibitory effect enhanced according the duration of the experiment, as shown in Table 1. For the three investigated g levels, an exposure time higher than 3 days resulted in a more obvious inhibitory effect; however, differences between the centrifuged populations after 17 or 30 days are not significant.

Centrifugation duration (days)	g level		
	2	5	8
3	0.88	0.64	0.70
17	0.66	0.54	0.51
30	0.70	0.49	0.50

Table 1. Effects of long term exposure to hypergravity. Relative variations of cell populations (all results are significant , $p < 0.05$).

2) The possibility of a secondary effect of hypergravity was studied, measuring the generation times of paramecia just after the centrifugation or 24 hours later. As shown in Table 2, the generation times of centrifuged paramecia was increased ($p < 0.05$) when cells were tested just after centrifugation.Twenty four hours later, the generation times of paramecia, exposed to 2.7 - 5 - 8 - 10 g for 3 days, was restaured.

3) In order to demonstrate that changes in cell proliferation could be related to a cell sedimentation, the cell displacement with regard to the centrifuge force was studied in cultures exposed to various g levels. Cells were housed in plastic tubes, including two compartments A and B, divided

by a rubber piece. Before centrifugation, 10 paramecia (3 hour-experiment) or 1 paramecia (3 Day-experiment) were introduced in each compartment, the rubber tube beeing clamped. The tubes were placed in an horizontal position, along the radii of the rotating platform of the centrifuge. The compartment A was placed near the centrifuge axis : cells were thus exposed to lower gravity levels than in B. The forceps were removed just before the centrifugation : thus, cells could shift from a compartment to another one. Just after centrifugation, the rubber tube was clamped and the cell shift was estimated by cell count in each compartment.

g level	Just after centrifugation		24 h after centrifugation	
	Control	Centrifuged	Control	Centrifuged
2.7	324 ± 1.85	330 ± 2.13 *	331 ± 1	330 ± 1
5	324 ± 1.85	341 ± 2.12 *	331 ± 1	328 ± 2
8	324 ± 1.85	343 ± 2.91 *	331 ± 1	326 ± 2
10	331 ± 1,86	364 ± 2.75 *	334 ± 0.98	336 ± 2

Table 2. Secondary effects of hypergravity. Generation times of Paramecium tetraurelia measured just after the centrifugation or 24 hours later (* significant differences, $p < 0.05$).

Table 3 shows that paramecia moved in an opposite direction to the centrifuge force when they were exposed, for 3 hours, to the lowest hypergravity levels. For 1.8 g in A and 2.4 g in B, cells remained in their initial position. At higher levels, a cell shift was noted : after 3 hours, cells moved according to the centrifuge forces. However all cells were not sedimented at 6 or 8 g. A cell sedimentation occured after 3 days at 4.7 - 7 g but about one third of paramecia remained in their initial position in populations exposed to 2.8 - 3.5 g. At 1.6 - 2 g half cells did not move from A to B.

CENTRIFUGATION DURATION	COMPARTMENT A		COMPARTMENT B	
	g level	Cell population		g level
3 hours	1.6	14.61 ± 0.61.	← 4.44 ± 0.53	2.0
	1.8	9.40 ± 0.50.	= 10.40 ± 0.6.	2.4
	3.1	5.00 ± 0.58	→ 14.00 ± 0.71	5.0
	6.1	3.10 ± 0.53	→ 16.40 ± 0.5	8.0
3 days	1.6	646.00 ± 36	→ 830.00 ± 76	2.4
	2.8	473.70 ± 108.6	→ 1088.00 ± 144	7.5
	4.7	1.00 ± 0.74	→ 475.00 ± 139	7.0

Table 3. Cell shift in a culture device including two compartments where the paramecia were exposed to different gravitational g levels. At the beginning of the experiments, each compartment included 10 paramecia (3 hour-experiment) or 1 paramecium (3 day-experiment).

Discussion

The most striking effect of space flights in Paramecia was an enhancement of the cell growth rate. As it was reported in this work, this gravity effect is confirmed by earth-based experiments : the growth rate is reduced when paramecia are exposed to various levels of hypergravity. Hypergravity has a transient effect, the cell proliferation was restaured several hours after centrifugation, even when cells were exposed to hypergravity for 30 days. The study of cells displacements at very low gravitational levels is in good agreement with the well known negative geotaxis of paramecia and demonstrates that they are adapted, not only to 1 g, but also to slightly higher gravitational levels. On the other hand, our results suggest that the reduced cell growth rate can be only partly related to cell sedimentation : after 3 day exposure to low g levels, a large fraction of cells is not shifted yet.

The mechanism of graviperception by Paramecium tetraurelia is still unknown. In contrast to Loxodes (5), a ciliated protozoan like Paramecium tetraurelia, the mechanoreceptors are still unknown. According to Kuroda (6), the negative geotaxis could be related to the location of the gravity center which is posterior to the center of buoyancy in Paramecium caudatum.

On the other hand, it can be pointed out that gravity influence depends on cell types. Thus, exposure to 10 g results in an enhanced activation of human lymphocytes (7) but it is obvious that gravity effects must be different in suspended or free swimming cells. In any cases, these results demonstrate that, besides the plant cells, gravity can have an influence at the cellular level.

Acknowledgements

This study was supported by the Centre National d'Etudes Spatiales. The authors thank Miss Brigitte Eche, Mr. René Rousseille for their technical assistance and Mrs Michelle Murat for her secretariat assistance.

References

1. Planel H., Tixador R., Nefedov I.G., Gretchko G. and Richoilley G. - Preliminary results of Cytos experiment flown in Salyut VI: investigations on Paramecium aurelia. Life Sciences and Space Research (1979), XVII:139-144.

2. Planel H., Tixador R., Nefedov Yu., Gretchko G., Richoilley G., Bassler R. and Monrozies E. - Space flight effects on Paramecium tetraurelia flown aboard Salyut VI in the Cytos 1 and Cytos M experiments. Adv. Space Research (1981), 1 : 95-100.

3. Richoilley G., Tixador R., Gasset G., Templier J. and Planel H. - Preliminary results of the "Paramecium" experiment. Naturwissenschaften (1986) 73 : 404-406.

4. Richoilley G., Tixador R., Templier J., Bes J.C., Gasset G. and Planel H. - The Paramecium experiment Biorack on Spacelab D.1. ESA SP-1091 (1987), 69-73.

5. Fenchel T. and Finlay B. - The structure and function of Muller vesicles in Loxodid ciliates. J. Protozool. (1986), 33 : 69-76.

6. Kuroda K., Kamiya N., Yoshimoto Y. and Hiramoto Y. - Paramecium behaviour during video centrifuge-microscopy. Proc. Japan Acad. (1986), 62-B : 117-121.

7. Lorenzi G., Bechler B., Cogoli M. and Cogoli A. Gravitational effects on mammalian cells. The Physiologist (1988), 31 : S 144- S 147.

ELECTROVESTIBULOGRAPHY IN EXPERIMENTAL ANIMALS.

Renaud Charlet de Sauvage, Gilles Dolivet, Jean-Paul Erre, and Jean-Marie Aran
INSERM Unit 229 - Audiologie expérimentale, Hôpital Pellegrin, 33076 Bordeaux. France

INTRODUCTION

Recording a compound response from a nerve trunk requires to trigger its fibres instantaneously, i.e. within about 1 ms for sensory fibres. With a time constant of several seconds, the vestibular system does not meet this requirement, thus accelerations in the physiological range cannot elicit a detectable gross response from the vestibular nerve. Electrical stimuli (ES) on the other hand, allow a perfect synchronisation of many fibres and have been successfully applied to the cochlear system by our group (1). A combination of electrical and acoustical stimulations enabled to record compound responses of the cochlear nerve to low frequency filtered clicks or to continuous sounds (2), in spite of the lack of synchronism between individual fibres. This paper reports on the application of the same principle to the vestibular system.

METHODS

Normal pigmented guinea pigs were chronically implanted with electrodes on the left round window and near the VIIIth nerve, with vertex and cerebellar references respectively. ES consisting either of a 100 Hz square voltage or negative current pulses with 300 us duration, were applied between round window and vertex. Recordings were made from the VIIIth nerve electrodes. Vestibular stimulations consisting of pendular or constant angular accelerations were applied in the plane of the left semi-circular canal (3) with the animal head down. The averaging window during which the signal was sampled could be shifted relative to the onset of the acceleration so as to explore various phases of the movement. The technique, summarized in figure 1, was based on a differential procedure.

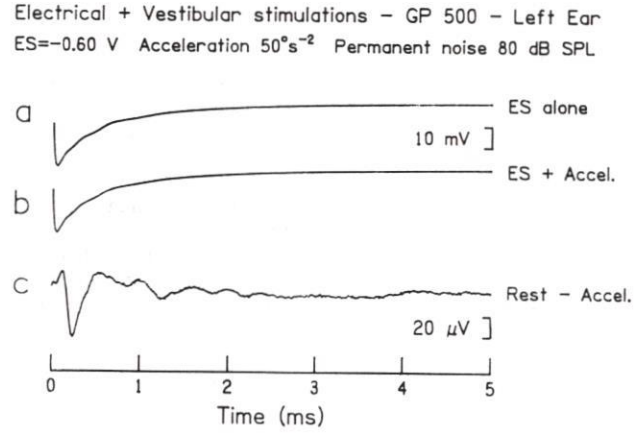


Fig. 1 : a) Artefact + unspecific physiological responses recorded near the VIIIth nerve when applying a 100 Hz square wave voltage on the left round window of the guinea pig in the resting condition. b) Same as in "a" but during constant left acceleration. A noise is permanently applied in a and b to eliminate any possible cochlear contribution. c) Difference a-b (2x100 sweeps repeated 5 times) leaving only the derived vestibular response (EVAP). Notice the amplitude ratio between response and artefact.

The ES being permanently applied, two averagings were performed, one during rest (top recording), a second one in the presence of an angular acceleration (middle curve). To cancel the possible effect of the noise generated by rotation, a continuous sound was permanently applied both at rest and during rotation. When subtracting the signals averaged during rest and rotation from each other, the artefact canceled, leaving a physiological response with a 0.3 ms latency (bottom curve). The procedure could be repeated several times to improve signal/noise ratio. The ES brought the synchronization necessary to observe the response, the vestibular stimulation allowed to derive a vestibular specific response without artefact. This response represents the change in electrical excitability of the vestibular fibres due to rotation, and will be refered to as Electrically evoked Vestibular Action Potential (EVAP).

RESULTS

The influence of amplitude and direction of the acceleration on the EVAP was investigated. Most commonly the polarity appeared negative for most responses derived with left accelerations and positive for right accelerations (fig. 2). In some guinea pigs the opposite was true, but in any case left and right accelerations always elicited responses with opposite polarities in the same animal. The input/output amplitude function of the EVAP appeared roughly linear, but left rotations tended to produce responses with bigger amplitudes than right rotations with the same absolute acceleration.

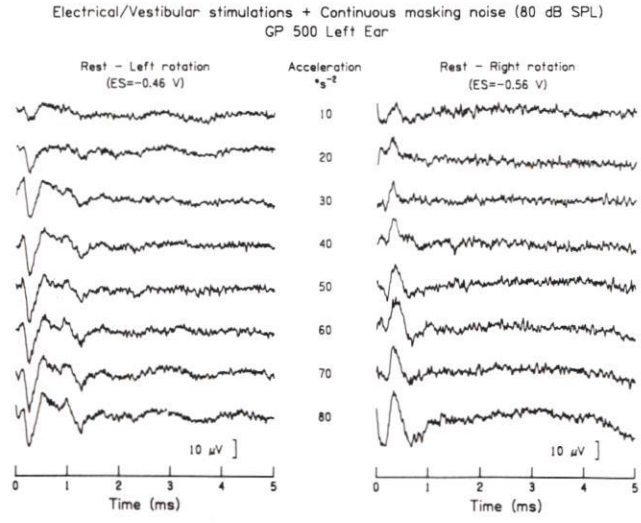


Fig. 2 : EVAPs derived as in fig. 1 as a function of acceleration in the left and right directions (2x100 sweeps repeated 10 and 5 times respectively).

Two experiments were performed using selective destructions of the cochlear and vestibular receptors. In a first experiment a single dose of amikacin at 500 mg/kg body weight was injected IM in a preimplanted guinea pig with initially normal cochlear and vestibular functions, followed by a single intracardiac dose of 50 mg/kg ethacrynic acid one hour later. Such a treatment was known to be mainly cochleotoxic and to produce a complete destruction of the organ of Corti within a few hours. It was indeed verified in this animal that 4 hours after the last injection no compound cochlear response could be recorded any more, whereas the EVAP was only slightly modified. In a second experiment a chronic treatment was performed using streptomycin, a mainly vestibulotoxic antibiotic. The animals received 21 injections 5 days per week at a dose of 200 mg/kg body weight. At the end of the experiment the cochlear potentials were still normal while EVAPs had disappeared at various degrees. In one guinea pig, for which the vestibular response was totally abolished, the histology confirmed the presence of severe lesions in the vestibular organs, particularly in the ampular crests, while the cochlear hair cells remained normal.

Post rotation EVAPs were studied in the following condition : a 100 sweep averaging was first performed with the guinea pig at rest. Then the animal was subjected to a progressive 99 deg.s^{-1} rotation maintained during 15 seconds, to allow the vestibular fluids to stabilize. The rotation was stopped with a deceleration adjustable from 99 deg.s^{-2} to 15 deg.s^{-2}, then a second 100 sweep acquisition was performed starting with a delay adjustable from 0.07 to 5 s after the end of rotation, and the second recording was subtracted from the first one. Figure 3 shows the influence of delay and deceleration level on EVAP amplitude in one guinea pig. Regression curves with an exponential decay appeared to yield the best fit with experimental data (r^2 in the range 0.84 to 0.96). Decelerations with opposite directions again yielded post rotation EVAPs with opposite polarities. EVAPs derived during an acceleration in one direction always presented a polarity opposite to that of EVAPs derived after stopping a movement in the same direction.

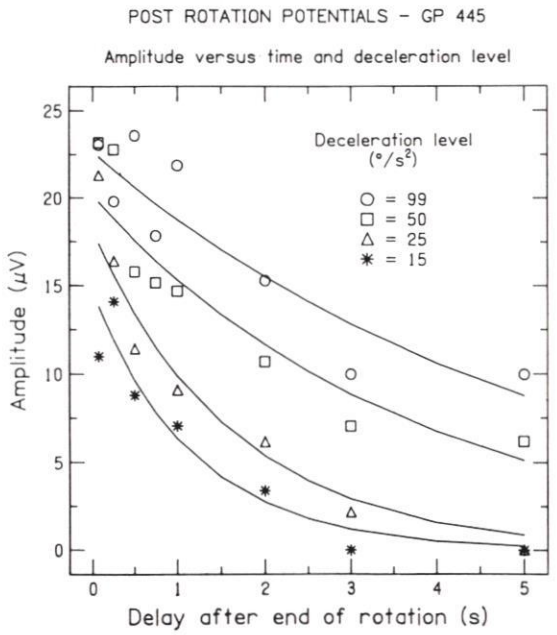


Fig. 3 : Post rotation EVAP amplitude as a function of time after the end of a stable 99 deg. s^{-2} rotation for various decelerations. Post rotation EVAPs were obtained by subtracting from each other the recordings performed before and after rotation (2x100 sweeps repeated 10 times). Best fit exponential regression curves for each series of data points are denoted by solid lines.

The influence of ES intensity on the EVAP was also investigated. The dependance of EVAP amplitude on stimulus intensity appeared almost linear, with a saturation level above which the amplitude of the response tended to decrease. The latency of the response decreased monotonically with ES intensity.

DISCUSSION

Lorente de Nò (4) demonstrated that the pattern of the response recorded near a nerve trunk could change drastically according to the position of the electrode. In the present experiment the active recording electrode is inserted stereotaxically near the vestibular nerve, with uncertainties about its localization due to anatomicalvariations. This can explain why EVAPs with variable polarities have been observed in some animals. On another hand, the variation of EVAP latency with ES current is probably due to the settling time of the stimulus. As the ES intensity is raised, the triggering threshold of the fibres is reached earlier and earlier, thus the latency tends to diminish. The change in polarity of the response with left and right rotations is a good argument about the vestibular specificity of the response. Excitatory stimulations (left accelerations in the present case) are known to increase the spontaneous discharge rate of the fibres (5) presumably reducing their electrical excitability at the same time. The opposite is true for right rotations. As expected, the response extracted in the rest-left condition thus has a polarity opposite to that recorded in the rest-right condition. The usually larger amplitude of the "rest-left" response seems to be related to the fact that the discharge rate of the fibres, about 39 spikes/s in guinea pigs (6), can increase several times with excitatory stimulations but cannot go below zero with inhibitory stimuli, thus bringing an assymetry in the response to opposite movements. The quasi linear relation between acceleration and response amplitude is to be related to the linear input/output function of canal fibres (7). Post rotation EVAPs are related to adaptive properties of the fibres (average time constant 3.5 s in the cat) (8), and to visco-elastic properties of the cupular movement with time constants between 20 and 30 s (9). The response presents an exponential decay with time and the numerical values of the observed time constants lie in the expected range, showing that the response has specific characters of vestibular apparatus behaviour, which again favours a vestibular origin of the response. Selective vestibular and cochlear destructions confirm the ability of the method to derive vestibular specific components.

In conclusion, the technique presented here seems to allow a specific investigation of the peripheral vestibular function, and as such could contribute to new developments in the field of vestibular research.

REFERENCES

1. CHARLET DE SAUVAGE, R., CAZALS Y., ERRE, J.-P. AND J.-M. ARAN. Acoustically derived auditory nerve action potentials evoked by electrical stimulation : an estimation of the waveform of single unit contribution. *J. Acoust. Soc. Am.* 73 : 616-627, 1983.
2. ARAN, J.-M., ERRE, J.-P. AND R. CHARLET DE SAUVAGE. Derived evoked potentials for continuous tones using a hybrid electrical-acoustical stimulation. *Hearing Res.* 20: 289-293, 1985.
3. CURTHOYS, I.S., CURTHOYS, E.J,. BLANKS, R.H.I. AND C.H. MARKHAM. The orientation of the semicircular canals in the guinea pig. *Acta Otolaryngol.* 80 : 197-205, 1975.
4. LORENTE DE NO, R. A study of nerve physiology. New-York Rockfeller Inst. Med. Res., 1947, two volumes 1044 pages.
5. CURTHOYS, I.S, BLANKS, R.H.I. AND C.H. MARKHAM. Semicircular canal functional anatomy in cat, guinea pig and man. *Acta Otolaryngol.* 83 : 258-265, 1977.
6. YAGI, T., UENO, H. J. AND YAMAGUCHI. Physiological characteristics of the primary horizontal canal neurons in guinea pig. In : *The vestibular system. Neurophysiologic and clinical research*, edited by M.D. Graham and J.L. Kemink. New York : Raven press, 1987.
7. GOLDBERG, C. AND J.M. FERNANDEZ. Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. I. Resting discharge and response to constant angular accelerations. *J. Neurophysiol.* 34 : 635-660, 1971.
8. BLANKS, R.H.I., ESTES, M.S. AND C.H. MARKHAM. Physiologic characteristics of vestibular first order canal neurons in the cat. II Response to constant angular acceleration. *J. Neurophysiol.* 38 : 1250-1268, 1975.
9. WILSON, V.J. AND G. MELVILL JONES. In : *Mammalian vestibular physiology*, edited by V.J. Wilson and G. Melvill Jones. New York and London : Plenum press, 1979.

Otolith versus Vertical Semicircular Canal Input in the Inhibition of Horizontal Postrotatory Nystagmus

E. Koenig, W. Dengler, M. Fetter, A. Hann, J. Dichgans

Dept. of Neurology, Univ. of Tuebingen, Fed. Rep. of Germany

Tilting the head away from its prior axis of rotation, reduces the dominant time constant of horizontal postrotatory nystagmus (PRN) (Benson and Bodin, 1966; Schrader et al. 1985,a,b) from about 15 sec to about 7 sec. (the time constant of the peripheral vestibular system; Büttner and Waespe, 1981). Thus it was suggested that the central vestibular velocity storage is discharged by the change in head position (Raphan et al. 1979,1981; Cohen et al. 1981). As this active change of head position simultaneously stimulates vertical semicircular canals (transiently), otoliths (transiently and continously) and neck afferents we tried to assess the relative contributions of static (mainly otoliths) and dynamic inputs (mainly semicircular canals) to this inhibition.

Methods:

To generate PRN 10 volunteers with normal vestibular function were abruptly stopped from a constant-velocity rotation of 90°/sec about the vertical head axis. Eye movements were recorded by DC-electrooculography during PRN I and PRN II (secondary phase of PRN into the opposite direction). Head tilts (forward pitch of 90° amplitude into the prone position) were actively performed by the subjects on verbal commands.

Exp. 0: As a control the head remained in the erect position during PRN.

Exp. 1: For a maximum contradictory static otolith input the head was tilted forwards in the 4th second after the stop (after allowing PRN to develop) and remained in this position until PRN II ceased.

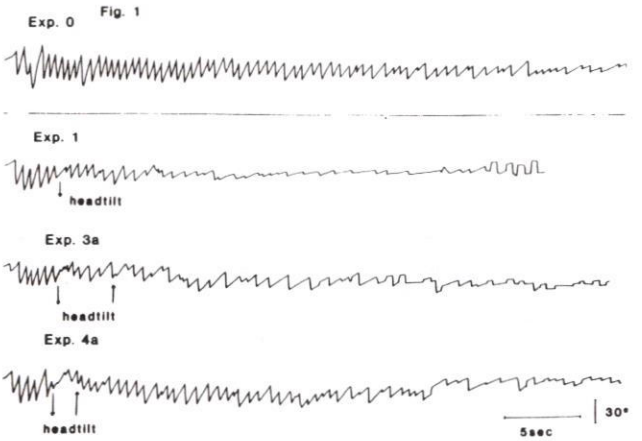
Exp. 2: To study inhibition after the presumed end of peripheral vestibular input the head was tilted forwards in the 22nd second (i.e. after 3 times the assumed peripheral time constant of 7 sec) and remained in this position until the end of PRN II.

Exp. 3: To study the effect of intermittent static inhibition the head was tilted
(Exp 3a) for 3 sec (from the 4th to the 7th sec) or
(Exp 3b) for 6 sec (from the 4th to the 10th sec) or
(Exp 3c) for 18 sec (from the 4th to the 22nd sec, i.e. for the remaining duration of input from the labyrinth) and thereafter the head was again upright.

Exp. 4: For maximum stimulation of the vertical semicircular canal with little otolith stimulation, subjects performed a forward pitch and immediately returned to the upright position. The transient pitching movements were performed
(Exp 4a) once in the 4th sec.,
(Exp 4b) once in the 22nd sec. or
(Exp 4c) five times from the 4th to the 10th sec.

Results:

Original EOG-recordings of one subject (Fig.1) demonstrate the inhibition of PRN I by tilts in the 4th sec of different duration compared to the control experiment without tilt (Exp. 0).



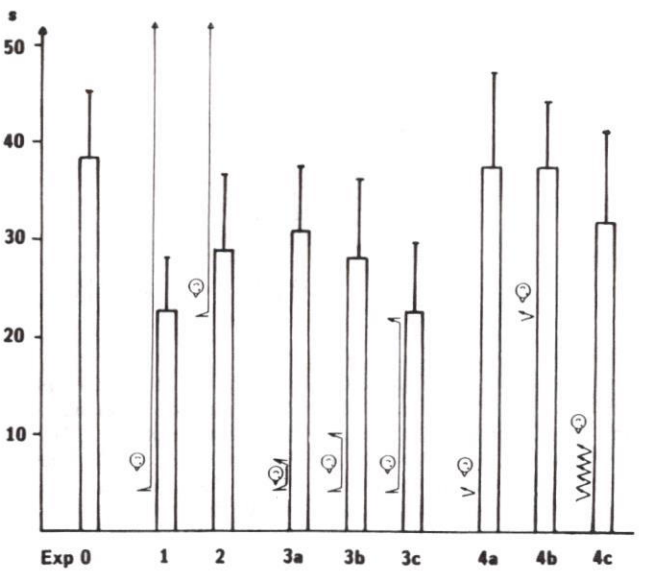


Fig. 2 Average duration (and one standard deviation of the 10 subjects) of PRN - I for the different experiments (see methods).

For evaluation of PRN I duration (Fig. 2), time constants (table 1), and cumulative amplitude (similar results as for duration), were measured as well as the maximum slow phase velocity at distinct time intervals after the stop (Fig.3).

Table 2 shows the significance of differences based on an analysis of variance.

PRN I was most strongly inhibited by a maintained forward head tilt starting shortly after the stop (Exp. 1). Tilting the head after the input from the labyrinth had presumably ceased (Exp. 2) did not result in a sudden loss of PRN; although PRN declined faster after the tilt it lasted on the average for another 7 sec. Intermittent head tilts of increasing duration (3, 6 and 18 sec; Exp. 3 a, b and c) led to increasing amounts of inhibition. One brief pitching movement, whether executed in the 4th (Exp. 4a) or 22nd sec (Exp. 4b), hardly reduced PRN I at all. Five pitching movements within 6 sec (Exp. 4c) reduced PRN less than maintained forward head tilt of the same duration (Exp. 3b), although dynamic stimulation (mainly of the vertical semicircular canals) by the repetitive movements far exceeded that elicited by a single head movement.

Table 1

Exp.	from	max	4th s	7th s	10th s
0	contr.	14,1 ±3,7	13,2 ±2,8	13,2 ±2,9	13,0 ±2,4
1	4th s		7,2 ±2,2	7,1 ±2,2	7,0 ±2,6
3a	4-7 s			11,4 ±2,5	10,4 ±2,7
3b	4-10 s				11,3 ±3,9
4a	4th s		13,6 ±3,6		
4c	4-10 s				11,6 ±3,9

Table 1 Averages of time constants of the decline of PRN I. For comparison time constants were also calculated starting at different time intervals after the stop (from the 4th, 7th, 10th and 22nd sec on, if possible). Because of a shortage of data (especially after tilts in the 22nd sec the remaining PRN was brief) time constants could frequently not be determined.

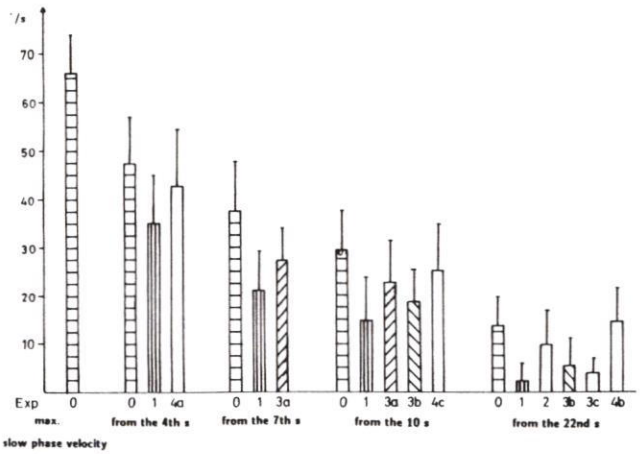


Fig. 3 Averages (and one standard deviation) of the slow phase velocity of PRN I measured at different time intervals after the stop (shortly after the stop for the control exp. (0), and after the 4th, the 7th, 10th and the 22nd second - for reasons of comparison the level of slow phase velocity in Exp. 0 and 1 are also shown at all time intervals after the stop).

Table 2

Exp.		0 davt	1 davt	2 davt	3a davt	3b davt	3c davt	4a davt	4b davt
Exp.									
1	4th s	++++							
2	22 s	+++	+-+						
3a	4-7 s	+++-	++++	-					
3b	4-10 s	+++-	+--+	--+	----				
3c	4-22 s	+++	---	+-+	+	+--			
4a	4 s	----	++-+	+	+	+	+		
4b	22 s	---	+++	+++	+	+++	+++	-	
4c	4-10 s	++--	++++	-	----	--+-	+	+	+

Table 2 Significant differences (p = 0,05) of PRN I are indicated as a result of an analysis of variance for the following parameters: duration (d), cumulative amplitude (a), maximum slow phase velocity (v) and time constant (t).

PRN II was evaluated by considering duration (Fig. 4) and cumulative amplitude (similar results as for duration). Experiments can be divided into 2 categories: those with the head upright during PRN II (Exp. 0, 3a,b,c and 4a,b,c) and those with the head in the prone position throughout PRN II (Exp. 1 and 2) which in general show a significantly weaker PRN II than experiments with the head upright.

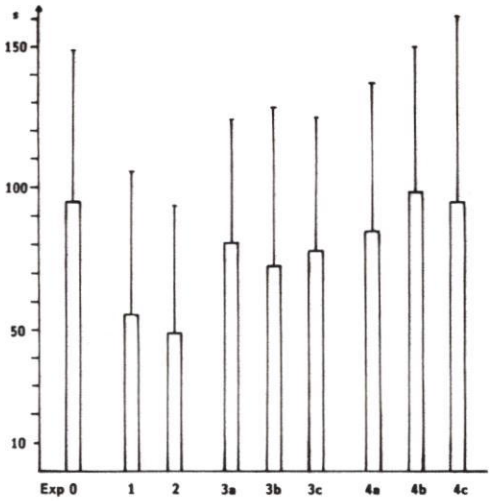


Fig. 4 Average duration (and standard deviation) of PRN II.

Conclusions:

The experiments show an inhibition of PRN which is mainly dependent on the time the head remains in the tilted position. This indicates that a continuous contradictory input from the otoliths is required for maximum inhibition. The time dependency demonstrates that this inhibition is not an all-or-none response like the loss of the subjective turning sensation after identical head tilting manoeuvres (Schrader et al. 1985a,b). The slight increase of the time constant after the end of the tilt from about 7 to about 11 sec (Exp.3a,b) suggests a partial recharge of vestibular velocity storage in the presence of a residual cupula deflection. The lack of a sudden cessation of PRN by tilts at the 22nd sec (after the assumed end of peripheral input) is in accordance with the findings of Lafortune et al. (1987) who found an incomplete discharge of optokinetic afternystagmus (without prior vestibular stimuli) by tilts of 5 sec duration. The minute effect of a brief head pitch (Exp. 4a) and the smaller effect of 5 tilts in 6 sec (Exp.3c) compared to a continuous head tilt of the same duration (Exp.3b) confirm the inefficacy of dynamic components of the movements (predominantly dynamic head tilts of course always contain some gravitational input). The failure of head tilts in 0 G to inhibit PRN again stresses the importance of static otolith input (DiZio and Lackner, 1988).

References:

Benson AJ, Bodin MA (1966) Comparison of the effect of the direction of the gravitational acceleration on post-rotational responses in yaw, pitch and roll. Aerospace Med 37: 889-897

Büttner U, Waespe W (1981) Vestibular nerve activity in the alert monkey during vestibular and optokinetic nystagmus. Exp Brain Res 41: 310-315

Cohen B, Henn V, Raphan T, Dennett T (1981) Velocity storage, nystagmus, and visual vestibular interactions in humans. Ann NY Acad Sci 374: 421-433

DiZio P, Lackner JR (1988) The effects of gravitoinertial force level and head movements on post-rotational nystagmus and illusory after-rotation. Exp Brain Res 70: 485-495

Lafortune SH, Ireland DJ, Jell RM (1988) Effect of active head movements about the pitch, roll and yaw axes on human optokinetic afternystagmus. Can J Physiol Pharmacol 66: 689-696

Raphan T, Matsuo V, Cohen B (1979) Velocity storage in the vestibulo-ocular reflex arc (VOR). Exp Brain Res 35: 229-248

Raphan T, Cohen B, Henn V (1981) Effects of gravity on rotatory nystagmus in monkeys. Ann N Y Acad Sci 374: 44-55

Schrader V, Koenig E, Dichgans J (1985a) The effect of lateral head tilt on horizontal postrotatory nystagmus I and II and the Purkinje effect. Acta Oto-Laryngol 100: 98-105

Schrader V, Koenig E, Dichgans J (1985b) Direction and angle of active head tilts influencing the Purkinje effect and the inhibition of postrotatory nystagmus I and II. Acta Oto-Laryngol 100: 337-343

CARDIOVASCULAR RESPONSES TO SUSTAINED ACCELERATION

David H Glaister

Biodynamics Division
Royal Air Force Institute of Aviation Medicine,
Farnborough, Hants.

As long as large animals with blood circulations remained confined to the oceans, vertical gradients of pressure caused by the weight of the blood were precisely balanced by comparable hydrostatic gradients in the surrounding sea water. Transmural pressures thus remained constant, and independent of depth of immersion, and of body orientation. Subsequent evolution onto dry land introduced a significant problem in that this balancing pressure gradient was lost, and any organ placed high in the body (such as the brain) then received its arterial blood supply at a pressure reduced from that at heart level by a factor of *hpg*, where *h* is the height above the heart, *p* the density of blood and *g* the gravitational constant. For man, an average heart to brain distance of 30 cm gives a pressure drop of some 22 mmHg when upright, while putting its head down to drink after browsing from a tall tree must be a source of a considerable headache for a Giraffe!

In a recent study on snakes, Lillywhite (11) showed significant differences in the anatomy and physiology of tree snakes compared to related sea dwelling species. The bodies of tree snakes were thinner and more tightly skinned, while their hearts were closer to their heads. Plethysmography demonstrated less pooling of blood in their tails when tilted head up, and injections of radio-isotopically labelled microspheres showed an increase in resistance to blood flow caudally, with maintenance of pressure and flow at head level, a logical response to the gravitational stress. These observations underline many of the problems and responses of man to hypergravity, and suggest at least two possible countermeasures - water immersion and a tightly fitting anti-G suit. This paper will consider the systemic and pulmonary circulatory responses to hypergravity and the physiology behind some of the methods which can be used to increase human tolerance to $+G_z$ acceleration (a headwards acceleration with the inertial force vector acting towards the feet).

Systemic Circulatory Response. From the factor *hpg* referred to earlier, it is clear that hydrostatic pressure gradients will increase in proportion to G (the ratio of the applied acceleration to the gravitational constant), so that pressures will tend to fall at levels above the heart and to rise in lower parts of the body, depending upon the sign and magnitude of *h* (Fig. 1). There is, therefore, a point of 'hydrostatic indifference' at which level pressure is independent of G. This lies at about the level of the aortic valve for the arterial system (10), with comparable levels within the venous vasculature and cerebrospinal fluid.

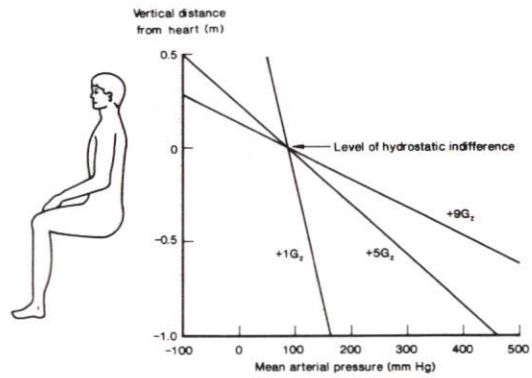


Fig. 1. Effect of $+G_z$ induced hydrostatic pressure gradients on mean arterial blood pressures down the body of a seated subject.

From figure 1 it is clear that arterial pressure at brain level will fall to levels critical for cerebral perfusion at $+5G_z$ or so while all intravascular pressures will be extremely high in the more dependent tissues. While the pressure drop will occur instantaneously with increasing G, the rises will be somewhat delayed as the distensibility of the vasculature will lead to an increase in its capacity and to an inflow of blood before equilibrium values are obtained. This passive dilatation will reduce the resistance to flow offered by the vasculature (particularly the arterioles); cause a transient decrease in venous return as the capacity vessels fill; and produce an effective reduction in circulating blood volume comparable in its effect to that seen with haemorrhage: cardiac output will be compromised by the latter two factors and arterial pressure at heart level by all three.

Fortuitously, evolutionary changes demanded by the move away from the buoyancy of the oceans, as well as those needed to maintain cerebral perfusion in the event of accidental haemorrhage, have led to the development of compensatory mechanisms which respond quite effectively to the novel stress of hypergravity. Thus, the fall in arterial pressure at head level is sensed by the baroreceptors of the carotid sinus which decrease their rate of firing and produce a reflex inhibition of vagal tone and stimulation of the sympathetic nervous system. These mechanisms lead to an increase in the rate and strength of contraction of the heart, and to an increase in peripheral resistance, but are relatively slow to become fully effective so that, for a rapid onset rate of $+G_z$ acceleration, a trough in pressure is seen after 7-8s with a subsequent recovery of pressure towards more normal levels (Fig. 2).

Other baroreceptors sited in the wall of the aortic arch will act to reinforce the initial response, but will subsequently act in opposition to the carotid baroreceptors as heart level blood pressure rises in an attempt to sustain cerebral blood flow. This situation is unique to the high G environment, when at $+9G_z$, for example, the pressures sensed at the two major baroreceptor sites could differ by up to 100 mmHg.

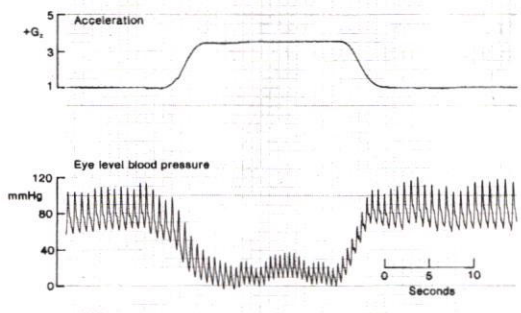


Fig. 2. Effect of $+G_z$ acceleration on arterial blood pressure referenced to the level of the eye.

While effective in raising blood pressure, a generalised vasoconstriction would act counter to the local requirements of some specific tissues, for example the active myocardium. Using radioactive rubidium, a gross redistribution of the cardiac output was demonstrated in dogs exposed to $+G_z$ acceleration (3). Figure 3 shows that blood flow to the gut at $+4.2G_z$ fell to about one third of its control value, while that to the myocardium was maintained at, or even above the control level, with up to one third of the cardiac output supplying the heart muscle in some individuals. Local flow control mechanisms were thus effective in ensuring an optimal distribution of the grossly reduced cardiac output (down by 54% at $+4.2G_z$).

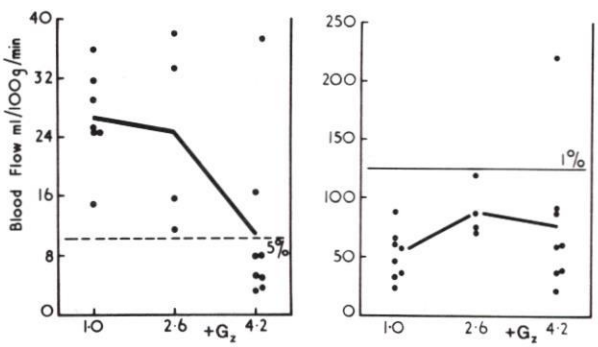


Fig.3. Blood flow to the gut (left panel) and heart (right panel) of dogs exposed to +2.6 and $4.2G_z$ (from Ref 3).

As blood pressures recover, further pooling occurs and, due to the extremely high capillary pressures, fluid is extravasated into the tissues with a further slow, but progressive, loss of circulating blood volume. Finally, the compensatory mechanisms breakdown with a dramatic slowing of the heart, generalised vasodilation and vasovagal syncope. The trigger for this failure in compensation in unknown, but is probably similar to that seen during exposure to lower body negative pressure (a comparable cardiovascular stress), for which cerebral oxygen insufficiency has been postulated (7).

Hormonal responses to G stress lead to the release of several vaso-active agents which will tend to increase blood pressure and so raise G tolerance. Blood levels of the catecholamines, adrenaline and noradrenaline, are increased, due both to direct simulation of the adrenal medulla and to leakage from sympathetic synapses, while hypotension and hypovolaemia afford a possible trigger for the observed increase in arginine vasopressin and cortisol (12). Though probably inoperative in the acute response to $+G_z$ acceleration, these agents may increase tolerance to more prolonged runs, or to repeated exposures.

The Pulmonary Circulation. West and his colleagues (1) first showed how blood flow distribution within the low pressure pulmonary circulation is influenced by gravity, and defined three zones according to differing relationships between arterial, alveolar and venous pressures. Comparable radioisotope techniques were applied in centrifuge studies and showed that the alveolar deadspace (Zone 1 in which arterial pressure is less than alveolar pressure with zero blood flow) is greatly increased by $+G_z$ acceleration. Below this level, due to the hydrostatic pressure gradient, flow increases at a rate proportional to the G level: in Zone 2 (the waterfall zone where venous pressure remains less than alveolar pressure) because of the increasing arterial-alveolar pressure difference; in Zone 3 (with all intravascular pressures exceeding alveolar pressure) because of passive distension and capillary recruitment (4). A fourth zone in the lowermost lung in which flow decreases despite a further increase in intravascular pressure is related to the closure of terminal airways, and is important in that any persisting perfusion is to non-ventilated alveoli, and so constitutes a right-to-left shunt with venous admixture and arterial desaturation. This will exacerbate an already embarrassed cerebral oxygen supply. It should be noted that, with the possible exception of Zone 4, there is no evidence for an active response of the pulmonary circulation to $+G_z$ stress, the changes described above being simply dependent upon the increased weight of the blood.

The cerebral circulation. In hypotension induced by haemorrhage, consciousness becomes disturbed when the mean arterial pressure at head level falls to around 50 mmHg. During exposure the $+G_z$ acceleration, however, consciousness is well maintained even during blackout when this pressure may fall to 20 mmHg, or less. The reason for this discrepancy appears to lie in the behaviour of the venous drainage from the brain, which acts to some extent like a siphon. Henry and co-workers (8) showed that, despite collapse of the superficial veins in the neck, the pressure in the jugular vein at its exit from the skull fell significantly below atmospheric pressure at $+4.5G_z$. An arterio-venous pressure differential was thus maintained, and cerebral blood flow facilitated. This venous siphon is not 100% effective, however, and unconsciousness results when the arterial pressure at head level falls to around zero mmHg.

The brain contains sufficient oxygen reserves to function for several seconds in the absence of blood flow. Using a cervical cuff to occlude blood flow to the brain, Rossen et al (14) showed that the eyes became fixated after 5-5.5s and collapse followed a second later. The unconsciousness produced by rapid-onset G exposure may occur marginally faster, in 4-5s, but any difference is explained by the fact that arterial occlusion traps blood in the brain and provides an extra store of oxygen in the form of oxyhaemoglobin while, with exposure to $+G_z$ acceleration, a considerable volume of blood drains rapidly from the cerebral tissues (5).

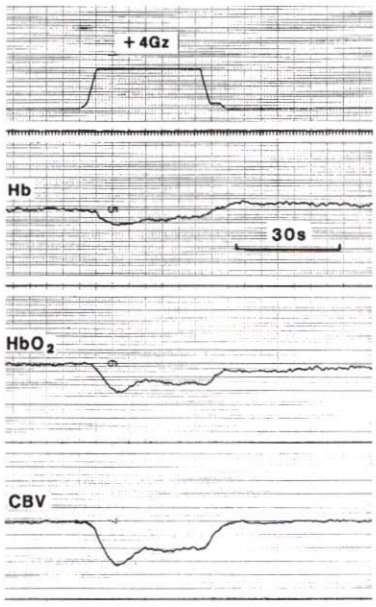


Fig. 4. Effect of $+4G_z$ (top trace) on Hb and HbO_2 concentrations in brain tissue (middle traces) and on the cerebral microcirculation blood volume (bottom trace).

Figure 4, obtained using multiwavelength near-infrared spectrophotoscopy, illustrates changes in cerebral blood content and oxygenation induced by exposure of a relaxed and unprotected subject to $+4G_z$. The bottom trace shows that blood rapidly drains from the brain, reaching a minimum at about 8s, then returns to an intermediate level while peak G is maintained, recovering promptly to normal on stopping the centrifuge. The middle two traces (of which the bottom trace is their sum) show, respectively, changes in the quantities of reduced and oxygenated haemoglobin. The relatively greater fall in HbO_2 indicates desaturation, while following return to normal gravity the HbO_2 trace remains below control levels and the Hb trace shows an overshoot. Thus, desaturation persists, actually for several minutes. These changes reflect an increased extraction of oxygen from the reduced cerebral blood supply caused by decreased flow, compounded by a generalised arterial oxygen desaturation due to shunting in Zone 4 of the lung, which persists until the collapsed areas re-open.

Increasing the G level to $+5G_z$ led to a more rapid drain off of cerebral blood volume (Fig. 5), with loss of consciousness and an overshoot in cerebral blood volume upon return to 1G, indicative of a reactive hyperaemia following a period of inadequate cerebral blood flow.

Raised Intrathoracic Pressure Breathing oxygen through an oro-nasal mask at a pressure of up to 70 mmHg (9.3 kPa) is a standard emergency procedure for protecting against hypoxia at altitude following loss of cabin pressurisation. It brings with it, however, problems of hyperinflation of the lung and syncope due to impaired venous return, unless appropriate counterpressure is applied to the trunk and lower limbs (2). The raised intrathoracic pressure is transferred directly to the heart and central vessels so that arterial pressure rises, and this has been demonstrated to be an effective means for increasing $+G_z$ tolerance (15).

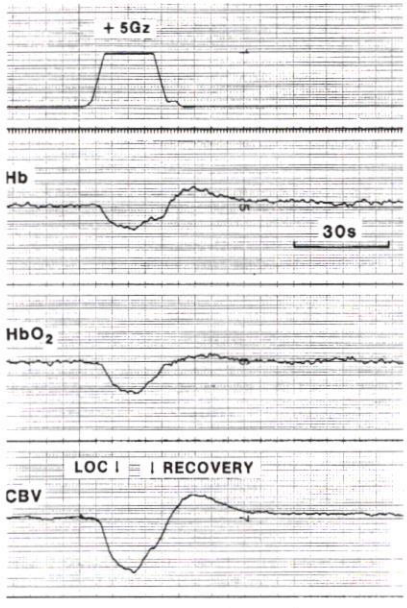


Fig. 5. As for figure 4, but at $+5G_z$ with G-induced loss of consciousness (LOC).

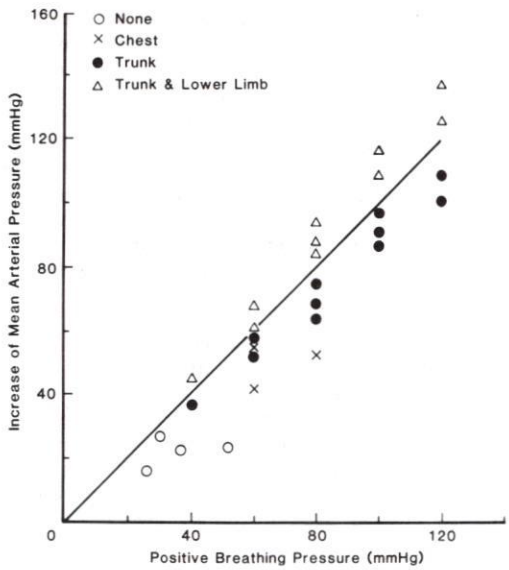


Fig. 6. Effect of pressure breathing at up to 120 mmHg (16 kPa) on mean arterial pressures for differing coverages of externally applied counterpressure (redrawn from ref. 2).

Figure 6 shows that the increase in arterial pressure depends upon the extent of the counter-pressure clothing. With the fullest coverage - trunk and lower limbs - the transmission of pressure from airways to arteries exceeds 100%. Assuming that a similar relationship applies during $+G_z$ acceleration, then positive pressure breathing at 60-65 mmHg should give some 3G increase in tolerance. Pressure breathing during G (or PBG) is the subject for another paper at this meeting (13). It is of interest to consider the role of the two baroreceptor sites under these conditions, as neither will actually respond to counteract the evoked hypertension. The baroreceptors are stretch receptors, sensitive to transmural tension, and for the aortic region, both intraluminal pressure and

the surrounding visceral pressure will have risen by approximately the same extent. Furthermore, the pressure sensed at the carotid sinus will have been reduced by the hydrostatic pressure gradient acting over a vertical distance of some 15 cm, sufficient at high G to negate the PBG induced rise. It would be expected, therefore, that the benefit of PBG will be maintained indefinitely, providing that the counterpressures applied is adequate to preclude peripheral pooling or fluid loss by extravasation. It might also be predicted that the evoked rise in sustained arterial pressure would be greater under conditions of high $+G_z$ than under normal gravity because the carotid sinus reflex is inhibited, though this does not appear to be the case (13) and other factors, such as increased displacement of blood to the periphery, must be involved.

Effect of head and neck pressure changes. The visual endpoint for $+G_z$ tolerance can be modified in either direction by altering extra-ocular pressure (9), a simple mechanical effect mediated through a change in the effective intra-ocular tension. Thus, applying suction to the eye decreases the resistance to retinal blood flow offered by intra-ocular tension, and tolerance increases by the expected 1G per 22 mmHg suction applied. Cerebral blood flow is of course unaffected by this purely local phenomenon. The application of suction to the neck, however, causes a decrease in tolerance determined either visually, or by the adequacy of cerebral perfusion, because the transmural tension sensed at the carotid sinus is effectively increased, and the baroreceptor response to the $+G_z$ stress thereby reduced, or even eliminated. Recent experiments have confirmed the expected relationships between pressure change and G tolerance, once allowance has been made for the incomplete transmission of suction through the tissues of the neck (6). This effect may be used to study the responsiveness of the carotid baroreceptors (in isolation) during $+G_z$ stress.

Application of suction to the head and neck together (but breathing at atmospheric pressure) produces an increase in $+G_z$ tolerance which is greater than the expected algebraic sum of the two separate effects considered above. The reason for this discrepancy is unknown, though it appears to offer a further means by which G tolerance may be enhanced in future protection systems.

References

1. Dollery C T, A Naimark and J B West. Distribution of blood flow in the isolated dog lung and its relation to arterial, venous, alveolar and pleural pressures. *J. Physiol.* 170:20-21P, 1963.
2. Ernsting J. Some Effects of Raised Intrapulmonary Pressure. AGARDograph No 106, Maidenhead : Technivision Ltd. 1966.
3. Glaister D H. The effect of positive acceleration upon cardiac output and regional blood flow in the dog. In : Aviation and Space Medicine. Ed. B Hannisdahl and C W Sem-Jacobsen. Universitetsforlaget, Oslo, 333-338, 1969.
4. Glaister D H. Effect of acceleration. In: Regional Differences in the Lung. Ed. J West. Academic Press Inc, 323-379, 1977.
5. Glaister D H. Current and emerging technology in G-LOC detection: Noninvasive monitoring of cerebral microcirculation using near infra-red. *Aviat. Space Environ. Med.* 59:23-28, 1988.
6. Glaister D H and J B Lenox. The effect of head and neck suction on G tolerance. *Aviat. Space Environ. Med.* 58:1075-1081, 1987.
7. Glaister D H and N L Lewis. Cerebral tissue oxygen status and psychomotor performance during lower body negative pressure. *Aviat. Space Environ. Med.* In press.
8. Henry J P, O H Gauer, S S Kety and K Kramer. Factors maintaining cerebral circulation during gravitational stress. *J. Clin. Invest.* 30:292-300, 1951.
9. Lambert E H. The physiological basis of "blackout" as it occurs in aviators. *Fed. Proc.* 4:43, 1945.
10. Lambert E H and E H Wood. Direct determination of man's blood pressure on the human centrifuge during positive acceleration. *Fed. Proc.* 5:59, 1946.
11. Lillywhite H B. Snakes, blood circulation and gravity. *Scientific American* : 66-72, December 1988.
12. Mills F J and V Marks. Human endocrine responses to acceleration stress. *Aviat. Space Environ. Med.* 53:537-540, 1982.
13. Prior A R J. Physiological Aspects of an Enhanced G Protection System. Eleventh Annual Meeting of the International Union of Physiological Sciences, Lyon, France. September 1989.
14. Rossen, R., H. Kabat and J.P. Anderson. *Arch. Neurol. Psychiat. Chicago.* 50:510-528, 1943.
15. Shubrooks S J. Positive pressure breathing as a protective technique during $+G_z$ acceleration. *J. appl. Physiol.* 35:294-298, 1973.

Acknowledgement. The author is indebted to Mrs Marion Mills for the preparation of this manuscript.

PHYSIOLOGIC BASES FOR INCREASED G-LEVEL AND G-DURATION TOLERANCES

Russell R. Burton
USAF School of Aerospace Medicine
Brooks Air Force Base, Texas 78235

Introduction

Environmental physiologists focus their attention as dictated by the results of their research. Consequently, they may be investigating the effects of an environment on the cardiovascular system at one time, eventually becoming involved with perhaps the physiology of the muscular system. So it is with acceleration physiologists whose interests are in protecting pilots of high performance aircraft (HPA). Several years ago as HPA capable of developing 9G sustained became operational, it was essential to understand the physiologic basis of G-level tolerances. Such information was necessary to develop G-protection methods that allowed pilots to tolerate such high-G levels.

G-Level Tolerances

Since G-level tolerance is primarily a function of the cardiovascular system, it obviously became the major focus of the human research at that time (5). That research clearly demonstrated that the anti-G straining maneuver (AGSM) was necessary to increase the arterial blood pressure (Pa) to levels required to counter the high hydrostatic pressures of the intravascular system from the acceleration forces.

This cardiovascular relationship to human high-G tolerances has been characterized with the following equation:

$$G_{LT} = ((Pa + P_I) / hd) + K \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

When: G_{LT} = G-Level tolerance level ($+G_z$)

Pa = Arterial blood pressure (mmHg)

P_I = Intrathoracic pressure resulting from the AGSM (mmHg)

h = Eye-heart vertical distance (mm)

d = Specific density of blood related to the density of Hg (1/13.6)

K = Anti-G suit constant of 1

This model, validated with existing acceleration data, is useful in predicting instantaneous G-level tolerances of subjects using anti-G suits and performing the AGSM (2). But since the AGSM requires maximal muscular tensing activity, fatigue rapidly limits the duration aspect of this tolerance; i.e., this model is not useful in defining G-duration tolerance. However, the development of a temporal model that predicts duration of tolerance with the accuracy that eq. 1 predicts G-level tolerance is not possible at this time because we do not know all of the physiological parameters nor their kinetics of the AGSM. These data must be made available if we wish to identify a specific research direction that will develop methods/systems useful in increasing G-duration tolerance; i.e., significantly reducing fatigue. Consequently, acceleration physiologists who were at one time investigating the cardiovascular system as the primary contributor to G protection methods now find it necessary to change their focus of attention to the physiologic and metabolic aspects of the physical components of the AGSM, principally the muscular system.

G-Duration Considerations

The concept that G-duration is an important parameter of human $+G_z$ tolerance is relatively new. It was not until about ten years ago that a method was devised to measure G-duration tolerance. This method, called the simulated aerial combat maneuver (SACM), although relying on the subjective endpoint of fatigue, remains the principal test for measuring this tolerance. Subjects are exposed to a continuous G profile of 15 sec of 4 G, then 15 sec of 7 G, back to 4 G, then back to 7 G, etc., until the subject becomes fatigued (Figure 1;4). Although this SACM has a subjective endpoint, plasma levels of lactic acid 3 min post-G offer some objective validation of the subject's fatigue endpoint (6). The SACM as a measure of G-duration tolerance has been useful in demonstrating the effectiveness of several anti-G methods: (a) physical conditioning, (b) positive pressure breathing during G (PBG), and (c) advanced technology anti-G suit (ATAGS).

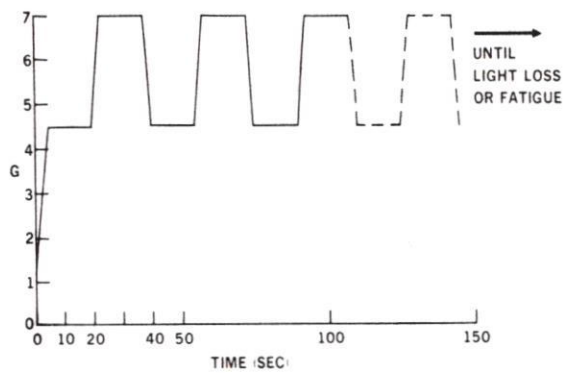


Figure 1. SACM tolerance time in sec is determined by this $+G_z$ profile.

The PBG is an interesting protective system that has been incorporated into the G-level model (eq 1) but has not been found to increase the maximum level of G tolerance. Its direct and immediate effect on Pa behaves in the model like the AGSM, but since the model assumes that only 100 mmHg of P_I is physiologically reasonable, PBG can only be substituted for the AGSM pressure. PBG is substituted for part of the AGSM because SACM (G-duration) tests demonstrate that PBG is much less fatiguing than the AGSM--yet has no additional advantage on G-level tolerance. The same is true for physical (muscle strength development) conditioning. Since P_I is limited, stronger people can do this maneuver with less effort; and, with greater anaerobic capacities that occur with certain muscle strengthening exercises, they can do it longer.

The G-level effect of the ATAGS as for other uniform pressure anti-G suits cannot be predicted with eq (1) because this equation does not include the physiologic basis of the anti-G suit; i.e., its function is only represented in this equation by a constant of 1 G. To incorporate anti-G suit effect on G-level tolerance, its effect on relevant physiologic parameters must be considered.

The anti-G suit has two physiologic functions: (a) raises the heart level by 30 mm (9), thereby reducing h of eq 1 by that amount, and (b) supports the vascular components below the heart that provide for "adequate" venous return (VR). The former increases G-level tolerance by about 1G--the basis for the 1G constant (K) for the anti-G suit effect in eq 1. Equation (2) has been modified for this effect on h by subtracting 30 mm from h. However, if the complete function of the anti-G suit is to be accounted for in this equation, VR must become incorporated, particularly as new anti-G suit designs improve leg coverage that should augment VR. An increase in VR results in an increase in cardiac output (CO) that causes an increase in Pa. An increase in Pa at 1G stimulates the pressure receptors and vasodilation occurs that now reduces the Pa. During G exposure, Pa is rapidly reduced, so now an increase in VR will only maintain the Pa and not increase Pa to a level that stimulates the baroceptors. Therefore, to accommodate for an increased VR, a Pa limit must be incorporated into this model. This limit cannot exceed 100 Pa; i.e., Pa should not increase to a level that produces a baroceptor response. Since Pa is a function of G (i.e., -25 mmHg/G) then VR should only be increased to a level that increases Pa by 25 mmHg/G if this anti-G suit is to be effective. The level of VR at various G levels that would do this is presently unknown. Nonetheless, involving VR in eq 1 and limiting Pa provides for this anti-G suit effect. Advanced anti-G suits that provide for more uniform lower body coverage do increase resting G-level tolerances (7,8):

$$G_{LT}=[(Pa+P_I)+P_{VR} \text{ (if } Pa<100 \text{ mmHg)}]/(h-30)d \quad ....(2)$$

When: G_{LT}, Pa, P_I, h, d same as eq. (1)

P_{VR} = increase in Pa that results from an increase in venous return flow (ml/min)

Although time is not directly included in this equation, clearly anti-G suit inflation that increases VR too rapidly will be ineffective. However, time is indirectly included when G onset rates are considered; i.e., G onset of 1 G/sec means that an anti-G suit should not increase VR more rapidly than the amount of venous return flow to the heart that will increase Pa by 25 mmHg.

G-Duration Tolerances

The onset of fatigue during G, limits G-duration tolerance. Obviously, fatigue is a metabolically based physiologic response. Consequently, G-duration tolerance has an energy (metabolic) basis.

$$G_{DT} = C_{an} \longrightarrow S_m \longleftarrow \dot{V}_m \quad .....(3)$$

When: G_{DT} = G-duration tolerance (sec)

C_{an} = Anaerobic capacity

S_m = muscular strength

$\dot{V}_m$ = blood flow through muscles used in AGSM

Anaerobic capacity and muscular strength are physiologic parameters with metabolic bases having an indirect relationship to the cardiovascular system that relates to G-level tolerance--the basis for P_I (AGSM) that supports Pa above 5 G. These parameters provide for and fuel the AGSM and are the basis for the development of fatigue (limiting G_{DT}).

Fatigue develops as a function of the decrease in C_{an} and a reduction in pH that is a function of V_m (greater blood flow in the muscles reduces the local muscle buildup of lactic acid maintaining a constant pH). A reduction in muscular strength (S_m) occurs directly with the development of muscle fatigue. The rate of loss of S_m is inversely related to its initial strength; i.e., stronger muscles require more time to fatigue (3).

As fatigue develops and S_m is reduced, the ability to perform the AGSM decreases which lowers P_I and therefore G_{LT}. Considering the fatigue rate of isometric contractions at about 1% per sec, G_{LT} is probably reduced at a similar rate so that after 25 sec G_{LT} would be lowered by 1 G. Blood flow (V_m) through muscles that removes lactic acid locally supports VR that (as shown in eq. 2) has a direct effect on Pa. As noted earlier, V_m removes lactic acid from the muscle which slows the onset of fatigue. Therefore, eqs. 2 and 3 can be combined as follows:

$$G_{LT} = [(Pa + P_I) + P_{VR} \text{ (if } Pa <100 \text{ mmHg)}]/(h-30)d$$

$$G_{DT} = C_{an} \longrightarrow S_m \longleftarrow \dot{V}_m \quad .....(4)$$

When: See eqs. (2) and (3) for symbol explanations

It appears, therefore, that VR becomes an extremely important cardiovascular parameter since it has two functions--its increase, because of greater blood flow in the muscles, delays the onset of fatigue and directly increases Pa via an increased cardiac output. Unfortunately, this parameter has not received the research attention that it has deserved in considering G-protection mechanisms. The first comprehensive basic cardiovascular physiologic study on VR during high G exposure was reported in 1986 by Burns et al. (1), who used miniature swine wearing an anti-G suit that performed an AGSM. That study showed how VR occurs against a hydrostatic pressure gradient during high sustained G (HSG).

Muscle strength (S_m) and anaerobic capacity (C_{an}) combined are measured as power in the Wingate Test. This measurement has been directly correlated with the SACM (Figure 2) by Wiegman et al. (10). Unfortunately, the specific relationships between S_m and C_{an} relative to G_{DT} have never been determined. These relationships should be measured since their relative importance in increasing P_I and therefore P_a would dictate the muscle conditioning requirements for the most effective AGSM.

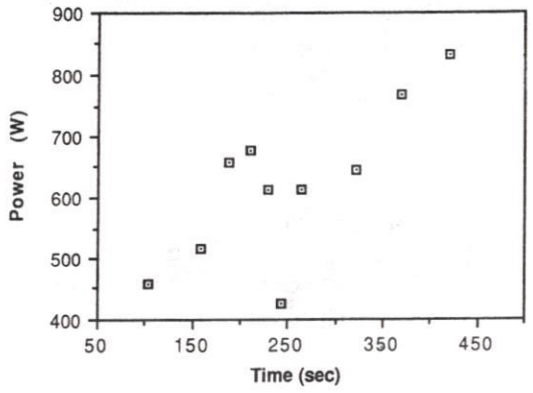


Figure 2. SACM tolerance time (see Figure 1) is significantly correlated (r = 0.77) with the 30 sec manpower (MP, an index of anaerobic capacity) Wingate cycle ergometer test.

Unlike the G_{LT} equation (1) that can be calculated with known values and can therefore predict exact G-level tolerances, the G_{DT} equation (3) cannot at present have any quantitation because these values (principally their kinetics) are unknown. Future acceleration research must be directed towards developing those values if a more precisely controlled anti-G suit inflation rate, an improved anti-G suit, a more effective AGSM, an optimal PBG pressure schedule, and a more efficient physical conditioning program are to be developed in support of advanced G-protection systems.

References

1. Burns, J.W., Parnell, M.J., and Burton, R.R.: Hemodynamics of miniature swine during $+G_z$ stress with and without anti-G support. J Appl Physiol 60:1628-1637, 1986.

2. Burton, R.R.: A conceptual model for predicting pilot group G tolerance for tactical fighter aircraft. Aviat Space Environ Med 57:733-744, 1986.

3. Burton, R.R.: Human physiological limitations to G in high-performance aircraft. Chapter 10, In: Physiological Function in Special Environments. Eds: V. Paganelli and L.E. Farhi; Springer-Verlag, N.Y., 1989.

4. Burton, R.R., and Shaffstall, R.M.: Human tolerance to aerial combat maneuvers. Aviat Space Environ Med 51:641-648, 1980.

5. Burton, R.R., Leverett, S.D., Jr., and Michaelson, E.D.: Man at high sustained $+G_z$. Aerospace Med 45:1115-1136, 1974.

6. Burton, R.R., Whinnery, J.E., Forster, E.M.: Anaerobic energetics of the simulated aerial combat maneuver (SACM). Aviat Space Environ Med 58:761-767, 1987.

7. Krutz, R.W., Burton, R.R., and Forster, E.M.: Physiologic correlates of protection afforded by anti-G suits. Aviat Space Environ Med (in press) 1989.

8. Prior, A.R.J.: Centrifuge assessment of the $+G_z$ acceleration protection afforded by full coverage anti-G trousers. Aviat Space Environ Med 60:504 (Abstract 165), 1989.

9. Rushmer, R.F.: A roentgenographic study of the effect of a pneumatic anti-blackout suit on the hydrostatic columns in man exposed to positive radial acceleration. Amer J Physiol 151:459-468, 1947.

10. Wiegman, J.F., Krock, L.P., Burton, R.R., and Forster, E.M.: Anaerobic power testing and $+G_z$ endurance. Aerospace Med Assoc Ann Mtg, 7-11 May 1989.

CARDIOVASCULAR FUNCTION OF MAN EXPOSED TO LBNP TESTS

A.A. Savilov, V.I.Lobachik, A.M. Babin

Institute of Biomedical Problems,
Moscow 123007, USSR

INTRODUCTION

It is well known that the cardiovascular function and responses to gravity-induced blood shifts are assessed by means of a tilt test or, when the test should not or cannot be applied (in simulated or real microgravity), its analogue, i.e. LBNP test, is used. Although LBNP effects on the cardiovascular system have been discussed in many publications, certain aspects still require further study. Among them, the LBNP effects on blood circulation and cardiac function which have been investigated using indirect methods whose yield and accuracy are limited. The object of this investigation was to clarify the pattern, degree and mechanisms of changes in the cardiovascular function in response to LBNP as well as to identify similarities and differences in circulation responses depending on its initial state.

METHODS

We used advanced methods of physiological measurements, viz, echocardiography and radioisotopes, that allowed detailed evaluations of intracardiac circulation, pumping and contractile function of the heart with reference to the topography of blood shifts. Echocardiography was performed using a commercial unit Echoview-80C according to the standard procedures (1,6). Radioisotope studies were carried out with the aid of a specially designed unit to measure isotope incorporation in the whole body and in various compartments, i.e. head, chest, stomach and legs (Fig. 1). Blood redistribution was measured using an indium generator isotope, In 113m (5).

LBNP tests were carried out using a vacuum bag the pressure in which was reduced by 25, 35, 40 and 50 mm Hg for 2, 3, 5 and 5 minutes, respectively, relative to ambient pressure; this approach was similar to the scheme used in cosmonaut examinations (2). Experiments were performed on 90 healthy men-volunteers, aged 24-46 yrs. The subjects were mostly people leading a sedentary mode of life.

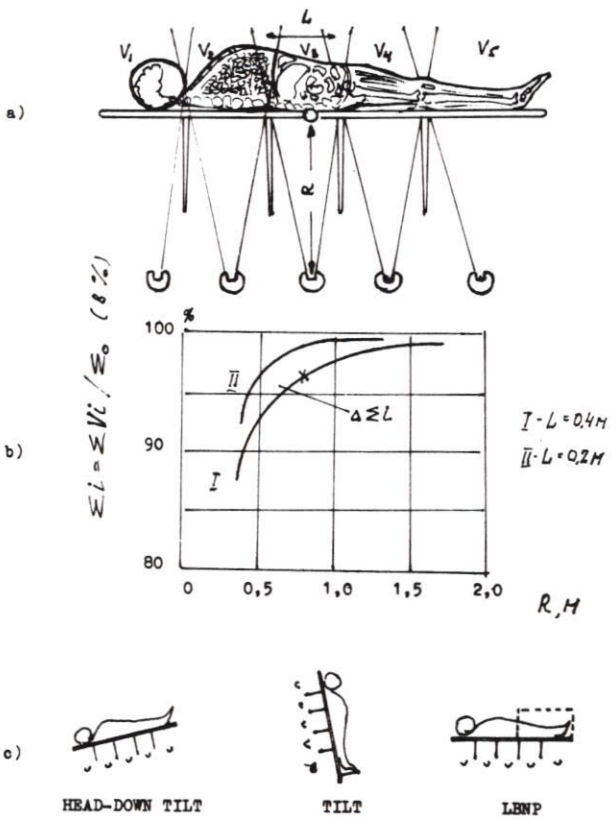


FIG. 1 RADIOMETRIC SET-UP. a) ARRANGEMENT OF SENSORS AND SHIELDS (SCREENS); b) COUNT EFFECTIVENESS AS A FUNCTION OF MEASUREMENT GEOMETRY; c) USE OF THE SET-UP: VARIOUS POSSIBILITIES.

RESULTS AND DISCUSSION

Good tolerance of LBNP

It can be seen from Fig. 2 that cardiovascular responses of the subjects to LBNP tests, who tolerated them well enough, involved noticeable changes in echocardiographic parameters. The changes included a progressive increase of heart rate (HR) (by 34% on the average) and a decrease of left ventricle systolic (ESV) and diastolic volume (EDV) which lead to a corresponding reduction of stroke volume (SV). In response to LBNP 35 mm Hg, ESV and EDV diminished by 15 and 16% and in response to LBNP 50 mm Hg (at the 5th minute), by 26 and 29% as compared to the baseline level. By the end of the LBNP test this resulted in a 31% decrease of SV. Due to a progressive HR increase cardiac output (CO) varied insignificantly. These parameters returned to normal within 1-2 minutes after pressure was increased to the barometric level.

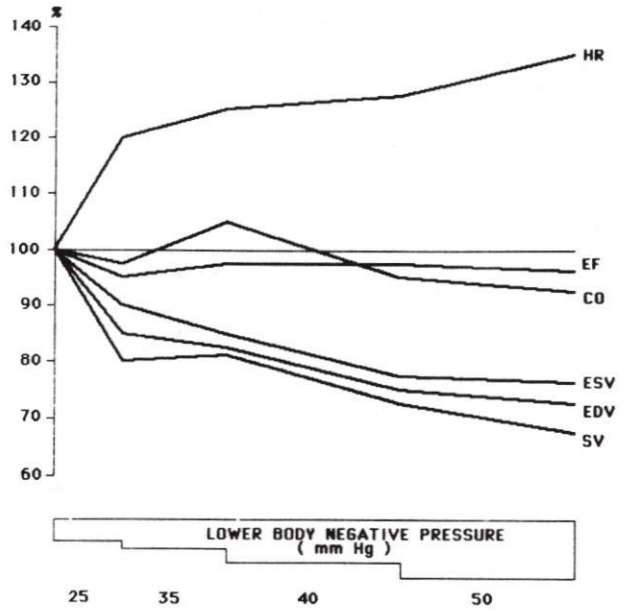


FIG. 2 EKG RESPONSES OF HEALTHY TEST SUBJECTS TO LBNP

(AS PERCENTAGE OF BASELINE VALUES)
HR - HEART RATE
EF - EJECTION FRACTION
CO - CARDIAC OUTPUT
ESV - END-SYSTOLIC VOLUVE
EDV - END-DIASTOLIC VOLUME
SV - STROKE VOLUME

Thus the specific cardiovascular response to LBNP tests was a significant decrease of EDV that correlated with pressure reduction. In spite of a compensatory decrease of the left ventricle residual volume, SV diminished significantly. However because of HR increase CO remained close to the baseline throughout the test. It is important to note that in the subjects, who well tolerated the test, the above changes were not accompanied by changes in the ejection fraction (EF) or other echocardiographic parameters of the contractility function; in other words, cardiovascular responses were adequate to the LBNP level and entirely compensated.

Radioisotope data shown in Fig. 3 demonstrated that the above variations of echocardiographic parameters were linearly correlated with LBNP-induced hemodynamic changes. It can be seen that about 30% of blood were shifted from the thoracic organs to leg capacitance vessels which amounted to 500-600 ml of blood during the test. It should be noted that the largest amount of blood was displaced (compared to the total amount of blood shifted) during the first 5 minutes of LBNP tests when the pressure was decreased only by 25-35 mm Hg. Later when the pressure was further diminished by 40-50 mm Hg, blood continued to pool in the legs but at a far slower rate. It appears that the reserves of leg capacitance vessels were largely exhausted and further blood shift occured due to their dilation. In other words, hemodynamic changes, in terms of time and rate, coincided with variations in the above echocardiographic parameters. Blood shifts from the head and abdominal cavity were comparatively smaller.

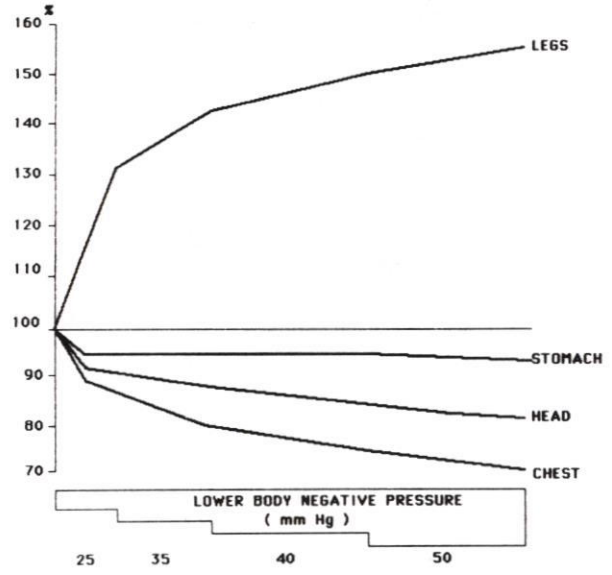


FIG. 3 BLOOD SHIFTS IN BODY OF HEALTHY TEST SUBJECTS TO LBNP ISOTOPE STUDIES. AS PERCENTAGE OF BASELINE VALUES.

Thus radioisotope studies have convincingly shown that one of the major factors responsible for the above shift in intracardiac hemodynamics was blood pooling in leg vessels and, consequently, diminished blood filling of intrathoracic vessels and blood return to the heart. According to the measurments performed using catheterization of different vascular areas (3,4), this may lead to a significant pressure fall in cardiac cavities and great intrathoracic vessels that reached 40-55%. It is interesting that the degree of pressure fall in the heart and great vessels was also correlated with pressure decrease in the LBNP bag. The latter seems to be one of the major mechanisms triggering compensatory reactions to LBNP effects that develop as a result of stimulation of reflexogenic baroreceptor areas of the heart and arterial bed. The final result of these reactions, in the case of good LBNP tolerance, was cardiac output that remained adequate to the body requirements at all levels of LBNP in spite of significant changes in systemic and intracardiac circulation.

It should be noted that after LBNP exposure HR diminished drastically (reaching bradycardia) while cardiac volumes, SV and CO increased when compared to both the values recorded at 50 mm Hg and the baseline. Radioisotope measurements revealed simultaneously a very significant increase of blood flow to the heart from leg vessels. This was accompanied by sensations (sometimes unpleasant) of a rapid blood rush to the head, blood pulsation in the temples and fever in the upper body.

Thus an LBNP test may exert two opposite effects: lower preload and afterload during exposure and higher preload and afterload immediately after it. The latter makes LBNP tests more informative and predictive to reveal cardiac reserve capabilities in response to gravitational and other effects that cause blood redistribution along the long axis of the body (e.g. head-to-feet acceleration).

Poor tolerance of LBNP

In the case of a poor test tolerance presyncopal states were preceded by a secondary increase of the residual heart volume and a pronounced decrease of other volume parameters as well contractility parameters (Fig. 4). Two typical patterns of response can be distinguished. The first pattern includes a gradual (taking several minutes) change of the above parameters and an insignificant HR increase. The second pattern is a similar but faster (30-45 seconds prior to collapse) change of ESV and contractility parameters against the background of a progressive and more significaat than in the first case HR rise. It should be noted that radioisotope measurements revealed, prior to collapse, a significant decrease of blood return to the heart. Some test subjects displayed ECG signs of myocardial hypoxia, probably, caused by a decrease of coronary blood flow. According to V.Katkov et al. (3,4), even at moderate LBNP (say, 30 mm Hg), coronary sinus flow and oxygen consumption diminished by 25% as compared to the baseline.

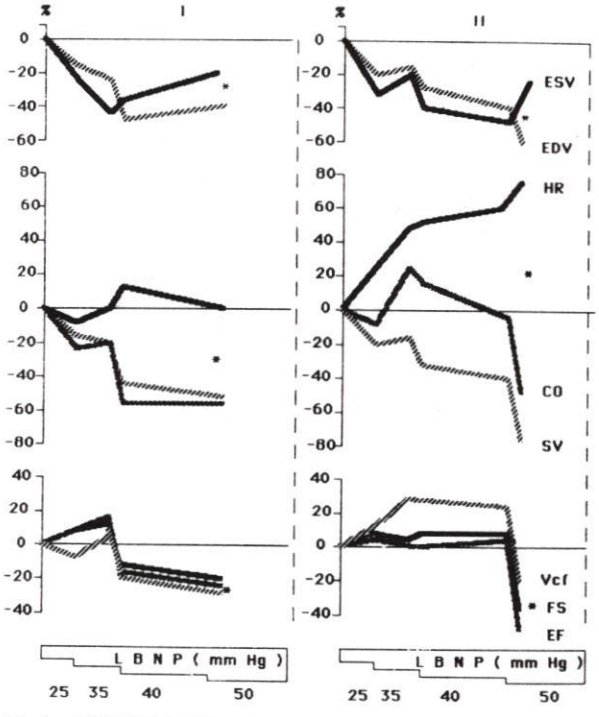


FIG. 4 DIFFERENT PATTERNS (I, II) OF POOR LBNP TOLERANCE AS DETERMINED BY EKG RESPONSES. * - SYNCOPAL EVENTS.

Radioisotope data presented in Fig. 5 helped identify three types of poor LBNP tolerance. The first type is characterized by a rapid blood displacement, primarily, to leg vessels during the first minutes of exposure without its stabilization later on.

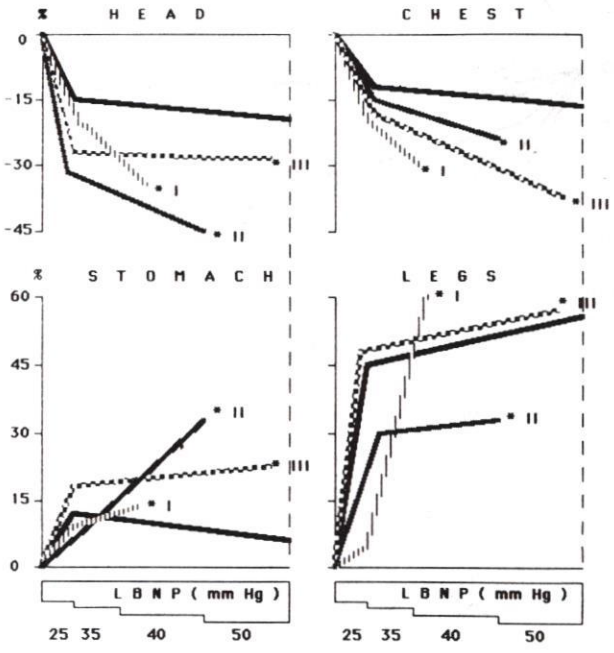


FIG. 5 DIFFERENT PATTERNS (I, II, III) OF POOR LBNP TOLERANCE AS DETERMINED BY ISOTOPE STUDIES.
— - GOOD TOLERANCE
* - SYNCOPAL EVENTS

The second type involves a similar, in terms of rate and degree, blood shift but directed to abdominal vessels. The third type is a combined blood shift in leg and abdominal vessels. The third type is a combined blood shift in leg and abdominal vessels (although less expressed in either area) which ends in a significant amount of blood displaced by the onset of collapse. It should be emphasized that, as shown above (Fig. 4), marked blood pooling in the abdominal area (especially at the beginning of the LBNP test) was not observed in the subjects who well tolerated the test and proved to be one of the most reliable prognostic signs of poor LBNP tolerance.

Our observations give evidence that circulation responses to LBNP may have substantial individual variations, especially in people with poor tolerance, which largely depend on the area from where blood was removed. It was found that hypotensive reactions mainly developed in parallel with cerebral circulation disorders, ECG signs of cardiac hypoxia, and predominant decrease of blood flow to the heart. Individual variations were, as a rule, seen during the first 5 minutes of LBNP exposure which allowed an accurate prediction of its outcome.

The effects of LBNP and tilt tests

One of the purposes of this investigation was to study similarities and differences of cardiovascular responses to LBNP and tilt tests using radioisotope methods. It can be seen from Fig. 6 that in response to both tests the amount of blood shifted was essential identical in all body compartments, except for the head. In both cases blood was mainly removed from the thoracic organs where by the end of the tests the blood volume diminished by 34 and 36%, respectively. This resulted in lower venous return to the heart. Blood volume in legs increased by 40-50% in both cases. However during the LBNP test blood volume in the head was almost two times less than during the tilt test. The difference can be attributed to the horizontal position the test subjects occupied during the LBNP test.

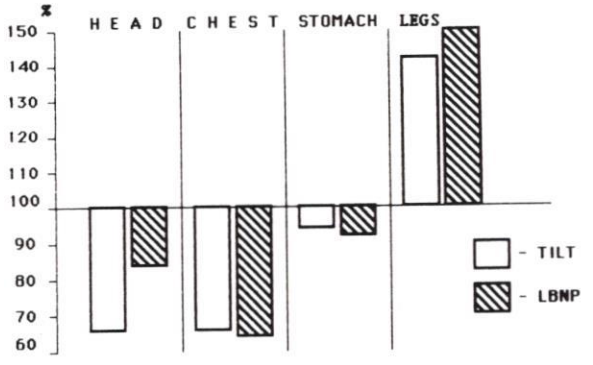


FIG. 6 BLOOD SHIFTS IN BODY COMPARTMENTS IN RESPONSE TO LBNP AND TILT TESTS (AS PERCENTAGE OF BASELINE VALUES).

LBNP during head-down bed rest

Our study also included an investigation of cardiovascular responses to LBNP tests at the end of a 120-day head-down experiment which is often used to simulate the effects of microgravity. Our findings indicate that blood shifts in response to LBNP tests on experimental day 120 were more significant than before the head-down study (Fig. 7). This can be ascribed to a lower tone and larger capacity of leg vessels. The amount of blood shifted to the legs was 20% greater than before the study which was accompanied by a more marked, by 9-10%, blood outflow from the head and chest.

Radioisotope data about hemodynamic changes were consistent with echocardiographic results. EDV, ESV and SV decreased to a greater extent than before head-down exposure. On experimental day 60 LBNP-induced decrease in cardiac volumes led to a significant (by 41%) reduction of stroke volume which was almost entirely compensated by an adequate increase in HR. As a result, CO during LBNP tests remained as diminished as before the tests, i.e. by 15%

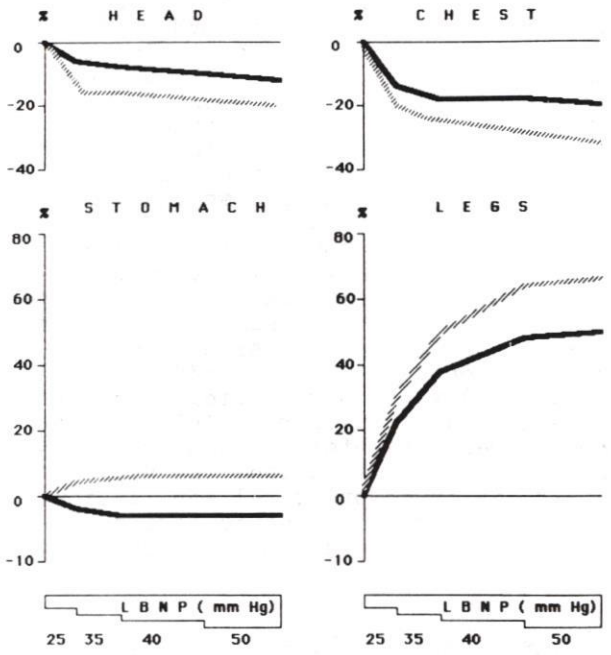


FIG.7 BLOOD SHIFTS IN BODY TO LBNP ON 120 DAY HEAD-DOWN TILT (////////), COMPARE OF BASELINE VALUES (——).

It follows from Fig. 8 that during the LBNP test performed on head-down tilt day 120 EDV, ESV and SV decreased more noticeably than on head-down tilt day 60 due to which CO diminished substantially (by 33%). This can be viewed as a symptom of lower capability of the cardiovascular system to tolerate peripheral blood displacement which is similar to what happens in cosmonauts upon their return to Earth's gravity.

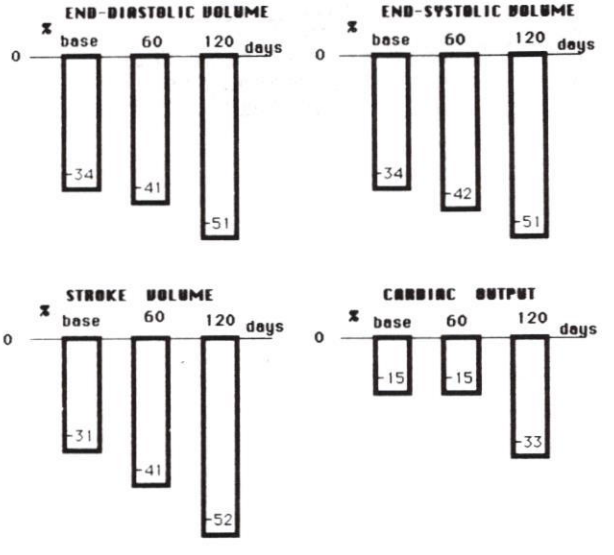


FIG. 8 EKG RESPONSES TO LBNP (5 min 40 mm Hg) DURING 120-DAYS HEAD-DOWN TILT (AS PERCENTAGE OF BASELINE VALUES).

CONCLUSTIONS

In summary, our observations show that it is very useful to apply echocardiographic and radioisotope procedures in combination during LBNP and other tests in ground-based simulation studies. This approach may help accumulate basically new and valuable information about the phenomenology and relationship of cardiovascular disorders developing in simulated microgravity; moreover it may help their quantitative and reliable assessment.

The above advantages of this combined approach permit it to be recommended for cosmonaut examinations before and after flight. This approach may help better evaluate cardiovascular deconditioning in response to gravity-induced blood shifts and develop more efficient countermeasures allowing for individual features of man's adaptation to microgravity. It can be stressed that the approach has already found practical application: echocardiographic diagnostic equipment has been used in recent manned missions.

REFERENCES

1. Feigenbaum H., Echocardiography. Philadelphia, Lea and Febiger, 1976.
2. Johnson R.L., G.W.Hoffler, A.Nicogossian and S.A.Bergman. Skylab experiment M-092: results of the first manned mission, Acta Astronautica, 1975, v.2, N 3/4, p.265-297.
3. Katkov V.E., Chestukhin V.V., Kakurin L.I., Coronary circulation of the healthy man exposed to tilt tests, LBNP and head-down tilt. Aviat. Space Envir. Med., 1985, v. 56, N.8, p.741-745.
4. Katkov V.E., Chestukhin V.V., Babin A.M. e.a. Central and coronary circulation on the normal man during orthostatic and LBNP tests. Aviat. Space Envir. Med., 1987, v. 58, N 9 Suppl., p. A55-455.
5. Lobachik V.I., Korsunsky V.N., Popov V.I. e.a. Isotope methods of blood distribution assesment. Kosm. Biol. Aviakosm.Med., 1987, v.21, N 5, p.65-67,
6. Mukharlyamov N.M., Belenkov Yu.N. Ultrasound diagnostic techniques in cardiology, Moscow, Meditsina, 1981.

THE EFFECT OF EXTERNAL INSPIRATORY RESISTANCE TO BREATHING ON MAN'S ORTHOSTATIC TOLERANCE

V.M. Baranov, V.P. Katuntsev,
T.A. Kabesheva, A.N. Kotov,
N.E. Panfyorova, M.V.Sazonova

Institute of Biomedical Problems,
123007 Moscow, USSR

Orthostatic intolerance is one of the worst consequences of man's exposure to microgravity. Immediately postflight horizontal-to-vertical transition causes tachycardia and arterial hypotension (5, 8,14,18), and sometimes syncopal events (4). Many authors attribute orthostatic hypotension and postural syncopes developing postflight to the lower venous return to the heart caused by gravity-induced blood pooling in the lower body (7,17,19).

Among the mechanisms that are responsible for venous return mention should be made of the chest sucking effect associated with the respiration act.

It is also well known that respiration against resistance loads induces a higher activity of respiration muscles and their greater efforts (1). Having in view these observations, we have postulated that inspiratory resistance to breathing applied externally may enhance the sucking action of the chest, and by increasing venous return, may exert a beneficial effect on man's orthostatic tolerance. The purpose of the present investigation was to verify this concept.

Methods

Experiments were carried out on 10 healthy male volunteers (age 31.7±1.8 yrs, height 176.7±1.3 cm, weight 72.0±4.0 kg). Orthostatic tests were performed using a tilt table equipped with a saddle for support. The tests in the head-up position at 70° lasted for 20 min. The tests were discontinued if any syncopal symptoms occurred.

Four experimental studies were carried out. The first series served as a control and was staged to identify the baseline orthostatic tolerance of every test subject. Immediately before orthostatic tests in the 2nd, 3rd and 4th series the test subjects were exposed to "dry" immersion for three hours in water at 38°C. For this purpose a transportable device was used which allowed the test subjects to be immersed and removed from the bath and then placed on the tilt table. The bath water temperature was maintained with an accuracy of ±0.2°C. Subjects lay in the bath on water-proof material to protect skin maceration.

In the 2nd experimental series the effect of immersion on orthostatic tolerance was investigated when the test subjects were breathing normally. In the 3rd and 4th series orthostatic tests were performed when the inspiratory resistance to breathing was 8.6 and 18.5 cm H_2O, respectively.

During all tilt tests the subjects used a mouthpiece connected to a valve box. Additional resistive resistance was generated using bladders of different diameter that were located in front of the inhalation valve in the box. Resistive resistance was calibrated in a routine method at flow rate of 1 l/s. The time interval between tilt tests for each subject was 4-5 days.

ECG was recorded in the 1st standard lead and heart rate (HR) was calculated from ECG tracings. Arterial pressure was measured in the shoulder area using Korotkoff sounds. Variations in thoracic blood volume (TBV) was measured with the aid of longitudinal tetrapolar rheography. Electrodes were fixed around the neck and on the chest at the level of the xiphoid process. Leg circulation variations were determined with respect to calf volume (CV). These measurements were performed plethysmographically using Whitney mercury-rubber transducers placed in the largest calf area. Respiration parameters were recorded with the help of Eric Jager equipment. Carbon dioxide partial pressure in alveolar air (P_ACO_2) was measured by a McGow PCM-1 mass-spectrometer. Body temperature in the oral cavity was measured by a medical electrothermometer with an accuracy of ±0.2°C. Body mass was measured at an accuracy of up to 50 g at the beginning and at the end of the experiment.

The results were processed by Student's method.

Results

In control tilt tests the health condition of all test subjects remained adequate. On the average, HR increased by 20 beats/min, systolic AP decreased by 2 mm Hg, and diastolic AP increased by 17 mm Hg, CV grew by 2.2% ($p < 0.05$). Lung ventilation ($\dot{V}_E$) increased by 1.5 l/min, and P_ACO_2 fell by 2.6 mm Hg ($p > 0.05$) (Figs. 1 and 2).

Three-hour water immersion at 38°C resulted in moderate hyperthermia, body temperature rise to 37.4°C, skin hyperemia, and profuse perspiration.

During water immersion body weight loss was approximately 0.930 kg and HR increased from 65.8 to 80.7 beats/min. After exposure absolute values of $\dot{V}_E$, tidal volume (V_T), respiration frequency (f) increased and P_ACO_2 decreased as compared to the pretest levels ($p > 0.05$).

Table 1 EFFECT OF EXTERNAL INSPIRATORY RESISTANCE TO BREATHING ON ORTHOSTATIC TOLERANCE

Syncope	Before immersion	After immersion		
	Inspiratory resistance to breathing, cm H_2O			
	0	0	8.6	18.5
During first 10 min of tilt test.	0	5	2	1
During last 10 min of tilt test.	0	3	2	4
No syncope.	10	2	6	5

Water immersion led to a significant decline of orthostatic tolerance of the test subjects (Table 1). Eight out of 10 test subjects developed syncope during tilt tests; moreover 5 of them showed syncope during the first 10 minutes. In the presyncope state the test subjects showed persistent tachycardia, decreased systolic and diastolic AP (to 94 and 62 mm Hg, respectively), reduced - by 10% - TBV, increased - by 5.6% - CV ($p < 0.05$). They also developed lung hyperventilation, with a $\dot{V}_E$ increase to 13 l/min, and hypocapnia with a P_ACO_2 decrease to 26.6 mm Hg.

When inspiratory resistance was applied (3rd and 4th series), the number of syncopal events was smaller than in normal respiration (2nd series). When inspiratory resistance was 8.6 cm H_2O, there were 4 syncopal events two of which occurred during the first 10 minutes and two, during the last 10 minutes. When inspiratory resistance was 18.5 cm H_2O, there were 5 syncopal events one of which occurred during the first 10 minutes and four, during the last 10 minutes of the tilt test.

As compared to normal breathing, HR and diastolic AP in the head-up position were lower during inspiratory resistance to breathing. In this situation TBV diminished and CV increased in a lower degree. During normal breathing TBV diminished by 3.8% and CV increased by 4.8% on the 6th minute of the tilt test. During resistive respiration at 8.6 cm H_2O TBV and CV changed by 1.8% and 3.6% and at 18.5 cm H_2O, by 1.2% and 3.4%, respectively (Fig. 1).

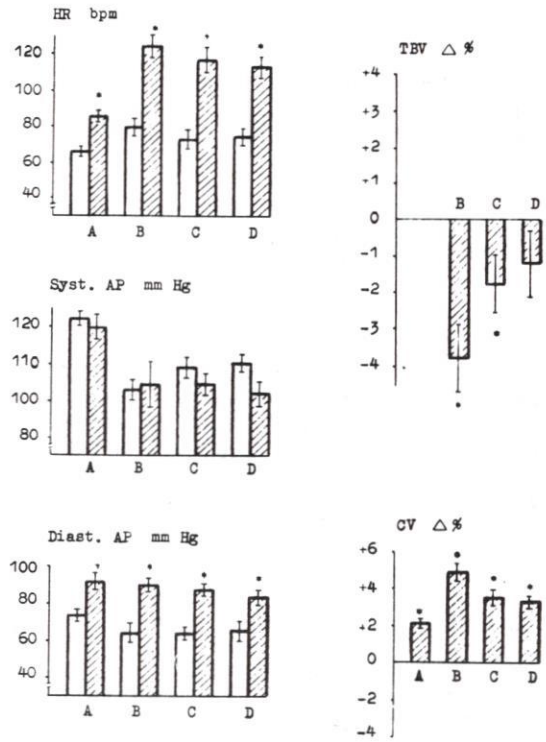


Figure 1 Effect of external inspiratory resistance to breathing on cardiovascular parameters during tilt test.

A - before immersion; B,C,D - after immersion; □ - baseline; ▨ - tilt test 6th min.

A,B - without external resistance; C,D - with inspiratory resistance of 8.6 and 18.5 cm H_2O, respectively.

* $p < 0.05$ as compared to baseline data.

When the cardiorespiratory function reached a steady state (6th minute of the tilt test), values of $\dot{V}_E$ in all post-immersion tests did not differ statistically. However, in respiration resistance Experiments at 8.6 and 18.5 cm H_2O $\dot{V}_E$ increased at the expense of V_T increments by 133 and 189 ml, respectively. At normal breathing V_T decreased by 98 ml and f increased by 2 cycles/min. As a result of this restructuring of the respiration pattern, P_ACO_2 was 36.3-37.7 mm Hg when inspiratory resistance was applied and 31.2 mm Hg when normal respiration was used (Fig. 2).

According to subjective estimates of all test subjects, inspiratory resistance of 8.6 cm H_2O did not prevent normal inhalation and did not produce unpleasant sensations after orthostatic tests. Inspiratory resistance of 18.5 cm H_2O gave

rise to the feelings of impaired inhalation during tests. After exposure most subjects voiced complaints saying that their cheek, neck, chest and stomach muscles felt tired.

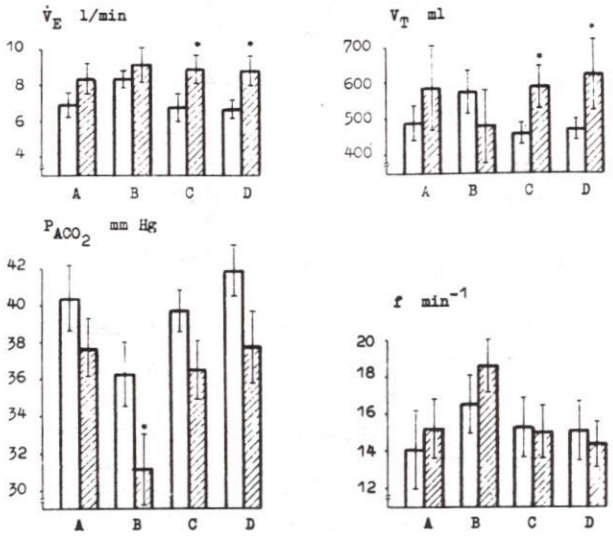


Figure 2 Effect of external inspiratory resistance to breathing on respiratory parameters during tilt test.

A - before immersion; B,C,D - after immersion; □ - baseline; ▨ - tilt test 6th min.

A,B - without external resistance; C,D - with inspiratory resistance of 8.6 and 18.5 cm H_2O, respectively.

* $p < 0.05$ as compared to baseline data.

Discussion

In the present investigation decrease in orthostatic tolerance was achieved through a combined action of two factors: immersion hypodynamics and increase in ambient temperature. After three-hour exposure the tilt test was discontinued in 8 cases because the subjects developed syncopal attacks.

Symptoms of orthostatic intolerance developed when central blood volume diminished drastically and blood pooled in leg vessels. From an analysis of cardiorespiratory responses during orthostatic tests it can be seen that the baroreceptor mechanism of AP regulation functioned less effectively after exposure. The test subjects developed a rapid decrease of preload which was not compensated by an increase in HR and peripheral resistance as a result of which AP started to diminish. The cardiorespiratory function was also challenged by body dehydration caused by profuse perspiration and presumably by plasma volume reduction. Besides, adequate relationships between the circulating blood volume and circulation capacity could not be maintained because of additional blood pooled in skin vessels dilated in response to hyperthermia. It should be borne in mind that skin circulation involved in the maintenance of temperature homeostasis is also controlled by arterial baroreceptors. However, the reflex constriction ability of skin vessels during postural tests cannot fully make up for their dilation that occurs in response to a high ambient temperature (6). Published data give evidence that, if body temperature rises approximately to the values recorded in our experiments, test subjects, as a rule, show reduced resistance to gravitational effects (2,11). The authors attribute the adverse effect of hyperthermia to a lower tone of peripheral vessels which causes blood pooling in lower extremities to increase and venous return to the heart to decrease (12,16).

Our results have demonstrated that application of external inspiratory resistance to breathing improves orthostatic tolerance. The cardiovascular deconditioning index (CDI) calculated according to the formula of Bungo et al. (4) diminished from 26.6 units in the 2nd series to 16.1 and 3.2 units in the 3rd and 4th experimental series, respectively. It is interesting to note that according to Bungo et al. (4) the astronauts who used inflight prescribed countermeasures, namely water-salt supplements, had the CDI equal to 21.4 ± 15.9 units; in other words, it was higher than in our experiments with additional resistive resistance. In our test subjects the frequency of orthostatic syncopal events decreased and the time of their occurrence shifted to the end of orthostatic tests.

Our results also show that external inspiratory resistance during orthostatic tests prevented a drastic reduction of intrathoracic blood volume and caused a less expressed fluid redistribution from the upper to the lower body. In our study we did not measure end-diastolic volume and cardiac output. However, there is evidence indicating less significant changes of intrathoracic blood volume in the case of lower HR and stable AP which suggests higher blood inflow to the heart (15), and, obviously, higher values of end-diastolic volume and cardiac output during inspiratory resistance to breathing.

In view of the fact that during our orthostatic tests the muscle pump effect was minimized, it can be assumed that chest respiratory excursions played an important role in the maintenance of venous return to the heart. The functional abilities of this mechanism were enhanced by artificial resistance to inspiratory gas flow. In order to make an inspiration in this situation, respiratory muscles had to make a greater effort which caused rarefaction in the intrathoraric compartment and augmented the sucking effect.

After horizontal-to-vertical transition $\dot{V}_E$ increment was accompanied by restructuring of the respiration pattern. The new respiration pattern that developed during the orthostatic test which included a deeper and, presumably, longer inspiration (1) enhanced to a greater degree the sucking function of the chest.

In the case of inspiratory resistance P_ACO_2 in the head-up position was higher than with unobstructed breathing. A higher level of PCO_2 in the alveolar gas and, presumably, arterial blood seems to help maintain adequate circulation in the head-up position (9), since a moderate rise of P_aCO_2 causes dilation of cerebral and constriction of peripheral vessels, increase of cerebral blood flow and improvement of oxygen utilization in the brain (10,13).

During orthostatic tests additional resistance to breathing was made up for by involvement of a number of regulatory mechanisms of varying complexity beginning with the capacity of intercostal muscles and diaphragm to enhance their contraction and increase their initial length and ending with the cortical influences (3). In our studies early signs of extreme load on the respiratory system emerged when inspiratory resistance to breathing applied was 18.5 cm H_2O in spite of the fact that P_ACO_2 was maintained at a level close to the baseline.

Our results may be used to advantage in the development of new countermeasures against adverse effects of microgravity on the human body.

References

1. Barer A.S., I.S.Breslav, G.G.Isayev, E.A.Sokol. The effect of increased respiratory resistance on human performance: a review. Kosm.Biol.Aviakosm. Med. 23(2):4-11, 1989.
2. Bjurstedt H., G.Rosenhumer, U.Balldin, V.Katkov. Orthostatic reactions during recovery from exhaustive exercise of short duration. Acta Physiol.Scand. 119:25-31, 1983.
3. Breslav I.S., V.D.Glebovsky. Regulation of respiration. Leningrad, 1981 (in Russian).
4. Bungo M.W., J.B.Charles, Ph.S.Johnson. Cardiovascular deconditioning during space flight and the use of saline as a countermeasure to orthostatic intolerance. Aviat.Space Environ.Med. 56: 985-990, 1985.
5. Gazenko O.G., A.I.Grigoriev, V.A.Degtyarev et al. Effect on the fluid-electrolyte metabolism as a countermeasure against orthostatic intolerance of the crewmembers of the second expedition aboard Salyut-4. Kosm.Biol. Aviakosm.Med. 13(3):10-15, 1979.
6. Johnson J.M., M.Niederberger, L.B.Rowell et al. Competition between cutaneous vasodilator and vasoconstrictor reflexes in man. J.Appl.Physiol. 35:798-803, 1973.
7. Kakurin L.I., B.S.Katkovsky, V.M.Mikhailov et al. Space flight effects on circulation and respiration during provocative tests. In: Space Flights on Soyuz Spacecraft. Biomedical Investigations. Moscow: Nauka, 230-265, 1976 (in Russian).
8. Kalinichenko V.V. Changes in orthostatic tolerance of cosmonauts after 2- to 63 day missions. Kosm.Biol. Aviakosm.Med. 11(3):31-37, 1977.
9. Katkovsky B.S., V.A.Gornago. External respiration and gas exchange in orthostatic syncopes. Bull.Exper.Biol. Med. 85(5):520-523, 1978.
10. Lassen N.A. The brain. In: Peripheral circulation ed. by P.Johnson. Moscow:Medizina, Ch.12, p.414-440, 1982 (Russian transl.).
11. Lind A.R., C.S.Leithead, G.W.McNicol. Cardiovascular changes during syncope induced by tilting men in the heat. J.Appl.Physiol. 25:268-276, 1968.
12. Marshak M.E. Meterological factor and hygiene of labour. Moscow, 1935 (in Russian).
13. Marshak M.E. Physiological role of carbon dioxide. Moscow, 1969 (in Russian).
14. Panfyorova N.E. Hypodynamics and cardiovascular system. Moscow: Nauka, 1977 (in Russian).
15. Sather T.M., D.S.Goldwater, L.D.Montgomery, V.A.Convertino. Cardiovascular dynamics associated with tolerance to lower body negative pressure. Aviat.Space Environ.Med. 57:413-419, 1986.
16. Shvartz E., A.Meroz, A.Magazanik et al. Exercise and heat orthostatism and effect of heat acclimation and physical fitness. Aviat.Space Environ.Med. 48:836-842, 1977.
17. Sokolkov V.I. Some peculiarities of external respiration and energy expenditures during orthostatic tests before and after 18-hour immersion experiment. Kosm.Biol.Med. 4(4):52-58, 1970.
18. Vorobyov E.I., O.G.Gazenko, N.N.Gurovsky et al. Preliminary results of medical investigations carried out during the second expedition on the orbital station Salyut-4. Kosm.Biol. Aviakosm.Med. 10(5):3-18, 1976.
19. Vorobyov E.I., O.G.Gazenko, A.M.Genin, A.D.Egorov. Medical results of Salyut-6 manned space flights. Aviat. Space Environ.Med. Suppl.1. 54(12): S31-S40, 1983.

COMPARATIVE ANALYSIS OF HYPERGRAVITY (2G) AND WEIGHTLESSNESS EFFECT ON THE BLOOD-FORMING SYSTEM OF MALE AND FEMALE MAMMALS

L.V.Serova, N.A.Chelnaya

Institute of Biomedical Problems, Moscow, USSR

The problem of the influence of the altered gravity to the living cells and cellular systems in vivo and in vitro is one of the general problems in gravitational biology. Comparative analysis of the materials, obtained in this area allows us to refer blood and blood-forming organs to those systems, which are very sensible to the change of gravity level.

The comparison of the reactions of the two periodically renovating cells systems of the same animals after 7- and 13-days space flight shows considerably higher sensitivity of blood system elements than the elements of spermatogenesis. For example, after 7-days space flight onboard biosatellite "Cosmos-1667", we did not find significant changes in the cytological picture of the spermatogenesis, via the cytological picture of the bone marrow which demonstrated significant decrease of the quantaty of the erythropoietic cells and lymphocytes and the increase of the quantaty of the granulocytes, plasmocytes and must cells. In many other experiments the exposure of rats to weightlessness was just accompanied by a decrease of the number of blood-forming bone marrow cells mainly due to the erythroid branch (1-4), as well as by the decrease in the CFUs number (5,6), changes in form of the erythrocytes (7) and decrease of their resistance etc. (8-10).

The influence of the hypergravity on blood and blood forming organs has been studied smaller. We may mention the investigation, performed by A. Vrabiescu (II) who has successed in studying of the bone marrow in rats after the exposure on the centrifuge at 4.5 and 6.5 G for 11 days. Becides this we would like to mention the paper of R.R.Burton et al., who used the lymphocytes number in blood as the sign of the stress reaction in the centrifuged animals (12) and our previous experiments with pregnant female rats exposed at 2G (13).

Our report includs the results of the investigation of the blood and bone marrow in male, female, and pregnant female rats of Wistar strain exposed on centrifuge at 2G in comparison with the results obtained in space flights of different duration onboard biosatellites of the Cosmos missions.

Fig. 1 shows us the data which characterize the erythroid branch of bone marrow (in percentage of common number of myelcaryocytes) in male rats after 7-, 14- and 22-days spaceflights. In all three cases we saw significant decrease of the erythropoietic cells in the flight groups. It was interesting that the changes were not dependent on the flight duration being the same in all three cases (7-9% of bone marrow myelocaryocytes).

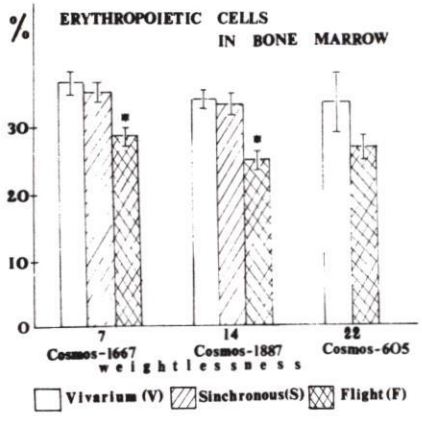


Fig.1

Unfortunatlly only in some space experiments we received the biomaterial which was necessary for calculation of the absolute number of bone marrow myelocaryocytes and the absolute number of erythropoietic cells. Fig.2 demonstrates such the materials for the rats' experiment onboard the biosatellite Cosmos-605. It was marked the decrease of the absolute number of myelocaryocytes in one femur by 50 mln cells ($p < 0.05$), herewith the spark of the erythropoietic cells was 26.4% in experimental group versus 33.4% in control group, and their absolute number was decreased by 37 mln cells. So the decrease of the cellularity of the bone marrow was perfomed in general by the erythropoietic cells.

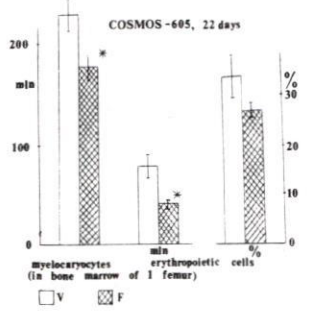


Fig.2

Fig.3 demonstates the results of the eperiments with the adult male and female rats, exposed on the centrifuge at 2G for 5 days (the rate of rotation was 33.3 rpm with a pause once a day from 9.00 to 9.30

a.m.) and examined after the experiments according to the same program. In both male and female experiments we saw the decrease of the spark of red bone marrow cells (9-10%), in this case the cellularity of bone marrow was decreased by 52-55 mln cells and the absolute number of the erythropoietic cells was decreased by 29-32 mln cells.

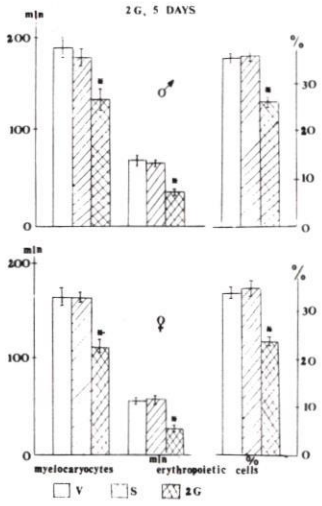


Fig.3

While the main function of the erythrocytes is to transport O_2 to provide the aerobic metabolism of organs and tissues, it was to be supposed that there must be the direct correlation between the energy expense and the value of red bone marrow. So in weightless conditions one might expect the decrease of the erithropoietic activity of bone marrow via its increase in hypergravity. But the real results have been found stright reverceble: hypergravity (2G) and weightlessness in the same duration of exposure (5-7 days) were followed by the same changes in the erythroid branch of bone marrow.

The same picture we have seen in the experiments with the pregnant female rats, exposed in weightlessness (Cosmos-1514) or on the centrifuge at 2G during gestation days 13 through 18-21 (Fig.4,5). In both cases we saw the decrease of the cellularyty of bone marrow, the decrease of the percent of the erythropoietic cells and, as a result, the decrease of the value of the erythroid branch of the bone marrow in 1 femur by 19 mln cells after space flight and by 25 mln cells after exposure at 2G.

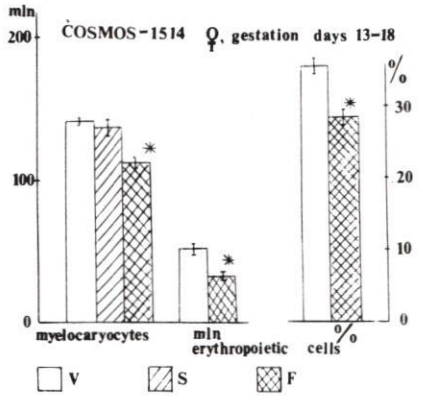


Fig.4

Previously we have described the results of our experiments in which we studied the influence of hypergravity (2G) on the different stages of the prenatal development of rats (14). In general they concluded that there were different biological mechanisms of the effects of hypergravity at different prenatal stages. When we breeded the rats at 2G, the only difference between the experimental and control groups was the considerable lengthening of the time between the setlting females to males and fertilization. When the female rats were exposed at 2G from the 7-th gestation day, the main difference between the groups consisted in the interruption of pregnancy in some animals of the experimental group. And finally, when female rats were centrifuged at 2G during gestation days 14 through 21, we did not observe any cases of interrupted pregnancy, but the fetuses of the animals of the experimental group were behind the controls in body mass and ossification level.

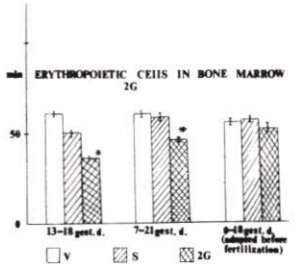


Fig.5

Fig.5 shows the data which characterise the erythroid branch of bone marrow of female rats from these experiments. The maximum decrease of the absolute number of the erythropoietic cells in bone marrow (by 25 mln cells) we have seen in rats exposed at 2G for 5 days (13-18 gestation days). In rats exposed at 2G for 14 days (7-21 gestation days) the decrease was only 14 mln cells, and finally in rats exposed on centrifuge for a month (before fertilization and then through 18-th gestation day) we have not seen any significant changes in experimental group as compare to the controls. Thus, in these experiments the changes in erythropoiesis were in the reverse dependence with the duration of exposure of the rats at 2G, being maximum in the experiment with minimum exposition.

Fig.6 shows the data which characterises the reticulocytes' concentrations in rats from all experiments described above. In all the cases, both at weightlessness and hypergravity the depression of red blood cells formation was in good correlation with the decrease of reticulocytes' level in blood. It is interesting to mention that in centrifuge experiments with pregnant rats we have seen the decrease of retyculocytes' level in blood as well as the decrease of the number of the erythroietic cells in bone marrow only in rats exposed at 2G during gestation days 13 through 18 and 7 through 21; and we have not seen these changings in rats exposed at 2G for a month before and after fertilization (Fig.7).

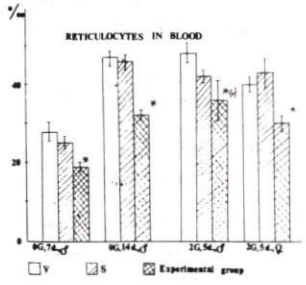


Fig.6

The changes of erythrocytes and hemoglobin concentrations were less synonymous, that apparently was defined by partial normalization of the values during several hours between the landing and the begining of the animals examination. In the experiments mentioned above the concentrations of erythrocytes and hemoglobin were not changed compared with the controls or were increased a little apparently because of the degydration of the blood. And only after the embryological experiment onboard biosatellite Cosmos-1514 the depression of red blood cells formation and decrease of reticulocytes' concentration were in good correlation with the significant decrese of hemoglobin concentration till 9.8g% via 12.9-13.4 in controls ($p < 0.001$).

Collecting together the results of different experiments, carried out in conditions of weightlessness and hypergravity, we should solve the difficult task to explain the unexpected fenomenon of the same changes in erythropoiesis both at weightlessness and hypergravity. Firstly we would like to mention that materials, presented above, which seemed to be so unexpected at first sight, really do not contradict to the literature data. Thus, A. Vrabiescu (11) studying the rats' bone marrow after 11-days exposure at 4.5 and 6.5G found the decrese of the part of the erythropoietic cells. A. Vacek et al. (5, 6) has found the decrease in CFUs number both at weightlessness and hypergravity.

As far as after the exposure at weightlessness or at hypergravity the changes in red bone marrow mentioned above are normalize rather quiqly, they should valued as the adaptive, Physiological reactions and so the courses of them should be looked for in normal mechanisms of erythron regulation. We may suggest that there are several courses of these changes because of the complexness of the erythron's regulation.

It is known that value of the erythropoietic branch of bone marrow is regulated by the organism's need in erythrocytes. Thus, while we belive that the physiological mechanisms of the regulation is in order, the question we should solve is what is the initiator which informates the bone marrow about the erythrocytes' abundance, and, as a result, allows the sequence of the reactions resulting in the depression of red blood cells formation both at weightlessness and hypergravity. For weightlessness such the initiator might be the increase of the peripheral hematocrit because of plasma volume decrease and erythrocytes' volume increase as a result of water loss (9,15, 16). The increase of hemoglobin concetration which we can see in some animals after landing is the indirect sign of such changes in rats. The some changes we have found out at hypergravity. Thus mice, exposed at 2G demonstrated the blood dehydration and hematocrite increase (Fig.8). These changes were like the changes taking place when we did not water mice, and were summerised in the variant: "2G+dehydration".

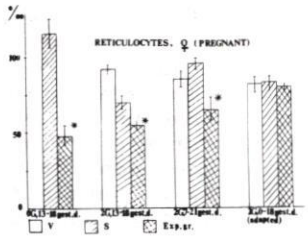


Fig.7

The information: " plenty of erythrocytes", appearing as a result of blood dehydration is practically false, while the real erythrocytes number at first is not increased and then is decreased because the decrease of their production. The mechanism, which determine the normalization of the changes in the conditions of hypergravity and nonprogressive changes during space flights is not clear. That fact is confirmed by the results of our experiments in wich the changes in red bone marrow were practically the same in 7-, 14- and 22-days space flights and in centrifuge experiments they were maximum in rats at 5-days exposure and were not seen at the animals which were at 2G during a month.

As far as the intensity of the erythropoiesis is significantly determined by different hormons, firstly by the sexual hormons, which operate via erythropoietin or directly (18), it might not be excluded that the change of the hormons' levels in the organism at weightlessness and hypergravity put some contribution into general picture of changes. At the same time although our point of wiew is that nonspecific stress-changes are the significant part in changes taking place both at 0G and 2G (19), we don't belive that changes in red bone marrow are from their number.

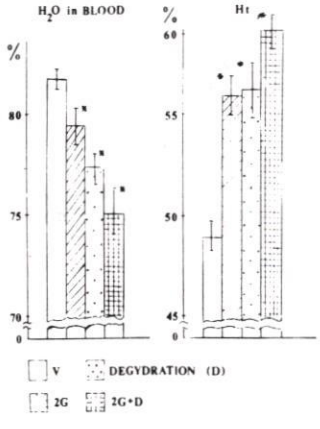


Fig.8

Long before the receiving of the real

experimental data from the animals exposed in space flight P. Korzuev (20,21) proposed that the decrease of the methabolic activity in weightlessness would lead to depression of the red blood cell formation in the bone marrow. At present we may say that the factual part of this hypothesis is aproved but the mechanisms of the development of the changes are different from those he has suggested. First of all the rats exposed at weightlessness did not demonstrate the decrease of energy expense. On the contrary it was marked the increase of their motion activity (22), the increase of their O_2 consumption (23) and the increase of their food assimilation (24). On the other hand the animals' adaptation in the first day of their exposure on centrifuge is connected with decrease of their motion activity. This fenomenon for the first time was described by H. Bengle (25) and we also regulary saw it at our centrifuge experiments. It is interesting to mention that many times we saw the same picture in rats during their readaptation period after space flights (26). This findings permit us to think that the changes of energy expense during the weightlessness and hypergravity are not quite contrary as we believed at first. Apparently they in general are determined by the animals' adaptive behaviour, firstly by the change of the animals' motion activity. In conclusion we want to mention that while studying the mechanisms of the animals' adaptation not only in gravitational physiology but in the general physiology too, we wrongly pay less attention to the possibility to adapt to different conditions by changes in motion activity and bechavior. At the same time in natural environment just these reactions has the main role.

References:

1. Shvets V.N., 1979, In: Effect of Dynamic Factors of Space Flight in the Animal Body, Moskow, Nauka, p. 180-183.
2. Kalandarova M.P., Rodyna G.P., Serova L.V., 1981, Problems of Hematology, v. 26, N 12, p. 26-30.
3. Chelnaya N.A., 1988, XXI Symp. Space Biol. Med., Warsawa, p. 41.
4. Chelnaya N.A., 1989, XXII Sump. Space Biol. Med., Varna, p. 271.
5. Vacek A., Tkadlecek L., Shvets V.N. et al., 1982, Cell Tissie Kinet., v.15, p. 643-649.
6. Vacek A., Bartonickova A., Rotkovska D. et al., 1983, Physiologist (Suppl.), v. 26, N 6, p. 131-132.
7. Sydorenko L.A., 1989, XXII Symp. Space Biol. Med., Varna, p. 223.
8. Leon H.A., Serova L.V., Cummins I. et al., 1978, Aviat. Space Environ. Med., v. 49, N 1, p. 66-69.
9. Leon H.A., Serova L.V., Landaw S.A., 1980, Ibid., v. 51, N 10, p. 1091-1094.
10. Serova L.V., Chelnaya N.A., 1982, VII Conf. Space Biol. Med., Kaluga, v. 2, p. 150
11. Vrabiescu A., Enachesku G., 1969, Aerosp. Med., v. 40, N12, p. 1300-1304.
12. Burton R.R., Sluka S.I., Beach E.L., Smith A.H., 1967, Aerosp. Med., v. 38, p. 1240-1243.
13. Serova L.V., Denisova L.A., Chelnaya N.A. et al., 1983, Physiologist (Suppl.), v. 26, N 6, p. 137-138.
14. Serova L.V., Denisova L.A., Pustinnikova A.M., 1985, Physiologist (Suppl.), v. 28, N 6, p. 5-8.
15. Kimzey S.L., Burns L.C., Fisher C.L., 1974, In: The Proceedings of the Skylab Life Sciences Symposium, v.2, p. 93-118.
16. Johnson P.C., Driscoll T.B., Le Blanc A.D., , Ibid, p. 69-79.
17. Dietlein L.F., , Ibid., p. 369-388.
18. Moiseeva O.I., 1970, Kidneys and erythropoiesis, Leningrad, Nauka, 123 p.
19. Serova L.V., 1977, Kosm. Biol. Aviakosm. Med., v.11, N 5, p. 25-32.
20. Korzuev P.A., 1963, Aviat. Space Med., Moscow, p. 284-287.
21. Korzuev P.A., 1971, Evolution, Gravity and Weightlessness, Moscow, Nauka, 152 p.
22. Klimovitsky V.J., Oganov V.S., Ilyin E.A. et al., 1979, In: Effect of Dynamic Factors of Space Flight in the Animal Body, Moscow, Nauka, p. 13-16.
23. Golov V.K., Ilyin E.A., 1977, X Symp. Space Biol. Med., Suchumy, p. 15.
24. Kondratyev Y.I., Ilushco N.A., Besedina E.G., 1979, In: Effect of Dynamic Factors of Space Flight in the Animal Body, Moscow, Nauka, p. 21-24.
25. Bengele H.H., 1969, Amer. J. Physiol., v. 216, N 3, p. 659-665.
26. Serova L.V., 1980, Physiologist (Suppl.), v. 23, N 6, p. 22-26.

RESULTS OF EXAMINATIONS OF THE +Gz ACCELERATION TOLERANCE USING DIFFERENT ACCELERATION PROFILES

W. Papenfuss, G. Kollande, B. Ullrich, and R. Eckardt

Institute of Aviation Medicine, Koenigsbrueck, GDR

According to the experience reported by other investigators, the results of tolerance examinations are influenced by the acceleration profile applied. This study informs on the results of an examination series, which was started in order to find appropriate methods for examination and evaluation.

Method

Examinations were made on a human centrifuge, which by computer control can create a clearly defined +Gz acceleration in all stages of the examination.

Subjects were exposed to three acceleration profiles. Profile 1 was an interval profil (IP 1). It consisted of accelerations increasing in steps of 0.5 Gz, each, starting from 2 Gz up to a maximum of 5 Gz. The onset rate of acceleration and the deceleration were 0.1 $G.s^{-1}$, respectively. The several G-levels and the intervening interval of +1.1 Gz were sustained 15 sec, each. The second profile was an interval profile, too (IP 2). In contrast to the preceeding profile, the onset rate was 0.5 $G.s^{-1}$, the deceleration 0.3 $G.s^{-1}$, and the first G-level 3 Gz. The third profile was an acceleration continuously increasing by 0.1 $G.s^{-1}$ up to 7 Gz, at most; the deceleration was again 0.3 $G.s^{-1}$ (linear profil, LP).

As tolerance criteria we defined the loss of the pulse amplitude photoplethysmographically measured at the earlobe, and the peripheral light loss. The peripheral light loss was determined with the help of a segmental perimeter. Subjects did not wear any anti-G suit and were asked not to make use of anti-G straining manoeuvres. Straining manoeuvres such as the L-1 and the M-1 were detected from the course of the spirogram. Such cases were not included into this study. Cases, where distinct functional disorders were the reason to stop the centrifuge exposure, were also excluded from this study.

Results and Discussion

Fig. 1 shows the percentage of the G-levels reached in both interval profiles. As you can see, in IP 1 with the onset rate of 0.1 $G.s^{-1}$ a smaller percentage of subjects than in IP 2 with the onset rate of 0.5 $G.s^{-1}$ reached the higher G-levels. This also concerns the percentage of those subjects who had not yet reached their tolerance end point, that means, who could have tolerated more than 5 Gz. In IP 1 this is the case with 25 % and in IP 2 with 54 %. In accordance with the corresponding literature, we consider the various times of exposure, which in IP 1 resulted in an early fatigue or exhaustion of the regulatory mechanisms in a relatively larger part of subjects, being the cause for this difference. On the other hand, despite the higher onset rate in IP 2, the time was obviously sufficient for the regulatory mechanisms to become effective in most of the subjects.

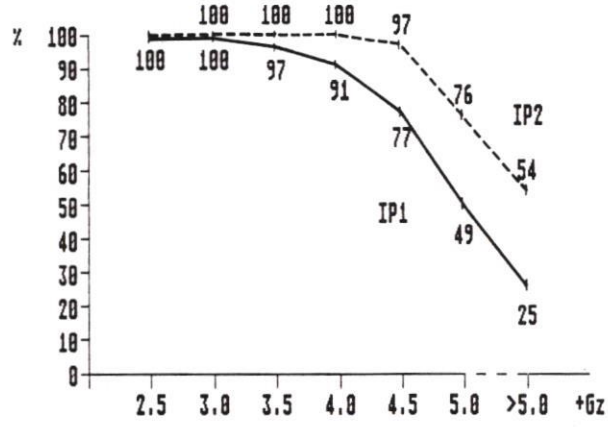


Figure 1. Percentage of the G-levels reached in the interval profiles.

The percentage of the several G-levels reached in IP 2 have been differentiated according to age. It is evident that subjects younger than 30 years remained under the mean values of the total population. Subjects at the ages of 31 to 35 years and over 40 years, by comparison, exceeded the average. But the age-dependent differences were statistically not significant (Fig. 2).

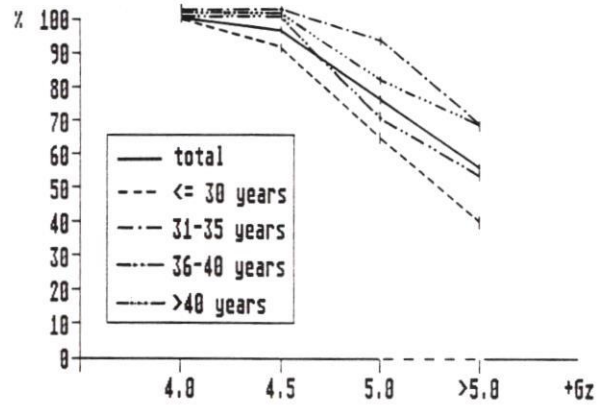


Figure 2. Age-dependent differences of the +Gz-acceleration tolerance in the IP 2.

A similar age group distribution - also without a statistical significance - occurred in the linear profile. What is remarkable is the relatively high acceleration tolerance of the more than 40year-old subjects. Anyhow 43 % of them had not yet reached their tolerance end point at 7 Gz. It can be supposed that these subjects not only had the greatest flight experience but that this age group was a positively selected one, that means they were the most stable ones as for their state of health and their acceleration tolerance and for this reason, they were able to maintain their fitness for flying up to this age (Fig. 3).

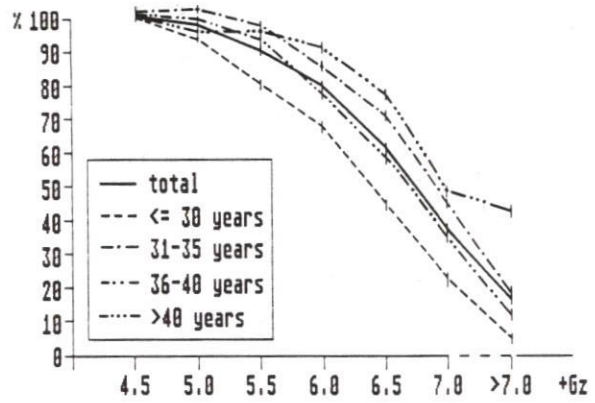


Figure 3. Age-dependent differences of the +Gz-acceleration tolerance in the LP.

As the results of the linear profile and IP 2 were achieved with the same subjects, it is possible to compare them to each other. This comparison shows that in IP 2 54 % of the subjects had not yet reached their tolerance end point at 5 Gz; in the linear profile, by comparison, more than 90 % tolerated more than 5 Gz. The intersubject variability was most distinct in the linear profile and least in IP 2. In summary, the comparison demonstrates that the continuous increase in acceleration with a low onset rate is better tolerated than intermittently occurring accelerations (Fig.4).

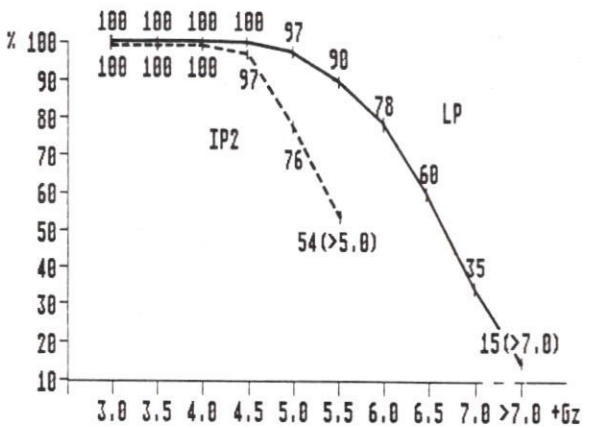


Figure 4. Comparison of the +Gz-acceleration tolerance in the LP and IP 2.

In Fig. 5 a review on the frequency of those tolerance criteria that gave reason to stop the centrifuge exposure in IP 2 and in the linear profile is given. According to this, the mentioned criteria loss of the ear pulse amplitude (LEPA) and peripheral light loss (PLL) were applied to 93 % of the cases in IP 2 and to 81 % in the linear profile. The other cases were in the main a sinus tachycardia with a heart rate of more than 180 beats/ minute. It is essential that in most of the cases, that means in 81 % and in 59 %, respectively, the objective criterion loss of the ear pulse amplitude was the cause to stop the exposure. In the linear profile, the criterion peripheral light loss was applied to an almost double number of subjects than in the interval profile.

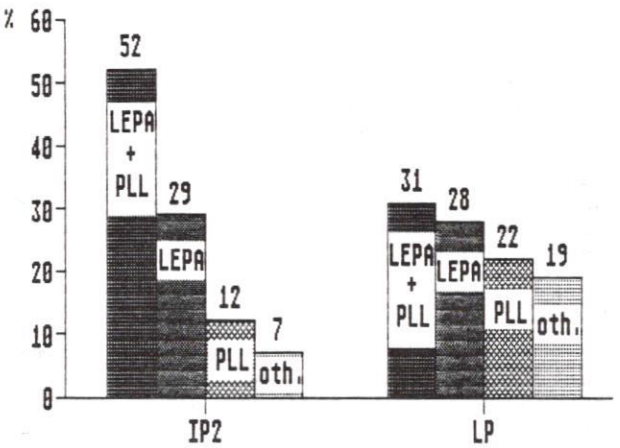


Figure 5. Frequency distribution of the tolerance criteria in the IP 2 and LP.

Conclusions

Regarding the results of the applied acceleration profiles the number of subjects reaching higher tolerance values increased with the shorter time of exposure. The resting intervals between the several increased G-levels did evidently not considerably reduce the beginning fatigue of the subjects. Age-dependent differences were statistically not significant. The relatively higher acceleration tolerance of the subjects older than 40 years can be attributed to a positive selection.

RESULTS OF EXAMINATIONS OF THE +Gz ACCELERATION TOLERANCE USING DIFFERENT ACCELERATION PROFILES

W. Papenfuss, G. Kollande, B. Ullrich, and R. Eckardt

Institute of Aviation Medicine, Koenigsbrueck, GDR

According to the experience reported by other investigators, the results of tolerance examinations are influenced by the acceleration profile applied. This study informs on the results of an examination series, which was started in order to find appropriate methods for examination and evaluation.

Method

Examinations were made on a human centrifuge, which by computer control can create a clearly defined +Gz acceleration in all stages of the examination.

Subjects were exposed to three acceleration profiles. Profile 1 was an interval profil (IP 1). It consisted of accelerations increasing in steps of 0.5 Gz, each, starting from 2 Gz up to a maximum of 5 Gz. The onset rate of acceleration and the deceleration were 0.1 $G.s^{-1}$, respectively. The several G-levels and the intervening interval of +1.1 Gz were sustained 15 sec, each. The second profile was an interval profile, too (IP 2). In contrast to the preceeding profile, the onset rate was 0.5 $G.s^{-1}$, the deceleration 0.3 $G.s^{-1}$, and the first G-level 3 Gz. The third profile was an acceleration continuously increasing by 0.1 $G.s^{-1}$ up to 7 Gz, at most; the deceleration was again 0.3 $G.s^{-1}$ (linear profil, LP).

As tolerance criteria we defined the loss of the pulse amplitude photoplethysmographically measured at the earlobe, and the peripheral light loss. The peripheral light loss was determined with the help of a segmental perimeter. Subjects did not wear any anti-G suit and were asked not to make use of anti-G straining manoeuvres. Straining manoeuvres such as the L-1 and the M-1 were detected from the course of the spirogram. Such cases were not included into this study. Cases, where distinct functional disorders were the reason to stop the centrifuge exposure, were also excluded from this study.

Results and Discussion

Fig. 1 shows the percentage of the G-levels reached in both interval profiles. As you can see, in IP 1 with the onset rate of 0.1 $G.s^{-1}$ a smaller percentage of subjects than in IP 2 with the onset rate of 0.5 $G.s^{-1}$ reached the higher G-levels. This also concerns the percentage of those subjects who had not yet reached their tolerance end point, that means, who could have tolerated more than 5 Gz. In IP 1 this is the case with 25 % and in IP 2 with 54 %. In accordance with the corresponding literature, we consider the various times of exposure, which in IP 1 resulted in an early fatigue or exhaustion of the regulatory mechanisms in a relatively larger part of subjects, being the cause for this difference. On the other hand, despite the higher onset rate in IP 2, the time was obviously sufficient for the regulatory mechanisms to become effective in most of the subjects.

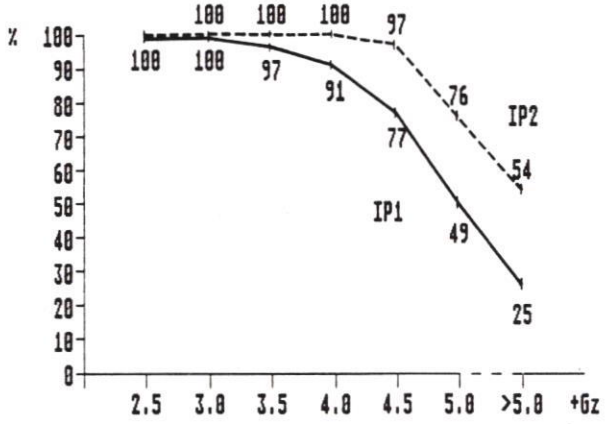


Figure 1. Percentage of the G-levels reached in the interval profiles.

The percentage of the several G-levels reached in IP 2 have been differentiated according to age. It is evident that subjects younger than 30 years remained under the mean values of the total population. Subjects at the ages of 31 to 35 years and over 40 years, by comparison, exceeded the average. But the age-dependent differences were statistically not significant (Fig. 2).

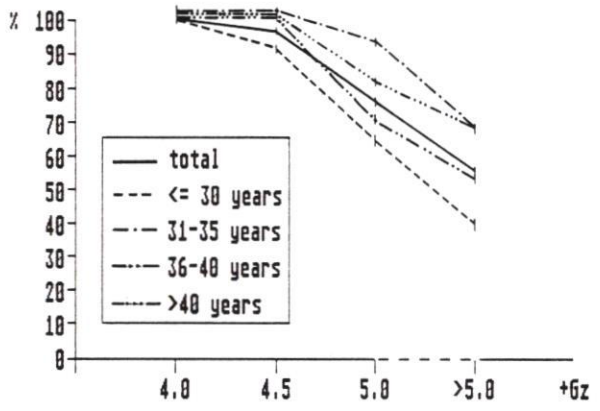


Figure 2. Age-dependent differences of the +Gz-acceleration tolerance in the IP 2.

A similar age group distribution - also without a statistical significance - occurred in the linear profile. What is remarkable is the relatively high acceleration tolerance of the more than 40year-old subjects. Anyhow 43 % of them had not yet reached their tolerance end point at 7 Gz. It can be supposed that these subjects not only had the greatest flight experience but that this age group was a positively selected one, that means they were the most stable ones as for their state of health and their acceleration tolerance and for this reason, they were able to maintain their fitness for flying up to this age (Fig. 3).

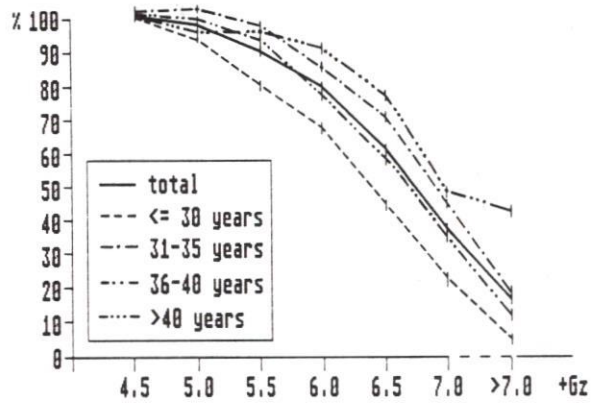


Figure 3. Age-dependent differences of the +Gz-acceleration tolerance in the LP.

As the results of the linear profile and IP 2 were achieved with the same subjects, it is possible to compare them to each other. This comparison shows that in IP 2 54 % of the subjects had not yet reached their tolerance end point at 5 Gz; in the linear profile, by comparison, more than 90 % tolerated more than 5 Gz. The intersubject variability was most distinct in the linear profile and least in IP 2. In summary, the comparison demonstrates that the continuous increase in acceleration with a low onset rate is better tolerated than intermittently occurring accelerations (Fig.4).

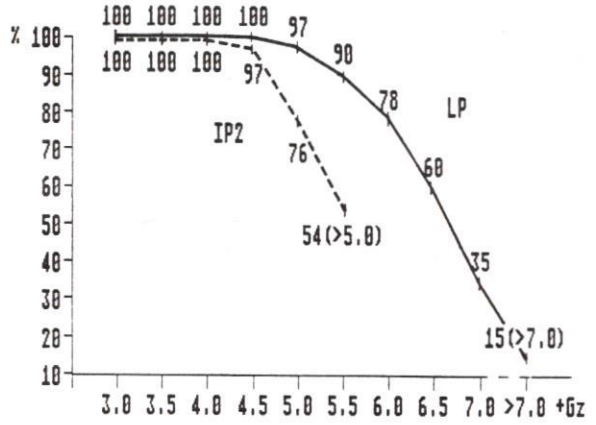


Figure 4. Comparison of the +Gz-acceleration tolerance in the LP and IP 2.

In Fig. 5 a review on the frequency of those tolerance criteria that gave reason to stop the centrifuge exposure in IP 2 and in the linear profile is given. According to this, the mentioned criteria loss of the ear pulse amplitude (LEPA) and peripheral light loss (PLL) were applied to 93 % of the cases in IP 2 and to 81 % in the linear profile. The other cases were in the main a sinus tachycardia with a heart rate of more than 180 beats/ minute. It is essential that in most of the cases, that means in 81 % and in 59 %, respectively, the objective criterion loss of the ear pulse amplitude was the cause to stop the exposure. In the linear profile, the criterion peripheral light loss was applied to an almost double number of subjects than in the interval profile.

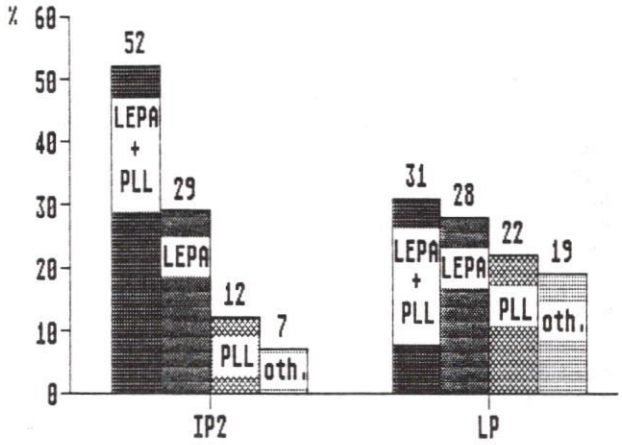


Figure 5. Frequency distribution of the tolerance criteria in the IP 2 and LP.

Conclusions

Regarding the results of the applied acceleration profiles the number of subjects reaching higher tolerance values increased with the shorter time of exposure. The resting intervals between the several increased G-levels did evidently not considerably reduce the beginning fatigue of the subjects. Age-dependent differences were statistically not significant. The relatively higher acceleration tolerance of the subjects older than 40 years can be attributed to a positive selection.

THE EFFECTS OF HYPERGRAVITATION, HYPODYNAMY AND THEIR COMBINATION ON THE QUANTITATIVE ULTRASTRUCTURE OF THE HEART MUSCLE OF JAPANESE QUAIL

BOZNER A.[x], BOĎA K., GAŽO M., DOSTÁL J.[x], JANKELA J., SABO V.

Institute of Animal Physiology, Slovak Academy of Sciences, Ivanka pri Dunaji, [x]Pharmaceutical Faculty, Comenius University, Bratislava, Czechoslovakia

In the year 1987 we reported on an experiment (3) in which the effect of chronic acceleration of 2 G hypergravitation, the hypodynamy and the simultaneous effect of chronic acceleration and the location in the centre of the turntable of the centrifuge on the protein fractions in skeletal muscles was studied. The ultrastructure of the heart muscle was examined as well.

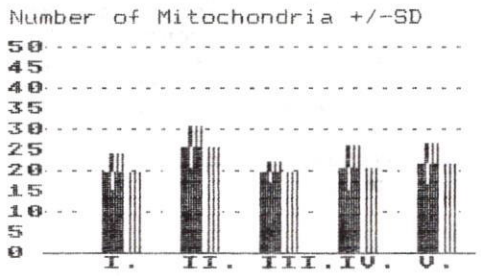
MATERIALS AND METHODS

Japanese quail cockerels (Coturnix coturnix japonica) of aged 48 days were exposed to 2 G chronic hypergravitation (group IV) in a 6,4 m diameter centrifuge, to hypodynamy (group II) and their combination (group V) respectively for 6 days. The hypodynamic state in group III was achieved by suspending the birds in jackets without contacting the floor. One group (group II) was located in the centre of the turntable of the centrifuge. The control group (group I) was kept under normal conditions. The birds were fed "ad libitum" a commercial mash prior to and during the experiments. The conditions of the experiment are described in our previous paper (3). In five animals from each group the ultrastructure of myocard was evaluated, by the method of Weibel (4). For the quantification of the ultrastructure served a grid projected on the ultrathin cuts prepared from the myocard. 4896 points of the test system achieved by this procedure from each bird were used for calculation. This method enables to determine the number, relative size and volume of mitochondria, volume of single mitochondria, defficiency of mitochondrial cristae, volume of myofibrils with an error of 15 % at $P < 0,05$.

RESULTS

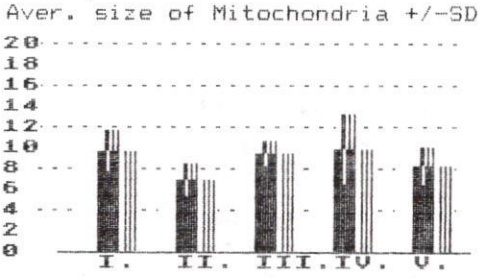
Number of mitochondria (Fig. 1). The most mitochondria were found in the preparation made from the myocard of birds located in the centre of the turntable of the centrifuge (group II). The value was significantly different from that of the control group (group I) as well as that of the hypodynamy group (group III).

Fig.1



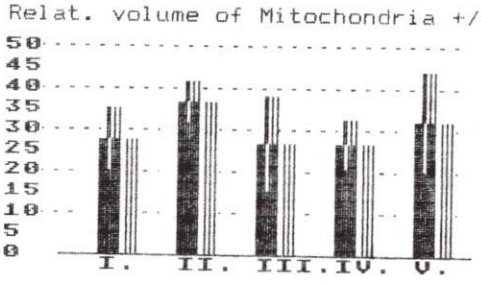
The average size of mitochondria (Fig.2). The smallest mitochondria were found in group II (centre of the turntable) and the largest in group I, and IV (control and 2 G-hypergravitation).

Fig.2



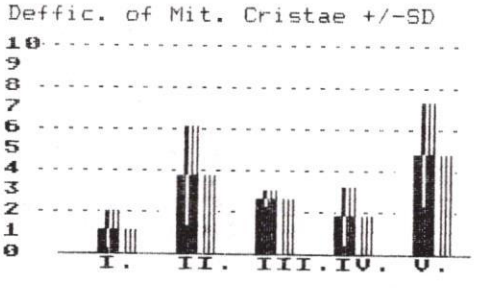
The relative volume of mitochondria in cytoplasma (Fig. 3). The highest value of this parameter was observed again in group II and then in group IV (centre of the turntable and 2 G-hypergravitation).

Fig.3



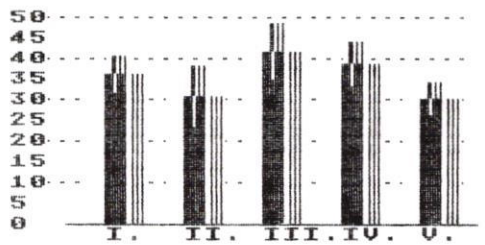
Defficiency of mitochondrial cristae (Fig. 4) was the highest in the myocard of the birds exposed to the simultaneous effect of hypodynamy and 2 G-hypergravitation (group V) and in group II in the centre of the turntable of the centrifuge. The lowest defficiency was found both in the control group and in the group exposed to 2 G-hypergravitation (group IV).

Fig.4



Relative volume of myofibrils (Fig. 5). In this case the hypodynamy not combined with hypergravitation (group III) showed the highest value, however, not significantly different from the control group (group I). The lowest value were observed in the group of birds simultaneously exposed to hypodynamy and 2 G-hypergravitation (group V).

Fig. 5



DISCUSSION AND CONCLUSION

In our experiment we tried to simulate the situation which occurs at the start of the space craft i.e. hypergravitation, hypodynamy and their combination. These effect were studied separately as well as simultaneously. In addition the effect of the location of the experimental birds in the axis of the centrifuge was also examined. As Japanese quail is suggested to serve as a source of animal proteins in long term space flights this species was the objective of our study. The biochemical investigation of muscle proteins (3) as well as their ultrastructure (1) was extended by the determination of the ultrastructure of the heart muscles.

In our experiments the most expressive changes in mitochondria of the heart muscles arosed unther the influence of the combination of hypergravitation and hypodynamy. Surprising were the findings in birds located in the centre of the turntable of the centrifuge (group II). The results of the majority of parameters of ultrastructure are in good coincidence with the corticosterone level in blood, which is one of the best marker of stress. Corticosterone values were highest particularly in these groups (3). From the interpretation of our results one can conclude that the differences in the birds exposed to hypergravitation and hypodynamy were anticipated. But the considorable defficiency of mitochondrial cristae as well as the size of mitochondria can be explained by the fact observed in the physiology of birds that the rotation around their own axis is an important stress factor in birds. In group II such a rotation around the own axis arosed. In literature no available information about the influence of the combination of hypergravitation and hypodynamy on the ultrastructure of the heart muscle could be found and hence it was possible to compare our results with those in other papers. These changes remind us in many a way of our original findings on the ultrastructure of the myocard under conditions of alpine acclimatization at a moderate hypoxy and hypobary in the High Tatras (2).

REFERENCES

1. Belák,M.,Kočišová,J.,Jankela,J.,Gažo, M.: Ninth Annual Meeting IVPS Commission on Gravitational Physiology, Nitra,Czechoslovakia,Sept.28-Oct.1, 1987,p.4
2. Bozner,A.:Pathological changes of myocard in electronmicroscopical picture (in Slovak),Osveta,Martin,1980,p.246
3. Gažo,M.,Boda,K.,Jankela,J.,Výboh,P., Juráni.M.,Baranovská,M.,Sabo,V., Strážnická,H.: Proceedings of the Ninth Annual Meeting of the IVPS Commission on Gravitational Physiology, 138-139,1987
4. Weibel,E.R.: Stereological Methods Academia Press.London,N,Y.,Sydney,San Francisco,1979

STRESSES IN THE BRAIN MASS AND IN THE PERIPHERAL FLUIDS UNDER GZ ACCELERATION. STUDY OF A SIMPLE MODEL.

P.LIEBAERT, D.GAFFIE, P.QUANDIEU

D.R.E.T.
26 ,Boulevard Victor
75996 , PARIS Armées

INTRODUCTION

The effects of high level + Gz accelerations on pilots have been studied for a long time.With gradual onset rate (GOR),symptoms are a narrowing visual field,followed by grey and black out.These symptoms are well accounted for by a hypoxic etiology.
With rapid onset rate (ROR) accelerations, it seems that alternative explanations for the observed phenomena (i.e. lack of black out) have to be considered.
In this paper,we shall show how a very simple model allows a purely mechanical explanation to be contemplated.

DESCRIPTION OF THE MODEL

The mechanical system considered is the following : the brain is considered as a visco-elas tic mass surounded by viscous fluid (cerebro-spinal fluid) in a rigid envelope (the skull), as shown in fig.1.

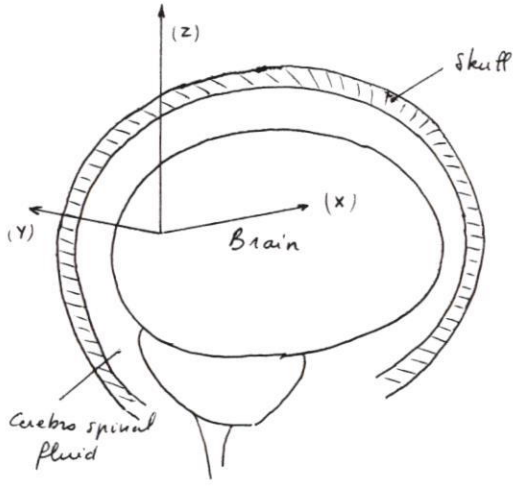


fig. 1

This system obeys the fundamental equation for deformable systems (Momentum equation)

$$\rho \, dU/dt = \mathrm{div}\, \Sigma + \rho \, f$$

where ρ is the local volumic mass,U the speed at one given point of the system,Σ the stress tensor and f the density of exterior forces per mass unit.

whether we consider the fluidic or the solid part of the system,we have a different additional relation between Σ and the gradient of U.In the case of the cerebro-spinal fluid,we assume its incompressibility and therefore add a mass conservation equation :

$$\mathrm{div}\, U = 0$$

These two equations are to be solved for every single point in the mechanical structure, and the consequent mathematical problem is by far too complex (in the case of the geometry of the human brain) for an exact mathematical solution.
This is why we made the following assumptions.

- the brain mass is to be considered as a cylindre,thus leaving only one space variable for the momentum equation in the case of the brain mass (along the z axis) and two variables for the equations in the case of the fluid (z axis and radius).

- since the study of the interactions between solid and fluid is also very complex,we shall first give results for two limit cases (solid or fluid alone),leaving the study of the interactions for a further step.

DESCRIPTION OF THE TWO LIMIT CASES

in both cases,the exterior forces will be supposed to vary linearly with time,following the relation

$$f = -\,(G + J\, t)$$

(G is the standard gravity and $J = dG/dt$)

First case (Solid alone)

in this case,the additional relation between Σ and U has the folowing form :

$$\Sigma = E \, dU/dz + \mu \, d^2U/dzdt$$

where E is Young's modulus for the brain tissue and μ the viscosity.

with these hypotheses, the equation giving the deformations of the solid could be solved exactly,but since in two or three dimensional cases this will not be possible anymore,a numerical solution has been obtained.The results are summarised on two graphs.

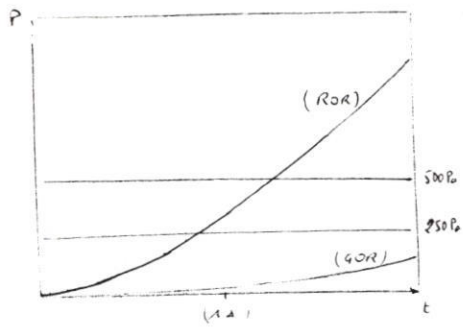


fig.2

in fig.2, the graph shows the stress at the base of the brain structure versus time in two situations (i.e. GOR and ROR).Two typical thresholds are shown (i.e. blood pressure in the veinous compartment and in the jugular vein.

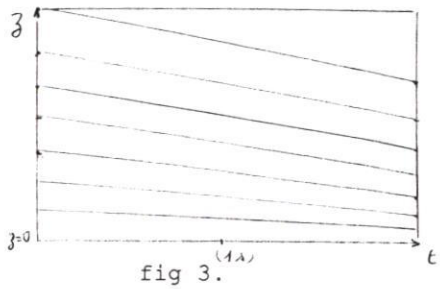


fig 3.

in fig.3,the deformation of the structure at different values of z is shown versus time in the case of ROR acceleration.

Second case (fluid alone)

In this case (in which we consider an infinite value for Young's modulus in the brain tissue),the additional relation between Σ and U is the following :

$$\Sigma = \mu \, \mathrm{grad}U + P \ .$$

where μ is the viscosity of the fluid and P the pressure.

If we consider a thin film (thickness h small compared to radius R) ,and neglect the acceleration of the fluid,we obtain a relation between the pressure distribution in the fluid and the vertical speed V of the upper surface of the fluid :

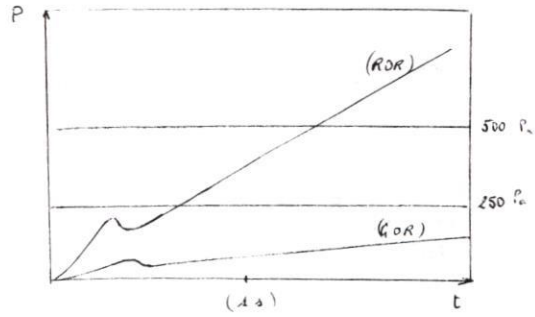
$$P(r) = -3 \ m \ V \ (R2-r2) / \ h3$$

After integration of P along the radius,we obtain the total force F applied to the solid by the fluid.Knowing the relation between the gravity forces and time,we obtain the equation for the movement of the solid (total mass M) :

$$M \ d2h/dt2 = F + M \ f$$

from which we obtain ,after numerical resolution, the increase in the pressure in the fluid.

in fig.4 is shown the maximum pressure in the fluid (obtained for r = 0) versus time.On the graph are shown the same twc thresholds as in the first case.



CONCLUSIONS AND FURTHER DEVELOPMENTS

These results,obtained in two extreme cases show that the stress and deformation levels reached in ROR acceleration simulations are important enough to lead to phenomena such as blood vessels collapse,at least as long as veins are concerned.
Of course,the model described here is by far too crude to have any other value than a simple warning (i.e. GLOC may indeed have other causes than hypoxia in certain circumstances).This is why another model was developped, taking into account a more complex geometry for the brain structure and the interactions between solid and neighbouring fluid.We expect to have more precise predictions from this model,and to realise an integration with Mr GAFFIE's mathematical model for blood flows, described in the same issue.

RESULTS OF EXAMINATIONS OF THE +Gz ACCELERATION TOLERANCE USING DIFFERENT ACCELERATION PROFILES

W. Papenfuss, G. Kollande, B. Ullrich, and R. Eckardt

Institute of Aviation Medicine, Koenigsbrueck, GDR

According to the experience reported by other investigators, the results of tolerance examinations are influenced by the acceleration profile applied. This study informs on the results of an examination series, which was started in order to find appropriate methods for examination and evaluation.

Method

Examinations were made on a human centrifuge, which by computer control can create a clearly defined +Gz acceleration in all stages of the examination.

Subjects were exposed to three acceleration profiles. Profile 1 was an interval profil (IP 1). It consisted of accelerations increasing in steps of 0.5 Gz, each, starting from 2 Gz up to a maximum of 5 Gz. The onset rate of acceleration and the deceleration were 0.1 $G.s^{-1}$, respectively. The several G-levels and the intervening interval of +1.1 Gz were sustained 15 sec, each. The second profile was an interval profile, too (IP 2). In contrast to the preceeding profile, the onset rate was 0.5 $G.s^{-1}$, the deceleration 0.3 $G.s^{-1}$, and the first G-level 3 Gz. The third profile was an acceleration continuously increasing by 0.1 $G.s^{-1}$ up to 7 Gz, at most; the deceleration was again 0.3 $G.s^{-1}$ (linear profil, LP).

As tolerance criteria we defined the loss of the pulse amplitude photoplethysmographically measured at the earlobe, and the peripheral light loss. The peripheral light loss was determined with the help of a segmental perimeter. Subjects did not wear any anti-G suit and were asked not to make use of anti-G straining manoeuvres. Straining manoeuvres such as the L-1 and the M-1 were detected from the course of the spirogram. Such cases were not included into this study. Cases, where distinct functional disorders were the reason to stop the centrifuge exposure, were also excluded from this study.

Results and Discussion

Fig. 1 shows the percentage of the G-levels reached in both interval profiles. As you can see, in IP 1 with the onset rate of 0.1 $G.s^{-1}$ a smaller percentage of subjects than in IP 2 with the onset rate of 0.5 $G.s^{-1}$ reached the higher G-levels. This also concerns the percentage of those subjects who had not yet reached their tolerance end point, that means, who could have tolerated more than 5 Gz. In IP 1 this is the case with 25 % and in IP 2 with 54 %. In accordance with the corresponding literature, we consider the various times of exposure, which in IP 1 resulted in an early fatigue or exhaustion of the regulatory mechanisms in a relatively larger part of subjects, being the cause for this difference. On the other hand, despite the higher onset rate in IP 2, the time was obviously sufficient for the regulatory mechanisms to become effective in most of the subjects.

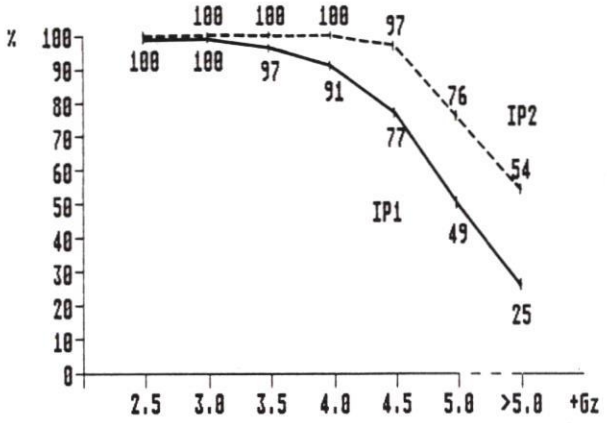


Figure 1. Percentage of the G-levels reached in the interval profiles.

The percentage of the several G-levels reached in IP 2 have been differentiated according to age. It is evident that subjects younger than 30 years remained under the mean values of the total population. Subjects at the ages of 31 to 35 years and over 40 years, by comparison, exceeded the average. But the age-dependent differences were statistically not significant (Fig. 2).

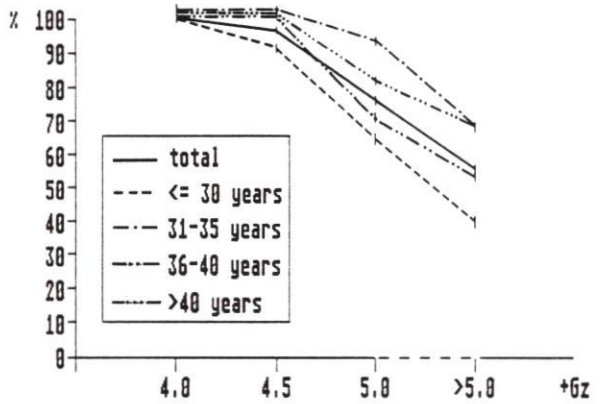


Figure 2. Age-dependent differences of the +Gz-acceleration tolerance in the IP 2.

A similar age group distribution - also without a statistical significance - occurred in the linear profile. What is remarkable is the relatively high acceleration tolerance of the more than 40year-old subjects. Anyhow 43 % of them had not yet reached their tolerance end point at 7 Gz. It can be supposed that these subjects not only had the greatest flight experience but that this age group was a positively selected one, that means they were the most stable ones as for their state of health and their acceleration tolerance and for this reason, they were able to maintain their fitness for flying up to this age (Fig. 3).

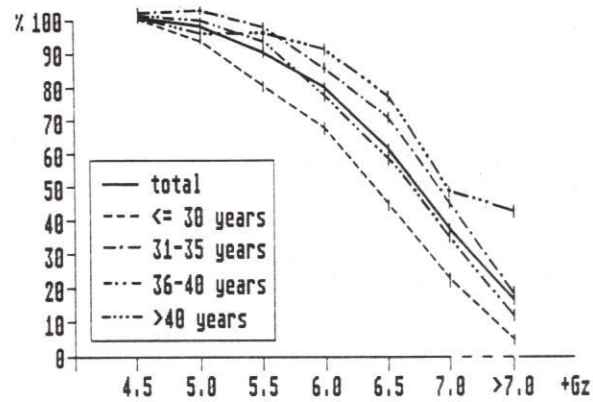


Figure 3. Age-dependent differences of the +Gz-acceleration tolerance in the LP.

As the results of the linear profile and IP 2 were achieved with the same subjects, it is possible to compare them to each other. This comparison shows that in IP 2 54 % of the subjects had not yet reached their tolerance end point at 5 Gz; in the linear profile, by comparison, more than 90 % tolerated more than 5 Gz. The intersubject variability was most distinct in the linear profile and least in IP 2. In summary, the comparison demonstrates that the continuous increase in acceleration with a low onset rate is better tolerated than intermittently occurring accelerations (Fig.4).

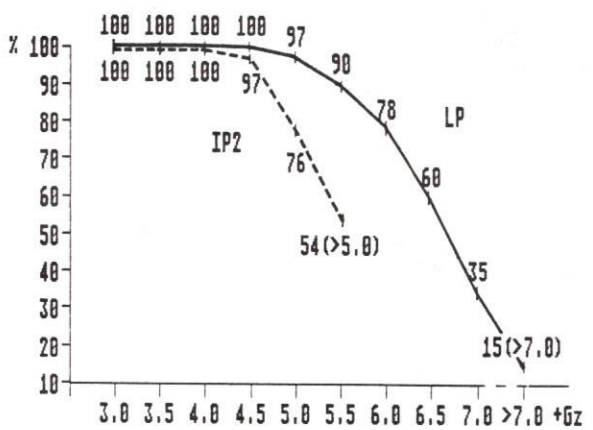


Figure 4. Comparison of the +Gz-acceleration tolerance in the LP and IP 2.

In Fig. 5 a review on the frequency of those tolerance criteria that gave reason to stop the centrifuge exposure in IP 2 and in the linear profile is given. According to this, the mentioned criteria loss of the ear pulse amplitude (LEPA) and peripheral light loss (PLL) were applied to 93 % of the cases in IP 2 and to 81 % in the linear profile. The other cases were in the main a sinus tachycardia with a heart rate of more than 180 beats/ minute. It is essential that in most of the cases, that means in 81 % and in 59 %, respectively, the objective criterion loss of the ear pulse amplitude was the cause to stop the exposure. In the linear profile, the criterion peripheral light loss was applied to an almost double number of subjects than in the interval profile.

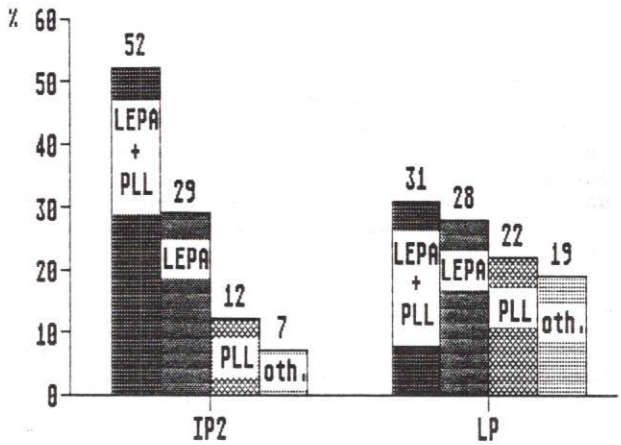


Figure 5. Frequency distribution of the tolerance criteria in the IP 2 and LP.

Conclusions

Regarding the results of the applied acceleration profiles the number of subjects reaching higher tolerance values increased with the shorter time of exposure. The resting intervals between the several increased G-levels did evidently not considerably reduce the beginning fatigue of the subjects. Age-dependent differences were statistically not significant. The relatively higher acceleration tolerance of the subjects older than 40 years can be attributed to a positive selection.

THE EFFECTS OF HYPERGRAVITATION, HYPODYNAMY AND THEIR COMBINATION ON THE QUANTITATIVE ULTRASTRUCTURE OF THE HEART MUSCLE OF JAPANESE QUAIL

BOZNER A.[x], BOĎA K., GAŽO M., DOSTÁL J.[x], JANKELA J., SABO V.

Institute of Animal Physiology, Slovak Academy of Sciences, Ivanka pri Dunaji, [x]Pharmaceutical Faculty, Comenius University, Bratislava, Czechoslovakia

In the year 1987 we reported on an experiment (3) in which the effect of chronic acceleration of 2 G hypergravitation, the hypodynamy and the simultaneous effect of chronic acceleration and the location in the centre of the turntable of the centrifuge on the protein fractions in skeletal muscles was studied. The ultrastructure of the heart muscle was examined as well.

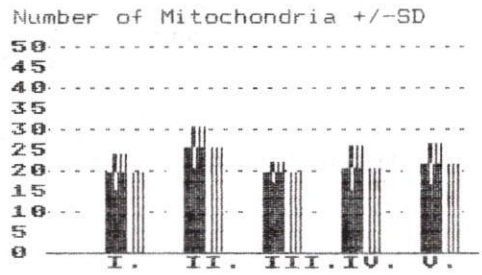
MATERIALS AND METHODS

Japanese quail cockerels (Coturnix coturnix japonica) of aged 48 days were exposed to 2 G chronic hypergravitation (group IV) in a 6,4 m diameter centrifuge, to hypodynamy (group II) and their combination (group V) respectively for 6 days. The hypodynamic state in group III was achieved by suspending the birds in jackets without contacting the floor. One group (group II) was located in the centre of the turntable of the centrifuge. The control group (group I) was kept under normal conditions. The birds were fed "ad libitum" a commercial mash prior to and during the experiments. The conditions of the experiment are described in our previous paper (3). In five animals from each group the ultrastructure of myocard was evaluated, by the method of Weibel (4). For the quantification of the ultrastructure served a grid projected on the ultrathin cuts prepared from the myocard. 4896 points of the test system achieved by this procedure from each bird were used for calculation. This method enables to determine the number, relative size and volume of mitochondria, volume of single mitochondria, defficiency of mitochondrial cristae, volume of myofibrils with an error of 15 % at $P < 0,05$.

RESULTS

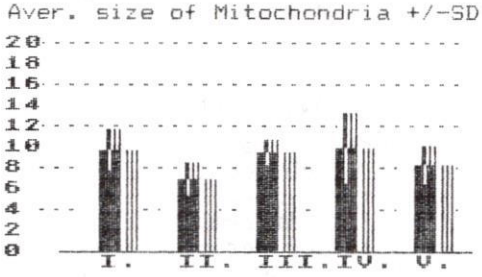
Number of mitochondria (Fig. 1). The most mitochondria were found in the preparation made from the myocard of birds located in the centre of the turntable of the centrifuge (group II). The value was significantly different from that of the control group (group I) as well as that of the hypodynamy group (group III).

Fig.1



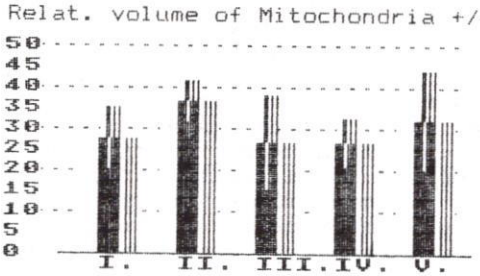
The average size of mitochondria (Fig.2). The smallest mitochondria were found in group II (centre of the turntable) and the largest in group I, and IV (control and 2 G-hypergravitation).

Fig.2



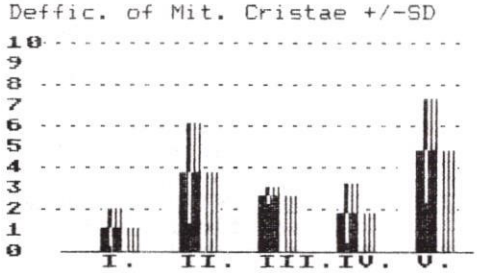
The relative volume of mitochondria in cytoplasma (Fig. 3). The highest value of this parameter was observed again in group II and then in group IV (centre of the turntable and 2 G-hypergravitation).

Fig.3



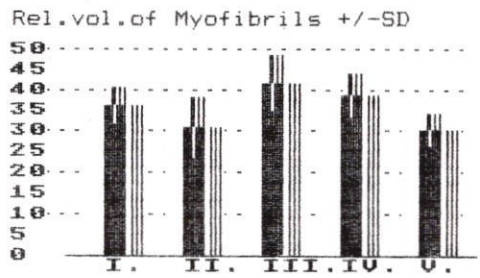
Defficiency of mitochondrial cristae (Fig. 4) was the highest in the myocard of the birds exposed to the simultaneous effect of hypodynamy and 2 G-hypergravitation (group V) and in group II in the centre of the turntable of the centrifuge. The lowest defficiency was found both in the control group and in the group exposed to 2 G-hypergravitation (group IV).

Fig.4



Relative volume of myofibrils (Fig. 5). In this case the hypodynamy not combined with hypergravitation (group III) showed the highest value, however, not significantly different from the control group (group I). The lowest value were observed in the group of birds simultaneously exposed to hypodynamy and 2 G-hypergravitation (group V).

Fig. 5



DISCUSSION AND CONCLUSION

In our experiment we tried to simulate the situation which occurs at the start of the space craft i.e. hypergravitation, hypodynamy and their combination. These effect were studied separately as well as simultaneously. In addition the effect of the location of the experimental birds in the axis of the centrifuge was also examined. As Japanese quail is suggested to serve as a source of animal proteins in long term space flights this species was the objective of our study. The biochemical investigation of muscle proteins (3) as well as their ultrastructure (1) was extended by the determination of the ultrastructure of the heart muscles.

In our experiments the most expressive changes in mitochondria of the heart muscles arosed unther the influence of the combination of hypergravitation and hypodynamy. Surprising were the findings in birds located in the centre of the turntable of the centrifuge (group II). The results of the majority of parameters of ultrastructure are in good coincidence with the corticosterone level in blood, which is one of the best marker of stress. Corticosterone values were highest particularly in these groups (3). From the interpretation of our results one can conclude that the differences in the birds exposed to hypergravitation and hypodynamy were anticipated. But the considorable defficiency of mitochondrial cristae as well as the size of mitochondria can be explained by the fact observed in the physiology of birds that the rotation around their own axis is an important stress factor in birds. In group II such a rotation around the own axis arosed. In literature no available information about the influence of the combination of hypergravitation and hypodynamy on the ultrastructure of the heart muscle could be found and hence it was possible to compare our results with those in other papers. These changes remind us in many a way of our original findings on the ultrastructure of the myocard under conditions of alpine acclimatization at a moderate hypoxy and hypobary in the High Tatras (2).

REFERENCES

1. Belák,M.,Kočišová,J.,Jankela,J.,Gažo, M.: Ninth Annual Meeting IVPS Commission on Gravitational Physiology, Nitra,Czechoslovakia,Sept.28-Oct.1, 1987,p.4
2. Bozner,A.:Pathological changes of myocard in electronmicroscopical picture (in Slovak),Osveta,Martin,1980,p.246
3. Gažo,M.,Boda,K.,Jankela,J.,Výboh,P., Juráni.M.,Baranovská,M.,Sabo,V., Strážnická,H.: Proceedings of the Ninth Annual Meeting of the IVPS Commission on Gravitational Physiology, 138-139,1987
4. Weibel,E.R.: Stereological Methods Academia Press.London,N,Y.,Sydney,San Francisco,1979

STRESSES IN THE BRAIN MASS AND IN THE PERIPHERAL FLUIDS UNDER GZ ACCELERATION. STUDY OF A SIMPLE MODEL.

P.LIEBAERT, D.GAFFIE, P.QUANDIEU

D.R.E.T.
26 ,Boulevard Victor
75996 , PARIS Armées

INTRODUCTION

The effects of high level + Gz accelerations on pilots have been studied for a long time.With gradual onset rate (GOR),symptoms are a narrowing visual field,followed by grey and black out.These symptoms are well accounted for by a hypoxic etiology.
With rapid onset rate (ROR) accelerations, it seems that alternative explanations for the observed phenomena (i.e. lack of black out) have to be considered.
In this paper,we shall show how a very simple model allows a purely mechanical explanation to be contemplated.

DESCRIPTION OF THE MODEL

The mechanical system considered is the following : the brain is considered as a visco-elas tic mass surounded by viscous fluid (cerebro-spinal fluid) in a rigid envelope (the skull), as shown in fig.1.

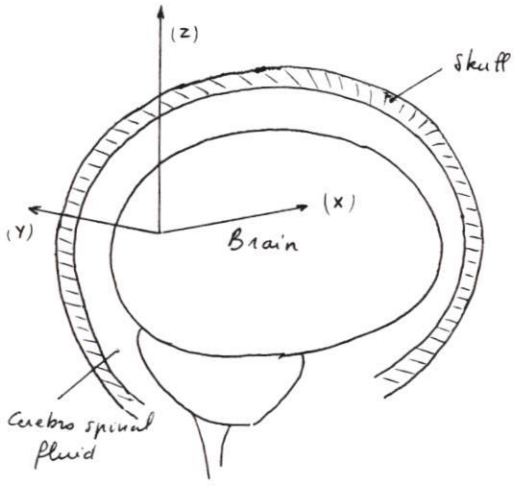


fig. 1

This system obeys the fundamental equation for deformable systems (Momentum equation)

$$\rho \, dU/dt = \mathrm{div}\ \Sigma + \rho f$$

where ρ is the local volumic mass,U the speed at one given point of the system,Σ the stress tensor and f the density of exterior forces per mass unit.

whether we consider the fluidic or the solid part of the system,we have a different additional relation between Σ and the gradient of U.In the case of the cerebro-spinal fluid,we assume its incompressibility and therefore add a mass conservation equation :

$$\mathrm{div}\ U = 0$$

These two equations are to be solved for every single point in the mechanical structure, and the consequent mathematical problem is by far too complex (in the case of the geometry of the human brain) for an exact mathematical solution.
This is why we made the following assumptions.

- the brain mass is to be considered as a cylindre,thus leaving only one space variable for the momentum equation in the case of the brain mass (along the z axis) and two variables for the equations in the case of the fluid (z axis and radius).

- since the study of the interactions between solid and fluid is also very complex,we shall first give results for two limit cases (solid or fluid alone),leaving the study of the interactions for a further step.

DESCRIPTION OF THE TWO LIMIT CASES

in both cases,the exterior forces will be supposed to vary linearly with time,following the relation

$$f = -\ (G + J\, t\ )$$

(G is the standard gravity and $J = dG/dt$)

First case (Solid alone)

in this case,the additional relation between Σ and U has the folowing form :

$$\Sigma = E\ dU/dz + \mu\ d^2U/dzdt$$

where E is Young's modulus for the brain tissue and μ the viscosity.

with these hypotheses, the equation giving the deformations of the solid could be solved exactly,but since in two or three dimensional cases this will not be possible anymore,a numerical solution has been obtained.The results are summarised on two graphs.

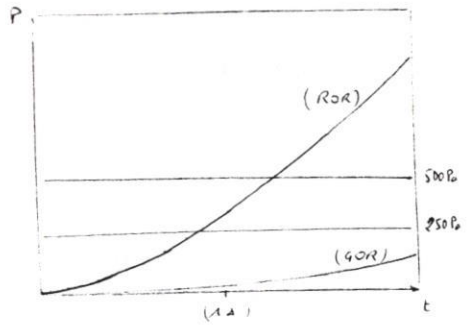


fig.2

in fig.2, the graph shows the stress at the base of the brain structure versus time in two situations (i.e. GOR and ROR).Two typical thresholds are shown (i.e. blood pressure in the veinous compartment and in the jugular vein.

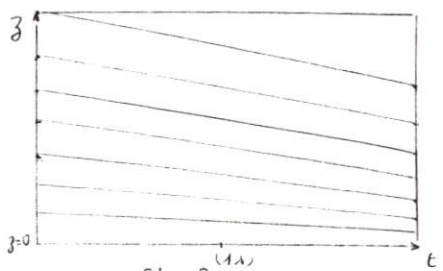


fig 3.

in fig.3,the deformation of the structure at different values of z is shown versus time in the case of ROR acceleration.

Second case (fluid alone)

In this case (in which we consider an infinite value for Young's modulus in the brain tissue),the additional relation between Σ and U is the following :

$$\Sigma = \mu \, \mathrm{grad}U + P \ .$$

where μ is the viscosity of the fluid and P the pressure.

If we consider a thin film (thickness h small compared to radius R) ,and neglect the acceleration of the fluid,we obtain a relation between the pressure distribution in the fluid and the vertical speed V of the upper surface of the fluid :

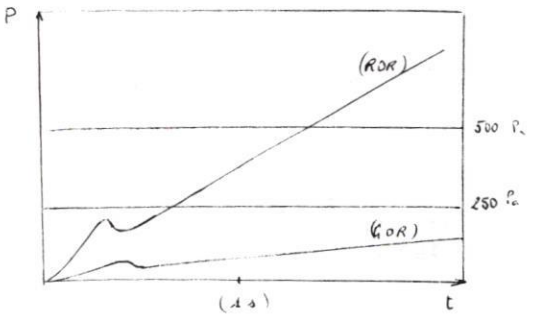
$$P(r) = -3 \, m \, V \, (R2-r2 \,) / \, h3$$

After integration of P along the radius,we obtain the total force F applied to the solid by the fluid.Knowing the relation between the gravity forces and time,we obtain the equation for the movement of the solid (total mass M) :

$$M \, d2h/dt2 = F + M \, f$$

from which we obtain ,after numerical resolution, the increase in the pressure in the fluid.

in fig.4 is shown the maximum pressure in the fluid (obtained for r = 0) versus time.On the graph are shown the same twc thresholds as in the first case.



CONCLUSIONS AND FURTHER DEVELOPMENTS

These results,obtained in two extreme cases show that the stress and deformation levels reached in ROR acceleration simulations are important enough to lead to phenomena such as blood vessels collapse,at least as long as veins are concerned.
Of course,the model described here is by far too crude to have any other value than a simple warning (i.e. GLOC may indeed have other causes than hypoxia in certain circumstances).This is why another model was developped, taking into account a more complex geometry for the brain structure and the interactions between solid and neighbouring fluid.We expect to have more precise predictions from this model,and to realise an integration with Mr GAFFIE's mathematical model for blood flows, described in the same issue.

extent in controls and +Gz trained baboons. Conversely, changes in relaxation and contraction-relaxation coupling were significantly different (table 2 : mechanical indices at 7.5 mM $[Ca^{2+}]_o$ are expressed as percentage of reference values at 2.5 mM $[Ca^{2+}]_o$; controls and +Gz trained animals were compared using the unpaired Student's t test after ANOVA ; * : $p<0.01$).

DISCUSSION

Our study showed modifications of intrinsic contractility after +Gz acceleration training in baboons . The significant increase in TPS and TPF observed in trained baboons (table 1), suggests abnormalities in excitation-contraction coupling. The modified Ca^{2+} responsiveness in +Gz trained baboons predominated on indices of both relaxation and contraction-relaxation coupling. The higher value of R1 at 7.5 mM $[Ca^{2+}]_o$ in trained baboons as compared to controls suggests alterations of Ca^{2+} sequestering membrane systems (Circ. Res.61 : 107-116, 1987). Papillary muscles were studied two days after the last training session , and this suggests that mechanical modifications were perhaps chronic. Increased Gz has been found to induce several direct and indirect cardiovascular hemodynamic effects . After +Gz acceleration, the decrease in preload and in atrial and ventricular diastolic pressure may be due to blood redistribution. Gz acceleration is followed by blood pooling in the legs . Gz training induces a fall in right atrial pressure , aortic flow and left ventricular end diastolic pressure (J. Appl. Physiol. 43 : 765-769.), and cardiac output (Aerosp. Med. 31 : 817-834 ; Acta Physiol. Scand. Suppl 276 : 1-61 ; Respir. Physiol. 62 : 141-151.). During exposure to +Gz , both the loss in venous return and preload may partly explain the decrease in cardiac output. Chronically repeated hemodynamic alterations induced by +Gz acceleration may cause myocardial cellular damage. Further histological, biochemical, metabolic, electrophysiological and mechanical studies are required to understand the effects of +Gz acceleration exposure on intrinsic cardiac behavior.

Table 1

	CONTROLS		TRAINED
Vmax	1.32±0.10	NS	1.06±0.08
ΔL	12±2	NS	14±2
TF/mm^2	2.46±1.00	NS	2.40±0.31
+dF.dt^{-1} max.mm^{-2}	9.2±2.5	NS	11.2±1.4
max.Vr	0.76±0.24	NS	1.09±0.09
-dF.dt^{-1} max.mm^{-2}	6.1±1.6	NS	9.3±1.3
t1/2	406±44	NS	336±29
TPS	522±15	+	578±21
TPF	482±23	+	560±22
R1	0.79±0.17	NS	0.54±0.07
R2	1.55±0.06	+	1.25±0.10

Table 2

	CONTROLS		TRAINED
maxVc	137±8	NS	157±12
maxVr	241±40	*	138±16
R1	97±5	*	143±17
+dF.dt^{-1} max.mm^{-2}	295±48	*	265±37
-dF.dt^{-1} max.mm^{-2}	316±43	*	212±19
R2	91±4	*	122±8s

MECHANICS OF CONTRACTION AND RELAXATION IN PAPILLARY MUSCLES OF HEALTHY AND CENTRIFUGATED BABOONS.

Yves LECARPENTIER, D. Cong Chi TRAN, Denis CHEMLA, Michel CLERGUE, Francine LAMBERT, Pierre QUANDIEU

INSERM U275-LOA-ENSTA- Ecole Polytechnique, 91120 Palaiseau and Laboratoire Central de Biologie Aérospatiale, Division Biomécanique, CERMA, 75731 Paris, FRANCE

INTRODUCTION

Exposure to +Gz acceleration has been shown to induce various direct and indirect transitory hemodynamic effects, particularly on venous return, preload, afterload and cardiac output. However, it is not known if +Gz training does or does not induce chronic modifications of intrinsic mechanical properties in isolated cardiac muscle. In order to answer this question, baboons were submitted to downward head-to-foot gravito-inertial force and mechanics of left ventricular papillary muscles of Gz trained animals were compared to those of control baboons.

METHODS

Acceleration (+Gz) training was performed on four baboons. Each baboon was anesthetized (ketamine IM,10mg/kg) and placed in the gondola of a centrifuge and was horizontaly seated, the head toward the rotational axis. The centrifuge of the "Centre d'Etudes et de Recherches de Médecine Aérospatiale" (CERMA) provided a radial acceleration of up to +10G maximum, with a jolt ranged from 0.5 to 2G/sec. The downward head-to-foot gravito-inertial +Gz force was imposed on the baboons in accordance with the following protocol : +1Gz during 15 to 30 seconds, then a sustained 8Gz level during 30 seconds ; the jolt was 1G/sec. The centrifuge training consisted of 3 consecutive runs at +8Gz, 2 times a week for 3 months. At the moment of training, the baboons were not anesthetized. Body weight of trained animals was 9.5 ± 0.4 kg before +Gz protocol and 9.3 ± 0.5 kg after + Gz protocol. Four control animals were also investigated. Age ranged from 3 to 4 years both in control and trained baboons.

Cardiac mechanics procedure :

The four baboons were sacrificed two days after the last centrifuge training. Each animal was anesthetized by means of ketamine IM (10mg/kg) and placed on a surgical table. The baboon was then intubated and ventilated with room air. After a thoracotomy through the fourth intercostal space, the pericardium was excised, the great blood vessels clamped and the heart rapidly extracted from the thorax. Six papillary muscles were excised from the left ventricle of the 4 trained animals. Six left ventricular papillary muscles of four control baboons were also studied. Each papillary muscle was quickly removed and vertically suspended in a bathing solution containing (in mM) : Nacl 118, Kcl 4.7, Mg $SO_4.7H_2O$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2.6H_2O$ 2.5 and glucose 4.5. The Krebs-Ringer solution was bubbled with 95% O2-5%CO_2, at 29°C, giving a pH of 7.4. Papillary muscles were electrically stimulated with rectangular pulses of 5 msec duration by means of two platinum electrodes at a stimulation frequency of 12 beats per minute. All experiments were performed at Lmax, the initial length corresponding to the apex of the length-active tension curve. Mechanical parameters were successively recorded at 2.5 and 7.5 mM extra-cellular calcium concentration $[Ca^{2+}]_o$.Mechanical parameters were determined from 3 twitches. Twitch 1 was loaded with preload only. Twitch 2 was abruptly clamped to zero-load just after the electrical stimulus. Twitch 3 was entirely isometric. Vmax and maxVc (Lmax/sec) were the peak shortening velocity of twitches 2 and 1 respectively. ΔL (%Lmax) was the peak amplitude of twitch 1 and was reached at time-to-peak shortening TPS (msec). TF/mm^2 (g/mm^2) was the isometric force normalized per cross-sectional area, and was reached at time-to-peak force TPF (msec). The indices + and - $dF.dt^{-1}$max. mm^{-2} (g/mm^2/sec) were the positive and negative peak force derivatives of twitch 3, normalized per cross-sectional area . maxVr was the peak lengthening velocity of twitch 1, and t1/2 (msec) the time to half -relaxation of twitch 3. R1 was the ratio of maxVc to max Vr, and R2 the ratio (+$dF.dt^{-1}$max) / (-$dF.dt^{-1}$ max) . These two ratios quantified the coupling between contraction and relaxation at low and heavy load respectively. The electronic device and force transducer have been previously described (Circ.Res.56 :331-339, 1985).

RESULTS

The mechanical results obtained from controls and trained animals at 2.5mM$[Ca^{2+}]_o$ are summarized in table 1 . Mechanical indices in controls did not significantly differ from those observed in trained animals excepted for TPS, TPF and R_2. In table 1, data are expressed as mean +SE ; controls and +Gz trained animals were compared using the unpaired Student's t test after ANOVA, + : $p<0.01$). Muscle Ca^{2+} responsiveness was tested by increasing $[Ca^{2+}]_o$ from 2.5 to 7.5 mM. The percentage increase in myocardial performance was significant in each group ($p<0.001$), and was of simular

MECHANICS OF CONTRACTION AND RELAXATION IN PAPILLARY MUSCLES OF HEALTHY AND CENTRIFUGATED BABOONS.

Yves LECARPENTIER, D. Cong Chi TRAN, Denis CHEMLA, Michel CLERGUE, Francine LAMBERT, Pierre QUANDIEU

INSERM U275-LOA-ENSTA- Ecole Polytechnique, 91120 Palaiseau and Laboratoire Central de Biologie Aérospatiale, Division Biomécanique, CERMA, 75731 Paris, FRANCE

INTRODUCTION

Exposure to +Gz acceleration has been shown to induce various direct and indirect transitory hemodynamic effects, particularly on venous return, preload, afterload and cardiac output. However, it is not known if +Gz training does or does not induce chronic modifications of intrinsic mechanical properties in isolated cardiac muscle. In order to answer this question, baboons were submitted to downward head-to-foot gravito-inertial force and mechanics of left ventricular papillary muscles of Gz trained animals were compared to those of control baboons.

METHODS

Acceleration (+Gz) training was performed on four baboons. Each baboon was anesthetized (ketamine IM,10mg/kg) and placed in the gondola of a centrifuge and was horizontaly seated, the head toward the rotational axis. The centrifuge of the "Centre d'Etudes et de Recherches de Médecine Aérospatiale" (CERMA) provided a radial acceleration of up to +10G maximum, with a jolt ranged from 0.5 to 2G/sec. The downward head-to-foot gravito-inertial +Gz force was imposed on the baboons in accordance with the following protocol : +1Gz during 15 to 30 seconds, then a sustained 8Gz level during 30 seconds ; the jolt was 1G/sec. The centrifuge training consisted of 3 consecutive runs at +8Gz, 2 times a week for 3 months. At the moment of training, the baboons were not anesthetized. Body weight of trained animals was 9.5 ± 0.4 kg before +Gz protocol and 9.3 ± 0.5 kg after + Gz protocol. Four control animals were also investigated. Age ranged from 3 to 4 years both in control and trained baboons.

Cardiac mechanics procedure :

The four baboons were sacrificed two days after the last centrifuge training. Each animal was anesthetized by means of ketamine IM (10mg/kg) and placed on a surgical table. The baboon was then intubated and ventilated with room air. After a thoracotomy through the fourth intercostal space, the pericardium was excised, the great blood vessels clamped and the heart rapidly extracted from the thorax. Six papillary muscles were excised from the left ventricle of the 4 trained animals. Six left ventricular papillary muscles of four control baboons were also studied. Each papillary muscle was quickly removed and vertically suspended in a bathing solution containing (in mM) : Nacl 118, Kcl 4.7, Mg $SO_4.7H_2O$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2.6H_2O$ 2.5 and glucose 4.5. The Krebs-Ringer solution was bubbled with 95% O2-5%CO_2, at 29°C, giving a pH of 7.4. Papillary muscles were electrically stimulated with rectangular pulses of 5 msec duration by means of two platinum electrodes at a stimulation frequency of 12 beats per minute. All experiments were performed at Lmax, the initial length corresponding to the apex of the length-active tension curve. Mechanical parameters were successively recorded at 2.5 and 7.5 mM extra-cellular calcium concentration $[Ca^{2+}]_o$.Mechanical parameters were determined from 3 twitches. Twitch 1 was loaded with preload only. Twitch 2 was abruptly clamped to zero-load just after the electrical stimulus. Twitch 3 was entirely isometric. Vmax and maxVc (Lmax/sec) were the peak shortening velocity of twitches 2 and 1 respectively. ΔL (%Lmax) was the peak amplitude of twitch 1 and was reached at time-to-peak shortening TPS (msec). TF/mm^2 (g/mm^2) was the isometric force normalized per cross-sectional area, and was reached at time-to-peak force TPF (msec). The indices + and - $dF.dt^{-1}$max. mm^{-2} (g/mm^2/sec) were the positive and negative peak force derivatives of twitch 3, normalized per cross-sectional area . maxVr was the peak lengthening velocity of twitch 1, and t1/2 (msec) the time to half -relaxation of twitch 3. R1 was the ratio of maxVc to max Vr, and R2 the ratio (+$dF.dt^{-1}$max) / (-$dF.dt^{-1}$ max) . These two ratios quantified the coupling between contraction and relaxation at low and heavy load respectively. The electronic device and force transducer have been previously described (Circ.Res.56 :331-339, 1985).

RESULTS

The mechanical results obtained from controls and trained animals at 2.5mM$[Ca^{2+}]_o$ are summarized in table 1 . Mechanical indices in controls did not significantly differ from those observed in trained animals excepted for TPS, TPF and R_2. In table 1, data are expressed as mean +SE ; controls and +Gz trained animals were compared using the unpaired Student's t test after ANOVA, + : $p<0.01$). Muscle Ca^{2+} responsiveness was tested by increasing $[Ca^{2+}]_o$ from 2.5 to 7.5 mM. The percentage increase in myocardial performance was significant in each group ($p<0.001$), and was of simular

extent in controls and +Gz trained baboons. Conversely, changes in relaxation and contraction-relaxation coupling were significantly different (table 2 : mechanical indices at 7.5 mM $[Ca^{2+}]_o$ are expressed as percentage of reference values at 2.5 mM $[Ca^{2+}]_o$; controls and +Gz trained animals were compared using the unpaired Student's t test after ANOVA ; * : $p<0.01$).

DISCUSSION

Our study showed modifications of intrinsic contractility after +Gz acceleration training in baboons . The significant increase in TPS and TPF observed in trained baboons (table 1), suggests abnormalities in excitation-contraction coupling. The modified Ca^{2+} responsiveness in +Gz trained baboons predominated on indices of both relaxation and contraction-relaxation coupling. The higher value of R1 at 7.5 mM $[Ca^{2+}]_o$ in trained baboons as compared to controls suggests alterations of Ca^{2+} sequestering membrane systems (Circ. Res.61 : 107-116, 1987). Papillary muscles were studied two days after the last training session , and this suggests that mechanical modifications were perhaps chronic. Increased Gz has been found to induce several direct and indirect cardiovascular hemodynamic effects . After +Gz acceleration, the decrease in preload and in atrial and ventricular diastolic pressure may be due to blood redistribution. Gz acceleration is followed by blood pooling in the legs . Gz training induces a fall in right atrial pressure , aortic flow and left ventricular end diastolic pressure (J. Appl. Physiol. 43 : 765-769.), and cardiac output (Aerosp. Med. 31 : 817-834 ; Acta Physiol. Scand. Suppl 276 : 1-61 ; Respir. Physiol. 62 : 141-151.). During exposure to +Gz , both the loss in venous return and preload may partly explain the decrease in cardiac output. Chronically repeated hemodynamic alterations induced by +Gz acceleration may cause myocardial cellular damage. Further histological, biochemical, metabolic, electrophysiological and mechanical studies are required to understand the effects of +Gz acceleration exposure on intrinsic cardiac behavior.

Table 1

	CONTROLS		TRAINED
Vmax	1.32±0.10	NS	1.06±0.08
ΔL	12±2	NS	14±2
TF/mm^2	2.46±1.00	NS	2.40±0.31
+dF.dt^{-1} max.mm^{-2}	9.2±2.5	NS	11.2±1.4
max.Vr	0.76±0.24	NS	1.09±0.09
-dF.dt^{-1} max.mm^{-2}	6.1±1.6	NS	9.3±1.3
t1/2	406±44	NS	336±29
TPS	522±15	+	578±21
TPF	482±23	+	560±22
R1	0.79±0.17	NS	0.54±0.07
R2	1.55±0.06	+	1.25±0.10

Table 2

	CONTROLS		TRAINED
maxVc	137±8	NS	157±12
maxVr	241±40	*	138±16
R1	97±5	*	143±17
+dF.dt^{-1} max.mm^{-2}	295±48	*	265±37
-dF.dt^{-1} max.mm^{-2}	316±43	*	212±19
R2	91±4	*	122±8s

		Pi	PC	ATP	PHi	LVDP
Ca 0.5 mM	C	0.36 ± 0.07	15.69 ±0.19	8.00	7.19 ± 0.03	34.40 ± 2.30
	R	0.83 ± 0.12	15.69 ± 0.19	7.60 ± 0.2	7.14 ± 0.03	30.50 ± 1.50
	Ce	1.40 ± 0.33	15.00 ± 0.39	7.70 ± 0.2	7.01 ± 0.04	20.90 ± 1.40
Ca 1.0 mM	C	2.00 ± 0.26	13.83 ± 0.34	8.2 ± 0.34	7.16 ± 0.02	58.00 ± 1.50
	R	2.40 ± 0.21	14.38 ± 0.36	7.30 ± 0.30	7.07 ± 0.04	54.00 ± 3.80
	Ce	3.63 ± 0.65	13.44 ± 0.68	7.20 ± 0.30	7.07 ± 0.03	38.90 ± 3.00
Ca 1.5 mM	C	2.62 ± 0.25	14.14 ± 0.36	7.40 ± 0.27	7.12 ± 0.01	65.10 ± 1.30
	R	4.07 ± 0.40	13.63 ± 0.49	6.40 ± 0.25	7.13 ± 0.02	63.00 ± 1.40
	Ce	3.49 ± 0.73	13.12 ± 0.50	6.70 ± 0.40	7.11 ± 0.02	48.70 ± 3.70
Ca 2 mM	C	3.96 ± 0.37	13.66 ± 0.48	6.40 ± 0.21	7.14 ± 0.01	65.90 ± 1.30
	R	4.68 ± 0.38	12.98 ± 0.29	6.50 ± 0.40	7.06 ± 0.04	65.60 ± 5.30
	Ce	3.83 ± 0.60	13.11 ± 0.66	6.60 ± 0.20	7.18 ± 0.03	51.60 ± 3.80

Table 1- Cytosolic concentrations of phosphorylated compounds, pHi and LVDP. (see text for abbreviations)

degradation of high energy phosphate compounds was correlated to an increase in myocardial Pi level which reached 230% and 388% of control value in restrained and centrifuged groups, respectively.

Intracellular pH was also altered in hearts of the centrifuged rats. A decrease of 0.2 pH unity was indeed observed in this group when compared to control value. pHi did not change in hearts of restrained rats. All there metabolic disorders diminished progressively during perfusion and were no longer detectable at the end of experiment, even at the highest extracellular calcium concentration when the workload was the highest. When perfused with a 0.5 mM calcium medium, hearts of the centrifuged group developed a left ventricular pressure which was 40% lower than that measured in restrained or control groups. When workload was increased by changing extracellular calcium concentration, the difference in LVDP, observed in hearts of centrifuged group in comparison with that measured in other hearts, decreased gradually. However, with a 2 mM Calcium medium, it remained significant (-22%).

DISCUSSION

As perfusions and NMR measurements were stretched over a week, all animals did not have the same recovery period at the end of centrifugation. However, it was previously demonstrated that cardiovascular disorders persisted for several weeks after repeated exposures to + Gz accelerations (2) and we postulated that myocardial bioenergetic perturbations induced by centrifugation also persisted in the present experiments.

Concerning NMR data, all cytosolic concentrations of myocardial phosphorylated compounds were calculated from an initial value of 8 mM for ATP concentration in controls as measured previously under similar conditions (1). The use of the reference value to calculate phosphorylate concentrations in the other groups is valuable only if it can be assumed that the sum of PC, Pi and ATP concentrations was similar in all three groups at the beginning of perfusion and that this sum remained unchanged during the whole perfusion. Such assumptions were not verified in the prsent experiments on account of the low number of animals but biochemical analysis have to be made in further experiments.

The observed degradation of myocardial ATP in restrained and centrifuged groups is probably imputable to the effects of catecholamines which are released under such conditions (4). The more pronounced degradation of high energy phosphate compounds in hearts of centrifuged animals could be due to an increase in the hormonal response when stress was increased by + Gz accelerations. As myocardial metabolic perturbations observed in the centrifuged group at the beginning of perfusion gradually diminished, then disappeared when cardiac workload was increased, there was probably no direct causal relation between these metabolic perturbations and the persistant decrease in contractility observed in hearts of this group. The present preliminary experiments provide no explanation of this loss of contractility. The explanation must probably be searched in further experiments, with histological and biochemical studies to detect possible structural modifications induced by + Gz accelerations.

REFERENCES

1. AUSSEDAT J., RAY A., LORTET S., REUTENAUER H. GRABLY S. and ROSSI A. Phosphorylated compounds and function in the isolated heart : a 31 P - NMR study (to be published)

2. BORREDON P., LISCIA P., HAZIOT A., QUANDIEU P. Expositions répétées aux accélérations + Gz de haut niveau : conséquences sur le myocarde et le système cardiovasculaire. Méd. Aéro. et Spat. 21, 84,420-424,1982.

3.DAWSON M.J. ,GADIAN D.J. and WILKIE D.R. Contraction and recovery of living muscles studies by 31P nuclear magnetic resonance. J. Physiol. 267 ,703-735, 1977.

4.LISCIA P.,DROGOU C., QUANDIEU P., BORREDON P. Accélérations +Gz de forte intensité et de longue durée et catécholamines plasmatiques. Méd. Aéro. et Spat. 27 ,108, 357-361, 1988.

31P -NMR SPECTROSCOPY STUDIES OF MYOCARDIUM SUBMITTED TO HYPERGRAVITATION

*MALLIAVIN M.J., *TRAN C.C., **AUSSEDAT J., **RAY A., **LORTET S., *QUANDIEU P., **ROSSI A.

* Laboratoire Central de Biologie Aérospatiale
Division de Biomécanique PARIS - FRANCE
** Laboratoire de Physiologie Cellulaire Cardiaque
URA CNRS 632 GRENOBLE - FRANCE

INTRODUCTION

Under hypergravitational conditions, human heart must preserve carotid blood pressure, in spite of an increase in hydrostatic pressure between the base of skull and aortic arch. The ability of the heart to increase its work level depends on the efficiency of the adjustement of energy production to energy requirements. The purpose of the present work was to study the consequences of repeated exposures to +Gz accelerations, on myocardial energy metabolism, in the rat. To meet this goal, an experimental model using 31P-NMR spectroscopy was applied to isolated perfused hearts of previously centrifuged rats. In this model, cardiac workload was modified by changing extracellular calcium concentration (1).

MATERIALS AND METHODS

Animals and treatment : 18 female WISTAR rats, weighting 220 to 280 g at the beginning of experiment, were used. A ventilated ring was used as a vivarium. Animals were distributed amongst several common cages.

A home-built restraint box (CERMA - France Technical Services) made up of two superposed rows of ten compartments, allowed the simultaneous centrifugation of the rats. When placed in a compartment, animal could not modify its head-tail axis orientation on accout of narrowness of the box. According to previous experiments (2), centrifugation was performed in a BAUDOT and HARDOLL centrifuge (arm 1.80 m) on the basis of three consecutive thirty seconds runs at 10 Gz four times a week, for four weeks. Each training period took place in the morning. Rats of the "centrifuged" group (Ce ,n=6) were weighted and installed into the centrifuge. At the end of centrifugation, rats were weighted again. Rats of the "restrained" group (R ,n=6) were handled in the same way, but the level of acceleration did not pass beyond 0.5G for this group. Rats of "control" group (C ,n=6) were weighted and placed again in the vivarium.

Perfusion and performance parameter acquisition:

All perfusions were performed within the week following the end of the centrifugation session, at a rate of 3 or 4 heart perfusions per day. Hearts of anaesthetized (Pentobarbital 50 $mg.kg^{-1}$b.w. , I.P.), then heparinized (1500 $U.l.kg^{-1}$b.w. , I.V.) and thoracotomized animals were excised and immediately submerged into ice-cold physiological solution. An aortic cannula was placed and a perfusion at a constant flow rate (10 $ml.\ min^{-1}$) at 37° C, was initiated. Millimolar composition of the basic perfusion solution was = NaCl 118, KCl 5.6, $MgCl_2$ 2.4, $NaHCO_3$ 21, $CaCl_2$ 0.5, glucose 9, pyruvate 2. pH was adjusted to 7.4 after bubbling the pertusion fluid with a O_2 : CO_2 (95 : 5 v/v) mixture. In order to evaluate cardiac mechanical performances, a drainage catheter was placed through the left ventricular wall at the apex and a latex balloon was inserted into the left ventricular cavity via the left atrium. The fluid-filled balloon was connected to a P23 DB Statham pressure transducer interfaced with a Gould 2000 recorder. Two fine silver electrodes were used to pace the heart at a constant frequency of 6 Hz. The perfused heart was then placed into a water-tight glass chamber (diameter 15 mm ; length 3O mm). Constance of preparation temperature was assured by a continuous flow of warm physiological solution (37°) through the glass chamber. At the end of a 30 minutes stabilization period, the end diastolic pressure was fixed at 8 mm of mercury by adjusting the volume of the balloon. Cardiac workload was assessed by measuring the left ventricular developed pressure (LVDP = systolic P. minus diastolic P.).

NMR measurements : 31 P - NMR spectra were performed at 81 MHz in a 4.7 T. vertical magnet (Oxford Instruments) recorded to a CXP 200 Bruker spectrometer. Each spectra resulted from the accumulation of 40 elementary signals provoked by 10 µs pulses separated by a 3 s delay.

Cytosolic concentration of cardiac phosphorylated compounds were estimated from the areas under the peaks of Phosphocreatine (PC), inorganic phosphate (Pi) and ATP (β–phosphate) (figure 1). Concentration of each compound was first expressed as a percentage of the sum (Pi + PC + ATP) which was assumed to remain constant during the whole perfusion period. According to previous experiments (1), ATP cytosolic concentration in the heart of control hearts was fixed at 8 mM. The concentration of each phosphorylated compound was then deducted from there assumption on each spectrum. Intracellular pH (pHi) was also estimated from the shift of the Pi peak (σ) relative to the PC peak using the formule :

$$pHi = 6.88 + \log [(\sigma - 3.35)/(5.6 - \sigma)]. \quad (3).$$

Experimental protocol : at the end of the 30 minutes stabilization period, 0.5 mM calcium basic solution was supplied alternatively with 1.0 mM, 1.5 mM and 2.0 mM calcium solutions for periods of 10 minutes. During each period, LVDP was measured and four NMR spectra were collected.

Statistical analysis : results were compared using variance analysis and Student's t test with a limit of significance of 0.05.

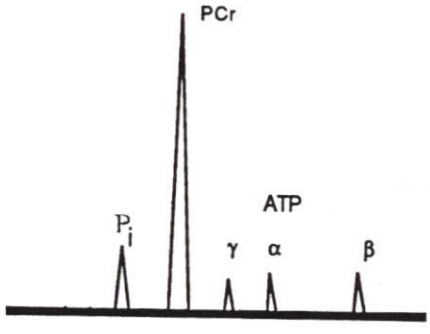


Fig.1- 31 P- NMR Spectra

RESULTS

Results are presented in table 1. Hearts of centrifuged animals showed some differences when compared to others at the beginning of perfusion. Myocardial ATP level was lower in restrained and centrifuged groups than in controls(-10%) .A PC concentration decrease was also observed in hearts of centrifuged groups (-5%). This

(M2) TIME PARAMETERS	CONTROL	+1 Gz	+2 Gz	+3 Gz	+4 Gz	+5 Gz	+6 Gz
Heart Rate /min	122	176	187	214	218	218	218
Ejection Time msec	180	160	146	130	98	95	95
Vmax cm/sec	104,5	86,5	76,2	64	46,5	47,5	44,5
Vmean cm/sec	62	48,3	39,1	31	24,7	24,8	22,5
Stroke Volume ml	16,7	11,6	8,6	6	3,6	3,5	3,2
Cardiac Output ml/min	2042	2040	1601	1294	792	770	699
Maximum Acceleration cm/sec²	2200	2174	2015	2270	1560	1872	1875

(O8) TIME PARAMETERS	CONTROL	+1 Gz	+2 Gz	+3 Gz	+4 Gz	+5 Gz	+6 Gz
Heart Rate /min	140	146	154	200	214	222	231
Ejection Time msec	200	155	165	95	85	80	80
Vmax cm/sec	78,5	56,25	52,25	45	41,6	41,5	41,25
Vmean cm/sec	49,4	32,5	34,9	23,4	20,8	20,6	20,3
Stroke Volume ml	16,3	8,3	9,5	3,7	2,9	2,7	2,7
Cardiac Output ml/min	2282	1214	1463	734	624	604	619
Maximum Acceleration cm/sec²	1880	1612	1526	1595	1600	1660	1628

Carotid measurements :

Table 3 & 4 show the variations of hemodynamic carotid parameters for M2 and O8. Like aortic parameters, carotid ones decrease with increasing load factor, and carotid blood flow stops from +4 Gz since the mid to the end of the plateau. Persistence of carotid blood flow at and above +4 Gz was only seen at the beginning of the plateau and only being due to systolic flow ; diastolic flow decrease since +1 Gz and always stops at + 4 Gz.

(M2) TIME PARAMETERS	CONTROL	+1 Gz	+2 Gz	+3 Gz	+4 Gz	+5 Gz	+6 Gz
Heart Rate /min	140	200	230	240	240	240	240
Vmax cm/s	36,7	29,2	17,2	6,8	0	0	0
Vmean cm/sec	15	11	5	1,5	0	0	0
Q carotid ml/min	44,2	32,4	14,7	4,4	0	0	0

(O8) TIME PARAMETERS	CONTROL	+1 Gz	+2 Gz	+3 Gz	+4 Gz	+5 Gz	+6 Gz
Heart Rate /min	110	140	150	180	180	190	/
Vmax cm/s	27,2	18,2	14,3	7,5	0	0	/
Vmean cm/sec	10,5	6,5	5	2,5	0	0	/
Q carotid ml/min	26,9	16,7	12,8	6,4	0	0	/

DISCUSSION

Methodological :

Accuracy of doppler technique to compute blood flows, like we did in this study, depends on :

-The knowledge and the constancy of the incidence angle between ultrasonic bean and vessel axis. This is realised with the probe design and is still valid under acceleration because probe and vessel wall move together keeping normal anatomical relation and location of sample volume.

-The doppler signal analysis : the reflected ultrasonic signal can be assessed either by Fast Fourrier Transform spectral analysis or zero-crossing hardware. We used the last device, whom accuracy depends on flow profile, with a better one if the profile is flat, like in the aorta, due to a smaller doppler frequency spectrum. On the contrary, error can reach 16 % in the carotid with its parabolic profile, because zero crossing detector measures root mean square frequency and not mean frequency.

-The measurements of a representative velocity of cross-sectional velocity : we explained in methods how we can assume it for the aorta and the carotid; flat profile in the ascending aorta has been supported by theorical and experimental results.

-The measurements of vessel diameter : they can be an important source of error since the diameter is squared in the cross-section area formula, and can limit the interpretation of blood flow absolute values. Greenfield and Patel(1) showed that the maximum change of aortic area during systole was 11 % (5.4 to 16.8 %), this result indicate that we can assume a circular and constant section of the aorta in our SV and CO calculations.

Results :

The results of the present study indicate the similar fashion of decreasing for aortic and carotid blood flow in hypergravity, with a threshold found in both cases at +4 Gz ; at this stage the diastolic carotid flow is always stopped while the systolic flow may persist during the beginning of the plateau. Repercussions of +Gz acceleration on diastolic carotid flow exist since +2 Gz and seem to be more important that on systolic flow probably because, diastolic resistance increase a lot in order to maintain the perfusion pressure, diastolic time is reduced by tachycardia and Windkessel effect is attenuated by the increased hydrostatic pressure. The relative independance of AM with level of acceleration, noticed by weak correlation coefficents, could indicate that the fall of CO is only related to changes of loading conditions, as V_{max} shows it, but not to inotropic state as AM suggests it (Payen et Al-4).

CONCLUSION

These preliminary results suggest that pulsed doppler with implantable microprobes, allowing continuous and phasic hemodynamic monitoring of local and systemic circulations without interfering with flowing conditions, could have important applications to study cardiovascular system in hypergravity and to assess the efficiency of countermeasures.

REFERENCES

1-Greenfield J.C, Patel D.J: Relation between pressure and diameter in the ascending aorta of man. Circulation Research, 1962, vol.X, 778-781.

2-Keagy B.A, Wilcox B.R, Lucas C.L, Hsiao H.S, Henry G.W, Baudino M. and Bornzin G: Constant postoperative monitoring of cardiac output after correction of congenital heart defects. J.Thorac.Cardiovasc.Surg., 1987, 93, 658-664

3-Payen D., Bousseau D, Laborde F., Beloucif S., Menu P., Compos A., Echter E, Piwnica A.: Comparison of peri-operative and postoperative phasic blood flow in aortocoronaty venous bypass grafts by means of pulsed doppler echocardiography with implantable microprobes. Circulation, 1986, 74(suppl.III), III-61.

4-Payen D.,Fratacci M.,Dupuy P., Laborde F.: Evaluation of left ventricular performance based on peak flow velocity and maximal acceleration of the blood with aortic implantable pulsed doppler probes. Circulation, 1988,78, no4,(Suppl.II),II-548

5-Payen D, Levy B.,Menegalli D.,Lajat Y., Levenson J and Nicolas F. : Evaluation of human hemispheric blood flow based on non invasive carotid blood flow measurements using the range-gated doppler technique. Stroke, 1983, vol.13, no3,,392-398.

STUDY OF AORTIC AND CAROTID BLOOD FLOW BY PULSED DOPPLER WITH IMPLANTABLE MICROPROBES IN HYPERGRAVITY

O. LANGERON *, C.C. TRAN *, F. PAILLARD *, D. PAYEN **, F. LABORDE ***, P. GALLIX *** and P. QUANDIEU*.

* Laboratoire Central de Biologie Aérospatiale (Paris)
** Hopital Universitaire Lariboisière (Paris)
*** C.E.R.A-Fondation de l'Avenir (Paris)

In hypergravity simulated by high sustained +Gz Graduate Onset Run (GOR) accelerations, grey and blackout may precede loss of consciousness (LOC). LOC seems to appear when the central nervous system blood flow is reduced to a critical level with brain hypoxia. Detailed informations concerning the cardiovascular response and compensatory mechanisms during +Gz stress exposure are still incomplete, mainly because informations can not be collected on a continuous way. The cardiac output continuous monitoring by pulsed doppler with implantable microprobes has been developped by some authors, who found a good correlation with thermodilution (r=0.89 Payen et Al-4) and electromagnetic flow probe (r=0.9 Keagy et Al-2), in human clinic during and after cardiac surgery because non invasive doppler method have often technical limitations in this context,with interposition of air or blood which hampers transmission of ultrasounds. With the same range-gated doppler technique Payen et Al (5) correlate well (r=0.73) in man the common carotid blood flow determined by pulsed doppler with mean hemispheric cerebral blood flow measured by intra-arterial injection of ^{133}Xenon.

The aim of this work is to assess repercussions of hypergravity on aortic and common carotid blood flows measured by pulsed doppler with implantable microprobes. The pulsed doppler method allows the beat to beat Stroke Volume (SV), cardiac output (CO) continuous monitoring and the common carotid output(Qcarotid)computing ; implantable microprobes get rid of fixing an external transducer on chest which could be a potential source of error by its displacement during centrifugation and changing the angle incidence between the ultrasound beam and the vessel axis.

MATERIAL AND METHODS

Animal model :

This study was performed on 2 males baboons M2 and O8 that were chronically instrumented under general anesthesia by right thoracotomy with 2 doppler microprobes : one on the ascending aorta above the aortic leaflets, and the other on common carotid. After a recovery period of 10 days without any complications, we started the experimental protocol.

Description of the flow probe :

The principle and design of the pulsed doppler microprobe are similar to a device previously reported for coronary and aortic flows measurements by Payen et Al (3,4). We used a 4 Mhz piezo-electric crystal for the aorta and an 8 Mhz for the common carotid, the dimensions of the probe were 6 mm width and 7 mm length. A silicone prism supported the crystal and was cut so that, when the probe is sutured to the adventicia of the vessel, the angle between the ultrasonic beam and the vessel axis equaled 60°.

Description of the range-gated doppler flowmeter :

A zero-crossing pulsed doppler flowmeter has been used for aortic and carotid measurements. The device has been previously described and validated (Payen et Al- 3,5). The main characteristic of this apparatus is the adjustable range-gated time system associated with the pulsed emission, which permits to select time delay from emission (depth of sample volume) and the length of reception duration (sample volume size). Adjustements of these times are made step by step of half microsecond with an incorporated pedal system in order to define the doppler sample volume location and size in the vessel lumen. This procedure is used to measure the diameter of the vessel (D), by choosing a small sample volume and moving it from the proximal to the distal wall, according to the difference between those two time delays :

$D=(t_2-t_1)C/2.\sin\theta$ with θ the angle of incidence, C velocity of ultrasounds in biological tissues (1540 m/s). The average doppler frequency shift (ΔF) was obtained by a zero-crossing system and converted into velocity with doppler equation : $V=(\Delta F.C)/(2.F.\cos\theta)$ with F emitted ultrasonic frequency (4 or 8 Mhz).

Blood flow measurements :

To compute blood flow the following parameters are needed :

- Cross Sectional Area (CSA) of the artery :

deduced from $\Pi.D^2/4$ with D diameter of the vessel calculated by the procedure described above.

- Mean blood velocity over CSA :

this parameter depends of the velocity profile in the vessel :

In the aorta : the profile is assumed to be flat in the ascending part ; so we sampled the velocity at the center of the aorta in a small portion of its section, in order to measure a velocity which reflects the velocity across the vessel. The mean velocity of blood flow during one cardiac cycle was calculated manualy on a digitizing tablet linked to a microcomputer. We report the average of ten consecutive beats in oder to take account of flow variations due to respiration. With this procedure we obtained the mean temporo-spatial velocity (V_{mean}) during ejection time (ET) :

$SV=V_{mean}.ET.CSA$ and $CO = SV.HR$ with HR: heart rate

We measured peak flow velocity (V_{max}) on paper tracing at the point of maximum systolic blood flow velocity, while the maximum acceleration of blood (MA) was calculated as the first derivative of the flow velocity during the first 40 msec of ejection.

In the carotid : the velocity profile is not flat but parabolic, and the gate duration must be adjust so that sample volume exactly encompasses the vessel diameter in order to record a doppler signal involving all streamlines. With this procedure the mean velocity over the vessel section (V_{mean}) is obtained, and the common carotid output computed : $Qcarotid=V_{mean}.CSA$

Experimental protocol :

Each animal was exposed to gravitoinertial forces of +1 to +6 Gz, with GOR during a plateau of 30 to 40 seconds. We have done two serials of runs on conscious animal to record continuously blood velocity in the aorta and then in carotid,with simultaneous ECG recording.

RESULTS

Aortic measurements :

Table 1 & 2 summarise the different measured and calculated parameters for the two baboons. Aortic diameters found for M2 and O8 were 1.38 and 1.45 cm. The fall of these parameters is proportionnal to the amplitude of +Gz acceleration, till +4 Gz level where the decrease is slowing down but with critical absolute values at this stage, in particular for SV. In fact diminution of SV is more pronounced than V_{mean} because of the simultaneous reduction of ejection time; decrease of CO is slightly attenuated by tachycardia. The inverse relation between AM and +Gz level is weak for M2 and O8 (r = -0.346 and -0.206).

EFFECTS OF HIGH SUSTAINED HYPERGRAVITY ON PLASMA CATECHOLAMINES.

*LISCIA P., *DROGOU C., *QUANDIEU P., *TRAN C.C., **BORREDON P.

*Laboratoire Central de Biologie Aérospatiale (PARIS), **Direction des Recherches Etudes et Techniques DRET (PARIS).

The purpose of this investigation using an animal model, was to test the specifity of the catecholaminergic response to +Gz accelerations compared to the same response induced by stress associated with the environment in general. The protocol included three experimental situations involving five primates which were their own controls 1) control animals sitting in the laboratory, 2) restrained stressed animals exposed to 0.5 Gz in a centrifuge, 3) animals to exposed to hypergravity. Exposure to hypergravitation included three plateaus at 8-9 Gz each of thirty seconds. Each plateau was reached with an onset rate of 1 $G.s^{-1}$. Plasma norepinephrine, epinephrine and dopamine were assayed in five blood samples drawn at fixed hours of the day. Results show that the three plasmatic hormone concentrations are significantly different in centrifuged primates, immediatly after acceleration, from that of the two controls groups. The discussion which takes into consideration the validity of the animal model, suggest that catecholaminergic response due to hypergravitation are different from response generaly observed during an emotional stress.

The changes in free plasma catecholamines levels in response to high sustained +Gz accelerations (more than 15 seconds and of an intensity of + 8 or 9 Gz) were studied.

The purpose of this paper was to know whether catecholamine changes to mechanical stress are different from catecholamine changes under stress in general in non human primates.

The hypothesis of a long term hypersympathicotonia has been forwarded following the studies of Pr. Borredon on an animal model : two primates under + Gz accelerations during four months had a transient increase in diastolic and systolic arterial pressure. Such an increase was maintained for one month after the end of the training period. Furthermore, Laughlin demonstrated an increase in peripheral resistance related to a visceral vascular bed vasoconstriction taking place in baboons in a G environment.

PROTOCOL

The experimental protocol we used was that developed by Pr Borredon (figure 1).

One exposure was made of three plateaus of 8 to 9 Gz lasting 30 to 40 seconds each. Each plateau was reached with an onset rate of 1 Gz/s. A recovery period of 2 to 3 minutes took place between every two plateaus. This protocol, performed to check the specificity of the catecholaminergic responses involved each primate in three experimental situations.

1. **The first situation** was animals sitting in the laboratory to assess the stress due to restraint.

2. **The second situation** was animals exposed to 0.5 Gz acceleration to check the stress related to a centrifuge environment.

3. **The third situation** was animals exposed to + 8 to 9 Gz accelerations.

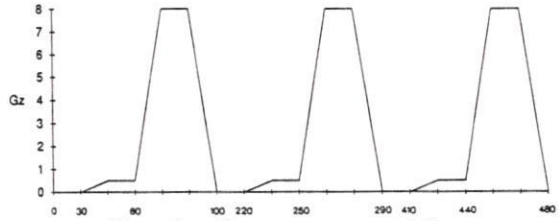


Fig. 1 - Gz exposure profil

The assessment of free plasma catecholamines levels was made on five blood samples, taken at fixed hours of the day.

Sample one : The animals were awake in a sitting position for 15 minutes. It was the resting level.

Sample two : Just before centrifuge exposure at 0.5 Gz, or 8 to 9 Gz, or at the corresponding time when the animal remained in the laboratory.

Third sample : Immediately after centrifuge exposure or at the corresponding time when the animal remained in the laboratory.

Fourth sample : thirty minutes after centrifuge exposures.

Fifth : two hours and a half after centrifuge exposures.

Blood samples were collected from the calf vein or from the tibial artery. Measurements were made using HPLC and electrochemical detection. Norepinephrine, epinephrine and dopamine molecules were simultaneously assayed.

RESULTS

Five animals were tested. Figure 2 (a) shows the kinetics of norepinephrine. On the graph, the ordinate are logarithmic ordinate and each point is the mean of levels obtained in various animals.

Sample N° 1 or resting levels are identical in the three situations. The mean value is 1,000 pg/ml.

Sample N° 3 which represents the immediate response to accelerations is very high : 4,500 pg/ml. This value is significantly different from values obtained in the two control situations (1,200 pg/ml).

Sample N° 5, two hours and a half after exposure, the level of + 8 Gz returns to normal, that is identical to levels obtained in the two control groups, i.e. a mean of 1,800 pg/ml.

Figure 2 (b) shows the kinetics of epinephrine.

Resting levels are identical in the three situations with a mean value of 600 pg/ml.

The level of sample N° 3 is 6,200 pg/ml. As for norepinephrine this value is significantly different from that of the two control groups (958 pg/ml). Two hours and a half after exposure (**sample N° 5**) the level is still significantly different from levels of the two control groups. It is as high as 3,200 pg/ml against 1,200 pg/ml for the two control groups.

Figure 2 (c) shows the kinetics of dopamine.

The resting levels of dopamine are identical in the three situations. They reache an average of 120 pg/ml.

Level of sample N° 3 reaches 400 pg/ml, and is significantly different from levels obtained in the two control situations (116 pg/ml).

In **sample N° 5**, level returns to normal and is identical to control groups with a mean of 100 pg/ml.

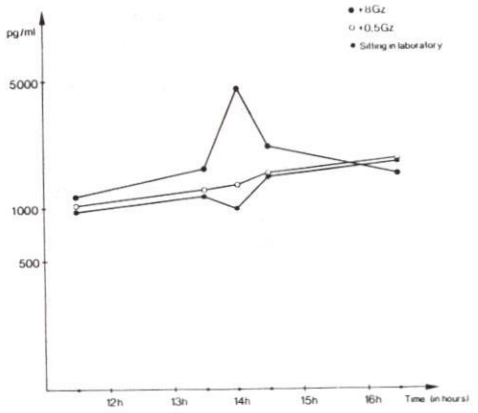


Fig. 2 (a)

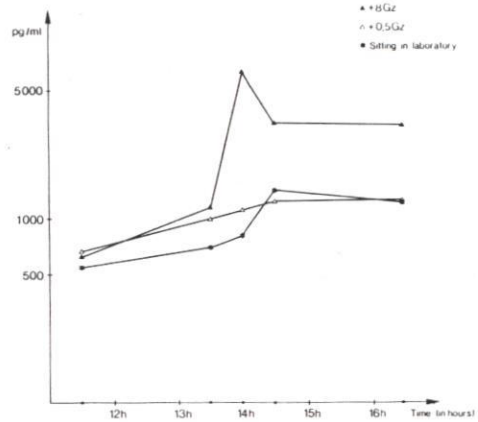


Fig. 2 (b)

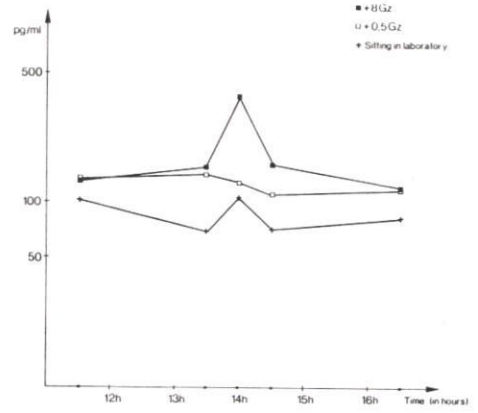


Fig. 2 (c)

Fig. 2 - Kinetics of plasma catecholamines in monkeys exposed to three experimental situations (+ 8 Gz + 0.5 Gz between 13.30 and 14.00, and sitting in laboratory).
a) norepinephrine b) epinephrine c) dopamine

Arterial and venous samplings were collected during 8 to 9 Gz accelerations. Figure 3 (a) shows the kinetics obtained for norepinephrine, epinephrine and dopamine from **venous samples.**

At rest, norepinephrine level 1,300 pg/ml, is higher than epinephrine level, 400 pg/ml. Dopamine level is 120 pg/ml.

Just after centrifuge exposure the epinephrine level is increased by a factor of 6.7 reaching 4,600 pg/ml and becomes higher than norepinephrine level with 3,700 pg/ml. Norepinephrine level is increased by 1.7. Dopamine level is also increased by 1.7 and reaches 250 pg/ml. Figure 3 (b) shows the kinetics from **arterial samples. At rest,** epinephrine level is higher than norepinephrine level respectively with 1,200 pg/ml and 900 pg/ml. Dopamine level is still 120 pg/ml.

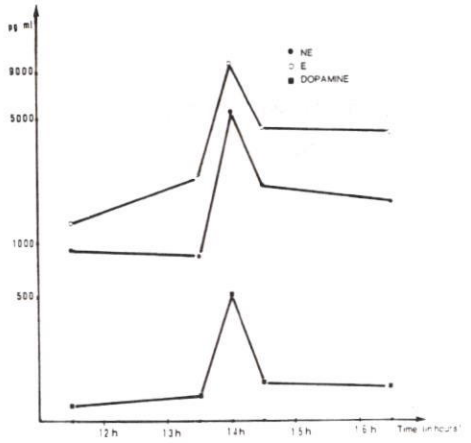


Fig.3 (a)

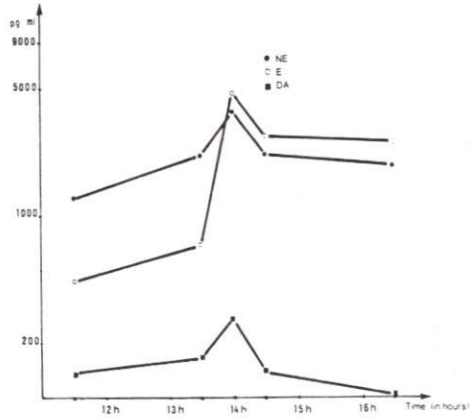


Fig. 3 (b)

Fig. 3 - Kinetics of plasma catecholamines in monkeys exposed to + 8 Gz accelerations between 13.30 and 14.00.
a) venous blood samples b) arterial blood sample.
Immediately after Gz exposure, norepinephrine level is multiplied by 4.7 and reaches 5,400 pg/ml and becomes higher than venous level. Dopamine level is increased 4.3 times and reaches 500 pg/ml and becomes higher than venous levels. Epinephrine level is multiplied by 4.3 and reaches9,700 pg/ml.

DISCUSSION

The comparison of kinetics seen in the three experimental situations demonstrate a specific stress due to + Gz accelerations.

This + Gz stress presents differents components : psychological stress (fear, etc...), blood shift and cardiovascular reaction and contractions of skeletal'muscles.

Epinephrine is only secreted by the centrally controlled medulla. The very high levels of epinephrine, both in venous and arterial blood, immediately and two hours and firty minutes after centrifuge exposure, shows the main role of the medulla.

The norepinephrine comes from the overflow of the sympathetic nerve endings and partly from the medulla. The very high arterial levels of norepinephrine immediatly observed after centrifuge exposure, when the arterial venous difference becomes positive, shows the important response to G-stress. The medulla involvement is probably more important when the animals are not trained to this experimental situation.

CONCLUSION The catecholaminergic response to + Gz exposure is clearly differentiated and shows the generalized sympathicoadrenergic response to a single exposure. The following studies with this animal model will concern the time course of the catecholaminergic response in combination with plasma renin activity, during four months training.

G-INDUCED LOSS OF CONSCIOUSNESS DETECTION LIMITS WITH TRANSCRANIAL DOPPLER MONITORING.

J.M. CLERE, D. LEJEUNE, G. OSSARD, A. LEGER, A. RONCIN*.

Laboratoire de Médecine Aérospatiale,
Centre d'Essais en Vol,
F-91228 Brétigny-sur-Orge Cedex;
* Laboratoire de Biophysique Médicale,
Université F. Rabelais,
F-37032 Tours Cedex; France.

INTRODUCTION.

Present generation high performance aircraft are capable of producing high sustained headward accelerative forces (G) on the human body and achieving these G levels at high onset rates. At their maximum, such performance exceeds the pilot's ability to tolerate the forces, even with current operational life support equipment. Sustained, intensive, air operations and repeated flights per day, further stress pilots by producing muscular fatigue which can further decrease G tolerance. G-induced loss of consciousness (G-LOC) is one consequence of intolerance to G and several fatal mishaps have been reported in aircraft such as the F-15, F-16, and F-18. High performance French combat aircraft like the Mirage 2000 and Rafale also present the risk of such mishaps.

Some aims of human acceleration research are to: (a) improve knowledge of the physiological mechanisms involved in G stress adaptation and G-LOC; (b) increase the efficacy of G counter-measures and develop new methods of anti-G protection; and (c) find methods for G-LOC detection which could be used in aircraft and effect aircraft control in the event of G-LOC. One possible detection method is analysis of cerebral blood flow using Doppler ultrasound technology (Whinnery et al. 1987).

In 1975, Krutz et al. used a Doppler method during G stress to investigate blood flow in superficial temporal artery. They demonstrated that G tolerance can be more accurately evaluated with Doppler than with the traditional techniques used in centrifuges that measure visual field changes. Since then, other groups (Crosbie 1984, Florence et al. 1989) have used ultrasound in physiological investigations of G stress.

To measure G tolerance and regional and systemic cardiovascular responses to G, studies combining Doppler and echographic measurements have begun in the Laboratoire de Médecine Aérospatiale of the Flight Test Center, Brétigny sur Orge in France. A transcranial Doppler unit was developped by the Laboratoire de Biophysique Médicale de la Faculté de Médecine de Tours. During one of our experiments in the human centrifuge, a subject experienced G-LOC without showing evidence of cessation of blood flow in a major cerebral artery. Interruption of cerebral perfusion is regarded as the prime mechanism producing G-LOC. Our observation raises the question about the applicability of Doppler for G-LOC detection, and the limits of Doppler for this purpose is the topic of this paper.

METHODS.

The experiment was conducted on a centrifuge with a 6 m long arm. Three 8 G runs with a 10 sec plateau following a 3 G, 20 sec warm up were performed. The G onset rate was 0.9 G/sec and the subject was instructed to keep his muscles relaxed during the run.

An electronic G-valve controlled by a computer was used to inflate the (ARZ 820) 5-bladder G-suit. Three schedules of G-suit inflation were used which developped the full pressure of 50 kPa in the suit : (a) 2 sec before the beginning of the G plateau ; (b) coincident with the start of the G plateau ; and (c) 2 sec after the beginning of the G plateau. The trials were randomized using double blind techniques.

An electrocardiogram was recorded for heart rate measurement and detection of ECG abnormalities. Visual field was assessed with the traditional light bar technique and the transcranial Doppler device was used to monitor blood flow in the middle cerebral artery (MCA). The probe was positioned against the scalp with a specific headmounted system. The Doppler gate was manually adjusted. The section of the vessel examined was assumed to remain constant and blood flow was calculated by integration of the velocity signal.

RESULTS.

The subject, a 27 yr old male, presented a G-LOC of approximately 3 sec in duration without reporting any peripheral light loss before the G-LOC. It was his third centrifuge run and the G-suit was inflated 2 sec before the beginning of the G plateau. Six seconds of the plateau had elapsed at the time of G-LOC. The centrifuge was stopped immediately. Heart rate of the subject was 85 beats/min before the run and 175 at G-LOC. The latter was similar to what had been observed during the previous 8 G plateau. No ECG abnormality was observed before or during the G-LOC.

The calculated blood flow during G-LOC in MCA was significantly decreased from the blood flow during rest. The systolic volume in the MCA was reduced to 64.5 %. The diastolic volume was reduced to 81.5 %. The systolic and diastolic flow were reduced to 27 and 62 %, respectively. The flow per beat in the MCA was reduced to 41 %.

DISCUSSION.

Spencer et al.(1989), using transcranial Doppler, found complete cessation of blood flow in MCA before G-LOC, an observation consistent with the traditionally regarded physiologic mechanism for G-LOC. In past experiments, we have observed momentary cessation of blood flow in the superficial temporal artery and these were not systematically followed by a G-LOC. The cessation generally occurred during the rapid inhalation phase of the anti-G straining maneuver. Brief periods of aperfusion without subsequent G-LOC is consistent with the notion that cerebral tissue has oxygen stores which maintain consciousness during momentary interruptions of oxygen delivery. We have now observed a G-LOC without cessation of blood flow in MCA. Indeed, blood flow was only marginally less than 41 %.

Therefore, three situations must be considered: (a) G-LOC with cessation of blood flow in MCA; (b) G-LOC without cessation of blood flow in MCA; and (c) short interruption of blood flow in the superficial temporal artery without G-LOC. Possible factors relevant to these phenomena are: (a) different vascularization of different areas of the brain during G, and (b) a threshold for brain ischemia. Xenon-133 clearance studies (Howard and Glaister, 1964) have shown that vascularization of white and grey cerebral matter in man under acceleration stress is different. Blood flow in the occipital area during G could be more reduced than in the middle cerebral artery area and G-LOC could occur without cessation of flow in the MCA. In contrast, uniform vascularization has been observed in the brain of baboons (Greenless et al., 1980), although the G level and G onset rate were lower than in the human studies.

Werchan (1989) has suggested that a threshold for cerebral ischemia exists on the basis of the appearance of EEG manifestations when blood flow was reduced to below 60 %. The blood flow levels in our subject were similary reduced and G-LOC is possible under this theory.

Other explanations are also possible. Quandieu (1989) proposed a theory suggesting that G-LOC is a result of biomechanically-induced dysfunctioning of the brain, related to a high rate of G onset. However, this theory would not explain the presented G-LOC which occured during a low rate of G onset.

The transcranial Doppler method, as used, may also have limitations. 1) MCA may not have an important role in G-LOC and other intra-cranial vessels should be studied. 2) Our calculations of blood flow assumed a constant vessel diameter from 1 G to 8 G. It is uncertain if variations in vessel size occur during G. A reduction in pressure in the MCA during G could produce a reduction in vessel diameter and a reduction of blood flow.

Until some of the points raised in this discussion are further studied, Doppler, with its presently simple signal processing, is unlikely to be accurate enough to be an in-flight detector of G-LOC.

CONCLUSION.

Doppler monitoring during an instance of G-LOC demonstrated reduced, but not completely stopped, middle cerebral artery blood flow. An explanation of the results considered the role of unequal brain vascularization and an ischemia threshold induced by low brain blood flow. Before acceptable as a possible in-flight detector of G-LOC, the Doppler signal will require more complex analysis.

ACKNOWLEDGEMENT.

The authors wish to acknowledge the contributions of Fred Buick Ph D., Head of Acceleration branch, DCIEM, Toronto, Ont., Canada.

REFERENCES.

Clère J.M., Arbeille Ph., Florence G., Marotte H. Tentative d'étude du débit de la carotide interne sous facteur de charge par vélocimétrie Doppler. J.E.M.U., 1989; 9 (3):97-101.

Crosbie R.J. Analysis of transcient response of temporal artery blood flow data to various anti-G suit pressure schedules. AGARD 1984; CP 371:8.1-8.11.

Florence G., Clère J.M., Marotte H., Léger A. Variations of common carotid flow with +Gz stress measured by echoDoppler. Aviat. Space Environ. Med. 1989; 60 (5):128.

Greenless K.J., Yoder J.E., Toth D.M., Oloff C.M. Radioactive microsphere study of cerebral blood flow under acceleration. AFAMRL-TR-80-50,1980.

Howard P., Glaister D.H. The effects of positive acceleration upon cerebral blood flow. J. Physiol. (London) 1964; 171:39P.

Krutz R.W., Rositano S.A., Mancini R.E. Comparison of techniques for measuring +Gz tolerance in man. J. Appl. Physiol. 1975; 38:1143-5.

Quandieu P. Gz et hypoxie! $Gz.s^{-1}$ et hypertension intracrânienne. Méd. Aéro. et Spat. 1989; 28 (111):190-4.

Spencer M., Werchan P., Davis D., McDaniel M., Burton R.R. Transcranial Doppler monitoring of middle cerebral artery flow velocities during +Gz accelerations. Proceedings for 3 rd International Symposium and Tutorial on Intracranial Hemodynamics. 13 Feb.1989, San Antonio, Tx. USA.

Werchan P.M. Physiologic bases of GLOC. 60th Meet. of Aerospace Med. Association. Washington DC. USA, May 7-11, 1989.

Whinnery J.E., Glaister D.H., Burton R.R. +Gz-induced loss of consciousness and aircraft recovery. Aviat. Space Environ. Med. 1987; 58 (6):600-3.

A MATHEMATICAL MODELING OF THE CEREBROVASCULAR SYSTEM

D. GAFFIE, P. LIEBAERT, P. QUANDIEU

O.N.E.R.A.

29, Avenue de la Division Leclerc

92320 CHATILLON - FRANCE

INTRODUCTION

The loss of conciousness in flight of fighter pilots is a phenomenon which cannot be explained by a single physiological interpretation. Indeed, from a mechanical angle, the brain is a complex system at once fluid and solid which encloses different functions and mechanics not always well known.

At the time of a submission to an important JOLT, this mechanical system undergoes a volume force field dependent on time, which produces motions and deformations modifying the stresses of the medium. Therefore, this source of perturbations generates parasitical phenomena which interfere with the usual motions of the cerebrovascular system produced by the action of the heart. The aspect not necessarily reproductible of the GLOC for a same intensity of the rate variation of the acceleration shows that the unsteady phenomena are predominate.

The classical equations of fluid mechanics associed with the knowledge of the physiological behaviours of the system are the starting point of every mathematical analysis of the problem.

PRESENTATION OF THE MODEL

The complexity of the studied problem makes a local analysis impossible. Then, in order to describe the system a compartmental approach is used. The elementary geometry of the latter is a single equivalent elastic tube in which a viscous incompressible fluid flows. This tube is submitted to the simultaneous action of:

(a) - a pressure longitudinal gradient dependent on time.

(b) - an external pressure distribution Pe applied on the lateral wall of the tube.

(c) - a volume force field F strongly dependent on time.

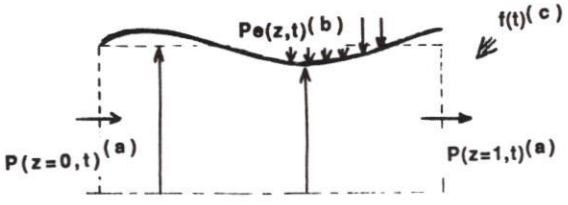


FIGURE (1) : Source of perturbations of the equivalent elastic tube.

The aim of this study will be to realize a comprehensive network to represent the whole of the system, and to observe the influence of different sources of perturbations numerically applied on the global working of the system.

ONE-DIMENSIONAL FORMULATION OF THE PROBLEM

The conservation laws of the mass and the momentum are applied to the fluid passing through an infinitesimal fixed control volume.

- masse : $\operatorname{div} \vec{V} = 0$ (1)

- momentum : $\rho \cdot \frac{d\vec{V}}{dt} = \operatorname{div} \sigma + \rho \vec{f}$ (2)

where $\vec{V}$ is the fluid velocity, $\vec{f}$ is the body force per unit mass. Body forces act at a distance and apply to the entire mass of the fluid (gravitational force, for example). div σ representes the surface forces per unit volume. These forces are applied by the external stresses (components of the stress tensor σ) on the fluid element. The stress tensor is frequently separated in the following manner:

$$\sigma = P.1 + \mathbf{P} \qquad (3)$$

where p is the pressure and $\mathbf{P}$ the viscous stress tensor. Equation (3) has to be completed by the data of the behaviour law of the fluid.

ASSUMPTIONS OF THE MODEL

In some particular flow cases, it is permitted to neglect certain terms of the local form of the equations.

Subsequently, we suppose that:

- the Reynolds number is not too large

- the flow has a main direction

For conveniency, we let:

ε = (RADIUS OF THE TUBE)/ (LENGTH OF THE TUBE) << 1

An analysis of the order of magnitude of the different terms of the equations with respect of ε allows us to deduct a simplified form of the system of equations. After that, the latter are integrated over any cross section of the tube to obtain the following one-dimensional formulation (c.f. Gaffie (1985)) :

$$\begin{cases} \dfrac{\partial U}{\partial t} + (1-\alpha)\dfrac{U}{S}\dfrac{\partial S}{\partial t} + \alpha U \dfrac{\partial U}{\partial z} + \dfrac{1}{\rho}\dfrac{\partial (P-P_e)}{\partial z} = \dfrac{1}{\rho}\dfrac{P_S}{S}\,\tau_p - \dfrac{1}{\rho}\dfrac{\partial P_e}{\partial z} + f_z \\ \dfrac{\partial S}{\partial t} + \dfrac{\partial}{\partial z}(SU) = 0 \end{cases} \qquad (4)$$

where S is the cross-section of the tube, U the mean velocity, P -Pe the transmural pressure, Tp the mean value of the shear stress component at the wall and α a corrective term of the momentum.

The determination of the two last quantities as a function of the other unknowns of the problem requires the introduction of a third and important assumption on the form of the longitudinal velocity profile:

$$Vz = U(z,t) \cdot f(r / R) \qquad (5)$$

where f is an analytical non-dimensional radius dependent function consistant with the boundary conditions and the definition of the mean velocity.

STATE LAW

The transmural pressure which appears in the equations (4) is related to the cross-section of the tube by a state law called "tube law". It is given in the following form:

$$P - Pe = K(z) \cdot T (S/So) \qquad (6)$$

where K is the rigidity of the tube dependent on the longitudinal direction, and T an analytical non-dimensional section dependent function. The figure (2) shows a graphical representation of the function T obtained experimentally.

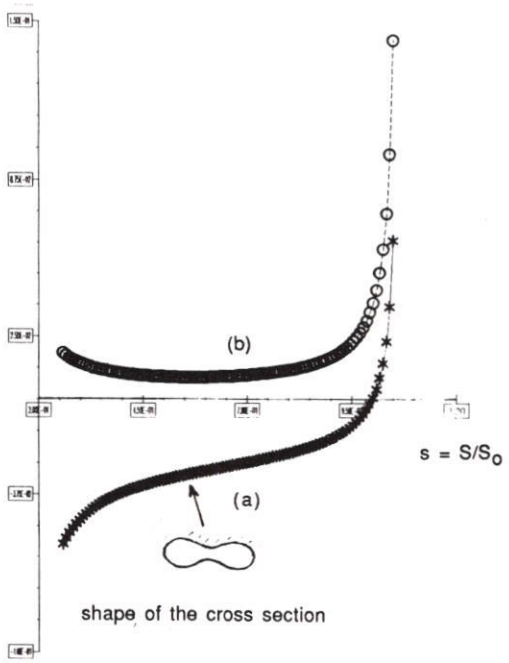


FIGURE (2) : Graphical representation of the tube law (a) and the velocity of the waves (b)

An interesting property of T is its nonlinear character which produces a strong dependency of the section on the speed of the waves propagating along the tube. Focusing of waves can appear to create shocks of transition described by Kamm and Shapiro (1979)

RESULTS AND CONCLUSION

The first calculations realized on the simplest case of a single equivalent tube have shown the existence of unsteady and nonlinear phenomena which can produce important perturbations of the flow as represented in figure (3).

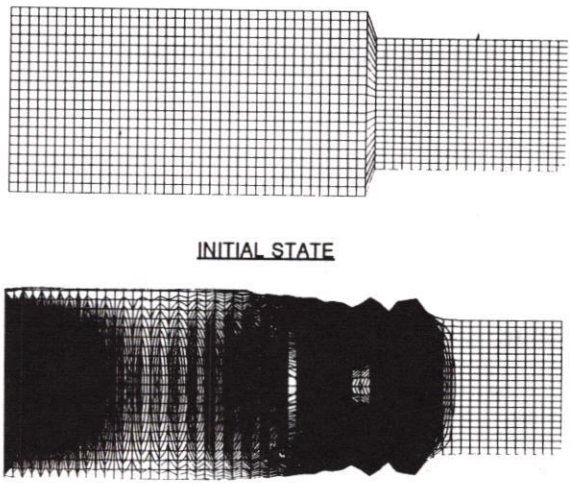


FIGURE (3) : Collapsus of the tube and propagations of waves created by the simultaneous action of surface or volume forces dependent on time and boundary conditions.

At the present time, this model is in validation for simples cases of flows. The next step of the study will be to extend the application of the model to a detailed network of equivalent tubes in order to analyse the effects of simultaneous perturbations on the behaviour of the cerebrovascular system.

REFERENCES

GAFFIE D. (1985)
"Modélisation d'un fluide visqueux incompressible dans un tube elastique"
Doctorat, Université Paul Sabatier - Toulouse.

KAMM D.K. SHAPIRO A.H. (1979)
"Unsteady flow in a collapsible tube subjected to external pressure or body forces"
J. Fluid Mech. 95, 1-78.

ULTRASOUND TECHNOLOGY and SPACE CARDIO-VASCULAR MEDICINE.

Ph . ARBEILLE , J M. POTTIER, F. PATAT, M. BERSON , A . RONCIN ,L. POURCELOT.

Unité INSERM 316 - C H U Bretonneau - 37044 -TOURS FRANCE

I Ultrasound methodology for cardiovascular studies :

Ultrasound methods allow the heart to be explored as well as the deep circulation (Aorta - Renal A) or the peripheral circulation (Carotid-Femoral A). The B mode imaging displays all the solid structures crossing the ultrasound beam generated by the transducers (fig 1). The structures which strongly reflect the ultrasound energy appear as bright points of high intensity. The liquids transmit all the ultrasound wave and appears in black on the image. Because a great number of beams are generated each second by all the transducers juxtaposed along the linear array probe or by the oscillating transducer of the sector scan probe each incidence is displayed in real time and the movements of the organs are clearly identified (cardiac contractions).

The heart chambers are visualized in real time by "B mode" imaging and the variations of their diameters can be evaluated by Time Motion mode (fig. 1). From this data the main cardiac parameters are calculated according to specific models necessary to extrapolate the chamber volume (ventricle) from one diameter. Most of the time the Teicholtz ellipsoidal model is used. This mode of calculation has been tested and proved during clinical and physiological studies and is routinely used in cardiology. On the basis of this formula we calculate the left ventricle volume in systole (LVSV) and in diastole (LVDV), the difference giving the stroke volume (SV). The cardiac output (CO) is obtained by multiplying the stroke volume by the heart rate, the ejection fraction (EF) is equal to the stroke volume divided by the diastolic volume. It is generally accepted that the precision of the results is no more than 10% .

A Doppler system integrated in the same device allows to record the blood velocity in different vessels or within the heart. Use of the Doppler effect consists in measuring the difference between the frequency of an incident ultrasound wave (F) and the frequency of the ultrasound waves backscattered by the particles in movement (F + dF). The frequency shift dF is proportional to the velocity (v) of the particles (Red cells) according to the following formula : $dF / F = 2 v \cos O / c$, with (c) the celerity of the ultrasounds and O the angle between the vessel axis and the Doppler beam (Fig 2). The continuous waves (CW) Doppler generates and receives continuously ultrasound beams and measures the Doppler effect produced by any particle in movement crossing the beam at any depth. This mode can be used only when the anatomy makes us certain to identify the vessel of interest (Superficial vessel : Carotid, Jugular ...). The pulsed Doppler (PW) generates short ultrasound pulses of some micro seconds and collect the backscattered waves only after a preselected lenght of time. In this case only the Doppler effects produced at a certain depth (within the Doppler sample volume) are recorded .

On the Duplex systems the Doppler beam axis is displayed on the B mode image as a bright dotted line, the extent of the Doppler sample volume along this line is delimited by two short segments (Fig 3). Therefore the Doppler recording can be performed selectively at any preselected depth within the body. This possibility is particularly useful for the exploration of deep thoracic and abdominal vessels (Renal artery and vein , Pulmonary A...) or for the identification of intracardiac flows at the level of the valves. For the exploration of these deep vessels it is recommended to use frequencies of 3 - 3.5 Mhz. By choosing a frequency adapted to the exploration of the superficial areas (5-7.5 Mhz) the system can be used for the assessment of the carotid, jugular or femoral flows .

The Doppler signal includes all the elementary Doppler frequency shifts due to all the red cells going through the vessel section insonated by the ultrasound beam. Therefore it becomes necessary to display this signal through a fast Fourrier transform analyser in order to obtain the distribution of the velocities (frequencies) among all the particles present within the section explored. The spectral analysis displays the signal separatly from the background noise, which significantly increases the accuracy of the measurements on the spectrum and provides information on the velocity profile. Finally from the ultrasound data we calculate the blood flow value (ml/mn) in the Carotid and the Femoral arteries and the Vascular Resistance in the different areas supplied by the vessels explored (Renal resistance - Inferior limbs resistance...). The assessment of the Cerebral blood flow requires a special Doppler system with a lower frequency (2 Mhz) and a higher energy than the one previously described because of the strong attenuation of the ultrasonic beam due to the thickness of the skull. The cerebral vascular resistances as well as the cerebral blood flow can be evaluated by transcranial Doppler . During all the ultrasonic sessions , measurements of the ECG and the Blood Pressure are

performed .
The vascular haemodynamic data are obtained from both the image and the Doppler traces. For the blood flow quantification we have to measure the vessel diameter and the angle between the Doppler beam and the vessel axis (on the image), and the mean frequency of the Doppler spectrum. For vessels of about 6 to 8 cm in diameter the precision is no more than 15%. In order to evaluate the vascular resistance changes in different areas we use a Doppler resistance index which expresses as R=S-D/S with S and D the systolic and D the diastolic amplitudes. Clinical and physiological studies have demonstrated that the variations of this parameter are closely linked with the vasomotor changes (vasoconstriction : R increases). When there is no forward diastolic flow within the vessel but a reverse flow (Femoral. A) we use an other index also correlated with the vascular resistance changes : R=D/S , with S the systolic amplitude and D the amplitude of the reverse flow .

II Ultrasound facility on board space vehicles :

Since 1982 we have been involved in several inflight cardiovascular ultrasound experiments in collaboration with the Soviets and the American Space Agencies : 1982 : 7 days flight onboard Saliout VII station -1984 : 237 days flight on board Saliout VII station - 1985 : 7 days flight onboard the Space Shuttle Discovery - 1988 : 30 days flight onboard MIR station. In 1985 the Nasa JSC Space Physiology Department has carried out an ultrasound cardiovascular experiment during a 7 days flight onboard the Space Shuttle Challenger .
For these experiments different ultrasound space facilities have been developed : A first generation space Echograph for cardiac and superficial vessels studies (1.3.4.5) was built up by Matra from a prototype developed in our ultrasound engineering department (1981). Several ultrasound functions were available on this device : a "B.mode" (real time imaging system) using linear arrays of 5 Mhz and 3.5 Mhz with electronic focusing, a "Time Motion mode" (3,5 Mhz pencil probe), a 4 Mhz "continuous Doppler" (pencil probe) and finally a "Duplex mode"coupling imaging and CW Doppler modes and using a 5 Mhz linear array with preoriented Doppler sensors at one of the extremities. Ultrasound data were stored on video tapes. With this first generation Echograph it was possible to study : the left ventricle function by measuring on the M mode, the ventricular dimensions the stroke volume, the cardiac output and the ejection fraction. The blood flow value and the vascular resistance on the peripheral areas (carotid, femoral) were evaluated by using the duplex probe. An ADR commercial machine was modified and adapted to space environment requirements (Nasa JSC program 1985) for cardiac studies during short term flights on board the space shuttle (2).This machine used one mechanical sector scan transducer (3 Mhz) for B mode, and Time Motion mode. A second generation Echo-Doppler system "As de Coeur" for cardiac, deep and superficial circulation studies was developed in our lab and built up by Matra (1988). This new device includes 2 Duplex modes using imaging curved arrays of 5 and 3.5 Mhz, the PW Doppler and the M mode beam being generated perpendicular to the surface of the array by any group of transducers. The maximum depth explored by the pulsed Doppler is 16 cm, the Doppler sample volume is adjustable from 0.5 to 2 cm. Time motion, Pulsed and Continuous Doppler pencil probes can be connected to the device. A transcranial pulsed Doppler (2 Mhz) is available for the intracerebral flow studies. All the Doppler informations are displayed through a high resolution fast Fourrier real time analyser. Other functions like the automatic blood pressure measurement, the evaluation of the central venous pressure (by venous stop flow method), and ultrasound phlethysmography have been integrated into the machine . All the information provided by the different modes are stored on video tapes, but can be transmitted to the ground by down-link video transmission. With the second generation echograph the main cardiovascular parameters can be assessed: the left and right heart dimensions, the stroke volume and cardiac output, the pulmonary flow and resistance, the intracardiac flows (Mitral,Tricuspid flows), the venous return (Inferior vena cava, Sus hepatic vein), the Renal circulation (Artery & vein), the peripheral flows (Carotid, Jugular, Femoral...) and the intracranial haemodynamics.

III Main Inflight Experiments and Results :

During Short term flights an increase in the Left Ventricle Diastolic Volume (LVDV) and in the Cardiac Ouput (CO) are commonly observed during the early exposure to 0g . These parameters recover within some hours or some days depending on the subject and tend to stay below the basal value during the rest of the flight. In addition no deterioration of the heart contractility is noticed. These observations suggest that ther may be a hypovolemia when the astrononaut is considered to have adapted to weightlessness. The cerebral flow is very well stabilized by the adjustement of the cerebral vascular resistance even when the cardiac output is significantly increased or decreased. On the inferior limbs the regulation is not so sensitive and the femoral flow changes, as does the cardiac output . The assessment of the inferior limbs vascular resistance and the vascular response to dynamic tests (effort test) provides information on the space vascular deconditioning. Post flight abnormal femoral flow response was associated with the development of an orthostatic intolerance syndrom.
During Long term flight only three astronauts were investigated by ultrasound. The Left Ventricle Diastolic Volume was significantly decreased for two of them

during the whole of the flight. The regulation of the cerebral blood flow was not observed (CBF decreased) and the inferior limbs resistance decreased. 30 days after landing the vascular parameters had not completely recovered .

Several ground Og simulations by Head Down Tilt (HDT) with ultrasound cardiovascular studies have been carried out. Initially the cardiovascular effects of the antiorthostatic position were assessed on volunteers at rest. In this case the cardiac parameters showed variations comparable to the one observed in flight but of weaker amplitude. More recently H D T simulations were used to study the vascular effects of dynamic tests such as L B N P or Fluid Loading and to check the efficiency of these tests when used chronicaly as space deconditioning countermeasures.

Finally the Ultrasound methods have enabled the early cardiovascular adaptation to 0g exposure to be determined and haemodynamic parameters to be validated for the assessment of the vascular deconditioning. The interest of these results will be emphasized when compared to the hormonal data involved in the regulation of the volemia and the hydroelectrical data. Moreover the possibility of following up quite continuously and atraumaticaly the inflight cardiovascular modifications will enable the different prophylactic countermeasures to be tested in order to make them more efficient.

REFERENCES

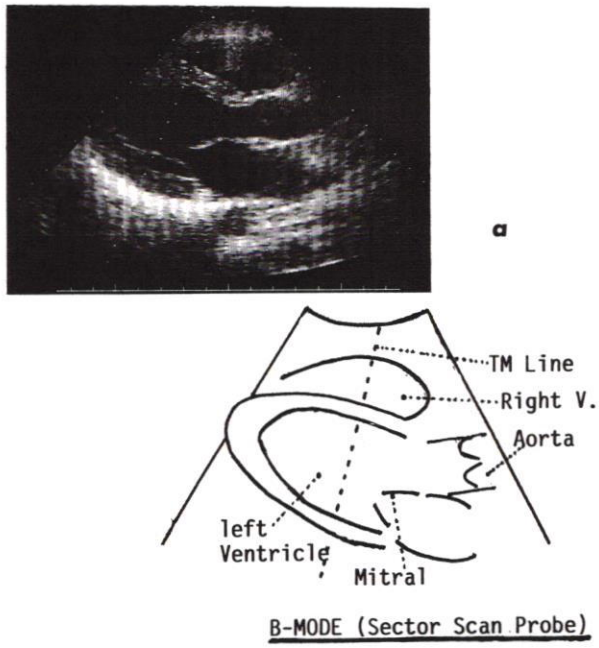
1 - ARBEILLE Ph, POTTIER JM, PATAT F, BERSON M, RONCIN A, LE TOULLEC Ch, MIGNE P, POURCELOT L, KOTOVSKAYA A, ATKOV O, STROGONOVA L, FOMINA G, KOKOVA N.
Cardiovascular adaptation to zero-G during a long- term flight (237 days) on board the Salyut-VII soviet space station in 1984 . E.S.A , S P - 271, pp. 134-146. 1987 .

2 - BUNGO HW, CHARLES JB.
The human cardiovascular system in the absence of gravity. I.A.F - 135. 1985.

3 - POTTIER JM, ARBEILLE Ph, PATAT F, et al.
Comparative study of the cardiovascular adaptation to zero g during 7 days space flights.
The physiologist 1988, suppl. vol 31, N°1.1988 .

4 - POURCELOT L , ARBEILLE Ph, POTTIER JM, PATAT F, MIGNE P, GUELL A, GHARIB G.
Ultrasonic study of early cardio vascular adaptation to Zero gravity. E.S.A SP - 212. 119-123. 1984 .

5 - POURCELOT L, ARBEILLE Ph, POTTIER JM, PATAT F, BERSON M, RONCIN A, LE TOULLEC Ch, GUELL A, GHARIB C.
Cardiovascular examination during STS 51 G Mission June (1985).
E.S.A. SP - 237. 3-17 ; 1985 .



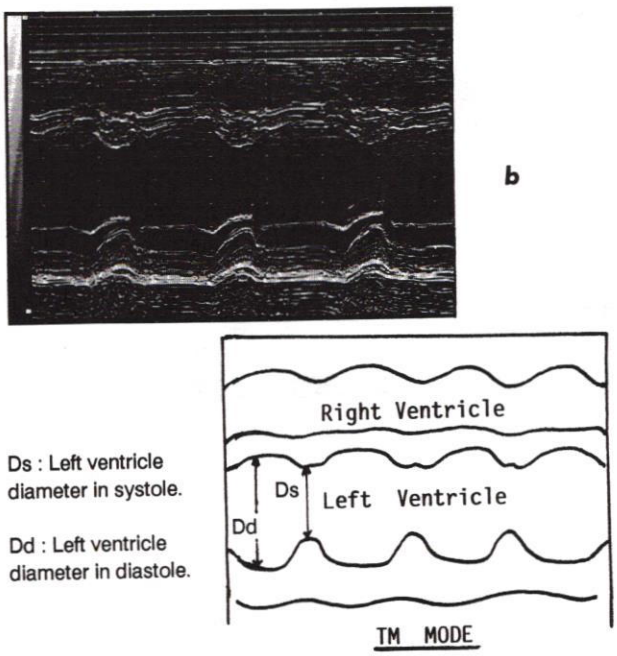


Fig.1 - a) Long axis view (B mode) of the heart , the bright dotted line on the image represents the direction of the ultrasound beam during the time motion sequence.

- **b)** Left and right ventricle wall movements displayed in time motion mode .

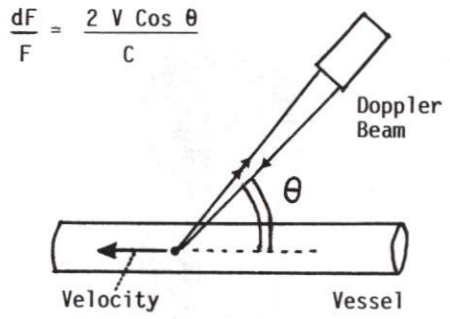
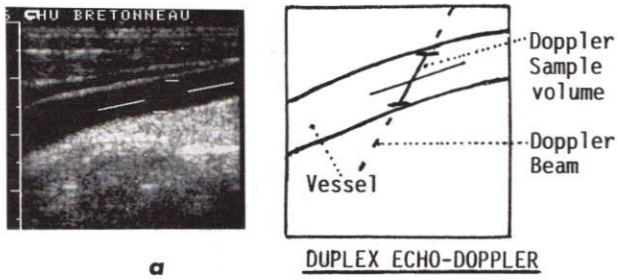
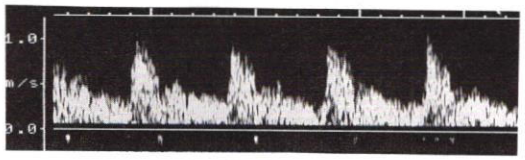


Fig. 2 - Relationship between the Doppler frequency shift (dF) and the velocity (v) .



a



b

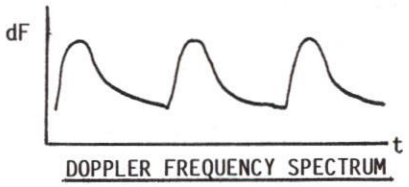


Fig. 3 - a) Longitudinal cross section of the carotid artery,the bright dotted line represents the Doppler beam direction. the doppler sample volume is delimited by the small segments on this line.

- **b)** Spectral analysis of the Doppler signal: Doppler frequencity shifts (dF) on the vertical axis, time on the horizontal axis.

EFFECTS OF A 10 - DAY PERIOD OF 6° HEAD DOWN TILT (HDT)

F. Baisch[1], L Beck[1], H. Maass[1], M. Heer[1], G. Plath[1], G. Blomqvist[2], A. Gaffney[2], J. Buckey[2], A. Hillebrecht[3], H. Schulz[3], M. Meyer[3], J. Kropp[4], D.J. Ten Harkel[5], J. Karemaker[5], F. Patat[6], PH. Arbeille[6]

[1] DLR -Institute for Aerospace Medicine, Cologne, FRG
[2] University of Texas, Southwestern Medical Center, Dallas, Texas, USA
[3] Max-Planck-Institute for Experimental Medicine, Göttingen, FRG
[4] Universität Bonn, Nuklearmedizin, Bonn, FRG
[5] Department of Physiology, Academic Medical Centre, Amsterdam, NL
[6] Université François Rabelais, Department of Medical Biophysic, Tours, F

Introduction

An international collaborative project, initiated by the DLR-NASA Life Sciences Working Group, led to the performance of an HDT study at the DLR Institute for Aerospace Medicine. Scientific and operational questions were addressed in preparation of the D-2 spacelab mission.

Principal areas of interest were cardiovascular regulation and fluid and electrolyte metabolism and control. Following fields were subject to specific examinations:

- Fluid and electrolyte turnover (see contribution M. Heer et al.)
- Compliance of the lower limb (see contribution J.C. Buckey et al.)
- Adrenergic receptors (see contribution H. Maaß et al.)
- Cardiovascular responses to intravenous fluid load (see contribution F.A. Gaffney et al.)
- Hemodynamic responses to LBNP (see contribution L. Beck et al.)
- Renal flow during fluid loading and LBNP (see contribution Ph. Arbeille et al)
- Carotid baroreceptor-cardiac reflex (see contribution D.L. Eckberg et al.)
- Blood pressure variability (see contribution D.J. ten Harkel et al.).

This contribution deals mainly with methodology and the long term adaptation. The study plan with the above mentioned interventions is depicted in figure 1.

Methods

The study group consisted of six normal male subjects with a mean age of 26 years and maximal oxygen uptake values of 42.5 ml/min/$kg_{body\ weight}$ (see Table 1). They all participated as volunteers and signed an informed consent form. The study plan was approved by the D-2 Medical Board in accordance to the rules of the NASA Human Research Policy and Procedure Committee.

6° head down tilt was chosen to produce redistributions of body fluids and adaptations in many organ systems similar to the phenomena which are expected to occur in microgravity [1]. During a baseline data collection period, a ten day period of continuous head down tilt and during six days of recovery, acute interventions were performed. Some of them caused changes in the magnitude and distribution of intravascular volume (fig. 1). Leg volume was measured utilizing both a circumferential and an ultrasound based method. Electrolytes were analyzed with flame-photometric technique emphasizing high accuracy up to a level better than 1%. Plasma and blood volume were calculated by determining the red cell mass by the ^{51}Cr method and measuring continuously the hematocrit value. Four segments of electrical body impedance were measured with the tetrapolar technique. Heart rate and blood pressure were determined by the finger plethysmographic method [6] (Finapres®). Pulmonary blood flow was measured with the acetylene rebreathing technique [7] at rest and during acute interventions in the three phases the study.

HDT '88 Test Subjects	dim	mean ± SD
age	years	26.0 ±4.4
length $_{body}$	m	1.76 ±.05
surface $_{body}$	m^2	1.87 ±.16
$mass_{body}$	kg	72.0 ±12
volume $_{blood}$	ml / kg	75.8 ±2.8
metabolism $_{basal}$	$kJ/m^2/h$ {$kcal/m^2/h$}	158.0 ±2.9 {38.0 ±0.7}
O_2 uptake $_{max}$	ml/min/kg	42.5 ±5.4

Table 1: **Test subject profile**

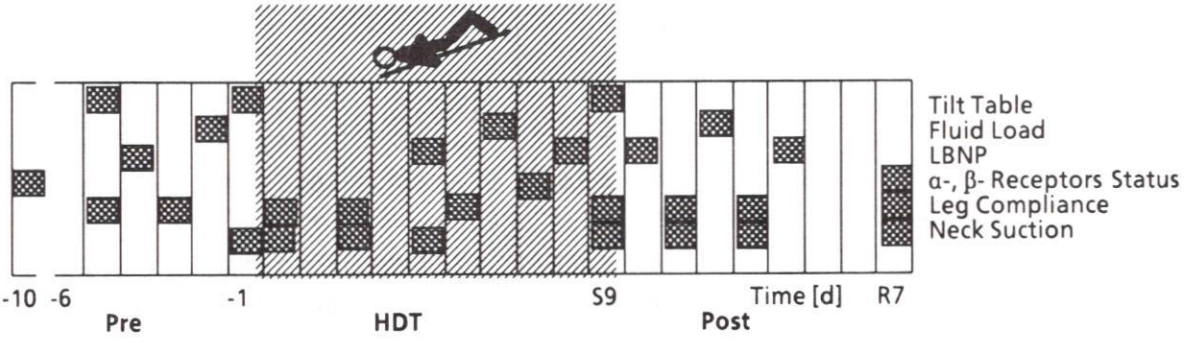


Figure 1: Interventions during the **HDT '88** study

10 days HDT: Shaded area

During the baseline data collection period it was also measured in different body positions. Total peripheral resistance was calculated as the quotient between mean arterial pressure and cardiac output.

Results and discussion

Table 2 offers an overview of the results described here. The period of 10 days continuous tilt with head down produced a body fluid redistribution [3]. During the first two days the leg volume measured at the belly of gastrocnemius decreased 6 % on average. A phase of a slower progressive volume loss followed. During the remaining days of head down tilt, the leg volume decreased up to 10%. During the first recovery day it increased 4 % and during the rest of the recovery period no significant increase could be observed. The leg volume remained below baseline levels as late as seven days after the end of the period of continuous tilt. The decrease at the beginning of the ten day period of head down tilt, had the same proportions as the increase after termination of it. The phase of the slower progressive volume loss at a relatively constant rate during the entire phase of head down tilt were accompanied by a constant loss of potassium [4]. This support the assumption that skeletal muscle mass was reduced during this phase of the study. The changes in leg volume were paralleled by the time course of the changes in total body weight. At the end of the immobilization 1.5 kg body weight were lost.

Head down tilt reduced the plasma volume by an average of 16%. The corpuscular volume of the red cells was also reduced. The blood volume reduction after two days of continuous tilt came up to 18% on average. During the rest of the ten days no further changes were observed.

Day by day measurements of segmental electrical impedance demonstrated significant changes only in the lower part of the body. The variations in the impedance of the torso segment were insignificant. The scatter of the respective values was around twice as big compared to the anthropometric method of limb volume measurement. Neither a rapid increase at the beginning of head-down tilt nor a decrease at the start of the recovery phase could be detected.

The observations of resting heart rate demonstrated no significant changes during the period of head down tilt. During the first three days of recovery there was a tendency for heart rate to increase.

The cardiac output measurements by acetylene rebreathing technique demonstrated a decrease during the HDT phase. Cardiac outputs measured at the end of HDT period were as high as the values obtained in an upright posture during baseline data collection. The cardiac output values computed with a relatively constant blood pressure level during all phases of the study yielded an increase in total peripheral resistance in the late HDT phase.

Change and magnitude of the variation from blood volume, electrolyte and anthropometric measure- ments [2,3,5] support the concept that head down tilt and microgravity produce similar effects on body fluid compartments.

References:

1. BLOMQVIST, C. G., AND H. L. STONE. Cardiovascular adjustments to gravitational stress. In: *Handbook of Physiology , Section 2, The Cardiovascular Systems, Vol. III.* 1025-1063, 1983.
2. JOHNSON, P. C., T. B. DRISCOLL, AND A. D. LEBLANC. Blood volume changes. In: *Biomedical results from skylab.* NASA SP-377. p235 - 241, 1977.
3. LEACH, C. S. A review of the consequences of fluid and electrolyte shifts in weightlessness. *Acta Astronautica.* 6: 1123-1135, 1979.
4. LEONARD, J. L., C. L. LEACH, AND P. C. RAMBAUT. Quantitation of tissue loss during prolonged space flight. *Am J Clin Nutr.* 38 (11): 667-679, 1983.
5. MOORE, T.P., E. WILLIAM, AND E. THORNTON. Space shuttle inflight and postflight fluid shifts measured by leg volume changes. *Aviat. Space Environ. Med.* 58 (9): A 91-96, 1987.
6. PEÑÁZ, J. Photoelectric measurement of blood pressure, volume and flow in the finger. *Digest 10th Int. Conf. Med. Biol. Engng.,* 104, Dresden, 1973
7. PIIPER, J. AND P. SCHEID, Model for capillary-alveolar equilibration with special reference to O_2 uptake in hypoxia. *Respiration Physiology* 46: 193-208, 1981.

Measurements		BDC mean ± SD	48 h HDT mean ± SD	end of HDT mean ± SD	1st d recovery mean ± SD	6th d recovery mean ± SD
Leg Volume	(%)	100	94.2 ± 2.3	90.1 ± 2.7	93.5 ± 2.7	92.8 ± 2.6
Blood Volume	(litre)	5.45 ± 0.86	4.74 ± 0.48	4.73 ± 0.65	5.22 ± 1.06	5.48 ± 0.93
Plasma Volume	(litre)	3.06 ± 0.43	2.56 ± 0.23	2.57 ± 0.30	3.02 ± 0.55	3.19 ± 0.51
Cumulative Potassium	(mmol)	0	-24.9	-99.4	-76	-45
Segmental Impedance	(litre)					
Torso		11.7 ± 0.9	12.4 ± 1.7	11.2 ± 1.9	12.3 ± 2.2	12.4 ± 2.6
Thigh		5.9 ± 1.9	5.2 ± 1.6	4.8 ± 1.4	4.7 ± 1.4	4.7 ± 1.4
Calf		1.8 ± 0.5	1.4 ± 0.6	1.2 ± 0.5	1.4 ± 0.5	1.4 ± 0.5
Heart Rate	(beats/min)	57.5 ± 5.1	58.3 ± 3.5	57.3 ± 3.7	61.3 ± 4.8	57.0 ± 3.2
Mean Blood Pressure	(mmHg)	71 ± 4	79 ± 5	76 ± 4	72 ± 8	71 ± 4
Pulmonary Blood Flow	(l/min)	6.8 ± 1.3	6.5 ± 1.0	5.6 ± 0.6	6.2 ± 0.8	5.8 ± 1.0
Total Periph. Resist.	(dyn•s/cm^5)	895 ± 155	1047 ± 133	1059 ± 117	936 ± 164	994 ± 255

Table 2: **Results overview**

EFFECTS OF 10 DAYS HDT ON FLUID AND ELECTROLYTE METABOLISM

M. Heer[1], C. Drummer[2], F. Baisch[1], R. Gerzer[2], H. Maass[1], G. Blomqvist[3]

[1] DLR -Institute for Aerospace Medicine, Cologne, FRG
[2] Universität München, Innenstadtkliniken, München, FRG
[3] University of Texas, Southwestern Medical Center, Dallas, Texas, USA

Introduction:
Head-down-tilt (HDT) and exposure to microgravity appear to produce similar changes in body fluid and electrolyte distribution, including loss of plasma volume (4,5). Changes in water balance can be caused by changes in dietary sodium intake (7,8) and variations in dietary protein intake (2). But no data have been previously collected under strictly controlled conditions. Therefore the test subjects were provided with a diet, which kept the daily water, protein and sodium intake constant.
We investigated the fluid and electrolyte turnover in the head down tilt position. Additionally, the impact of an acute intravenous saline load on the water and sodium turnover was determined in the prephase, the HDT phase and the recovery phase, respectively. Other aspects of the study have been described by Baisch et al..

Methods:
During the 22 days study period the test subjects were provided with a standardized diet, including a daily water intake of 40 ml/$kg_{body\ weight}$ and a daily sodium intake of 2.2 mmol/$kg_{body\ weight}$. Urinary volume and the excretion of electrolytes and cyclic GMP were measured over the whole study from 12 h samples. Caloric intake during the HDT period was 10 % above basal corresponding to about 2000 kcal/day. During the prephase and recovery periods it was 30 % above basal metabolism (about 2600 kcal). Basal metabolism was calculated from body surface, sex and age according to Fleisch (3). The body weight of the test subjects was monitored every 12 hours. During the prephase (-02), the HDT phase (S06) and the recovery phase (R02), each volunteer received an intravenous infusion of 0.9% NaCl (22ml/$kg_{body\ weight}$) within 20 minutes. This amount of 1500 ml saline solution on average was given in addition to the daily oral water intake. During three hours after these fluid load experiments, urine flow and the urinary excretion of sodium and cyclic GMP were determined as well as the plasma levels of atrial natriuretic peptide (ANP) and cyclic GMP.
Water and sodium balances were calculated from the oral water and sodium intake, metabolic water and saline infusions, from the renal excretion and evaporative water losses. The evaporative water losses were estimated according to Davidson and Passmore (1). Cumulative balances were determined by adding up the daily balances.

Results:
Figure 1 shows the mean changes of body weight and mean body water loss of the six test subjects. The

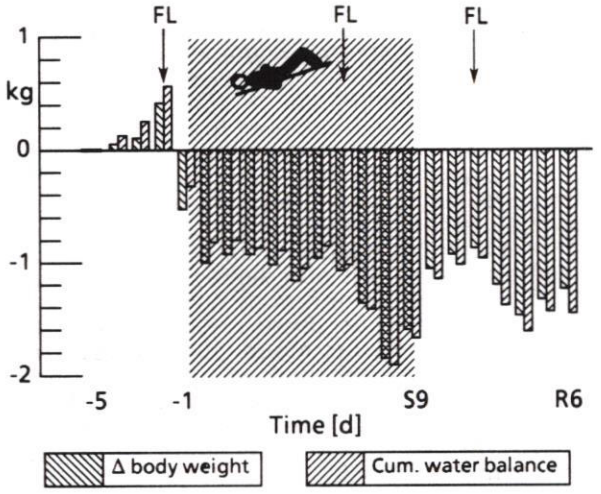


Figure 1: Comparison of mean body weight changes and cumulative water balance. Shaded area = HDT

posture change to HDT caused a mean weight change of 1.0 kg body weight. After termination of the HDT phase the volunteers regained about 0.6 kg. At the end of the study the body weight had diminished by about 1.24 ± 1.03 kg (mean ± SD).
Two days after the HDT and the recovery fluid load experiment (days S08 and R04) body weight had decreased by 0.7 kg on average. A comparable effect seems to occur after the first fluid load experiment but the posture change to prolonged HDT two days after the i.v. infusion caused an interference.
The cumulative water balance revealed a body water loss of 1.40 ± 0.24l (mean ± SD) after the posture change to HDT. After retilting 0.94l ± 0.25l SD body water was restored. At the end of the study the cumulative water balances revealed an absolute water loss of 1.46 ± 0.15l (mean ± SD).
The i.v. infusion provoked positive water balances on the days -02, S06 and R02 and resulted in net water losses of 0.7 liters on average 48 hours after the HDT- and recovery infusion experiment. The water loss after the prephase infusion was interfered by the tilting maneuver.

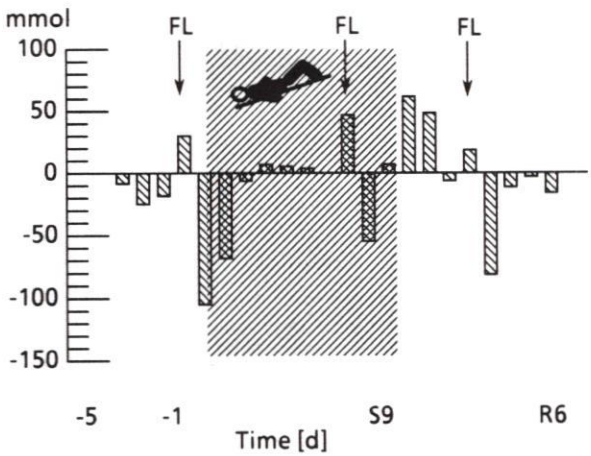


Figure 2: Mean sodium balance

After tilting to HDT a mean sodium loss of 120 mmol was observed. Again the opposite effect, the restoration of about 100 mmol sodium occured at the beginning of the recovery phase. At the end of the study the cumulative sodium balance revealed a sodium loss of about 180 mmol.
The rapid infusion of saline solution caused a positive sodium balance on the days of infusion, whereas the balance became negative during the following 24 hours (fig. 2). The prephase infusion resulted in an absolute sodium loss that could not exactly be separated from the tilting effect.
Urinary volume and sodium excretion (fig. 3 and 4) were increased for more than 48 hours after each fluid load experiment. Urinary excretion of cyclic GMP also increased following the same time course. In contrast, plasma ANP only slightly increased at the end of the rapid i.v. infusions and were back to normal 60 minutes later. Plasma levels of cyclic GMP were significantly increased 90 minutes after infusion onset and also back to normal one hour later. There was no difference in plasma ANP and plasma cyclic GMP responses between the prephase, the HDT-phase and the recovery fluid load experiment, respectively.

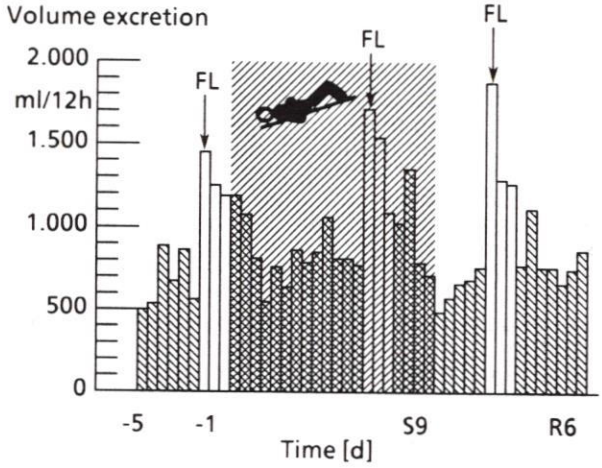


Figure 3: Mean urinary volume excretion. The open bars show the effects of rapid intravenous infusion on volume excretion

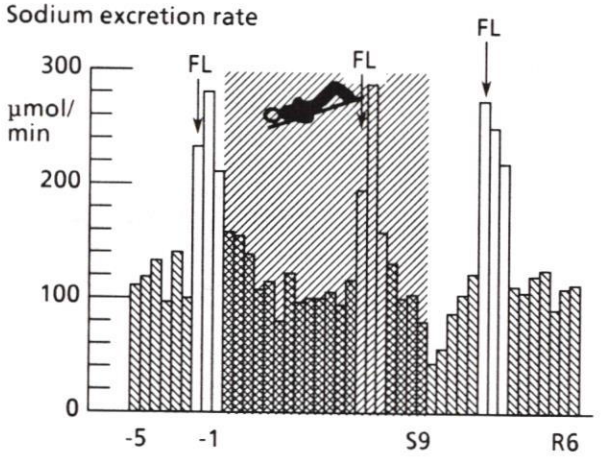


Figure 4: Mean urinary sodium excretion. The open bars show the effects of rapid intravenous infusion on sodium excretion

Discussion:
Changes in total body weight during and after HDT conformed to the expected pattern (4,5,6). Caloric intake was strictly controlled and the weight loss was most likely caused by a fluid loss. Furthermore, the rapid i.v. infusion of about 1.5 litres of isotonic saline solution, independently of the study phase, always caused increases in urine flow and sodium excretion for more than 48 hours and resulted in absolute losses of body water and sodium.
The mechanisms for the prolonged diuretic and natriuretic responses after the acute saline infusions cannot be explained by the present study. The shortterm increases in plasma levels of ANP and cyclic GMP might be an argument against the primary role of the cardiac hormone and its second messenger in the blood volume regulation. However, since urinary cyclic GMP excretion and in parallel the excretion of sodium was increased for more than 48 hours the present data suggest that renal cyclic GMP plays a role in volume loading-induced diuresis and natriuresis.
HDT provokes a fluid redistribution that includes an increase in central blood volume. Rapid i.v. infusion causes a transient increase in central blood volume. Our data suggest that the same mechanisms that are responsible for the prolonged diuretic and natriuretic responses after a rapid i.v. infusion are involved in the water and sodium losses during the adaptation to the HDT body position.
Since the reactions on the rapid i.v. infusions persist for more than 48 hours, the first fluid load experiment was, retrospectively, conducted too close to the tilting maneuver. One would be tempted to speculate that total body water and sodium content were in some way preadapted to the HDT conditions and only minor adaptive changes were still necessary.

References:

1. DAVIDSON M.A. and R. PASSMORE Human nutrition and dietetics. Churchill Livingstone. Edinburgh. 1989
2. ELMADFA I. and C. LEITZMANN. Ernährung des Menschen. Ulmer Verlag. Stuttgart. 1988
3. FLEISCH A. *Helv. Med. Acta.* 18: 23, 1951
4. GRIGORIEV A.I. Ion regulatory function of the human kidney in prolonged space flights. *Acta Astronautica.* 8: 987-993, 1981
5. LEACH C.S. Fluid control mechanisms in weightlessness. *Aviat. Space Environ. Med.* 58: A74-9, 1987
6. LEONARD J.I. Understanding metabolic alterations in space flight using quantitative models: fluid and energy balance. *Acta Astronautica.* 13: 441-457, 1986
7. LUFT F. C., L. I. RANKIN, R. BLOCH, L.R. WILLIS, N. S. FINEBERG and M. H. WEINBERGER The effects of rapid saline infusion on sodium excretion, renal function, and blood pressure at different sodium intakes in man. *Am. J. Kidney Dis* 2 (4): 464-470,1983
8. SAGNELLA G.A. and N.D. MARKANDU, Hormonal responses to gradual changes in dietary sodium intake. *Am. J. Physiol.* 256: R 1171, 1989

EFFECTS OF HEAD DOWN TILT FOR 10 DAYS ON THE COMPLIANCE OF THE LOWER LIMB

Jay C. Buckey, Lynda D. Lane, Gernot Plath,*
F. Andrew Gaffney, Friedhelm Baisch,*
C. Gunnar Blomqvist

University of Texas Southwestern Medical Center, Dallas, TX and *Deutsche Forshungsanstalt für Luft Und Raumfahrt, Köln, FRG.

INTRODUCTION

The orthostatic intolerance seen after head-down bedrest and spaceflight cannot be completely explained by the loss of blood volume seen with both these interventions. An increase in leg compliance could produce a greater movement of blood from the thorax to the legs with standing and aggravate orthostatic intolerance. The purpose of this investigation was to measure lower leg compliance before, during and after six degree head-down tilt to answer the question--is leg compliance increased by head-down bedrest?

METHODS

This study was conducted as part of HDT '88, a multinational six degree head down bedrest study sponsored by the Deutche Forschungsanstalt für Luft und Raumfahrt (DLR). Six normal male subjects, mean age 26, were studied before and after a 10 day period of head down bedrest.

Leg compliance, using venous occlusion plethysmography, was measured five days and three days prior to bedrest, on the first, third, sixth and ninth days of bedrest, and one, three and seven days afterward. Two different plethysmographs were used simultaneously. One device, the System for Venous Occlusion Plethysmography (SVOP), uses a mylar band that fits snugly around the calf. Calf expansion moves the band and turns the shaft of an optical shaft encoder, which produces a digital pulse for each 0.02mm of expansion. The device correlates well with mercury-in-silastic "Whitney" strain gauge measurements, but underestimates the Whitney result (1).

The other device is an ultrasonic plethysmograph designed and built at the DLR. This device uses ultrasonic transducers to transmit and receive ultrasound at three points around the calf. In this way the area of the triangle created by the transducers can be monitored. This device has been described and used previously (6).

The subjects rested in a room where temperature was controlled between 25 and 28 degrees centigrade. Before and after tilt the subjects lay supine for twenty minutes prior to testing. During head down tilt they were tested head down. Both legs were elevated above heart level by cushions at the heel and under the thigh. Calf circumference was measured with a measuring tape. The SVOP band was placed over the largest portion of the calf. Water soluble gel placed under the SVOP band smoothed band movement on the leg. The ultrasonic transducers were placed on the calf next to the band.

Occlusion cuffs were placed above the ankle and knee. Leg compliance was measured by inflating the thigh cuff sequentially to 20, 40, 60, 80 and 100 mmHg. After reaching a plateau in the tracings, pressure was increased to the next level without deflation. Resting flow and maximal hyperemic flow were measured after compliance.

RESULTS

As expected, calf circumference declined significantly during the head down period (Fig. 1). The mean calf circumference had not reached its pre-bedrest value even after seven days, although the difference between the pre and post bedrest values are not statistically significant. Even so, this suggests that muscle atrophy may have occurred leading to tissue, as well as fluid, loss during the head down period.

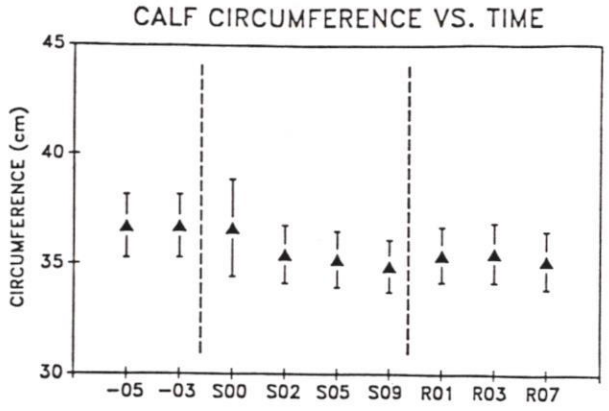


Figure 1. X axis: -(pre); S(during); R(post) tilt in days.

A comparison of the two plethysmographs showed a good correlation. The final calf expansions (in ml/100ml) achieved at each occlusion level during all measurement sessions for every subject were compared for each device using linear regression analysis (Fig. 2).

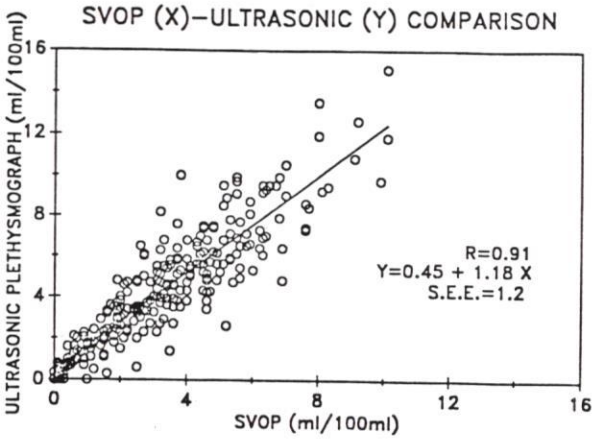


Figure 2.

The correlation coefficient was 0.91 with a standard error of 1.2 ml/100ml. Similar to the experience when compared to Whitney gauges, the SVOP values were approximately 70% to 80% of the ultrasonic values. One possible explanation for this is that the SVOP is relatively insensitive to volume changes in the anterior portion of the leg which constitutes approximately 20-25% of the muscular volume of the leg.

To make comparison of the total compliance (or distensibility) of the leg easy over time, a single number to represent compliance was defined. This number, the compliance integral, is the area under the curve produced when leg volume change in ml/100ml is plotted versus occlusion pressure. This number roughly corresponds to the total amount of fluid put into the leg during the compliance measurement. Statistical analysis on these numbers revealed the same results as on the individual pressure levels.

Figure 3 shows the results from the study. For both the SVOP (SVOP2 on the graph) and the ultrasonic device (ULT) there is a clear trend toward greater leg compliance during and after head down tilt. Analysis of the individual records revealed a confounding variable. The time to reach a plateau in the compliance records showed a tendency to be greater in the late bedrest and recovery measurements. To compensate for this, measurements were taken two minutes into each pressure level. This data is shown on Figure 3 as SVOP1 data. A repeated measures ANOVA on the SVOP1 data revealed that R07 compliance was significantly greater than the pre-bedrest values ($p<0.05$). Also, if the pre-bedrest data is combined and compared to the combined R01 and R03 data, then post-bedrest compliance is significantly greater than baseline ($p<0.05$ Mann-Whitney U test).

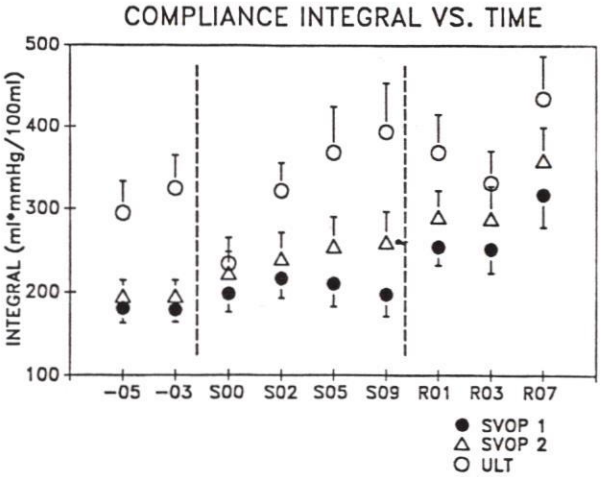


Figure 3.

DISCUSSION

Leg compliance or distensibility as measured by venous occlusion plethysmography is a complex function of the amount of tissue in the leg, the turgor of this tissue as well as venous distensibility. Convertino et al. (3) studied several factors that might affect leg distensibility and found that calf cross sectional area and calf volume were the two factors that correlated best with measurements of compliance in their study.

Also, the deep veins in the leg are very thin walled and do not have significant sympathetic innervation (7). Ludbrook (5) using subcutaneous epinephrine and Buckey (2) using MRI images of the leg have shown that the deep veins contain most of the venous blood in the leg. During a compliance measurement approximately 50-60% of the increase in calf volume is due to filling of the deep veins.

This is significant because the compliance of these venous spaces and indeed of the calf as a whole is probably determined to a great extent by the distensibility of the surrounding muscular tissue. If this tissue should atrophy, as would occur during bedrest or spaceflight, this could lead to increase leg compliance and aggravate the orthostatic intolerance seen after spaceflight. The data from this study tend to support this conclusion, but are not conclusive.

The data do show, however, a clear tendency toward a greater compliance in the recovery period. If the longer cuff inflation times did lead to greater tissue filtration, this would be expected to decrease compliance, and not lead to an artifactual increase in compliance. The data at R07 are more difficult to explain. If tissue atrophy had led to a increase in leg compliance, then this should be improved seven days after head down tilt. The degree of vasoconstriction in the leg is not known, but resting flow on R07 was not different from pre-bedrest values.

In conclusion, both this study and the study by Louisy et al. (4), show a tendency for increased compliance after head-down tilt. Although the existing data does not allow this change to be definitively ascribed to muscle atrophy, this scenario is likely and points to the need for more, carefully performed studies of the legs with weightlessness.

REFERENCES

1. Buckey JC, Watenpaugh DE, Kim LT, Smith ML, Gaffney FA, Blomqvist CG: Initial experience with a new plethysmograph for zero-G use. Physiologist, 28(6):S145-146, 1985.
2. Buckey JC, Peshock RM, Blomqvist CG: Deep venous contribution to hydrostatic blood volume change in the human leg. *Am. J. Cardiol.* 62:449-453, 1988.
3. Convertino VA, Doerr DF, Flores JF, Hoffler GW, Buchanan P: Leg size and muscle functions associated with leg compliance. *J. Appl. Physiol.* 64(3):1017-1021, 1988.
4. Louisy F, Gaudin C, Oppert JM, Guezennec CY: Leg venous hemodynamics and bedrest. Effect of lower body negative pressure. *In: Proceedings 11th Annual Meeting IUPS Commission on Gravitational Physiology*, Lyon, 1989.
5. Ludbrook J, Loughlin J: Regulation of volume in postarteriolar vessels of the lower limb. *Am. Heart J.* 67: 493-507, 1964.
6. Muller E, Hohlweck H, Plath G, Baisch F: Leg volume changes. Responses to LBNP during 7 days of 0-g simulation. Proc. 2nd Eur. Symp. Life Sci. Res. Space, Porz-Wahn, Germany, pg. 159-162, 1984. (ESA Rep. SP-212).
7. Shepherd JT and PM Vanhoutte: Veins and their Control. Philadelphia: W.B. Saunders, pg. 53-56, 1975.

RESPONSES OF ADRENERGIC RECEPTORS TO SIMULATED WEIGHTLESSNESS (6° HEAD DOWN TILT)

Hartmut Maass, Joao Transmontano, Friedhelm Baisch

DLR-Institute for Aerospace Medicine, D-5000 Cologne, FRG

Stress related hormones under simulated weightlessness, their production pattern and influence have been investigated in the past. It has been demonstrated that the excretion rate of epinephrine is reduced under bedrest conditions (1) reflecting altered activities of the autonomic nervous system. In general, if hormone levels change for a longer period, the function of their corresponding target cells may also change noticeably. The function of target cells is very efficiently regulated by the availability of binding sites for the circulating hormones.

Both the receptor density and responsiveness to the appropriate hormone contact can change in reaction to altered conditions.A reduced catecholamine concentration as observed during bedrest could therefore lead to an altered state of those cells carrying adrenergic receptors with respect to the density and responsiveness of these receptors.

Methods and Results

During the international head-down-tilt (HDT) study 1988 at the DLR-Institute for Aerospace Medicine (the study's design in detail is published elsewhere) $alpha_2$- and $beta_2$-receptors were investigated. For this purpose blood samples of the six young, male volunteers were collected on days S-5 (i.e. 5 days before HDT), S7 (i.e. day 7 of the HDT period), R7 and R155 (i.e. 7 days and 155 days after HDT, on day R155 for beta-receptors only). Blood platelets were chosen for the model of cells carrying alpha-receptors, lymphocytes represented cells carrying beta-receptors (8).

The isolation of the desired cellular fractions folowed methods described in the literature (2-7).Diluted heparinized blood was centrifuged on a Ficoll-Paque (Pharmacia,Freiburg) gradient. The lymphocyte layer was harvested and separated from the platelet rich plasma above, resuspended and spun again. The platelet rich plasma and the supernatants were used for the isolation of the platelets by vigorous spinning. Finally, the washed lymphocytes were counted and adjusted to the desired concentration. Viability tests with Trypan blue revealed in average 95% of the lymphocytes being alive. The platelets were washed, counted and adjusted to the wanted final concentration.

The binding assay for $alpha_2$-receptors was performed by radioligand titration with (^{3}H)-O-methyl-yohimbine (0.5 - 10 µM). The calculation of max. binding sites followed Scatchard's plot (7). The densities of the alpha-recetors on platelets are given in Table 1. Although there is a broad variety of individual densities among the volunteers, an increase during the HDT phase and a decrease afterwards are observed.

Table 1 gives an overview for the mean $alpha_2$-binding sites per platelet.

Day	$alpha_2$-binding sites/platelet mean ± SD
S-5	89 ± 24
S 7	177 ± 36
R 7	46 ± 8

The binding assay for $beta_2$-receptors was carried out by radioligand titration with (^{125}I)(-)-3-iodocyanopindolol (10 - 500 pM) and subsequent calculation of the max. high affinity binding sites according to Scatchard. The densities of the $beta_2$-receptors on lymphocytes are shown in Table 2. A lower level of binding sites is seen only before the HDT phase, whereas the densities during and after HDT are only slightly changed with highest mean densities shortly after HDT.

Table 2 gives an overview for the mean $beta_2$- binding sites per lymphocyte.

Day	$beta_2$-binding sites/lymphocyte mean ± SD
S -5	549 ± 221
S 7	1391 ± 556
R 7	1665 ± 537
R155	1250 ± 578

The responsiveness of the beta-receptors was measured by the production of cyclic adenosine-3'-5'-monophosphate (cAMP) after maximum stimulation of living lymphocytes by adding (-)isoproterenol bitartrate. The control samples received buffer instead. The stimulation was stopped by adding distilled water and immediate boiling of the samples. After spinning the debris the cAMP concentrations of the supernatants were done by a radioimmunoassay (DuPont,Dreieich).

The recovery of added cAMP was more than 98%.

Table 3 gives the responsiveness to stimulation by the factor obtained from the cAMP production with stimulant against cAMP production without stimulant.

Day	Responsiveness factor of $beta_2$-receptors (cAMP)+stimulant/(cAMP)-stimulant mean $\pm$ SD
S -5	2.51 $\pm$ 0.49
S 7	2.46 $\pm$ 0.85
R 7	2.67 $\pm$ 1.18
R155	1.95 $\pm$ 0.71

A significantly altered responsiveness of the $beta_2$-receptors during the HDT study is not detectable. Lowest responsiveness is observed 5 months after the study.

Though the results are obtained from a small group of subjects aggravating statistical evaluation, reduced densities of adrenergic receptors prior to the HDT period and increasing during HDT are noticed. This coincides inversely with the catecholamine excretion rates being high before and lower during HDT. Cortisol levels are elevated throughout that period. Therefore it is suggested that on day S-5 the adrenergic receptors reflect a rather stressful situation before HDT resulting in a down-regulation of the receptors. Gmünder et al. (to be published) found similar results investigating the lymphocytes responsiveness as activation to concanavalin A. The responsiveness was severely reduced beyond the end of the HDT phase. On day R155, months later, and without any other manipulations anticipating, the subjects show higher densities of $beta_2$ binding sites revealing a different, higher baseline.

The $alpha_2$-receptors density decreases after HDT and may suggest some up-regulation during HDT. But this might reflect also the short half-life of the platelets. The $beta_2$-receptors mean densities give onlya tendency that a slight up-regulation effect during HDT exists, though the receptors responsiveness is unchanged throughout the study.
In conclusion one might speculate about an up-regulation of adrenergic receptors during simulated weightlessness and even beyond due to decreased catecholamine levels. From the few data, however, it is not possible to give firm evidence. The effects may be masked by different levels of stress during the investigation.

References

1. Samel,A. et al.:J.Autonomic Nervous System,Suppl.205-207,1986
2. Böyum,A.:Scandinav.J.Clin.Lab.Invest. 21(Suppl. 97):77-89,1968
3. Brodde,O.E. et al.:J.Cardiovasc.Pharmacol.7(Suppl.6):162-167,1985
4. Perper,R.J. et al.:J.Lab.Clin.Med.72: 842-848,1968
5. Hiatt,W.R.:J.Receptor Research 5:419-429,1985
6. Brodde,O.E.:J.Receptor Research 3:151-162,1983
7. Scatchard,O.:Ann.N.Y.Acad.Sci.51:660-672,1949
8. Brodde,O.E. et al.:Science 231:1584-1585,1986

THE EFFECTS OF A 10-DAY PERIOD OF HEAD-DOWN TILT ON THE CARDIOVASCULAR RESPONSES TO INTRAVENOUS SALINE LOADING.

FA Gaffney[1], JC Buckey[1], LD Lane[1], A Hillebrecht[2], H Schulz[2], M Meyer[2], F Baisch[3], L Beck[3], M Heer[3], H Maass[3], PH Arbeille[4], F Patat[4], CG Blomqvist[1].

[1]University of Texas Southwestern Medical Center, Dallas, Texas, [2]Max-Planck-Institute, Göttingen, FRG, [3]DLR-Deutsche Forschungsanstalt für Luft- und Raumfahrt, Cologne, FRG, [4]Université Francois Rabelais, Tours, France.

Introduction.

The effects of a 10-day period of -6° head-down tilt (HDT) on the cardiovascular responses to intravenous saline loading were examined during a recent international study performed at DLR. The basic study design, subject characteristics, and various physiological findings are presented in several companion papers included in this symposium.

We tested the hypothesis that relative dehydration and hypovolemia as produced by actual and simulated microgravity alter the response to intravenous saline loading. A larger fraction of the infused volume is retained, and the acute hemodynamic responses are magnified.

Isotonic saline was selected in preference to any colloidal solution (a) to minimize potential interference with other physiological studies, and, (b) to reflect the use of saline loading during spaceflight as a countermeasure against orthostatic hypotension on return to 1 g (1,2). I.V. infusion was preferred over oral administration to control tightly the volume entering the intravascular compartment.

Methods and Procedures.

Six normal male subjects, mean age 26 ± 4 years, were studied 2 days before HDT, on the 6th day during HDT, and on the 2nd day of recovery. Isotonic saline, warmed to 37 degrees C, was infused over 20 minutes at a constant rate determined individually to produce a total volume of 22 ml/kg. Cardiac output was measured by the foreign gas rebreathing technique using acetylene as the soluble and helium as the insoluble indicator gas (3). Heart rate was monitored by ECG recording and arterial pressure was measured by the Finapres technique. Baseline red cell mass was measured by the standard 51Cr method. Plasma volume was monitored indirectly by combining the data on red cell mass and frequent measurements of hematocrit.

Results.

All subjects tolerated the saline fluid load well and reported no symptoms. Saline infusion produced significant ($p<0.05$) but transient increases in plasma volume and total blood volume, stroke volume, heart rate, and cardiac output (Table 1).

TABLE 1. HEMODYNAMIC RESPONSE TO BLOOD VOLUME EXPANSION BEFORE, DURING AND AFTER HDT.

		-5 min	+20 min	+60 min
BV (l)	Pre	5.21	5.91	5.47
	HDT	4.52	5.19	4.77
	Post	5.09	5.89	5.37
HR (beats min)	Pre	66 ± 3	71 ± 4	56 ± 12
	HDT	60 ± 2	64 ± 3	60 ± 10
	Post	62 ± 2	67 ± 3	60 ± 2
SV (ml)	Pre	81 ± 8	103 ± 6	101 ± 17
	HDT	85 ± 6	108 ± 7	103 ± 18
	Post	84 ± 7	102 ± 5	94 ± 5
$\dot{Q}$ (l/min)	Pre	6.62 ± .49	8.10 ± .39	7.03 ± .62
	HDT	5.79 ± .33	7.51 ± .40	6.68 ± .42
	Post	6.01 ± .46	7.45 ± .45	6.23 ± .40

BV = total blood volume, HR = heart rate, SV = stroke volume, and $\dot{Q}$ = cardiac output. Pre = 2 days before HDT, HDT = 6th day during HDT, and Post = 2 days after HDT.

Mean arterial pressure was unchanged while total peripheral resistance decreased. All changes were maximal at the end of the 20 min infusion. Heart rate had returned to control values within 1 hr, while blood volume, stroke volume, and cardiac output tended to remained elevated. Baseline hemodynamic states differed significantly with the phase of the study, but the hemodynamic responses to fluid loading before, during, and after HDT were qualitatively and quantitatively similar.

The dynamics of the blood volume expansion are documented in Table 2. The magnitude and time course of the changes were again independent of HDT phase. Table 2 reflects the lumped data from all 3 studies. Approximately 45% of the infused saline remained in the intravascular space at 20 min, and 15% to 20% was retained at 60 and 120 min after the start of the infusion.

TABLE 2. INTRAVASCULAR VOLUME EXPANSION

	Time, Min.	Volume, ml.	Per Cent
Infusion	0 - 20	1,600	100
Plasma Vol. Expansion	+ 20	720	45
	+ 60	270	17
	+120	290	18

(Lumped data from all phases of the HDT study).

Discussion.

Rapid infusion of a large volume of normal saline can be performed safely in healthy subjects and produces transient changes in hemodynamic parameters and intravascular volume. Only a small fraction of the infused volume is retained after 1 hour.

Plasma volume was equally well regulated in all 3 experimental conditions, including the hypovolemic state produced by HDT. The resistance to volume expansion and the maintenance of the contracted volume and during HDT indicates that a new set-point or operating point for intravascular volume control had been established. Infusion during HDT also produced virtually identical acute hemodynamic responses as pre-HDT. The subjects remained in a state of relative dehydration and hypovolemia until resuming predominantly upright position post-HDT.

The presence during HDT of a new operating point for the regulation of intravascular volume has important implications for the design of countermeasures intended to counteract microgravity-induced hypovolemia and orthostatic hypotension. It is unlikely that an equal volume given as oral saline loading would have a greater effect than an intravenous infusion. If only 15% of the infused saline remains in the intravascular space after 1-2 hours, the hemodynamic effects are likely to be marginal. Data from several sources (4) suggest that a blood volume expansion of about 300 ml. is required to produce a measurable increase in orthostatic tolerance.

The infused saline disappears from the intravascular space by two primary mechanisms: filtration into interstitial space and renal clearance. Most of the early loss of the infused volume is accounted for by tissue filtration. Urinary losses are quantitatively less important during the early phases of volume loading. Unpublished data from our laboratory (Watenpaugh et al.) from a similar study infusing 3,000 ml. showed 38% retained intravascularly at 20 minutes, with 55% transferred to interstitial space, and 12% eliminated by the kidneys. Corresponding percentages at 60 min. were 15, 55, and 28%. Insensible loss accounted for the remaining 2%.

Our data indicate that a sizable portion of the infused volume does not leave the body during the early post-infusion period, and may therefore be mobilized later to maintain an increased intravascular volume for a relatively long duration. However, it is likely that saline loading needs to be supplemented by other procedures to produce optimal hemodynamic benefits. Maximal exercise and lower body negative pressure do not cause immediate plasma volume expansion, but these interventions induce a delayed and prolonged salt and water retention. These effects combined with saline loading, may constitute an effective anti-orthostatic countermeasure following bedrest and exposure to microgravity.

References

1. Grigor'yev AI, Stepantsov VU, Tishler VA, Mikhaylov VM, Pometov YuD, Dorokhova VR: Results of medical research performed on board the "Salyut-6-Soyuz" orbital scientific research complex. Moscow: Nauka, 1986, pp. 125-145.

2. Bungo MW, Charles JB, Johnson PC, Jr.: Cardiovascular deconditioning during space flight and the use of saline as a countermeasure to orthostatic intolerance. *Aviation, Space, and Environmental Medicine*, 56:985-990, 1985.

3. Triebwasser JH, Johnson RL, Jr, Burpo RP, Campbell JC, Reardon WC, Blomqvist CG: Noninvasive determination of cardiac output by a modified acetylene rebreathing procedure utilizing mass spectrometer measurements. *Aviation, Space, and Environmental Medicine*, 48:203-209, 1977.

4. Blomqvist CG, Stone HL: Cardiovascular adjustments to gravitational stress. *IN: Handbook of Physiology*, (Eds.) Shepherd JT, and Abboud FM. Section 2: The Cardiovascular System. Volume III: Peripheral Circulation and Organ Blood Flow, Part 2. Bethesda: American Physiological Society. 1983, pp. 1025-1063.

EFFECT OF A 10-DAY HDT ON THE HEMODYNAMIC RESPONSE TO LBNP

L Beck[1], G. Blomqvist[2], PH. Arbeille[5], F. Baisch[1], J. Buckey[2], A. Gaffney[2], A. Hillebrecht[3], J. Karemaker[4], F. Patat[5], H. Schulz[3], D.J. Ten Harkel[4].

[1] DLR -Institute for Aerospace Medicine, Cologne, FRG
[2] University of Texas Southwestern Medical Center Dallas, Texas, USA
[3] Max-Planck-Institute for Experimental Medicine, Göttingen, FRG
[4] Department of Physiology, Academic Medical Centre, Amsterdam, NL
[5] Université François Rabelais, Department of Medical Biophysic, Tours, F

Introduction

Head down tilt (HDT) bed rest alters the response to the application of a negative differential pressure to the lower body (LBNP). It reduces the tolerance to this stress. Several reasons are usually invoked to explain this fact: reduced intravascular volume; altered volume / pressure relation in the lower limbs facilitating fluid pooling; and altered pattern of neurohumoral control. These are the same mechanisms invoked to explain the impairment of orthostatic tolerance found after space flight.

Methods

Study framework and other methodological aspects are detailed in the contribution by F. BAISCH et al. in this issue. LBNP was applied in the three phases of the study. The sessions were conducted in the pre-tilt phase on day -04, in the HDT phase on days S04 and S08 and in the recovery phase on days R00 and R04. Both in the pre-tilt and in the recovery phase, LBNP was performed in the 6° head down position. The posture was adopted one hour before application of the stress. The Δp profile applied after a 30 minutes resting period for control measurements was as follows:
-15 mmHg during 15 minutes
-30 mmHg during 05 minutes
-40 mmHg during 15 minutes
Ten minutes were allowed for post LBNP data collection. The test is quite rigorous and the load adds up to 975 mmHg x minutes. Different measurements were performed during LBNP. Of relevance for this report are the following:

- pulmonary flow by the acetylene rebreathing technique, that will be referred to as Cardiac Output (CO), was measured in the resting period, at Δp 15 and 40 and in the post-stress control period;
- blood pressure (BP) by the finger plethysmographic method and
- electrocardiogram (EKG)

BP and EKG were recorded continuously.

Results

In all three phases of the study the application of LBNP resulted in a decrease in stroke volume, an increase in total peripheral resistance and heart rate, and a decrease in cardiac output. Only four of the six test subjects were able to complete the LBNP protocol at the end of the HDT phase.

The **heart rate** response to LBNP was conformed to the expected pattern (2) and was much larger at the end of the ten day head-down tilt period and during early recovery than at baseline studies before tilt. The control values pre LBNP were similar in all three phases of the study. The heart rate increase during maximal LBNP load represents about 21% from the control in the pre-tilt phase and of 36% during maximal LBNP in the HDT phase

Baseline **stroke volume** was reduced by 15% during the HDT phase with respect to the pre-HDT phase stroke volume. During LBNP at -40 mmHg it was reduced by 33 per cent relative to pre-tilt measurements.The increase in heart rate under maximal LBNP load did not compensate for the marked decrease in stroke volume leading to a noticeable decrease in **cardiac output.** Measurements during HDT showed significantly reduced cardiac output values at rest and at all levels of LBNP.

Both during the pre and post HDT phase the **mean arterial pressure** remained fairly constant. In the HDT phase there was a more pronounced increase in mean pressure during maximal level of LBNP.

In the three phases of the study **total peripheral resistance** increased during LBNP application and returned to control after release of the negative pressure. The increase represented 20% and 32% of the control during the pre-tilt phase and the HDT phase respectively.

The LBNP response pattern during the recovery period was intermediate.

Discussion and Conclusions

The initial LBNP-level (Δp = 15mmHg) caused a response that has already been described as being mediated by low pressure baroreceptors. This response is characterized by a decreased stroke volume and cardiac output, increased total peripheral resistance but constant blood pressure and heart rate (1). With increasing levels of negative pressure there is also an increase in the heart rate and a further increase in total peripheral resistance. These changes are thought to express the progressive involvement of the arterial baroreceptors. This pattern of response was found in all three phases of the study. However, the magnitude of the response was markedly larger in the HDT period and in the early phase of recovery than during the baseline pre-tilt period.

Pre-LBNP data were collected in the pre- and post HDT phases of the study after one hour in the 6° head down position. This may have magnified the differences between the responses before and during tilt. Central blood volume and cardiac filling were probably increased acutely during the control phase before but not during tilt when the system also has to deal with reduced intravascular volume.

In fact, blood volume decreased by 18% during the HDT phase (see F. BAISCH et al in this issue). This resulted in a decreased cardiac output under resting conditions pre LBNP at the end of the HDT phase. Its values were comparable to cardiac outputs measured in the pre-tilt phase of the study in the upright posture.

The increase in heart rate during LBNP was not sufficient to compensate for the decrease in stroke volume induced by head-down tilt which eventually led to the occurrence of pre-syncopal symptomatology and premature interruption of the LBNP test in two test subjects during the late tilt phase. The decreased intravascular volume associated with decreased cardiac filling and reduced resting stroke volume seems to be to a great extent responsible for the more pronounced reaction found in the HDT phase.

Nevertheless, other changes induced by HDT may have contributed as e.g. the increased ability of the lower extremities to pool volume (see J. BUCKEY et al. in this issue) and the altered neurohumoral regulatory mechanisms (cf. D. ECKBERG et al. in this issue).

These results document that HDT produced orthostatic intolerance (see also J.D. TEN HARKEL et al. in this issue) and provide further support for the use of HDT as a means of simulating microgravity during studies of cardiovascular function.

REFERENCES

1 AHMAD, M., C.G. BLOMQVIST, CH. B. MULLINS, AND J. T. WILLERSON. Left ventricular function during lower body negative pressure. *Aviat. Space Environm. Med.* 48 (6):512-515, 1977.

2 BAISCH, F., J. BEIER, U. GEBHARDT, J. R. HORDINSKY, AND H. MORSBACH. Lower body negative pressure (LBNP) - a method of controlled circulation disorder. *Proceedings of a workshop held at Toulouse, France, March 1982*, p. 43-75 (ESA SP-180).

VARIATIONS OF THE RENAL FLOW IN RELATION WITH THE VOLEMIA

Ph . ARBEILLE *, D. LEBOUARD * , F . PATAT * J.M . POTTIER *, J.BUCKEY***,L . BECK **,G. BLOMQVIST *** ,C.GHARIB **** , L. POURCELOT *

*Unite INSERM 316 - CHU Bretonneau - 37044 - Tours - France .
** Institute Aerospace Medicine (DLR)D.5000 - Koeln 90 - Germany.
*** Cardiovasc. Physiol Laboratory. South Medical School. Texas Univ. - Dallas, USA.
**** Dept Physiol .UER. Grange Blanche -69373- Lyon -08- France.

I - INTRODUCTION

Weightlessness induces a significant fluid shift from the legs toward the cephalad part of the body (5.10). This transfer of liquid simulates a sudden central hypervolemia and triggers neurohormonal systems which try to regulate the flow distribution and to reduce the volemia. Within some hours or some days the cardiovascular system of the astronaut reaches a new and quite stable haemodynamic equilibrium characterized by a hypovolemia (2.3.8.13.15.16.).

Several physical tests has been suggested to simulate (on the ground), the cardiovascular effects of zero g environment and to study the mechanisms involved in the regulation of the cardiovascular function. The Head Down Tilt (HDT) is the most widely used method for 0g simulations (4.6.7.9.12.17) . This test leads to a hypovolemia as observed during space flights. The LBNP (Lower Body Negative Pressure) induces a central hypovolemia which provokes a flow redistribution in different organs, such as brain, kidneys...(1.11) . On the other hand intravenous infusion of a saline solution (fluid loading) generates a hypervolemia which also disturbs the regional flows.

Previous ultrasound doppler studies have demonstrated that the cerebral flow remains quite stable even during significant modifications of the volemia and the cardiac output because the cerebral vascular resistance change very quickly and control the inflow toward the brain (2.13.).The aim of this paper is to study the renal flow response to these different situations of hypo or hypervolemia.

II - MATERIAL AND METHOD

The renal flow was investigated by pulsed doppler ultrasound method using duplex ultrasound systems combining B mode, and time motion or doppler.

The right kidney was vizualized in a transverse incidence performed just below the costal border on the right lateral side of the subject. The pulsed doppler sample volume was located at the level of the main trunk of the renal artery, close to the hile. The doppler signal was displayed through a frequency spectrum analyser. The doppler spectrum of the renal artery shows an important diastolic flow due to the fact that the kidney is an organ with very low vascular resistance. The amplitude of this diastolic component is closely related to the importance of the vascular resistance in the area supplied by the artery (14). The renal vascular resistance is evaluated by using the resistance index Rr = S-D/S with S and D the maximal systolic and end diastolic frequency amplitude, on the spectrum (fig.1). This parameter has been used in various pathologies (on adults, infants and fetuses..) and is considered to change in proportion to the vascular resistance . This index is generally used for the detection of any change in the arterial vasomotricity such as the cerebral vasodilatation on patient breathing Co2 or the cerebral vasoconstriction on hyperventilated infants.

The renal flow was investigated in four different conditions on which the volemia was supposed to change :

a) A one month HDT experiment (CNES,87-88), with 6 subjects resting, and 6 subjects submitted to repeated LBNP tests throughout the HDT period : (2 to 4 LBNP of 20 mn long per day at - 30 mn Hg). The objective of this experiment was to evaluate the long term effect of repeated LBNP on the cardiovascular parameters and the vascular deconditionning. The cardiac output and the volemia were maintained on the subjects under LBNP, and decreased on the control group (at rest). The renal flow was investigated 5 times for each of the 12 subjects approximately each week during the one month HDT and at least 2 hours after any LBNP session. The results were interpreted according to the data concerning the volemia and the cardiac function.

b) A 10 days HDT (DLR-88) during which each of the 6 subjects had two LBNP sessions (20 mn long, at - 20 to - 40 mn Hg) and intravenous fluid loading (22 ml/kg = 1 to 1,5 l in 20 mn). In this case the purpose of the cardiovascular examination was to study the acute response of the renal flow to physical maneuvers (LBNP-Fluid Loading) inducing hypo or hypervolemia. The measurements were performed during and after these test. The subjects had LBNP and Fluid Loading sessions before, during and after HDT.

c)The 25 days French-Soviet spaceflight (Aragatz.88) on board the Mir Station. Six inflight measurements were taken at +4, +5, +15, +18, +20 and +24 days on one subject in order to study the renal flow adaptation to the hypovolemia induced by the zero g environment.

III - RESULTS AND DISCUSSION

a) Renal flow changes during LBNP :

During the LBNP test (at 15 mn, - 40 mn Hg) the renal resistance index (Rr) significantly decreases (-13% +/- 5) and recovers 5 mn after the end of the test (fig.2). At the same time the left ventricle diastolic volume (LVDV) as well as the cardiac output (CO) decrease (LVDV : - 20% Co : - 25% approx) which is in favour of a central hypovolemia. The renal response to LBNP remained the same before, during, and after the 10 days HDT period.

b) Effect of chronic LBNP on the renal circulation :

The renal resistance index (Rr) remains stable and slightly elevated on the subjects submitted to repeated LBNP, all during the one month HDT period (+5%+/- 5) (fig.3). On this group the left ventricle diastolic volume (LVDV) and the cardiac out put (CO) stay above the basal value measured before the HDT (LVDV + 5% ; CO : +15%). On the group without LBNP treatment the cardiac parameters are decreased (LVDV: 5%- Co: 5%) as well as the renal index Rr (-10% +/- 5) (fig.3).

c) Renal flow adaptation to 0g exposure :

During the flight, the renal resistance index (Rr) stays below the basal value (Pre flight) (fig.4). The left ventricle diastolic volume (LVDV) is decreased (-10%) during the whole of the flight however the cardiac out put (CO) remains above the basal value (+5%) due to the increase in the heart rate.

d) Renal flow changes during fluid loading :

The intravenous infusion of about 1,5 liters of saline solution increases the volemia and the cardiac parameters (LVDV: +20% - CO: +25% approx). At the same time the renal resistance index (Rr) increases significantly during the infusion (+12% +/- 4) and does not recover immediately after the end of the infusion. (fig.5) .

Finally we observe that in the case of central hypovolemia induced either by zero g environment or by long term HDT or by LBNP maneuver the renal vascular resistance decreases significantly. This variation could be interpreted as a vasoactive response of the renal vascular area (vasodilatation) in order to

maintain the renal perfusion. The same haemodynamic adaptation has already been observed for the brain. In the case of hypervolemia induced by Fluid Loading or by repeated LBNP test, the renal vascular resistance index increases which suggests an arterial vasoconstriction in the renal vascular bed probably to prevent any increase in the renal flow despite the elevation of the volemia. The renal flow response seems to be very sensitive to the variations of the volemia. The follow up of the renal haemodynamics will probably be of great interest for the assessment of the cardiovascular disturbances induced by microgravity and for the evaluation of the efficiency of counter measures like LBNP or Fluid Loading or exercices intended to reduce the cardiovascular deconditioning.

REFERENCES

1 - AHMAD M. , BLOMQVIST CG , MULLINS CB , WILLERSON JT. Left ventricular function during lower body negative pressure.Aviat. Space Environ. Med. 1977 ; 48 : 512-5.

2 - ARBEILLE Ph., POTTIER JM, PATAT F., BERSON M., RONCIN A., LE TOULLEC Ch., MIGNE P., POURCELOT L., KOTOVSKAYA A., ATKOV O., STROGONOVA L., FOMINA G., KOKOVA N. Cardiovascular adaptation to zero-G during a long-term flight (237 days) on board the Salyut-VII soviet space station (1984). ESA 1987, SP-271, pp. 134-146.

3 - BUNGO HW, CHARLES JB. The human cardiovascular system in the absence of gravity. IAF - 1985 - 135.

4 - BLOMQVIST CG, NIXON JV, JOHNSON RL. JR, MITCHELL JH. Early cardiovascular adaptation to zero gravity simulated by head-down tilt. Acta Astronautica, 1980, 7, 543.

5 - BONDE-PETERSEN F. et al. Aspects of cardiovascular adaptation to gravitional stresses. Physiologist 23, sup. 1980, S7-S10.

6 - GAFFNEY FA, NIXON JN, ERLING S., KARLSSON S., CAMPBELL W., DOWDEY C., BLOMQVIST G. Cardiovascular deconditioning produced by 20 h of bed rest with Head down tilt (- 5°) in middle aged healthy men. Am. J. Cardiol. 1985 - 56 ; 634-638.

7 - HINGHOFER-SZALKAY H. Zero-G simulation for ground-based studies in human physiology, with emphasis on the cardiovascular and body fluid systems. ESA, Estec. 1982, SP-180 : 81-102.

8-JOHNSON.RL,HOFFLER.GW,NICOGOSSIAN.AE,BERGMAN.SA. Skylab experiment M 092 : Results of the first manned mission . Acta Astronautica : 1975 - 2 . 265 - 296 .

9 - KATKOV VE, CHESTUKHIN VV, NIKOLAYENKO EM, GVODZEV SV, RUMYANTSEV VV, GVOZDEV SV. Central circulation of a normal man during head-down tilt and decompression of various body parts. Aviat. Space Environ. Med. 1983, 54 : 524-30.

10 - KIRSCH KA, VON AMELN H. Current views and future programs in cardiovascular physiology in space. Acta Astronautica, 1981, 8, p. 939-950.

11 - LOOLGEN H., KLEIN KE, GEBHARDT U. et al. Hemodynamic response to LBNP following 2 hours HDT (- 6°). Aviat. Space Environ. Med. 1986. 57 - 406-12.

12 - NIXON JV, MURRAY RG, BRYANT C, JOHNSON Jr RL, MITCHELL JH, HOLLAND OB, GOMEZ-SANCHES C, VERGNE-MAMRINI P, BLOMQVIST CG. Early cardiovascular adaptations to simulated zero gravity. J. Appl. Physiol.1979. 46: 451-8.

13 - POTTIER JM, ARBEILLE Ph, PATAT F. et al. Comparative study of the cardiovascular adaptation to zero g during 7 days space flights.The physiologist 1988, suppl. vol 31, n° 1.

14 - POURCELOT.L . Applications techniques de l'examen doppler transcutané . In : Velocimetrie Ultrasonore Doppler .Edition Inserm Paris . 1974 . 213 - 240 .

15 - POURCELOT L., ARBEILLE Ph., POTTIER JM, PATAT F., MIGNE P., GUELL A., GHARIB G. Ultrasonic study of early cardio vascular adaptation to Zero gravity. ESA SP 212. 1984. 119-123.

16 - POURCELOT.L .Cardiovascular examination during STS 51 G mission , June 1985 .ESA . SP . 237 .1985 .13 - 17 .

17 - SANDLER H. Effects of bed rest and weightlessness on the heart. In : Bourne GH, ed. Heart and heart-like organs. New-York : Academic Press, 1980 2 : 435-524.

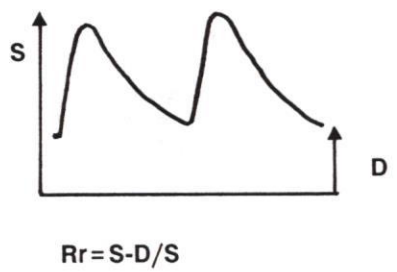


Fig 1 Evaluation of the Renal Vascular Resistance.(Rr)

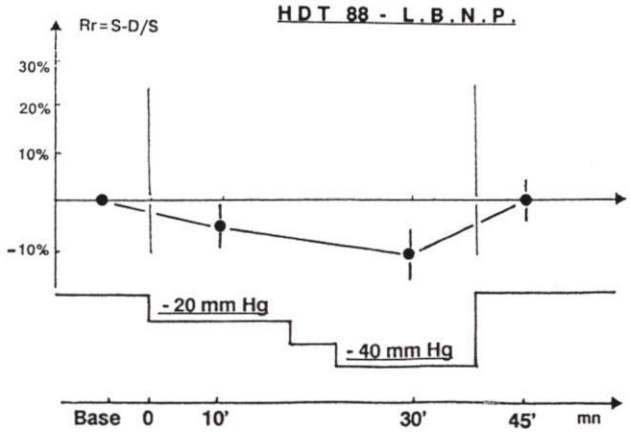


Fig 2

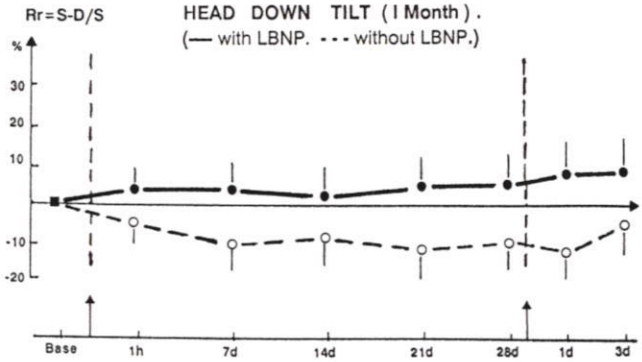


Fig 3

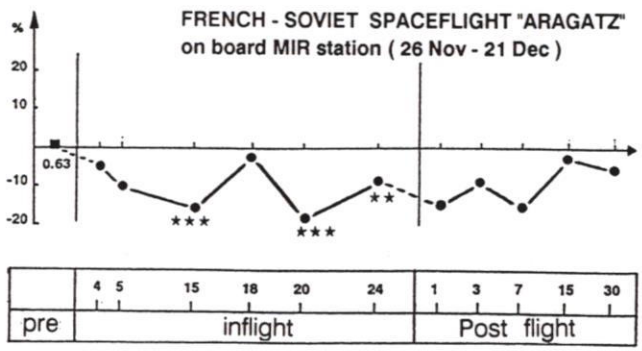


Fig 4

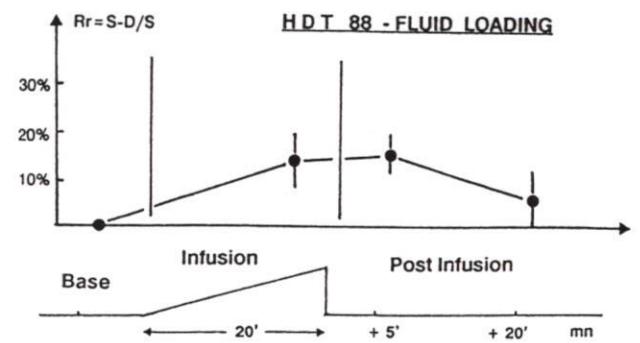


Fig 5

CAROTID BARORECEPTOR CARDIAC-VAGAL REFLEX RESPONSES DURING 10 DAYS OF HEAD-DOWN TILT

Dwain L. Eckberg and Janice M. Fritsch

Department of Medicine, V.A. Medical Center and Medical College of Virginia, Richmond, Virginia 23249

Astronauts experience post-flight orthostatic hypotension and may have impaired baroreflexes. We studied changes of carotid baroreceptor cardiac reflex function in 6 men before and during 10 days of 6° head-down tilt. During held expiration, 40 mmHg of pressure was applied to a neck chamber, followed by 15 mmHg, R-wave triggered decrements to -65 mmHg. R-R intervals were plotted against carotid distending pressures. The slope of the response decreased from 5.0 ± 0.4(SEM) msec/mmHg (control) to 3.6 ± 0.7 (day 10, P=0.11) and increased to 4.0 ± 0.8 by day 7 of recovery. The range of R-R interval responses decreased from 263 ± 31 (control) to 182 ± 18(day 10) (p=0.03) and returned to 215 ± 40 msec during recovery. These results suggest that head-down tilt (widely used as a model of microgravity) may impair normal blood pressure control mechanisms by reducing both the gain and the range of carotid baroreflex responses.

INTRODUCTION

Astronauts experience a variety of changes of cardiovascular function during space travel, including elevations of heart rate, systolic and mean arterial pressures, and reductions of diastolic pressures. Although usually asymptomatic during flight, some astronauts become symptomatic upon return to earth. Primary symptoms provoked by cardiovascular dysfunction may include orthostatic hypotension, tachycardia, presyncope or syncope, and reduced exercise capacity.

Although loss of blood volume may account for some of the changes in cardiovascular function, this mechanism is probably not a sufficient explanation, because comparable blood volume reductions in terrestrial man do not provoke the same changes recorded after space travel, and restoration of blood volume after simulated microgravity does not restore cardiovascular function to normal. Another explanation may be that normal autonomic cardiovascular control mechanisms may be disrupted due to the hemodynamic changes that occur during exposure to microgravity. One of these mechanisms, the carotid baroreceptor-cardiac reflex response, has been the focus of many of our investigations. The hypothesis of the current study was that head-down tilt, a generally accepted model of microgravity, impairs this reflex.

METHODS

We studied carotid baroreceptor-cardiac reflex function in 6 healthy young men before, during, and after 10 days of 6° head-down tilt. During held expiration, 40 mmHg of pressure was applied to a tightly sealing neck chamber and held for 5 seconds. Then, the next 7 R-waves each triggered a 15 mmHg decremental step in neck pressure, until -65 mmHg was reached, and was then released. This stimulus sequence was repeated 7 times and the data were averaged. R-R intervals were measured from the electrocardiogram and plotted against carotid distending pressures (systolic - neck chamber pressure). The data are reduced to the following variables: minimum R-R interval; pressure at minimum R-R; maximum R-R interval; pressure at maximum R-R; range of R-R interval responses; maximum slope (linear regression applied to each set of three consecutive points to find the segment with the steepest slope); pressure at maximum slope; and operational point [(Control R-R - minimum R-R)/range x 100%]. Measurements were made before tilt, on the 1st, 3rd, 6th, and 10th days of tilt, and on the 1st, 2nd, 4th, and 7th days of recovery. All variables were analysed using a Wilcoxon signed rank test with contrast for each hypothesis between control and each experimental day.

RESULTS

Systolic and diastolic pressures did not change, but baseline R-R intervals were significantly increased by the 10th day of tilt. Control R-R intervals were 933 ± 24 msec before tilt and increased to 1065 ± 35 msec on tilt day 10. Maximum slope decreased (insignificantly) from 5.0 ± 0.4 msec/mmHg during control, to 3.6 ± 0.7 on day 10 ($p = 0.11$), and increased to 4.0 ± 0.8 by day 7 of recovery ($p = 0.50$) The range of R-R interval responses decreased from 263 ± 31 msec during control, to 182 ± 18 msec on day 10 ($p = 0.03$), and returned to 215 ± 40 by day 7 of recovery ($p = 0.35$). Other characteristics of the reflex response did not change.

CONCLUSIONS

These results suggest that head-down tilt may impair normal blood pressure control mechanisms by reducing the range and possibly the gain of the carotid baroreceptor-cardiac reflex response. These changes during simulated microgravity may have relevance to the orthostatic intolerance experienced by some astronauts upon return to earth.

THE AUTONOMIC NERVOUS SYSTEM IN BLOOD PRESSURE REGULATION DURING 10 DAYS 6° HEAD DOWN TILT

A. D. J. ten Harkel, F. Baisch, L. Beck, J. M. Karemaker

Departments of Internal Medicine and Phsyiology, Academic Medical Centre, University of Amsterdam, The Netherlands
Deutsche Forschungsanstalt für Luft und Raumfahrt e. V. Cologne, West-Germany.

Re-exposure to normal gravitation after a period of zero gravity often leads to orthostatic intolerance. This problem has been attributed to both central hypovolemia and to deconditioned cardiovascular regulatory mechanisms (1). To study orthostatic tolerance and the contributory role of the autonomic nervous system after a period of simulated weightlessness, 6 healthy male volunteers were studied before, during and after 10 days 6° head down tilt (HDT).

The parasympathetic part of the autonomic nervous system was tested by the heart rate (HR) response to forced breathing (Forced Respiratory Sinus Arrhythmia, FRSA). The efferent sympathetic part of the autonomic nervous system was tested by the blood pressure (BP) and HR responses to sustained handgrip (SHG) and mental stress (MS). The BP and HR responses to the Valsalva manoeuvre (VM) were used to assess both efferent sympathetic and parasympathetic nervous control. Orthostatic BP control was investigated by a passive 70° head up tilt (HUT) manoeuvre on a tilt table with foot support before and at the end of 10 days HDT. In order to compare the prolonged effects of fluid shifts induced by HDT with those acutely taking place during normal daily-life we also assessed the influence of posture on the BP and HR responses to FRSA, VM, SHG and MS. These four manoeuvres were tested before the HDT-phase in the 6° HDT, the supine, the sitting and the standing position.
The responses were quantified as the change in BP and HR during the respective manoeuvres compared to the average of a 30 s period before the manoeuvre.
Finger BP was measured continuously non-invasively by means of Finapres™ (3,4). HR was determined from the electrocardiogram.
During the 10 days HDT baseline BP decreased, while no change in baseline HR

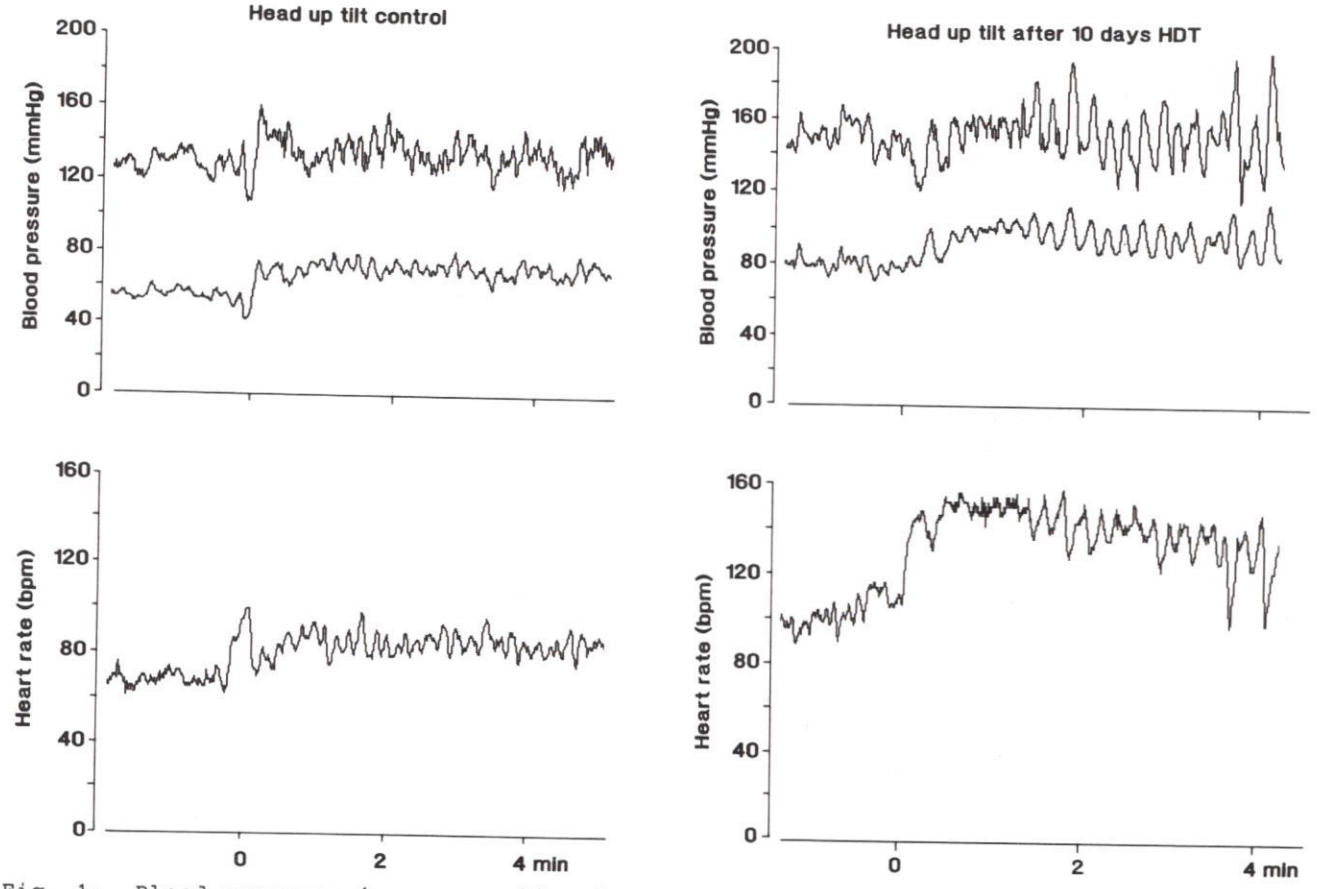


Fig. 1: Blood pressure (upper panel) and heart rate (lower panel) responses to head up tilt before (left panel) and at the end of 10 days head down tilt (right panel) in one subject.

was found. The responses to MS and SHG did not change during prolonged HDT nor by change of posture. HR variation to FRSA did not change either, while the BP variation to forced breathing was larger in the standing position compared to supine. BP variation to forced breathing did not change during prolonged HDT. Posture change from 6° HDT to standing and prolonged HDT caused qualitatively similar changes in BP and HR responses to VM (Fig. 2).

The HR increase upon 70° HUT at the end of 10 days HDT was larger, compared to the increase during control HUT. BP changes were comparable on the two occasions, while BP variability during HUT was larger at the end of 10 days HDT (Fig. 1)

Spectral analysis of the BP variability by FFT (2) showed that the increased variance was confined to a significantly augmented 0.1 Hz peak.

The responses to MS and SHG were not influenced by posture, which indicates that fluid shifts did not influence these responses. Therefore, they may be used as indicators of efferent sympathetic reactivity independent of changes in fluid distribution. The unchanged BP and HR responses to MS and SHG during prolonged HDT indicate that efferent sympathetic reactivity was unchanged. The vagal efferent reactivity was unchanged as well, as evidenced by the HR variation to forced breathing.

A previous study attributed the influence of posture on the responses to VM to fluid redistribution (5). As posture change from 6° HDT to standing and prolonged HDT caused qualitatively similar changes in BP and HR responses to VM this might indicate that similar fluid shifts occur during prolonged HDT as by changing posture to standing.

The changed responses to HUT (increased HR and increased 0.1 Hz BP variability) indicate an increased sympathetic nervous reactivity. This might also be explained by the influence of fluid shifts on the responses to HUT.

We therefore conclude that autonomic nervous reactivity upon manoeuvres that are not influenced by fluid distribution was unchanged, while an increased sympathetic reactivity was shown upon manoeuvres that are dependent on blood volume and fluid distribution. This was probably due to decreases in plasma volume and central blood volume under prolonged HDT.

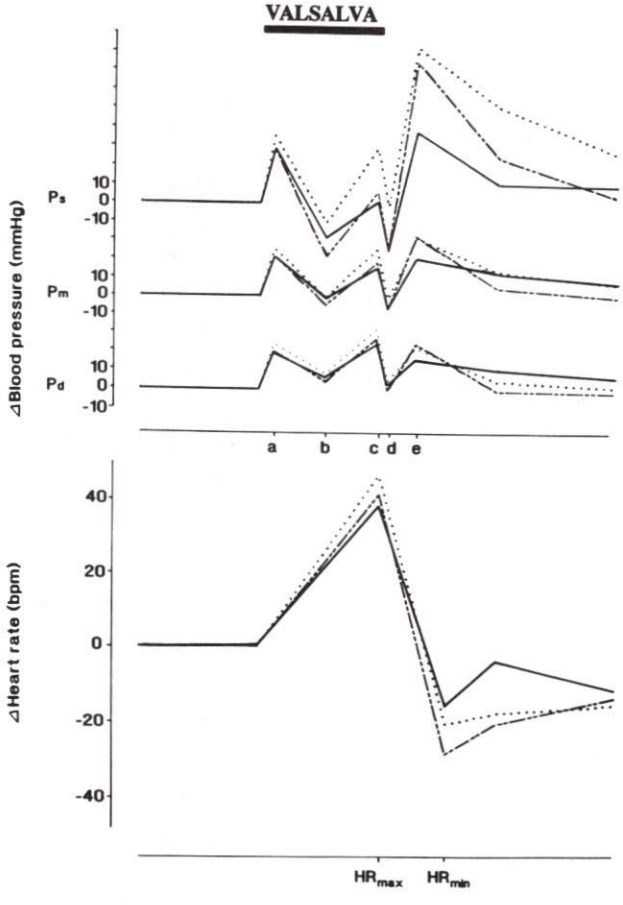


Fig. 2: Blood pressure and heart rate responses to the Valsalva manoeuvre: influence of posture and prolonged head down tilt (average of six subjects)

———— control;
———— — — — standing;
..... prolonged HDT.

ACKNOWLEDGEMENT

This study was supported by grant SRON-MG020 from the Netherlands Organization for Scientific Research (NWO).

REFERENCES

1. Blomqvist, C.G. and H.L. Stone. Response to stress: gravity. In: Handbook of Physiology, Peripheral Circulation and Organ Blood Flow, J.T. Shepherd and F.M. Abboud (Eds). American Physiological Society, 1984, pp 1025-1063.
2. De Boer, R.W., J.M. Karemaker and J. Strackee. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. Am. J. Physiol. 1987; 253: H680-689.
3. Imholz, B.P.M., G.A. van Montfrans, J.J. Settels, G.M.A. van der Hoeven, J.M. Karemaker and W. Wieling. Continuous non-invasive blood pressure monitoring; reliability of Finapres device during the Valsalva manoeuvre. Cardiovasc. Res. 22: 1988, 390-397.
4. Imholz, B.P.M., J.J. Settels, A.H. van den Meiracker, K.H. Wesseling and W. Wieling. Noninvasive continuous finger blood pressure during orthostatic stress compared to intra-arterial pressure. Cardiovasc. Res. 1989, in press.
5. Ten Harkel, A.D.J., J.J. Van Lieshout, E.J. Van Lieshout, W. Wieling. The assessment of cardiovascular reflexes: influence of posture and period of preceding rest. J. Appl. Physiol. 1990, in press.

IUPS Commission on Gravitational Physiology

Cumulative Author Index, 1979-1990

1979 *Physiologist* 22(6): Supplement (New Orleans)
1980 *Physiologist* 23(6): Supplement (Budapest)
1981 *Physiologist* 24(6): Supplement (Innsbruck)
1982 *Physiologist* 25(6): Supplement (San Diego)
1983 *Physiologist* 26(6): Supplement (Moscow)
1984 *Physiologist* 27(6): Supplement (Lausanne)
1985 *Physiologist* 28(6): Supplement (Niagara Falls)
1987 *Physiologist* 30(1): Supplement (Tokyo)
1988 *Physiologist* 31(1): Supplement (Nitra)
1989 *Physiologist* 32(1): Supplement (Montreal)
1990 *Physiologist* 33(1): Supplement (Lyon)

Volume no. given in parentheses, followed by page no.

N

O

P

Q

R

S

T

U

V