Vasilyi V. Parin
1903–1971

Proceedings
of the
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of the
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Orr E. Reynolds, Guest Editor

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Commission on Gravitational Physiology

The *Proceedings* of the 9th Annual Meeting of the IUPS Commission on Gravitational Physiology are dedicated to Vasilyi V. Parin (1903–1971), a world-renowned physiologist who made a significant contribution to different branches of life sciences such as circulation physiology, medical electronics and biological cybernetics, clinical physiology, and space medicine. This long list shows the diversification of his scientific interests. However, he was a dedicated researcher who concentrated on one specific area—the functioning of a living system in the boundary state, i.e., between norm and pathology. His was a search for new methods that could reveal the earliest signs of pathology, a search for the limits of the living system and an exploration of its adaptive and compensatory mechanisms, and a search for approaches, procedures, and techniques that would help man maintain his health and work capacity.

The characteristic feature of Parin's exploratory work was a comprehensive approach to any problem known as a systemic approach. He had a striking capability of effectively applying his expertise accumulated in one area of research to the solution of problems arising in other areas.

Parin started his career as a researcher when he was a student of the Perm University under the guidance of V. V. Verigo, a famous electrophysiologist, and then took postgraduate courses at the Kazan University under the guidance of A. V. Samoilov, one of the founders of electrocardiography. At the age of 30, Parin became the head of the Normal Physiology Department of the Sverdlovsk Medical Institute. Parin was elected the first Executive Secretary of the USSR Academy of Medical Sciences, which was established in 1944, and later, i.e., in 1963–1966, was elected its Vice-President. Parin was very well known not only in the USSR but also in many other countries of the world. He was a member of various international conferences and congresses for physiology, medical electronics, aviation, and space medicine, e.g., IAF International Astronautical Federation and COSPAR (Committee for Space Research) sessions.

In the 1930s to early 1940s, Parin's findings concerning neuronal regulation of spleen contractility associated with cardiovascular adaptation to environmental variations became generally recognized. These responses were largely determined by pulmonary vessel reflexes. Parin's investigations of receptors of pulmonary vessels are still considered classic (1). They resulted in the identification of the unloading reflex of pulmonary circulation, which protects the right ventricle when the pulmonary artery pressure increases and which is referred to in the literature as "Parin's reflex." This reflex is very important for gravitational physiology investigations. More recently, Parin was actively involved in circulation physiology and clinical physiology investigations, having made a significant contribution to this area (2).

The late 1950s and 1960s witnessed intense penetra-tion of electronics, mathematics, and cybernetics into physiology and medicine. Parin was a great enthusiast and a leader of these developments (3). He identified new trends in physiological sciences, taking into account new possibilities of elucidating mechanisms of physiological regulation. At that time, Parin took part in the development of new methods and electronic devices for biomedical application, in the design of early automatic systems to evaluate and control the health condition of man.

Parin was one of the founders of space medicine, in which he fully exploited his talent as a scholar and organizer.

Parin participated in the preparation for the first man-in-space flight made in 1961. After the flight, it became clear that the human factor would be of crucial importance in manned missions. To increase the duration of manned missions, it was necessary to utilize to advantage the data from clinical medicine, applied physiology, hygiene, psychology, and exact sciences. It was also necessary to provide close ties between different scientific disciplines and to develop new research methodology. At that time Parin initiated the development of key components of space biology and medicine such as space biotelemetry, space cardiology, and medical prediction. The works of Parin and his assistants and followers devoted to circulation physiology occupy an important place in space medicine. It is known that microgravity-induced blood shifts toward upper body are in the focus of gravitational physiology. Parin viewed the cardiovascular function as an indicator of adaptive reactions of the whole body (4). This approach still remains very useful, being widely used in other branches of medicine. Some cardiological methods that were developed under the guidance of Parin found a practical application in his lifetime, viz., mathematical analysis of the cardiac rhythm to study circulation regulation and seismocardiography to evaluate the contractile function of the heart. Parin did a lot for the development of ballistocardiography, the technique used to measure cardiac work and coordinated contractions of the right and left heart. His dream was to record a ballistogram in space flight, which is an ideal environment for investigating cardiovascular forces, to streamline the method when used on the Earth. Although ballistocardiography was performed for the first time in December 1977 during the Salyut-6 flight, it was actually a practical realization of Parin's scientific concepts.

Parin paid a lot of attention to various aspects of medical prediction in space flight. This area of research, which is acquiring a greater practical importance from year to year, began with Parin's paper "Prediction in space biology" published in 1968 (5). This paper discussed the basic aspects of medical prediction and prognosis aimed at maintaining good health and high performance of crewmemebers in space missions of increasing duration. Better than anyone else, Parin could distinguish future in the present and properly

evaluate every research work; he knew very well that a breakthrough in science was always based on regular and tedious work. This is particularly true of space medicine in which successful achievements of crewmembers visible to everyone result from great efforts of numerous ground-based terms that remain invisible to the broad public. Parin was a very nice person: benevolent, correct, and kind. The image of Parin as a scholar and person, researcher, and organizer still remains a source of inspiration for many people who devotedly serve science.

References

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3. Parin V. V., and R. M. Bayevsky. *Introduction to Medical Cybernetics.* Moscow-Prague: Medizina, 1966.

4. Parin V. V., R. M. Bayewsky, Y. N. Volkov, and O. G. Gazenko. *Space Cardiology.* Leningrad; Medizina, 1967.

5. Parin V. V. Prediction in space biology. In: *Prediction of Advances in Science and Engineering.* Moscow: Znanie, 1968, p. 31–36.

PRELIMINARY ANNOUNCEMENT

IUPS Commission on Gravitational Physiology—Tenth Annual Meeting
9–13 October 1988
Montreal, Canada

The Tenth Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences will be held in Montreal, Canada, 9–13 October 1988, in conjunction with the Fall Meeting of the American Physiological Society.

The Commission Meeting will consist of open sessions for slide presentations of voluntary papers dealing with the effects on physiological systems of humans, animals, and plants of changes in magnitude or direction of the force environment. Included are the effects of the weightlessness during space flight, acute and chronic acceleration, vibration, and the various forms of simulated weightlessness. Also included is consideration of evolutionary consequences of gravity and the role of gravity in the manifestations of scale effects in animals and plants.

Symposia by invited speakers will be sponsored on the following topics:

Recent Space Flight Results in Gravitational Physiology, I. B. Kozlovskaya, Moscow, USSR, Organizer;

Current Concepts in Gravitational Physiology, N. Pace, Berkeley, California, USA, and H. Bjurstedt, Stockholm, Sweden, Organizers;

and The Lung and Gravity, J. B. West, San Diego, California, USA, Organizer.

It is planned to publish the Proceedings of the Tenth Annual Meeting as a supplement to *The Physiologist.* The Proceedings will contain both voluntary and symposium papers presented at the meeting.

Your participation in the Commission Meeting is welcomed. If you are interested in the particulars, please contact Orr E. Reynolds, Ph.D., Commission Business Officer, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814, USA, by 1 May 1987 for abstract, registration, and housing information.

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Members of the IUPS Commission on Gravitational Physiology

Introductory Remarks

It is a great pleasure for me, as Chairman of the International Commission of IUPS on Gravitational Physiology, to welcome all of you to another of the Commission's Annual Meetings, the ninth of its kind. For the Commission it is especially gratifying to note that in spite of gravitational physiology being a young offspring of the physiological family, it has shown remarkably rapid growth and development into maturity. Not only have our annual meetings been well frequented from the start, but we like to think that because of our Commission's pioneer activities during the first years, interest in the problems of gravitational physiology rapidly gained a sufficient degree of permanency to justify the establishment of other scientific societies, national and international, to further encourage and promote research in the area.

During the Commission's latest Business Meeting in Tokyo in 1986 the Commission was happy to note the interest shown by the Slovak Academy of Sciences in our activities. We greatly appreciate having been kindly invited by the Academy to hold this year's Annual Commission Meeting in the beautiful region of West Slovakia, in the town of Nitra, which I understand is today the all-Slovakian center of agriculture and at the same time represents the old culture and history of Slovakia, with the Nitra Castle being the dominant feature and a clinical monument.

The Commission on Gravitational Physiology was established in 1974 by a decision of the International Union of Physiological Sciences. The purpose of the Commission is, among other things, to promote scientific communication concerning the effects on physiological functions of changes in magnitude or direction of the gravitational force environment. Included are the effects of the earth's gravity, of the weightlessness of space flight, of acute and chronic acceleration and of vibration and the various forms of simulated gravity and weightlessness. Also included are the evolutionary consequences of the earth's gravity and the role of gravity in the manifestations of scale effects on animals and plants.

During the course of the four days ahead of us a great number of papers will deal with results from investigations in most of these problem areas. They will be distributed over 13 sessions. As the program shows, three of these are symposia consisting of invited papers, and 10 sessions are devoted to voluntary papers. The first symposium will be held this morning, and will include presentations of recent space flight results in gravitational physiology. The second symposium will be devoted to gravitational effects on the cellular level and is scheduled for tomorrow morning's session. The third symposium will highlight certain current concepts in gravitational physiology and will be held on Wednesday morning.

In the 10 sessions of voluntary papers a considerable variety of topics will be discussed. These will deal with effects of normal gravity as well as of true and simulated weightlessness and of high-G exposure on physiological functions on many levels. These functions include those of the respiratory system, thermoregulation, muscle and bone tissue, sense organs, plants and single cell organisms, and hormones and metabolism. In addition there will be reports on gravitational effects on animal development and on the relationships of biological rhythms and gravity. There will be a session on the effects of gravity on the avian organism and one on new projects and methods for the study of the physiological effects of the G factor.

All the invited papers and other papers presented here will appear as the Proceedings of this meeting and will be published in *The Physiologist*, a serialized journal of the American Physiological Society. Manuscripts are to be prepared on special photocopy mats, which have already been mailed to the speakers.

I would like, at this point, to extend the Commission's great appreciation and gratitude to the Slovak Academy of Sciences for graciously hosting this meeting. I especially wish to express our thanks to Professor K. Boda and Professor L. Macho as representatives of the Academy and to Professor M. Pospisil of the Intercosmos Program. We are greatly indebted to the Local Organizing Committee and its Chairmen Drs. M. Jurani and R. Kvetnansky for all the efforts they have expended in making the preparations necessary for the successful realization of the meeting. Finally, let me also thank, on behalf of the Commission, all our speakers and their colleagues for their work and the time they have spent in preparing their papers to make this meeting a stimulating and memorable experience for all of us. Let me conclude these remarks by introducing Professor Boda, Director of the Institute of Animal Physiology of the Slovak Academy of Sciences.

H. Bjurstedt

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

MEDICAL INVESTIGATIONS RESULTS OBTAINED
IN 125-DAY FLIGHT ON "SALYUT-7" AND
"MIR" ORBITAL STATIONS

A.Egorov, O.Anashkin, O.Itsehovsky,
I.Alferova, L.Golubchikova, V.Lyamin,
A.Polyakova, V.Talavrinov, V.Turchani-
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In 1986 in USSR medical investigati-
ons were continued in 125-day flight on
"Salyut-7" and "Soyuz-T-15" orbital sta-
tions and on "Mir" basic block. Medical
program consisted of medical control du-
ring active parts of flight and works in
open space, profound medical examinations
in rest and in function tests, metabolism
and its regulation analysis, hygienic es-
timation of environment, estimation of
prophilactic methods to prevent unfavou-
rable influence of weightlessness on hu-
man organism. Cosmonauts felt well in all
parts of flight on the dates of radio ex-
change, TV-seances, self-and each other
control. Profound medical investigations
discovered several individual changes in
parametres of every cosmonaut's blood
system. This charactaral changes appeared
not in rest only, but with the influence
of loading test and depended on initial
hemodynamic status before the test.

In 1986 in USSR medical investigations
were continued in flight on "Salyut-7"
and "Soyuz-T-15" orbital stations and
on "Mir" basic block (a new generation
of stations).
Cosmonauts felt well in all parts of
flight on the dates of radio exchange,
TV-seances and medical questionnaires,
tiredness was absent. The first adapta-
tion period to microgravity was taking
its normal course, cosmonauts were men-
tioning the rush of blood to head du-
ring first days, space movement illness
symptoms were absent.
By cosmonauts' reports physiological
indices fluctuated within usual rate du-
ring the flight: body temperature 36.0-
36.8°C, arterial pressure - systolic
pressure 108-120 Hg mm, diastolic pres-
sure 60-75 Hg mm; hand indices 50-55 kg
were almost the same as before the
flight.
At the beginning of the flight du-
ring two months cosmonauts' body mass
didn't distinguish from pre-flight mass,
but in further period it grew from initi-
al mass to 4.5 and 0.9 kg. The deficien-
cy of the leg volume was 18-22%.

Medical program consisted of the fol-
lowing parts: medical control, some meta-
bolism indices investigations and metabo-
lism regulation researches, cardio-vascu-
lar investigations, hygienic estimation
of environment, estimation of prophilac-
tic methods to prevent unfavorable influ-
ence of microgravity on human organism,
medical investigations during the work
in open space.
Heart rate changed according to the
influencing factors and the whole situa-
tion in all parts of flight. Heart rate
rose in one hour period on every active
part of the flight during docking, un-
docking, flight from one station to ano-
ther and work in open space.
Work-and-rest regime was kept during
the whole flight. The twenty four hours
were divided in two periods - work and
everyday necessities. Work period formed
8 hours 30 min., the period of everyday
necessities - 15 hours 30 min. The dura-
tion of sleep-time and spare time was
not less than 9 hours.
Cosmonauts were doing regular train-
ing exercises one a day during 1 hour
and 80 min.
Neuro-psychological sphere of cosmo-
nauts was in good state in all parts of
the flight. Emotional reactions against
the background of moderate tension were
adequate to the situations.
Cosmonauts' work efficiency was high,
emotional reactivity was stable on the
whole, the mood was equal. Moving acti-
vity was usual, without disturbances.
Facial expression and gesticulation
were alive, speech activity had an indi-
vidual manner.
The following methods were used for
the estimation of the blood circulation:
electrocardiography with 9 or 12 conven-
tional leads and with DS-lead; reography
to calculate the heart stroke volume and
minute blood volume and to investigate
the state of cerebral hemodynamic in in-
ner carotid artery basin and vertebro-ba-
silar system basin; venous-arterial pul-
sogram from jugular fibro-vascular ban-
dle and tacho-oscillogram from upper arm
to register arterial pressure indices.
Physiological parameters were studied in
rest and during dosed physical exercise
(D.Ph.E;) and under the influence of low-
er body negative pressure (LBNP). The
D.Ph.E. had two stages: 125 Watt during
5 min., and 175 Watt during 3 min. with
one minute pause. The LBNP test was exe-
cuted in the following regimes: 25 Hg mm
during 1 minute, 35 Hg mm in 3 minutes,
45 Hg mm in 3 minutes. Each cosmonaut
was examined 10 times including functio-
nal tests through the whole flight.

Investigation Results

At rest haemodynamic indices changes
may be divided relatively into 3 stages.
At the first stage (30-40 days at the
beginning of the flight) several indices
of both cosmonauts changed similarly in
quality but with differencies in quanti-
ty (fig. 1). Sinking of several indices
was observed, among them heart rate (HR),
minute blood volume (MBV), arterial pres-
sure (AP) and peripheric vascular resis-
tance (PVR). Stroke volume (SV) didn't

change or increased by 21%. Absolute meaning of arterial pressure indices and PVR of one of the cosmonauts were lower than those of the other, but meanings of haemocirculation volumes were higher both in flight and before it.
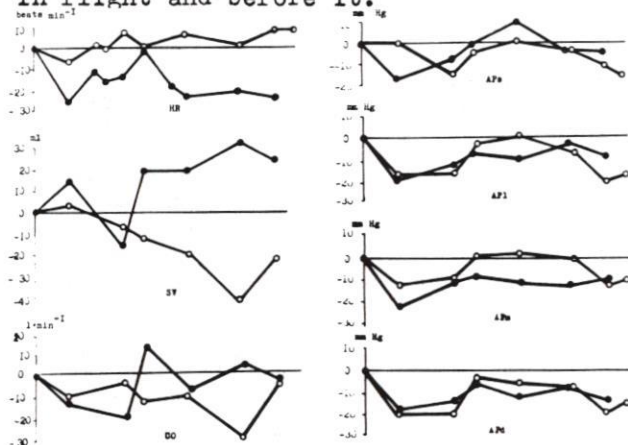
Fig.1. Cardiovascular changes at rest in the "Mir-Soyus- 125-day flight
Note: —.—. fluctuations of mean value inflight

At the next stage (by the beginning of the 4th month) there was a period when both cosmonauts' indices changes had different character. One cosmonaut demonstrated HR lowing and SV incresing after its previous lowing on the 41st day of flight. MBV dynamics had unexpressed remitting character. HR of the other cosmonaut grew moderately, SV sank progressively (especially on the 96th day of flight), MBV was constantly lower comparing to the pre flight index. AP indices of both cosmonauts had tendency to recovery, but such indices of one of the cosmonauts didn't achieve the pre-flight meaning (except for increased final systolic pressure-, the other cosmonaut's indices corresponded to the pre-flight meanings (excluding sinking minimal AP).

The third stage longed approximately 3 final weeks of the flight. At that time SV had tendency to return to initial pre-flight meanings (one cosmonaut's index after constant sinking, the index of the other cosmonaut after increasing), the meanings of MBV returned to initial ones with simultaneous sinking of PVR indices and repeated decrease of AP indices.

At some stages of the flight (16-41 days of flight, 112, 122 days) one cosmonaut had marked signs of hypotonic status in AP, volume of haemocirculation and PVR lowering. Minimal AP achieved 42 Hg mm, average dynamic pressure was 66 Hg mm, lateral systolic pressure - 81 Hg mm, final systolic pressure - 108 Hg mm. Pulse AP and the meaning of haemodynamic shock (as difference between final and lateral AP) didn't change significantly comparing to the pre-flight data.

The investigations at rest showed individual character of adaptation to flight conditions: one cosmonaut had symptoms of hypervolemia in upper part of the body, mainly increased HS and simultaneous decrease of HR, AP and PVS. The other cosmonaut had no signs of blood redistribution to the upper part of the body and decrea-

se of the haemocirculation volume with indications of hypotonic syndrome. We may suppose that the volume of the circulating blood was reduced. The results of functional tests are inderect evidence to this conclusion.

The character of haemodynamic reactions of both cosmonauts to DPL changed. In tests with DPL on 51st day HR grew significantly compared to the pre-flight meanings; one cosmonaut showed increase of HR by 18-24 strokes/min. the other- 9-10 strokes/min.; RWC-170 of the first cosmonaut decreased by 24% of the other - by 14% (Table 1).

Table I.

Cardiovascular changes during exercise tests (bicycle ergometry)

Crewmembers	Parameters	Preflight			Inflight					
					51-st day			71-st day		
		Rest	125w	175w	Rest	125w	175w	Rest	125w	175w
1	HR	77	128	138	76	156	162	55	118	132
	APd	70	70	65	60	58	-	55	58	57
	APm	101	117	100	77	79	-	74	81	72
	APl	118	135	118	97	105	-	95	108	95
	APs	140	162	155	125	150	-	135	157	183
	SV	64	99	89	87	64	-	86	102	63
2	HR	68	123	134	69	132	144	74	130	156
	APd	64	55	58	58	53	50	56	43	44
	APm	81	92	92	80	72	70	81	-	70
	APl	102	102	104	97	96	95	100	-	102
	APs	122	143	147	119	140	158	125	188	193
	SV	119	148	142	99	99	86	91	85	99

Note: HR = heart rate (Beats·min⁻¹)
APd = diastolic arterial pressure (mm Hg)
APm = mean arterial pressure (mm Hg)
APl = lateral systolic arterial pressure (mm Hg)
APs = systolic arterial pressure (mm Hg)
SV = stroke volume (ml)
CO = cardiac output (l·min⁻¹)

Both cosmonauts demonstrated decrease of relatively initial level of HS after test by 26% and 14% correspondingly; MBV increased not so sharp compared to pre-flight testing and was formed due to the growth of HR.

The cosmonaut with lower meanings of minimal and average dynamic AP at rest after DPE test showed further decrease of these indices with simultaneous growth of final systolic pressure to more high level (pre-flight meaning of this index was 147 Hg mm).

In tests with DPE on 71st day several blood indices changes of one cosmonaut during the load and right after it were close to the pre-flight meanings: HR didn't exeed 118-132 beats/min. and returned to initial meanings easily enough, PWC practically corresponded to the pre-flight meaning. However, as in the first test SV decreased by 27% after the second stage. The other cosmonaut's reaction to the load was more expressed than on the 51st day. It revieled itself in greater increase of HR especially on the second stage HR was 22 beats/min. stronger than pre-flihgt. RWC-170 decreased by 35% compared to the ground. HS didn't change practically. The growth of final systolic AP was more relevant than pre-flight and achieved 183-188 Hg mm (155-162 Hg mm pre-flight).

Thus there was intensification of cardio-vascular reactions to the physical load though it appeared in different

flight periods: one cosmonaut had them in earlier and the other in later periods of the flight.

In this flight, as in many previous, characteristic for weightlessness peculiarities came to light in dynamics of blood indices: absence of growth and even tendency to decrease of HS at DPE; less expressed compared with pre-flight data increase of MBV, to ground data final systolic pressure enlarged the expence sharp grow of hemodynamic stroke, dyastolic and average dynamic AP decreased

The results of test with DFE got in present flight conform to earlier assumptions cardial chrotropical functions increasing directed to compensate venous blood return less intensive in microgravity. Evidently, hemodynamic reorganization in flight under physical load is connected with some volume deficiency of circulating blood, reorganization is directed to its more effective and economical delivery to working organs.

LBNP provoked adequate cardio-vascular reaction in both cosmonauts, test endurance was estimated as good. Side by side with this the attention was paid to several peculiarities of blood indices under vacuum. The use of LBNP promoted normalization of reoencefalogram form (against the background of lowered small cerebral vascular tone), indicating the growth of vascular tone (particularly of small vessels) to its whole normalization. This phenomenon was observed in many cases with different cosmonauts, but not with everyone. Hence LBNP, being selected strongly for individual use may be used only as prophilactic method for training of orthstatic steadiness but as a factor of cerebral circulation normalization (Table 2).

ves quality, increased moving activity of examined persons could be supposed. Evidently, LBNP through a short period of time (during 1 minute) provoked change for the worse of person's state in connection with cerebral circulation disturbances which manifested in marked vasodilatation and decreased energy of systolic throw. Examined individual eliminated momentary appesred deficiency of VCB and normalized cerebral blood circulation by his legs moving and starting up muscular pumps. It is important that in this case such indices as haemodynamic stroke and dicrobic index REG turned out to be the most informative for extraordinary prognosis of orthostatic steadiness lowing to other haemodynamic indices. High diagnostic significance of REG indices in similar conditions was noted by other researches.

Thus conducted investigations showed the functional state of cardiovascular system of the 5th crew on "Salyut-7"and "Mir" stations to be well enough, though blood circulation indices fluctuated within wide limits through the flight. Haemodynamic changes had adaptive character and were formed on the whole by microgravity factor, which caused the redistribution of organism's liquid medium from the one hand, and facilitated the load sinking on cosmonaut's muscules and the deconditioning to some extent. The data received in this flight supplement and define more exactly hypotetic schemes suggested before.

Table 2.

Cardiovascular changes during LBNP tests

Crewmembers	Parameters	Preflight			Inflight (94/96-th day)		
		Rest	-35 mm Hg	-45 mm Hg	Rest	-35 mm Hg	-45 mm Hg
I	HR	73	88	91	58	66	74
	APd	71	73	72	58	-	61
	APm	90	94	91	73	-	80
	APl	107	107	103	102	-	100
	APs	138	128	131	122	-	120
	SV	64	55	59	99	79	79
	CO	4,7	4,8	5,2	5,6	5,7	5,7
2	HR	65	80	88	69	81	93
	APd	61	62	63	55	63	59
	APm	77	81	85	79	88	84
	APl	93	94	97	96	94	95
	APs	119	120	119	120	112	111
	SV	103	78	70	70	61	54
	CO	6,6	6,3	6,1	4,7	5,1	5,3

Note: see table I.

Let's consider REG data of the other cosmonaut. There was observed momentary displacement to isoline on the second minute of LBNP test when rarity was 45 Hg mm. This fact indicates sinking of small vascular tone. Simultaneously haemodynamic stroke decreased sharply. Other indices didn't change significantly. At the same time judging by registrated cur-

BIOMECHANICAL CHARACTERISTICS OF BONE STRUCTURE CHANGES FOLLOWING REAL AND SIMULATED WEIGHTLESSNESS

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As a result of 18,5-19,5-day space flights the mechanical characteristics of the femur heads of the rats were significantly degraded. Following 20-42 days of the bed rest of the subjects who have been treated in these conditions and died of the suddenly developed complications, the mechanical properties of the vertebrae were also degraded as a result of a calcium concentration decrease in the vertebrae ash residual. Defence mechanisms against bone structure resorption may exist in the human spinal column. Spinal column resistivity to the impact exposure is decreased not only due to a partial loss of the strength by the vertebrae but as a result of its changed dynamic reaction.

Raised with the advent of manned space flights, a question concerning the possibility of maintaining, under weightless environment, the typical bone properties opposing the gravity and hypergravity effects, has become, when solved, a very complex problem required the conduct of a deeper fundamental research. And this is not a chance event. First, an answer to a simple, at first glance, question is difficult to get on the basis of examining the cosmonauts, therefore, an experimental-theoretical approach to solving the problem is a more acceptable one.

Second, consideration must be given to the variability of weightlessness effects, depending on biological species, characteristics of an individuum as well as on bone as a multifunctional organ (weight-bearing, non-weight-bearing, spongiosus, compact). Third, it is necessary to study a bony substance at various structural levels in order to get a full understanding and probable mechanisms of the changes.

On the basis of such methodological approach, it is possible to do the theoretical generalizations with respect to etiology, mechanisms of bone changes, their species, individual and topological characteristics, an integrated alteration of the structure and the mechanical properties of the bone, which show the ways of preventing the shifts detected.

It is clear that some particular questions relating to different trends in research can be resolved either in experiments with animals or on the basis of examining the test subjects and the cosmonauts or with an application of the analytical methods. Revealing of the general regularities of structural alterations is possible using total consideration of the data obtained.

Summarizing the data so gained it is possible to state that under real and simulated weightlessness there develops an involved complex of bone changes which, as a whole can be classified as osteodystrophy. It shows itself as a decrease in the volume content of bone mass and hence of mineral substances, as a slight fall in a degree of mineralization of the organic matrix of the bone, as a small decline of calcium concentration in a bony ash residual. In this case, there observes a dissociation of mineralization of bone substance: low mineralization of the organic matrix, formed under hypogravic conditions and hypermineralization, of the old structures (4, 5).

Osteodystrophy is developing more rapidly in the weight-bearing bones of the body and an intensity of the changes in the spongy structures is substantially higher than in the compact ones. In an inter-species aspect, a degree of alterations in spongy bone varies, depending directly on an intensity of metabolism and inversely on an initial density of the bone structure and the relation of the variables mentioned may be expressed by one equation (5).

The manifestation of heterotopia of the bony changes may be explained, on the one hand, by a significant difference in the alterations of force exposure for various bone structures (weight - and non-weight-bearing), on the other hand - by a different rate of their physiological rearrangement.

The first circumstance explains an actual selectivity of the changes of the bony skeleton with respect to its non-weight-bearing compartments. The second one explains a delay in the development of osteodystrophy in the metabolically more inert structures in relation to the ones characteriezed by a high metabolic rate.

From theoretical point of view and in an effort to afford manned spaceflight safety, it is important to know about an effect of the osteodystrophic changes on the bone strength and especially on the spongy bone.

The studies of mechanical properties of the bone have been conducted in two directions. The first direction, having chiefly a theoretical significance, focused on revealing a tendency of the shifts of these properties, based on the findings of studying bone material of the rats flown aboard Biosatellites "Cosmos-782, 936 and 1129". The femurs have been examined. The mechanical properties of the head of the femurs when compressed degraded significantly and constantly (Fig.1).

ULTIMATE STRESS, kp/mm^2 — MODULUS OF ELASTICITY, kp/mm^2 — ELASTIC ENERGY, $kp \cdot mm/mm^3$
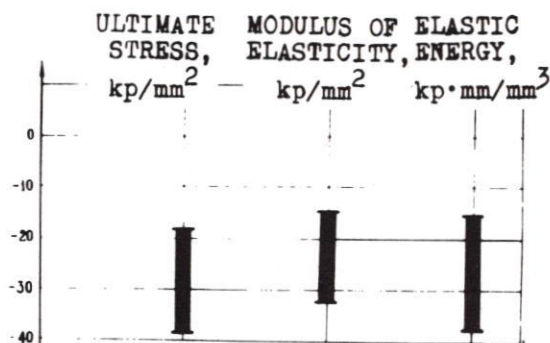
Figure 1. Change of mechanical characteristics of heads of the femurs in the rats as a result of 18.5-19.5 day space flights.

It related both to the indices of the strength and modulus of elasticity, and specific energy. A degradation of the characteristics of mechanical properties was associated with a severity of osteodystrophy in a spongy substance of the heads what was seen from comparing the indices of the strength and volume content of mineral substances. The relationship had an exponential pattern - $y = 0.524 \cdot 58.9^x$ and was sufficiently tight ($r=0.982$). Such a relationship could be also seen for other mechanical features studied, namely, modulus of elasticity and specific energy with somewhat lesser correlation coefficients (0.958 and 0.820 respectively).

It should be particularly emphasized that the dependences derived are of general character both for normal and osteoporotic bone as well. From this it follows that decrement of bone mass plays a key role in lowering the indices of mechanical properties of animals exposed to weightlessness.

However, resorption of a part of bone substance is not the only cause of a partial loss of the strength by spongy bone. Such a conclusion follows from the results of studying the characteristics of a substance of the heads of the femurs in the rats flown on board the biosatellite "Cosmos-1129" after a 6 - day recovery period. By this time, judging from an index of the heads density, there was a slight trend of bone mass

increment. However, the strength continued to decrease (Table 1).

Table 1.

Relationship of some indices of heads of the femurs in the rats after space flight and 6-day recovery (% of control)

Groups	Density of dry heads	Calcium content in ash residual	Strength limit
Space flight	94,0	95,5	81,1[*]
Recovery	95,0	94,0	72,3[*]

[*] Here and further there are significant differences as related to control.

Together with this, there was a tendency towards futher declining the calcium concentration in an ash residual of the bone. Hypothetically, it is precisely this fact that explains a decrease of the bone strength. Loss of calcium ions being on the surface of the crystals of hydroxyapatite can weaken the force of a physicochemical bond of a "collagen - crystal" complex and may result in decreasing the resistivity of bone substance to a mechanical load. Since the shifts presented in Table 1 occurred at early stage of the recovery after the space flight it is possible to assume that any activation of bone rearrangement associated with a change of the external conditions towards both hypo - and hyperfunction of bone may result in a decrease of its mechanical properties.

Thus, a weightlessness-induced osteodystrophy causes a considerable loss of characteristics of mechanical features of the spongy bone. At the same time, it appears that a progressive decline of the strength independently on the density of bone structure may arise as a result of the changes occurred at the lower structural levels of the bone.

From the data presented and their interpretation, it follows that in order to predict the changes of the mechanical properties of human spinal column as applied to an exposure to weightlessness of a particular duration there need be at least the following characteristics of osteodystrophy: change of bone structure density and calcium content of a mineral component. The solution of both tasks come across the methodological difficulties related to vital determination of appropriate parameters. Therfore, concurrent with the experimental works, a theoretical substantiation of possible levels of bone mass loss by the various compartments of skeleton has been done. Thus, based on a hypothesis of a directly proportional dependence of losses upon the rate of physiological rearrangement, we obtained the values of the bone mass loss for various compartments of the skeleton, depending on magnitude of negative calcium balance (2). It is seen from the figure that spinal column may be subjected to the more marked changes. As for

another compartments of skeleton, the severity of osteodystrophy may vary considerably. The theoretical level of losses has a good agreement with the converted literature data for hipbone (3) and heel bone (2). However, for lumbar vertebrae such an agreement is not reached. According to the literature data (1), the losses of mineral substances in the spinal column are about 4 times lower than theoretical ones. What is the reason for such differences? Based on a conception of conditionality of bone dystrophy by a reduced exposure to force on skeleton under real and simulated weightless environment then this suggests that for the spinal column this exposure may change to a lesser extent than for other bones of the skeleton. Let us consider this question in detail. The anatomofunctional property of vertebrae is their ability to conjugate with intervertebral disks which are highly tight and rigid systems. The strength is provided by the collagen fibers having complex architectonics. They are also responsible for a regidity to which a high hydration of the disks, particularly its gelatinous nucleus, is made a contribution. Hydration is provided essentially by a high osmotic pressure of the organic substances, among which proteoglycanes, play a leading role. In man when upright an osmotic pressure is balanced by the one produced by the above body mass and body muscular tension. In on equilibrium state, the pressure within the lumbar disks amounts to 6.5 kgf/cm^2 (6). It is clear that just this pressure is transmitted to end lamellae of the vertebrae.

During weightlessness or bedrest, an equilibrium is disturbed at the cost of weight unloading of the vertebra. After an immediate change of man's posture from vertical to horizontal one, the pressure within the lumbar disks is decreased up to 3.3 kgf/cm^2 (6). However, during a prolonged stay in such position the pressure should reach a level corresponding to an osmotic self-pressure at the expense of extra hydration of the disk, i.e. should approach 6.5 kgf/cm^2. It appears that an elevated hydration may explain an increase, under weightlessness and bedrest conditions, of height of the disks and length of the human torso as a whole. Stabilization of length increment occurs on 1st-6th days. It appears that by this time an appropriate intradisk pressure is reached. A presented hypothesis should be checked, however, it shows the ways of scientific search. Indeed, it is known, for example, that an increase of the length of trunk varies individually. The differences in the levels of self-pressure in a disk can be judged, based on this fact. And if so, it is possible to expect the different effects of an exposure to real and simulated weightlessness on the spinal column. It is anticipated that in individuals with a pronounced hydration of the disks there will be even an increase of the density of bony struc-

ture as a result of a 24-hour effect of pressure on the vertebrae in amounts corresponding to vertical position of the body on earth. And conversely during defficient hydration in case of osteochondrosis of spinal column, an increment of intradisk pressure in real and simulated weightlessness may be inadequate, therefore, the losses of the bone mass close to a theoretical level will occur. It might be well point out the fact that distinction of gelatinous nucleus diminishes from the lumbar to mid - and superthoracic compartments of the spinal column. Therefore, a relative loss of the bone mass in a particular man in different compartments may vary due to the differences in an anatomo-functional state of the disks.

In an effort to evaluate the significance of particular changes in the spinal column for its biomechanical characteristics, the experiments with a static and dynamic loading of its elements have been conducted. The single vertebrae $T_{10} - L_3$, taken from 9 cadavers of human beings aged between 22 and 45 years, were subjected to static testing. Of these humans, 5 persons before their deaths have been under conditions of bedrest for 20-42 days and died as a result of the suddenly developed complications. The vertebrae from 4 persons died in an accident have been used as a reference material.

The changes in the density of the bone structure of vertebrae by an index of volume content of mineral substances during bedrest have not been observed (Table 2). However, the calcium concentration in ash residual was diminished ($p < 0.05$).

Table 2.

Characteristic of volume content of mineral substances in the vertebrae and calcium concentrations in an ash residual during 20-42 day bedrest.

Groups	Volume content of mineral substances g/cm^3	Calcium content in ash residual %
Control	0.250$\pm$0.050	36.72$\pm$0.56
Bedrest	0.254$\pm$0.030	34.46$\pm$0.53
% of control	101.6	93.8*

The values of strength, modulus of elasticity and specific energy in a bedrested group were considerably lower than these observed in control (Table 3).

In other words, in this case there was a picture identical to that observed in the rats after a 6-day postflight recovery. A decrease of the strength of vertebrae in an absence of signs of osteoporosis but with a decline of calcium concentration of mineral component mainly is indicative of the calcium loss from the surface of hydroxyapatite crystal and of loosening its adhesion with collagen. Does this loosening associate with onset of rearrangement of the bone

towards decreasing or increasing the bone mass-is difficult to judge, based on the findings of this study.

Table 3.

Change of mechanical features of human vertebrae during 20-42 day bedrest.

Groups	Mechanical features		
	Limit of strength kgf/cm^2	Modulus of elasticity kgf/cm^2	Specific energy kgf/cm^2
Control	68.9 2.1	1700.2 111.1	1.48 0.01
Bedrest	47.1 1.2	1169.4 70.0	1.01 0.05
% of control	64.8*	68.8*	68.2*

Two segments $T_{11}-L_3$ were subjected to dynamic loading. One was isolated after the death of patient who was bedrested for 74 days. Another segment was taken from the cadaver, after a sudden death of patient, and was identical with the first one according to the parameters of body mass and density of bone structure of the vertebrae. The segments have been fixed in succession on a platform simulating the case of reentry capsule and did load by mass corresponding to the one of an above-located compartment of the body. The whole system was subjected to a graded impact exposure by its dropping on arresting device what as a whole simulated an interaction of recoverable capsule with the ground under some landing conditions. Duration of an impact impulse was about 60ms. The results of the studies are presented in Table 4.

Table 4.

Characteristics of the material tested and data of dynamic experiments with segments of vertebrae $T_{11} - L_3$.

Segment	Volume content of mineral substances in vertebrae, g/cm^3	Loading, mass, kg	Platform load, units	Mass load, units	Δ,%	Acting force, kgf
Control	0.267	41	21.2	21.7	1.9	890
Bedrest	0.269	40	19.0	25.0	31.6	1000

With the same density of the bone structure of the vertebrae bodies for both segments in a control experiment, an acceleration on the platform and loading mass practically coincided while in the basic experiment an acceleration on the segment considerably exceeded an appropriate index on platform. As a result strength acting upon a segment in the second case substantially increased which resulted in a destruction of T_{12}.

Static strength testing of the T_{10}, isolated after 74-day bedrest demonstrated its decrease as in the cases of

20-42 day bedrest by 30% of the proper value (81.5 and 115 kg/cm^2 respectively).

Thus, a complex of osteodystrophic changes, developing in real and simulated weightlessness, result in decreasing the resistivity of spongy bone to an effect of static and dynamic loads. A leading factor of the changes of its mechanical characteristics is the loss of the bone mass. Another factor can be a mobilization of calcium from the surface of the hydroxiapatite, accompanying by loosening a "collagen-crystal" bond, this reaction may be more rapid, being a component of changing the rearrangement of the bone in accordance with a variable of functional conditions. In the spinal column of man may exist the defence mechanisms against the resorption of the bone structure at the cost of the anatomo-functional and biomechanical properties of intervertebral disks. But in this case it may also occur a decrease of durable, elastic and energy-absorbing features of the vertebrae at the expense of declining the calcium concentration in hydroxyapatite. Resistivity of the spinal column to the impact exposures decreases not only due to a partial loss of strength by the vertebrae but as a result of its changed dynamic reaction.

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NEURONAL ACTIVITY OF NUCLEUS VESTIBULARIS DURING COORDINATED MOVEMENT OF EYES AND HEAD IN MICROGRAVITATION

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ABSTRACT

In this issue the authors describe the surgical and technological methods, which provided stable recording of electric activity of neurons of vestibular nuclei in monkeys during the flight of the biological satellite "Kosmos-1667".

The vestibular system depends on gravity more than any other system. Its work in microgravity breaks sharply. This was assumed from the experience of cosmonauts and indirect experimental results that were obtained in the vestibular-motor investigation. There are certain data which testify the change of static and dynamic excitation of vestibular system in weightlessness, but these are only indirect evaluations of the system state as a whole.

We have no knowledge of the activity of different parts of vestibular system in weightlessness. Only direct recordings of bioelectrical activity of vestibular structures in intact animals during natural motor acts can give the data for a detailed description of vestibular disorders in weightlessness.

Traditionally, in order to place an element on the animal's head, one removed the skin and soft tissues from the skull and then fixed the necessary elements right on the skull. This method has certain shortcomings. Thus, there is great possibility of inflammation of tissues on the head on the edges of the injured area, the electrodes and constructive elements in plastmass attached to the skull cannot be changed; the electrodes are likely to be damaged when the animal is kept in cage for a long time. These shortcomings were done away with due to a new method (1,2,3). According to this methoda light plastic base was fixed on the skull with the help of screws without removing the muscles and skin, then all the electrodes and technological elements were attached onto this base.

Under narcosis 10 holes (1.5 mm in diameter) were radially made in the skull; the skin and muscle tissues in these points were slightly cut. The screws made of medical non-rusting steel were driven into the holes. The rounded ends of the screws protruded into the inside of the skull at less than 1 mm, dura mater did not suffer. The screw heads were tied by a wire (0.5 mm in diameter) and served as a framework for the plastic base and as the ground. The plastic mould was placed under the wire carcass which had special cutouts for radially inserted screws, and the fast polimerisation plastmass was casted in. The lower part of the plastic base was stuck to its upper part with the help of the same plastmass. Nuts were inserted into the upper part of the base in order to fasten technological elements and to fix the monkey's head in the special chair frame. The base was raised 3-5 mm above the skin.

Such method of attaching the base to the head did not cause traumas or inflammations in any monkeys of the flight group. The rigidness of the base-head fixation was so high that the animal's head could be regularly gripped in a special chair frame during the experiments and also for the correction of elements and electrodes without narcosis. There were no cases of the loss of recording due to the break in the electrode fixation, there were no pathological changes in the skull bones.

All the elements were fixed on this base: transducers, connectors, preamplifiers, electrodes. These elements were protected by a cap made of plastic. The aluminium foil stuck to the inside of the cap shielded the recording circuits. Figure 1 schematically represents all technological elements on monkey's head in assembly.

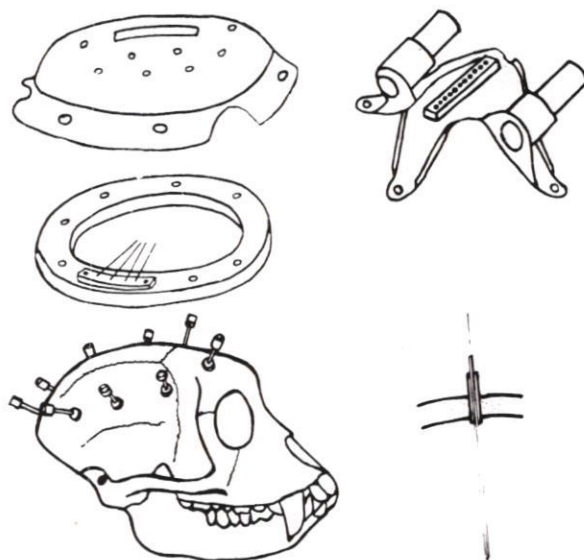Figure 1. Set for fixation of technological elements on the monkey's skull.Screw positions on the skull bones, plastic base with electrode arms, protecting cap and frame with two preamplifiers and connectors. Bottom right - scheme of stereotaxic insertion of microelectrode in the brain: isolated electrode is in the guiding glass tube,which is in stainless steel canule, stereotaxically protruded through the bone.

3-4 weeks after the base was attached to the head, the second stage of the operation took place. Under the narcosis holes (1 mm) were stereotaxically drilled in the skull bone through the skin and muscle tissues. Steel canules were inserted into the holes, so that the dura remained safe. The outside diameter of the canule was equal to that of the hole, which provided for the stereotaxicity of the canule fixation. The canules were filled with steril wax, so that in the postoperational period the epidural area should not be connected with the external side. The canules were united and connected with the base by plastmass insertion. More than 50 canules could be inserted into the skull bone. The canules guided microelectrodes during their penetration into the brain.

The electrooculografic and the encephalografic electrodes were implanted according to standard procedure.

On the animal's head there were: 2 preamplifiers, the connector, the arm for the fixation of electrodes, the frame and the cap.

Preamplifiers were mounted on the connectors and could be removed. The input resistance of these amplifiers was 20 Mom, the level of noise - 5-7 mcV and the gain amounted to 10. A special light metal frame was used for placing two preamplifiers and the connector. The frame was attached to the base by screws. Such disposition of technological elements made the possibility for the change of elements and for the control of troubles.

The electrode arms were made of a thick wire, fixed to a small isolating plate. This plate was screwed to the base.

The standard caps were made of acrylic plastic (3 mm wide). 5 holes (of 3 mm diameter) were made in the top of the cap for the ventilation inside it.

Standard tungstem electrodes in varnish isolation were used for extracellular recording of neural activity in the experiment on the biological satellite "Kosmos-1667". The diameter of the electrode trunk with isolation was 80 mcm.

The electrodes were inserted into the monkey's brain without narcosis. The head of the monkey, sitting in the chair, was fixed in the special frame. The recording electrode was placed inside the glass guiding tube. The outside diameter of the tube was just a little less than the inside one of the steel canule, stereotaxically inserted into the skull bone. When the guiding tube penetrated through the canule into the brain, its end was 5-8 mm above the required point. Then the tube was clewed to the canule and a recording electrode was protruded from the guiding tube with help of the manual microdriver.

It was very important to know the focus of recording and the position of the electrodes. The monkey's head was fixed in the frame and the chair was oscillated in the horizontal plane. The activity of neurons was recorded as the electrode penetrated into the brain. Thus we found the population of neurons that changed their activity in the rhythm of vestibular disturb. When such neuronal activity was found in the required point we concluded

that this is a neuronal population of medial vestibular nucleus, connected with horizontal vestibular channels (Figure 2).
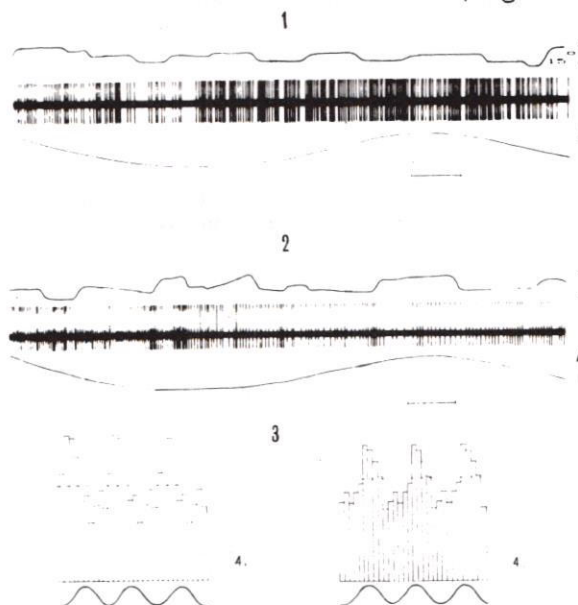
Figure 2. Activity of medial vestibular neurones during head oscillations. The monkey was sitting in the chair and its head was fixed in special chair frame. 1,2 - top trace - EOG, neuronal activity, bottom - head position (two different monkeys); callibrations: EOG - 15 grade, head position-80 grade, time-200 ms. 3 - hystograms of neuronal activity, modulated in oscillation rhythm.

The activity of medial vestibular nuclei was recorded before the flight, during it and after the landing in two experiments: when the animal performed rapid gaze fixation reaction and lift reaction. During the gaze fixation reaction the animal turned its head. This movement induced the activity of receptor apparatus of the semicircular channels. The vestibular afferentation provided for the coordination of the rapid gaze fixation reaction.

The described method made the possibility to get the data of the electric activity of vestibular nuclei during the real cosmic flight of the monkeys (4).

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PROBLEMS IN ANALYSIS OF DATA FROM
MUSCLES OF RATS FLOWN IN SPACE

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Comparison of hindlimb muscles of rats flown
on Spacelab-3 or tail-traction-suspended showed
that 11-17 h reloading post-flight might have
altered the results. Soleus atrophied, plantaris,
gastrocnemius and extensor digitorum longus grew
slower, and tibialis anterior grew normally. In
both flight and simulated soleus and plantaris,
higher tyrosine and greater glutamine/glutamate
ratio indicated negative protein balance and
increased glutamine production, respectively, rel-
ative to controls. Aspartate was lower in these
muscles. Reloading generally decreased tyrosine,
but increased aspartate and glutamine/glutamate.
These data showed that at 12 h of reloading after
flight is characterized by reversal to varying
extents of effects of unloading.

Analysis of tissues from animals subjected to
spaceflight provides an opportunity for under-
standing the adaptation of biological organisms to
microgravity. Using models for hindlimb unloading
on earth, in conjunction with space studies,
offers a means for investigating further the
effects of unloading on muscle and bone. Hence
investigators have developed tail-cast (1,2),
harness (3) and tail-traction (4) suspension
models. The real test of whether these unloading
models are useful for accelerating investigations
of the effects of unloading in microgravity is the
comparison of data from muscles unloaded by sus-
pension and in spaceflight.

Rat hindlimb unloading by suspension or micro-
gravity induces physiological and biochemical
changes in muscle. The soleus is most responsive
of the hindlimb muscles (1,5,6,7). Next in respon-
siveness are the gastrocnemius and plantaris mus-
cles, followed by the extensor digitorum longus
and tibialis anterior muscles, which may show
little or response (1,2,6,7). Histochemistry of
muscles from flight or restrained rats showed
similar changes and pathologic development in the
soleus (5,8). Comparison of the soleus of tail-
casted, suspended rats (2) with rats from Cosmos
936 (9) showed a diminution in maximum isometric
tension of both muscles. Distribution of iso-
enzymes of lactic dehydrogenase in rat soleus
muscle shifted from types I and II to types IV and
V after 2 days flight on Cosmos 605 or after 15
days of cage restraint (10). Unloading by micro-
gravity (11,12) or by suspension (13) also leads
to increased glycogen concentration in the soleus.

Acquisition of muscles from rats flown on
Spacelab-3 (SL-3) (May 1985) afforded us the
opportunity to study the effects of spaceflight on
muscle mass, protein and amino acids in growing
rats (190-250 g). Muscles studied included the
soleus, plantaris, gastrocnemius, extensor digi-
torum longus and tibialis anterior. Tyrosine was
measured because it is neither synthesized nor
degraded by muscle and thus serves as a qual-
itative indicator of changes in muscle protein
turnover (14). Aspartate and glutamine are of
particular interest because of their important
roles in muscle as a source of nitrogen for the
purine nucleotide cycle (15) and in the removal of
nitrogenous waste, respectively. A previous study
from this laboratory showed that unloading by
tail-cast suspension altered the metabolism of
these amino acids (16). Soleus muscles of adult
rats (360-410 g) flown on SL-3 exhibited lower
amounts of aspartate and asparagine, glutamine and
glutamate, glycine, histidine and lysine than in
control muscle (17). The muscle ratio of gluta-
mine/glutamate used in this study to evaluate
potential effects of unloading on muscle glutamine
metabolism provides a qualitative indication of
glutamine production in muscle (16).

Because the flight animals were subjected to
11 to 17 h of reloading and food deprivation on
earth following the flight, a study using tail-
casted suspended hindlimbs was conducted at the
NASA Ames Research Center by Dr. Emily Holton
using the same strain of rats and following a
similar protocol to which the flight rats were
subjected. Data for muscles acquired from this
latter study are used here as the ground-based
study for comparison.

MATERIALS AND METHODS

Flight animals. Six male albino rats (Taconic
Farms) flown on the 7 day SL-3 mission were
specific pathogen free, caged in separate filter
cap vivarium cages, and provided Teklad L 356 diet
as pellets or food bars (in-flight only) and water
ad libitum prior to and during flight. Control
animals were maintained similarly in simulation
cages. After landing, animals were flown to
Kennedy Space Center with access to gel-paks only
for 11-17 h before being decapitated. The controls
were transported in a van but not in an aircraft
and were also provided only with gel-paks for 11-
17 h before being killed. Right leg muscles were
excised and weighed by NASA technicians, then
frozen in liquid nitrogen and shipped on dry ice
to this laboratory within 24 h for processing the
day of receipt. Flight animals initially weighed
197 $\pm$ 4 g and 241 $\pm$ 9 g after landing compared to
control values of 211 $\pm$ 2 g and 258 $\pm$ 5 g. Muscle
and animal weights were provided by Dr. Christo-
pher Schatte, NASA Project Scientist for SL-3.

Suspended animals. A study conducted at NASA
Ames Research Center by Dr. Emily Holton simu-
lated, using their model of tail-traction suspen-
sion (4), the general conditions to which the
flight animals were subjected with the exception
of no transportation during reloading. Rat strain
and size, cage conditions and food were matched to
flight conditions as closely as possible. Groups
of suspended animals included those subjected to
no recovery of 11-17 h recovery (reloading) prior
to killing. The sequence for tissue dissection
paralleled that used for the flight animals.

Muscle preparation. In a cold (4°C) room, frozen muscles were weighed and then sliced into weighed pieces for protein and amino acid analysis. Ratios of fresh weight (provided by NASA) to frozen weight for each muscle were used for correcting the weights of the cut pieces in calculating protein concentrations. Tissues were homogenized in cold 0.2 N $HClO_4$ (12 to 20 mg tissue/ml). After centrifugation (10 min; 5000 x g; 4°C), the supernatant was removed and neutralized to pH 6 to 7 using 2.5 N KOH, 0.1 M piperazinediethanesulfonic acid. The protein pellet was washed once with 10% (w/v) trichloroacetic acid and then twice with ethanol:ether (1:1), and then was solubilized in 1 N NaOH (10-20 mg tissue/ml).

Assays. Protein (18), aspartate (19), glutamate (20), glutamine (21), and tyrosine (22) were assayed by standard procedures.

Statistics. Significance of differences between concentrations were tested by the unpaired Student's t test or between ratios of glutamine/glutamate by the Wilcoxen Sum Rank Test.

RESULTS AND DISCUSSION

Response of protein. Since it is not possible to obtain directly initial muscle mass or protein content to calculate their changes during unloading, an alternate approach has been used for evaluating the magnitude of muscle atrophy and growth inhibition (1). Estimated initial protein content can be calculated from the initial body weight, plots of normal muscle weight versus body weight, and initial protein concentration (1). Plots of body vs. muscle weight were performed for muscles of 22 animals (185-260 g) used in the NASA flight-simulated studies yielding equations given in the legend to Table 1. Such analyses were used to compare the changes in protein content of hindlimb muscles during unloading in space or by tail-traction suspension (Table 1). Controls for the flight and suspended rats grew by 21-26%. These determinations showed that only the soleus atrophied when unloaded by either method. In both flight and suspended animals, the plantaris, gastrocnemius, and extensor digitorum longus muscles showed reduced growth. The tibialis anterior grew more normally than other muscles.

TABLE 1. COMPARISON OF PROTEIN CHANGES

Muscle	SL-3 Animals	Simulation Animals
	percent change from day 0	
Soleus	-11 + 6	-18 + 6
Plantaris	+14 + 3	+ 3 + 2
Gastrocnemius	+11 + 5	+ 2 + 4
Extensor digitorum longus	+14 + 6	+ 8 + 3
Tibialis anterior	+21 + 5	+10 + 3

The equations for calculating muscle weight, where X = body weight were: soleus: 0.43X - 11.8; plantaris: 1.42X - 85; gastrocnemius: 5.75X -175; extensor digitorum longus: 0.49X - 13.7; tibialis anterior: 2.0X - 32.

Response of amino acids. Previous work showed a number of significant changes in amino acids in the unloaded soleus. The soleus of both flight and simulated-recovering rats exhibited a greater concentration of tyrosine (Table 2), lower concentration of aspartate (Table 3), and a greater ratio of glutamine/glutamate (Table 4).

Since fumarate is formed anaplerotically from aspartate via the purine nucleotide cycle (15), we also measured levels of fumarate, as well as malate, in the flight soleus to determine if changes in these metabolites coincided with the change in aspartate. Fumarate (0.25+0.02 nmol/mg protein) and malate (1.98+0.09 nmol/mg protein) were lower (P<0.05) in flight soleus than in control muscle (0.34+0.03 and 4.96+0.17 nmol/mg protein, respectively). Therefore, the lower aspartate in flight muscle likely reduced the anaplerotic formation of fumarate.

In the simulation study recovery diminished or reversed the response to unloading. Tyrosine was much greater in the muscles without recovery. Tyrosine, which is neither synthesized nor degraded by skeletal muscle, has been used in vitro as an indicator of changes in net protein breakdown (14). Therefore, the increase of tissue tyrosine with unloading undoubtedly reflects the negative protein balance of the unloaded soleus. Conversely, the fall in muscle tyr with reloading suggests a diminished contribution of protein-derived amino acids to the general amino acid pool, yet aspartate rose significantly.

Aspartate is important in muscle in the purine nucleotide cycle for the reamination of IMP to AMP after exercise (15). Therefore, availability of aspartate in a weight-bearing muscle may be essential, while with reduced activity aspartate becomes less important. Electrical stimulation or exercise is associated with increased aspartate and malate (23-25). Following tetanic train, the large increase in malate is diminished when flux through adenylosuccinate lyase in the purine nucleotide cycle diminishes (26). Therefore, it is possible that the increase of aspartate with reloading and its diminution with unloading could be functions of changes of muscle activity.

Most responsive was the glutamine/glutamate ratio. There are two reasons why faster synthesis of glutamine might be expected in reloaded muscles of flight animals. The mild stress of unloading by suspension leads to increased glutamine synthetase activity, likely due to elevated circulating corticosteroids (16). Since unloading also leads to a greater maximum binding capacity of glucocorticoid receptors in load-bearing muscles (27), glutamine synthetase, at least in the soleus, may be more responsive to any given plasma level of corticosterone. Similar responses may occur in flight muscles, as well. The 12 h of weight-bearing post-flight might have promoted sufficient production of ammonia, which seems limiting in unloaded muscle (16), to allow for the synthesis of glutamine. Secondly, production of glutamine seems to be especially sensitive to reloading of the soleus. Indeed, net release of glutamine by isolated soleus was near normal within only 4 h after reloading and the muscle ratio of glutamine/glutamate increased to control values within 12 h (Jaspers and Tischler, unpublished observation). Hence reloading may have increased greatly the ratio of glutamine/glutamate in the flight soleus.

TABLE 2. COMPARISON OF TYROSINE RESPONSES TO UNLOADING AND RECOVERY

Muscle	SL-3 Animals		Simulation Animals		
	Weight-bearing	Flight	Weight-bearing	Suspended	
				No recovery	12 h recovery
	nmol/mg protein	difference	nmol/mg protein	difference	
Soleus	0.61 + 0.03	+38%	0.54 + 0.03	+109%	+35%
Plantaris	0.80 + 0.05	NS	0.46 + 0.01	+ 87%	+28%

NS means not significant

TABLE 3. COMPARISON OF ASPARTATE RESPONSES TO UNLOADING AND RECOVERY

Muscle	SL-3 Animals		Simulation Animals		
	Weight-bearing	Flight	Weight-bearing	Suspended	
				No recovery	12 h recovery
	nmol/mg protein	difference	nmol/mg protein	difference	
Soleus	8.1 + 1.0	-78%	8.8 + 0.6	-88%	-74%
Plantaris	1.4 + 0.1	-26%	0.9 + 0.1	-68%	-31%

TABLE 4. COMPARISON OF GLUTAMINE/GLUTAMATE RATIO RESPONSES TO UNLOADING AND RECOVERY

Muscle	SL-3 Animals		Simulation Animals		
	Weight-bearing	Flight	Weight-bearing	Suspended	
				No recovery	12 h recovery
	nmol/mg protein	difference	nmol/mg protein	difference	
Soleus	3.3 + 0.1	+76%	2.3 + 0.1	-26%	+61%
Plantaris	7.0 + 0.3	+31%	5.0 + 0.4	NS	+14%

NS means not significant

Other responses to reloading. Other studies in this laboratory have pointed to the potential problems which may be encountered with reloading even for short periods (Henriksen and Tischler, manuscript submitted). One of our more remarkable observations was the triphasic response of glycogen metabolism to reloading. Within 15 min the activity ratios of glycogen synthase and phosphorylase rises significantly and over the first 2 h there is a marked fall in the concentration of soleus glycogen. Thereafter between 2 and 24 h, there is a sharp increase in glycogen concentration to 2 times normal ("supercompensation of glycogen") followed subsequently by a return to normal over the next 48 h. Hence during the 11-17 h of reloading following flight it is conceivable that muscle glycogen declined and then increased again to the high levels reported previously post-flight (12).

There is also a considerable response of glucose uptake to reloading. After 3 days of unloading, basal uptake of 2-deoxy[1,2^{3}H] glucose (2-DG) climbs to 60% above control within 12 h of reloading (Henriksen and Tischler, submitted). There is also a diminution during this time of the enhanced insulin sensitivity reported for unloaded soleus (13). Even after only 24 h of unloading, reloading for just 24 h enhanced 2-DG uptake by 45%.

Concluding remarks. These various findings point to the potential problems we face in interpreting data from muscles subjected to post-flight recovery. Even the 20 min of weight-bearing during re-entry may produce some significant changes. Hence it is imperative to develop research programs which will allow more extensive experimentation entirely within a space laboratory.

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COMPARATIVE STUDY OF THE CARDIOVASCULAR ADAPTATION TO ZERO g DURING 7 DAYS SPACE FLIGHTS.

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SUMMARY :

The assessment of the cardiovascular function was performed by the help of the Space Echograph (MATRA) onboard Saliout VII and the Space Shuttle. During short flight we noted a moderate increase (maximum + 30 %) of the left ventricle and the cardiac output followed one day or a few days later by the decrease (maximum - 15 %) of these parameters.
At the same time, the cerebral circulation remained quite stable whereas the femoral circulation changed with the cardiac output. After landing, most of the haemodynamic parameters were transiently increased but showed large oscillations all along the recovery period. The cardiovascular parameters returned to their basal value within in a few days (6 days). The variations of the main haemodynamic parameters presented as a percentage of the basal value will be compared, preflight, inflight and post flight.

I - INTRODUCTION :

The assessment of the main cardiac and vascular parameters have been performed during space flights with ultrasound methods.(2. 3. 11. 12.) Two experiments were carried out onboard Saliout VII 1982, (7 days flight) and onboard the Space Shuttle : 1985 - (7 days flight). The same device (MATRA ECHOGRAPH) was used with the same protocol during this two flights on which 3 astronauts were explored.
The purpose of that study is to compare the evolution of the different cardiac and vascular parameters during short exposure to zero g.

II - MATERIEL AND METHOD :

- The main cardiac parameters assessed were the left ventricle systolic and diastolic volume, the cardiac output (CO), the heart rate, the left ventricle diameter shortening and the ejection fraction.
- On the peripheral circulation, we measured the carotid and femoral flow (Q), the indices of cerebral and femoral resistance (R).(1. 10.)

III - RESULTS :

The variations of the main cardiovascular parameters are expressed as the percentage of the basal value : cardiac parameters on figure 1, carotid parameters figure 2 and femoral parameters figure 3.

IV - DISCUSSION :

First results demonstrated that the cardiac and the vascular parameters did'nt show the same evolution and did'nt change in the same amplitude on the different astronauts.

IV.1 - Left heart parameters :

The left ventricle volume in diastole (LVDV) and the cardiac output (CO) were increased during 5 days on astronaut A1 whereas for A5 and A6 these parameters showed only a slight increase on flight day one followed by a significant decrease (same day) and remained stable during the rest of the flight. For some measurements the heart rate increase participated to the increase of the CO but the variation of the CO were always related to the variations of the stroke volume and the cardiac cavities size. None of the three astronauts did'nt show any disturbances of the cardiac contractility.
After landing the left ventricle volume in diastole was decreased however the cardiac output was strongly elevated on all of the 3 astronauts. These parameters recovered their basal value within approximatly 5 to 10 days. The blood pressure did not change significantly.

Finaly, it seems that for most of the astronauts, we can hypothesize that there was a transient increase of the cardiac volume and output which recovered within one day or a few days and then decreased (-10 to - 20 %). This observation is in agreement with the results of the echocardiography experiment performed in 1985 onboard the shuttle (STS 51E) on 4 american astronauts (HW BUNGO, JB CHARLES) and Bed rest studies (3. 5.). The increase of cardiac output could be related to the fluid shift toward the cephalic area which simulate an hypervolemia. The adaptation of the cardio vascular system will consist in a transfert of liquid from the vascular compartment towards the interstitial area on the upper part of the body (lungs ?) and the loss of liquid by the urinary tract the whole lot inducing an hypovolemia (6. 7. 8. 9.).

IV.2 - Peripheral circulation :

- On astronaut A1 we noted a slight increase of the carotid blood flow (+ 10 %) despite a marked elevation of the cardiac output but at the same time, an increase of the cerebral vascular resistance. The carotid blood flow was weakly increased on A5 and A6 (+ 15 %) all during the flight despite a decrease of the cardiac output. At the same time the cerebral vascular resistance decreases. These three cases show that a strong vasomotor regulation of the cerebral circulation exists, the CO changes being compensated by the adjustement of the distal vascular resistance.
- The femoral blood flow was slightly elevated on A1 when the CO increased however the vascular resistances increased. On astronaut A6 we observed a slight decrease of the femoral flow associated with a decrease of the CO and an increase of the vascular resistances. It seems that on the inferior limbs the blood flow regulation is not as accurate as in the cephalic area the elevation of the resistance index could be related to the fluid shift and the relative empting of the limb venous system.

- After returning in one g gravity the blood flow and the vascular resistance changed strongly, on A1, A5 and A6 :
- the carotid and femoral flows were increased during several days and progressively returned to the basal value,
- the cerebral vascular resistance was decreased on A5 but remained quite constant on A1 and A6,
- on day 1, the femoral resistance was strongly decreased on A1 and A5 (inferior to the basal value and the last in flight value), then showed large oscillations and recovered

after a longer delay than for the cardiac or carotid parameters. On A6, the femoral index was higher after landing than during the flight and remained superior to the last inflight value during several days.

When the astronauts come back to the one g environment there is a strong fluid shift toward the inferior limbs, therefore one can expect an increase of the vascular resistance on the inferior limbs in order to compensate a possible hypotension.(4) This phenomenon is well observed on A6 who has no problem to stand up a long time or when doing a squate up test (hypotension test). On the contrary on A1 and A5, we noted that the femoral resistance index after landing was lower than in flight.

This was associated to the existence of big troubles to keep the stand up position or when doing the squate up test. Finaly the femoral resistance index could be considered as a wittness of the disadaptation of the vascular vasomotricity.

Because of the large individual variations of each parameters, it is necessary to extend such an experiment to a larger number of astronauts, but we have to keep in mind all the troubles we have to face nowadays to carry out such human inflight experiment.

Moreover, the study of the left ventricle function and the peripheral circulation will be completed by the investigation of the right heart, the deep arterial circulation (pulmonary artery, renal artery, ...) and the follow up of the main hormons involved in the cardiovascular regulation.

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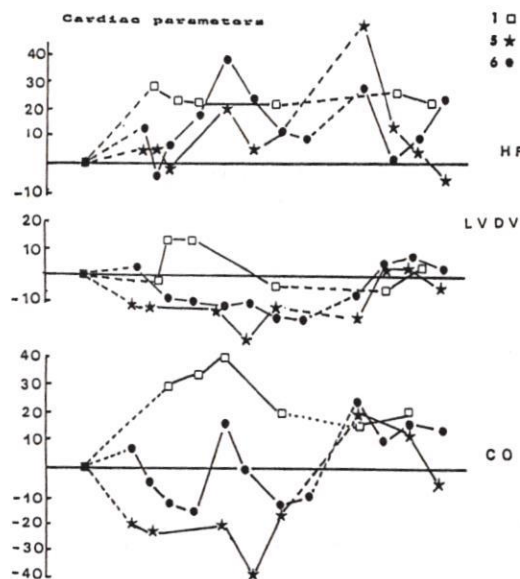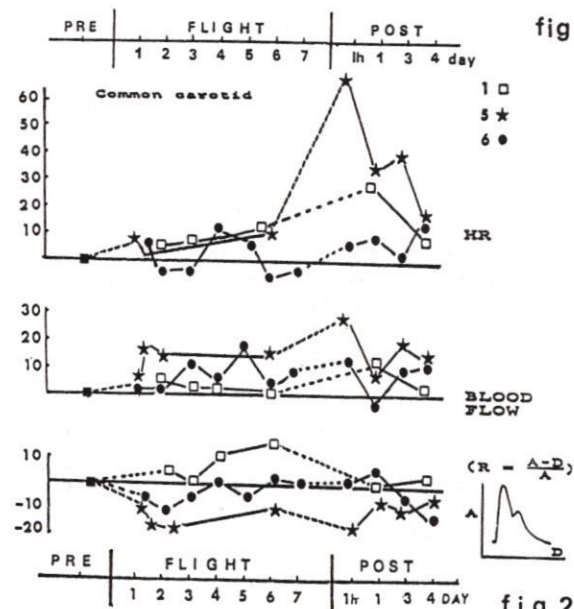
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fig 2
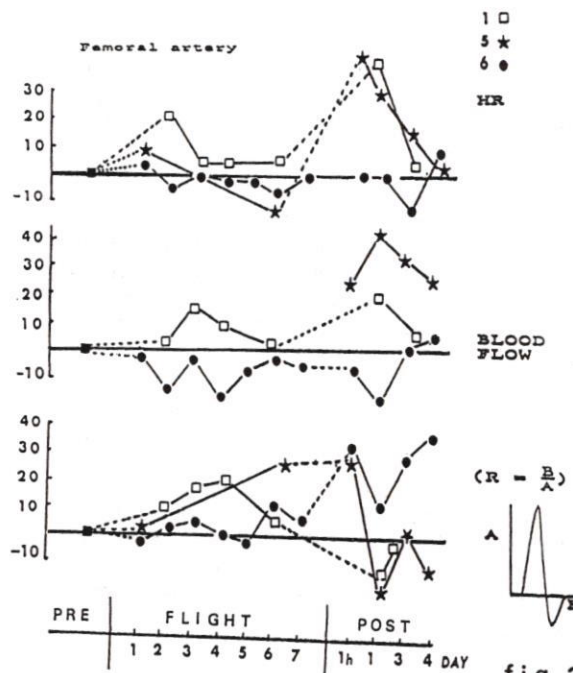
fig 3

EFFECTS OF LEG POSITIVE PRESSURE ON CARDIO-RESPIRATORY ADJUSTMENTS TO DYNAMIC LEG EXERCISE

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Several experiments planned for the coming Shuttle/Spacelab flights will deal with physiological responses to dynamic leg exercise. One factor that should be considered when interpreting such experiments is the role of reduced blood flow in the working muscles which is to be expected in the weightless condition. In ground-based experiments, exercise performance is usually assessed with the subject conducting cycle ergometry in the upright posture. In this condition the action of the normal force of gravity adds large hydrostatic columns to arterial pressure which increases perfusion pressure in the working leg muscles (Folkow et al. 1971). In the weightless condition, however, gravitational vectors are non-existent, and muscle perfusion pressure is consequently lower. This reduction in perfusion pressure will probably lead to restriction of the blood flow in the working muscles which, in turn, may influence the physiological responses to exercise.

To facilitate the study of the influence of reduced muscle blood flow on cardiorespiratory adjustments to exercise, the perfusion pressure was further decreased in the present investigation. This was done by exposing the working legs of supine subjects to a supraatmospheric pressure of 50 mm Hg (Leg Positive Pressure, LPP).

Methods

The experiments were conducted with the subject positioned supine in an opening to a pressure chamber with the legs inside the chamber. Hermetic sealing at the level of the crotch was accomplished by the use of a rubber diaphragm with two holes and short self-sealing sleeves for the legs. Shoulder supports were used to prevent craniad displacement of the body upon application of the pressure. Graded leg exercise was performed on an electrically braked cycle ergometer, with the axis of the pedals at the level of the heart.

Heart rate (HR), breath-by-breath inspiratory minute ventilation ($\dot{V}_I$) and end-tidal PCO_2 ($P_{ET}CO_2$) were recorded using standard techniques. Systolic arterial pressure (SAP) was measured by the auscultatory method from a brachial artery. Oxygen uptake ($\dot{V}O_2$) was determined using the Douglas bag technique, blood lactate concentration was determined in samples drawn from a fingertip.

The experimental protocols were as follows:

In a first series of experiments 8 subjects performed incremental-load cycling with and without LPP at 50 mm Hg. The work load was increased in steps of 30-50 W every 4 min until the point of exhaustion. HR, SAP, $\dot{V}_I$, $P_{ET}CO_2$, $\dot{V}O_2$ and blood lactate concentration were measured during the last min at every work load.

In a second series of experiments $\dot{V}_I$, $P_{ET}CO_2$ and blood lactate concentration were measured during constant-load cycling at 120 W with temporary application of LPP during a 5 min period.

Results

Incremental-load exercise: Work capacity was markedly impaired by LPP, the peak load that could be attained in this condition (mean-SE= 141-5 W) being only 61 % of control peak load (235-13 W). Exposure to LPP augmented the exercise responses for HR and SAP, the difference between LPP and control values amounting to 13% for HR and 19% for SAP at LPP peak load (Fig. 1). At all work loads, LPP induced a greater exercise $\dot{V}_I$ than in the control condition with higher ventilatory equivalent for O_2 and lower $P_{ET}CO_2$ values than during control exercise. Exercise values for blood lactate concentration were increased by LPP, with a 56 % higher lactate concentration at LPP peak load.

Constant-load exercise: As Shown in Fig. 2 temporary application of LPP induced increases in $\dot{V}_I$ and blood lactate concentration and a drop in $P_{ET}CO_2$. A sudden release of LPP induced a rapid fall in $\dot{V}_I$ and an immediate and sustained elevation of $P_{ET}CO_2$ in spite of a concomitant increase in the blood lactate concentration.

Discussion

The present results show that exercise performance during supine dynamic leg exercise was severely impaired by an experimentally induced reduction of the muscle perfusion pressure, and that this impairment occurred in spite of an exaggerated pressor response, which to a large extent counteracted the LPP-induced drop in muscle perfusion pressure. Thus, it seems that muscle perfusion pressure is critical for work performance during supine cycling.

That the exercise-induced increases in SAP and HR were exaggerated when muscle blood flow was restricted by LPP may be explained by increased activation of muscle chemoreflexes by accumulation of metabolites. The increased pressor response would then act to reduce the existing blood-flow error (cf. Mitchell & Schmidt 1983).

Exercise-induced responses of the pulmonary ventilation were markedly exaggerated by LPP. In part, this effect is attributable to a humorally mediated increase in respiratory drive resulting from the increased rate of lactate formation consequent to LPP-induced restriction of muscle blood flow. However, Fig. 2 shows that a sudden release of LPP during constant load pedaling induced a prompt and rapid fall in $\dot{V}_I$ and an increase in $P_{ET}CO_2$ in spite of a marked, sustained increase in the blood lactate level.

This finding strongly suggest that muscle chemosensors play an important role in the development of exercise hyperpnea in conditions of flow-restricted exercise.
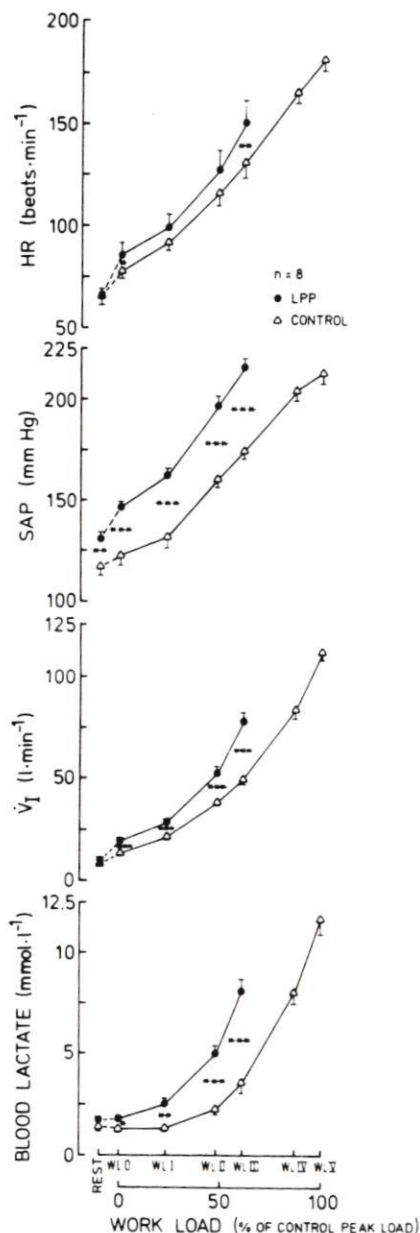
Fig. 1. Comparisons (from above downwards) of heart rate (HR), systolic arterial pressure (SAP), inspired minute ventilation ($\dot{V}_I$) and blood lactate concentration at supine rest and during supine exercise in the control and in the LPP conditions. Work loads are given in per cent of the peakload attained in the control condition with WL O-V representing 0, 23, 48, 61, 87 and 100% of this load and WL III indicating the highest load that could be managed in the LPP condition. *p<0.05, **p<0.01, ***p<0.001.
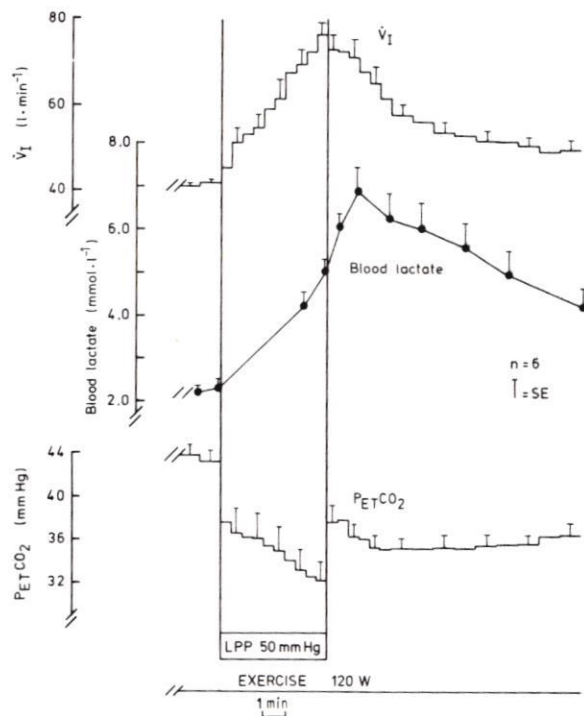
Fig. 2. Effects of temporarily (5 min) applied LPP at 50 mm Hg on inspired minute ventilation ($\dot{V}_I$), blood lactate concentration, and end-tital PCO$_2$ (P$_{ET}$CO$_2$) during supine, constant-load pedaling at 120 W. Initial values represent data obtained during min 4 and 5 after onset of exercise (from Acta Physiol Scand, Vol. 131, Suppl. 566, 1987).

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EFFECT OF ACUTELY EXPOSURING TO 40 mm Hg LBNP ON CARDIOVASCULAR RESPONSES DURING REST AND MILD EXERCISE AFTER 6 hrs REST IN 5° HEAD DOWN TILTING (HDT)

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To investigate the tolerance of sympathe-
tic control to 40 mm Hg LBNP acutelly ex-
posed after 6 hrs bed rest at head down
tilting (HDT) and the effect of mild pe-
dalling (40 % VO2max) on restoring car-
diovascular functions broken, 6 female
students participated as subjects. The
tolerance was determined by the crite-
rions given in the study. The averaged
tolerable time was 10 min., which was li-
ttle shorter than it given by NASA pro-
cedure (12 min.). At the intolerance po-
int, VO2 and fore-arm blood flow did not
change, but pulse pressure was suddenly
fallen with strikingly decreasing SAP and
HR from the peak values during LBNP. But
exercising brought the pressur to restore
over the control levels and further the
normal exercising levels in LBNP, altho-
ugh HR increased and SV, TPR, FBC dec-
reased. LBNP tolerance might be due to
decreasing sympathetic control, but it
should be restored by light exercise.

In the previous studies (1), cardiovascu-
lar functions in woman were broken the sy-
mpathetic control by average 44 mm Hg LBNP
as the LBNP was gradually loaded gy means
of NASA procedure after 6 hrs bed rest at
5° head-down tilting (HDT), although the
tolerable LBNP could be above - 50 mm Hg
as exposed to LBNP after 15 min. rest in
the supine position. The tolerance time
was depend on lean body mass as well as
maximum oxygen uptake.

This study is therefore concerned with
the tolerance of sympathetic control to LB
NP in woman as a 40 mm Hg LBNP was acutel-
ly exposed after 6 hrs bed rest in 5°HDT,
and the effect of mild pedalling exercise
on restoring the sympathetic control bro-
ken after reaching the intolerance of the
cardiovascular functions.

Methods

Six healthy female students participated
to the study as subjects. Mean age, height,
weight, and VO2max were 23.7 years old,
160 cm, 53.7 Kg, and 42.78 ml/kg/min, re-
spectively. After 15 min. supine rest in
a LBNP box with a bicycle ergometer, sub-
jects were tilted at 5°head down for 6 hrs
Thereafter, they were acutely exposed to
40 mm Hg LBNP in the HDT untile breaking
sympathetic control. Immediately after re-
aching the intolerance of sympathetic con-
tol, a mild peadlling exercise (about 40
% VO2max) was performed for a 15 minutes
period. The criterions of determining the
intolerance of sympathetic control against
LBNP in the present study were the same as
the NASA criterions except for arterial
pressures. That is, the criterions of the
pressures were that below 90 mm Hg systo-
lic pressure and/or below 20 mm Hg pulse
pressure were continuously recorded two
times. During the experiments, VO2 was de-
termined by a metabolic analyzing system
which was consisted of mass-spectrometer,
gas flowmeter, and computer. EKG and HR
were continuously observed by a EKG moni-
tor. Arterial blood pressure in the left
upper arm was measured by the ausculation
method. Cardiac output (CO) was measure by
means of athetylen rebreathing method.
Fore-arm blood flow (FBF) in the right arm
was measured by Whytney's mercury rubber
strangauge plethysmogragh (2). The experi-
mental protocol was shown in changing time
courses of the measurements in Fig. 1.
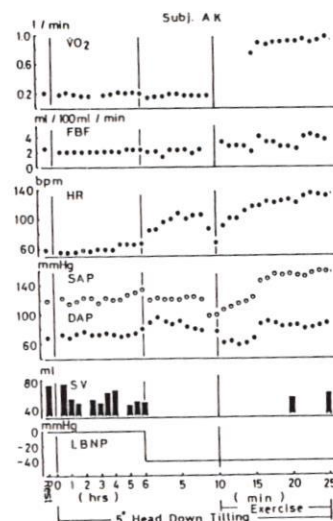
Fig.1
Experimental
protocol and
one case of
Changing time
courses of
cardiovascular
responses.

Oxygen uptake
(VO2), fore-
arm blood flow
(FBF), heart
rate (HR), sy-
tolic pressure
(SAP), diasto-
ric pressure
(DAP), and st-
roke volume(
SV)

In the control study, exposing LBNP was in
accordance with NASA procedure following
HDT, and as performing pedalling exercise
for 15 min., LBNP was released at 10 min.
point.

Results and Discussion

Showing in Fig.2, the averaged tolerable
time of 10 minutes against acutely loaded
LBNP was not significant difference from
the 12 minutes given by the control study,
although the tolerable pressure of LBNP
in the control was -44 mm Hg in average.

Constant load Increasing load
of LBNP   of LBNP
after 6hrs Tilting rest

Fig.2
Tolerable time
against acute-
ly exposing to
40 mm Hg LBNP,
and respecive
against gradu-
ally exposing
to LBNP (ave-
rage tolerable
LBNP;-44mm Hg)

In Fig.3, the averaged (+SE) changing values from the levels given during supine rest in cardiovascular responses are shown in the two experimental conditions. At 6 hrs point during HDT, there were not different from the resting values in VO2 and HR in the both conditions, but SAP and DAP were slightly increased and FBF was decreased. At intolerance point against LBNP in each condtion, VO2 was not different from the resting level and FBF decreased, but HR and DAP were significantly increased and pulse pressure (PP) and SAP were decreased. Despite of shorter tolerable time in acutely exposing condition than in the control the changing values of PP, SAP, HR, and FBF were greater in the control, slightly, while the changing DAP given at the intolerable point was not different in the two conditions.
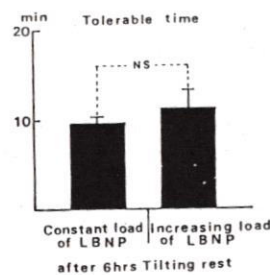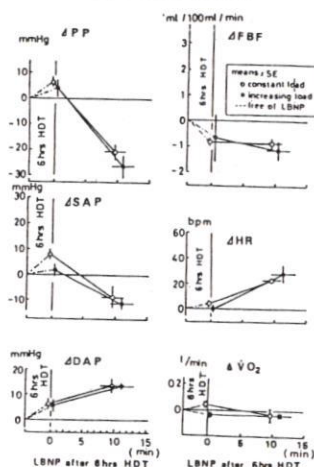

Fig.3
Changed values in VO2, HR, FBF, DAP, SAP, PP from the levels given during supine rest.

The values plotted at 6 hrs HDT and intolerance point against LBNP were mean +SE of subjects in each condition.

These results shown in Fig, 1, and 2, suggest that the tolerance against LBNP loaded should be broken by decreasing sympahtetic control to cardiovascular functions, because despite of maintaining a higher DAP, SAP and HR were rapidly fallen from the peak values given during LBNP and thus PP was suddenly lowered to the intolerable level. As assuming, the falled PP may be due to decreasing central blood volume and thus become less of adaptation to baroreceptor activity. According to these facts, LBNP tolerance related with sympathetic control is probably broken by decreasing central blood volume with combining the both of the intensity of and the exposing lenght to LBNP given.

On the other hand, cardiovascular responses at 10 min. during exercise were rapidly restored with increasing VO2 as shown in Fig.4. As comparing with the values given at the intolerance point against LBNP in HDT, VO2, HR, mean arterial pressure (MAP), and fore-arm volume condactance(FVC) given at 10 min. during exercise were about 400 %, 150 %, 12 %, and 10 % higher, respectively. The increase in cardiovascular responses due to exercise means that a light exercise made sympathetic control broken by LBNP stimulation restore to the normal level even in LBNP condition. However, the LBNP stimulation brought to increase HR and FVC during exercise to maintain MAP, as observed when LBNP was released on the way of exercise.
In Fig. 5, the influence of releasing LBNP on cardiovascular responses during exercise was shown by changing ratios after
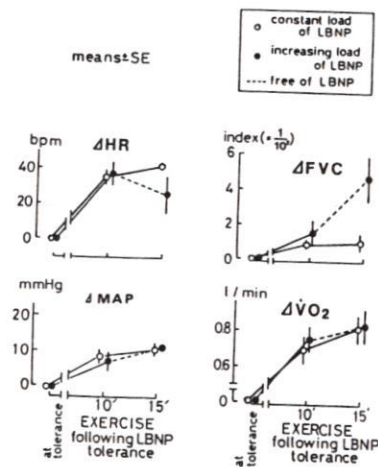
Fig.4
Exercising effect on HR, mean arterial pressure(MAP), fore-arm volume conductance(FVC), and VO2 altered at intolerance against LBNP after 6 hrs HDT.

Values; delta values, and mean+SE of all subjects.

the releasing or the non-releasing against the values given at 10 min. in exercise. By releasing LBNP, HR increased and stroke volume(SV), total peripheral resistance(TPR), and FVC were decreased, while mean arterial pressure (MAP), CO, and VO2 were not changed. These facts suggest that in order to maintain arterial pressure and CO with the object of keeping VO2 level during exercise, TRR was decreased by increasing peripheral conductance and HR was increased with decreasing SV by LBNP stimulation.
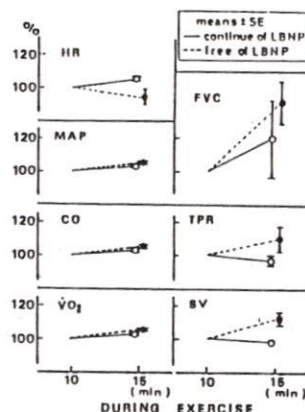
Fig.5
Effect of LBNP on cardiovascular responses during mild exercise.

Values; ratio against the values given at 10 min. during exercise, mean +SE of all subjects.

In conclusion, the tolerance against LBNP should be due to the decreased sympathetic control to cardiovascular functions. Especially, by failing to maintain SAP and HR with decreasing baroreceptor activity, PP is suddenly fallen until intolerable level for LBNP. However, the decreased or broken sympathetic control might be restored to normal level by muscle contraction with a light pedalling exercise and further to the normal exercise level. That is, to have and maintain blood pressure adjusted to exercise, baroreceptor activity maybe increase in HR with decreasing SV and decrease in TPR and FVC in LBNP condition.

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EFFECT OF STATIC HAND-GRIP CONTRACTION
(HGC) ON CARDIOVASCULAR RESPONSES TO
CHANGING IN HYDROSTATIC PRESSURE IN WOMAN

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To investigate the cardiovascular res-
ponses to changing hydrostatic pressu-
re during rest and HGC in the upright
position, 6 female students were parti-
cipated as subjects. The pressure was
altered with water immersion(WIL) in a
water bath with 31 C. 5 different WILs
were apillary, diaphragm, os ilium, pate-
lla, and non water points. Through expe-
riments, mean arterial pressure (MAP),HR,
VO2, cardiac output, and fore-arm blood
flow (FBF) were measured. During HGC wi-
th 15 % MVC, HR, MAP, and FBF at zero
hydrostatic situation assumed by diaph-
ragm-WIL were higher in the standing po-
sition than in the upright position wi-
th floating legs. When changing in WIL
the degree of hydrostatic pressure inf-
luenced so that MAP, HR, and total peri-
pheral resistance was gradually increased
and stroke volume decreased with increa-
sing the pressure, and in special these
responses were more significant at pate-
lla-WIL and non WIL in the standing. So,
the cardiovascular responses to hydros-
tatic pressure should be affected by a
muscle activity and central blood volume.

In the previous study(Refer), a light LBPP
(about 20 mm Hg) given by a pressure ti-
ghts brought to increase venous return
suggested by stroke volume (SV), but mean
arterial pressure (MAP) was maintained al-
most the same level as it without the ti-
ghts during exercise, while heart rate(HR)
was decreased with a little bit lower car-
diac output (CO) in the tights. Then,
it was considered that the maintained MAP
should be controled by sympathetic reac-
tion due to baro-receptor activity adju-
sting to exercise and LBPP, as increasing
in venous return and/or central blood vo-
lume with strengthing muscle pump in the
effect of the tights. According to the
discussion, the increase in central venous
pressure and/or central blood volume with
increasing venous return may be correlat-
ed with baro-receptor activity to maintain
the controled MAP during muscle contrac-
tion. Because venous return and thus cen-
tral blood volume are strongly related
with hydrostatic pressure in hemodynamic
control system, therefor, there is inte-
resting in the relationship between arte-
rial blood pressure and venous return (SV)
in the cardiovascular responses during

HGC in several hydrostatic pressure condi-
tions where will be altered by water im-
mersion procedure in the upright position.

Methods;

Six female students perticipated to the
present study as subjects. Their averaged
age, body height, body weight, and maximum
VO2 were 22 year old, 161 cm, 53 Kg, and
42 ml/kg/min, respectively. These values
were included in Japanese standard values
of the same age woman. The subjects were
informed the aim and details in the prese-
nt experiments, after taking a light bre-
akfast at over two hours before experiment.
Then, they clothed in swim-suit and rest-
ed for 15 min. at each given condition of
five kinds of water immersion level (WIL)
in the upright position. After each rest-
ing a sustained HGC of 15 % MVC intensity
was performed for 5 min. period. WIL in
a bathtub with a 31 C water temperature in
28 -31 C room temperature regulated were
as follows ; apillary-WIL, diaphragm-WIL,
os ilium-WIL, patella-WIL, and non-WIL.
There were two upright positions in expe-
rimental conditions. One was the standing
with the legs on the floor of the bath (St
P), and other one was the upright position
with freeing legs from the floor(Up-FLP)
which was made by using a saddle chair so
that floating the legs. As compared, the
experiemnts with the same protocols and
measurements were also performed in the su-
pine position. Through experiments, VO2
was measured by a metabolic analysing sys-
tem which was consisted of a mass-spectro-
meter, gas flow-meter and computer. EKG
and HR were continuously observed on the
visual face of EKG monitor and then reco-
rded. Arterial blood pressure in the left
upper arm was measured by the ausculation
method. Fore-arm blood flow (FBF) was al-
so measured in the left fore-arm by means
of Whytney's mercury rubber straingauge
plethysmograph after measuring the blood
pressure. CO was determined by means of
athetylen rebreathing method with the mass
-spectrometer.

Results;

In Fig.1, MAP(= (systolic arterial pres-
sure(SAP) - diastoric arterial pressure(D
AP)) / 3 + DAP), VO2, and HR which were
averaged in the subjects were shown by
comparing between rest and HGC, and bet-
ween three different conditions of zero
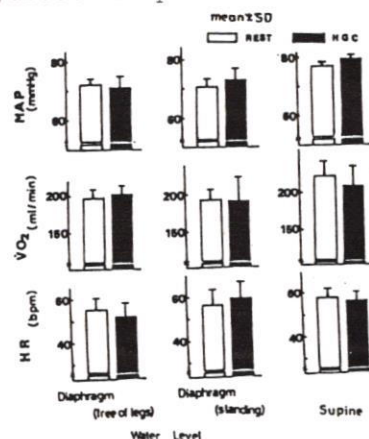hydrostatic pressure situation. That is,

Fig.1
Averaged MAP,
VO2, and HR
during rest
and HGC at
two diaphra-
gm-WILs in St
P and Up-FLP,
and in the
supine posi-
tion.

Values; mean
+SD in six
subjects.

diaphragm-WIL is physiologically defined as zero hydrostatic pressure situation in the hemodynamic system as well as it in the supine position. During rest, HR was similar with each other in the three conditions, but VO2 and MAP were little lower in StP and Up-FLP than in the supine position.During HGC, HR was little decreased in Up-FLP below resting level and increased in StP but its difference was not shown in the supine position. At that time, VO2 was little increased in Up-FLP and similar to the resting level in StP. Also, MAP was similar to the resting it in Up-FLP but in the other conditions little higher.

In Fig. 2, also, the averaged SV, FBF, and total peripheral resistance (TPR = MAP/CO) were compared between rest and HGC, and between the three conditions. During rest,
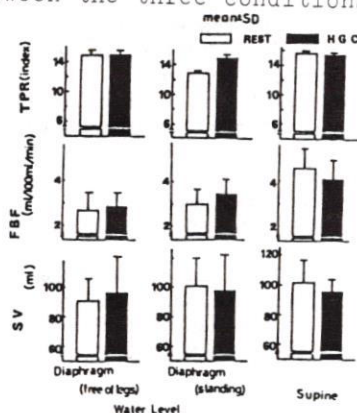
Fig.2
Averaged SV, F BF, and TPR during rest and HGC at two diaphragm-WILs in StP and Up-FLP, and in the supine position.

Values; mean ± SD in six Subjects.

SV was little lower in Up-FLP than in the other conditions, FBF was similar in the both StP and Up-FLP but about 150 % higher in the supine position than in the other, and TPR was lower in StP. As comparing to the levels given during rest, SV was little higher during HGC in Up-FLP, while in the other conditions it was slightly lower. FBF was decreased below the resting level in the supine position, however it was rather increased in the other conditions. TPR was increased more than the resting level in StP, but similar to each other conditions' values.

In Fig.3, averaged VO2 and HR changed from the values given at diaphragm-WIL during rest and HGC were shown in StP and Up-FLP, respectively. As comparing to the level at diaphragm-WIL, VO2 was increased during
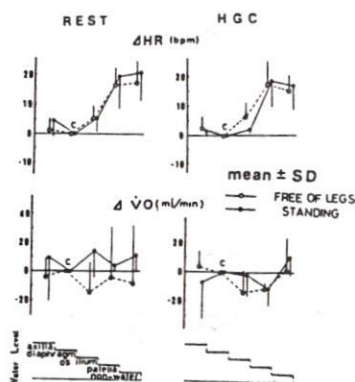
Fig.3
Averaged VO2, and HR changed from the diaphragmWIL levels during rest and HGC in all of other WILs in StP, and Up-FLP, respectively.

Values; mean+ SD in all subjects.

rest at each other WIL in StP, but it was inversely decreased in Up-FLP, while it was similarly changed during HGC in the two conditions except for the case of axilla-WIL. HR was significantly increased at each WIL against at diaphragm-WIL in the bo-

th StP and Up-FLP, but the increment became higher with lowering WIL during HGC as well as it during rest, and further it was higher at patella-WIL and non-water in StP than in Up-FLP.

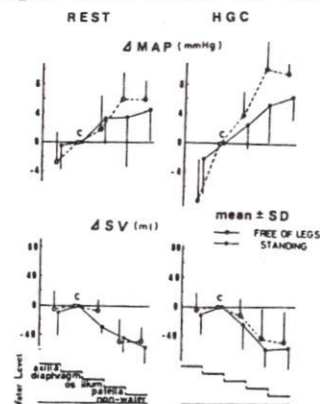In Fig 4, also the averaged MAP and SV changed were shown as well as in Fig. 3.

Fig.4
Averaged MAP, and SV changed from the diaphragm-WIL levels during rest and HGC in all of other WILs in StP and Up-FLP, respectively.

Values; mean ± SD in all subjects.

SV during the both rest and HGC was given the peak value at diaphragm-WIL in StP and also in Up-FLP, and the changing manner was similar in the two conditions, while during HGC the decrement in SV was lower in Up-FLP than in StP. MAP was increased with lowering WIL during rest and HGC in StP and also in Up-FLP, but at patella-WIL and non-water it was higher during the both rest and HGC in Up-FLP than in StP. TPR was increased with lowering WIL, but there was not different in the changing degree between StP and Up-FLP conditions. FBF was given the peak value during rest and HGC at diaphragm-WIL in StP and also in Up-FLP, and further the changing degree against the peak was not different between the two conditions.

Discussion;

In the two upright positions, cardiovascular responses during rest were not significantly different at diaphragm-WIL in spite of the slight difference, although MAP and FBF were lower at the WIL than in the supine position. On the other hand, during HGC, HR, MAP, and FBF were higher in StP than in Up-FLP. These differences are probably due to the level of sympathetic activity to keep HGC and the body position, because little higher FBF and TPR with increasing MAP during HGC and due to different body positions were shown likely to be related with each other despite of physiologically zero hydrostatic pressure.

When changing in WIL and thus hydrostatic pressure, the cardiovascular responses are exactly influenced by the degree of the pressure. In fact, the influenc was shown in the increased MAP, HR, and TPR and the decreased SV with lowering WIL. In special, these responses were much clearly shown in StP. That is, at patella-WIL and non WIL MAP and HR increased and SV decreased were respectively more great during rest and HGC in StP than in Up-FLP. In conclusion, the cardiovascular responses to the levels of hydrostatic pressure should be affected by muscle activity with HGC and supporting the position and also by changing in central blood volume assumed by SV.

Reference; J.Nagano, et al., Effect of LBP P....., Physiologist,30-1, Suppl.,1987

BODY FLUID SHIFTS IN SPINAL CORD TRANSECTION AND SIMULATED MICROGRAVITY

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It has been suggested that spinal cord transection could be used as a model for studying the physiological effects of weightlessness. This hypothesis is tested in the present study by comparing the effects of spinal cord injury and head-down tilt (HDT) on body fluid shifts. A study on healthy men and men suffering from traumatic spinal cord injury using a radioisotope technique showed that in the stable phase after spinal cord injury there is an increase of the interstitial space. Using an electric impedance technique in another study it was found that the ratio Z1.5/Z150, which is an index of the relative size of the interstitial space, is decreased. A decrease in this ratio indicates an increased interstitial space. Thus, the radioisotope and the electric impedance technique give results which can be given the consistent interpretation that the interstitial space is increased after spinal cord injury. In the HDT experiment measurements by electric impedance indicated a decrease in interstitial space volume. These findings strongly suggest that body fluid distribution after spinal cord injury and HDT change in opposite directions.

Spinal cord transection has been suggested as a possible model for studying the physiological effects of weightlessness[8]. This suggestion is probably rooted in the fact that persons suffering from traumatic spinal cord injury present several physiological alterations[3] which are similar to those observed during space flight or simulated gravity. One of these alterations is the change in the distribution of body fluids[6,9]. It is not clear, however, whether the change in the distribution of body fluids in spinal cord injury and in weightlessness is the same in both circumstances.

The purpose of the research here reported was: 1) to compare a non-invasive technique of assessing body fluid shifts with the radioisotopic technique and 2) to study and compare the body fluid shifts in the spinal cord injured (SCI) person with those observed in healthy subjects submitted to head-down tilt (HDT).

Methods

The two techniques for assessing body fluid shifts utilized in this study were the radioisotopic and the electric impedance techniques. Total body water (TBW) determinations were made using tritium oxide (HTO). Extracellular water (ECW) was determined using radio sulfate ($Na^{35}SO_4$). Plasma volume (PV) was determined using ^{125}I-HSA. Red cell volume (RCV) was determined using ^{51}Cr labeled autologous red cells. Radioassay for gamma radiation was accomplished by pulse-height analysis in a Packard Auto-Gamma Instrument. Beta radiation was assayed by liquid scintillation in a Packard Liquid Scintillator. The dilution principle was used in each case to estimate fluid volumes[3].

Electric impedance measurements of the whole body were done at the two frequencies of 1.5 kHz (Z1.5) and 150 kHz (Z150) using an input signal of 100 µA. The recording instrument utilized was developed in the Department of Medical Engineering of the Karolinska Institutet, Stockholm, Sweden[10]. The power source of the instrument was optically isolated from the subject. A tetrapolar technique was employed. Input spot electrodes were placed in the palm of the left hand and the instep of the right foot. Detector electrodes were placed in the inner surface of the left wrist at the carpal-metacarpal line and on the anterior surface of the right ankle at the line joining the external and internal malleoli. Readings of impedance (in ohms) were taken directly from the instrument panel. Such measurements were taken after the subject had been resting in the supine position for at least one hour. In the HDT experiment the readings were taken immediately after assuming the HDT position every 15 min. up to 6 hrs. and the plateau of the curves thus obtained was used as the stable impedance reading.

Three experiments are reported. The first consists of a comparison of body fluid measurements in healthy and quadriplegic subjects using the radioisotope technique. The second compares electric impedance measurements in healthy and quadriplegic subjects for the purpose of relating them to the radioisotope measurements. The third reports the results of the HDT experiment on healthy subjects for the purpose of comparing electric impedance changes between healthy and quadriplegic subjects.

Results

1. Radioisotopic measurements[1,2]. The mean and standard deviation of age, height, and weight and standard weight of 12 healthy men and 23 quadriplegic men are shown in table 1. The mean and standard deviation of TBW, ECW, radio ECW/TBW, PV and RCV are given in table 2.

Table 1. Radioisotope Study. Anthropometric data of healthy and quadriplegic subjects. (Values are mean±sd).

Group	Age(yr)	Weight(kg)	Height(cm)	St Weight(kg)
Healthy n=12	30.0±7.6	80.7±12.1	179.9±6.9	73.6±5.7
Quad n=23	27.1±10.8	59.2±9.6	177.9±6.4	72.1±5.7

Table 2. Radioisotope Study. Determinations of total body water (TBW), extracellular water (ECW), their ECW/TBW ratio, plasma volume (PV) and red cell volume (RCV). (Values are mean±sd)

Group	TBW(1)	ECW(1)	ECW/TBW	PV(ml)	RCV(ml)
Healthy n=12	49.3±7.3	19.2±4.7	0.394±0.097	3266±362	2188±400
Quad n=23	34.9±7.4	15.7±3.7	0.463±0.124	2519±537	1626±324

2. Electric Impedance Measurements[4]. The mean standard deviation of age, weight, height and standard weight of 20 healthy and 20 quadriplegic men are given in table 3. The ratio $(Z1.5/Z150)$ of the impedance measurements is also given in this table.

Table 3. Impedance Study. Anthropometric data and impedance ratio of healthy and quadriplegic subjects. (Values are mean±sd, except for the ratio which is mean±se).

Group	Age(yr)	Weight(Kg)	Height(cm)	St Weight(kg)	Z1.5/Z150
Healthy n=20	32.5±8.4	75.0±2.5	175.5±6.7	70.8±7.6	1.366±0.011
Quad n=20	27.7±8.0	69.5±10.6	179.9±6.8	74.3±6.4	1.164±0.012

3. Head-down tilt study. The HDT experiment was conducted on 6 healthy men. The mean and standard deviation of age, weight, height and standard weight of the group is given in table 4. The last two columns of this table show the initial and the plateau values of the impedance measurements (ratio $Z1.5/Z150$).

Table 4. Head-Down Tilt Study. Anthropometric data and impedance ratio of the six subjects who participated in the study. (The ratio is relative to initial value. Values are mean±sd, except for the ratio which is mean±se).

Age(yr)	Weight(kg)	Height(cm)	St.Weight(kg)	Z1.5/Z150
42±16	81.9±13.1	175.9±3.7	70.9±2.9	1.036±.007

Discussion

The radioisotope studies show that the ratio ECW/TBW was higher in quadriplegic than in healthy subjects. The extracellular space is then abnormally increased after spinal cord injury. Table 2 shows also that quadriplegic patients had plasma volumes lower than those in healthy subjects. This leads to the conclusion that the space which is increased is the interstitial space.

The electric impedance studies show that the ratio $Z1.5/Z150$ was lower in quadriplegic than in healthy men. A lower ratio suggests a larger interstitial space[5]. Since impedance measurements are inversely related to fluid volumes and the impedance technique utilized does not measure fluids in blood, bladder or the intestines,[7,10] a lower ratio indicates an increase in interstitial space. Thus, radioisotope and electrical impedance measurements are consistent in suggesting that the interstitial space of men suffering from traumatic spinal cord injury is increased.

The HDT experiment showed a consistent pattern in all subjects. The ratio $Z1.5/Z150$ increased monotonically with time tending to an asymtotic value, practically reaching a plateau 2 1/2 hours after in the HDT position. The mean increase in the ratio after six hours in HDT was approximately 3.6%. Thus, this study shows that in the stable phase following spinal cord injury the ratio $Z1.5/Z150$ is decreased suggesting a relative increase in the extracellular space. This result is consistent with measurements of the ECW/TBW ratio obtained with radioisotopes. The HDT experiment on healthy subjects shows on the contrary, that there was an increase in the $Z1.5/Z150$ suggesting a relative reduction of the extracellular space, and more concretely, the interstitial space. From these

results it must be concluded that the alteration in fluid distribution that follows spinal cord injury is not the same as the change in fluid distribution that follows HDT. It should be pointed out, however, that in the case of the quadriplegic men the situation was a chronic one, whereas in the case of the healthy men it was acute. Impedance measurements performed immediately after spinal cord injury would provide a more appropriate basis for comparison and possibly a better understanding of the dynamics of body fluid changes.

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THE STUDY OF BARORECEPTOR REFLEX FUNCTION BEFORE AND AFTER BED REST

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In order to investigate the mechanism of the lowering of orthestatic tolerance (OST) after exposure to weightlessness (WL), the changes of baroreceptor reflex function (BRF) of the two groups, 2° head-down group (n=7) and sit-up group (n=7), were studied before and after the bed rest (BR). The open-loop gain (G) was calculated during the head-up tilt (HUT) and HUT plus LBNP (-5333 Pa) before and after BR. The frequency spectrum changes of R-R intervals (RI) were also compared during neck positive pressure and LBNP between the two groups. The result was that the changes of G and RI and lowering of OST were different between the two groups after BR. Thus it indicated that the declination of BRF, which probably resulted from the change of function state of CNS, was one of the important causes responsible for the lowering of OST after WL or simulated WL.

Introduction

The mechanisms of orthostatic intolerance (OSI) after WL have been studied by many authors, but the exact biological mechanisms have not been established [1]. We consider that the state of stimulation of baroreceptor may be changed, if the conditions of lack or decrease of hydrostatic pressure and the cephalad redistribution of intravascular fluid during WL or SWL are maintained for a considerably long time, and that the changes of the BRF may occur and cause OSI. So the purposes of this paper were: 1) to observe the change of BRF before and after BR; 2) to study the mechanism of OST after WL in order to offer the theoretical basis of countermeasures in future space flights.

Method

14 healthy young men, aged 18-22, were divided into two groups. Each consisted of 7 subjects. All of them were on bed for 15 days. The subjects in Group A remained strictly in lying position with 2° head-down, while those in Group B were permitted to sit up for 50' and lie down for 10' every hour during day time.

Four kinds of examination were done pre and

post BR (Table 1). The recorded indices included heart rate, blood pressure and cardiac output. The open-loop gain G, heart rate g_H and peripheral resistance gain g_T were calculated with Mei's model [2] which was supposed to reflect the characteristics of cardiovascular regulation of various individuals during HUT and HUT plus LBNP.

Table 1. Examinations before and after BR

	Time	Day of examination
5333 Pa NPP*	2'	15th day in BR
-2667 Pa LBNP	4'	15th day in BR
HUT** 75°	20'	1st day after BR
HUT 75°+LBNP(-5333 Pa)	20'	11th day after BR

NPP: neck positive pressure
HUT: head-up tilt

Result

1. Orthostatic tolerance of the two groups pre and post hypokinesia. The tolerances of the two groups to HUT and HUT+LBNP were same before hypokinesia, but different after different kinds of hypokinesia (Table 2). The tolerance time of Group A decreased significantly after BR, while that of Group B did not. It was clear that influence of the BR, with subjects being in a strictly lying position, on cardiovascular function was more marked than that of the BR, with subjects alternating sitting with lying.

Table 2. Tolerance time of the two groups (A,B) pre and post BR (min.)

n		HUT			HUT + LBNP	
	pre	post	P	pre	post	P
A 7	20 ±0	11.49 ±7.26	<0.01	8.25 ±2.96	2.87 ±1.28	<0.001
B 7	20 ±0	16.87 ±4.78	>0.1	8.32 ±4.43	8.70 ±5.74	>0.9

2. Influence of the two types of BR on BRF. After BR, in simple HUT, which was a light orthostatic load, the G, g_H, g_T of Group A increased significantly, while those of Group B did not. In HUT+LBNP, which was a heavy stress, the three indices of Group A decreased, while those of Group B increased. In order to clarify whether the changes of the three indices wwere caused by different grades of stimulation, the changes of P in Wei's model before and after BR were compared between the two groups. In the model, P_1 was the value to be regulated by baroreceptor reflex. The result was that there were no differences in P_1 both between the two groups and within a same group pre and post BR. That meant the differences in gains were caused by the regulation system itself and had nothing to do with P_1.

Table 3 showes the changes of gains before or at the termination of examinations. Just at the termination, three gains decreased significantly. It indicated that the syncope of orthostasis was caused by the decrease of regulative capacity of the reflex system.

3. The changes in NPP and LBNP. The major difference of cardiovascular indices between the two groups was the frequency distribution of R-R interval during NPP and LBNP. After BR in both

of the groups, total spectrum, C and D waves decreased, and the percentage of A+B in total waves increased. Most of the parameters of Group A changed significantly, while those of Group B did not.
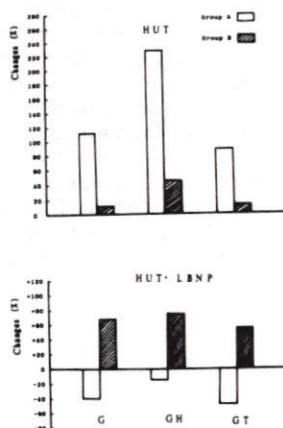

Fig.1 The changes of gains of BRF
in two groups after BR

Table 3. Changes of gains before
or at the termination

	G	g_H	g_T
Before	3.23±1.47	3.27±2.20	0.81±0.63
At	1.44±0.70	1.66±0.91	0.39±0.48
P	<0.001	<0.001	<0.02

Discussion

In order to study the influence of blood redistribution on BRF, different types of hypokinesia were used in the two groups of subjects. The influence of cephalad blood redistribution on BRF in WL was simulated on Group A. Group B was allowed to maintain a stimulation of normal erect posture on BRF for periods of time every day.

The result showed that the OST and the gains of BRF in both groups were different (Fig. 1 and Tab. 2). The decrease of OST and the change of gains in Group A were significant. Those in Group B were not. The P_I value showed that the difference between G in the two groups was not caused by different stimulations, but by the change of functional state of the body. The changes of BRF showed close relations with the changes of OST. This indicated the change of RBF was an important factor causing OSI after BR. Many factors in BR could influence the parts of BRF (receptor, CNS, effector). Among these factors, the stimulation state of baroreceptor and the state of brain circulation in the two groups were different. The change in Group A was more marked than in Group B. They would give different influences on CNS. It would cause more evident incoordiation of CNS after BR in Group A than in Group B. It appeared in Group A that after hypokinesia only power spectrum of R-R interval showed changes during a small local interference (NPP and LBNP). It indicated that CNS activity only increased slightly. During medium interference (HUT), increased G indicated the increase of regulation of CNS. Under a big interference (HUT+LBNP), decreased G indicated incoordination of the regulation function of CNS. But in Group B there were

no changes under both small and medium interferences. G increased only under big interference.

In a word, there were many factors causing OSI during BR. It was also showed in this experiment that the disorder of CNS caused by the cephalad blood redistribution was the most important factor. The cardiovascular deconditioning in a space flight may be prevented if cephalad blood redistribution is prevented, or if the baroreceptor stimulation similar to that on the earth is maintained. We believe that Chinese "Qigong" may be used as a good countermeasure. Some experiments [3] [4] in China have proved that the blood redistribution and increase or decrease of blood pressure can be controlled by the person who is exercising "Qigong" with his mind concentrated in certain parts of his body. It is possble to enhance the adaptation to WL and readaptation to the earth gravity if blood is regulated to the lower body during WL and prevented from being pooled in the lower part of the body on returning to 1G condition by using the Chinese "Qigong". Meanwhile, "Qigong" can enhance the adaptation of body to external environment and keep the coordination of regulative function. This kind of exercise needs no special equipments. So it is convenient to be used in a space flight. We suggest that "Qigong" be an effective measure to prevent the influence of WL on cardiovascular system. It, however, needs to be further studied before it can be put into practical use.

Conclusion

1. The OST and BRF were influenced by a 15-day hypokinesia. The influence on Group A was stronger than on Group B. The result showed that the change of BRF, especially the disorder of regulative function of CNS, was the important factor causing OSI after BR.

2. To change the blood distribution or to regulate the stimulation to baroreceptor during WL or SWL might be effective means to prevent the cardiovacular deconditioning during space flight.

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THE ROLE OF PHYSICAL TRAINING IN
INCREASING +Gz ACCELERATION TOLERANCE
IN THE INITIAL PHASE OF AVIATION
TRAINING

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The effects of L-1 and M-1
maneuvers depend on regular training,
developing contractile force in certain
groups of skeletal muscles, in
co-ordination with a respiratory maneuver.
This is connected with developing force
and co-ordination in some muscular
groups. Great effort, necessary to
perform the above mentioned maneuvers,
requires subjecting the pilot to a
properly oriented physical training
regime. The earlier results /Baldine
1984/, Cete 1985, Epperson et al. 1985,
Tesch et al. 1983/ suggest that
strength and endurance exercises,
improving the performance of those
muscles which are involved in Valsalva's
maneuver, are of particular importance
in the early phase of pilot training.
Since certain respiratory indices
/including inspiratory capacity IC/
play a significant role, it seemed
necessary to take also spirometric
measurements in the subjects undergoing
the respective training.

The aim in view was to examine
the effects of selected physical
exercises, performed in a regular
schedule, upon acceleration tolerance
and certain respiratory indices in
pilots during the initial stage of
their training.

Materials and Methods

The subjects were 135 young pilot
candidates, aged 19-20, who had never
exceeded the limit of 5,2 G in the
0.1 G/s GOR program without visual
disturbances during their entrance
centrifuge selection. These men were
subject to physical training /described
below/ by daily schedule /Fig.1/,
throughout a period of 8 weeks. After
that period they had their acceleration
tolerance evaluated again, up to the
black-out point. The results revealed
that 21 subjects had not reseached the
limit of 5.7 G, the classification
minimal standard for high performance
aircraft in our aviation. In this

group the training was continued for
the further 6 weeks, to be followed
by another acceleration tolerance
examination series. Before and after
the training, during each centrifuge
test, such measurements were taken as:
expiratory thrust time at 30 mm Hg of
force /Flack's test - PF/; Hewlett -
Packard instrumented spirometry,
including /IC/, vital capacity /VC/,
peak flow /PF/ and the last first MVV
/maximal voluntary ventilation/
coefficient, i.e. the ratio between
the value of ventilation during the
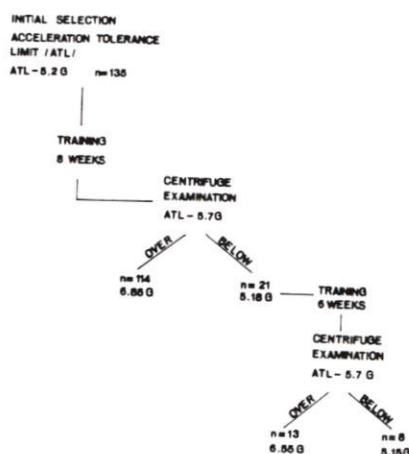last 5 seconds and that of the first
5 seconds.

Fig.1. Examination plan

Physical training

The program included 10 exercises
/Fig. 2/, mainly isometric ones,
developing muscular force in upper
and lower limbs, abdominal muscles,
dorsal and femoral extensors, and
the thoracic musculature. In most
of the exercises the subjects performed
apnoea and expiratory thrust upon
partly or completely closed glottis.

The point of Exercise 1 was
changing from upright standing to
upside-down position, in order to
trigger the vasomotor responses
compensating for the orthostatic
changes in blood pressure distribution.
This exercise produces muscle tone
in cervical musculature, spinal
extensors and flexors, and in respira-
tory muscle /mm. intercostales and
diaphragm/. In Exercise 2, apnoea is
sustaind for 25 seconds /without
thrust/, causing blood shifts from
cephalic and pulmonary regions, which
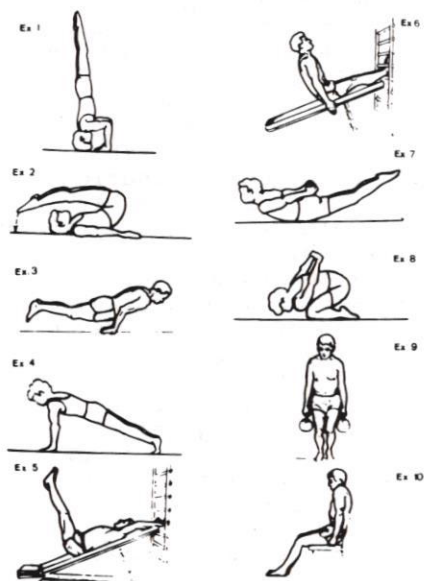
is turn develop systemic anaerobic reactions.

Fig.2. Exercises applied in the physical training program

In Exercise 3, a quick, deep inspiration is performed, to be followed by air retention in the lungs. The cycle is repeated several times. This forced manner of breathing develops responses typical for the L-1 maneuvre which compensate for the considerable blood pressure fluctuations in intrathoracic and cephalic vessels through subsequent phases of breathing.

Exercise 4 is based on a possibly fast change from the supported horizontal /on all fours/ to standing position and back again. It develops the force and speed of abdominal muscles contraction.

Exercises 5 and 6, as proposed by Balldin et al. /1/, were supplemented in our training program by respiratory exercises. When lifting up his trunk, the subject performs the maneuvers M-1 and L-1, thus improving the co-ordination of abdominal muscles and femoral extensors with the specific mode of breathing. Intermittent breathing in combination with strong expiratory trust produced periodical, breathing--conditioned increases of blood pressure in supracadiacally located vascular regions. This exercise improves the functions of the diaphragm and venous pump.

Exercise 7 develops the static endurance of spinal extensors. In Exercise 8 elements of the L-1 and M-1 maneuvers are performed in parallel with tensing skeletal muscles. The subject's posture enhances the thoracic muscles tension and affords possibilities for producing high values of intrathoracic pressure. Resting the abdominal wall against the thighs enables to attain a high increase of intra-abdominal pressure. In Exercise 9 the subject had to hold 17.5 kg weights /in both hands/ with his knees half-bent, and then bend his knees. This developed the strength and endurance, both static and dynamic, of primarily femoral extensors. In Exercise 10, the M-1 and L-1 maneuvers were fully executed, and the position was close to that of the pilot's in his cockpit.

Results

In the group of 135 men subjected to physical training for a period of 8 weeks, 114 /Group A/ gained acceleration tolerance ranging from 5.7 to 8.0 G, mean 6.85 G. The mean Flack's test result was 54.9 s. High IC values were also resulted in these subjects. However, 21 subjects failed to reach the limit of 5.7 G. Their mean results /Table 1/ were lower by 32% for acceleration tolerance, by 25.3% for Flack's test, and by 17.8% for IC.

TABLE I. SUBJECT DATA

GROUP	Number of subject	Ht cm	Wt kg	ATL G	FLACK s	PF l/s	IC l	VC l	MVV LAST/FIRST
A G ≥ 5.7	114	174.5 ±4.5	71.5 ±3.7	6.85 ±0.51	54.9 ±6.9	6.81 ±0.62	3.69 ±0.26	4.95 ±0.42	1.01 ±0.06
B G < 5.7	21	177.4 ±5.7	72.6 ±8.3	5.18 ±0.32	43.8 ±6.2	6.59 ±1.64	3.13 ±0.56	4.66 ±0.87	0.96 ±0.07
A:B%		-1.2 ns	-1.1 ns	+32.2 **	+25.3 *	+3.4 ns	+17.8 **	+6.2 ns	+5.3 ns

The remaining indices were statistically insignificant, although, in comparison to the pre-training test

data, the PF index was also higher . Such positive changes achieved through applying the exercises encouraged the authors to repeat thus oriented training programs. After a 6-weeks' period of training, based mainly on isometric exercises combined with special breathing techniques, the subjects underwent another centrifuge examination series, accompanied by spirometric measurements. The results are presented in Table II.

TABLE II. SUMMARY OF THE POST-TRAINING EFFECT OF GROUP B, WHICH WAS DIVIDED AT ATL= 5.7 INTO TWO GROUPS C /n=13, ATL ≥ 5.7 / AND D /n= 8, ATL< 5.7 /

	TRAINING BEFORE	TRAINING AFTER	P. Value
	B n= 21	C n= 13	
		D n= 8	
Ht cm	177.4 ± 5.70	177.2 ± 5.40	n s
		176.3 ± 7.70	
Wt kg	72.5 ± 8.30	73.2 ± 6.50	n s
		70.3 ± 9.40	
ATL G	5.18 ± 0.32	6.55±0.60	C:B < 0.01
		5.15±0.52	D:B n s / C:D < 0.01
FLACK s	43.8 ± 6.20	49.6 ± 5.60	C:B < 0.05
		38.2 ± 4.40	D:B < 0.02 / C:D < 0.01
PF l/s	6.59 ± 1.64	5.88± 1.71	n s
		4.74 ± 2.45	
IC l	3.13 ± 0.56	3.41± 0.40	n s
		3.17± 0.42	
VC l	4.56 ± 0.57	4.57± 0.73	n s
		4.02± 0.82	
MVV LAST/FIRST	0.96 ± 0.07	1.00± 0.11	n s
		0.97± 0.08	

On the premises of the acceleration tolerance limit = 5.7 G, 13 subjects showed results exceeding this value, and 8 subjects failed to reach it. Accordingly, they were classified in two subgroups: Subgroup C and Subgroup D . The mean acceleration value for Subgroup C was 6.55 G, whereas in Subgroup D it remained on the same level. The subjects' results varied from 4.4 to 7.3 G. Significant differences were also found in Flack's test data, since in the higher acceleration tolerance subjects /Subgroup C/ the resulted time was prolonged by 13.2% /p 0.05/, whilst in the subjects of Subgroup D the result was worse by 12.8% in comparison to the values for all the subjects in Group B. Similar results, although statistically insignificant, were indicated for the IC values /this index increased by 8.9% in Subgroup C/. Unlike the above mentioned, the PF values decreased after the repeated training series, and the VC index remained unchanged. Similarly, the MVV index, which affords possibilities

for evaluating the capacity of respiratory muscles under intensified breathing, surprisingly, remained unchanged.

Discussion

The presented data indicate a relatively high increase in acceleration tolerance as a result of the applied orientation of training program. This improvement is primarily connected with the fact that for many young pilot-candidates it was the first acceleration tolerance examination in their lives. In spite of their high personal motivation, the candidates were not always able to tolerate easily acceleration values beyond 5 G. The presented series of exercises could have partly affected the improvements achieved for these values, since some candidates managed to increase their results, e.g. from 4.8 to 7.8 G. The very fact of learning for the first time about the conditions of human centrifuge examination, and knowing the mechanism of behavior during such tests, could also have been of considerable importance. Nevertheless, the positive effects were more marked in those young pilots who had better Flack's test and IC results. However, since 21 pilots had negative results in the second examination series at 5.7 G, another 6-weeks' training series produced in 62% of the pilots a marked increase of G-tolerance. A parallel effect of the exercise series was prolonging the resulted Flack's test time, but without any significant modifications in spirometric indices.

The data suggest that a combination of respiratory exercises with isometric traning for certain groups of muscles provides a good starting point for the future pilot--training programs, towards developing those muscle which are important in respiratory straining maneuvers, and thus indirectly improve G-tolerance.

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COMPARATIVE MORPHOMETRY OF FIBERS AND CAPILLARIES IN SOLEUS FOLLOWING WEIGHTLESSNESS (SL-3) AND SUSPENSION

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This work is a continuation of efforts to assess responses of rat skeletal muscle to weightlessness and earthside laboratory experiments with unloading of hind limbs. The soleus is a slow twitch, load bearing (antigravity) muscle. Both exposure to weightlessness (W) and to the hypokinesia/ hypodynamia of whole body suspension (WBS) results in soleus atrophy. Cross sectional areas of both slow and fast twitch fibers decrease during 7 days of W, and 7 or 14 days of WBS. Density and area changes tended to reverse to control levels during 7 days of recovery (R) following WBS. Capillary density was increased with 7 days of W, and 7 or 14 days of WBS. During 7 days of R the capillary density returned toward control levels. In summary, the reduction in fiber cross sectional areas and increase in fiber and capillary densities support the hypothesis that in both forms of disuse, i.e., W and WBS, there is a loss in soleus muscle cell mass and not in fiber numbers.

INTRODUCTION

The soleus, a slow twitch load bearing muscle, responds to conditions of weightlessness (W) and to the hypokinesia and hypodynamia (H/H) of unloading during suspension with a loss in mass and cellular protein. In COSMOS flights 605, 690 and 1129 rats were exposed to microgravity for periods from 15.5 to 22 days. Ilyina-kakueva and Portugalov (1977), Gayevskaya et al (1979), and Rapcsak et al (1983) reported losses in muscle mass and protein. More recently, Henricksen et al (1985) and Steffen and Musacchia (1986) reported that with one week of exposure to microgravity in the Spacelab-3 (SL-3) flight there was also a significant loss in muscle mass and protein.

Jaspers et al (1985) and LeBlanc et al (1985) using tail cast suspension and Musacchia et al (1983) and Steffen et al (1987) using whole body suspension (WBS) unloaded the hind limbs for periods for one week or longer. These investigators reported losses in muscle mass and reduction in protein content. The loss in protein content has been credited to both increased catabolism and to decreased protein synthesis. Steffen and Musacchia (1986) recently reported that during a one week exposure to microgravity, the relationship of protein to cellular DNA concentrations suggested a loss in cell mass without a loss in cell numbers. This was deduced from unaltered DNA contents following the one week of microgravity in SL-3.

To date many of the experiments concerned with muscle metabolism have used young, growing rats. We now propose the need for adult animals in WBS experiments to simulate space flight responses, particularly if aspects of disuse atrophy are to be extended to the human subject.

In brief, we decided that there was not only a need to do experiments concerned with cellular changes in adult rats, but also the need to pursue experiments that assess the changes in fiber morphometry. We reasoned that if our contention that disuse atrophy was seen as a loss in mass, i.e., intracellular protein, and not in fiber numbers, then morphometric studies would assist in confirming this hypothesis. Another objective of this investigation was to make comparisons between ground based simulations i.e. (WBS), and flight experiments. Lastly, we initiated experiments in recovery from WBS in order to compare results and to predict responses following exposure to microgravity.

MATERIALS AND METHODS

Male Sprague Dawley rats (360-410gm flight and flight controls and 350-400 gm for WBS and WBS controls) were used. The SL-3 flight lasted approximately 1 week plus an additional 12hr post flight period of loading prior to euthanasia. Halothane anesthesia and decapitation were used for the flight subjects and their controls. An overdose of pentobarbital sodium (65mg/100gm) was used with the WBS and WBS control subjects. The soleus muscle was surgically removed, fixed at an in situ length (placed on a piece of index card), wrapped in aluminum foil and frozen in 2 methyl-butane cooled to the temperature of liquid nitrogen.

The muscles were prepared for frozen sectioning by embedding in gum tragacanth and sectioned at 10um. The frozen sections were treated with ATPase stain adjusted to an alkaline pH. Muscle sections were pre-incubated at pH 9.4 for fiber typing and at pH 3.8 for capillary staining. These methods are described in detail by Dubowitz and Brooke (1973) and Sillau and Banchero (1977).

Cross sectional areas (um^2), fiber densities (fibers/mm^2) and capillary densities (capillaries/mm^2) were determined using a computerized image analysis system. Both slow and fast twitch fibers were differentiated and data were analyzed by t-test (flight experiments) and ANOVA (WBS experiments). When significant differences were noted by ANOVA, appropriate post hoc tests were employed.

RESULTS AND DISCUSSION

Microscopic examination of the soleus from rats that were flown on the seven day SL-3 mission showed a significant reduction (30%) in cross sectional areas of both slow and fast twitch fibers. This resulted in a significant increase in density (30 and 60%, respectively) of both slow and fast twitch fibers. We interpret these results as indicative of a loss in fiber mass but not in numbers of fibers. In effect, there was a more dense concentration of fibers per unit of muscle after a week of microgravity exposure. The capillaries in the soleus also showed a significant increase in density (50%) after the 7 day flight. The increased density of capillaries raises the question of potential alteration in blood flow to the disused muscle.

The next question focused on the suitability of an earth bound experimental model (i.e., WBS)that could be used to simulate responses seen in flight subjects. We also assessed the responses seen after 7 days of recovery, following 7 days of WBS. After 7 and 14 days of WBS, the soleus showed a significant reduction in cross sectional areas of slow twitch fibers, about 14% and 26%, respectively. After 7 days of recovery there was a significant recovery of slow twitch fiber areas to control levels. Although the fast twitch fibers are fewer in number, there was also a significant reduction in cross sectional area after 7 and 14 days, about 20 and 40%, respectively. In fast twitch fibers there was recovery in cross sectional area to a level of the controls.

The densities of slow twitch fibers were significantly increased after 7 and 14 days of WBS, (20 and 60%, respectively) and after 7 days of recovery there was a reversal to control levels. There were no significant changes in the fast twitch fibers after 7 and 14 days of WBS. The capillary density increased in parallel with slow twitch fiber densities.

These morphological changes in the soleus parallel and compliment the changes reported earlier in terms of muscle mass, protein and protein/DNA ratios. In addition, cellular biochemical changes may be used to predict changes in muscle fiber morphometry.

CONCLUSIONS:

We concluded that weightlessness and WBS results in true muscle atrophy, viz. loss in slow twitch and fast twitch fiber cross sectional areas and increase in fiber and capillary densities. Also, there is significant recovery within 7 days following removal from WBS. Seven day changes in muscle morphometry induced by H/H of WBS appear to be comparable to those induced by space flight.

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POSTURAL EFFECTS
ON MUSCLE SYMPATHETIC NERVE RESPONSIVENESS
TO SUSTAINED MUSCLE CONTRACTION

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ABSTRACT

To confirm the dominancy of the effect be-
tween postural change and muscle contraction on
muscle sympathetic nerve activity (MSNA), MSNA was
recorded before, during and after a sustained
handgrip exercise in a lying and in a standing po-
sition by means of microneurography. At rest,
MSNA was markedly higher in the standing position
compared with that in a lying position. However,
during handgrip, MSNA did not increase in the
standing position, and significantly increased in
the lying positon, although MSNA was lower during
handgrip in the lying than that in the standing
position at rest without exercise. It is con-
cluded that the gravity dependent postural effect
dominates the MSNA responsiveness to exercise.

INTRODUCTION

Previously, we reported that muscle sympa-
thetic nerve activity, as an indicator of autonom-
ic nerve activity, was reduced under weightless-
ness simulated by head-out water immersion (1),
while being increased by head-up tilting from a
lying to a standing position (2). MSNA also
increased by sustained muscle contraction in the
lying position (3,4). In this study, we attempted
to determine the dominancy of the effect between
postural change and voluntary sustained muscle
contractions on MSNA.

SUBJECTS AND METHOD

1) Subjects

Five healthy male subjects aged from 19 to 41
years old, who gave their consent to take part in
the experiment, were investigated.

2) Muscle contraction

Sustained muscle contraction was performed
for two minutes using a handgrip, at a tension of
20-30% of the maximum voluntary contraction which
was determined before the experiment in a supine
position. The tension in the handgrip was moni-
tored by a cathode rays oscilloscope (Fig. 1)
during this exercise to maintain a constant
tension.

3) Change in posture

The posture of the subjects was changed from
lying to standing using a tilt table. MSNA re-
sponses to a sustained handgrip were compared in
these two different postures. The effect of an
abrupt postural change from lying to a 30° head-up
tilted position on the handgrip which induced
changes in MSNA was also analyzed. The postural
change was carried out using a tilt table.

4) Physiological measurements

Electrocardiogram (ECG), electromyogram (EMG)
of the forearm and arterial blood pressure (BP)
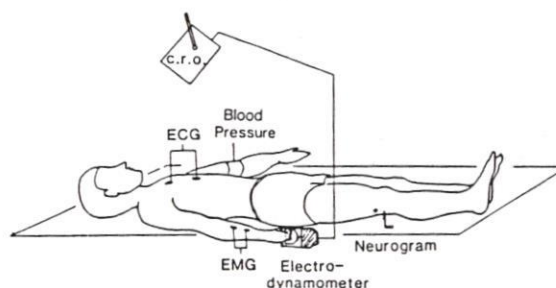were measured throughout the experiment (Fig. 1).

Fig. 1. Experimental setup.

5) MSNA recording

MSNA (Neurogram) was recorded from the tibial
nerve at politeal fossa using a tungsten micro-
electrode (Fig. 1). The tungsten microelectrode,
with a tip diameter of about 1 μm, a shaft diame-
ter of 100 μm, and impedance of 2-5 MΩ was in-
serted manually and percutaneously without anes-
thesia. MSNA was recorded on a magnetic tape with
other signals and analyzed after the experiment.
MSNA was expressed quantitatively as burst rate,
which means the number of bursts for one minute,
being counted from the peak of the full-wave rec-
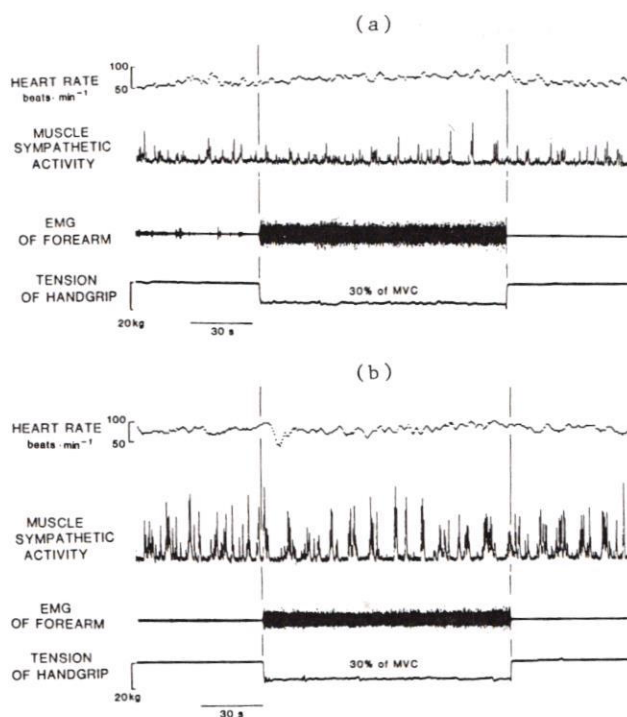tified and integrated MSNA trace recorded on a pen
recorder.

Fig. 2. Typical recordings of heart rate (HR),
MSNA, EMG and tension of handgrip in lying (upper
panel; a) and standing positions (lower panel; b).

RESULTS AND DISCUSSION

1) MSNA response to a handgrip exercise in both standing and lying position

In a lying position, MSNA and heart rate increased during the handgrip exercise (Figs. 2a and 3), though the resting value of the MSNA burst rate was clearly lower as compared to that in a standing position, as shown in Figs. 2b and 3. In a standing position, the MSNA burst rate and heart rate at rest were higher than those in a lying position. After commencement of the handgrip, it showed a tendency to decrease slightly but the heart rate increased (Figs. 2b and 3). In both positions, BP increased gradually during muscle contraction.

During handgrip exercise, MSNA burst rate increased in a lying position, however, the maximum value of the MSNA burst rate during handgrip in the lying position did not exceed that in the standing position at rest without exercise (Fig. 3).
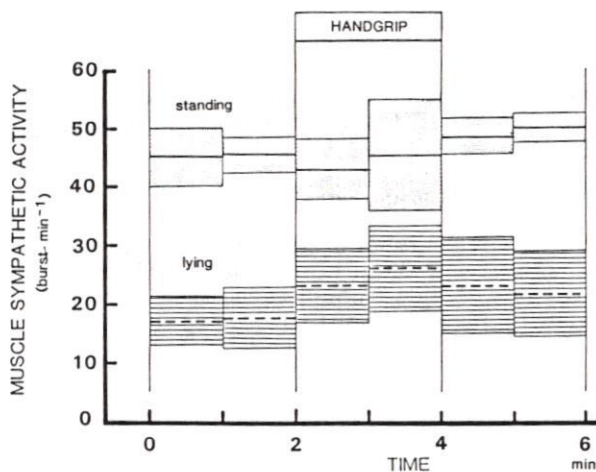
Fig. 3. Comparison of average MSNA burst rate in standing and lying positions. Shaded areas indicate ± 1 SD.

2) Effect of abrupt changes in body position on MSNA responsiveness during handgrip

In a standing position, the contraction of the antigravitational skeletal muscle in the leg should occur. It might also activate MSNA. In order to exclude the effect of muscle contraction in the leg, we carried out another experiment, in which an abrupt postural change from lying to a 30° head-up tilted position without support of the both foot was performed.

When the position was changed abruptly during handgrip from lying to a 30° head-up tilted position and from a 30° head-up tilted to a lying position, MSNA was activated in the former condition and reduced remarkably in the latter condition (Fig. 4).

The higher activity in the sympathetic nerve to the skeletal muscle during a standing than during a lying position, which has also been reported by other authors (5), may be related to increased venous pooling in the leg by gravitational force. On the contrary, MSNA suppression during a lying position seems to be connected with the cephalad fluid shift, activating the baro- and volume receptors.
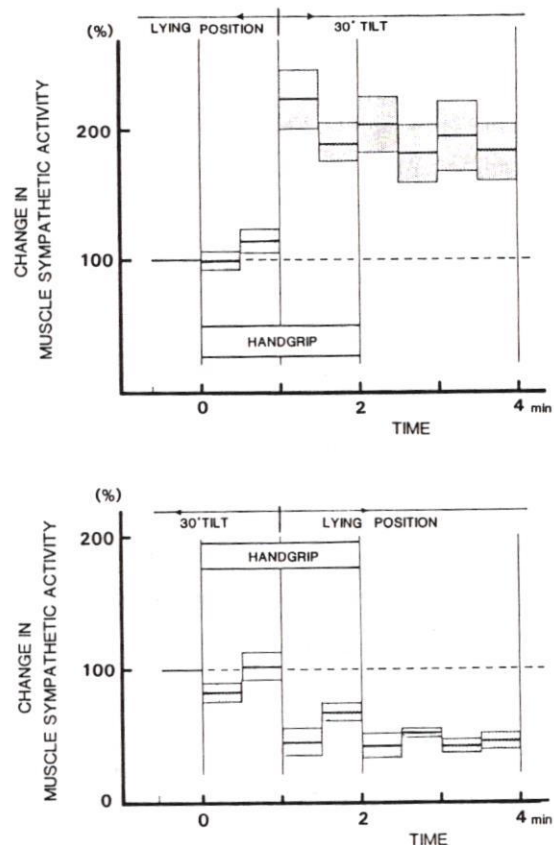
Fig. 4. MSNA responses to changes in body position during handgrip. From lying to 30° head-up tilted position (upper panel) and from 30° head-up tilted to lying position (lower panel).

The muscle contraction which stimulates the peripheral chemoreceptor might activate MSNA during a lying position (4), but it could not increase MSNA to a value exceeding the resting level in a standing position. These results suggest that MSNA during a standing position is too strong to be increased more by muscle contraction.

CONCLUSION

In conclusion, the muscle sympathetic nerve activation to sustained muscle contraction in humans is influenced by the posture of the subjects. The gravitational input seems to dominate MSNA responsiveness during sustained muscle contraction.

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EFFECT OF SPACEFLIGHT ON COLLAGEN PEPSIN SOLUBILITY AND COLLAGEN TYPE DISTRIBUTION IN FEMORAL BONE AND SKIN OF RATS

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Samples of femoral bone and skin of male Wistar rats, subjected to a 7-day spaceflight on the biosatellite Cosmos--1667, were analyzed to reveal possible effects of weightlessness on collagen pepsin solubility and collagen type distribution. The analysis has shown the following effects of spaceflight as compared with controls: in bones - the increase of collagen solubility and the presence of collagen type III; in skin - the decrease of collagen solubility and a higher percentage of collagen type III.

Material and Methods

Specific pathogen-free male Wistar rats, aged 100 days, were used. Flight animals were placed in specially constructed boxes and kept during 7 days of the flight under housing conditions enabling a regular automatic loose diet feeding, clear air supply and removal of excreta. Synchronous control animals were kept in model spaceship conditions. The vivarium control animals, kept under routine conditions in an experimental room, were also used. Four to eight hours after the landing of the biosatellite the rats were killed by decapitation and samples of bones (femurs) and skin from a dorsal depilated region were taken for analysis.

Samples of both tissues, deep frozen with liquid nitrogen, were mechanically homogenized and subjected to a standard procedure of pepsin digestion (twice for 24 h, 0.5 mol/l acetic acid with 1 mg of pepsin "Boehringer" per 10 mg of wet tissue, at 4 °C). The insoluble fraction was subjected to cleavage with cyanogen bromide as described by Epstein E.H. (J. biol. Chem. 249: 3225, 1974). The cyanogen bromide-liberated peptides were separated using the "Fast Protein Liquid Chromatography" system. Peptides characterizing type I and type III collagen were identified and for calculation of the proportion of both collagen types the procedure of Hanson A.N. and Bentley J.P. (Anal. Biochem. 130: 32, 1983) was used. The soluble fraction was subjected to "Zone Precipitating Chromatography" according to Ehrlich H.P. (Prep. Biochem. 9: 407, 1979). Col-

Fig.1
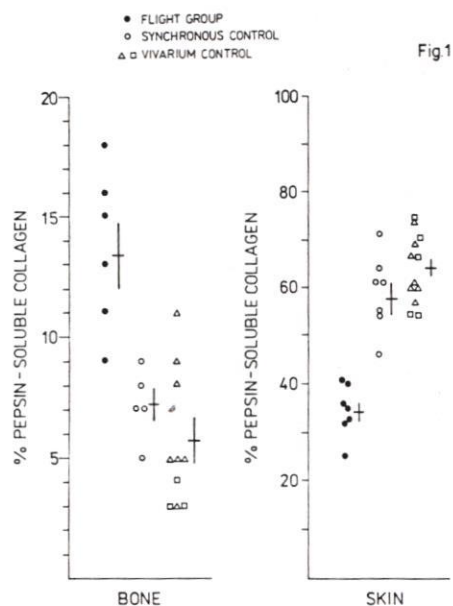
lagen was determined as hydroxyproline (Stegemann H., Hoppe-Seylers Z. physiol. Chem. 311: 41, 1958).

Results and Discussion

The total amount of collagen (soluble plus insoluble fraction) per 100 mg of wet weight of bone decreases significantly by about 35 per cent in flight animals as compared to both controls. The proportion of pepsin soluble collagen in bones is significantly increased in the flight group (Fig. 1). Fig. 2 gives the results of collagen type analysis in both the pepsin soluble and insoluble fractions. As expected, in bones of control groups collagen type I predominates, collagen type III is absent (with the exception of two control, pepsin soluble samples). In bones of the flight group the presence of collagen type III was demonstrated in both fractions.

Analysis of skin samples did not reveal changes in the total amount of collagen per wet weight. However, the proportion of soluble collagen is significantly decreased in the flight group (Fig. 1). As expected, collagen type III is present in the skin of control animals in both fractions. In the flight group, this type of collagen was found in a significantly higher percentage (Fig. 3).

The results have thus shown evidence of spaceflight effects on the collagen matrix of femoral bones and skin. A decrease of the total collagen content in weight-bearing bones may be a sign of complex reactions of this tissue to weightlessness, additive to calcium loss and conditioned by a suppression of the proteoanabolic processes and/or prevalence of enzymatic degradation effects. Calcium may be a factor stabilizing collagen structures. If so, the increased pepsin solubility of bone collagen in flight
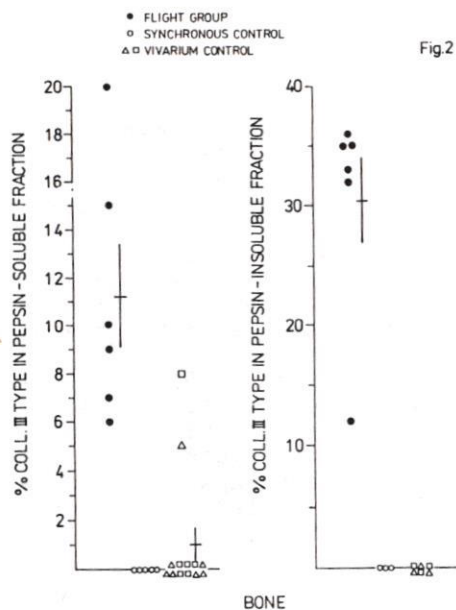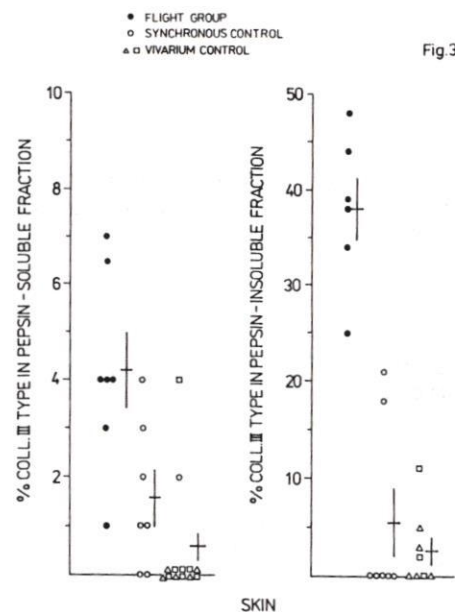
Fig.2

Fig.3

BONE

SKIN

animals could be due to calcium loss. The opposite finding, i.e. the decreased pepsin solubility of skin collagen in flight conditions is interesting, but the interpretation of this effect has to wait for further experiments.

The main result of this work is the evidence of the presence of collagen type III in bones of flight animals and an increased proportion of collagen type III in the skin of these animals. It should be mentioned that similar findings, i.e. the occurrence of type III collagen in femoral bones of pregnant rats orbiting for 5 days aboard the biosatellite Cosmos-1514, were reported by ourselves earlier (Pospíšilová J., Serova L.V., Pospíšil M., Scripta medica, Brno, 59: 277, 1986). Collagen type I is the major structural component in bones, tendons and the skin. Collagen type III is thought to comprise the reticulin fibres described by histochemists; it was originally detected in fetal skin and is a collagen of young, growing tissue. Fragile bones of patients with osteogenesis imperfecta, a disease characterized by defects in the chains of type I collagen, contain collagen type III. Thus it seems that our findings, indicating an increased content of collagen type III in the bones and the skin of rats subjected to a spaceflight, signalize active remodelling processes and possibly a pathological situation in the organic matrix of connective tissue. The presence of this effect in the bones and the skin indicates the role of some systemic factors. The significance of these findings for the physiology and pathophysiology of man subjected to spaceflight conditions remains to be further analyzed.

THE INFLUENCE OF A ONE-WEEK SPACE FLIGHT ON TEETH AND JAW BONES OF WISTAR-RATS (COSMOS I5I4 AND COSMOS I667)

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The carbonatapatitconcentration
of teeth and upper and lower jaw bones
of 5 male and 5 pregnant rats in Soviet
biosatellites was measured. While the
carbonatapatitconcentration in male rats
during the flight significanly decreased,
this effect was absence in pregnant rats.

INTRODUCTION

Cosmical medicine and biology take
only a small notice of the influence of
hypogravitation on teeth up to now.
Erben (I986) investigated the influence
of hypokinesia on jaw bones and teeth of
rats. Erben demonstrated that a 60 day
hypokinesia result in alteration of
growth-processes of alveolar bones.
Following results were found:
- osteoporosis and inhibition of bone
  growth
- reduction of cementocytes, osteocytes
  and blood vessels of alveolar bones
- reduction of the cytoplasma of osteo-
  cytes.
These changes were found in spite of a
free flexible head of rats, during the
period of the 2 month hypokinesia. In
results of these mode. experiments, it
was the aim to investigate the influence
of a space flight on teeth and jaw bones
of rats.

METHODS

The carbonatapatitconcentration of
dentin and cement of molares and in-
cisivi and of lower jaw bone and upper
jaw bone of male and pregnant rats was
investigated.Rats (two groups of 5
animals) exists under free movement in a
box (660x220xI60 mm) on board of Soviet
biosatellites (3). Fife female rats
were 5 days in space during the I3[th] to
I8[th] day of pregnancy in biosatellite
"COSMOS I5I4". Fife male rats were 7
days in space on board of biosatellite
"COSMOS I667". The rats were killed
immediately after landing. After that
the jaw bones were denudated the teeth
extracted and cement and dentin were
isolated according to (5). The bio-
materials were pulverized during a
period of 90 minutes under vacuum condi-
tions and 40°C temperatur. The deter-
mination of carbonatapatitconcentration
was carried out by infrared spectros-
copy according to (3).

RESULTS

Under control conditions male rats
continously showed higher levels of
carbonatapatitconcentration than preg-
nant rats. We didnt find significantly
differences between the carbonatapatit-
concentration of molares, incisivi and
jaw bones in pregnant rats of both
groups. (Tab. I). In opposite to preg-
nant rats in male rats we found signi-
ficantly reduced carbonatapatitconcen-
trations in all investigated bio-
materials.

DISCUSSIONS AND CONCLUSION

This results demonstrate, that
cosmical conditions cause important
changes of teeth and jaw bones in male
animals. The reduction of carbonatapa-
titconcentration in teeth and jaw bones
of male animals can be discussed as an
example for the special sensitivity of
male organism against microgravitation.
Sex dependent reaction on rats obtained
by Poppei and Hecht I977 in different
functions in organism in complete hypo-
kinesia influenced by chronical stress.
In this case the male animals were more
sinsitive against stress than the
female rats. The resistance of the
pregnant organism against microgravi-
tation and the changes of dentin and
cement is discussed as an protective
mechanism caused by relation between
hormonal regulation and mineral meta-
bolism. The table reflect also thet
lower carbonatapatiteconcentration of
teeth and jaw bones of female rats in
opposite to male rats. Further in-
vestigations are necesary to find out
the mechanisms of these processes.

Table I. Carbonatapatiteconcentration of dentin and cement of molares and
incisivi and of lower and upper jaw bone of male and pregnant rats
(% in mean)

+ control versus flight $p < 0,05$

	N	molares		incisivis		jaw bone
		cement	dentin	cement	dentin	
male						
control	IO	$37,3 \pm 3,I$	$28,I \pm I,3$	$4I,7 \pm 3,4$	$33,6 \pm I,2$	$32,6 \pm 0,5$
flight "Cosmos I667"	IO	$32,2 \pm I,5$ [+]	$25,8 \pm 0,I$ [+]	$36,3 \pm I,8$ [+]	$27,6 \pm 0,6$ [+]	$25,8 \pm 3,2$
pregnancy						
control	IO	$33,9 \pm 3,6$	$25,4 \pm 3,6$	$39,2 \pm 4,6$	$28,4 \pm 0,7$	$29,2 \pm 0,5$
flight "Cosmos I5I4"	IO	$32,6 \pm 2,8$	$25,6 \pm 0,6$	$36,7 \pm I,8$	$27,6 \pm 0,4$	$28,8 \pm 3,I$

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A NEW DRUG IN TREATMENT OF OSTEOPOROSIS

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The term osteoporosis designates a group of diseases with different aetiologies, all characterized by the disappaerance of the normal bone structure as the main symptom. The frequency of this multifactorial bone disease is a function of age and it is associated with a great number of diverse disorders such as malnutrition and malabsorption, various dysfunctions of the kidneys, endocrine disorders and with changes in skeletal mechanical factors. It has been a long clinical experience that inactivity may also leads to osteoporosis. As has been shown recently in astronauts, weightlessness can also result in osteoporosis (1, 2). It has been repeatedly demonstrated that rats flying on a spacecraft develop demineralization and disordered growth of their bones (3, 4). In recent studies we have shown that plaster cast immobilization is capable of inducing marked osteoporosis in rats (5, 6, 7). The aim of present experiments was to study the effect of Ipriflavone on the osteoporosis induced by immobilization.

Materials and methods

The right hind limb of 15 albino Wistar strain about 200 g of body weight was immobilized by plaster cast in slightly flexed position. The plaster cast was continuously checked and strengthened or replaced when it was necessary. 10 animals received daily Ipriflavone. This new drug (7-isopropoxy--isoflavone) is product of Chinoin Pharmaceutical and Chemical Work Ltd., Budapest (Fig. 1). Ipriflavone suspension was prepared in the following manner: 500 mg Ipriflavone was suspended in 100 ml physiol. sodium chloride solution containing 1 ml of Tween 80. This suspension was administered to rats via a gastric tube at a daily dose of 40 mg/kg body weight for 8 weeks. Five rats served as untreated controls. At the end of experiment the tibia was removed and halved in the midsagittal plane, fixed and decalcified in Susa solution, 8-10 u thick sections were stained with haematoxylin-chromotrop, Goldner and Azan's methods, then qualitative and quantitative histomorphometric measurements were carried out.
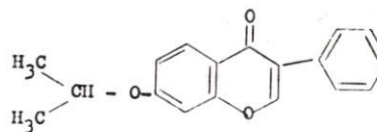
Empirical formula: $C_{18}H_{16}O_3$
Molecular weight: 280,33
Synonym: FL-113
Registered Trade name: Yambolap
Generic name: Ipriflavone

Figure 1.

Results and discussion
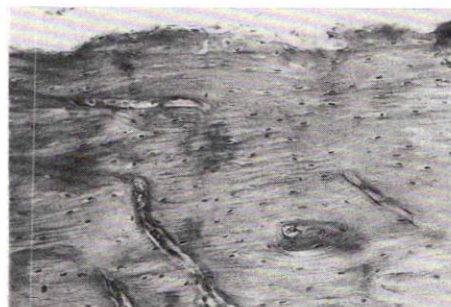
Figure 2. shows the cortical bone of normal untreated control (Goldner's staining, 130x)

Figure 3. After 8 weeks number of Howship's lacunae filled with osteoclasts and osteon disintegration were present (Goldner's staining, 130x)
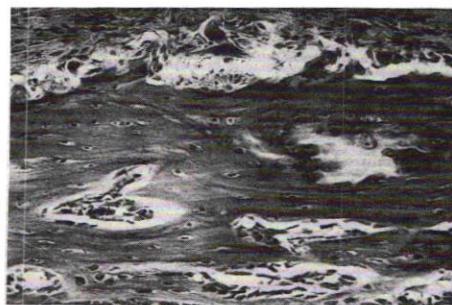
Figure 4. The structure of the bone was altered, rarefication and osteocytic osteolysis was also prominent (Goldner's staining, 130x)
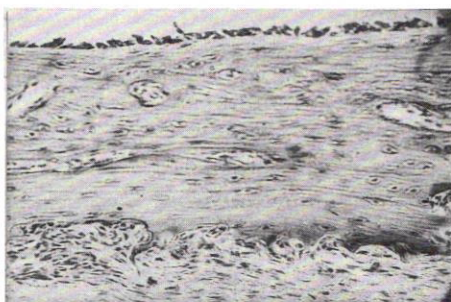
Figure 5. After Ipriflavone treatment it was can see Howship's lacunae without appearance of osteoclasts, enlarged osteocytic capsules, vascularization and endosteally osteoblasts (H.E.-staining, 130x)
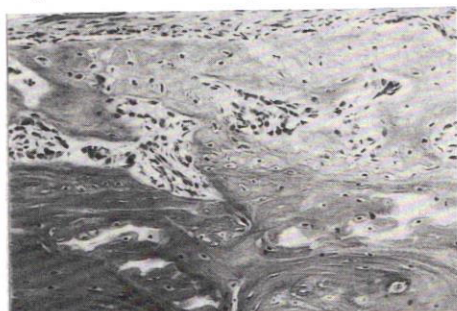

Figure 6. shows an area of subperiosteal osteogenesis in cortical bone (Goldner's staining, 130x).

On the basis of histomorphometric examinations of tibia the results of Ipriflavone treatment are the following (see Table I. and II.)

Table I.
M e t a p h y s i s

	Control	Immobil.	Immobil.+drug treatment
Sv	2915+198	2195+138	2741 + 194
Vv%	22+ 1	15+ 1	18 + 2
OB%	52+ 8	27+ 3	35 + 2

Sv mm^2/cm^3 = trabecular surface,
Vv% = trabecular volume, OB% = osteoid interface.

Table II.
D i a p h y s i s

	Control	Immobil.	Immobil.+drug treatment
Sv	1616+48	1126+66	1662 + 91
Vv%	12+ 1	7+0.4	13 + 1
Oc-index	5+ 1	31+ 1	6 + 2

OC index = osteoclast index

The decrease in density and volume of spongy trabecules was less apparent in both meta- and diaphysis, in the metaphysis osteoblastic activity was considerably enhanced; bone resorption and cortical thinning were decreased. The Ipriflavone treatment did not prevent the development of osteoporosis but significantly diminished it.

This pathogenesis of trabecular bone loss in osteoporosis is still the subject of numerous controversies. The mechanismus leading to bone loss have been successively attributed to an decreased formation. Mechanical loading

perhaps acting through a direct or an indirect stimulation of osteoblasts thereby producing a positive remodeling balance may be important in attainment of bone mass and in subsequent rates of loss. According to our present investigations the Ipriflavone treatment decreased the bone alterations produced by immobilization. Recent results indicate that in osteoporosis produced by streptozotocin, glucocorticoid treatment or ovarectomy Ipriflavone markedly suppresses bone resorption (9, 10, 11). It has been suggested that calcitonin release from the thyroid gland is stimulated by estrogen and Ipriflavone enhanced the action of estrogen stimulated calcitonin secretion (12). Our experimental results yield a hopeful possibility in the treatment of osteoporosis. - Full details will be published in Acta Morphol. Hung. (13).

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THE SIMULATION OF THERMAL MICROCLIMATE IN THE GARMENT SIMILAR TO THOSE OBSERVED IN THE WEIGHTLESSNESS

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Deviation of thermal conditions in
the chest region and the shin different
from those observed in the real weight-
lessness are described in the experiment
with the HDT body position -12° which is
often used on earth for the simulation
of weightlessness on the cardiovascular
system. The metodical approach for the
possible improvement of this inadequacy
is discussed.

Introduction

In weightlessness, by the almost ab-
sence of the gravitation force condition-
ing on the earth surface the thermo-
boyance of the heated air, increases the
thermal insulation of the surface layer
of the air attached to the surface of all
bodies (2). Increased thermal insulation
of the air's surface layer impedes the
heat transfer from the body by means of
the spontaneous convection. As demonstra-
ted in the experiment Heat exchange-2
accomplished on board of the Soviet orbi-
tal laboratory Salyut-6 by members of
the Interkosmos crews V. Remek (CSSR),
A. Gubarev (USSR), M. Hermaszewski (PLR)
and P. Klimuk (USSR) there are signifi-
cant differences between the values of
the skin temperatures obtained under the
terrestrial conditions and in the weight-
lessness.

The values of the skin temperature
in weightlessness are in a dressed man
almost of 2 to 3°C higher in the chest
region and about 2°C lower in extremities
than in the similar conditions in the
laboratory on earth surface (3). For the
simulation of the influence of weight-
lessness on the heart and the blood cir-
culatory system by means of the head-down
tilt body position (HDT) applied in the
laboratories on earth it follows, that
the experimental conditions should be
completed also by the adequate changes
of the heat output. To verify the reality
we examined the question: what are the
values of the skin temperature and the
heat transfer conditions in man under
HDT body position?

Methods

Experiment was carried out in a
thermal chamber with the air temperatures
of 28 and 40,5°C. Seven trained healthy
man were dressed in a pilot clothing ven-
tilated with the volume rate of 125±5
l p m of air.

The skin temperature was measured in
5 spots together with the rectal tempera-
ture and heart rate in a five minutes
intervals. The local conditions of the
heat transfer by convection were measured
on the chest and the shin by means of
two miniprobes of the electric dynamic
katathermometer, model EDK-IK-2M (1) and
evaluated in the form of the operative
temperature T_o (4). The subjective feel-
ings of thermal state were assessed
according to ASHRAE scale.

The experiment was divided into three
periods. In the first control period (C)
the subject was sitting in the chamber
with the air temperature of 28°C; air
ventilated into the clothing 23°C. At the
beginning of the second period the subject
changed its position to HDT (-12°) and
remained in it until the end of the expe-
riment in the 180 min. In the beginning
of the third period (90-180 min) the tem-
perature of the chamber was elevated to
40,5°C, so that the subject was exposed
to the thermal stress (TS-HDT). The mean
and SD were calculated from data obtained
during the last 30 minutes of each period.
The significance of differences were
examined by means of the t-pair test.

Results

As demonstrated in Fig. 1 the HDT
body position decreases significantly the
heart rate and the rectal temperature
and increasis the temperature of the
forehead and the value of the mean skin
temperature calculated according to
Vittes' formula. From it follows the
significant decrease of the core-skin
thermal gradient and no change in the
longitudinal thermal gradient between the
rectal temperature and the skin tempera-
ture in the shin. The subjective feeling
of thermal state remain unchanged.

The skin temperature on the chest
(Fig. 2) increases significantly and in
general its average values are compatible
with those measured by V. Remek in the
weightlessness. On contrary the skin tem-
perature on the shin remained in the
average unchanged and its absolute value
is close to the values measured by
V. Remek on earth, not to those measured
in weightlessness.

Compatible with the changes of the
skin temperature are the changes of the
operative temperature (Fig. 2). The sig-
nificant steep increase of the under-
clothing operative temperature in the
chest indicates the substantial decrease
of the convective heat output joined
probably with the local changes of the
ventilated air distribution which could
be the sequence of HDT body position.
In the shin region no similar changes
were observed. The reduction of the heat
output by convection in the chest region
is expressed also by the increase of the
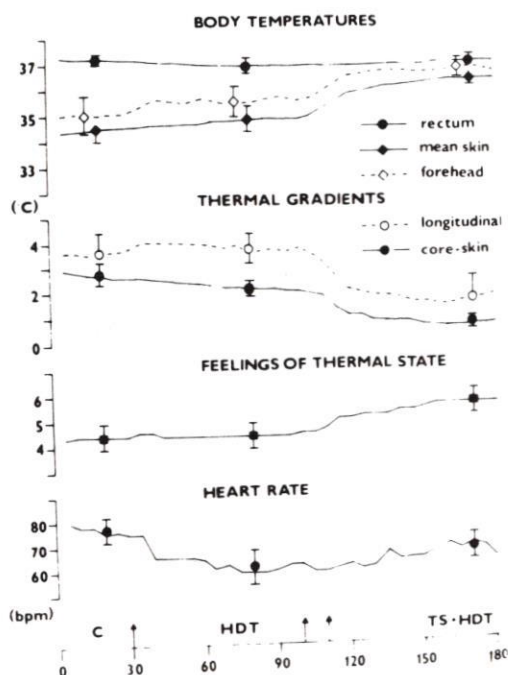
BODY TEMPERATURES

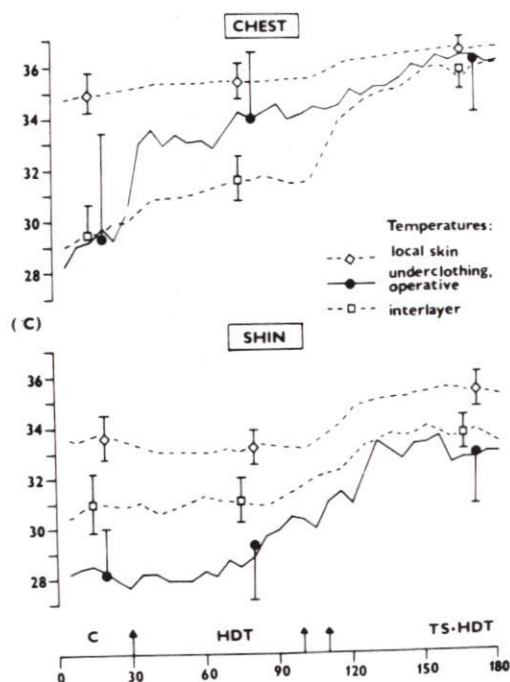Fig. 1 Heart rate and temperature changes

Fig. 2 Operative temperature in the chest and the shin

clothing's interlayer temperature (Fig.2) which however has a smaller difference and a very slow change in comparison with the change of the operative temperature.

The effect of thermal stress in the TS-HDT interval from 120 to 180 minute aggravated in general the heat output of the experimental subject. This unfa-

vorable condition is expressed by the significant increase of the heart rate, the rectal temperature, the temperature of the forehead (Fig. 1) and the increase of the skin temperature in the chest and the shin.

The almost nivelation of operative temperature with the skin temperature and the interlayer temperature in the chest indicates a further dramatic decrease of the heat output by convection (Fig. 2). Similar changes in the increase of the skin temperature, the operative temperature and the interlayer tempera-ture in the shin (Fig. 2) are compatible with the increase of thermal discomfort to the value of 5,6.

Discussion

The increase of the body heat storage of +1,6 kJ/kg during 90 minutes indicate that the used pilot clothing ventilated by the air tempered 26,5°C could maintain thermal equlibrium even during the applied thermal stress. However at the price of the increased circulatory strain and the middle level of thermal discomfort. No dissociation however of the skin temperatures in the chest and the shin similar to those in the weightlessness was observed.

We may conclude therefore, that for the better simulation of the effects of weightlessness on the cardiovascular system by means of the HDT-body position in the lab on earth, it wold be desirable from the point of wiew of the convective heat output in the chest region and the extremities, the additional ventilatory heating or cooling controled by means of the EDK minisensors.

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Stability of cell polarity under various gravitational forces

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Application of centrifugal forces (10^3 g) to growing cress roots causes separation of lipid droplets and protein vacuoles in meristematic cells and loss of structural polarity in statocytes. Cell division, however, is not affected. The original structural situation in statocytes is restored under 1 g conditions after some minutes, in meristematic cells after several hours. Division of meristematic cells and structural polarity in statocytes is, in general, not affected by hypogravity conditions ($< 10^{-3}$ g). There are, however, remarkable effects with lipid droplets, protein vacuoles, starch grains and ER membranes in meristematic cells and statocytes respectively. The results demonstrate the high stability of cell polarity in a wide range of gravitational forces. Furthermore, they show that hypogravity conditions are more effective in plant cells than hypergravity conditions are.

Introduction

The establishment of cell polarity is one of the most important steps during the development of organ polarity and asymmetry. Up to now it is widely unknown which molecular mechanisms are involved in this process. It is even unclear how far and to what extend internal and/or external factors influence cell polarity. From zygotes of the brown alga Fucus for example it is well known that cell polarity can be induced by unidirectional illumination, at least during some hours after fertilization. One of the first measurable responses after onset of illumination is an external asymmetrical current pattern of K^+ ions (for a review see 9). Higher plants are characterized by an extreme polar organization of their organs. In connection with this polar organization roots and shoots are able to perform gravity controlled growth. In these organs meristematic cells are the basis for different cell types, like statocytes or parenchyma cells etc. which are polarly organized. This polar organization goes probably back to the state of early embryogenesis. If the external factor gravity interferes with this process of cell and organ polarization is unclear. This paper investigates the stability of cell polarity under various gravitational forces during the development of meristematic cells from cress roots.

Material and methods

Dry seeds from cress (Lepidium sativum L.) were activated on Earth and accelerated at 10^3 g (hypergravity conditions) or they were activated in orbit and cultivated at gravitational forces $<10^{-3}$ g (hypogravity conditions). The samples were chemically fixed for electron microscopy on the centrifuge and in orbit respectively (for electron microscopy of cress roots see 6).

Results and discussion

1. Meristematic cells and statocytes under 1 g conditions

Meristematic cells of plant roots produce a number of different cell types. In connection with gravity controlled growth, statocytes as the site of graviperception and parenchyma cells as probable site of graviresponse are the most important ones. In meristematic cells (Fig. 1a) the nucleus is localized in the center of the cell. Other organelles like proplastids, endoplasmic reticulum, dictyosomes, mitochondria, protein vacuoles, and lipid droplets are randomly distributed in the peripheral cytoplasm. Statocytes (Fig. 1b) are characterized by a polar arrangement of their cell organelles. The nucleus is always located at the proximal cell pole whereas a system of endoplasmic reticulum (ER) is visible at the distal pole of the cell. Starch filled statoliths (amyloplasts) are sedimented on this membrane system. Dictyosomes, mitochondria and small vacuoles are mainly observed in the center of the cell. Lipid droplets are mostly in close connection to ER membranes (for a review compare 7). Both cell types meristematic cells and statocytes were investigated under different gravitational forces.

2. Meristematic cells
2.1 Hypergravity conditions

12 h after activation cress seedlings were accelerated for 18 h at 10^3 g in axial direction of the root. By this procedure the storage material, i.e. lipid droplets and protein vacuoles, in meristematic cells are significantly affected. Lipid droplets are floating at the centripetal cell pole whereas protein vacuoles are sedimented mainly in the centrifugal part of the cell (Fig. 2, upper cell). With other cell organelles the situation is not as clearly pronounced. However, mitochondria and ER membranes show a tendency for floating, too. Acceleration at 10^3 g has no influence on formation, orientation and location of the new cell wall (Fig. 2, lower cells). This result indicates that the process of cell divi-

sion, i.e. caryokinesis and cytokinesis, is not influenced by hypergravity but it is strictly controlled by internal mechanisms. Furthermore, it suggests that cytoskeletal elements like microfilaments and microtubules which are involved in the process of cell division are not affected by hypergravitational forces.

The result of cell division during accelerations at 10^3 g are two cell types which are completely different in their content of organelles. The centripetal daughter cell is rich in lipids, mitochondria and ER membranes whereas the centrifugal daughter cell is especially rich in protein vacuoles (Fig. 2, lower cells). If roots, after this treatment, grow for 24 h under 1 g conditions further cell divisions occur and as a result meristematic cells show a distinct pattern of 2 "lipid cells" followed by 2 "protein cells" and so on. In spite of these significant differences at the structural level this pattern has disappeared after 4 to 5 cell divisions. This again proves the extreme stability of plant cells toward hypergravitational forces (compare also 1). In connection with this stability plasmodesmata probably play an important role. The frequency of these intercellular plasmatic connections is particularly high in axial direction of the root (4).

2.2 Hypogravity conditions

Dry seeds from cress were activated in orbit (spacelab mission D1) cultivated for 26 h in a humid chamber and were than chemically fixed (compare also 8). It becomes evident that cell division, especially formation, orientation and location of the new cell wall is in general not affected by hypogravity conditions (Fig. 3a). The number of irregular cell plate formation, however, are remarkably high. Sometimes the orientation of the new cell wall is changed by 90°, a situation which has never been observed under 1 g (Fig. 3b) and hypergravity conditions. Between 2 and 3 % irregular oriented cell walls were reported for microspores from Tradescantia if they are activated in orbit. Cell wall orientation has changed exclusively by 90° (2). These results indicate that hypogravitational forces interfere more effectively with the process of cell division than hypergravitational forces do.

Furthermore, small lipid droplets show a clear tendency to confluence to one or two large droplets. This situation has never been observed under hypergravity and very seldom under 1 g conditions. This result indicates changes in the hydrophobic/hydrophilic interface by hypogravitational forces.

Finally the mobilization of storage proteins from protein vacuoles is obviously enhanced. This is indicated by a higher number of small vacuoles in comparison to 1 g control samples.

3. Statocytes
3.1 Hypergravity conditions

Cress roots were cultivated for 24 h under 1 g conditions. Then roots were accelerated for 20 min at 10^3 g and chemically fixed during centrifugation. After this procedure the polar arrangement of cell organelles as it was described previously is completely lost (compare 5). The position of ER membranes has changed from the distal cell pole to longitudinal cell walls. The nucleus is located in the distal part of the cell. The statoliths (amyloplasts) are sedimented close to the distal plasma membrane. The position of other cell organelles like mitochondria, dictyosomes, and vacuoles is not influenced by this treatment. If roots of this structural characteristic are further cultivated under 1 g conditions reestablishment of the polar organization starts approx. 7 min later and it is completed after 20 min in 1 g environment. This reestablishment of cell polarity, however, is prevented by the additional treatment of the roots with drugs like cytochalasin. From these results it has been concluded that microfilaments play an important role in the establishment of cell polarity in statocytes (10; compare also 3). Similar results were obtained with lower values of acceleration between 10 and 100 g (10). These results prove that hypergravity forces affect cell polarity in root statocytes drastically. The effects, however, are reversible under 1 g conditions. Thus one can conclude that cell polarity in statocytes is distinguished by a high plasticity probably due to the cytoskeleton of the cells.

3.2 Hypogravity conditions

The polar arrangement of cell organelles is principally realized under hypogravity conditions (compare 8). The nucleus is positioned at the proximal cell pole, ER membranes are located at the distal pole. Statoliths are not sedimented but distributed at random as it was expected. This result demonstrates that structural polarity in statocytes is determined by internal factors. Hypogravitational forces, however, cause remarkable modifications on amyloplasts and on ER membranes. Amyloplasts show a more roundish shape, starch grains are less clearly pronounced and smaller, the amount of membranes within the statoliths is increased. On the other hand ER membranes are running less parallel, they are more loosened. The overall quantity of ER membranes is increased. These results indicate again that hypogravity shows a more pronounced influence on cell structures than hypergravity does.

Conclusion

- Polarity of cells from cress roots show a high stability toward gravitational forces in the wide range between 10^{-3} and 10^3 g.

- cytoskeletal elements like micro-filaments and microtubules play an important role in the establishment of cell polarity.

- hypogravitational forces are more effective on cell structures than hypergravitational ones. Especially lipid droplets, protein vacuoles, and starch grains are affected by hypogravity. This might be important for further developmental steps of plants in a hypogravity environment.

- at present it is completely unclear whether the influence of hypogravity affects the plant cell at the membraneous, the cytoskeletal, the metabolic, the energetic or the regulatory level.
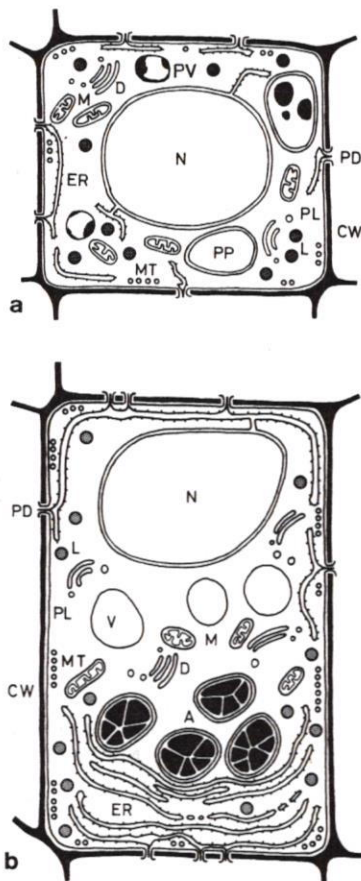
Fig. 1a and b. Schemes of a meristematic cell (a) and a statocyte (b). In meristematic cells organelles are randomly distributed except for the nucleus which is localized in the center of the cell. Statocytes show a polar arrangement of cell organelles. N=nucleus, ER=endoplasmic reticulum, A=amyloplast (statolith), PP=proplastid, PV=protein vacuole, L=lipid droplet, M=mitochondrium, D=dictyosome, PL=plasma membrane, PD=plasmodesmos, MT=microtubule.
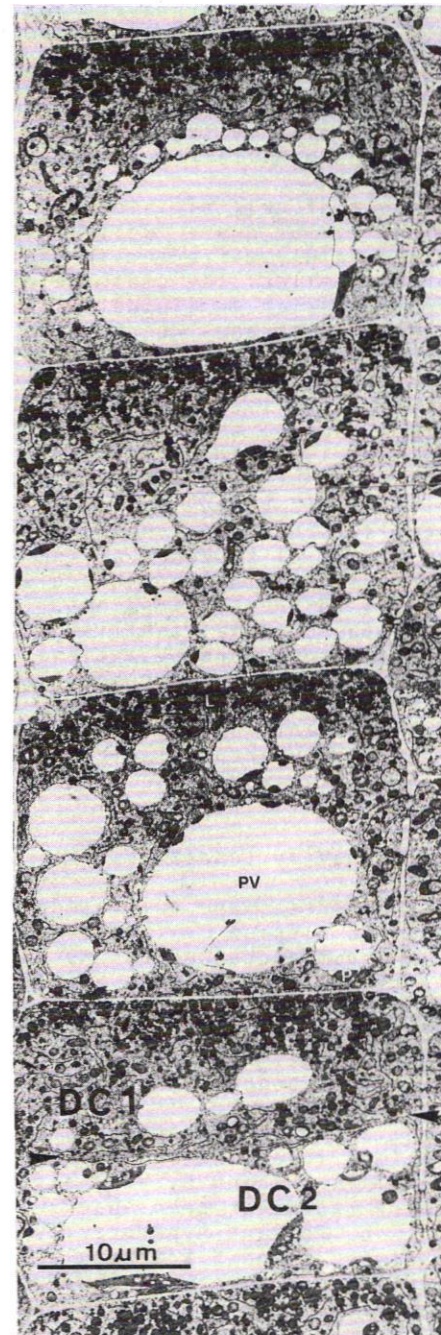
Fig. 2. Meristematic cells after 18 h of centrifugation at 10^3 g in axial direction of the root. Acceleration causes a separation of the storage material. Lipid droplets are floating in the centripetal part of the cell, protein vacuoles (PV) are sedimented in the centrifugal part. Cell wall formation (arrows) is not affected by hypogravity. The results of cell division during acceleration are two cell types. One daughter cell (DC1) is rich in lipids, the other daughter cell (DC2) is rich in proteins.

Fig. 3a and b. Meristematic cells from cress roots cultivated under hypogravity ($<10^{-3}$ g, a) and 1 g (b) conditions. - Cell division is principally the same under both conditions. The number of irregular cell plate formations are higher under hypogravity. Furthermore, small lipid droplets show the tendency to confluence to large droplets (L). The mobilization of proteins from protein vacuoles (PV) is enhanced under hypogravity conditions.

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GROUND-BORNE METHODS AND RESULTS IN
GRAVITATIONAL CELL BIOLOGY

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Dedicated to Prof. Dr. Karl-Ernst Wohlfarth-
Bottermann, Bonn, FRG, on the occasion of his 65th
birthday

Some minimal physical principles of g-effects on cells

When considering the possibilities existing in a
laboratory on the ground to study the effects of
gravity on cell structure and function it seems
useful to define some minimal physical principles
for interactions between gravity and the cell. In
earlier papers, I have proposed to use the term
"smallest functional units" (SFUs (1, 2, 3)). For
understanding gravity effects in organisms the
application of SFUs has an heuristic value, even in
ecophysiology (2, 3). We can speak about a direct
gravity effect if the interacting mass or the
primary receptor is located inside an SFU(type I);
we can speak about an indirect effect of gravity if
the interacting mass of the organism is located
outside an SFU (type II). This definition can be
used for analyzing gravity effects in multicellular
and cellular systems as well; here I would like to
concentrate on single cells where I have defined
the respective SFUs as "infra-SFUs" (ISUF I, II;
(3); see Fig.1). A cell may be deformed or flatten-
ed by its own weight; I will call this phenomenon a
direct effect on the shape of the cell (shape taken
as a special SFU (3)). This reaction may be an ac-
tive or a passive one. If the cell would try to
compensate the flattening effect, it would have to
measure mechanical forces and would react actively
with the aid of its SFUs II (discussion of an exam-
ple in (4)). The basis of the reaction may be the
cytoskeleton together with other functional ele-
ments of the cell. Without using a specialized g-
receptor, the cell theoretically has - after a cer-
tain computation - information about magnitude and
direction of gravity! - Real gravireceptors can be
found in certain free-living cells of the family
Loxodidae (ciliates); according to the definition
these receptors are ISFUs I (see Fig. 1). On the
other hand, there may exist also unspecialized or
unproved specialized SFUs I for active g-reactions
(geotaxis in Physarum (4), or the centriole (2,5)).
- Sometimes the discrimination between an active
or passive reaction will be difficult. Therefore, I
would like to propose the following definition: An
active reaction is the attempt of the cell to re-
gain a disturbed (actual) value of a regulated
structure or function (with feedback, second order
regulation). In a passive reaction, the cell is not
able to make a correction of a changed or disturbed
actual value of a regulated structure or function
(without feedback, first order regulation), also

Cell morphological units (membranes, cytoskeleton) of the ISFU type I and II with feedback (second order) regulation
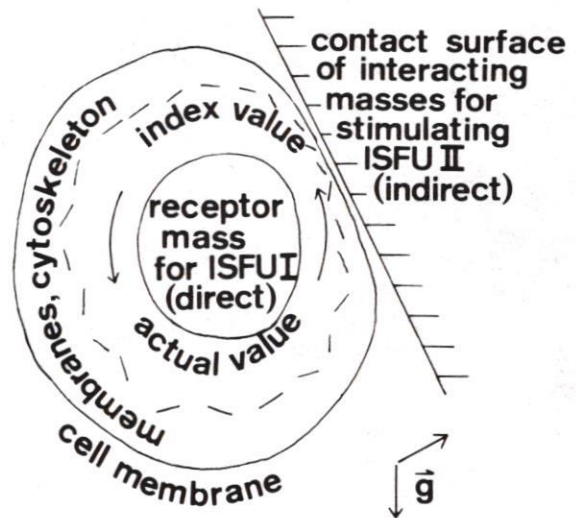
Fig. 1: Scheme of a cell which may perceive directly
or indirectly the force of g. The g-inter-
acting masses may be located inside (direct
influence) or outside (indirect influence)
of the sensitive cell matrix. For direct
effects the primary receptor mass has to be
more dense than the rest of the cell,
whereas for indirect effects the density of
the interacting mass is of no importance.
In both cases the sensitive matrix may be
an actively regulated configuration of the
cytoskeleton/membrane system which "feels"
mechanical stress. If the cell compensates
such a stress it simultaneously has infor-
mation about direction and magnitude of
gravity. See also (16).

because the cell will get no information of that
disturbation.

There may be also direct or indirect effects of
gravity on the "milieu externe" (environment) of a
cell. Examples for such environmental direct and
indirect effects on cells suspended in a liquid
medium are: density convections in the vicinity of
cells caused by metabolic processes (direct effect
on the environment), hydrostatic pressure, and
stratification in the liquid medium (indirect
effects on the environment) (3).

In addition to the interaction of gravity with SFUs
(I and II) two other minimal physical principles
are of interest:
- the relative incompressibility of liquids and
cells by weak hydrostatic pressures; this results
in a relative weightlessness at least for the
shape of suspended cells;
- the existence of a threshold for an acceleration
sensitivity of every imaginable abiotic process
and regulated function in a living cell. In cells,
acceleration influences which can only be effec-
tive on dense particles (including ions), organ-
elles, and compartments, have to be taken into

account. Stoke's law, (describing the scale effect in the microscopic range!) and Brownian movement of such dense elements allow the calculation of threshold values, below which there exists a functional 0g-state (6, 7). For the mechanics of small elements of the cell this threshold value may be in the range of 1g or even higher (8).

EARTH-BORNE METHODS FOR ANALYZING G-EFFECTS

Bearing in mind the above-mentioned minimal physical principles of g-effects it is relatively easy to define cell experiments which may reveal or may be helpful in analyzing g- or 0g-effects. Theoretically, gravity may enhance or inhibit different cell functions. Therefore, by changing the influence of g, beneficial or detrimental effects should be observed. At least two different methods of changing the g-influence on cells can be used:

- Variation of the hydrostatic pressure (Veldhuizen (9): Reactions of bone cells;)

- Variation of the direction of the g-vector or increasing its magnitude.

Concerning the last point a system of experimental conditions may be established (cf. Fig.2):

A. Observation of cell cultures on free surfaces in lying, hanging or vertical arrangements.
   1. Observation of cells on a horizontally positioned substratum before and after an upside-down turn; a vertically tiltable light microscope will be useful (Todd, cited in (9): Orientation of the mitotic apparatus; Block et al. (8): Change of contraction rhythm in Physarum).
   2. Observation of surface cultures on a vertically positioned substratum during its turning - performed in intervals - about a horizontal axis; this can also be performed by using a horizontal microscope, (Wolke et al., cited in (4): Geotaxis of Physarum).

B. Observation of freely swimming autonomously moving cells.
   1. Cells contained in a vertically tiltable test tube with isotropic conditions excluding gravity (Jennings, cited in (14): Geotaxis in Paramecia).
   2. Use of a horizontal microscope and its turning in intervals about its optical axis (Fenchel et al., cited in (14): Geotaxis in Loxodes).

C. Investigation of correlated gravi- and phototaxes by adjusting an organism - possibly with the aid of a third taxis (rheotaxis) - to a vertical position. During stimulation with light from alternating directions in a horizontal plane, geo-and phototaxes are decoupled.
   1. Experiments conducted with fish (von Holst, cited in (10): Dorsal-light reaction; Seibt (10): Circadian rhythm of performance).
   2. Attempt to apply this principle to ciliates (and Physarum).

D. Experiments performed on continuously rotating platforms.
   1. Platforms with vertically positioned wheels.
      a. Plant seedlings on a horizontally positioned disk (Knight 1806: Proof of gravitropism).
      b. Cells attached to a horizontally posi-

GRAVITY RELATED EXPERIMENTS PERFORMED ON THE GROUND

Turns in intervals of the specimen and/or substratum
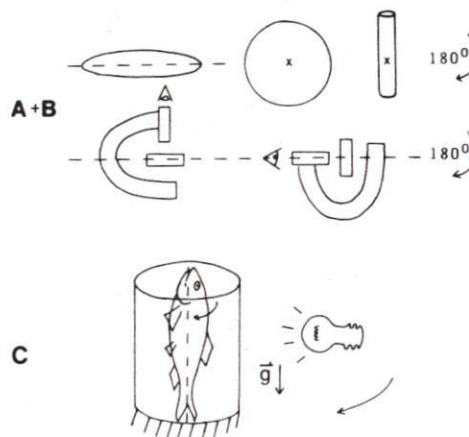
Steady turns of the specimen and substratum
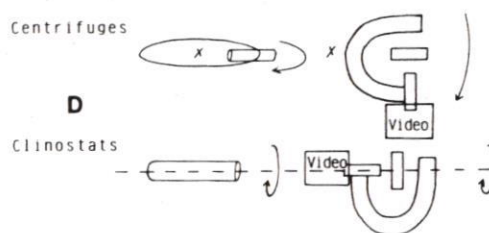
Fig. 2: Scheme of possible variations of the influence of outer accelerations on g-sensitive (cellular) systems. An exception is shown in C where there is no relative movement of the g-vector with respect to a g-sensitive turning system. See also text!

tioned disk (free surface) or suspended in a cuvette; investigation of a preference behavior of motile cells within the acceleration gradient (Wohlfarth-Bottermann (11): Experiments with Physarum). Combination with 2.!
      c. Conventional cuvette centrifuges (Schatz et al., cited in (2): High g-tolerance of embryogenesis of Ascaris; Cogoli (12): Lymphocyte activation).
      d. Low and high speed centrifuge microscopes (Kuroda et al.,cited in (14): Geotaxis of Paramecia; Moroz (13), Briegleb: Technical proposals).

   2. Platforms with horizontally positioned wheels = "Clinostats"
      a. "Slow-rotating clinostats" (Pfeffer 1897: Elimination of gravitropism; Hensel et al., cited in (8): Destruction of statocytes).
      b. "Fast- or rapidly-rotating clinostats" (Muller, cited in (7): 0g-simulation for the vestibular function of the human; Briegleb et al. (1): Clinostat microscopy, amphibian embryogenesis, genetics of insects; Block et al. (8): G-sensitivity of Physarum; Hemmersbach et al. (14): G-sensitivity of Paramecium; Cogoli (12): Inhibition of lymphocyte

activation; Sobick, cited in (2, 4):
Threshold for geotropism and correlated
cell structure; shortening of mitotic
phases).
  c. Clinostats with several wheels (Gordon
     et al., cited in (15).
3. Combinations of D.1. and D.2. (Gordon et al.,
   (15): Threshold of geotropism).

Out of the above-mentioned techniques or methods
the tiltable or turnable microscopes need the
greatest exertion. To date it seems that world-wide
a tiltable vertical microscope does not exist;
until now not a single centrifuge microscope seems
to exist in Europe! For preparing cell experiments
in space and for performing them in space low-speed
centrifuge microscopes appear to be extremely
necessary. I have proposed the construction of a
low-speed centrifuge microscope to the German
space authority. In my laboratory two clinostat
microscopes and different cuvette- or test tube-
clinostats have been constructed which are working
well since several years.

The fast-rotating clinostat

Because of the great interest the fast-rotating
clinostat has gained today, I would like to give
- in qualitative terms - a short survey of its
physical principles and its applications; the re-
spective mathematical calculations were made by
Schatz (7) and Silver, cited in (8). I would like
to start with the following statement:

Under ideal conditions the clinostat produces a
condition of perfect functional weightlessness.

How do I define functional weightlessness? It is
achieved, when an observed function behaves theore-
tically and by observation in the same manner as
under real weightlessness. - Here I have to make
two assumptions: In a living cell, the influence of
gravity only leads to a certain relative displace-
ment of different dense particles or compartments,
which may result in pressures, tensions, or a
changed topology. Both practice and calculations
demonstrate that the fast-rotating clinostat can
prevent those dislocations which otherwise would be
above a threshold of perception. As already men-
tioned every imaginable process occurring within a
cell has an acceleration threshold given by the
thermal activity of its cytosol. - A second assump-
tion is made: A functional 0g-state on the clino-
stat is only achieved, if weak hydrostatic pres-
sures will not influence cell function. Then, also
the shape of a submerged cell is not influenced by
gravity. Now, if there is any effect of gravity on
the distribution of dense particles inside the cell
above the threshold given by the thermal activity
of the cytosol, the clinostat will eliminate this
effect.

How does the clinostat work? The main precondi-
tion for the functioning of the clinostat is that
the observed system can be described as a suspen-
sion. The contents of a cell may also be described
as a suspension. If a suspension is kept in a stiff
and hermetically closed container and is rotated
about a horizontally positioned axis, then parti-
cles with a density higher than that of the liquid
of the suspension will start to move and will
follow the g-vector. If the density differences
between the particles and the liquid are small and
the liquid has a certain viscosity, then, at a low
revolution speed of the clinostat, gravity will
force the sedimenting particles on circular paths.
If the rotation speed increases, the diameters of

those circles will approach zero. Simultaneously,
the new unidirectional or radial vector of the
centrifugal force will drive the particles to the
periphery of the container. This force, which de-
pends on the speed of rotation and the distance of
the particles from the rotation axis, may be smal-
ler than 1g at the periphery of the container and
- at any speed - it will tend to go to zero near
the center of rotation. So, in a certain area
which depends on the physical characters of the
suspension and the rotation speed, the centrifugal
forces will be much smaller than the forces of the
thermal activity of the liquid. In such an area, no
conceivable g-sensitive processes, may they be
abiotic or biotic, can be affected by gravity or
can "perceive" a gravity effect. Consequently, the
diameter of a rotating cylinder, in which these
conditions are fulfilled, depends on the accelera-
tion sensitivity of the observed system. For cellu-
lar systems, which have no morphologic specializa-
tion in this respect, the diameter of the cylinder
will be a few millimeters (8). For specimens with
specialized g-receptors, e.g. embryos of verte-
brates, the 0g-simulation on a fast-rotating
clinostat is rather imperfect, but tiny nematodes
may be of interest. On the contrary, nearly every
kind of cell culture can be examined in thin tubes
on the fast-rotating clinostat.

For examinations of cells in the clinostat micro-
scope we have developed special chambers which
allow nearly all kinds of observations under condi-
tions of functional weightlessness just as in the
normal vertically positioned microscope. - With the
aid of the test-tube clinostat as well as with the
clinostat microscope we have found certain changes
of functions in different types of cells (1, 4, 8,
14). Most of these effects were not detrimental and
in case comparable experiments were performed in
space, the effects were of the same quality,
e.g. (12).

Two typical objections repeatedly are brought for-
ward against the fast-rotating clinostat:
- Sedimenting particles should be accelerated in
  sinusoidal intervals! This is not the case as
  long as the particles sediment with a steady
  speed within the liquid of the suspension. Only
  if the particles still move on circular paths
  (slow rotating clinostat) and if they move near
  the wall of the cuvette a sinusoidal acting force
  produced by superposition of gravity and the
  centrifugal force is pressing them to the wall.
- Cells in suspension will not rotate with the same
  velocity as the liquid! This is only true for a
  fraction of a second after the clinostat has
  reached its final rotation speed; this situation
  can be a little bit changed depending on the
  density of the cells and the liquid.

Conclusion: The fast-rotating clinostat has - for
physical reasons - the same exceptional position
for investigating certain abiotic and biotic speci-
mens as a real space flight. Gravisensitivities of
cells may be revealed by using the methods in the
above-mentioned system of methods including the
slow-rotating clinostat; but the behavior of cells
in (functional) weightlessness can only be investi-
gated on the fast-rotating clinostat or in space.
Gravisensitivities detected with methods other than
these latter ones have nothing to do with a pos-
sible g-dependency of cell functions, in case the
cell may function quite normally in weightlessness.
- For investigating the effects of g on evolution
of cells only flown and experiments on the fast-
rotating clinostat are useful.

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DOES VECTOR-FREE GRAVITY SIMULATE MICROGRAVITY? FUNCTIONAL AND MORPHOLOGIC ATTRIBUTES OF CLINOROTATED NERVE AND MUSCLE GROWN IN CELL CULTURE

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Cocultured Xenopus neurons and myocytes were subjected to non-vectorial gravity by **clinostat rotation** to determine if microgravity, during space flights, may affect cell development and communications. Clinorotated cells showed changes consistent with the hypothesis that cell differentiation, in microgravity, is altered by interference with cytoskeleton-related mechanisms. We found: increases in the myocyte and its nuclear area, "fragmentation" of nucleoli, appearance of neuritic "aneurysms", decreased growth in the presence of "trophic" factors, and decreased yolk utilization. The effects were most notable at 1-10 rpm and depended on the onset and duration of rotation. Some parameters returned to near control values within 48 hrs after cessation of rotation. Cells from cultures rotated at higher speeds (>50 rpm) appeared comparable to controls. Compensation by centrifugal forces may account for this finding. Our data are consistent, in principle, with effects on other, flighted cells and suggest that "vector-free" gravity may simulate certain aspects of microgravity. The distribution of acetylcholine receptor aggregates, on myocytes, was also altered. This indicates that brain development, in microgravity, may also be affected.

Introduction

Xenopus nerve and muscle cocultures were grown under conditions of vector-free gravity by use of clinostat rotation. Horizontal rotation, of cultured cells, resembles microgravity encountered in space because of the cancellation of the vector of gravity consequent to continuous averaging. Thus, the substrate-attached cell surface, normally exposed to the unidirectional force of gravity, now experiences this force as a vector-free field. We used this preparation to determine whether this simulation of microgravity might affect cellular development and cell-cell communications between neurons and their target cells.

Our studies show that clinorotation is associated with significant changes in cytomorphometric attributes of embryonic spinal neurons and myocytes. Comparison of these results to published data on other cell types, after exposure to real microgravity in space flights (e.g. Cogoli, Planel, Briegleb, Montgomery, Talas), reveal many similarities and are consistent with our working hypothesis that exposure of developing cells to microgravity alters their development by interfering with intra- and trans-cellular mechanisms which may be mediated via gravitational perturbations of the cytoskeleton.

Materials and Methods:

Nerve and muscle cells were isolated from Xenopus larva at stages 17-21 and grown in culture as previously described (Anderson & Cohen, 1979; Gruener et al. 1980; Gruener, 1985). Cells were allowed to attach to collagen-coated glass coverslips, which comprise the floors of the chambers, for 4-6 hours prior to clinorotation. Cells were grown in plating medium containing serum or in defined medium (Lechleiter & Gruener, 1985) with and without supplementation with trophic substances [embryo extract (Harris et al., 1985); laminin (Manthrope et al., 1983; Lander et al., 1985)]. Microgravitational simulation is carried out using clinorotation about the horizontal plane at speeds of ranging from 1 to 100 rpm. Cells rotated parallel to the vector of gravity were indistinguishable from stationary controls, and served as motional and vibrational controls. Exposure to clinorotation lasted from 16-60 hours. This time is sufficient to allow cells to proceed through their ontogenetic maturation in culture, produce striations or neurites and develop functional synaptic contacts (Kidokoro & Gruener, 1979). Cells are observed immediately after removal from the clinostat using phase-contrast video microscopy. Data were obtained from at least 120 experimental runs and sample size, for a given condition, exceeded 600 for morphometric analysis and 80 for assessment of synaptic contact formation. To determine whether development in vector-free gravity alters synaptic developments the following regime was used. Six hours after seeding of the myocytes on collagenated coverslips, dissociated spinal neurons were added and allowed to produce neurites for an additional 6 hours. At this time, neuritic growth was normally not sufficient to result in nerve-muscle cell contact. The sealed culture dishes were then mounted in the holder of the clinostat and rotated at either 1 or 10 rpm for a period of 24-60 hrs. Because these cells are richly endowed with yolk platelets, which provide the cells with metabolic energy, cell growth, maturation and functional interactions take place under virtual anaerobic conditions at room temperature. After rotation, cultures were incubated with rhodamine-labeled α-bungarotoxin (Collaborative Research, Eugene, OR). Acetylcholine receptor aggregates, in hot spots and in nerve-induced patches, were visualized by fluorescence microscopy. Quantitative morphometry was accomplished by use of computer-assisted software (Bioquant[R]; R & M Biometrics, Nashville, TN). Statistical significance was assessed by single and multi-variate ANOVA and by Student-t tests.

Results

The following changes were consistently observed subsequent to clinorotation: increase in cell and nuclear area, "fragmentation" of nucleoli, appearance of neuritic "aneurysms", decreased neurite growth despite the presence of "trophic" factors, and decreased utilization of yolk. We also found that clinorotation of non-innervated muscle cells is associated with a marked reduction in the presence of fluorescently labeled α-bungarotoxin sites. In addition, innervated myocytes were only sparsely labeled with this probe for the Acetylcholine receptor, at the contact region between the nerve and the myocytes.

These effects were most pronounced at low rotation rates (1-10 rpm), and depended on the time of onset and the duration of rotation. Interestingly, some parameters appeared to return toward control values within 48 hrs after cessation of rotation. Table I shows changes in cell attributes after 24 hrs of clinorotation.

Table I: Effects of Clinorotation on Cell Morphometry

	CTL	1rpm	10rpm	50rpm	
Myo Area	3673	4051	4418	3630	μm^2
Nuc Area	307	337	376	302	μm^2
Ncl Area	36	41	44	35	μm^2
Nrt Lngt	134	96	105	94	μm
Anr Freq	8	21	18	--	#/mm

Myo=myocyte; Nuc=nucleus; Ncl=nucleolar; Nrt=neurite
Anr=aneurysm; Lngt=length; Freq=frequency;
CTL=control

Note that rotation at 50 rpm showed virtually no significant changes in cell attributes when compared to controls. Changes in these cell attributes were linked to the time of clinorotation, at a given speed. For example, table II shows changes in myocyte area as a function of the duration of rotation, and as percent of the time-matched control values.

Table II: Effects of Duration of Rotation on Myocyte Area

	10hrs	20hrs	40hrs	60hrs
1rpm	108	117	121	124
10rpm	140	112	136	128

Data are shown as percent of control values.

Similar trends were observed for other parameters. For example, the frequency of aneurysms (large, irregular varicosities) along neuritic shafts increased from a value of 129% of control after 10hrs rotation to a value of 384% after 40hrs rotation at 1rpm. Addition of trophic factors (e.g. Xenopus embryo extract; laminin, 20μM) did not prevent the changes induced by rotation of cells in the normal plating medium (containing 5% serum alone). In contrast, allowing cultures to recover from rotation by leaving them stationary for various "rest" periods, after cessation of rotation, appeared to permit the return of altered cell attributes toward control values. This effect is shown in table III for myocyte area. Cultures were rotated for 36 hrs and examined after the "rest" period indicated.

Table III: Myocyte Area in Rotated and Rested Cultures

Rest time:	0hrs	48hrs	96hrs
1rpm	117	114	101
10rpm	136	110	108

Cell area expressed as percent of control after 24 hr clinorotation.

In order to assess the ability of myocytes to synthesize acetylcholine receptors (AChRs) and organize their distribution, required steps for the formation of synaptic interactions, we measured the number and area of "hot spots", naturally-occurring small aggregates of AChRs in non-innervated myocytes after clinorotation. We found that hot spot numbers and areas were reduced to 49% and 30% of control values after rotation at 1 and 10 rpm, respectively, for 24 hrs. More significantly, when we examined nerve-contacted myocytes for the presence of AChR patches along the region of contact between the two cells, we found a profound reduction in the presence of synaptic receptors. Thus, 71% of nerve-contacted control myocytes had clearly-identifiable nerve-induced AChR patches. In contrast, only 23% of contacted myocytes from cultures rotated at 1 rpm had detectable nerve-induced patches. Similarly, only 35% of such myocytes, from cultures rotated at 10rpm had nerve-induced patches. A parallel reduction in the area of the AChR patches was also seen. Nerve-induced patch areas, in myocytes from 1rpm and 10 rpm cultures, were reduced to 32% and 41% of control values, respectively.

Discussion

Our results demonstrate that clinorotation, at low speeds, is associated with changes in cell morphometry and in the ability of myocytes to adequately retain synaptic receptors and to accumulate such receptors near the synaptic zone. These changes indicate that cell growth in a vector-free environment alters cell structure and function and its intercellular interactions. If clinorotation is accepted as a faithful model of microgravity, then it is predicted that cellular processes during development may be severely affected consequent to exposure to microgravity. For example, our findings concerned with the distribution of acetylcholine receptor patches in both innervated and non-innervated cells raise the possibility that the ability of muscle cells to insert such receptors in their membranes will be reduced and their ability to respond to neuronal cues to accumulate such receptors at the developing synapse may also be compromised. It is also possible that the capacity of neurons to induce receptor accumulation, to the point of nerve-muscle contact, may also be impaired. It is noteworthy, however, that for these cells, clinorotation at higher speeds (>100rpm) and "resting" of cells after rotation, permits recovery of some of the altered morphologic parameters to near normal values. These findings suggest that the "microgravity-induced" effects, we observed, are reversible. Such findings are consistent with the efficacy of muscle exercise against a load virtually eliminating the atrophy observed in flight crew after prolonged exposure to microgravity in space.

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IN VITRO INTERFERON PRODUCTION BY HUMAN LYMPHOCYTES DURING SPACEFLIGHT

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The experiment was carried out during the Hungarian-Soviet spaceflight and repeated by the Roumanian-Soviet crew aboard the Space Station Salyut-6. Human lymphocytes separated from blood of healthy donors and interferon inducers were placed separately into a special equipment. Interferon induction was carried out by the cosmonaut in the space laboratory. Interferon titers of the flight samples proved to be 4-8 times higher than   those of the synchronous ground controls. Lymphocytes isolated from cosmonauts after spaceflight showed decreased activity.

Introduction

The study was based on the hypothesis that speceflight conditions, i.e. lowgravity, may change the functions of cells and thus modulate interferon production. The effect of complex conditions of spaceflight on interferon production „in vitro" by human lymphocytes isolated from healthy donors was investigated.

Further, the effects of extreme conditions of spaceflight on the interferon producing capacity and natural killer activity of blood lymphocytes isolated from cosmonauts before and after spaceflight were studied. These examinations render a basis to estimate non-specific resistance of the organism in general, and especially to viral infections.

Materials and methods

The equipment „Interferon" was constructed by „Medicor Works". „Interferon" equipment consisted of a small metallic box with a metallic rack where 10 plastic tubes were placed. Eight tubes were provided with head-reservoirs and plungers, two tubes were closed by screw caps. The head-reservoir was separated from the tube by a one-way valve opening into the direction of the tube by rotating the plunger head.

The experiments were performed under spaceflight conditions and on the Earth simultaneously (ground control) using materials of identical origin.

Seven hours before launching the tubes of „Interferon" equipments were filled with human lymphocyte suspensions, and the head-reservoirs with interferon inducers. The flight equipment was transported to space laboratory Solyut-6 by the cosmonauts. Interferon induction was carried out by the cosmonaut in the first hour following arrival to the space laboratory, and synchronously on the ground. Interferon inducers were added to the lymphocyte suspensions by rotating the plunger heads. Then „Interferon" equipments were kept in thermostats at $37^{\circ}C$, in experiment 1 for 6 days, in experiment 2 for 4 days. As the flight thermostat was switched off for 8 hr daily (period of rest of cosmonauts), the ground device was switched off at the same time.

After 6 days aboard Solyut-6 the flight equipment was brought to Earth.

Lymphocyte separation was accomplished according to A.Böyum;(Scand.J.Clin. Lab.Invest.21,Suppl.97,51-76,1986). Cell viability was controlled by the trypan blue exclusion test in a haemocytometer.

The following interferon inducers were applied: polyriboinosinic-polyribocytidylic acid (poly I:C) (Calbiochem)-600

/ug; polyriboguanilic-polyribocytidylic
acid (poly G:C) (Institute of Macromole-
cular Compounds, USSR)-500 /ug; purifed
protein derivative (PPD) of tuberculin
(Institute of Vaccines and Serum control,
Moscow)-500 /ug, and Newcastle Disease Vi-
rus, UV-inactivated (NDV-UV)-$10^{7,3}LD_{50}$/ml.
NDV, Hertfordshire strain, was inactiva-
ted by UV-rays (100 W, 32 cm distance)
for 4 min.

Interferon induction in cosmonauts'
lymphocytes „in vitro" was carried out
as follows: venous blood from the cubital
vein of the cosmonauts was taken twelve
days before spaceflight, and 1 and 6 days
after returning to Earth. A suspension of
$5x10^6$/ml lymphocytes in Parker's medium
containing 2% foetal calf serum was prepa-
red. NDV-UV was used as inducer in a dose
of $10^{6,3}LD_{50}$/100 /ul.

Assay of interferon: antiviral activi-
ty of lymphocyte supernatants was carried
out by the adapted cytopathic effect-in-
hibition test described in detail earlier
(M.Benczur, et al.Clin.Exp.Immunol.39,
657-662,1980).

Natural killer (NK) activity of lympho-
cytes was determined by the method of Ry-
kova et al.(Immunologiya 3,17-21,1981).

Results

Assay of antiviral activity of flight
and ground-control samples showed that in
every tube where inducer was added inter-
feron production could be demonstrated.
Comparing the antiviral activity of flight
samples to that of their corresponding
ground controls, an increased interferon
production in the flight samples could be
observed. Interferon titers of the flight
samples were 4-8 times higher than those
of controls, independently of the inducer
used.
Viability of lymphocytes of flight
samples on the 1st day after landing was
50%, in the synchronous ground controls
70%, in comparison to 98% viability of
cells in suspension before filling the

tubes. These results were confirmed by
the experiment carried out in the second
spaceflight (Soviet-Roumanian).

Interferon production in vitro of lym-
phocytes of cosmonauts was investigated
before and after spaceflight. One day af-
ter landing a decrease of induced inter-
feron production was observed; in samples
of both cosmonauts interferon levels were
found to be markedly lower than before
flight. Six days after return to Earth
one cosmonaut's lymphocytes showed a ten-
dency to normalize interferon production;
almost the same level of interferon pro-
duction as 12 days before flight could be
observed. Lymphocytes of the second cos-
monaut produced low quantities of inter-
feron 6 days after flight; the same level
as 1 day after landing was measured.

Natural killer activity of the cosmo-
nauts' lymphocytes isolated before and
after spaceflight showed individual dif-
ferences. Comparing the natural killer
activity 1 month before orbital flight
and 1 day after landing, an about 4 times
lower value was observed in both pilot's
samples after flight - independently of
the levels before flight.

The experiments performed proved that
human lymphocytes are sensitive biologi-
cal objects to study low gravity effects
on a cellular level. The results obtained
show that factors of cosmic flight inf-
luence interferon production by human
lymphocytes.

CELL BIOPROCESSING IN SPACE: APPLICATIONS OF ANALYTICAL CYTOLOGY.

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Abstract

Cell bioprocessing in space consists of the preparation, cultivation, purification and investigation of cells and their products in the microgravity environment of orbital space flight. Inertial acceleration is used as an independent variable to explore the limits of specific bioprocessing functions, such as cell growth and secretion, gravity-dependent phenomena in cell bioreactors, cell fusion, the influence of thermal convection on processes at cellular dimensions, the electrophoretic separation of cell subpopulations and subcellular particles, and two-phase partitioning of cells, bioparticles, and macromolecules. Analytical cytology techniques are under development for on-orbit application to future cell growth and separation experiments, such as those anticipated in the Space Station era.

Introduction

Cell bioprocessing in space consists of cultivating cells in vitro and separating suspended cells into subpopulations having various functions (see Abstract, above). In both cases, such features as morphology, secretion, viability, metabolism, synthesis, and growth are studied before and after processing.

Review

Eukaryotic cells that have been cultured in space or separated in space have, to date, undergone rather little quantitative evaluation on the ground prior to flight and no evalutaion in space prior to processing. End-point evaluations, often limited, have been performed on the ground after flight, in many cases also after a storage period. In the anticipated cell growth experiments on long-duration orbiting craft, such analyses will have to be done on board. Table 1 lists eukaryotic cell cultivation experiments performed to date showing pre-flight and post-flight analytical end-points. Table 2 lists eukaryotic cell separation experiments performed to date showing type of cell studied and separation experiment objective.

There is a need for on-board cell analytical capabilities that are sufficiently versatile to serve experiments of the types listed in Tables 1 and 2 and of more advanced experiments, such as cell fusion experiments. Methods are therefore under development that

exploit the power of flow cytometry as a technique for rapid, semi-automatic quantitative analysis of large numbers of specimens of suspended eukaryotic cells and bioparticles. Some results of current experiments are therefore presented below and followed by a discussion of in-space cell analysis requirements.

Table 1. Eukaryotic cell cultivation experiments in space.

CELL TYPE	END POINTS	REFERENCE
human diploid fibroblasts	growth, morphology, cytogenetics, glucose consumption	(1)
Paramecium aurelia	growth, morphology, density, metabolism	(2)
human lymphocytes	blastogenic response to mitogen	(3)
human lymphocytes	interferon production	(4)
rat pituitary cells	growth hormone and prolactin release	(5)
human kidney cultures	attachment to beads morphology	(6) (7)

Table 2. Eukaryotic cell separation experiments in space.

CELL TYPE	OBJECTIVE	REFERENCE
erythrocyte mixture	test resolution	(8,9,10)
lymphocytes	test resolution	(8)
lymphocytes	isotachophoresis	(11)
lymphocytes	free flow test	(12)
human kidney	functional tests	(8,13)
canine pancreas	functional separation	(13)
rat pituitary	functional separation	(13)

Materials and Methods

Cells
Human embryonic kidney (HEK) cell cultures and rat anterior pituitary cell suspensions were used in these studies. Culture methods and treatments were as described in earlier publications on cell processing in space experiments (13,14,15).

Cell Separation
Cell separation experiments utilized the Continuous Flow Electrophoresis System (CFES) of McDonnell-Douglas Astronautics Co. (13). In the case of HEK cells fractions were collected into 10 ml teflon bags during Space Shuttle flight STS-8 (13), plated at 30,000 cells/cm^2, and subcultured once before being subjected to analysis by flow cytometry. In the case of rat anterior pituitary cells fractions were collected during ground-based separation and fixed with formalin for staining with antibodies.

Flow Cytometry

All experiments were performed using a Coulter Electronics, Inc. "EPICS V" flow cytometer equipped with a 5-W argon-ion laser tuned to 488 nm and normally operated in the 50-300 mW range. Two-parameter light scattering experiments were performed on living cells suspended by enzymatic digestion as previously described (13,16,17). Collection angles for forward-angle light scatter (FALS) and perpendicular light scatter (PLS) intensity measurements were, respectively 2.5-19 and 70-110 degrees. In addition, the width of the unintegrated PLS signal pulse ("time of flight" (TOF)) was used as an indicator of cell diameter (18). Rat anterior pituitary cells were stained fluorescently with antibodies against growth hormone (GH) or prolactin (PRL) using the method of Hatfield and Hymer (19); in some cases the same cells were stained with propidium iodide to identify them as DNA-containing cells (20).

Results

Functional Staining of HEK Cells

Flow cytometry can be used to identify plasminogen activator containing cells by using a fluorescent staining method developed by Dolbeare and co-workers (21). The fluorogenic substrate CBZ-gly-gly-arg-methoxynaphthylamide (obtained from Enzyme System Products, Inc.) was mixed with HEK cells of strain "1593" (obtained from MA Bioproducts, Inc.). Cleavage of the arginyl amide bond releases 4-methoxy-2-naphthylamine, which can be stimulated to fluoresce by the 488 nm light from the argon ion laser. Fluorescent light was detected through a 590 nm band pass filter. Distributions of cell number vs. fluorescence intensity revealed subpopulations of stained and unstained cells (Figure 1). Unstained cells in control and stained populations had approximately the same intensity distributions (Figure 1, top and bottom panels). Incubation in serum-free "production medium" (13) decreased cell fluorescence, presumably due to the secretion of plasminogen activators in this medium (Figure 1, middle panel).

Light Scattering by Separated HEK Cells

The integrated PLS intensity reflects the internal structure of cells, including granulation, nuclear size, and shape. For example, granular cells scatter more light perpendicularly than do non-granular cells. FALS intensity is a measure of particle size and, to a lesser degree, refractive index. Single-cell suspensions obtained from 7 electrophoretic fractions after one subcultivation from the kidney cell separation experiment on Shuttle flight STS-8 were analysed for their light scattering patterns without fixing or staining. Bivariate histograms were obtained using integrated PLS vs. TOF, integrated PLS vs. FALS, and integrated PLS vs. PLS. Histograms from two such fractions are compared in Figure 2, in which integrated PLS (granularity) and TOF (diameter) is displayed for each cell. The cell fraction on the left has more large, granular cells, while the fraction on the right has more large, non-granular cells.

Light Scattering by Pituitary Cells

Cytoplasmic granulation constitutes a dominant feature of pituitary cells (8), and this feature can be used to advantage when scattering of laser light is used as a probe.

As in the previous section, FALS and PLS intensities were measured for each cell and plotted against each other in contour plots such as that in Figure 3, which is an isometric display of the distribution of two-parameter signals produced by the total mixture of living pituitary cell types. When the ridges marked "A", "B", and "C" were selected by cell sorting and subsequently analysed by antibody staining, it was found that 82% of the "C" cells contained GH, 70% of the "B" cells contained PRL, and the "A" cells were follicular cells, macrophages, or endothelial cells (16,17).
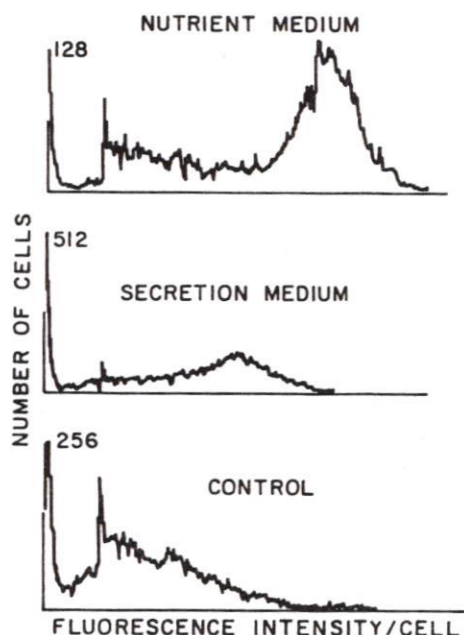
Figure 1. Fluorescence intensity distributions of cultured HEK cells stained for plasminogen activator with fluorogenic substrate after cultivation in complete medium (top panel) or after 3 days in serum-free "production medium" (middle panel), compared wtih unstained cells (bottom panel).
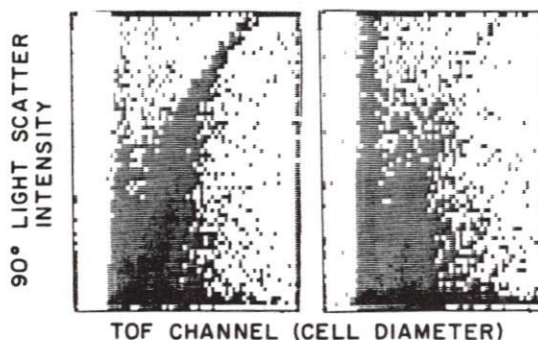
Figure 2. Two examples of bivariate contour plots of cell number vs. integrated PLS (granularity, vertical axis) and TOF (diameter, horizontal axis). Left: low-mobility fraction (CFES #91); Right: High mobility fraction (CFES #113).

Functional Staining of Pituitary Cells
after Separation

Results of preliminary experiments in
which the potential of the flow cytometer in
analyzing pituitary cells after CFES was in-
vestigated are shown in Figures 4 and 5. The
electrophoretic mobility profile of the total
cell population is shown in Fig. 4 (top).
Cells that stained with fluorescent antibodies
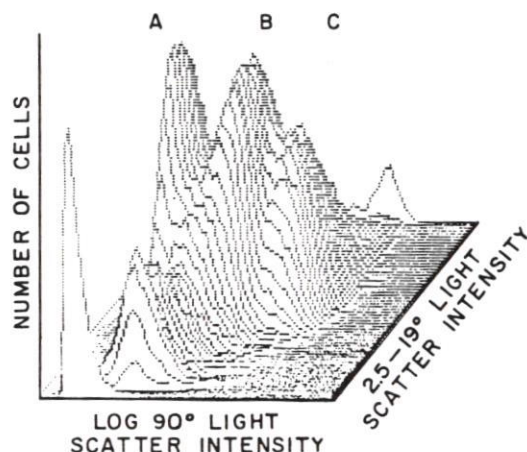
Figure 3. Isometric plot of cell number (verti-
cal axis) vs. FALS (horizontal axis) and PLS
(30 degree axis) for freshly dispersed cells of
male rat anterior pituitary. FALS was measured
in the 2.5-19-degree angular interval, and PLS
was measured in the 70-110-degree interval.

against GH tended to be found in two regions
(fractions 11-15 and 16-20, middle panel),
while cells that stained with anti-PRL anti-
bodies were found in lower electrophoretic
mobility fractions (6-14, bottom panel). Fig-
ure 5 reveals information concerning the re-
lationships between hormone content and cell
morphology. A contour plot of the forward
angle light scattering (FALS) intensity of each
cell counted vs. the logarithm of its green
fluorescence intensity (LPGFL, proportional to
content of antibody to GH) is shown for frac-
tions 5 and 19 from the profile at the top of
Fig. 4. The cells were also stained with pro-
pidium iodide, and only particles with red
fluorescence (showing that they contain DNA)
were counted by the cytometer. The predominant
difference between GH containing cells in frac-
tions 5 and 19 is that a majority of GH cells
in fraction 19 are large (higher FALS inten-
sity) and constitute a well defined population
(contour area marked by arrow) whereas smaller,
less intensely stained GH cells are common to
both fractions (identified by *).

Conclusion

Bioprocessing experiments of the type
designed for space research are served by flow
cytometry as their central analytical tool.

Discussion

It is assumed that cell bioprocessing
experiments of the type described above or
similar ones will be performed on the inter-
national Space Station. If so, an advanced
cytometer will be a vital part of the Space
Station on-board analytical capability. This
important, expensive capability should not be
reserved for a small number of bioprocessing
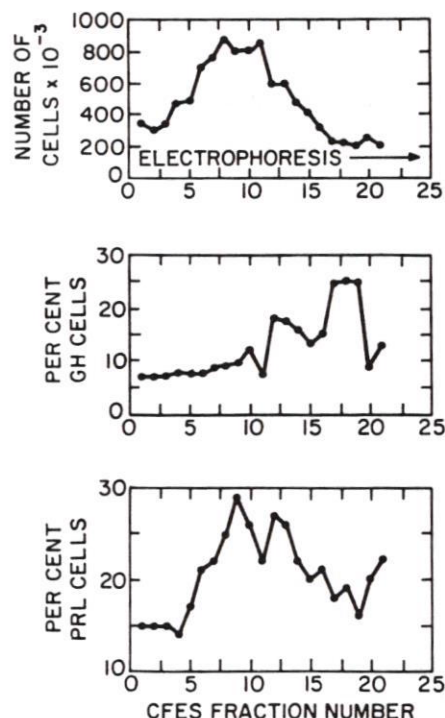
Figure 4. Top panel: Distribution of cells in
fractions after separation by CFES. Middle and
bottom panels: Distribution of per cent GH
cells and of per cent PRL cells, respectively,
in fractions after CFES, as determined by
counting 30,000 cells by flow cytometry.

CFES GROWTH HORMONE CELLS
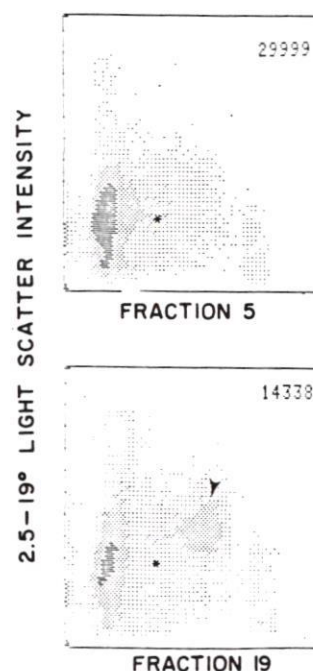
FLUORESCENCE INTENSITY/CELL

Figure 5. Bivariate contour plot of distri-
bution of cells from fraction 5 (top) and
fraction 19 (bottom) vs. FALS (vertical axis)
and fluorescent GH antibody staining intensity
(horizontal axis, log scale). Cells were
obtained from fractions described in Figure 4.

experiments but should be made available for a
wide variety of applications, some of which are
listed in Table 3.

Table 3. Applications of a flow cytometer in long-duration space flights.

CATEGORY	APPLICATIONS
space medicine	hematology and immunology
	microbiology
	endocrinology
	cytogenetics
	body fluids experiments
	cabin particulates
biology	hematology and immunology
	animal endocrinology
	cell biology and kinetics
	plant reproduction
	plant cytogenetics
	protozoology
	microbiology
bioprocessing	cell growth
	bioreactor technology
	cell separations
	cell fusion
	cell and particle assays
	biorheology

An advanced cytometer for Space Station installation could not resemble today's traditional 300 kg, high-power laser-based system. It must be custom fabricated using microfabrication techniques to produce integrated electro-optics circuits sufficiently small and sensitive to fit into a 0.5 cubic meter space, use 200 watts (efficiently), and be "intelligent" enough to put the versatility implied in Table 3 into the hands of any operator. Preferably, it should even include an imaging capability. These features are useful in several other settings besides the Space Station environment, so this requirement should result in a revolution in cytometer design and applications.

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THE INFLUENCE OF WEIGHTLESSNESS ON CELL SKELETON

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The influence of weightlessness on cytoskeleton expressing in cell changings in form and size, redislocation of nucleus and vacuolies leading to disturbance of genom activity and cell differentiation was discovered in experiments on microspores of Tradescantia paludosa performed aboard spaceships "Vostok 3,4,5,6", "Voshod 1", "Cosmos 110", "Cosmos 368", "Zond 5". The quantity of such cells was low but it grew with increasing of flight duration. It is suggested that this is a result of genome work according to new programme comparing with norma. Direct and indirect roots of weightlessness influence on genetic apparatus of a cell are considered.

Recently the increased interest to study of biological effects of space flight factors and, first of all weightlessness on cell level has been marked (1,2).

The action of this factor on cell level has been exposed even in the first experiments performed onboard satellites and manned spaceships. In particular it has been shown on microspores of tradeskantia that weightlessness may cause the changes in form and size as well as some disturbances in mechanizm of mitosis in small amount (2-3%) of cells (3,4,5). Although these data was supported in the last experiments (6,7,8,9) they were the results of single observations. Now, basing on the experience of genetic researches during space missions we suggest that this problem deserves special attention.

The analysis of collected facts from the point of view of modern conceptions of cells' architecture permits us to make some conclusions on the effect of weightlessness on cytoskeleton. The last one includes a whole complex of those structures which are responsible for spatial cell organization. It defines a cell form takes part in cell replacements, determines the process of its division, participates in intercell transport of organells and visicula, provides structural base of metabolism, etc. (10). Cytoskeleton is one of the most important parts of cell gomeostasis.

Therefore if in the conditions of weightlessness the form and sizes are changed, swells are formed, nucleus dislocation and spindle turn on 90° are registered that leads to genom activity change and cell differentiation, we have, obviously, every reason to assert that this factor has an influence on cell skeleton.

It naturally arises a question about not only possible mechanizms of this influence, but also the approaches in the research of this difficult task which is closely connected with the problem of cell adaptation in weightlessness. Some opinions about possible mechanizm of weightlessness influence on cell level are known (1) but they did not concern the role of genetic cell apparatus in this process. In our paper such hypothesis is suggested and discussed.

The analysis of results of researches for 30 years since the flight of legendary Laika, the launching that opened the era of biological studies in prolonged weightlessness has showed that the best results were achieved in the field of investigation of mechanizm of adaptation to weightlessness on system and organizm level and, certainly, less ones were achieved on cell and subcell levels.

On modern stage of development of space biology and medicine this level seems to be the most important.

If in short-term flight adaptation is mostly realized on system level and cell level turns out to be in the background: in long-term flight cell level is in the foreground. It is necessary to underline that this problem has not only biomedical aspect but is closely connected with fundamental researches and solutions of a number of practical points of space biotechnology.

It is known that for 30 years period in space flights a great number of various experiments has been carried out on different types of cells - of procaryotic and eucaryotic unicellular organizms, cells of plant and animal origin in culture and cells, functioning within one multicell organizm.

However, as it was stated above, the effects of weightlessness on cell level were discovered only in several experiments (3,4,5). They were not found in the majority of flight experiments. This can be connected with the fact that adequate objects and research methods for solving rather difficult methodical task weren't always chozen and necessary conditions of flight not sustained. Hardware level of biological experiments wasn't rather high either.

It is necessary to underline that the effect of weightlessness was found in actively metabolazing cells, cells undergoing differentiation. It is obvious that cell form, cell size, organell size and subcell structures play a certain role in its reaction to weightlessness. Thus, success in finding weightlessness effect mostly depends upon the choice of the ob-

ject and research methodiques. We agree with A.Cogoli's opinion (1) that in the condition of weightlessness Montgomery P. O.B. et all (11) didn't receive marked changes in such indices as proliferation, mobility, morphology, etc. of human embryo lung cells because these cells are not suitable objects for investigation of this factor as they grow on substrate and unable to differentiate.

Our researches were performed on Tradeskantia paludosa microspores. We think it is rather suitable object for weightlessness effects study on cell level. Allow me to discuss its characteristics in short.

At cells' division in microsporegenesis this clon practically doesn't form spontaneous chromosomes aberrations and genome mutations. We have studied in details the way of microsporegenesis at + 10°C, + 15°C, + 20°C, +30°C, +35°C. The optimal temperature was + 30°C when the cell development cycle is lasting for 7 days, while at + 20°C it increases up to 10 days, etc.

While watching microspore development under + 30°C we describe 15 stages which were distingueshed morphologically, The duration of every stage was defined, a form of nuclei, chromosomes and their position in cell were discribed. Considerable difference between stages according to these parametres were discovered.

E.g. compare early interfase and early profase. Duration of the first one is 24 hours, of the second one is 4,43 hours. The cell length in early interfase is varying from 32 to 34 mkm, nuclei diametre is 10-12 mkm, cell length is incresing to 52 mkm in early profase, nuclei diametre is 26 mkm.

It is known that according to a number of reasons it is impossible to measure the length of chromosomes in interfase but one can suppose it will be considerably more than in metafase (10-14 mkm).

Then hametogenese section was divided into 6 fases from formation of two-nuclei pollen to the ripe one.

Such detailed information about the object was necessary to plan flight experiment and analyse received data, in particular, to evaluate what fase was effected by launching, landing factors and weightlessness in each case.

The experiments were performed onboard of space apparatus "Vostok 3,4,5,6", "Voshod 1", "Cosmos 110", "Cosmos 368" and "Zond 5". Either plant or cutdown stem with inflorescence were taken aboard. The duration of the flight varied from 1 to 22 days. More detailed discription of conditions and methodiques of experiment conduction is given in our works (4, 12).

Some results are shown in table 1. This table demonstrates that in all flight experiments the analysis of microspores in profase, metafase and telofase, two-nuclei pollen discovered cells differed from normal by form and size. These are gigantic one-nucleus, two- and multi-nuclei cells, rather little cells and cells with swells (fig.1). Many cells have swells, especially one-nucleus ones. Gigantic cells are 80-101 mkm in length, which is thrice more than the length of a normal one being in early interfase and twice more in early profase. The length of swells varied from 1 mkm to 37 mkm.

Table 1

Amount of cells with altered shape in Tradescantia paludosa
(experiments aboard ships "Vostok-6", "Voshod-1", "cosmos - 110")

Ship, time of flight	Samples	Time of fixation	Amount of cells	Gigantic cells						Small Cells
				In all	% ± m	mono-nuclei Nr. %	with swells Nr. %	two-nuclei Nr. %	multi-nuclei Nr. %	Nr. %
Vostok-6 72 h	1	4,5 after landing	1000	5	0,50±0,22	2 0,20	- -	2 0,20	1 0,10	1 0,10
Voshod-1 24 h	1	1,5 before landing	1000	1	0,10±0,10	- -	- -	1 0,10	- -	2 0,20
	2	2 h 15 min a.l.	1000	2	0,20±0,14	1 0,10	1 0,10	- -	- -	- -
	3	24 h a.l.	2000	5	0,25±0,11	- -	4 0,20	1 0,05	- -	- -
	4	48 h a.l.	1000	1	0,10±0,10	- -	1 0,10	- -	- -	1 0,10
	5	120 h a.l.	1000	1	0,10±0,10	1 0,10	- -	- -	- -	- -
Cosmos-110 528 h	1	48 h after landing	2000	33	1,65±0,28	10 0,50	7 0,35	14 0,70	2 0,10	17 0,85
	2	120 h a.l.	1921	28	1,45±0,27	13 0,67	- -	15 0,78	- -	18 0,93
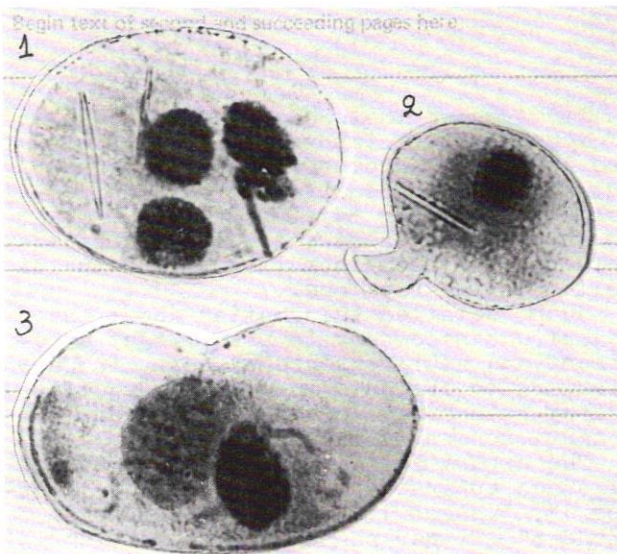
Fig.1. Disturbances of form and sizes of cells in weightlessness.

1 - four-nuclei cells; 2- one-nucleus cell with a swell; 3 - gigantic two-nuclei pollen of wrong form.

Cell development in the first postmeiotic mitos inside pollen-sack goes so synchroneously that finding even separate cells dropping behind or passing ahead in the development is regarded as digressin from normal. Especially as the cell development of special form and sizes which are not met in the control is certainly to be pathology.

Finally, degenerating abnormal cells finish their development by abortive pollen, eliminate without giving new generation.

The further examination of the material draws attention to at least two circumstances. Firstly, only several cells were registered, secondly, the number of such cells is increasing with flight duration. At the same time laboratory experiments on study of the influence of vibration on tradeskantia microspores showed that these factors in studied parametres didn't cause such changes of cell forms and sizes. This allows us to suppose that weightlessness is the factor which can cause changes of cell gomeostasis.

It is necessary to mark that all abnormal cells have not only forms and sizes differed from normal but special nuclei position. The role of this circumstance is vividly displayed in turning spindls on 90° during the process of tradescantia division.

As it is known the first postmeiotic mitosis in microsporegenesis is a bright illustration of differentiating mitosis. Vegetative and generative cells result from this mitosis. In this process an axis of spindle is strictly oriented towards thickened zone of cell capsule (fig.2). In a number of flight experiments the turn of spindle on 90° was registered; it was also registered in Sparrow A.' experiments lately (14). Besides a good comformity of a number of cells with ori-

ented spindle metafase and a number of cells of abortive pollen with undeveloped generative cells was discovered (tab.2).
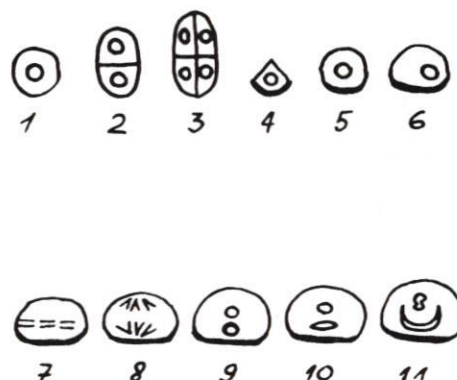
Fig.2. Microsporegenese scheme at Tradescantia paludosa.

1 - mother cell of pollen; 2 - diada; 3 - tetrade; 4,5 - interfase in microspore; 6 - metafase; 7 - anafase; 8 - two-nuclei pollen after formation; 9 - two-nuclei pollen a day after formation; 10,11 - ripen pollen.

This can be presented schematically as following. Because of reorientation of mitotic spindle on 90°, two-nuclei pollen is formed, which at first goes along the line of some differentiation of these nuclei, however delay in development takes place and generative cell doesn't form then (fig.3).
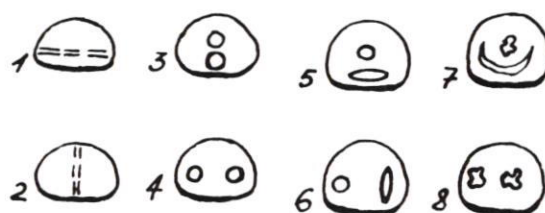
Fig.3. Scheme of microspores and two-nuclei pollen with normal development and after spindle reorientation.

1,2 - metafase in microspores; 3,4 - two-nuclei pollen after formation, 5,6 - two-nuclei pollen with differentiating generative nucleus, 7 - two-nuclei ripen pollen with developed generative cell; 8 - abortive pollen.

Naturally, a question appears how in the condition of weightlessness during microsporegenesis abovementioned cells come into existence. Certainly this is a result of genome activity on a new programme comparing with the norma. Let's examine possible mechanizms of this phenomenon.

Table 2

Amount of microspores with disturbed spindles and
amount of cells (two-nuclei pollen) with undeveloped
generative cell in Tradescantia paludosa

Ship, time of flight	Time of fixation	Analysis of metaphase in microspores				Analysis of two-nuclei pollen				md
		In all	disturbed spindles			In all	undeveloped generative cells			
			Number	%	$\pm$ M		Number	%	$\pm$ M	
Vostok-5 120 h	120 h after start	1032	13	1,26	0,35	1000	11	1,1	0,12	0,36
Vostok-6 72 h	45 h after landing	2249	23	1,02	0,21	1000	11	1,1	0,12	0,24
Cosmos-110 528 h	48 h a.l.	703	9	1,28	0,18	2000	25	1,25	0,06	0,18
Cosmos-368 144 h	31 h a.l.	805	14	1,74	0,46	1000	15	1,5	0,15	0,48

Note: md - reliability of difference between M
metaphases and M two-nuclei pollen.

There is a whole hierarchy of regulatory systems influencing the work of every genome. Besides specific mechanizm of genetic activity regulation, eucaryot in contrast to procaryot have mechanizm of unspecific regulation the essence of which consists of taking down condensation blocks from chromosome areas. Taking down heterochromatine blocks with heterochromatic euchromatin brings lead-in additional number of genes in polygen system in the state when transcription occurs. As a result stimulation of processes in cell takes place. However, for all this, genes with harmful mutation could be freed from heterochromatization and imperfect changes appear in such cells.

Heterochromatine is a labile component of chromosomes. Its large areas can be eliminated and then restored. But there is a limit when the loss or another position of heterochromatic locuses along the length of chromosomes prove to be irreversible and then irreversible changes take place in a cell. Probably it is rightfully to speak about a type of mutations which don't affect genes and unique sites of chromosome, but mutation rebuilding locuses heterochromatine into chromosomes; locuses acting as unspecific gene regulators. Heterochromatization of chromosome areas and then taking down heterochromatine block is an index of adaptation in a cell or organizm to this or that factor.

Schematically adaptation of the process to extreme factors at this level may be described as follows. At first there are no changes in nucleus, an additional reproduction of heterochromatine and intensified heterochromatization of euchromatic areas take place owing to which stability to unfavourable factors is increasing. More prolonged influences lead to the fact that heterochromatine begins to exhaust and free from heterochromatization additional genes in poly genetic systems, however, then mutagenetic genes can be freed. Such nucleus reaction to stress mobilizes a cell, but makes its chromosomes more sensitive to stress.

In optimal conditions of organizm development cell nuclei of certain tissues have peculiar position of condensation places. Various differentiation is mainly caused by this unspecific apparatus of genetic regulation.

At the same time nucleus chromatization depends on its position in cell. We introduce a term "nucleus position effect" attaching great importance to nucleus topology in regulation of genetic activity, processes of differentiation, etc. It's known for a long time that various differentiated cells have specific sizes, cell form, position of a nucleus in it, organell position, etc., owning distinctive features for each differentiation. A "nucleus position effect" is a necessary element for cell hemostasis.

In weightlessness a small part of cells acquired specific form, position of nucleus and functions were changed. They were unable to divide by mitosis. Most of them began to grow and looked like round gigantic cell which had swells. One can suppose that due to weightlessness all cells of Tradescantia microspores got an impulse to change form. However, their hemostasis in most of the cells continued owing to normalizing effect of various regulation systems. It's known that cell homeostasis depends upon the order of genes transcription and is formed by reserve connection between a nucleus and cytoplasma, and intercell and organizm regulators. The same cell which escaped "norma" became to follow the new structure-functional way at once.

According to this general discussion we can assume as one of possible mecha-

nisms of appearing of abnormal cell a me-
chanism connected with "removal of" bloc-
ked heterochromatine by weightlessness and
further change of genome work. In a number
of cases this can be expressed in the ap-
pearance of mutant cells. Apparently this
effect is connected with the change of
nucleus position in a cell, this was the
primary event.

The explanation is certainly simplifi-
ed and schematical in reality weightless-
ness effect is presented by multicanal
systems of influence.

Examining different mechanism of weig-
htlessness action on cell genom, it is ne-
cessary to take in account a cell type:
its size, form and function. There are com-
mon moments in these mechanisms peculiar
to all types of cells but there is a dif-
ference mainly connected with difference
in systems regulating genetic activity ex-
hists.

We can speak about direct and indirect
effect of weightlessness on the cell.
E.g. Pollard E. (15) considers that the
cells the size of which exceeds 10 mkm in
diametre must be the target of weightless-
ness. According to this calculations, it
is clear that nuclei, vacuolies and other
big organoids exceeding this measusize
must also react to weightlessness. Trades-
cantia microspores used in our experiment
have the size of nucleus and chromosome
more than 10 mkm, and, hence, if we sup-
pose that Pollard E. is right in his cal-
culations, we can't exclude their direct
interaction with weightlessness.

We have better understanding of the
mechanism of indirect weightlessness ac-
tion on the cell, genom through "replace-
ments" caused by this factor in physico-
chemical and other parametres of environ-
ment. "Environment" is not only natural
habitat of one-cell organisms but a mul-
ti-cell organism as well whick is a media
for existing of every separate cell tis-
sues and organs of this organism.

The change of membrane permeability,
ion relations, more or less intense irri-
gation leads to replacing of structures
in cell and nucleus, hence, changing the-
ir function as spatial nucleus position in
a cell and genes in nucleus plays a cer-
tain role in their functioning.

Displacements in exchanging Ca^{++}, mar-
ked in the cells and tissues of organism
being in the conditions of weightlessness
present special interest. As known this
element forms a part of chromosomes and
plays a great role in their integrity.

In multi-cell organism these processes
are in a consiquence of changes in func-
tions of some regulatory systems and or-
gans caused by weightlessness and in this
case we ought to speak about indirect se-
condary action of the factor on the cell.
Population of genes is an extremely im-
portant link in general chain of events
taking place in cell. However, genom un-
dergoes a reverse influence from the or-
ganism and such a reverse connection me-
ans creation of an organism as a unity.

So, discovered influence of weightles-
sness to cell skeleton in Tradescantia
microspores expressed by a new form nuc-
leua and vacuoly dislocation and leading
to change of genom activity and cell dif-

ferentiation may be realized indirectly.
At the same time we can't exclude direct
influence of weightlessness, e.g., with
cytoskeleton which can be considered as
a gravireceptor in this case.

It's quite clear that both first and
second ways need to be proved.

Concerning further study of mechanism
of weightlessness effect on cell, mecha-
nism of its adaptation to this factor we
must underline advisibility to use comp-
lex approaches taking in account all ty-
pes of cells, their forms, sizes and fun-
ctions. Side by side with genetic methods
considered in this paper, it is necessary
to search for intercell organells, sen-
sitive to gravitation, analyse mechanism
of biochemical regulation of cell behavi-
our, its morphology during gravitation
stresses. It's expedient to continue the
study of membrane permeability, ion in-
terrelation and albumen biosynthesis.

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EFFECT OF SPACE FLIGHT AND HYPOKINESIA ON PLASMA HORMONE LEVELS AND LIPID METABOLISM IN RATS

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Important changes of plasma levels of hormones involved in the regulation of metabolic processes (catecholamines, cortisol, growth hormone, insulin and thyroxine) were observed in crew members at the end of space flight (1,2). Therefore the aim of the present experiments was to study the effects of space flights on plasma hormone levels and on metabolism in fat tissue of experimental animals. Since hypokinesia was used as a model for simulation of the effects of stay in microgravity (3) the changes in lipid metabolism were investigated also in rats after the exposure to hypokinesia for various period.

Material and Methods

Adult male Wistar rats (body mass 330--350 g) were devided into three main groups: 1. intact control rats (C), 2.flight rats (F)-group of animals subjected to space flight on biosatellites Cosmos series for 18,5 days (Cosmos 936 and 1129), or 7 days (Cosmos 1667), 3. rats in model experiments (SM), simulating the conditions of space flight but weightlessness (4). A group of rats (6-8 animals) was sacrificed at the landing site of the biosatellites, 6-10 hours after landing, the blood was collected, the epididymal fat pad was removed and the slices of fat tissue were immediately incubated without and with the addition of norepinephrine (NE 10^{-5} and 10^{-3} mol l^{-1}) and the release of free fatty acids (NEFA) was determined (5).

The concentration of corticosterone, insulin, epinephrine and norepinephrine was determined in plasma (6).

In experiments with hypokinesia the animals were immobilized in special adjustable cages for 1, 7 and 60 days, the lipolysis in fat tissue was determined (5) and the binding of ^{125}I-monoiodoinsulin was estimated (for references see 5,7).

Results

An increase of plasma corticosterone concentration was found in animals subjected to space flight for 7 and 18,5 days (Fig. 1). This increase was due to state of weightlessness, because in rats exposed to arteficial gravity (1 g) during the space flight on a board of biosatellite no such elevation of plasma corticosterone level was noted (Fig. 1).

An increase of plasma insulin level was found in flight animals (Fig. 2) after short and longer space flight.

An augmentation of epinephrine and norepinephrine plasma levels were noted in rats after space flight in weightlessness and also in arteficial gravity in comparison to intact control and rats in model ex-

periment (Fig. 3).
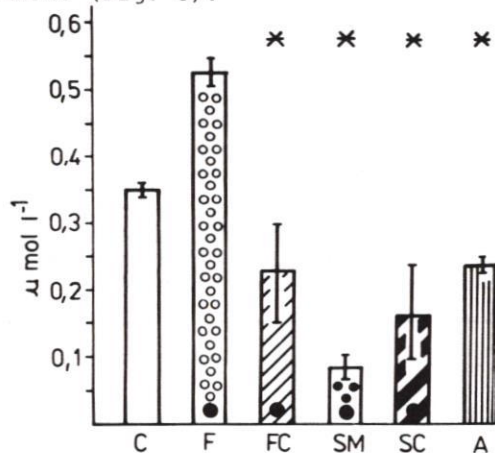
Fig. 1. Plasma corticosterone levels in rats. (C, A - controls, F - flight; Cosmos 936, FC - flight in (1 g), SM, SC - model experiment ● p<0.05 to C, ** p<0.05 to F.
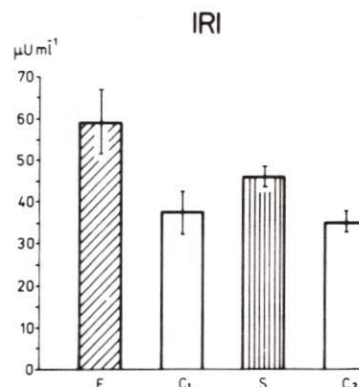
Fig. 2. Insulin plasma levels of rats after space flight (F), in model experiment (S) and in controls (C_1; C_2) (Cosmos 1667).
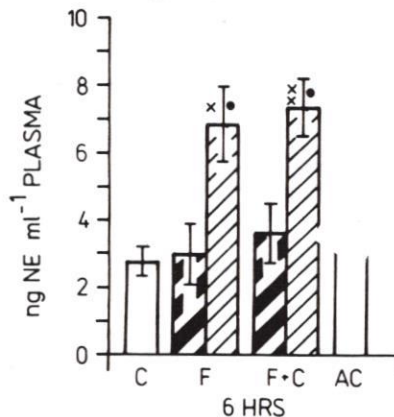
Fig. 3. Norepinephrine plasma levels in rats exposed to space flight (F), in arteficial gravity (F+C). C, AC - control groups ▨ SM groups (Cosmos 936) (6).

Since these hormones are involved in the regulation of the metabolism in adipose tissue the changes of lipolysis were studied in rats exposed to space flights. Only slight increase or no changes in the release of NEFA from adipose tissue were observed in the first hours after the space flights. However, the stimulation of lipolysis by norepinephrine was lower in flight animals (Table 1) and this lower response was observed in flight rats also after 6 and 21

days of recovery period.

The marked increase of lipolysis was found in rats exposed to hypokinesia for short (1-7 days) and long period (60 days) (Table 2). Similarly as in animals after space flight a decrease of the response of adipose tissue to norepinephrine was found in rats exposed to hypokinesia (Table 2).

Table 1. Stimulation of lipolysis by norepinephrine (NE) in rats after space flight (18.5 days) on Cosmos 936 (expressed in % of lipolysis without hormone) *p<0.05, C : F

	0	NE 10^{-5} mol 1^{-1}	NE 10^{-3} mol 1^{-1}
Controls	100±7	405±69	650±230
Flight	100±18	126±16*	150±20*

The studies of the processes of lipogenesis in animals exposed to hypokinesia showed that under basal conditions there are no significant differences in the rate of incorporation of glucose into lipids after 1 and 7 days, the significant increase was found after long term hypokinesia (5). The stimulation of lipogenesis in adipocytes was lower in rats exposed to hypokinesia for 1 day, no significant changes were observed in rats immobilized for 7 days, however in animals after long term hypokinesia an increase of stimulatory effect of insulin was noted (Table 3).

Table 2. Stimulation of lipolysis (μmol g^{-1} of FFA) in rats exposed to hypokinesia (HK).

	Controls	Days of hypokinesia		
		1	7	60
Basal	2.1±0.3	3.2±0.45*	2.9±0.25*	4.3±0.3*
NE 10^{-5} mol 1^{-1}	+4.2±0.5	+3.3±0.4*	+0.9±0.4*	+1.9±0.5*
NE 10^{-3} mol 1^{-1}	+7.3±0.8	+5.1±0.7*	+2.3±0.6*	+2.6±0.3*

Means ± SE; C : HK - * p < 0.05.

Table 3. Stimulation of incorporation of ^{14}C-glucose into lipids by insulin after hypokinesia (in % of control), *p<0.05 C:HK.

Group		Insulin 10 mU	100 mU
1	C	182±26	302±82
	HK	153±17	173±31*
60	C	114±8	120±12
	HK	133±16	228±44*
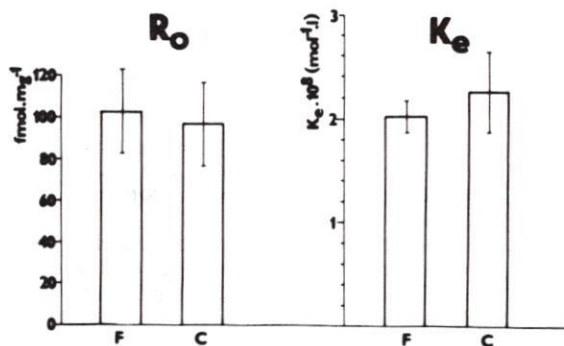
Fig. 4. The insulin binding capacity (R_O) and apparent affinity constant (K_e) of insulin binding in liver of rats.

The determination of insulin binding to

adipocytes showed that in rats exposed to hypokinesia for 1 day the binding capacity and insulin receptor density is decreased (7), no changes were found after 7 day hypokinesia, however an increase was demonstrated in animals immobilized for long time (60 days). In agreement with the result in adipocytes also the binding capacity of insulin receptors in liver is similar in control and 7 day-hypokinetic animals. Similarly in animals after space flight for 7 days (Cosmos 1667) no changes in insulin binding in liver were observed (Fig. 4).

Discussion

The results of present experiments showed an important increase of plasma levels of hormones also in rats exposed to space flight. Hypercorticosteronaemia is followed by activation of several enzymes involved in the metabolism of aminoacids in liver(9). Further the changes in the hormonal regulation of lipolytic processes in flight rats were noted not only immediately after flight but also during the recovery period. Also the changes in stimulatory action of catecholamines on lipolysis and of insulin on lipogenesis were found in hypokinetic rats suggesting that hypokinesia and space flight have important effects on the processes of hormonal regulation of lipid metabolism in adipose tissue. The results of our previous (5) and present experiments showed that these changes could be partially connected with the changes of binding of hormones to specific receptors in adipocytes.

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EFFECTS OF HYPOKINESIA ON HORMONAL REGULATION OF INSULIN RECEPTORS IN RAT ADIPOCYTES

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Hypokinesia is an experimental model for the study of the effects of movement restriction or diminished motor activity of animals and is used for the simulation of the state of weightlessness in laboratory conditions. Previously we had reported changes in lipolytic as well as lipogenic activity of adipose tissue as the result of hypokinesia. One day lasting hypokinesia decreased lipolytic response to norepinephrine stimulation in rat adipose tissue (1), but simultaneously an increase of beta-adrenergic receptors in isolated adipocytes was observed (results not published). The investigation of lipogenic activity had shown diminished insulin effect on glucose metabolism in adipose tissue (2) and subsequently a decline of insulin receptors was found as the effect of 1 day lasting hypokinesia. The movement restriction is a strong stress situation for an animal and is accompanied by permanent elevation of corticosterone and catecholamines during the first 24 hours of hypokinesia (3). Decreased or unchanged insulin binding to human and rat adipocytes after short or long in vitro exposure to catecholamines and glucocorticoids were described (4,5,6,7). The purpose of this study was to investigate in vivo role of catecholamines and corticosterone in regulation of insulin receptors in rat fat cell after short term (24 hours) lasting hypokinesia.

Materials and Methods

Adult Wistar male rats (300 g body mass) were exposed to hypokinesia for 24 hours in special adjustable cages. Control animals were allowed to move freely in standard cages. In one group of animals catecholamines were eliminated by medullectomy and by subsequent treatment of the animals with guanethidine (Ismelin Sulfate, Ciba) in the dose 3 mg per 100 g of body weight for 5 weeks, starting 18 days after the surgery (group MEDEX+GUAN). In the other group of rats corticosterone was eliminated by adrenalectomy performed 14 days before the experiment (group ADREX). Isolated adipocytes were prepared from epididymal fat pads by collagenase digestion (Collagenase cruda, SEVAC, Prague) according to Rodbell (8). The binding studies were based on competition of unlabelled monocomponent insulin (NOVO, Denmark) to ^{125}I-monoiodoinsulin. The binding capacity was calculated by computer fitted multilinear regresion curve according to DeMeyts and Roth (9). (Model of one binding site with negative cooperativity). Determination of plasma norepinephrine and epinephrine was according to the method previously described (10). Modified protein binding method for the plasma corticosterone estimation was used (11).

Results

The plasma corticosterone level of control rats subjected to hypokinesia is by 3 times higher in comparison with intact rats (Fig. 1A). Adrenalectomy decreased corticosterone level in intact rats and this low concentration persists also after hypokinesia, what proves correct surgical elimination of adrenals in this experimental group. Rats without endogenous catecholamines had significant higher corticosterone level under basal condition and hypokinesia evoked futher slight elevation of hormone concentration. Hypokinesia significantly increased plasma norepinephrine levels in control animals (Fig. 1B), while in ADREX group both basal and stimulated concentrations are elevated in comparison with control rats. Surgical removal of adrenal medulla in combination with administration of guanethidin resulted in undetectable values of norepinephrine in intact rats and slight, unsignificant increase after hypokinesia was observed. These results give the proof of a successful surgical and chemical elimination of endogenous catecholamines. Plasma epinephrine is elevated in control rats after 24 h of hypokinesia (Fig. 1C), while in ADREX and MEDEX+GUAN groups low concentrations under basal conditions are uneffected by hypokinesia. The upper part of fig. 2 shows the insulin binding capacity of isolated adipocytes. In control animals hypokinesia significantly decreased insulin binding capacity. The same effect of hypokinesia is observed in adipocytes of rats without endogenous catecholamines, however, adrenalectomy protected the decline of insulin binding after hypokinesia. Calculation of insulin binding capacity in terms of the insulin receptor number per 1 μm^2 of cell surface area (density) is shown in the lower part of fig. 2. The tendency of changes are preserved after hypokinesia, it means decline of insulin receptors density in control rats and in adipocytes of animals without endogenous catecholamines.

Discussion

It is well known that insulin receptor number is negatively regulated by plasma insulin concentration. In our experiments we did not find any changes in plasma insulin and glucose (data not presented here), neither as the result of hypokinesia, nor the effect of surgery. Thus the fall of insulin receptors as the effect of hypokinesia must be the result of other factors e.g. insulin contraregulating hormones. The in vitro decreasing effect of catecholamines and glucocorticoids on insulin binding was described (4,5,6,7). Permanent elevation of the above hormones during 24 hours lasting hypokinesia led us to investigate the role of catecholamines and corticosterone in regulation of insulin receptor in vivo. Decreased insulin receptor number in adipocytes of animals with high concentration of corticosterone and without epinephrine and norepinephrine is identical with the fall of receptors in control rats after being exposed to hypokinesia. On the other hand, no changes of insulin binding were observed in adrenalectomized animals after

hypokinesia. These results point out the significant role of high corticosterone levels in regulation of insulin receptors in rat adipocytes in vivo. Described fall of insulin receptors after hypokinesia is very probably related to the lower response of adipose tissue to stimulatory effect of insulin on lipogenesis as it was reported previously (2). The results suggest that 24 h permanent elevation of corticosterone during hypokinesia plays a role in the regulation of insulin receptors in rat adipocytes.

Fig. 2. R_O - insulin binding capacity (fmol/10^6) in isolated adipocytes and density of insulin receptors per 1 um^2 of cell surface (calculated from R_O values) of control, ADREX and MEDEX+GUAN rats under basal conditions and after 24 h hypokinesia

Fig. 1. Plasma corticosterone (A), norepinephrine (B) and epinephrine (C) under basal condition and after 24 h hypokinesia in control, ADREX and MEDEX+GUAN rats.

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ADAPTATION TO RESTRAINT IN THE RAT

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ABSTRACT Use of telemetry in physiological research is still in early stages. Cardiovascular studies planned for gravitational work during the next few years on primates or squirrel monkeys depend on restraint devices. Two rat cardiovascular experiments (US Space Lab SLS I and SLS II) use unrestrained rats for resting cardiovascular measurements (chronic intravascular cannulas and implanted blood flow probes), but employ semi-restrained animals for microcirculatory investigations. Prior to weightlessness, these instrumented rats will be exposed a number of times to semi-restraint. In order to ascertain if there is adaptation to restraint (decreased toward normal of plasma stress hormones), the rats were exposed 18 times to restraint chambers, but the level of stress hormones stayed elevated.

INTRODUCTION

A large increase in plasma stress hormones (ACTH, corticosterone, prolactin, catecolamines and others) is observed when a rat or other experimental animal is exposed to stress (increased external or increased body temperature, electric shock, etc.). Restraint represents a stress, too. During repeated restraint, an experimental animal is struggling during early exposures but later becomes quiet and docile. The question arises: is stress of restraint still present even in the latter case? In order to study this problem, rats were exposed to restraint twenty minutes each day during a period of eighteen days. Microamounts of arterial blood were withdrawn from the animals four times during this period and analyzed for two stress hormones, ACTH and corticosterone. The obtained results were compared with the level of the same stress hormones before the animals were exposed to stress and to the results obtained in control, non-restrained rats. The blood was withdrawn through chronically implanted aortic cannula (Popovic et al, 1963).

METHODS

Forty adult male Sprague-Dawley rats (200 + 9 g) with chronically implanted aortic cannula were used in the experiments. Twenty five rats were placed in restraining plastic boxes (permitting some movement but not turning of the animal) once each day for eighteen days. Each twenty minute exposure was between 9 and 11 AM. The arterial blood was sampled five times from each animal. Only 0.2 ml of blood was sampled which was sufficient for two plasma stress hormone determinations (ACTH and corticosterone). The blood was sampled before exposure to repeated stress and four times during the exposure. Blood was withdrawn from the animals that were at rest for at least 10-15 minutes. Levels of plasma hormones were determined by radioimmunoassays.

RESULTS

Figures 1 and 2 summarize the obtained results. The repeatedly stressed rats were growing at a slower rate than the control animals (Fig 1).

During the early part of repeated stress exposure, the stress level of both hormones was greatly elevated. Several days later, plasma ACTH and plasma corticosterone decreased somewhat. The decrease was greater for plasma ACTH than for plasma corticosterone. But even after eighteen restraints, stress hormone level was very high, much higher than in control animals or in the same animals before exposure to restraint.

Adaptation to repeated stress has been already studied. Rats were exposed to signalled, unpredictable, short lasting stress once or several times per day for a number of days, and microamounts of blood were sampled from the animals to measure the level of plasma stress hormones. Some investigators sacrificed animals after one or more stressful exposures and studied secretory activity of a particular endocrine gland (for instance intermediate and anterior lobe of the pituitary for ACTH synthesis) as well as determined the level of plasma stress hormones. Pollard (1983) found that stress significantly raised ACTH synthesis in pituitary. After several stress exposures ACTH synthesis returned to normal, but plasma corticosterone level was elevated at all times studied. The initial (extreme) rise of corticosterone was observed between the first and fifth day of exposure. The plasma corticosterone decreased to an intermediate level by the day 40 and persisted thereafter at that level. This observation is in accord with other studies (Pollard, Bassett & Cairncross, 1976) demonstrating a similar corticosterone time curve. The same researchers saw anterior lobe corticotrophic activity initially increased, with a return to a control condition after 20 days of repeated stressing. An increase in the total number of corticotrophs and in the total number of chromophobes was seen as well. The chromophiles are believed to be an ACTH source. Presumably these cells secrete ACTH, a process seen at the later part of stressing (Pollard, 1983).

Lacombe and Seylar (1984) excluded struggling and movement of animals (curarized rabbits) as factors that led in stress and to an increased muscle blood flow, an increased level of plasma stress hormones and an increased

cardiac output. While muscle blood flow was not increased during such an immobilization stress, the rabbits showed a marked increase of brain blood flow. The increase was large, between 40 and 80 percent. A similarly increased cerebral blood flow was found in stressed rats (Carlsson et al, 1977).

Burchfield et al (1980) studied adaptation to stress. The rats received chronic or repeated acute cold stress during a period of up to three months. The authors found that chronically-stressed rats had an elevated resting plasma corticosterone level, as much as seen in the control animals during an acute stress. On the other hand, plasma ACTH levels remained at the resting level. Similarly, Sakellaris and Vernicos-Danellis (1975) found that after adaptation to chronic stress, and despite continued exposure to the stressor, the pituitary-adrenal system regulates plasma corticosterone concentration as before exposure to repeated stress. The pituitary secretion returned to control value after the adaptation to stress was completed.

Our own results indicate that after eighteen repeated exposures of rats to a semi-restraint device, plasma stress hormones (ACTH and corticosterone) are elevated. We conclude that adaptation to restraint stress does not exist. (Supported by NASA contract NAS2-10527).

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Fig. 1. Plasma ACTH in rats exposed to daily 20 minute long semirestraint (triangles, full-line) and in unrestrained rats (circles, broken line). Arrow indicates when the daily restraints were initiated.
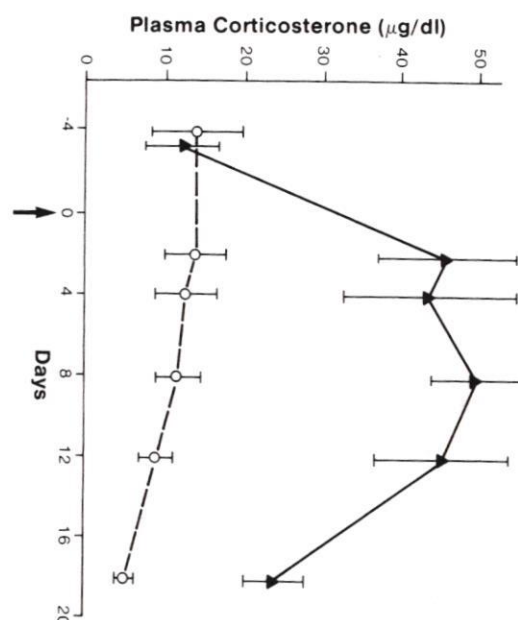
Fig. 2. Plasma corticosterone in semi-restrained (triangles, full-line) and in unrestrained rats (circles, broken line). Arrow indicates initiation of daily 20 minutes long restraint.

EFFECT OF SPACE FLIGHT ON PLASMA LEVELS OF GROWTH HORMONE, PROLACTIN, CORTICOSTERONE AND INSULIN IN PREGNANT RATS AND THEIR OFFSPRINGS DURING ONTOGENESIS

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Abstract: Plasma levels of growth hormone
(rGH), prolactin (PRL), corticosterone
(B), insulin (IRI) and blood glucose were
measured in pregnant female rats flown on
the board of COSMOS 1514 between day 13
and 18 of pregnancy and in their offsprings
in day 15, 30 and 100 of age. Flown preg-
nant females showed enhanced PRL secretion,
reduced IRI and blood glucose and reduced
variation in rGH when compared to synchro-
nous and intact controls. No changes in
B were observed. Offsprings of flown fema-
les did not show any changes in PRL levels,
GH was in day 30 and 100 diminished. No
substantial changes in all other parame-
ters were found. These results suggest
that space flight represent a stress sti-
mulus for pregnant females being manifes-
ted by enhanced PRL release. In offsprings
of those rats the decrease in GH remains
to be clarified.

Space flight accompanied by microgra-
vity and following overload represens
complex of stress factors exerting possib-
le effects on the organism. After prolon-
ged space flight an activation of sympatho-
adrenomedullar system was observed, however,
since the activity of catecholamine synthe-
tizing enzymes were not changed, this
effect was due to acute stimulus during
the landing and not due to the prolonged
weightlessness (Macho et al., 1980). Also
other endocrine parameters of stress such
as ACTH, GH, cortisol, IRI in men as well
as PRL, TSH, FSH, MSH in rats remained
uninfluenced after the space flight (Ka-
lita et al., 1986, Grindeland et al.,1979).
Different situation can occur during preg-
nancy. It has been known that pregnancy is
associated with specific changes in endo-
crine functions which can alter the sensi-
tivity to environmental stimuli. One of
the characteristcs of pregnancy is mutual
regulation of pituitary adrenocortical
axis between mother and fetus (Macho, 1979
Review). The aim of presen experiment was
to reveal the effect of space flight on
the endocrine profile of female rats in the
last third of pregnancy and its possible
consequences on their offsprings during
ontogenesis.

Materials and Methods: We investigated the
levels of rGH, PRL, B, IRI and blood glucose
in female Wistar Stolbovaja rats subjected
to 5 day space flight on the biosatelite
COSMOS 1514 between the 13th and 18th day
of pregnancy. One part of the rats was sa-
crificed immediately after the landing,
other part of females delivered pups, ma-
les of them were selected and kept under
standard animal room conditions and sacri-
ficed in day 15, 30 and 100 of age. The
same protocol was used for synchronous
controls i.e. animals kept under simulated
flight conditions and for intact controls.
rGH and PRL were estimated by radioimmuno-
assay using the materials kindly supplied
by National Pituitary Agendy, NIADDK,
Bethesda. Concentrations of IRI were mea-
sured by radioimmunoassay using the kits
RIA OPIDI, Poland. Blood glucose was
measured by ortotoluidine method. For B
determination the protein-binding method
was used. Nonparametrical test of Mann-
-Whytney or analysis of variance were used
to evaluate rGH data, the unpaired Student's
t- test was used for calculation of statis-
tical significance of PRL, B, IRI and
blood glucose values.

Results and Discussion: Mean plasma levels
of rGH (Fig.1) did not differ between
flown and both control groups of pregnant
rats, but when evaluated the standard
deviation by the analysis of variance, a
significantly lower (p< 0.01) variation
of values in flown rats compared to syn-
chronous controls was found.
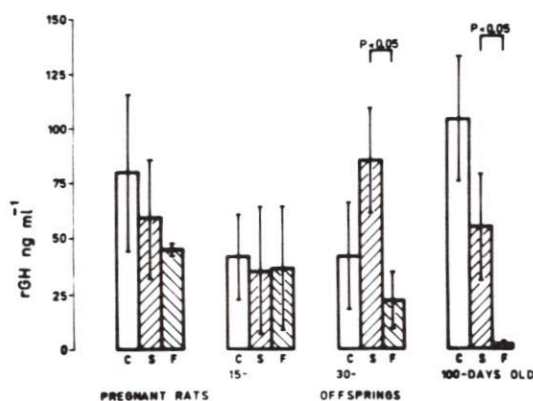
Figure 1. Plasma rGH levels in 18 days
pregnant female rats of flown (F), syn-
chronous experiment (S) and intact control
(C) groups and plasma rGH in 15- 30- and
100 days old offsprings of those mothers.

It may represent an impairment of the phy-
siological balance between somatostatin
and GH releasing hormone due to the flight.
Lessened secretory activity of somatotrophs
with higher content of intracellular rGH
of flown rats was recently observed (Grin-
deland et al., 1987), suggesting higher
tone of somatostatin during flight which may
result in disturbing the physiological rGH
pulses as well. Lower rGH levels in 30 and
100 days old offsprings of flown mothers

can represent a muted secretory activity of these rats, however, an effect of non-specific stressor before decapitating the rats cannot be excluded. In 15 days old rats the rGH levels did not differ between individual groups and it is to be considered that in this age group is the rGH raction to acute stressors lacking which fully recovers after the 21st postnatal day (Štrbák et al., 1985).
Plasma B (depicted in Fig. 2) was enhanced in all 3 groups of pregnant females which is a normal physiological phenomenon in the 18th day of pregnancy(Macho 1979, Review). No differences in B levels between the offsprings of flown and synchronous control mothers in all age intervals were observed pointing out that the prenatal flight is not a severe stressor for the rats.
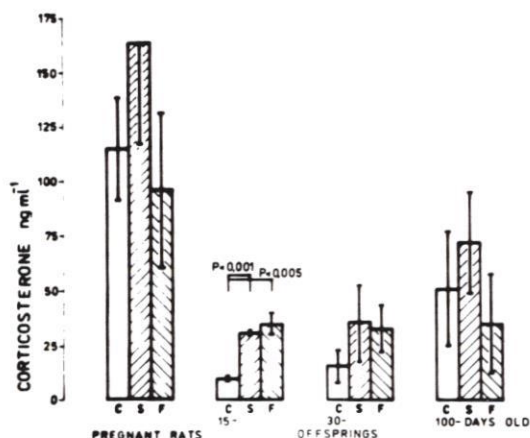
Figure 2. Plasma levels of B in pregnant rats and in the offsprings. Legend see Fig. 1.

Plasma IRI was decreased in flown mothers against synchronous controls (18.5 + 5.1 vs. 34.6 + 2.7 μU ml^{-1}; p < 0.05) and so was blood glucose (4.5 + 0.4 vs. 7.4 + 0.3 mmol l^{-1}; p < 0.001). These values are a consequence of the rhythm of feeding rather than of a stress response, since during the stress exposure the release of IRI is blocked with a concomitant hyperglycaemia (Vigaš et al., 1973).
The enhanced PRL levels depicted in Fig. 3 can be ascribed to the synergistic effect of flight and circulating estrogens.
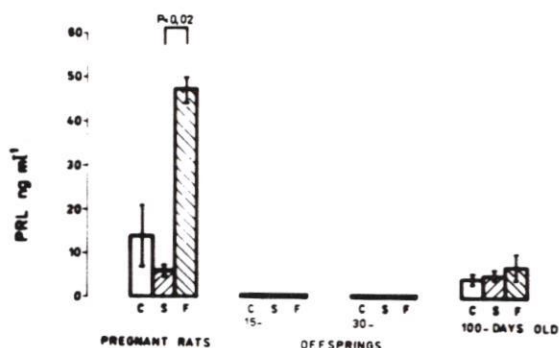
Figure 3. Plasma PRL in pregnant females and in the offsprings. Legends see Fig. 1.

In the 18th day of pregancy a high level of estrogens in blood was observed (Kolena et al., 1977), and estrogens potenciate the sensitivity of the lactotrophs to mild stimuli (Leong et al., 1983). In this case the stressogenic effect of the flight could have been vizualized. We assume that the activation of PRL was evoked by the final phase of the flight (overload, landing maneuver) and not by microgravity, for enhanced PRL levels reflect the status of acute stress. After chronic stress no increase in PRL can be observed (Neill,1970). In the offsprings no effect of prenatal flight on PRL was observed.
We conclude that space flight represents a stress stimulus for pregant female rats which becomes manifest by enhanced PRL levels and disturbed rGH pulses. The enhanced PRL levels are due to the last phase of the flight. In the offsprings of flown rats the lessened levels of rGH remain to be clarified.

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THE SYNTHESIS AND CONCENTRATION OF THYROID HORMONES IN RATS AFTER SPACE FLIGHT

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The aim of the study was to investigate the influence of space flight on the synthesis of thyroid hormones in the thyroid gland and their concentrations in serum in adult rat and pregnant rats which delivered babies after the flight. The percentage of 3,5,3-L-triiodothyronine T_3 and thyroxine /T_4/ in the thyroid gland of adult rats after space flight was unchanged. The concentration of thyroxine in rat serum was not changed in all groups studied, however increased gravitation resulted in a significant increase of T_3 in comparison to control and flight group. No marked changes were found in concentration of T_3 and T_4 in thyroid glands of flight mothers and their babies delivered. Space flight appears does not change significantly the thyroid gland activity in rats.

The thyroids of rats returning from space flights showed certain histological alterations suggesting a reduction of the thyroid activity. Five to twelve hours following landing, thyroidal cells showed smaller nuclei, the epithelial layer was attenuated, and the C cells nuclei were smaller in both their volume and numbers. These changes returned to normal within 25 days following the landing. However, cosmonauts returning from 13 days lasting space flight aboard Apollo showed, on the day of landing, elevated plasma concentrations of thyroxine which entirely normalized within 24 hours after the landing (Sheingeld M., Leach C.S. et al. 1975). The authors believed that these results might suggest enhanced activity of the thyroid during the flight rather than changes in thyroxine binding plasma proteins. Similar results have also been obtained with astronauts aboard the orbital station Skylab, the only defference being that following space flights for 28, 56, and 84 days, blood concentrations of T_3 decreased (Leach C.S., Johnoson P.C. 1977). These differences in thyroxine and triiodothyronine levels have been explained as be-

ing due to enhanced activity of the thyroid; the reduced levels of T_3 may result from decreased release from the thyroid into the cicrculation, or from decreased conversion of T_4 to T_3 in the peripheral glands.

The present work was aimed at studying biosynthesis of both the thyroid hormones and their precursors, of thyroxine, triiodothyronine in the blood of adult rats, pregnant females and their litter after the completion of space flights.

The experiments were performed on male rats (SPF Colony, Bratislava), weighing 200-250 g. The animals from Cosmos 936 were decapitated 6 h or 25 days after completing an 18,5 day space flight. Aboard the biosatellite, the rats were kept in individual cages in the state of wieghtlessness. Another group of animals aboard the same spacecraft were also placed in a centrifuge simulating gravitational force of 1 g almost throughout the flight. Another experiment was performed on pregnant females spending 5 days, namely days 13 through 18 of gestation, in space. Following the landing, some of the pregnant females were decapitated, the rest were kept on Earth until delivery, and the litter was sacrificed on days 15, 30 or 100, of postnatal life. The thyroids were removed, freezed in liquid nitrogen, and transported to the laboratory on dry ice. Homogenates were prepared from unpooled thyroids and assayed by pronase hydrolysis, iodinated amino acids were separated by paper chromatography, and hormones in spots vere quantitatively determined. Radioimmunological methods were used in a parallel experiment to determine concentrations of the thyroid hormones directly in the hydrolysates of the thyroid.

Blood concentrations of the thyroid hormones were determined using the radioimmunological technique.
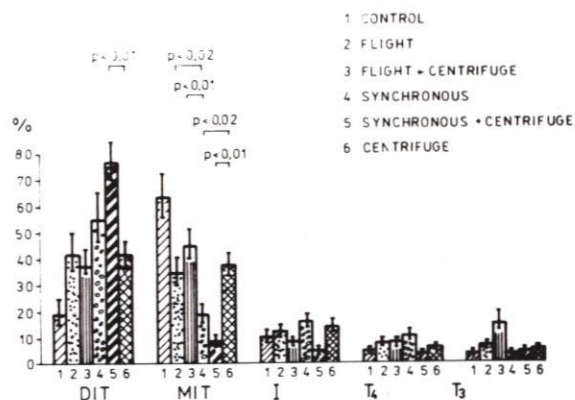
Fig. 1 shows percentual participation of iodinated compounds in the thyroids of adult rats. As you can see, the thyroid hormone precursors, diiodothyrosine and monoiodthyrosine, were significantly elevated in the parallel group subjected to centrifugation. Percentual values of thyroxine and triiodothyronine remained unchanged in all the age groups studied. However, the iodothyrosine /iodothyronine ratio which reflects synthesis of the thyroid hormones, was found to be decreased as compared to controls/. These results suggest that the thyroid hormone biosynthesis was reduced.
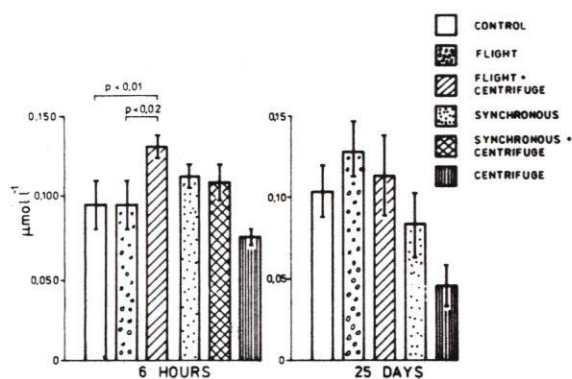
Fig. 2 shows that thyroxine concentrations in the blood of rats exposed to gravitation during the flight were elevated immediately after the landing as compared to those measured in controls and in animals having spent the flight under conditions of weightlessness. No differences in thyroxine concentration were detectable 25 days after the landing.
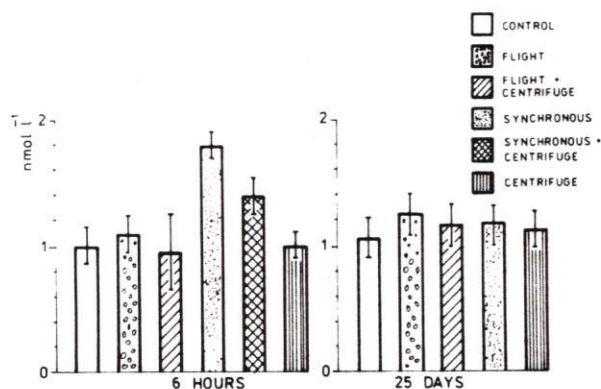
It is clear from the Fig. 3 that triiodothyronine concentrations were identical in the blood of rats of all the groups examined with the exception of the synchronous control that showed elevated concentrations of this hormone.

Effects of a 5-day space flight were studied in another experiment performed with pregnant females and their litter delivered after the landing and sacrificed on days 15,30 and 100 of the postnatal life.

Tab. 1

T_3 ng/mg protein

	mother	15 days	30 days	100 days
flight	500+120	20+5	20+ 6	180+40
control	350+ 70	40+4	30+ 4	80+40
synchronous control	110+ 20	40+4	60+13	200+ 4

This table 1 shows results of T_3 determinations in the thyroids of rats of various age and in the thyroids of the mothers. T_3 concentations in the thyroids of mothers participating in the space flight did not change statistically significantly as compared with control animals. A statistically significant increase was found between the synchronous control group on one and the mother control and flying group on the other hand.

The thyroids of the young during the postnatal period showed significantly decreased concentrations of T_3 in 15-day-old animals as compared to both the control and the synchronous group. Lower T_3 concentrations were observed also in the thyroids of 30-day-old animals as compared to the synchronous group.

Tab. 2

T_4 ng/mg protein

	mother	15 days	30 days	100 days
flight	1500+420	250+50	80+ 20	270+120
control	1400+330	200+20	120+ 40	150+ 80
synchronous control	210+ 9	140+40	260+110	230+ 80

The concentrations of T4 (Table 2) in thyroids of mothers flying in the space as well as those in the control group were higher as compared to the synchronous control. The young of these mothers did not show considerable differences in T_4 concentrations in the thyroid.

It is generally known that the hypothalamo-pituitary system activity is enhanced in rats between days 12-16 of the postanal life. Also, changes in specific activities of iodinated thyrosines and thyronines have been reported. Changes in concentrations of both T_3 and T_4 in mothers and the young of mothers flying in the space are statistically significant; They however do not allow any unambiguous interpretation. It should be noted that the hormone production itselfdoes not say anything about the metabolic activities of these hormones in the tissues, and that the mechanism of activity starting at the receptor level in the cell nucleus is rather intricate. Moreover, during the postnatal development, organs and tissues of rats undergo considerable changes in their structure and functions with the thyroid hormones also playing a role in them.

It may be concluded based on our results obtained adult rats and their voung that the production of T3 in the thyroid decreases during space flight and that these changes are not prolonged and are reversible.

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EARLY PLASMA ATRIAL NATRIURETIC FACTOR
CHANGES IN THE RAT DURING ANTIORTHOSTATIC
HYPOKINETIC SUSPENSION.

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Abstract -

To investigate the releasing mechanisms of
atrial natriuretic factor (ANF) during
weightlessness simulation, rats were suspended
of 1, 2, 6 and 24 hours. At the same time,
plasma and atrial immunoreactive ANF (IR-
ANF) were measured using a radioimmunoassay.
Plasma and atrial IR-ANF increased after two
hours of suspension, and returned rapidly to
the basal levels at 6h. These results suggest
that ANF may contribute to the natriuresis
reported in weightlessness.

Introduction

Within the first few hours of head-down
bedrest, fluid shift from the extremities to
the thorax and head leads to an increase in
central venous pressure. Simultaneously,
increased pressure levels in both atria leads
to hormonal modifications characterising the
Gauer-Henry reflex (4) : inhibition of
secretion of the renin-angiotensin-aldosterone
system (RAAS) and vasopressin (AVP). In
addition, another factor appears to play a
role : the atrial natriuretic factor. It has
been speculated that ANF is involved in the
regulation of extracellular fluid volume and
electrolyte balance (3, 1). In man, this fluid
shift is well documented. In animals,
specially rats, the tail suspension model
(antiorthostatic hypokinesia= AOH) is a well
documented maneuver to simulate weightlessness
(2). For these reasons, we investigated
whether the ANF synthesis and release are
modified during early adaptative response to
weightlessness.

Methods

Male Wistar rats (230-250 g)(Iffa Credo)
were used. They arrived in the vivarium 9 days
prior to the experiment. Thereafter, they were
divided into groups: twelve population cage
(PC), 12 isolated in separate cages (I), 24
were attached by the tail using Morey's model
(11) as modified by Roberts (12) : A plastic
hole drilled disc was wrapped with adhesive
tape around the tail and connected to a
pulley plastic bar. These rats were remained
in the horizontal position (attached
horizontal = AH) for 7 days before the test.
On the eighth day, after the adaptation
period, half of the rats which were attached
horizontal, were suspended for 1, 2, 6 or 24
hours (a head-down suspension of $30-35°$ was
used). Then rats were anesthetized with
pentobarbital sodium (60 mg/kg body weight
i.p.). Blood was collected by aorta puncture.
One ml, taken separately, was processed for
plasma Na^+, K^+, protein and osmolality
analysis. The micro-hematocrit was also
measured. Two ml of blood were placed in ice -
chilled plastic tubes containing the pro-

tease inhibitors EDTA (10^{-5} M), pepstatin

(10^{-5}M) and phenylmethylsulfonil fluoride
(PMSF 3.10^{-5} M) for plasma IR-ANF
determination (99.126). ANF was extracted from
plasma by means of Vycor glass beads (Corning
Glass Works Corning, WK) and measured by
radioimmunoassay (RIA) (6). The heart was
removed immediately after sacrifice. The right
and left atria were dissected separately and
atrial ANF concentration were measured by RIA
(7). The data were evaluated by two way
analysis of variance with repeated measures to
globally test the time effect, the group
effect and the group interaction by time
(F.test). If a significant effect was
detected, Newmann and Keul's test was used to
determine which treatment means were
significantly different. Results are expressed
as means±SEM.

Results

No difference was found in plasma sodium,
potassium, proteins, osmolality and hema-
tocrit. After two hours of suspension, plasma
ANF concentration was significantly higher in
AOH rats than after one hour of suspension,
being 16.6±2 pg/ml in the former and 10.9±1.5
pg/ml in the latter ($p < 0.05$). A significant
increase was also noted between the AOH, AH
and I rats at 2 hours of suspension (16.6±2
pg/ml; 7.5±1 pg/ml and 7±1 pg/ml respectively)
Six hours after suspension, no difference
between the three groups was found. For all
experimental stages, plasma ANF in PC was
significantly higher than in the other 3
groups ($p < 0.01$). The atrial ANF concentration
was higher in the right atrium of AOH rats

after 2h of suspension compared to 1h of suspension. No difference in the left atrium was noted between any of the groups.
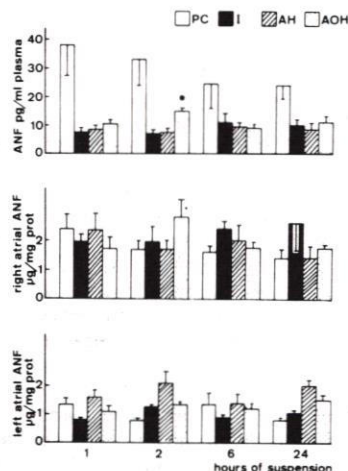
Effects of AOH suspension on plasma ANF and right and left atrial ANF concentrations.

Discussion

To further examine the potential participation of ANF in adjustments to fluid volume, we studied the effects of acute simulated weightlessness on plasma and atrial ANF. The early hormonal changes induced by simulation studies in the rat are not well documented. We have also reported (5) in a head-down simulation at 9° an increase in ANF plasma level in human. This plasma elevation was very rapid (maximum at 30 minutes) and by 1 hr plasma ANF had returned to pretilt levels. We recorded an increase in plasma IR-ANF concentration after two hours of suspension compared to orthostatic hypokinetic or isolated animals. This increase in plasma ANF was transient. A higher ANF concentration was observed in the right atrium after two hours of suspension compared to one hour of suspension. It has been demonstrated that the release of ANF directly correlates with changes in right atrial pressure in water immersed rats (9). Volume expansion, by enhancing venous return, could elevate right atrial pressure and increase the concentration of right atrial ANF. Thus, these results are consistent with those of Shellock et al concerning central venous pressure changes in the rat during the head-down suspension.(13)

The second problem of this study was to choose an appropriate control. We have previously reported (personal results) that the ANF plasma of rats housed five in a standard cage was much higher than in those isolated in plastic cages. The baseline values in orthostatic hypokinetic or isolated rats are lower than those reported by some investigators (10, 8). Our baseline levels in rats housed five in a standard cage are very similar to those reported by Horky (8) in anesthetized rats. Whether the stress of the rats housed together caused elevations in

baseline plasma ANF levels remains to be clarified.

In summary, the antiorthostatic hypokinetic rat therefore appears to be a good model for the study of the hormonal system during orthostatic manipulations.

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SPECIFIC BINDING OF ATRIAL NATRIURETIC FACTOR
TO RENAL GLOMERULI DURING DAY OR NIGHT ANTI-
ORTHOSTATIC-HYPOKINETIC SUSPENSION.
(AOH)

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Abstract -

The purpose of this study was to compare the
day and night atrial natriuretic factor (ANF)
responses to AOH and to determine whether the
renal glomerular ANF receptors are modified by
the duration of AOH. We observed a significant
increase in plasma ANF in AOH rats after 2 h
of day or night suspension. The glomerular ANF
receptor population was slightly lower in AOH
than in attached horizontal rats (AH). During
night ANF receptors were significantly redu-
ced in AOH as compared to AH animals. We
conclude that the attenuation of the
natriuretic and diuretic response during
night is associated with elevated plasma ANF
levels and decreased ANF receptor density.

Introduction

There are three main hormonal systems
involved in blood volume regulation :
arginine-vasopressin (AVP) renin-angiotensin-
aldosterone system (RAAS) and atrial
natriuretic factor (ANF). We observed (6) in
man, for head-down tilt during night and day-
time the same inhibition of AVP and RAAS and
the same increase in ANF. But the natriuretic
and diuretic responses are very different in
primates when this manoeuver is produced
during day or night time (9). Therefore, the
three main hormonal systems involved in blood
volume regulation are not directly involved in
the attenuated renal response during night.
Specific receptors of ANF have been
demonstrated in rat renal glomeruli (1, 2) and
may vary inversely with circulating levels of
ANF. The antiorthostatic hypokinetic
(AOH) model is currently used in rat for
simulating weightlessness (4).

For these reasons, we investigated whether
the density and affinity of ANF glomerular
receptors are modified differently during day
and night in the rat. Another problem was the
choice of controls for such experiments where
stress could play a major role.

Materials and Methods

Male Wistar rats (230-250g) (Iffa Credo)
were suspended in individual cages using
Morey's tail suspension model (10) as modified
by Roberts (12) After 2 hours of suspension
animals were sacrificed by sodium pento-
barbital anesthesia during the day or night.
Blood was withdrawn by aorta puncture and
kidneys were excised from the renal capsules.
Blood and kidneys from orthostatic rats (AH)
or rats was housed five to a standard cage,
designated Population cage (P.C), were used to
determine control parameters. ANF was
extracted from plasma with Vycor glass beads
and measured by radioimmunoassay (RIA)(7). To
determine glomerular ANF receptor density and
affinity, glomeruli were isolated from the
kidneys (3). The binding assay was performed
as described elsewhere (5).

The results, expressed as means $\pm$ SEM, were
evaluated by two-way analysis of variance. "A
posteriori" comparisons were made with Newmann
and Keul's test. The binding data were
analyzed by processing raw data with the
computer-based program EBDA (8)and then the
density and affinity of binding sites was
determined using the computer-based LIGAND
program (11). The results were assessed by the
unpaired Student's t-test and were considered
significant at $p < 0.05$.

Results

Plasma ANF (Table II) was significantly
higher after two hours of suspension during
day and night (19 ± 2 vs 9 ± 1 pg/ml and 18.3 vs
10.2 ± 3 pg/ml respectively, $p < 0.05$). For all
experimental stages, plasma ANF in P.c was
significantly higher than for AOH rats
($p < 0.01$). The number of ANF-binding sites
(Bmax fmol/mg proteins) in renal glomeruli
was lower in AOH after two hours of suspension
(429 ± 12 fmol/mg protein vs 507 ± 5 fmol/mg
protein, $p < 0.05$) during day. During night a
significantly lower number of ANF receptors
was observed in AOH animals (168 ± 2 fmol/mg
protein vs 455 ± 3 fmol/mg protein, $p < 0.001$).
Glomerular receptor affinity (k_d) was
unchanged.
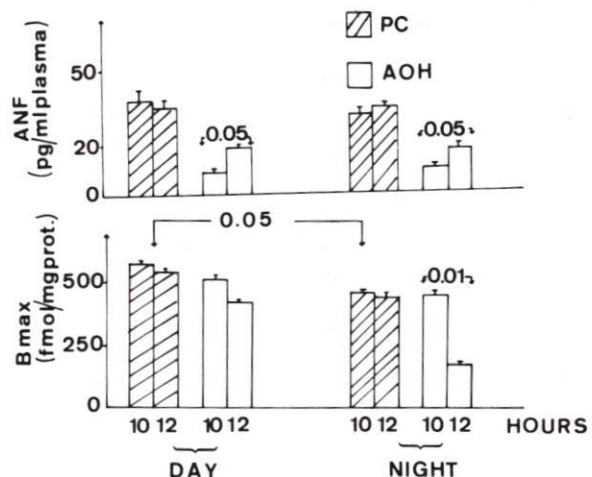
Plasma ANF and ANF receptors in rat renal
glomeruli during day or night antiorthostatic
hypokinetic suspension.

Discussion

The present work was undertaken to define the role of ANF in the development of AOH natriuresis. We have already demonstrated for ANF plasma the same response during both day and night if the same fluid modification occurs (6). The aim of the present study was to compare the ANF response to AOH during day and night in the rat and to determine whether the renal glomerular ANF receptors are involved. During the day, we recorded an increase in plasma IR-ANF concentration after two hours of suspension as compared to that of orthostatic hypokinetic animals (Gauquelin et al, unpublished results). It has been reported that the number of ANF specific receptors in renal glomeruli can be modified by chronic sodium intake and that these changes are inversely correlated with plasma ANF levels (1, 5). We found the same increase during the night as during the day for plasma ANF. We also found that the number of specific ANF-binding sites in glomeruli from suspended rats during the night is lower than in the orthostatic rats. The glomerular ANF receptor population was slightly lower in AOH than in AH during the day. The K_d was the same during day and night. This finding suggests that the regulation of glomerular ANF receptors, by changing the renal responsiveness to ANF, may play an important role, as do plasma ANF levels, in maintaining sodium and water homeostasis.

The second problem of this study was to choose an appropriate control. We have observed (G. Gauquelin et al, unpublished results) that the ANF plasma of the rats housed five in a standard cage was much higher than in those isolated in plastic cages. Whether the stress of the rats housed together caused elevations in baselin plasma ANF levels remains to be clarified.

In summary, we can conclude that the natriuretic and diuretic response not observed during night can be associated with a decreased ANF receptor density.

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EFFECT OF HYPERGRAVITY (2G) ON THE REGENERATION OF RAT LIVER

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It is well known that after 2/3 hepatectomy an increase in DNA synthesis and mitotic activity in rat liver occures with the maximum at 18-24h and 30-36h, respectively. The cellularity of regenerating liver quickly increases during the first 3 days and regeneration is nearly complete at about 21 day after treatment. In the regenerating liver of rats exposed to hypergravity (2G) the concentration of nucleic acids, especially of ribonucleic acid was increased at 24-30h in comparison with non-exposed rats. However, in connection with slower growth of regenerating liver mass the increament in total contents of DNA and RNA as well as the cellularity of organ was lower mainly at the 72h. The inhibition of mitotic activity was evident at 30h after partial hepatectomy. Exposure to hypergravity had no significant effect on the ratio of prophase to metaphase counts and chromosomal abnormalities.

Introduction

It is well known, that the proliferative activity of cells in intact liver of adult rats is on a very low level. Most cells in normal adult liver are in a reversible resting stage G_0, since they maintain the ability to DNA synthesis and mitotic activity increasis synchronously with a maximum at the 18-24h or 30-36h, respectively (1,2). The most intensive increas in cellularity occurs within first 3 days and full regeneration is achieved at about day 21 after surgery (3).

There fore liver regenerating after partial hepatectomy (PHE) is often used as a model for studying process of growth under various experimental conditions.

In this work we have investigated the effect of hypergravity (2G) on liver regeneration of rats within the first three days after PHE. We have investigated liver weight, quantitative changes in DNA and RNA, cellularity, mitotic activity and chromosomal aberration frequency.

Methods

Male rats of Wistar train weighting about 220g were used in experiment. 2/3 partial hepatectomy according to standard method was performed. One part of animals was centrifuged immediately after the PHE with acceleration of 2G, the second part served as controls. Rats of both groups were examined 24, 30 and 72 hours after PHE and hypergravity or PHE alone. Quantitative changes in DNA and RNA were estimated by the method of (5). Mitotic activity and frequency of chromosomal aberrations were determined by evaluating squash slides stained according to Feulgen method. Cellularity per mg of tissue and total cellularity of liver was investigated by counting of nuclei in suspension using a Coulter Counter model ZF.

Results and discussion

In regenerating liver of rats exposed to hypergravity the concentration of nucleic acids namely of RNA (Fig. 1) was enhanced of first two intervals compared with that after PHE alone.
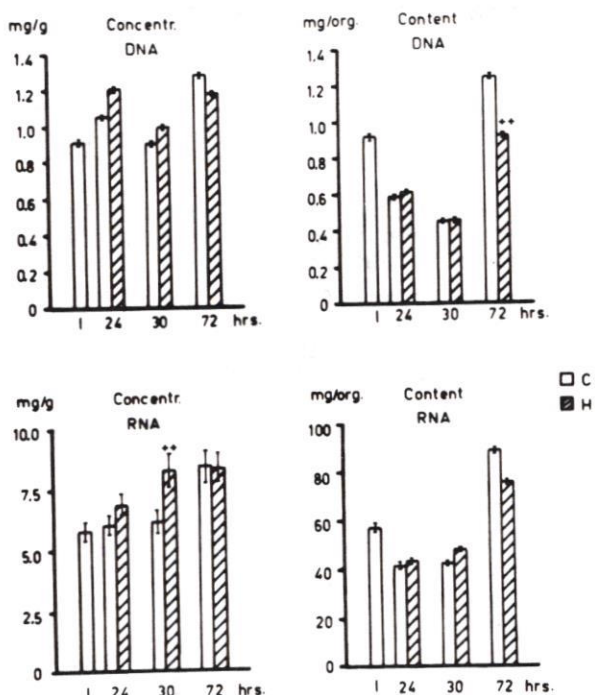
Fig. 1. Regenerating liver of rats after partial hepatectomy. I - intact liver; C - control; H - hypergravity (2G).

Total DNA and RNA contents were lower than control values especially at hour 72. Lower nucleic acid content at this time was caused by slower increase in the weight of regenerating liver.

For the same reason (i.e. for slower increase in regenerating liver weight) the total cellularity per organ in particular at hour 72 was more influenced by hypergravity than cellularity per mg (Fig. 2).
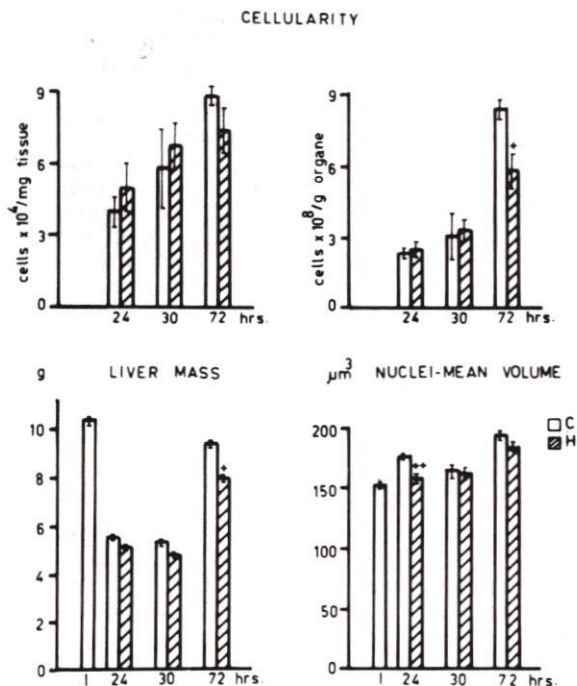
Fig. 2. Regenerating liver of rats after partial hepatectomy. I - intact liver; C - control; H - hypergravity (2G).

Nucleus mean volume in rats exposed to hypergravity didn't increase within 24 hours contrary to the controls.

Mitotic index of regenerating liver in control animals increases rapidly from hour 24 showing a maximum 25,20 ± 0,51 ‰ at hour 30 after PHE (Fig. 3).
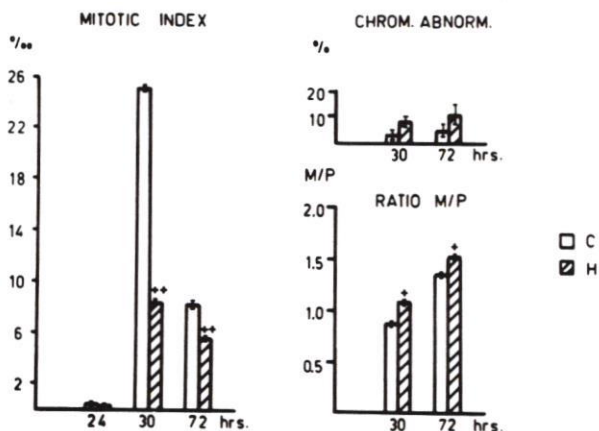
Fig. 3. Regenerating liver of rats after partial hepatectomy. C - control; H - hypergravity (2G).

According to the known schema, till the third day the mitotic index decreases more than by two-thirds of maximal values. Inhibition of mitotic activity induced by hypergravity was manifested by marked reduction in the number of mitotic figures particularly 30 hours after surgery. In this period mitotic index was reduced by two-thirds compared to control values.

This is in agreement with findings of (4). They have found delayed start of mitotic activity and mitotic index value reduced to one-third or one-quarter 28 hours after treatment.

Increased metaphase to prophase ratio indicates the metaphase prolongation caused by the hypergravity. Changes in number of chromosomal aberrations in post metaphase were not significant.

Conclusion

Presented results suggest, that shortly after PHE hypergravity can activate synthetic processes wich is manifested by increased nucleic acid concentration. However a remarcable inhibition of mitotic activity was found resulting in retardation in DNA and RNA content increament, total cellularity and the weight of regenerating liver in rats exposed to hypergravity at later interval.

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THE EFFECT OF SUPPORT UNLOADING

INDUCED BY MICROGRAVITY IMITATION

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INTRODUCTION

Among the attempts to disclose the ca-
use-and-effect relationships of microgravi-
ty effect on a living organism, the predo-
minant concept at present is that of a lea-
ding role played by cardiovascular and ves-
tibular shifts.The role of motor system is
still poorly known. However, the close in-
terrelationships among vestibular, motor
and visual systems suggest that peculiari-
ties of proprioceptive activity may explain
the major changes in sensory interaction and
vegetative functional support. The most im-
portant of them is the motion sickness syn-
drome which develops during the first ho-
urs and days of manned orbital spaceflights.

Our previous studies with microgravity
simulation by dry immersion showed that sup-
port unloading in this case caused a decre-
ase in muscular tone and in muscular cont-
raction strength,as well as muscular hypper-
reflection and coordination disturbances.
All this decreased the accuracy of reprodu-
ced muscular efforts and changed the biome-
chanical structure of locomotion (1). The
above symptoms were very similar to those
found earlier during the postflight period
in the cosmonauts after 7-day flights onbo-
ard the "Saljut-6" station (1,2).

Imitation of support "loading" by pe-
riodical and prolonged pressures on the fo-
ot via the "support" device during real we-
ightlessness in one of the crew members ca-
used a marked decrease of motion sickness
symptoms, and, therefore, blocked the expe-
cted microgravity effects during the post-
flight period (2).

We were able to achieve the closest
imitation of weightlessness effect with the
help of a new microgravity model - costume
immersion. This method allows a new level
of support input unloading with free motion
as well as of oriented vertical and hori-
sontal rest during up-to-the-neck water
immersion in a hydrocostume with an inflat-
ed head restrain.

Besides, it should be taken into acco-
unt that in performing specific integral
motion programs, as well as, in mechanisms
of sensoryfunction shifts adaptation an im-
portant role in ensuring sensory interactions
is assigned to the cerebellum (3-5).

The aim of the present study was to
determine the effects of support unloading
during microgravity simulation by costume
immersion, to compare them with those ob-
served during real weightlessness and with
pathological disturbances of propriocepti-
on and cerebellar control.

METHODS

The motor effects of microgravity we-
re studied in primates (Rhesus-monkeys)
during flights along parabole of Kepler
(pK) and onboard the "Cosmos-1514" biosate-
llite. Two months before the weightlessness
experiments electrodes were placed for re-
gistering ECG,EEG,EOG in two leads, as well
as EMG of flexor and extensor muscles of
the lower limbs. The fixation systems allo-
wed for sinusoidal swinging of the animal
in the vertical plane on-board the aircraft
and the biosatellite. During parabolic
flights weightlessness lasted about 20-30s,
such seances were repeated 10-12 times, the
duration of exposure on board the biosate-
llite was 5 days. Postflight studies of mus-
culo- skeletal system, forcevelocity charac-
teristics of shin muscles, orthostatic to-
lerance (as shown by stabilography) in
cosmonauts were carried out on 22 crew mem-
bers of " Saljut-6 " and " Saljut-7 "stati-
ons. These research methods were described
in more detail earlier (6). Stabilographic
studies were carried out on 12 patients
with motor disturbances and cerebellar ata-
xy. They were compared with a control group
of 15 healthy males from 24 to 34 years of
age.

The costume immersion studies were do-
ne in 10 male volunteers of medium physical
fitness, aged from 24 to 34. The total time
of balanced floating rest (the horisontal
posture) and of semi-immersion (the verti-
cal posture) was 72 hours. Before and after
costume immersion (CI) stabilographic data
were studied as well as corrective respon-
ses of locomotor tests (walking on a soft
and firm surface); the status of the ves-
tibular system was evaluated by EOG, senso-
ry and vegetative responses to a standard
acceleration of Coriolis and by the nature
of vestibulo-motor interaction during gaze
fixation reactinos.

RESULTS

Effect of real microgravity on motor and
vestibular reactions. The transfer of the
monkeys to weightlessness during parabolic
flight was accompanied by marked changes in
EMG muscle activity in the hind limb; a
high-amplitude (up to 800 mkV) component
of burst activity of up to 600 ms duration,
followed by moderate activation which un-
derlay repeated short bursts of decreasing
amplitude. The total duration of the EMG
reaction was 3-3.5 s (Fig.1). During repe-
ated weightlessness seances the amplitude
of EMG reaction decreased, but their dura-
tion was almost unchanged. With the eyes
closed, the amplitude of EMG activity mar-
kedly increased. EOG during the first weigh-
tlessness period registered single weak
nystagmoid movements of up to 50 mkV ampli-
tude, which after 6 or 7 seances become
marked high-frequency (up to 20 b/s) nys-
tagmoid reaction of up to 80 mkV amplitude

and total duration of about 2.5 or 3s (Fig 2 A).
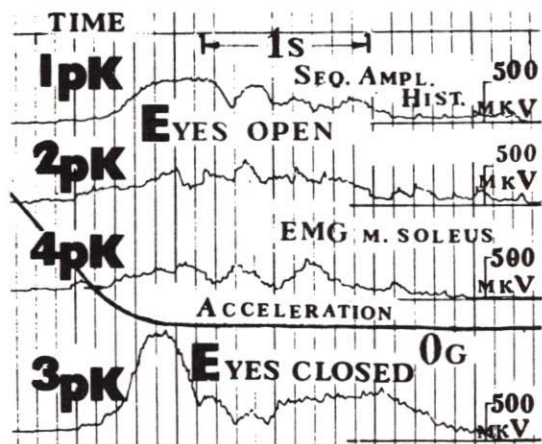
Fig. 1. Changes in EMG activity of monkey as a result of repeated effect of microgravity. 1-4 pK - numbers of parabolas of Kepler. The horisontal line - Seq.Ampl. Histogr. of EMG activity.
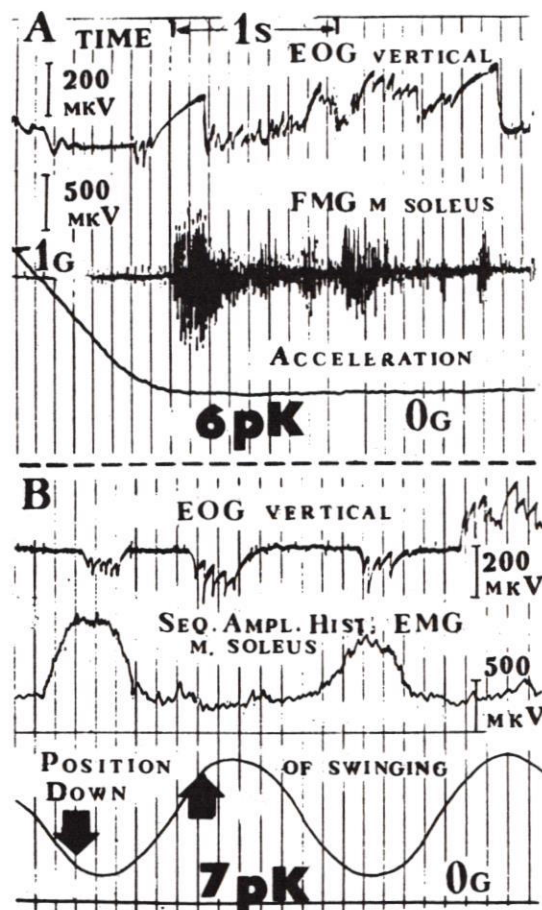
Fig. 2. Effect of repeated real microgravity - A and vestibular stimulation - B on pattern of EMG and EOG activity of monkey. 6,7 pK - numbers of parabolas of Kepler.

The EMG responses to vestibular stimuli (swinging) during microgravity were significantly facilitated, particularly if registered with initially increased muscular activity. During swinging we observed synchronous modulation with periods of maximal accelerations that appeared during the downward movement of the animal capsule (the tailward direction).Simultaneously there appeared nystagmoid eye movements on the EOG (Fig.2 B).

The latency period of the lift reaction in the monkey flown on-board the biosatellite was practically unchanged for the posterior muscles (72 ms vs 69 ms preflight) and somewhat increased for anterior muscles (70 ms vs 58 ms baseline).Latencies of the second and the third components of the EMG responses of the lift reaction increased markedly - to 133 and 144 ms vs 80 and 112 ms baseline, respectively.
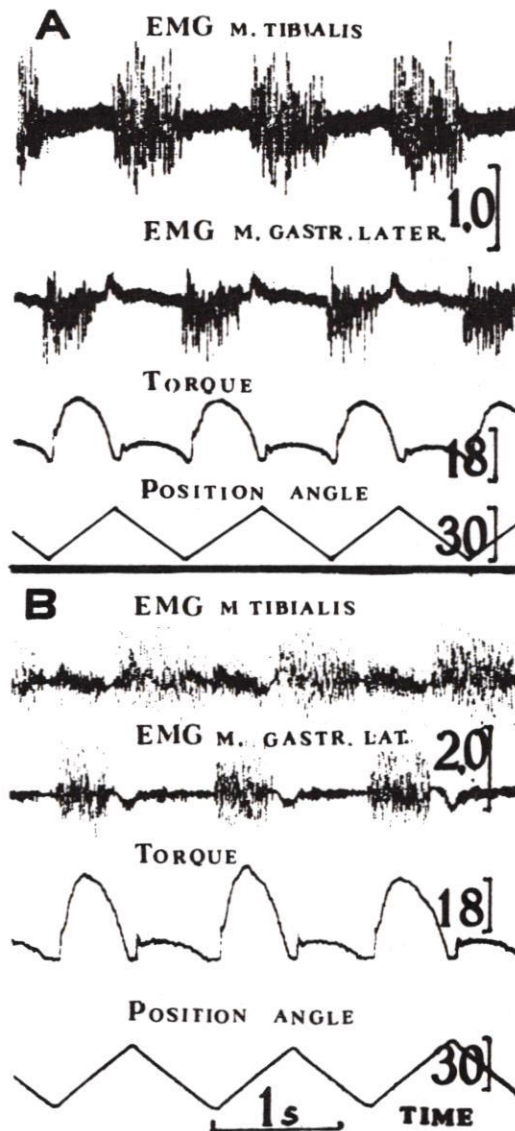
Fig. 3. EMG activity and forcevelocity characteristics of lower limb muscles in a participant of "Saljut-6" expedition: A-pre and B-post flight periods.Testing with the isokinetic "Cybex" dynamometer with the velocity of 120 deg/s.Cal. for EMG-mV;torque--ft/lbs; velocity-deg/s; position angle of the foot-deg.

The effects of 7-day microgravity on the cosmonauts' motor system were seen in a

lowering of the muscular tone, hyperreflexion of both skeletal and muscular reception.The skeletal reception thresholds, as shown by perception of foot zones vibration,were lowered more than twofold.There was a rise of muscular sensitivity thresholds as shown by Achillov tendon reflex parameters.During isokinetic dynamometry there was a decrease of maximal moments of force in the gastrocnemius muscle by 20-30 per cent in all test ranges.EMG showed the myographic cost of the effort of graded exercise increased 1,5-2,0 times (Fig. 3). In some cases the EMG of anterior muscles during exercise with test loading showed coactivation of the antagonist muscles. This activity was lower than their own EMG exercise responses, but it reflected constant participation in antagonistic cycles, causing easy fatiguability (Fig. 3 B).

EYES CLOSED
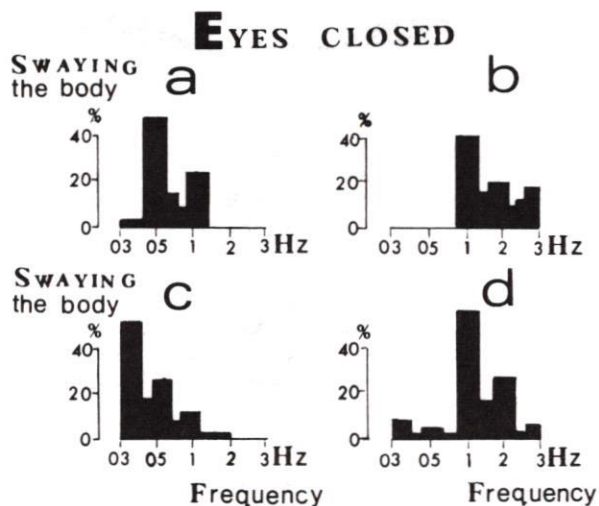
Fig. 4. Effects of real and imitated (CI) microgravity on human vertical tolerance in the Romberg position.The frequency spectrum of general center of gravity oscillation.On (a,b)-7 days pre- and post- space flight; (c,d)-before and after 3 days of CI.

These effects were short-term: they were maintained till the second day.A decreased orthostatic tolerance (as shown by stabilography)(Fig. 4 a,b) was seen in standard postures, which was characterized by an increased oscillation amplitude of the general center of gravity (GCG) from 0,5-1,0 Hz to 3,0 Hz.The shifts of posture regulatory mechanisms were reflected in an 1,5-fold increase of baseline EMG activity in shin muscles.On closing the eyes and assuming the Romberg posture EMG activity grew still higher, and muscular tremor appeared.

A study of vestibulo-motor reactions after the flight was followed by an increase of vestibular canal excitability indicated by an increase of the gain of vestibulo-ocular reflex.

Effect of imitated microgravity on motor and vestibular reactions.During the 3-day exposure to CI all the subjects showed marked vestibulo-vegetative disturbances,

including extreme manifestations (vomiting, aversion for food).These symptoms were close to those seen during the acute period of adaptation of cosmonauts to weightlessness.During the first hours after CI, as studies in the vertical position in standard postures (free posture, Romberg's posture with open and closed eyes) show, there was a marked decrease of orthostatic tolerance.This was manifested by an increase in the amplitude of GCG oscillations in the frontal and sagittal planes, the magnitude of high-frequency (1-2 Hz) oscillations, growing in number and amplitude.The histogram of frequency oscillations in the free posture with the eyes closed (Fig. 4 c,d) shows a resetting from frequencies of 0,3 - 0,5 Hz,characteristic of the baseline, into the frequency range of 1,0 - 3,0 Hz.The decrease in tolerance was characterized by a marked increase (up to 60-80 mkV vs 15- 20 mkV) in EMG cost of the vertical posture with predominating activity in anterior shin muscles.When closing the eyes and assuming the Romberg posture vertical tolerance became still lower: there was a loss of eqvilibrium with swaying in the frontal plane in 10-15 s, and the EMG cost of the posture increased to 80-100 mkV (Fig. 5).
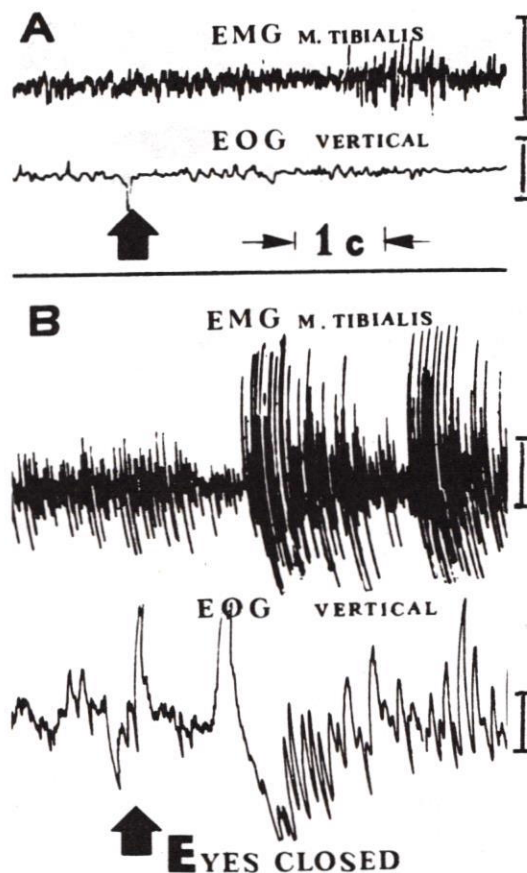
Fig. 5. The pattern of vertical tolerance as shown by EMG and EOG activity before-A and after -B 3 day CI.Testing of the standard posture (Romberg) position.Cal.-200 mkV

It should be noted that EOG responses in the baseline period during vertical tolerance test, were insignificant.There were only solitary low-amplitude eye deviations in the vertical plane (Fig. 5 A).After CI in the Romberg posture and locomotor tests with the eyes closed we registered marked nystagmus reactions in the vertical plane.They were clearly correlated with anterior muscle reactions to maintaining the vertical position (Fig. 5 B).The amplitude and duration of these EOG reactions reflected individual sensitivity of the subjects to the Coriolis acceleration tests.
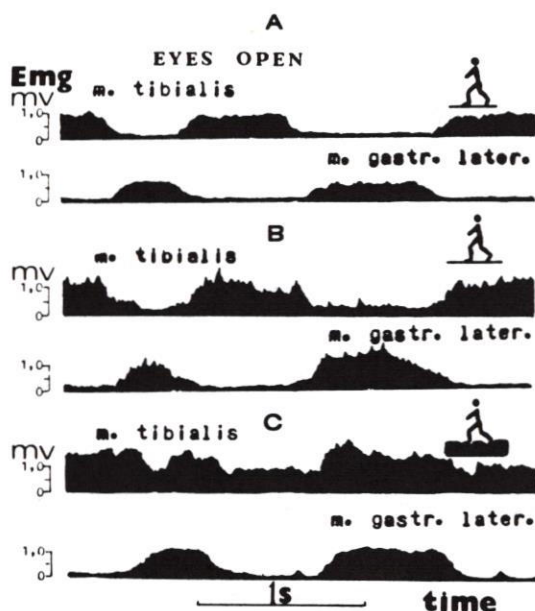
Fig. 6. EMG activity in shin muscles during the walking test on a firm and soft surface A- before and B, C- after CI.The horisontal line - Seq. Ampl.Histogr. EMG activity.Tne right-hand symbols show the degree of firmness of support.

After CI locomotor reactions (walking tests with different degrees of firmness of support) with the eyes opened and closed,were significantly changed.All the subjects showed an increase in EMG cost of the motor cycle with a greater shin muscle EMG amplitude (up to 1,5-2,0 mV) and duration (up to 900-1 200 ms)- control values 0,9-1,1 mV and 500-600 ms respectively (Fig. 6 A,B).A major CI effect was levelling of amplitude of EMG responses when walking on a firm surface alternated with a soft surface.During the baseline period the transfer from a firm support onto a soft one was differentiated by support perception; the response EMG on soft surface was always higher by 200-300 mkV than on firm surface.After CI this differentiation was qone, suggesting a change in support perception thresholds.Walking with the eyes opened on soft surface was accompanied by clear sinphasic response of anterior shin muscles with an amplitude up to 1,2-1,5 mV (Fig. 6 C).
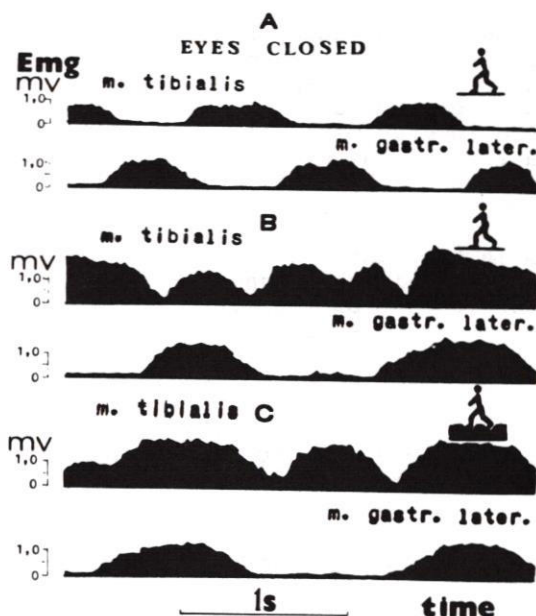
Fig. 7. Relationship of shin muscles EMG activity with visual control during the walking test.A -before and B,C -after CI. (For symbols see Fig. 6).

With the eyes closed, all the subjects showed uncertain shaky gait; synphasic EMG responses of anterior shin muscles were seen when walking both on soft and on firm surface (Fig. 7 B,C).

Posture regulation in patients with motor function disorderes.Vertical tolerance as shown by stabilography in healthy subjects was small GCG oscillations in the frontal plane.There were no significant differences between stabilograms reflecting the initial positions in the free posture and in the Romberg posture.Closing the eyes increased the differences both for the frontal and sagittal stabilograms.In spite of the significance of mean velues, the response of GCG oscillations to closing the eyes was variable.The stabilographic index (per cent ratio of mean amplitudes of GCG oscillations with the eyes closed and opened for each of the planes) was 120% for the free posture and 140% for the Romberg posture.

In patients with motor disturbances and cerebellar ataxy the baseline values of the amplitude of GCG oscillations with the eyes opened, as shown by stabilography, were greater and there was no significant differences between the two postures (Fig. 8 B,C).Closing the eyes caused a significant increase in the mean values of the amplitude of GCG oscillations (from 6,0-6,5 to 11,0-13,0 mm) in both planes (Fig. 8 B,C).Along with the growth of the amplitude of GCG oscillations, there were changes in the frequency spectrum of stabilografic responses.During the baseline study with eyes opened the predominating frequency was 0,3-0,7 Hz (like in healthy persons), and with the eyes closed the freq-

uency of GCG oscillations grew significantly up to the 1,0 - 3,0 Hz range (Fig. 8 B,C).The responses to closing the eyes were much more pronounced in subjects with motor disturbances, including cerebellar ataxy.This was reflected in stabilographic index values which were 160 and 210 % (the frontal and sagittal planes, respectively) for the free posture, and 180 and 230% for the Romberg posture respectively.
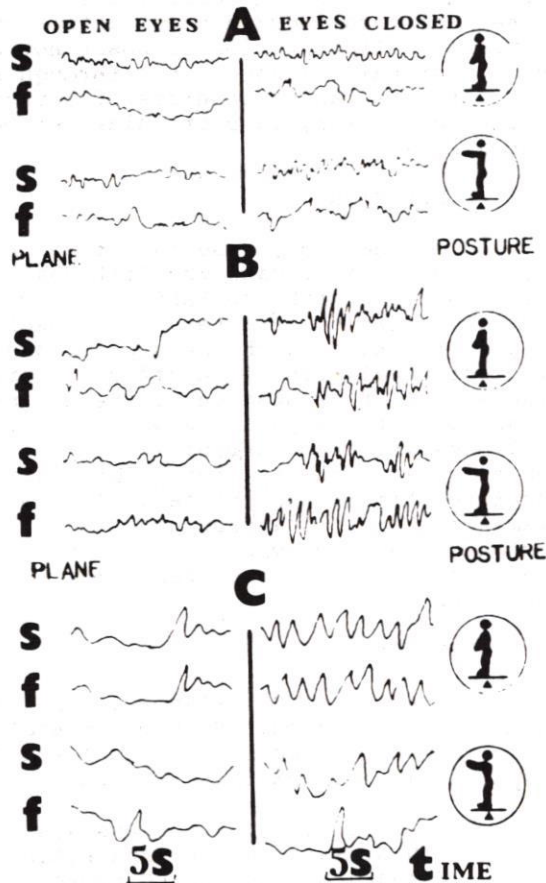
Fig. 8. The effect of visual control on the vertical tolerance pattern in standard postures A- control group, B- patients with cerebellum disturbances, C- disturbances of proprioception.The circles show the position tested.(s,f)-sagittal and frontal planes, as shown by stabilogram.

DISCUSSION

The results of animal studies during natural microgravity show that the irregularity and inadequacy of motor and behavioral responses seen on the EMG during the early period, vanish rather quickly. The absense of visual control of the body position aggravates motor disorientation and deteriorates the adaptation processes. However, the nature of adaptive changes is different for different sensory systems. For the motor system, each subsequent seance of microgravity (pK) is characterized by a transfer from stormy muscular hyperactivity to the development of efforts or movements of a more adequate EMG cost. In

the vestibular system, on the contrary,there were growing changes testifying to a rise of the threshold of its sensitivity.The joint analysis of EOG and EMG data showed that beginning with the 5-th or 6-th weightlessness seances, with already marked adaptation of motor reactions, there appear at first isolated nystagmus eye movements then clear nystagmus reaction, accompanying the EMG reactions to postural changes. It can be supposed,that during the acute period of adaptation, such a growth in vestibular sensitivity reflects not so much in the vestibular system itself and in its receptors as a decrease of proprioceptive input with no support and gravity. The inhibitory nature of proprioceptive effects on the vestibular system under normal conditions was noted by Gernandt (7) during his electrophysiological research studies of systems of spinal control of motor reactions.Ito (8) obtained the same results on a vestibular nuclei level. Results of the "Support" experiment the Cuban cosmonaut T.Menzes during a 7-day space flight confirm a biological nature of such interrelations(2). Imitated support by artifical pressure on the receptor zones of both feet,fully suppressed his spatial illusions and vestibular discomfort, caused a sense of weight in the "lower" extremities. The appearence of a "top-bottom" feeling made orientation much easier, and also facilitated movement and work on-board the station. Taking the device away for 1 or 2 hours renewed illusion of a turn-over of the body and caused growing symptoms of vestibular discomfort.

It can be supposed that microgravity alters the initial character of the vestibulomotor relationship which underlies spatial orientation and supporting the body in space. Under these conditions more pronounced motor responses correspond to vestibular stimuli. During swaying of the animal in weightlessness, we noted an accentuation of the EMG response and synchronous modulation with periods of maximal acceleration in the tailward direction, which was practically absent under natural condition. These effects are not limited by the sensory and somatic systems,but also spread to the vegetative system. This is suggested by such phenomena as the developement in most cosmonauts of motion sickness during transfer from the smaller transport vehicle volume to the greater volume -due to additional vestibular stimuli appearing in the process of transition.

The significance of microgravitational shifts in the motor system is seen from the postflight data on cosmonauts and subjects in model experiments. In the absense of gravity it becomes unnecessary to make the usual muscular efforts for maintaining the posture relative to the gravity vector, which removes the objective reason for muscular work under antiphasic (agonists - antagonists) conditions. Adaptation to the new situation of moving during weightlessness was accompanied by the developement of a new type of responses - not antagonistic, but joint. Traces of this shift were seen during the post-effect period in cosmonauts when testing the motor system as coactivation of anterior shin muscle acti-

vity during the posterior muscle responses, in synphasic responses of shin muscles during corrective reaction to maintaining the posture (1,2). The EMG cost of muscular efforts was changed, and quick-velocity motor reactions were lost as inadequate for the new environment. Slow, smooth movements prevailed, which was confirmed in the studies of force-velocity characteristics of shin and hip muscles after flight (2). It should be noted, that in imitating microgravity with dry immersion hypokinesia, we failed to find the described pattern. This was probably due to the peculiarities of this model-hypokinesia and a lesser degree of support input unloading. Studies with our microgravity model - CI - allow a qualitatively greater support unloading without limiting the subjects'movements during many days of floating. On the contrary, these studies allowed the closest imitation of real microgravity. Synphasic responses of shin muscles were monitored during walking on soft and firm surface. This was accompanied by changes in muscular and support reception, and a growth of EMG cost of maitaining the vertical position. The frequency spectrum of GCG oscillation moved into the high frequency range and resembled that seen in cosmonauts. It should be noted that the motor system adaptation to CI was accompanied in most subjects by motion sickness symptoms. During the adaptation period this was seen as involuntary slowing natural motor action and limitation of sharp head and eye movements.

This, CI, without a direct effect on vestibular receptors, created support unloading effect, and allowed imitation of motor and vestibular sensory responses similar to real weightlessness effects. This may cast doubt on a causal relation between motion sickness in cosmonauts and a direct vestibular effect of microgravity. However, adaptation of the motor system to microgravity becomes impossible after removal of the cerebellum (9,10) - a system controlling and regulating coordinatory patterns of muscular contributory activity. During support unloading effects are seen which result from proprioceptive insufficiency. The motor analyzer practically falls out of the evolutionary pattern of proprioceptive vestibular control, facilitating or stimulating inadequate reflexes. Practically this is well seen in patients with disordered deap muscular sense and cerebellar ataxy (multiple sclerosis,tabies dorsalis, Fridreich's disease). Such patients during standing, particularly with the eyes closed, show swaying oscillations of the body, stimulating vestibular activity to compensate for sensory visual and proprioceptive information deficit. The reverse effect is seen with dominant proprio-tactile flows that not only block vestibular activity itself, but also the reaction caused by it: illusions, nystagmus (11), vegetative disturbances (12). In animals maintaining constant contact with the support, the labirynth overturning reflex was fully inhibited, with the eyes both closed and opened (13). In the case of real short-term microgravity, the loss of contact with the support markedly facilitated vestibular and vestibulo-motor reflexes in response to lift motor stimuli (14). However,

prolonged exposure of animals to microgravity on-board the biosatellite did not cause disruption of the functional vestibular reflex chain. As seen from EMG responses of oculomotor muscles and limb muscles, the latency period of lift reactions did not change significantly from the preflight period. Similar results have been obtained earlier during a post-flight study of rats which had been flown on biosatellites (10). But the latency period of these reaction, judged by real multistage movement,was markedly greater than the control values. This means that the labirynth-induced motor action during the post-flight period is upset not because of vestibular changes, but through a muscular effector and mechanisms of suprasegmental regulation, in particular cerebellar mechanisms.

CONCLUSION

Based on the study results, it can be supposed that most of microgravitational changes connected with the labirynth, are pseudovestibular in character. They result from loss of contact with the support and proprioceptive afferentation deficit. This proprioceptive afferentation is the dominating factor in the regulation of many, if not all, vestibular phenamena.

During microgravity, the primacy proprioceptive stimuli and the cerebellar mechanisms of their regulation underlie vestibulo-somatic, - sensory and - vegetative changes, including their extreme form - motion sickness.

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VESTIBULAR - VISUAL CONFLICT DURING STANCE CONTROL AS A SIMULATION OF SOME EFFECTS OF MICROGRAVITY

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Abstract

A sinusoidal galvanic stimulation of the labyrinths and the visual stimulation by sinusoidal tilting room were given in a variable phase relationship. The value of the phase between visual and vestibular stimuli evoking maximal body sway and stressful control of upright posture was examined. The vestibular stimulation had an expressive postural effect in lateral direction more frequently than visual stimulation. Analysis of lateral stabilograms during stance on 10 cm foam rubber with bimodal vestibular and visual stimulations indicates a stronger stimulating effect of the stimuli which are in phase or nearly in phase. In some subjects this simulated sensoric conflict caused a loss of orientation and evoked vegetative effects similar to initial stage of motion sickness.

Introduction

Information about actual position of the body originates mainly in visual, vestibular and somatosensory systems. This integrative afferent information is utilized by the upright postural control system for maintaining the body center-of-mass within the foot support area probably in two modes. First is a dynamic posture stabilization by the control of reflex-like action of muscles to a rapid pertubation of posture. In the centre of our interest is the second mode which includes the static postural adjustment of the equilibrium point of upright posture system. It has been indicated that afferent information is not used significantly for rapid postural control. But the postural low frequency adjustment of body position is highly dependent on visual, vestibular and a proprioceptive feedback (Diener et al. 1986) and therefore is more suitable to be influenced by sinusoidal afferent stimuli (Bles et al. 1977). From this point of view it is interesting that the early (pre 500 ms) EMG characteristics in response to a disturbance in the posture

of the subject remained apparently unchanged after 10 days of weightlessness. However, the late (post 500 ms) response showed higher amplitudes than was found pre-flight (Kenyon and Young, 1986).

In the present study we observed the stressful influence of combined vestibular and visual stimuli given in mismatching mode on both body balance and orientation control systems.

Methods

Effect on postural stability of sinusoidal lateral tilting of visual surroundings together with vestibular galvanic stimulation was studied in 8 healthy subjects (Fig. 1). The tilting room was closed (2.5m x 2.5m x 2m) but for a hole in the centre of the floor which admitted the stabilometer for body sway recording. The subject stood on the stabilometer and he was instructed to stand upright relaxed.
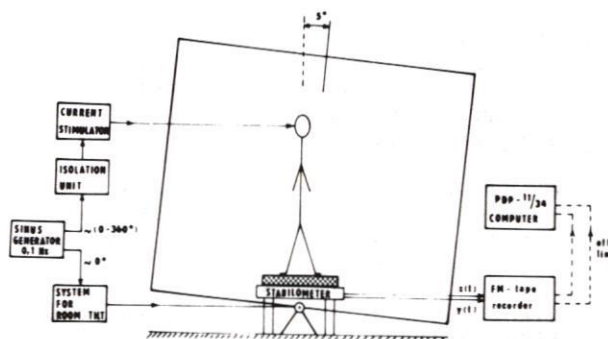
Fig. 1. A block diagram of stance control during bimodal vestibular and visual stimulations with variable phase lag (0 - 360°).

The visual stimulus was a sinusoidally varying lateral tilt of the room with an amplitude of 5° to either side. As a vestibular stimulus we used a bipolar binaural stimulation of the labyrinths by sinusoidal current with amplitude -1mA (peak to peak 2mA). The frequencies of the both sinusoidal stimuli were the same (0.1 Hz). The phase lag between both stimuli was randomly taken from the values: 0, 45, 90, 135, 180, 225, 270 and 315 degree. The measurement for on experimental situation with fixed phase value lasted 50 s. From tape records the variance of anteroposterior (APsd) and lateral (LRsd) stabilograms and power spectral density of stabilograms (PSD) in both direction were computed. Experiment was carried out in four situations of the stance control:

Control - stance without stimulation
GS - galvanic stimulation only
TR - tilting room only
GS + TR - bimodal stimulation with variable phase lag during stance with or without 10 cm thick foam rubber

Results and discussion

A single vestibular galvanic (GS) or visual stimulation by tilting room (TR) evoked an increase of amplitude of body sway in lateral direction. (Hlavačka, and Njiokiktjien, 1985.) This effect was manifested by increase of the lateral stabilogram variance. Simultaneously were registered significantly higher PSD values of lateral stabilograms within the frequency range 0.1 Hz what confirmed a specific influence of the mentioned stimuli on postural control. The galvanic stimulation showed a marked postural effect more frequently than visual stimulation.

During bimodal stimulation (TR + GS) the amplitude of evoked body sway in lateral direction was influenced by the value of the phase lag between stimuli. It is of interest, that the relationship between the value of the stimuli phase lag and the variance of lateral body sway was highly individual. It seems likely, that demand to stabilize upright posture in unusual conditions for the activity of afferent systems iniciates a new and therefore individual solution. Similar fact of the large variations in individual styles for the processing of sensory orientation signals in unusual situation during weightlessness was reported (Young et al., 1986).
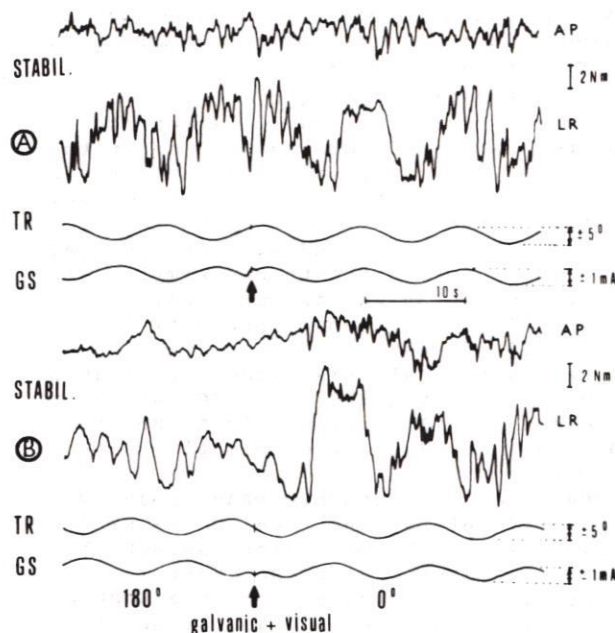
Fig. 2. The records of body sway in lateral LR and anteroposterior AP direction during stance on foam rubber. Common action of galvanic stimulation (GS) and tilting room (TR) is shown on two subjects A, B. The point from which stimuli are in phase (0°) - "disorientation", is indicated by arrow.

To get a more pronounced effect of vestibular - visual stimulation, in experiment with two subjects the lower limb proprioceptive information was reduced by the stance on the foam rubber.

A dramatic sensoric conflict was observed during the stance on the foam rubber in the situation when vestibular and visual stimulations came in phase (Fig. 2). The subjects showed considerable lateral body sway and perceived that the tilting of the room was stopped. This "disorientation" was repeatedly confirmed. When experimental situation was stopped, slight vegetative symptoms of motion sickness were observed.

From our results can be concluded, that the bimodal vestibular - visual stimulation showed a stronger common action on posture control when stimuli were in phase or near to this condition. Loss of orientation during stance control or some vegetative symptoms of motion sickness were observed only when all three afferent systems (vestibular, visual and proprioceptive) were attacked and worked in unusual conditions. Similar unusual activity of afferent systems is all time actual in microgravity and therefore we can consider this type of sensoric conflict as one possible source of space motion sickness.

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FOLLOW UP OF THE GASTRIC EMPTYING (GE) BY ULTRASOUNDS AFTER A STRESS (ROTATING CHAIR). INTEREST OF THIS METHOD IN SPACE DURING SPACE MOTION SICKNESS.

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SUMMARY :

- During motion sickness the gastric emptying can be disturbed before the clinical symptoms (nausea, headache, vomiting) arise. The gastric emptying duration can be follow up and quantified by ultrasound imaging method.
- The experiment try to evaluate the incidence of a physic stress (rotating chair) on the gastric emptying.
The first results obtained on the 8 subjects showing motion sickness symptoms (nausea, retching...) demonstrated a significant increase of the gastric emptying time after the rotating test : 256 +/- 48 mn (157 +/- 22 mn at rest). This parameter allows a quantification of the disturbances induced on the gastric emptying.
- The echographic method would be very usefull to study the gastric emptying during space flight and to test the efficacy of drugs devoted to prevent or to reduce space motion sickness.

INTRODUCTION

- The proximal stomach regulates intragastric pressure and thereby controls gastric emptying (GE) of liquids. Distal stomach retains gastric solids while at the same time triturating them and has a minor role in GE of liquid (3, 4, 7).
- During motion sickness digestion function is impaired more or less. The main symptoms observed are anorexia, nausea, vomiting, pallor, headache... (8). In previous observations it has been reported that nausea always precedes vomiting (rotating-chair test, zero g parabolic flights in aircrafts). On the contrary, during space motion sickness vomiting are frequently sudden in onset without any nausea or malaise before (10).
- The sensory conflict theory (unusual informations given by sensory receptors mainly visual and vestibular) is now accepted as the starting point of motion sickness.
- The mechanism of stimulation of the vomiting center is very complex and remains hypothetic (1, 5, 6). However the vomiting center may be stimulated by different ways like the cathecolamines (epinephrine, dopamines), the vestibular efferences, the vagal nerve... and can disturb the gastric function leading to an increase of the gastric emptying time or to a sudden vomiting..
- The aim of this study is to investigate further the relationship between motion sickness symptoms and induced gastric emptying delay (before vomiting occur) with the help of an echographic method for measuring the GE time.

II - MATERIAL AND METHOD :
    II-1 Volunteers :
8 subjects (5 men, 3 women) between 23 to 38 years old (mean = 28) were involved in the study. None of them reported any gastro intestinal or central venous system desease.
The subjects fasted for at least 12 h before the test.
    II-2 Echographic measurements :
The ultrasound examinations were performed with a high resolution real time scanner (Aloka 256) with a 3,5 MHz linear array.
Like in Bolondi and coll study (2), the cross sectional area of the gastric antrum was measured at two levels : S1 on the sagittal plane passing through the superior mesenteric vein, S2 on the sagittal plane crossing the limit between the antrum and the lesser curvature of the stomach (fig. 1).
For the evaluation of the Antrum volume changes during the test, we used the expression $V = ((S1 + S2) / 2)$.

    II-3 Rotating test :
The test duration was limited to 8 minutes, with a first run of 2 mn on the positive way (speed : 180 °/sec) a second run of 2 mn on the negative way a third run of 2 mn in the positive way, the subject bending over his head each 5 sec and a fourth run similar to the third one on the opposite way.
    II-4 Experimental protocol n°1 GE at rest
- The test meal was made of liquid yoghourt : 375 g 370 ml, 300 cal, 68 % glucides, 18 % lipides 12 % protids, PH = 3,7 to 4,2, 1000 osm per liter of water.
- One basal echographic measurement was performed before the meal. The second measurement 30 mm later showed the maximal distension of the antrum. The echographic measurements of the antrum were performed each 30 mn after this second measure till the antrum volume recovered completly.
    II-5 Protocol n°2 :"GE after a rotation test"
- Same test meal ; one basal echographic measurement before the meal. 20 mn after meal the rotation test began : the test was stopped when the subject mentionned unusual sensations related to motion sickness (nausea, retching) but before any vomiting occured.
- At the end of the rotation (30 mn after the meal), the second echographic measurement was performed. The following

echographic measurements were performed each 30 mn after the second measurement and till the antrum volume recovered completly.

III RESULTS

III-1 Protocole n°1 : "Normal GE"
- The mean time of GE on the 8 subjects was of 157,5 ± 22 mn (fig. 2).

III-2 Protocol n°2 "GE after rotating test"
The mean time of GE on the 8 subjects (256,5 ± 48 mn) was significantly increased in comparison to the normal values (p < 0,001) (fig. 2, 3).

IV - DISCUSSION :

- Till now the motion sickness symptoms were discribed with qualitative terms. Presently the gastric emptying time is an objective parameter to assess and quantify the motion sickness effects even before any clinical sign appears. The significant increase of this parameter on subject under motion sickness may be related to a disturbance of the proximal stomach tone. Moreover this gastric parameter would be very usefull to test the efficacy of drugs against motion sickness.

- The echographic evaluation of the antrun volume is a safety , accurate and reproductible method for the assessement of the gastric function. This mode of investigation of the stomach will probably be of great interest to study the gastric function adaptation to weightlessness. At last, this method is easy to practice in most circunstances espicialy in space stations (9) where ultrasounds has been used for several years.

a) SAGGITAL CROSS SECTION FOR S1 MEASUREMENT

b) CROSS SECTION S1 AND S2

FIG. 1 : EVALUATION OF THE ANTRUM VOLUME (V) BY ECHOGRAPHY (V S2 + S2 / 2).
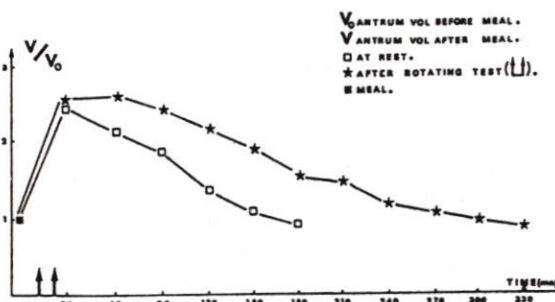
FIG. 2 : MEAN GASTRIC EMPTYING CURVE AT REST AND AFTER A ROTATING TEST FOR 8 SUBJECTS.
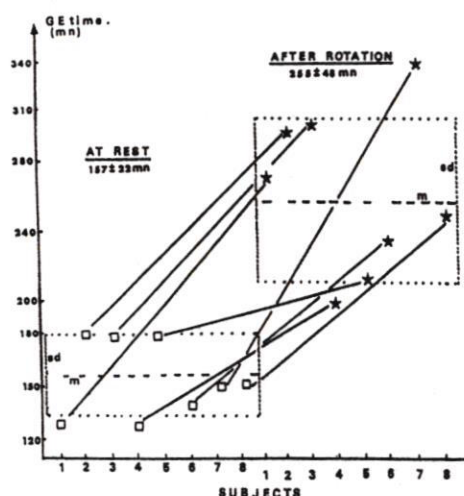
FIG. 3 : VARIATION OF THE GASTRIC EMPTYING TIME FOR EACH SUBJECT AT REST AND AFTER A ROTATING CHAIR TEST.

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CELL MORPHOLOGY AND ULTRASTRUCTURE OF MAIZE ROOT MERISTEM IN MICROGRAVITY

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ABSTRACT

An experiment called "root" was carried out on the biosatellite "Cosmos-1667" in June 1985. The test material consisted of 7-day old maize seedlings.

The following parameters of "flight" and "control" roots were compared: the size of the root cap, the size of the meristems zone, cell numbers in these parts along root axis, the size of periblem cells and that of nuclei in them and, at last, the nucleoplasmic ratio was determined in the cells. Moreover, the mitotic index was defined in periblem cells of the root meristem.

The electronmicroscopic study of the root cap cells took place on the ultra-thin sections.

The cytomorphological and ultrastructural investigations of maize roots grown in flight revealed some difference in their organization as compared with those control.

An experiment called "root" was carried out on the biosatellite "Cosmos-1667" which was functioning in the orbit of the earth in June 10-17, 1985. The experiment was developed and fulfilled by the researchers of the Institute of Medico-Biological Research, Ministry of Health of the USSR. The afterflight investigations of the test material (maize roots) were made at the Laboratories of Biosystematics and Anatomy of Komarov's Botanical Institute, Academy of Sciences of the USSR.

The test material consisted of 7-day-old maize seedlings (roots) grown in microgravity ("flight") and on the earth ("control"). Air-dry seeds were placed in an organic-glass container on a synthetic polyvinylformal-foam substrate (PVFF) put into a device BB-IM. Before the container was placed in the biosatellite and 36 hours prior to the start, 200 ml of water had been poured into it. Thus, the seeds swelled up on the earth and the roots started developing in the first hours of the space flight. After the experiment had been completed, at the landing place,

the fixation of the test, objects was carried out for further investigating with light and electron microscopy.

The investigations were aimed at comparing the characteristics of the root meristem cells and ultrastructure of the root cap cells of maize grown in state of weightlessness and under normal terrestrial gravitation conditions.

MATERIAL AND METHOD

The maize roots to be tested under light microscope were fixed in the mixture of formalin, acetic acid and alcohol (FAAA, at 7:7:100 ratio), dehydrated in alcohols and chloroform and embedded in paraffin. 8 to 11 -thin microtomic sections were put on slides, stained according to Feulgen with an additional alcian blue staining. The measuring of the parameters of tissues and cells, the counts of dividing cells and other procedures were always made under microscope at the least magnification x 900.

The following parameters of "flight" and "control" roots were compared: the size of the root cup, the size of the meristems zone, cell numbers in these parts along the root axis, the size of periblem cells and that of nuclei in them and, at last, the nucleoplasmic ratio was determined in the cells. Moreover, the mitotic index (MI) was defined in periblem of the root meristem.

The size of the root cap and meristem was determined on the longitudinal sections cut through the central part of roots, and namely, that of the root cap was measured from the end of a root up to the cap border and quiescent centre; the basal meristem border was defined in periblem according to the cell extension. The basal border was considered to be the "site" in which cells were twice as long as the remainder ones in the same meristem row. After the meristem borders had been established the mean cell amounts per a row of periblem cells were counted. The cell numbers in a root cap were calculated along the main axis of a root from the end of the root cap up to the border between calyptrogen and the quiescent centre of meristem.

The size of periblem cells (their area) was measured on longitudinal sections, in the 2nd to 6th cell layers of cortex at the same distance from the starting root meristem. On the longitudinal sections, the cells looked like rather rectilinear rectangles, that allowed to determine their area but with two parameters taken into consideration, viz. its height and length along the root axis. Since a cell row had, in fact, the same sectional area throughout the cell meristem, the area of the cells, and not their volume, could, with good reason, be compared in test and in control.

The parameters of 100 and more cells of each specimen were applied to determining their mean size (x) and square error (S x). The surface of nuclei was measured in the same cells. The sections of nuclei looked like rather regular circles, that allowed to calcu-

late their areas according to their diameters ($\frac{D^2}{4}$). The nucleoplasmic ratio was defined as the ratio of nucleus area to cell area. Mitotic index (MI) was determined in the apical part of meristem (from 0 to 0.4 mm) and, in addition, in the periblem of the whole root meristem (from 0 to 1.0 mm). Such determinations of the meristem parameters and meristem cells are, evidently, rather relative but quite admissible for comparative tests.

For the electronmicroscopic study, root pieces were fixed in 3% glutar aldehyde with 0.1 M buffer phosphate, then washed in the same buffer, post fixed in 2% OsO_4 and embedded in eponaraldit mixture. Ultrathin sections were stained with uranilacetate and Pb citrate.

RESULTS

Cytological tests have shown that the roots developed outside of gravitation or, more precisely, in state of microgravitation do not, in fact, differ from those grown under normal terrestrial gravitation (Table 1, Figs.1,a,b). The single evident difference of the roots grown on the biosatellite is an approximately 30% reduction of the meristematic zone (Table 1). These results have also been confirmed with the cell numbers in periblem cell rows from the border between the root cap and quiscent centre up to the starting "point" of the cell extension in the rows, viz. 176 cells in control and 131 cells in "flight". The other parameters of cells and meristem under investigation resemble those in control.

The investigations of mitotic index have revealed a decrease in the number of dividing cells both throughout the meristematic zone and its apical part (Table 2).

The data presented above suggest that, in "flight", the elongation of meristem cells starts somewhat earlier than usually. Meristem cells seem to undergo the elongation more rapidly, i.e. with one division missed, as compared with those in control. This idea appears to be the more especially true as, in state of microgravitation, the other parameters of meristem cells remain similar to those in control.

Two reasons might be responsible for 15 to 30% reduction of mitotic index in "flight" (Table 2): firstly, the diminished proliferation pool and, secondly, a change in the ratio of dividing cells to interphase ones, i.e. an irregular change in their persistence, e.g. accelerated mitosis. The first reason, i.e. 15-30% reduction of the proliferation pool, is unlikely since the release of so many cells from the pool should result in the destabilization of the morphological structure of meristem on account of an irregular cell growth. Some special additional tests should be carried out to establish the true reasons of reducing the mitotic index in "flight".

The meristem cells of the root cap are small and have thin cell walls, nucleus with nucleolus occupying their central part and major volume. In dense hysloplasm, it is possible to discern mitochondria, dictyosomes, small vacuoles, plastids, often leucoplasts without the starch, though sometimes also amyloplasts. At a more distant level, there exists a zone of differentiating statocytes. In this zone, cells, are slightly elongated along the root axis, amyloplasts around nucleus increase in number but they are rather small. The zone of differentiating statocytes is followed by the zone of graviosensitive cells displaying evident polarity in the location of organelles, i.e. the zone of statenchyma. The cells of this zone are more extended, their cell walls thickened. Nucleus is displaced to the proximal end of a cell and often it is lobed. Plastids, i.e. large amyloplasts, are centered under nucleus. The plastids contain complex starch grains, peripheral reticulum is discernible along their envelope. The cisterns of granular endoplasmic reticulum are located between the plastids and cell walls. Vacuoles increase in size, but no central vauole has been found. Mitochondria and dictyosomes are distinctly seen (Figs. 2,3).

Two or three outer cell layers form so-called secretory zone. In secretory cells, nucleus is usually located either in the centre of a cell or near its proximal end. Amyloplasts are situated around of the nucleus, but some plastids occur also in other parts of a cell. Dictyosomes and segregated vesicles sharply increase in number, the ends of dictyosome cisterns strongly being swollen with secretory product. The slime deposits released by dictyosomes are accumulated outside the plasmalemma. They can occupy a significant part of a cell volume, which becomes compressing. Hyaloplasm is very dense in such cells. Vacuolisation is in the progress, a central vacuole being arised. The amounts of granular endoplasmic reticulum increases, some of its cisterns being located along plasmalemma.

In "flight" material, no significant differences have been found out in the meristem zone. In the statenchyma zone, amyloplasts have been found in different parts of a cell. Vacuolization is poor. Nucleus is usually located above plastids. Under the plastids, the cisterns of granular endoplasmic reticulum are discernible. But the amounts of plasmalemmasomes and the number of lipid drops increase significantly in all the above-mentioned root cap zones. Electrodense granules arise in the secretory zone, and especially, in the cells along the root cap border, often along the plasmalemma border on the side of cell walls.

An increase in plasmalemmasomes in the "flight" material suggests an increased membrane material, that may result either from some changes in membrane characteristics or from a disproportion in growth rates of cell walls on one side and plasma membrane.

Table 1. Cytological comparison of the parameters of the maize roots developed in weightlessness ("flight") and under normal gravitation ("control")

	Size of root cap (mm) x + S x	Size of meristematic zone (mm) x + S x	Area of periblem cells (mcm^2) x + S x	Area of nuclei (mcm^2)	Nucleus area cell area ratio
Control	0.36∓0.02	2.1∓0.14	113.4∓4.1	37.7∓1.6	0.33
"Flight"	0.30∓0.03	1.4∓0.14	112.6∓3.6	36.6∓1.2	0.34

Table 2. Mitotic index in periblem of the maize roots developed in weightlessness and under normal gravitation

	Mitotic index measured from the initial meristem border at the distance	
	from 0 to 0.4 mm	from 0 to 1.0 mm
Control	15.3	13.4
"Flight"	10.7	11.2
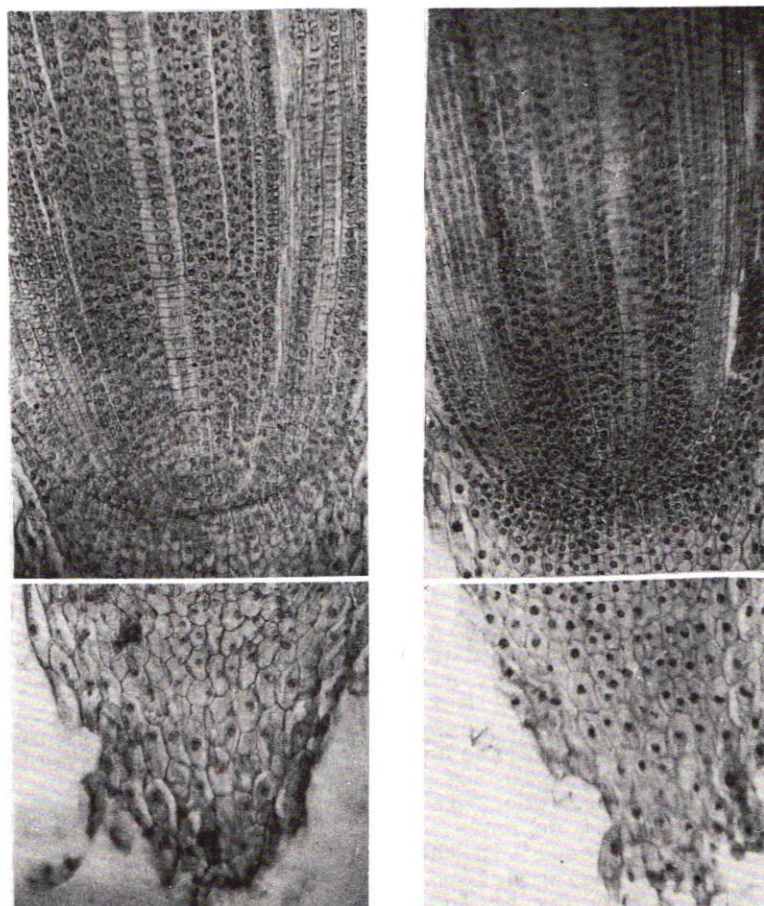
Figure 1. Longitudinal median sections of maize root tips.

a - control, b - flight
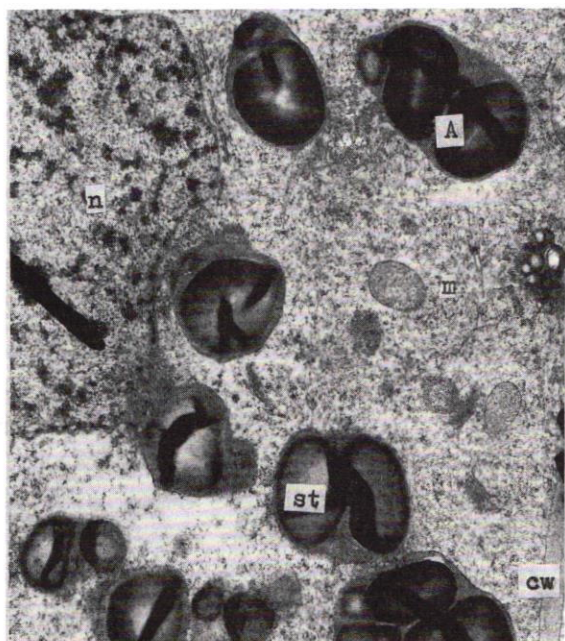
Figure 2. Parts of gravity-perceiving cells (statocytes) from maize root caps.

Figure 3. Parts of secretory cells of maize root caps.

(For both figs.) a - control, b - flight

A - amyloplast; cw - cell wall; d - dictyosome; ld - lipid droplet; m - mitochondrion; pl - plasma-lemma; ps - plasmolemmasoma; st - starch; t - tono-plast; v - vacuole; n - nucleus; nl - nucleolus; s - slime; g - granules.

Thus, the cytomorphological and ul-trastructural investigations of the maize roots grown in a space flight revealed some difference in their organization as compared with those control, which are needed the more detailed investigations.

DEVELOPMENT OF CARDIOVASCULAR SYSTEM AND THE GRAVITY.

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ABSTRACT

The Earth gravity influented on the development of cardiovascular system during all of stages of life evolution. In vertebrates, this system is evolutinary adapted to maintain the blood supply of vital important organs in spite of gravitational blood displacements. The mechanisms of this adaptation include the peculiarities of heart structure and its pump function as well as the autoregulation in some regional vascular systems.The Earth gravity forces together with replacement of vertebrates from water media to dry earth had the influence on anatomical and functional organisation of lesser circulation and on the principle of venous return of blood.The common natural law of development of all parts of the circulatory system is, primary, the establishment of quantitative parameters of control processes and then the perfection of its quality.

The force of Earth gravity is one of the main factors which had a constant influence on living organisms during all period of life development.This force, in its turn, have had an great influence on the development of many physiological system in vertebrates (Parfenov,1983) such as sceleton architecture and bone structures, muscles, circulatory system, respiration, digestion etc.

This can be illustrated by archeological reconstructions (Osten,1978), which show that the initial development tendency of terrestial animals was the tendention to vertical body position. This is suggested from asymmetry of extremities in reptilies which adapted to live on dry land about 225 millions years ago.However, 200 - 150 millions years ago, the symmetry of upper and lower extremities suggests that antient reptilies with vertical body position proved not to be adopted to Earth gravity and this force, in full sense of the word, pressed the vertebrates to the Earth.

The transition to vertical position demanded the reconstruction of support and locomotive structures and of other systems. Among them the circulatory system have been especially reconstructed.In fact, the blood is the fluid that fills in vessels and could move passively according gravity forces. So, the majority of vertebrates could not adopt perfectly to vertical position.Only a few mammals partially to du this process, and only human did it completely.Therefore, human vertical position is the evolutionary second but successful attempt (Fig.1):

MIL. YEARS
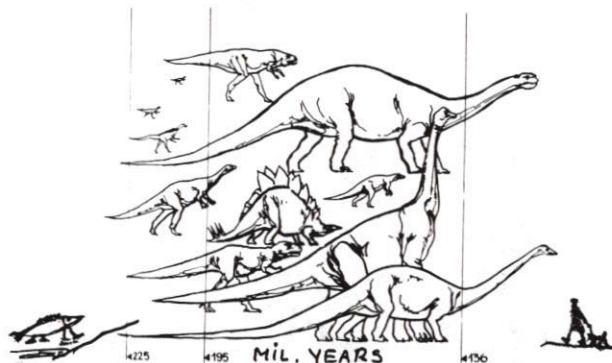
Therefore,in conditions of constant influence of Earth gravity, the functional tasks of cardiovascular system are:

- Adequate blood inflow to different vascular regions,

- Complete outflow from organs and tissues,

- **Preservation** of fluid balance in the tissues in the face of high water permeability of microvessels.

The blood disposition under gravity stress is counteracted by the complex memechanism which moves the necessary blood volume to particular directions independently to gravity vector direction.This mechanism consists of some elements which can be grouped, by their nature, as follows:

(1) FUNCTIONAL: Regulation of cardiac output, regional blood flow autoregulation, control of vascular permeability.

(2) STRUCTURAL: Thickness of vascular wall, valves of main veins, special regio-

nal blood vessels architectonic.

(3) BIOPHYSICAL: Using of arterial pulsations for regional blood outflow.

The development of this mechanism consisted in both improvement of pump function of circulatory system and venous return as well as regulation viscoelastic and permeability properties of vessels, active regulation of their radius against intraluminal pressure.

Conversion of vertebrates to vertical position was not the first factor in development of pump function in circulatory system(Moskalenko,1985).That was preceded by convertion from many - hearts invertebrates to two - chamber heart in fishes; then to three - chamber - because of change of enviroment, and later - to four - chamber, due to increase of respiration, muscular activity, particulary, in vertical plane, but the adaptation to mainly vertical body position was taken place in mammarians with four chamber heart (Fig.2)

OPEN SYSTEM   CLOSED MULTIHEART SYSTEMS
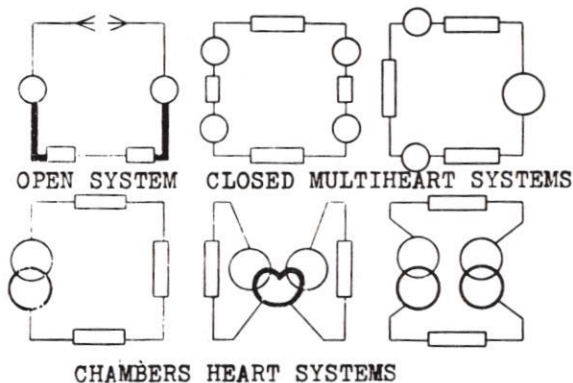
CHAMBERS HEART SYSTEMS

Fig.2. Stages of evolution of cardiovascular system.

Thus, the transition to vertical body position is predicated, in many parts, by previous stages of heart evolution.

The influence of gravitational force on adaptation to vertical position leads only to some increase of myocardial mass; that could be supported by involution of myocardium in weightlessness (Sandler,1979) and the fact that in animals with vertical body position myocardial mass is comparatively large (Hargens,1987).Venous return is well developed also in vertebrates.One of main factors in that mechanism is the presence of valves in big veins and energetic transformation of arterial pulsation

to venous system, that is an additional force for venous return against gravitational pressure gradient.

Such mechanism is the most pronounced in vessels of the organs included into the rigid capsula,e.g. brain, kidney (Moskalenko et al.1980).

Excert venous return to the heart, the specific transformations took place also in the microcirculation bed with the aim to prevent the shift of water across capillary wall due to absorbtion - filtration processes.Results of this are a special structure of microvessels and development of regulation system for control of microvessels permeability.

During the adaptation for vertical body position a significant transformation was arrowed to control processes in cardiovascular system.As a result, the special compensatory and autoregulatory mechanisms were developed which guarantee an independent from changes of systemic hemodynamic parameters and constant blood flow in vital important organs within some ranges. This includes the independentce of organ blood flow during changes of body axis versus gravitational vector.Realisation of this function, in its turn, demanded the coordination of vascular control based on the complex neuro - humoral mechanism, which is characterized by the marked structural and functional heterogenity of localisation of various receptor and efferent zones, Fig.3:
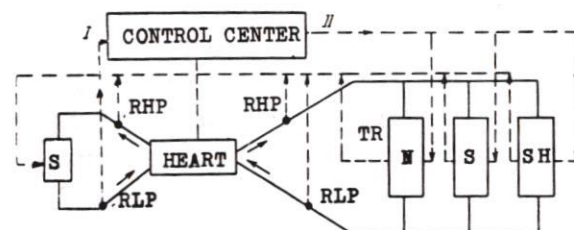
N - Nutritious vessels,
S - Serving vessels,
Sh- Shunting vessels.

RHP - Hich pressure receptors,
RlP - Low pressure receptors,
TR - Tissue chemoreceptors.

Presented on Fig.3 functional - structural organisation of cardiovascular control system gives the opportunity not only to regulate the blood flow in separate vascu-

lar regions, but also to redistribute the
the circulating blood volume during chan-
ges of body position (Moskalenko,1985).

In this respect the cerebral circulation
system is of special interest.The process
of adaptation of this system to changes of
gravity vector coincided with the process
of intense development of brain as a main
regulatory system of the organism and with
the passage its metabolism from anaerobic
to aerobic principle, but the last needs
constant and intense blood supply.Apparen-
tly, in connection with this the quantita-
tive limits of cerebral blood flow autore-
gulation were established in early phylo-
genesis of vertebrates (in amphibians)but
qualitative characteristics of this pro-
cess (time of engaging of compensatory me-
chanisms, effectiveness of compensations)
were developed in the course of adaptati-
on to vertical position.

The analogous regularity can be shown
in ontogenesis of child during its first
postnatal months, when it adapt to verti-
cal body position (Moskalenko et al.1983).
In the process of evolution of vertebra-
tes one more property of the cerebral cir-
culation system was developed - the gravi-
tational stability, based on peculiariti-
es of structural - functional organisati-
on of the system (Moskalenko et al.1980).

The gravitational stability of cerebral
circulatiry system manifests that the com-
pensation limits of gravitational stress
intensivity are significantly different
among classes of vertebrates.These limits
are restricted in amphibians and reptiles,
however in some mammals they are large
enough to compensate ever the hypergravi-
ty stresses (Moskalenko,1967).The human
beings can compensate the longitudinal
gravity stress up to 2G,(Fig.4).

The gravitational stability of cerebral
circulation, therefore, the product
of complicated evolutionary process, which
developed together with evolution of nerve
system.

All foregoing suggest that gravitational
factor played an important part in the de-
velopment of circulary system of verteb-
rates. It manifisted in development of

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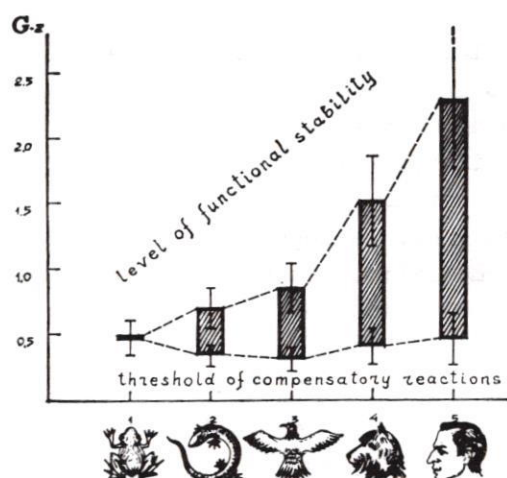
Fig.4

special mechanisms which promote the mo-
ving of blood circulation and regulate both
blood flow and blood volume redistribution
depend on functional significance and me-
tabolic demands of various vascular regions.

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HUMAN PHYSIOLOGY IN MICROGRAVITY: CONCEPTS IN
EXPERIMENTAL DESIGN

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The selection of space experiments is -
and should be - made on the basis of scientific
merit, space relevance and feasability. Once
selected the experimental design and hardware
must conform to a large number of operational
restrictions. The purpose of this paper is to
discuss if there may be potential conflicts
between these restrictions and the scientific
quality. One prerequisite for quality is that
the design of a particular experiment is suited
for the scientific problem under study.

The scientific problem, which is to be
addressed in a study can be classified, with
regard to its complexity as listed below in
Table 1.

TABLE 1: PROBLEM DEFINITION

	Simple	Complex
Theoretical basis	Well-founded hypothesis	Explorative
Response under study	Binary	Continuous function
Non-G influences	None	Significant

Also the experimental design can be clas-
sified according to the complexity of a number
of elements as listed below in Table 2.

TABLE 2: EXPERIMENTAL DESIGN

	Simple	Complex
No. of subjects	Single	Multiple
1G control	Preflight parallel	Inflight
Methods	Established	New
No. of observations	Single	Multiple

Usually, the more complex or the less
well-defined the experimental problem, the more

complex is the required experimental design.
The validity of this simple rule of thumb is
recognized in the experience of most physiol-
ogists.

Thus an all-or-none type of response can
be studied successfully on one occasion and on
a few individuals provided the problem is well-
defined, that it is based on a well-established
hypothesis and that conventional methods are
used. In contrast an exploratory type of inves-
tigation aimed at the study of gradual changes
with potential non-G influences, requires a
large number of observations and a complicated
set of controls.

In the design of past and planned space
experiments the above rules are not always
applied. One mechanism behind these violations
of common sense may be that the scientists lose
control of their experimental design in the
gradual process of converting ground-based
laboratory routines to space experiments.

The most critical factor in human physio-
logy is the limited number of subjects that can
be studied in each flight. This should be com-
pared to the number of subjects that must be
studied in order to detect significant changes
in a typical physiological experiment. Table 3
shows some relevant examples.

TABLE 3: OPTIMAL GROUND-BASED LAB CONDITIONS:
TILT EXPERIMENT

Variable under study	No. of subjects required to detect a significant change
Cardiogenic oscillations	1 - 2
Heart rate	5
Stroke volume	5 - 10
Cardiac output	5 - 10
Pulmonary tissue volume	10+

Thus, most variables require more obser-
vations than can be obtained during one single
flight. Accordingly reflight of experiments
therefore has been planned for most experiments
in human physiology. However, reflight oppor-
tunities have not been provided as originally
planned. This seriously reduces the value of
the scientific results obtained sofar, and
therefore represents a waste of scientific
effort and capital.

The above reasoning illustrates the
necessity for a close coordination between the
agencies and the scientists with regard to
flight opportunities and experimental design so
that results from the various experiments per-
formed by for example American, European and
Soviet scientists can be used together. The
value of such a coordinated effort will be much
higher than the sum of its parts.

PHYSIOLOGICAL RESPONSES OF SKELETOMUSCULAR SYSTEM TO MUSCLE EXERCISES UNDER LONG-TERM HYPOKINETIC CONDITIONS

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INTRODUCTION

As it is known, reconstructive and metabolic activities of body tissues are reduced under hypokinetic conditions and thus the catabolic processes become predominant during long-term hypokinesia. Atrophic changes in this case are developing the most intensively in tissues of the skeleto-muscular system. A marked decrease in the optic density of cosmonauts bones has been noted already in the first space flights (1,2). These observations have been supported and supplemented by the results of later studies in which more elaborate methods of bone tissue analysis were used (3,4). Muscle atrophy, manifested by a significant decrease of the muscles mass, alterations of their structure and a reduction of the contractile properties, is a regular consequence of a muscle load reduction (5,6) and gravitational load decrease (7-12).

Changes in bone and muscle tissues due to the gravitational load decrease are followed usually by a system rearrangement of metabolic processes including water-salt metabolism and its control system (13).

One of the most effective means of the atrophic processes countermeasure under conditions of a gravitational load decrease constitute physical exercises (14,15). Physical exercises used by Soviet cosmonauts in long-term space flights diminished substantially the rate of atrophic processes in muscles and bones helping to save their mass and structure (12). However a large variability of work load along with the great difficulties of evaluation of physical exercises used by cosmonauts lower considerably the possibilities to analyse quantitatively the efficiency of different types of physical exercises in space flights. A number of other important questions have also no replies. In particular, the results of 120-day bed rest studies have shown that the mechanisms of muscle properties alterations developing during the first and successive month of hypokinesia differ considerably (Fig.1). A decrease of the muscle contraction force observed during the first month was determined mainly by the muscle atonia and during the successive period - by the atrophic processes (16). It is evident, that an efficiency of equal physical exercises within these two (and possibly more) periods should be different.
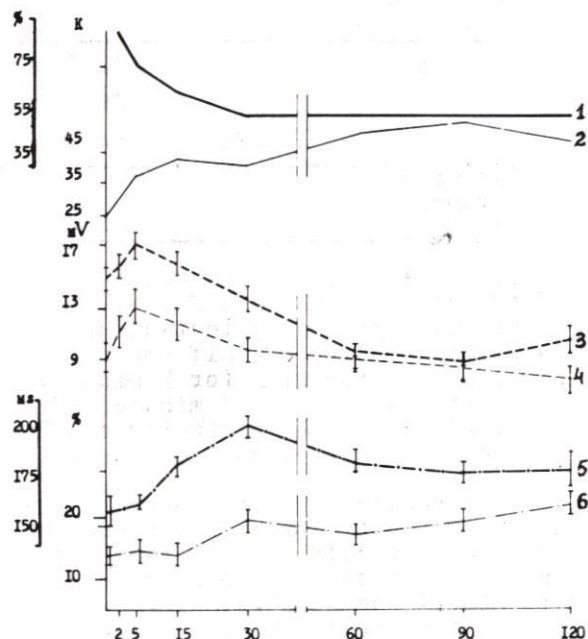
Figure 1. Dynamics of changes of different motor system characteristics during 120 days anti-orthostatic bed rest (ABR). Abscissa shows days of ABR. Muscle stiffness (1), coefficient of electro-mechanical efficiency (2). M- and H- responses amplitudes (3,4), motor units interspike intervals and their variation coefficient (5,6) are shown by solid and interrupted thick and thin lines.

Two questions mentioned above, namely: an efficiency of physical exercises of different kind at the same stage of hypokinesia and an efficiency of physical exercises of the same kind at different stages of hypokinesia were objects of studies in the 120-day anti orthostatic bed-rest (ABR) experiments with 21 test subjects participants.

Experimental procedures and methods

All test subjects were bed rested in the head down (-4.5°) position for 120 days. 9 of them, constituted a control group (C), did not use any preventive measures; 12 others, divided into 3 groups by 4 subjects each, by contrast, have used different countermeasures and have formed in accordance with a measure applied groups of pharmacological (PP), physical training (PT) and complex (PP+PT) countermeasures. 4 types of physical training were used. 3 of them were active - velocity, strength-velocity and strength oriented (see Table 1), the fourth one was represented by a passive muscle stretching. All 3 active regimes of training have included: i)exercises with elastic cords for the muscles of calf, thigh, back and neck performed in static

Table 1. Load characteristics in different groups of physical training

Day of cycle \ Mode of exercises	Velocity (I)		Strength-velocity (II)		Strength (III)	
	Dynamic (KGM)	Static (KGM)	Dynamic (KGM)	Static (KGM)	Dynamic (KGM)	Static (KGM)
1	3638	4200	3210	420	2730	4200
2	5457	4846	4815	4860	4173	4860
3	7276	5480	6420	5480	5564	5480
Number of movements for 20 s	16-18		14-16		12-14	

and dynamic modes with an intensity reaching 70% of voluntary maximal force; special exercises reproducing instantaneous high intensity loading of calf and thigh muscles; ii)hand bicycling for 5 minutes (1 minute-without load and 4 minutes of 100 watt loading) at the beginning of PT; and iii)breathing exercises - at the end of it.

Passive regime of PT included, besides hand bicycling and breathing exercises, stretching of the calf, thigh, back and neck muscles in succession performed by trainers. Durations of stretching of each muscle was close to 1 minute. As a whole, active physical training lasted for 60 minutes, passive one - for 40 minutes.

Each of PT regime has been used by every test subject for a month. To minimize effects of ABR stage and consequences of previous PT regime 8 test subjects of PT and PT+PP groups were divided into 4 subgroups of 2 individuals each, which have been trained according to their own schedules so that each regime of PT was tried at each stage of ABR following every regime of PT (Fig. 4).

The extensive clinical and physiological examinations have been performed at the end of each month aimed to reveal functional as well as structural alterations of different body tissues, organs and systems including bones, muscles and fluid-electrolyte metabolism.

RESULTS AND CONCLUSIONS

Long-term ABR was followed as usually by pronounced changes in calcium balance: a significant increase of calcium excretion by the kidney has been observed in 7 of 9 control group subjects; 6 of 9 revealed a definite decrease of calcium intestinal absorbtion along with elevation its fecal excretion. Total calcium loss for 120 days averaged in the control group close to 20 g with the extreme values ranged from 8 to 34 g.

The dynamics of calcium loss in the control ABR group was of the progradient character with relatively slow development at the beginning (up to 36-38 days of ABR) and steep one subsequently(Fig.2). The calcium loss dynamics in the experimental PT groups was more uniform. It did not differ significantly from the control data during the first 48 days of ABR but did differ during the second stage show-
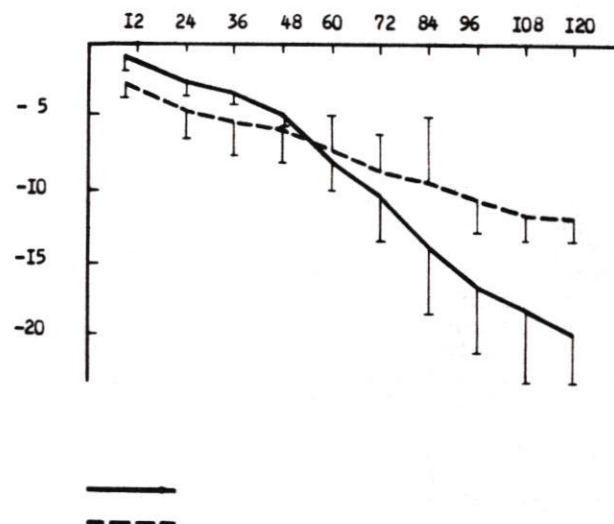
Figure 2. Dynamics of calcium loss during 120 days ABR.
Days of ABR are shown on the abscissae. The data of the control and experimental groups are represented by solid and interrupted lines correspondingly.

ing much (two fold) slower rate of calcium loss. Thus being not very effective to stop calcium leakage at the first stage (prior to 48-60 days) of ABR, physical exercises become effective thereafter.

An efficiency of PT with respect to potassium ions was distributed in time in analogous fashion (Fig.3).

Similar results have been obtained when strength and velocity properties of triceps surae muscle (ST) were tested by isokinetic dynamometry. As Fig.4 shows, during the first month of ABR none of the PT regimes used was sufficiently effective: a decreases of TS strength and velocity properties in the control and PT groups were similar. Later, however, an efficiency of exercises increased markedly: strength and velocity deficiencies increasing prominently from month to month in the control group did not increase and diminished slightly in subjects of the PT and PT+PP groups.

The comparative analysis of efficiency of 4 PT regimes under study (Fig.4) indicated that 3 active regimes were almost equally effective: in each case in one half of test subjects (4 of 8) strength parameters of TR when compared to previous month data remained unchanged
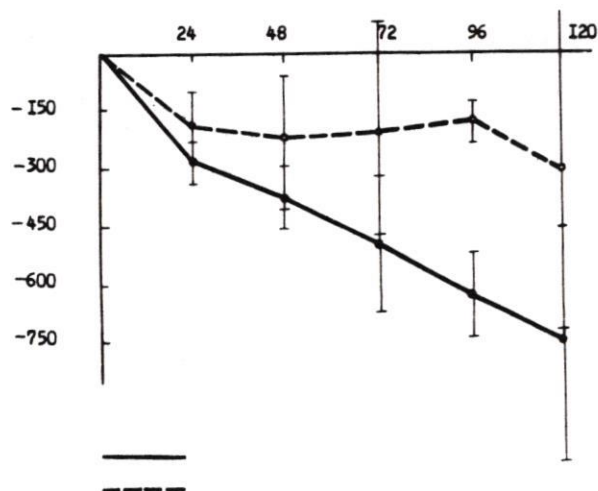
Figure 3. Dynamics of potassium loss during 120 days ABR.
Days of ABR are shown on the abscissae. The data of the control and experimental groups are represented by solid and interrupted lines respectively.

or even increased. Slightly more uniform in this respect were the results obtained with the passive training (muscle stretching): a decrease of muscles strength was observed in this case only in 2 of 8 test subjects. These findings seemed to be in a good agreement with those obtained in post ABR TS muscle strength testing performed by means direct tendography (Table 2,B).

Distinct differences in a muscle fibers status and their responses to physical exercises during the first (up to 60 days) and subsequent stages of ABR were revealed also by the results of morphological studies (Table 2,C). As it is seen, a significant decrease of muscle fibers size being more pronounced in slow (type I) fibers was observed after first 60 days of ABR. Atrophic changes proceeded further in type I fibers during the second stage of ABR, while in type II fibers the atrophyc tendency was followed by the hypertrophic one. Changes of activities of substances linked to the activity of oxidative processes in muscle fibers (NAD.N-TR) were indicative of an aerobic oxygenation processes increase (predominantly in slow myons) during the first 60 days of ABR and further stabilization of their activity.

Physical training compensated to a large extent for morphometric effects of hypokinesia in fibers of both types so that the magnitudes of size alterations looked almost equal in fibers of the first and second types, as well as for the increased activity of NAD.N-TR in slow fibers. In fast fibers PT resulted in an initial increase of NAD.N-TR activity followed by its progressive decrease as well as in a reversed dynamics of LDH activity not observed in the control data.

Thus, having confirmed experimentally a notion of an efficiency of physical exercises as countermeasure of muscle and bone changes under conditions of gravita-
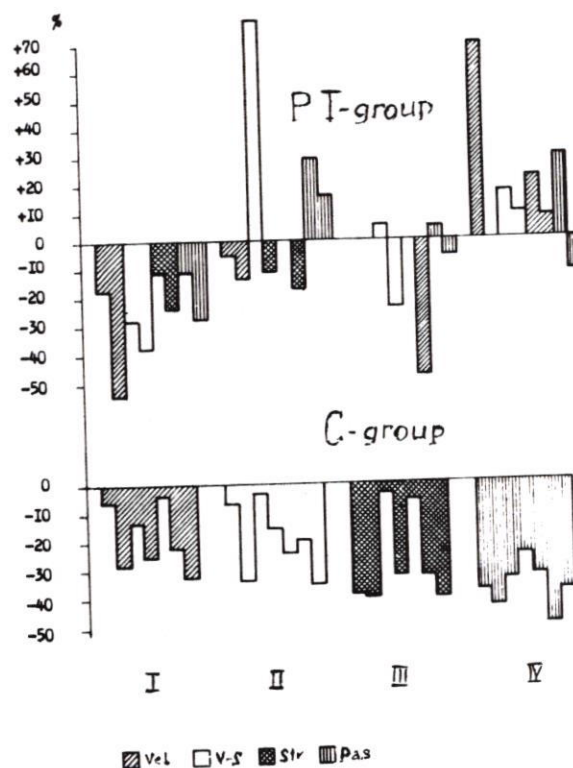
Figure 4. Alterations of the calf muscles contraction strength during ABR at different time intervals.
I-IV show months of ABR. Bars represent individual changes of strength (in percentage) occurred during month interval. Types of physical training is marked by shading pattern.

tional unloading, the results of the studies revealed an obvious dependence of PT efficiency upon stage of hypokinesia. During the first stage, which we characterize as reflectory "one", the efficiency of PT of any type was low; later on, when "atrophic" processes became predominant any type of training was on the opposite quite effective. Lack of distinct differences in efficiency of physical exercises of different types suggests that the retention of the muscle mass under hypokinesia is determined largely by the volume and intensity of exercises and to a lesser extent by their specific features.

Table 2. Magnitudes of changes /%%/ of strength-velocity properties (A,B) and triceps surae structure (C) after 120 days ABR.

A. Isokinetic dynamometry

Experiment-al group \ Speed of movement	0 deg/s	60 deg/s	120 deg/s	180 deg/s
Control	-42.7 ± 6.7	-35.3 ± 3.5	-25.3 ± 5.2	-30.7 ± 5.6
PT and PT+PP	-22.8 ± 2.1	-18.1 ± 3.6	- 8.2 ± 5.4	- 2.5

B. Tendography

Experimental group \ Parameter measured	Single contraction strength	Tetanic contraction strength	Voluntary contraction strength
Control	-23.5	-21.2	-36.8
PT and PT+PP	- 4	-16	-28

C. Morphometry and hystochemistry

Experimental group	Fibers type	Fibers size I	Fibers size II	NAD.N-TR activity I	NAD.N-TR activity II	LDH activity I	LDH activity II
Control	60 days	67	80	156	94	133	122
Control	120 days	88	139	160	94	87	83
PT and PT+PP	60 days	93	90	98	118	136	85
PT and PT+PP	120 days	115	110	89	85	132	117

Note: All parameters of morphometric and histochemical studies are presented in percents of a baseline level.

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EVOLUTION OF GRAVITATIONAL TOLERANCE IN THE SEA

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JUMPING

Fig. 1. A two-axis accelerometer was fastened to a wide belt placed around the waist of a young adult male who bent his knees, jumped upward, and landed on his feet. The record shows 1 G standing, 0.3 G bending the knees, 3 G extending the legs, 0 G with feet off the ground, 3 G landing, and 0.6 G flexing the legs on landing. An integrator circuit registered velocity. Compare with fish acceleration (Fig. 2).

ABSTRACT

Land animals have the muscle strength, skeletal structure, and circulatory reflexes that allow different postures, running, and jumping despite gravity. But fish, the ancestors of land animals, were supported by water from the beginning of their evolution. However, hydrodynamic forces oppose locomotion of fish, which require body structures and a circulatory system able to withstand the stress of swimming. Dogfish show poor circulatory tolerance for gravity. But bluefish have the bony strength and circulatory responses necessary to accelerate 3 G in water, withstand large transmural pressures during swimming, compensate for hemorrhage, and tolerate head up tilting in air to 30° or even 60° for 30 minutes. Sympathectomy attenuates the vascular responses. Cross-tolerance for gravity may have evolved before or simultaneously with emergence from the sea.

INTRODUCTION

Land animals possess attributes that enable them to withstand the force of gravity which is 1 G at rest, but is greater when running or jumping, for example, 3 G in man. In keeping with this, land animals have strong muscles and bones, and a circulatory system adapted to returning blood from the lower extremities to the heart despite alterations of posture. Because these characteristics are needed on the earth's surface, it could be argued that they were not, or are not presently, needed by fish which evolved solely in the sea. If that were true, then when fish are out of water, they might have little or no tolerance for gravity. Their muscles would be weak, their bones low in mineral content, and their circulatory systems incapable of restoring adequate venous return to the heart if challenged by gravity. They would, in effect, possess the defects found in humans who have undergone prolonged bed rest, immersion, or weightlessness.

HYDRODYNAMIC PRESSURE

A simple calculation showed that the force of water impacting on the head of a fish during swimming would flatten the brain and collapse the spine unless the fish had a hard head and strong vertebral column. Pitot pressure, in grams per square centimeter, or cm water, approximately equals water speed in miles per hour squared. More exactly, $P = (m.p.h./0.98)$ squared. Fish can swim ten body lengths per sec. A two foot fish can swim 12 m.p.h. for a short time. Pitot pressure would be about 144 cm water. It seems that body structure and function would have had to evolve in water to sustain forces of this magnitude, and if true, the resulting strength of the body and circulatory system would be capable of overcoming large forces directed along the axis of the body, and might have created a cross-tolerance for the force of gravity directed in the head to tail direction of fish, or head to foot direction of their descendants.

STRESSES ON THE BODY

The above hypothesis was tested in several different ways (DuBois, et al, 1974, 1978). Pressure distribution on the body surface of blue-fish was measured with small plastic tubes implanted in the body, at speeds such as 4 m.p.h. Compression strength of the vertebral bodies was measured using an Instron vice and load cell. Accelerometers were implanted in the body. Tail thrust was measured using small flat gauges sewn on the tail, and compared to forward acceleration of the fish. The results showed that pressure on the front of the fish exactly equalled Pitot pressure. Pressure on the wide part of the body and gills was negative compared to adjacent pressures in the surrounding water, in keeping with the Bernoulli principle. The fish were capable of accelerating at 3 G when frightened, and the vertebrae resisted crushing until about 20 kg/sq. cm, which is about the same as for mammals. These findings showed that fish had need of strong bodies, and that their bodies did, in fact, possess such strength.
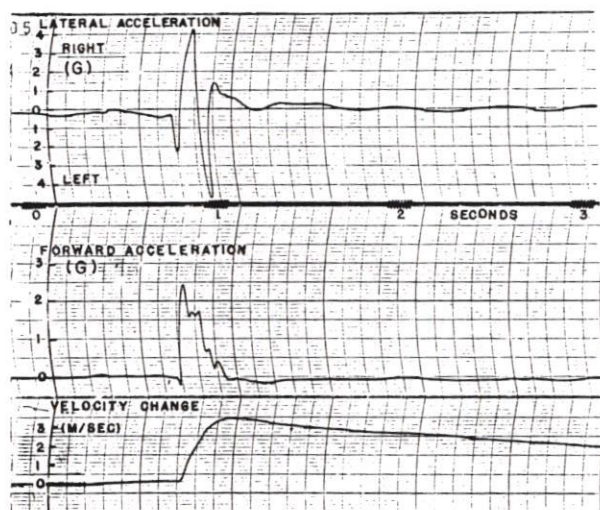
Fig. 2. A two-axis accelerometer is placed near the center of gravity of a bluefish swimming in a pool. The fish is frightened with a broomstick and moves the body to one side, thrusting the tail to the side, causing forward acceleration of 2.4 G, and an increase of forward velocity to 3.5 meters per sec. Compare with man jumping (Fig. 1).

CIRCULATORY RESPONSES

Preliminary studies of the circulatory system of bluefish swimming in a water tunnel, or tilted head up in air while seawater was supplied to the gills, indicated that the blood pressure and pulse pressure had become adapted to the needs of the body during locomotion, and also were tolerant for the stress of gravity on the circulation (DuBois, et al., 1975).
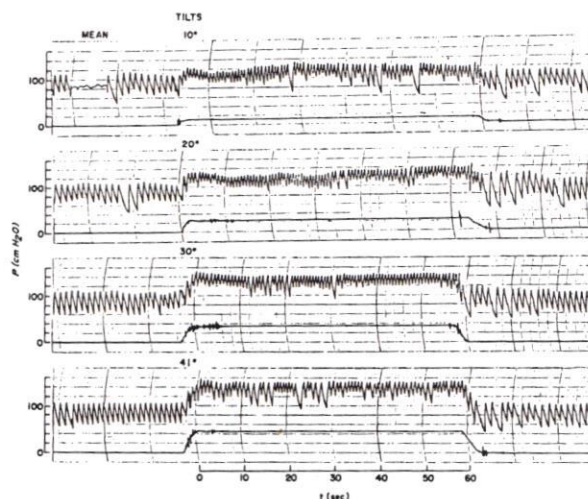
Fig. 3. Pressure is measured in the ventral aorta of a bluefish. Reference pressure (lower tracing) is measured from a water tube opposite the ventral aorta. The fish is on a V-board, and its gills supplied with running salt water containing tricaine as an anesthetic. The V-board is tilted head up in air for periods of a minute in these records or up to 30 minutes in other records. Blood pressure is well sustained, suggesting possibility of compensatory mechanisms, tested subsequently.

COLLOID OSMOTIC PRESSURE

Bluefish do not develop dependent edema during tilting for 30 min, whereas dogfish show obvious swelling of the lower body under these conditions (Ogilvy and DuBois, 1982). The dogfish appear to have porous capillaries, lack innervation of their blood vessels, and lack plasma albumin and therefore have a low plasma colloid osmotic pressure (Tremml and DuBois, 1984). As a result, the pulse pressure diminishes, the eyeballs soften, and the tail swells when dogfish are tilted.

Plasma Protein vs Colloid Osmotic Pressure
(Prather Curve with Tremml points)
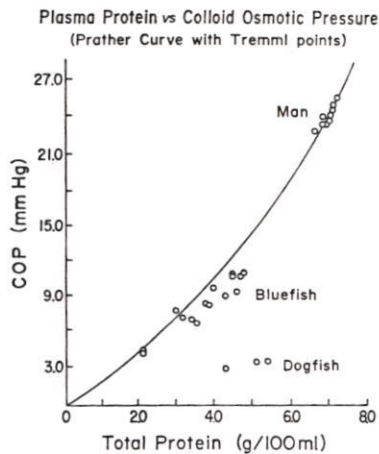
Fig. 4. Plasma colloid osmotic pressure of bluefish, dogfish, and man as obtained by Tremml.

ACUTE HEMORRHAGE

The next question was whether the vascular system of bluefish would show evidence of vaso-constriction when challenged by hemorrhage. Preliminary experiments, in which rapid hemorrhage was followed by rapid reinfusion of the blood, did not show that the bluefish could sustain its blood pressure during the initial phase of rapid hemorrhage (DuBois and Tremml, 1984).

Blood Volume vs Blood Pressure
During Rapid Hemorrhage & Reinfusion
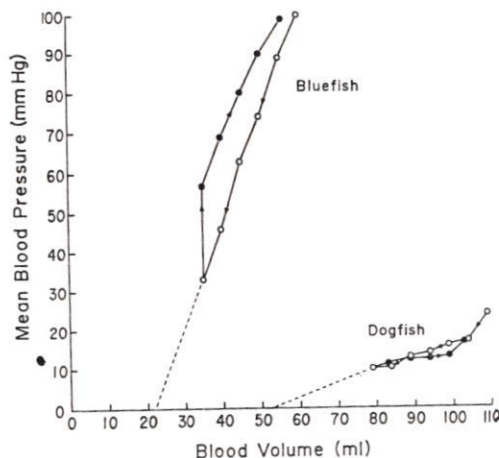
Fig. 5. Blood was withdrawn 5 ml at a time through an 18 gauge thin wall spinal fluid needle in the ventral aorta of bluefish or dogfish. Blood pressure was measured between aliquots removed. Blood was reinjected stepwise within two or three minutes of the onset of hemorrhage. This was too fast for anything other than minimal compensation to occur.

It seems that there are no published data on whether land animals sustain their blood pressure during the first minute of hemorrhage, because it is hard to remove large volumes of blood that fast. The important question is what happens during the next 15 minutes? The other question is whether vasoconstriction, if it does occur after hemorrhage or during head up tilting in air, can be prevented or abolished by pharmacological or surgical blockade of the sympathetic system?

Ogilvy and DuBois (1987) rapidly removed 17 percent of blood volume from unanesthetized blue-fish and found an immediate decrease of blood pressure in the ventral aorta, but restoration of blood pressure during the next five minutes after hemorrhage. However, this recovery of blood pressure was almost completely inhibited by prior administration of phentolamine to the bluefish. This suggested that the early phase of recovery of blood pressure had been due to vasoconstriction that was initiated by the fall in blood pressure, and that the vasoconstriction had been mediated by the sympathetic nervous system. There were indications that epinephrine and norepinephrine had been released during the recovery from hemorrhage. Small, repeated hemorrhages at twenty minute intervals produced progressive hemodilution, and restored the blood volume. These results should be considered tentative until published.

TRANSECTION OF THE SPINAL CORD

The other question recently examined was whether fish would retain their ability to sustain their blood pressure and pulse pressure during head up tilting in air, if the spinal cord were transected near the brain stem. Burnstock (1969) had cited evidence for adrenergic nerves supplying the arteries of teleosts, and Nilsson (1976) localized the sympathetic chain ganglia that supply fibers which run forward and then down the vagus of codfish as being located between the first cervical vertebra and the medulla. It is not known whether the anatomy of the sympathetic system in bluefish is similar to that in codfish, or whether the blood vessels are under this sympathetic control. However, the following experiments are compatible with that assumption (Fox, Ogilvy, and DuBois, 1987). Bluefish were anesthetized with tricaine methanesulphonate and placed on a V-board, and running seawater circulated through their gills. Blood pressure was measured in the ventral aorta, and compared to pressure in a reference tube whose water level was kept opposite the ventral aorta. The V-board was tilted at angles of 10 or 20 degrees for 2 minutes, or 30 degrees for 5 minutes. In this initial series of tilts, the blood pressure was maintained, or it returned to pre-tilt levels during the period of tilting. The region of the head over the medulla and first cervical vertebra was opened, and the spinal cord was transected 3 mm caudal to the obex or 7 mm caudal to the obex. Tilts similar to those made prior to cord section did not lower the blood pressure of the fish with the cord sectioned 7 mm caudal to the obex (at the first cervical vertebra), but definitely caused an immediate decrease of blood pressure as soon as tilting began if the cord had been transected 3 mm caudal to the obex (at or below the medulla). The region between the medulla and the first cervical vertebra is the area in which the sympathetic chains of codfish seem to be vulnerable. Therefore, it appears that vasoconstriction occurs during tilt board experiments in bluefish, and that it is sympathetically mediated.

EVOLUTION OF GRAVITATIONAL TOLERANCE

The evidence indicates that fast swimming requires a strong skeletal structure as well as adequate musculature, and that over the millenia fish evolved the body structures required to swim fast. Circulation of modern fish overcomes the inertial force of acceleration of the body, the transmural pressure across the skin during swimming, and the need for increased venous return during exercise. The blood vessels of at least some teleosts have a sympathetic supply and can withstand gravity so long as the cervical sympathetic chains are not cut. Although these characteristics evolved in fish, it is not possible to say whether they developed before or after the fish emerged to become the ancestors of land animals. It would be interesting to do tilt board experiments on a Coelacanth (Latimeria chalumnae) to find out whether that species (descended from the species that was the ancestor of terrestrial tetrapods) is tolerant of gravity. However, even though they might vasoconstrict during hemorrhage or tilting, they might not have the plasma constituents that retain fluid in the circulation and prevent dependent edema such as that found in dogfish.

Speculation about how bluefish evolved their body form and function leads to the following diagram in which an attempt is made to relate stress, strain, and the adaptation of species to withstand them.

LINKS BETWEEN MECHANICAL AND METABOLIC ENERGY

INPUT: CHEMICAL ENERGY

OUTPUT: WORK & HEAT
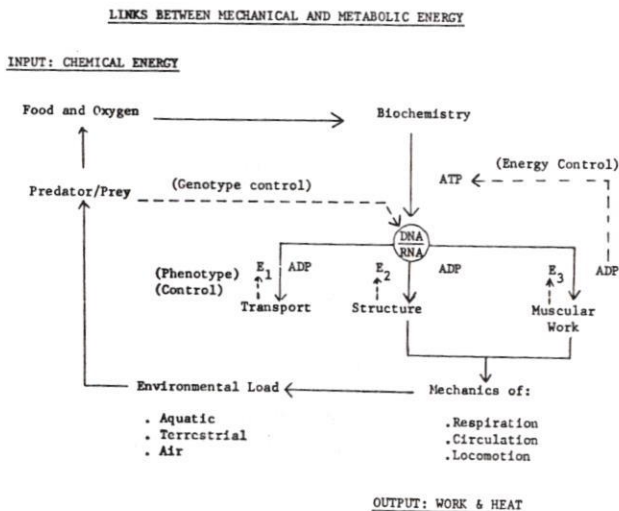
Food and oxygen, available in the environment of the fish, are metabolized to produce body protein and extracellular supporting tissues whose quantity and location are controlled by nucleoprotein and enzyme concentrations influenced by body demands and metabolic energy supply. These affect the respiration, circulation and locomotion of the fish, overcoming environmental loads producing greater or less success in catching prey or avoiding predators. Competition selects the most successful fish, leading to a predominance of those with the most apt genotype. A dashed line is used to show this selection process because it is not clear how so many features of body structure and function can be optimized simultaneously. This scheme has not been tested or evaluated critically.

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THE RELATIONSHIP BETWEEN CARDIOVASCULAR RESPONSES AND STRESS TOLERANCE BEFORE AND AFTER BED REST

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Introduction

A few years ago, we made a HUT plus LBNP test on 20 healthy young males. According to their differences of cardiovascular responses, the subjects were divided into four types as (1) vascular type (Increase of peripheral resistance was higher and of heart rate was lower during test), (2) mixed type (Both increases of heart rate and peripheral resistance were higher during test), (3) cardiac type (Increase of peripheral resistance was less and was mainly regulated by increase of heart rate during test), and (4) less regulating type (Both increases of heart rate and peripheral resistance were much less during test). The objectives of this bed rest test were: to testify whether the result of the HUT plus LBNP test in this experiment was similar to that obtained in the experiment made on the same subjects sometime before; to observe what changes of the features of cardiovascular responses in the subjects of the four types had taken place during and after exposure to the hypokinetic bed rest; to see if the tendency of the changes was consistent; and to evaluate which kind of cardiovascular response could better adapt to the hypokinetic environment and readapt to normal environment.

Procedure of Experiment

According to the four different types of cardiovascular response, 7 healthy males, aged 19-20, were chosen out of the 20 young men who had been subjected to the HUT plus LBNP test a few years ago. The 7 selected subjects were strictly restricted on bed for 20 days with their heads being 2° lower than their feet. The subjects in Bed 1 and 2, Bed 3 and 4, Bed 5 and Bed 6 and 7 were the ones of vascular, mixed, cardiac and less regulating types, respectively. The cardiovas-cular indices were recorded every other day during bed rest. The control period was 7 days before bed rest. The recovery period was 14 days after getting up.

The subjects were exposed to the stress of HUT plus LBNP just the day before bed rest. After getting up, they were subjected to the same test on the 5th and 10th day. The pressure of lower body chamber was maintained at -40 mmHg and the inclination of head-up tilt was kept at 75°. The longest exposing time was 20 minutes, but experiment should be stopped immediately when pre-syncope symptoms appeared in the subjects. The indices recorded included heart rate, blood pressure, cardiac output, ECG. According to the values of blood pressure and cardiac output, peripheral resistance could be calculated.

Results and Analyses

The consistency of the characteristics of cardiovascular responses obtained in the two experiments. There was an interval of 11 months between the two experiments. It was indicated that the features of cardiovascular responses from the 7 subjects to HUT plus LBNP before bed rest in the 2nd experiment were basically the same as those in the same subjects in the 1st one. These features were fundamentally consistent with the individual standards and the repeatability was high (Table 1).

Thus, it could be seen that under the stress of HUT plus LBNP, cardiovascular responses were mainly regulated by nerve and fluid. In a certain period, for an individual subject, the effect of nerve and fluid was relatively stable [1], but among the subjects there existed individual differences, which reflected the individual features of cardiovascular responses.

Individual differences of the subjects' adaptation to the hypokinetic bed rest. All the 7 subjects suffered from their various responses to the hypokinetic environment. The responses of the subjects in Bed 1 to 3 were slight. They suffered from light dizziness, abdominal distension and lumbago early in the bed rest, felt fidgety in the middle and accommodated themselvs to the circumstances late in the test. Apart from the above responses, the subjects in Bed 4 had the feeling of nausea and vomiting late in the test. The subjective responses of the subjects in Bed 5 to 7 were severe. They suffered from the responses of fullness in head, headache, dizziness and stomach. They felt their whole bodies discomfortable in the middle and late part of the test. They were also found difficult to fall asleep and easy to get excited. Such symptoms were even sustained till the end of the bed rest.

The change of subjects' tolerance to HUT plus LBNP pre- and post bed rest and the changing tendency of cardiovascular responses. It was recorded for the

Table 1. Comparisons of cardiovascular responses to HUT plus LBNP obtained in different experiments

			Period of endurance (min,sec)	Peripheral resistance Value(dyne.sec. millimeter)	Changing rate (%)	Heart beat 4' value beats/min	10' value beats/min	Slope
Vascular type		Criterion	>15'	>2400	>170	<100	<110	<1.5
	Bed 1	1st experiment	20'	2209	186	71	87	1.50
		2nd experiment	20'	1925	254	78	90	1.49
	Bed 2	1st experiment	20'	2755	225	98	108	0.95
		2nd experiment	18'	2652	199	93	101	1.36
Mixed type		Criterion	>10'	>2400	>170	>100	>110	1.5-2.5
	Bed 3	1st experiment	20'	4218	340	85	119	2.37
		2nd experiment	20'	5323	429	86	106	2.56
	Bed 4	1st experiment	15'15''	2765	184	104	114	1.43
		2nd experiment	11'12''	2840	243	125	138	2.64
Cardiac type		Criterion	<10'	<2400	<170	>100	>110	>2.5
	Bed 5	1st experiment	9'50''	1976	156	108	-	2.50
		2nd experiment	4'15''	1624	147	116	-	2.90
Less regulating type		Criterion	<10'	<2400	<170	<100	<110	<2.5
	Bed 6	1st experiment	2'20''	2123	142	-	-	-
		2nd experiment	3'05''	1670	146	-	-	>.25
	Bed 7	1st experiment	9'	1956	170	88	-	1.76
		2nd experiment	4'16''	1611	143	94	-	1.37

Note: The changing rate refers to the percentage of changes of peripheral resistance during stress as compared with those before the stress. There was an interval of 11 months between 1st experiment and 2nd one.

first time after getting up that the tolerances to the stress of HUT plus LBNP decreased significantly after bed rest. None of the 7 subjects could finish the 20 minutes' test. It was found that they recovered a lot when the tolerances were measured for the second time. The subjects of cardiac type recovered more quickly. But the changes in their main indices were still different from those of pre-bed rest ($P < 0.05$). This showed that the subjects did not recover to the pre- bed rest level 10 days after getting up. Anyway, it could still be seen that the changing tendency of cardiovascular responses pre- and post bed rest were basically the same. The t-test results of the increasing rate of heart rate and peripheral resistance of different types of cardiovascular response pre- and post bed rest indicated that the comparisons of different responses between pre- and post bed rest were almost consilient.

In addition, the relation coefficient of the order of the 7 subjects equaled 1, which showed that the order (fine to poor) of cardiovascular regulating functions post bed rest of each of the subjects was not different from that pre-bed rest.

Results obtained through an analysis by "fuzzy mathematics". The characteristics of different cardiovascular responses to HUT plus LBNP before bed rest and the readaptational ability after hypokinesis

reflected the difference of regulatory compensation ability mobilized by cardiovascular system among the subjects. The regulatory compensation ability was a "fuzzy concept". We used the "fuzzy concept" to further approach and observe the differences of regulatory functions of the subjects of different cardiovascular responses [2].

We used 11 cardiovascular indices which could indicate the regulatory compensation. They were heart rate, systolic pressure, diastolic pressure, mean pressure, peripheral resistance, stroke volume, cardiac output, cardiac index, work by heart, work index and cardiac ejection work. These indices constituted one "fuzzy set", while the subjects with different responses constituted another "fuzzy set". The changes in the values of above indices reflected the capabilities of regulatory compensation.

First the "weight" of various indices was calculated. Under the stress of HUT plus LBNP, the conformability of changes of the each index with subjective responses was the "weight" of individual index! Substituting the "weight" value and the changing rate of indices into a certain formula, we obtained the calculation of Z at different moments during the HUT plus LBNP tests pre- and post bed rest. The compensatory changes of the subjects with different responses to HUT plus LBNP pre- and post bed rest are listed

in Table 2.

were observed (Fig. 2).

Table 2. The mean value Z̄ of the features of 4 types of cardiovascular responses to HUT plus LBNP pre- and post bed rest

Features of cardio-vascular responses	Pre- bed rest	Post bed rest(1)	Post bed rest(2)
Z̄ value of vascular type	22.1	-2.0	20.0
Z̄ value of mixed type	20.4	2.4	11.2
Z̄ value of cardiac type	4.4	-5.7	10.0
Z̄ value of less regulating type	-1.2	-11.5	-1.5

Note: Post bed rest (1) refers to the first test of HUT plus LBNP on the 5th day after getting up. Post bed rest (2) refers to the second test of HUT plus LBNP on the 10th day after getting up.

According to the mean value Z̄, we classed the subjects' regulatory compensation abilities into good, moderate and poor "fuzzy Z sets", with G representing the "fuzzy set" of the subjects who had compensatory functions and worked out a standard, which corresponds to Fig. 1.

Fig. 2 Curves of Z value of 4 subjects exposed to HUT plus LBNP pre- bed rest.

•——• bed 1 (vascular type)
····· bed 4 (mixed type)
•——• bed 5 (cardiac type)
•——• bed 6 (less regulating type)

Fig. 3 illustrates the curves of Z value of the same subjects subjected to HUT plus LBNP for the second time after bed rest.

Fig. 1. The distribution of the order of regulatory compensation.

It could be calculated from Fig. 1 that in the test of the first time the regulatory compensation abilities of the 7 subjects exposed to HUT plus LBNP after bed rest decreased by 50 to 80%, while in the test of the second time the compensation abilities returned to 60 to 90% of the levels of pre- bed rest. The compensation abilities were arranged in order (from optimal to poor) as vascular, mixed, cardiac and less regulating types.

The differences of Z values of the subjects in Bed 1, 4, 5 and 6 exposed to HUT plus LBNP pre- and post bed rest

Fig. 3 Curves of Z value of 4 subjects exposed to HUT plus LBNP for the second time after bed rest.

•——• bed 1 (vascular type)
—— bed 4 (mixed type)
•—·—• bed 5 (cardiac type)
•—·—• bed 6 (less regulating type)

Under stresses, cardiovascular responses are related with many factors. The neural regulatory function plays very important role. Several previous studies

have pointed out that the tolerances to hupokinesis of athletes are poorer than those of ordinary people. The cause Klein et al [3] found was that athletes' sympathetic nerve functions were not predominant. Stegemann [4] determined sensibility of carotid sinus to heart rate and pressure reflex of blood pressure and found that athletes were not so sensible as ordinary people and the gain of their reflexes was lower than that of ordinary people. In addition to neural regulatory function, the regulatory effect of fluid on cardiovascular system cannot be neglected. Haber [5] suggested that the renin activities of those who could maintain their blood pressure and not develop syncope during a test increase by 283% on the average, while the renin activities of those who could maintain their blood pressure and develop syncope only increase by 49% on the average. Although the mechanism of effect of HUT or LBNP on cardiovascular regulation has been studied. it is still not completely clear and therefore further studies are needed.

Conclusion

1. Under the stress of HUT plus LBNP, the subjects' cardiovascular responses might be divided into four characteristics: vascular type, mixed type, cardiac type and less regulating type. When the stresses, environments and health conditions were basically the same and without additional strong stresses, the features of individual response were relatively stable.

2. During the 20 days of bed rest, as for the subjects of vascular and mixed types, the subjective responses were slight, the capacity for heart to do work decreased, the compensatory responses were slight, and the adaptation to hypokinetic environment was good; while, as for the subjects of cardiac and less regulating types, the subjective responses were severe, the capacity for heart to do work increased, the compensatory responses were great, and the adaptation to hypokinetic environment was poor.

3. As to the responses to the HUT plus LBNP stress, the regulatory compensation and the readaptation, among the four types, the vascular type was optimal, the mixed type came second, the cardiac type was the third, and the less regulating type was the poorest. This order remained the same pre- and post bed rest.

4. "Fuzzy mathematics" was used to further evaluate the individual differences of cardiovascular regulatory function quantitatively during the test of pre- and post bed rest. Thus the evaluation of those differences was quantized.

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PHYSICAL PERFORMANCE AND Gz TOLERANCE

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A fighter pilot is under extreme physical and psychological stress when in combat. This paper discusses some of the physiological reactions occurring in response to acute exposure to high Gz forces; the influence of individual state of training and various physical training programs on Gz tolerance and some possible mechanisms explaining why Gz tolerance may be altered in response to specific physical conditioning programs.

Acute exposure to high Gz forces is associated with a substantial increase in sympathetic activity. Accordingly plasma catecholamines are increased manyfold during acceleration. Hence the epinephrine concentration after a 1-minute exposure to 6 G increases abruptly and 4-fold (1). Also the maximum heart rate response to Gz stress is directly related to the amount of Gz stress (2). Thus, heart rate increases linearly with the Gz force. Interestingly there is a corresponding increase in the pre-acceleration heart rate with the anticipated G-stress. Thus the peak acceleration heart rate and the pre-acceleration heart rate increase in parallell with increasing Gz load (2).

Although the increase in sympathetic activity partially can be attributed to mental stress, acceleration per se and the associated straining maneuver contributes to the exaggerated heart rate response seen during acceleration. In simulated aerial combat maneuver (SACM) markedly increased plasma lactate levels have been demonstrated. Using either a progressive Gz profile or traditional ACM-profiles lactate levels usually exceeded 4 mmol/l (3, 4, 5). The increased plasma lactates at high Gz forces during these experimental conditions suggested that lactate production, as a result of straining, was exaggerated due to muscular work and/or the catecholamine-induced increased rate of skeletal muscle glycogenolysis.

It is not surprising that G-tolerance is improved subsequent to simulated combat training in human centrifuge (2) although the underlying mechanism is not fully understood. Likewise, fighter pilots display higher G-tolerance than non-pilots (6). Yet, there is an appreciable variation in G-tolerance among experienced fighter pilots (2, 3, 4, 6). Hence, in some pilots the limit may be set already at 7 G using a standardized profile of gradual onset rate (GOR) or rapid onset rate (ROR). Due to the impressive power and maneuverability of modern fighter planes it is therefore not too speculative to suggest that the pilot´s G-tolerance could be a limiting factor in future combats.

Is G-tolerance among pilots influenced by their physical characteristics or physical work capacity? Recently, various measures, reflecting work capacity and physical characteristics, were assessed in non-pilots accustomed to SACM (7). G-tolerance, measured using a 3.5- 5.0 G-profile and defined as time to exhaustion, ranged 185-940 s. Although no single measure of physical work capacity correlated with G-tolerance it was observed, using a multiple regression analysis, that 92% of the variance in G-tolerance could be accounted for by the combined effect of body fat percentage, muscle fiber area of the m. vastus lateralis, supine heart volume and body stature. Thus, relatively short and lean individuals who possessed greater muscle fiber area and heart volume than the group mean also demonstrated greater G-tolerance. We therefore conclude that G-tolerance could be predicted from some physiological background variables. It is also apparent that aerobic capacity or power is not enhanced in individuals showing superior G tolerance (3, 4, 7, 8, 9, 10).

Although the anti-G-suit provides some G-protection by increasing peripheral resistance and venous return and by preventing blood to plasma extravasation the execution of straining maneuver enhance performance even more. Repeated, forceful straining of the abdominal and peripheral skeletal muscles may, however, induce premature muscle fatigue. In recent experiments electromyographic (EMG) activity was measured in fighter pilots in the human centrifuge using a standard 3.5-5.5 G-profile without anti-G-suit (11). EMG was recorded by surface electrodes from m. vastus lateralis (VL), m. tibialis anterior (TA) and m. rectus abdominis (RA) while the pilots were instructed to perform M-1 straining maneuver. EMG activity, was expressed relative to the integrated EMG activity (IEMG) during a maximal voluntary contraction performed at 1 G. At 3.5 G IEMG was low for TA and VL but exceeded 40% for RA. At 5.5 G there was a markedly increased activation of VL. It therefore appears that during straining when performing SACM there is a substantial activation of m. quadriceps femoris and m. rectus abdominis.

The physiological adaptations taking place in response to endurance training (ET) are very different to those occurring consequent to weight training (WT) (12); a fact, which has been neglected when discussing effects of physical training on physiological responses to changed gravitational forces.

Recent data suggests that WT but not ET improves Gz tolerance (8, 10, 13, 14). A positive relationship was also demonstrated between leg strength and G-tolerance (14). Thus the greater the strength the greater is the G-tolerance. Fighter pilots were examined before and after 11 wks of a progressive but low intensity, low frequency strength training program (3). They performed leg presses, leg curls, sit-ups and bench presses. The program improved strength and anaerobic power of quadriceps muscles and Gz tolerance as well. In a recent study two groups of pilots, competitive athletes and non-athletes, underwent a 30 month weight training program. Both groups increased muscular strength and G-tolerance and the athletes displayed greater

muscle strength- and power and Gz tolerance pre and post training (14). Anecdots and reports from various laboratories have described decreased G-tolerance in runners carrying out excessive volume of low intensity running. It appears though that this effect is not present if the intensity of the training, and therefore also the cardiac stress, is maintained high (14).

The hemodynamic changes typically occurring consequent to endurance training would suggest a decreased capacity in sustaining high G-forces. Endurance training reduces catecholamine levels during exercise at the same absolute or relative power output. Also, when given β-blockade, trained subjects display less of decrease in heart rate compared to untrained subjects. It also appears that endurance training causes increased cardiac parasympathetic activity (15). Altogether these changes in cardio-vascular response to exercise, noticed after endurance training, may impair the pilot in combat. What then governs an increase in G-tolerance after weight training? Long-term and intense strength training results in skeletal muscle hypertrophy and a concomitant decrease in muscle capillary density (12). In our studies (3, 14) there were no significant increases in muscle mass, probably due to the relatively low load, intensity and frequency of training. Skeletal muscle innervation, however, is typically improved following training resulting in increased muscular strength and enabling a greater mechanical compression of blood vessels. This may in turn prevent excess leg blood pooling. It cannot be ruled out though, that changes in sympathetic activity or control of circulation is influenced by weight training. Long-term weight training, however, is not associated with increased systolic or mean arterial blood pressure at rest or during standardized cycle ergometry exercise or heavy lifting exercise (16, 17).

In conclusion, it appears that a high aerobic power is not a prerequisite for a high G-tolerance. Studies, rather suggest that excessive endurance training may impair G-tolerance. On the contrary, weight training has shown to be effective in order to enhance G-tolerance.

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MORBIDITY REDUCTION OF IN-FLIGHT ACCELERATION INDUCED LOSS OF CONSCIOUSNESS*

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Introduction

Studies on the Mayo human centrifuge (1-3) documented that zero arterial pressure at head level and the simultaneous loss of the ear opacity pulse uniformly preceded loss of consciousness (GLOC) by a period of 3 to 10 seconds as illustrated in the right panel of Figure 1.
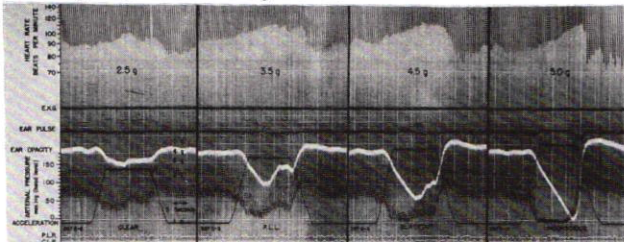
The capability by combined use of an anti-G suit and muscular-respiratory straining maneuvers developed during World War II of producing the systolic arterial pressures of 200-300 mm Hg at heart level required to maintain cerebral perfusion during sustained exposures in the 6 to $9G_z$ range, have, until the recent advent of very maneuverable high speed fighter planes, provided reasonably adequate protection against GLOC (4-6).

However, realization that an unacceptably high fraction of the fatal crashes of F-16 and other highly maneuverable aircraft have been caused by GLOC has evolved gradually from the late 1970's up to the present (7-9). The recent fatal crash of an advanced fighter plane flown by an experienced engineering test pilot wearing a well fitted USAF anti-G suit highlights this problem (10). Multiple incidents of this type coupled with the admission by the U.S. Air Force that, in the upright or partially supinating position, there is no known operationally acceptable method for eliminating GLOC (11,12), emphasizes the importance of reducing the morbidity of GLOC, i.e. (incidents of GLOC induced fatal crashes) $\div$ (total incidents of GLOC).

At least three possibilities of reducing the probability of a crash following an inadvertent occurrence of GLOC (in-flight) have been proposed.

The first is the use of an on-board special purpose computer to activate a plane control takeover system when calculations based on real time input of height above the ground, air speed, plane attitude, etc., indicate that the plane is nearing the height from which crash avoidance is approaching the impossible (15).

The second, more sophisticated approach, is to activate such a takeover system on the basis of real-time monitoring of a physiologic variable(s) changes in which are a uniformly reliable indication of impending loss of consciousness (11,15,16).

The third, and most easily implemented, is to utilize induced GLOC training on a human centrifuge to reduce the duration of the period of incapacitation that follows GLOC and thus the likelihood of an uncontrolled crash (10,14,15).

Theoretically, if a completely reliable automatic ground impact crash avoidance takeover system could be devised, barring mechanical, electronic and/or computer failures, ground impact crashes would be eliminated by the first of these three listed possibilities. Since development of this apparently ideal solution is underway (15) and also

is beyond the authors' area of expertise, it will not be discussed further herein.

However, data collected during human centrifuge and in-flight studies of the blackout and unconsciousness problem during World War II are of significance in relation to the second and third possibilities. Intensive tests of a variety of anti-G suits, self-protective maneuvers and other anti-G stratagems were carried out in the 1942-1945 period (17-23). The more than 300 healthy young men and women who served as volunteer subjects for these studies experienced approximately 10,000 exposures to centripetal acceleration ranging from 2 to 10G on the Mayo human centrifuge and in specially instrumented dive bombers (Figure 2).
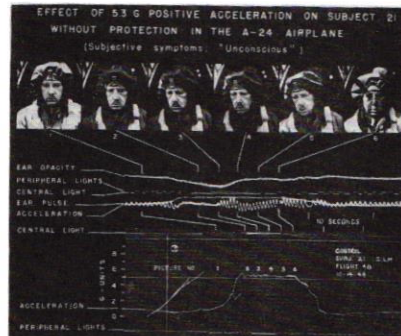
Because accurate multiparameter physiologic recordings were obtained during most of these exposures (Figures 1-4), considerable information concerning the physiologic alterations, which invariably occur prior to acceleration induced loss of vision and/or loss of consciousness as well as data concerning acute and possible residual damage from such exposures, were obtained. A description of these data, which are of potential value relative to the use of: (A) Physiologic monitoring to activate an automatic plane control takeover system prior to or coincident with pilot loss of consciousness and, (B) the possibility of acute or chronic injury from induced episodes of GLOC for training purposes follows (14,18).

A: Morbidity Reduction by Prodromal Monitoring

Photoelectric plethysmographic techniques for non-invasive measurement of changes in the opacity (blood content) of the ear and pulsatile changes in ear opacity (blood content) produced by each heartbeat were recorded routinely in all centrifuge and in-flight studies by two modified Millikan type oximeter earpieces (24-28) held in place on the ears by specially designed universal clamps mounted on a head band or for increased convenience and comfort of repetitive volunteer subjects, a custom made plaster skull cap was used particularly by the laboratory personnel (24,28).

Figure 3 is an example of ear opacity and ear opacity pulses and other variables recorded during a series of exposures of a healthy young man to progressively higher, 15 second duration, plateau levels of acceleration.
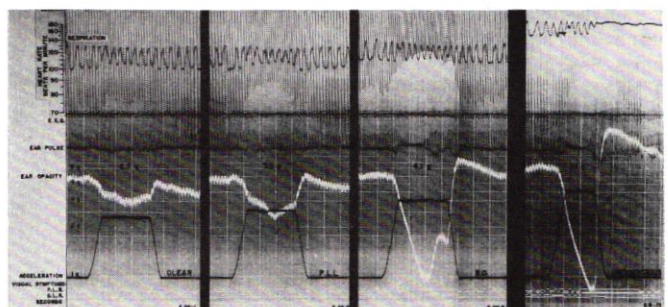
Note particularly (right panel) that the ear opacity pulse was obliterated throughout the exposure to 5G but that failure to respond to the peripheral and central light signals was delayed 5

and 6 seconds, respectively, after the attainment of 5G and, most importantly, relative to a possible on-line prodromal GLOC warning system, arterial ear opacity pulsations were absent for 8 seconds before evident loss of consciousness occurred.

Figure 4 is a recording of the changes in ear opacity and ear opacity pulses along with simultaneous intra-arterial pressures at base of brain and heart level during exposures to increasing plateau levels of acceleration. Note that loss and recovery of directly recorded intra-arterial systolic pressures at head level occurred simultaneously, i.e. on the same heartbeat as the loss and recovery of the ear opacity pulse.
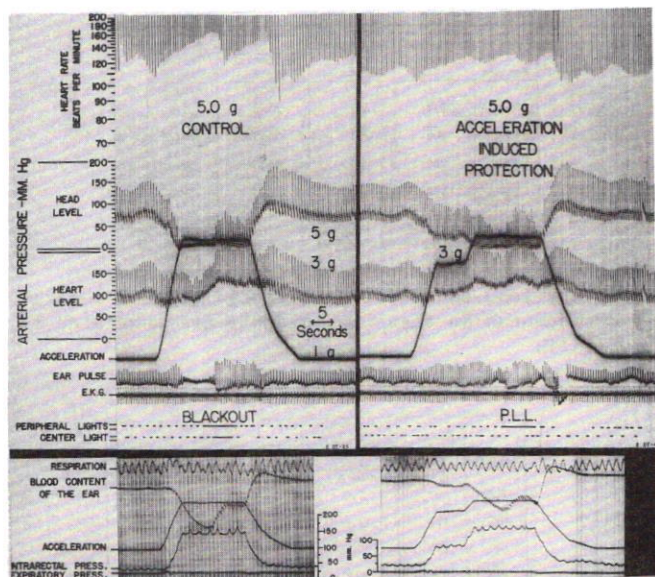
That loss of the ear opacity pulse during G_z acceleration is associated with zero arterial pressure at head level, has been an invariant finding in simultaneous recordings of arterial pressure at head level, and the ear opacity pulse during multiple exposures of healthy young men to G_z accelerations ranging from 4 to 7G (24-28).

Furthermore, in the more than 50 incidents of loss of consciousness observed from 1942 through 1945 on the Mayo human centrifuge and in-flight (Figures 1-4), a period of at least 4 seconds of zero or very near zero ear opacity pulse invariably preceded loss of consciousness. These findings support the belief that loss of the ear opacity pulse could be used as a forewarning of possible loss of consciousness.

Visual documentation of this possibility was achieved back in 1943 by use of electronic circuitry, designed and built by Ralph E. Sturm (29) which, in real time, illuminated and modulated the intensity of a red light in proportion to the amplitude of each photoelectric ear opacity pulsation detected within a subject's ear before, during and after exposures to G_z acceleration.

This photoelectrically activated light was mounted in an assembly juxtaposed to the subject's head, as illustrated in Figure 5(a) of a single frame from a motion picture of a subject in the cockpit of the Mayo human centrifuge.

The upper (b) dial and lower (c) dials indicated elapsed time in seconds and acceleration in G units, respectively. Two lights mounted between these dials, the left (c) covered with a green and the right (d) with a red filter, were illuminated synchronously with light signals in the peripheral and central regions of the subject's field of vision, respectively, and were turned off each time the subject responded (h) to these respective peripheral and central light signals.

fig 5
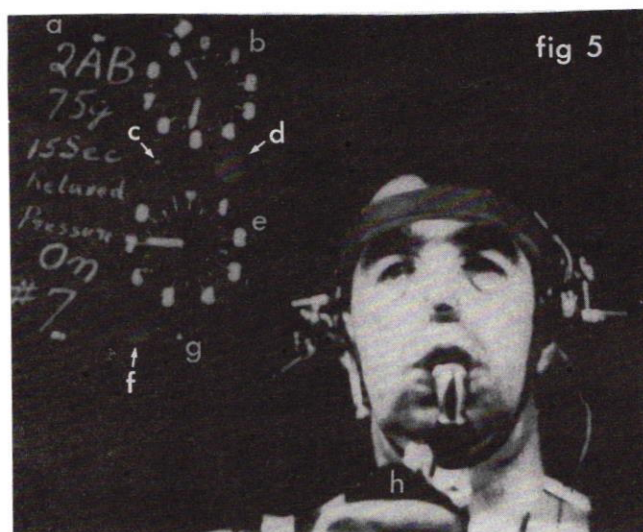
The small white light (g) below the accelerometer dial flashed every second. A red filtered light (f) to the left of the seconds flasher was illuminated and its intensity modulated in proportion to the amplitude of each ear opacity pulse detected by the electronic circuitry described elsewhere (16,24-28).

Viewing of the series of cine films obtained on the Mayo Human Centrifuge during World War II (30-33) and published synchronous recordings of the ear opacity pulse and other variables during multiple incidents of blackout and/or GLOC on the Mayo Human Centrifuge or in-flight (16-28), provide convincing conformation that acceleration induced loss of vision (i.e. blackout) is frequently preceded by a several second period of loss of ear opacity pulse, particularly in unprotected individuals and, if a nearly concomitant or subsequent loss of consciousness occurs, it is invariably preceded by loss of ear opacity pulse ranging from about 3 to 10 seconds prior to the slumping in the seat which occurs with muscular relaxation at the onset of the unconscious state.

These data document the possibility that a loss of ear opacity pulse of longer than 2-3 seconds duration could be used to activate: 1) A visual or auditory warning signal to the pilot of the danger of impending loss of consciousness or, 2) an automatic plane control takeover system.

There are, however, operational and physiological considerations which decrement the apparent advantages of use of such a system.

One of these is illustrated by the relationship of minimal systolic arterial pressure at head level to symptoms experienced in 18 subjects during sustained exposures to G_z acceleration on the Mayo Human Centrifuge (Figure 6).

LOWEST SYSTOLIC ARTERIAL PRESSURE AT EYE LEVEL WHEN SYMPTOMS OCCURRED DURING G
18 SUBJECTS
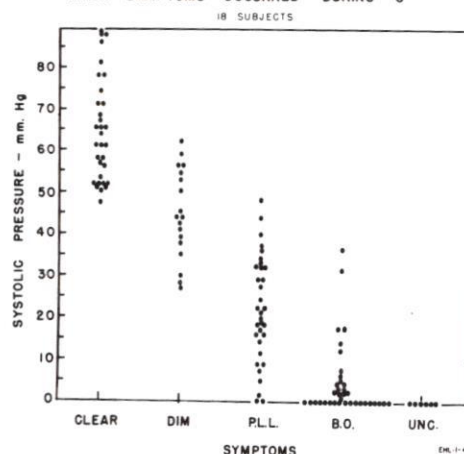
These direct arterial pressure data indicate that zero systolic pressure at head level for one or more heartbeats is always associated with significant loss of vision.

However, although the six instances of loss of consciousness were all preceded by a 3 or more second period of zero systolic pressure at head level, in 24 instances, GLOC did not occur in spite of periods of zero arterial pressure at base of brain level ranging from 1 or 2 heartbeats to as long as 7 seconds.

A more complete picture of the relationship between minimal systolic pressure at head level and simultaneously recorded ear opacity pulses is provided by Figure 7.

RELATION OF MINIMUM EAR PULSE AMPLITUDE TO MINIMUM INTRA-ARTERIAL SYSTOLIC PRESSURE AT HEAD LEVEL DURING EXPOSURE TO POSITIVE ACCELERATION
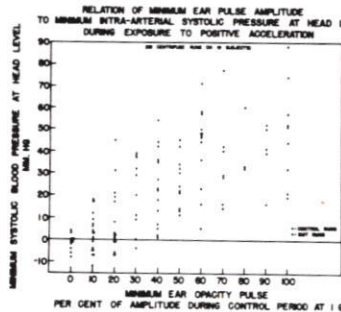
These data document the fact that loss of the ear opacity pulse is a reliable index of simultaneous zero or very near zero systolic pressure at head level. However, when the ear opacity pulse and arterial pressure at head level are maintained throughout G_z exposures, the relationship between these two parameters is quite variable. For example, in different individuals, the amplitude of an ear opacity pulse, generated by a heartbeat resulting in a 50 mm Hg peak arterial pressure at head level, may range from 40 to 100% of the ear opacity pulse recorded at 1G just prior to the exposure. Never-the-less, when the amplitude of a given ear opacity pulse was less than 30% of the control value, the arterial pressure of that particular beat at head level was invariably less than 45 mm Hg. Furthermore, if this situation pertained for longer than the ischemic anoxic latent period of the retina, some degree of visual impairment invariably occurred.

Conclusions

During G_z acceleration, loss of the ear opacity pulse and zero systolic pressure at ear level are simultaneous phenomenon. Consequently, loss of the ear opacity pulse can be used as a reliable, non-invasive prodromal warning of visual loss and, if sustained for more than 2-3 seconds, as a danger sign of possible impending loss of consciousness.

B: GLOC Morbidity Reduction by Pilot Training

The potential value of GLOC training is based on Mayo Centrifuge results and more recent, extensive data by Whinnery and colleagues (13) which indicate that the duration of the period of disorientation as to time and place, which always follows GLOC, is reduced an average of 8.5 seconds during subsequent incidents of loss of consciousness in experienced centrifuge subjects. This reduction in recovery time could be the difference between life and death if an inadvertent incident of GLOC occurs during a simulated high G, low altitude combat maneuver such as commonly practiced by fighter pilots (14).

Since G_z acceleration induced complete loss of vision, i.e. blackout, is usually associated with very low or zero arterial pressure at head level and loss of consciousness is always preceded by zero arterial pressure at brain level (Figures 1-4, 6), the possibility that multiple incidents of blackout and/or loss of consciousness might produce residual retinal and/or cerebral damage merits investigation.

During the October 1942 to October 1945 war years, four members of the Mayo centrifuge staff experienced multiple exposures to accelerations of 2.5G to 10G, which were sustained routinely for 15 or more seconds and ranged in number from 246 for subject RLE to 1,198 for subject EHW (18). Symptoms ranging from loss of peripheral vision to loss of consciousness occurred in 70 percent of these exposures resulting in accumulated losses of central vision of more than one-half hour in two of these subjects (Table 1).

ACCUMULATED TIME OF LOSS OF PERIPHERAL AND CENTRAL VISION BY MAYO CENTRIFUGE PERSONNEL

October 1942 to October 1945

Subject	Number of exposures above 2.5 g	Number of exposures with symptoms*	Accumulated time of loss of:	
			Peripheral vision (minutes)	Central vision (minutes)
RLE	246	169	15.3	5.1
CFC	364	286	38.9	20.7
EHL	985	749	76.0	34.0
EHW	1198	890	62.3	31.0

* Dimming of vision, loss of peripheral vision only, loss of central vision, loss of consciousness.

The number of instances and accumulated times of complete loss of vision experienced without loss of consciousness in these individuals are tabulated in Table II.

INSTANCES OF COMPLETE LOSS OF VISION BY MAYO CENTRIFUGE PERSONNEL

October 1942 to October 1945

Subject	Number of instances	Average duration of loss* of:		Accumulated time of loss of:	
		Peripheral vision (seconds)	Central vision (seconds)	Peripheral vision (minutes)	Central vision (minutes)
RLE	47	7.5	4.9	5.9	3.9
CFC	140	11.7	8.8	27.2	20.4
EHL	274	10.7	6.6	49.0	30.0
EHW	299	7.9	6.0	39.9	29.8

* On the average peripheral vision was lost at 5.2 seconds and central vision at 7.8 seconds after the attainment of the maximal g level at the start of the exposure.

The incidents of loss of consciousness which ranged from 3 to 23 in these four individuals are listed in Table III.

INSTANCES OF LOSS OF CONSCIOUSNESS BY MAYO CENTRIFUGE PERSONNEL

October 1942 to October 1945

Subject	Number of instances	Average duration of loss* of:		Accumulated time of loss of:	
		Peripheral vision (seconds)	Central vision (seconds)	Peripheral vision (minutes)	Central vision (minutes)
RLE	6	11.6	11.5	1.2	1.2
CFC	3	9.8	7.5	0.5	0.4
EHL	23	15.9	12.7	6.1	4.9
EHW	8	12.5	10.5	1.6	1.4

* On the average peripheral vision was lost at 5.3 seconds and central vision at 6.5 seconds after the attainment of maximal g level at the start of the run.

INSTANCES OF COMPLETE LOSS OF EAR OPACITY PULSE* DURING EXPOSURES TO POSITIVE ACCELERATION OF MAYO CENTRIFUGE PERSONNEL

October 1942 to October 1945

Subject	Number of instances	Average duration of ear pulse loss (seconds)	Accumulated time ear pulse lost (minutes)	Estimated total time ear pulse lost (minutes)
RLE	34	5.3	3.0	3.4
CFC	27	6.3	2.8	3.5
EHL	68	5.4	6.1	7.7
EHW	151	5.4	13.7	15.6

* Under these circumstances the systolic blood pressure at head level is zero.

Because the ear opacity pulse was recorded throughout most of these exposures, it is possible by measuring the total accumulated time of zero pulse amplitude, to estimate that the total accumulated time of zero arterial pressure at head level in these individuals ranged from 3.4 to 15.6 minutes (Table IV).

At this time, 42 years later, it is of interest that these individuals have normal vision for persons in their age group and are as active physically and mentally as one could expect after enduring more than their allotted 3 score and 10 years of existence on planet earth.

We conclude, therefore, that subjecting pilots to several incidents of human centrifuge induced losses of consciousness for training purposes has a high degree of safety and is a potentially life saving exercise. Consequently, inclusion of this procedure as an integral part of the training program for fighter pilots merits serious consideration by the military (10,14,15).

A much more certain crew and plane saving solution of the GLOC problem is, however, described elsewhere (16,23,34,35).

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TISSUE ADAPTATIONS TO GRAVITATIONAL STRESS: NEWBORN VERSUS ADULT GIRAFFES

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Load-bearing tissues of the giraffe present important material for developmental studies of adaptive mechanisms to gravity. Fetal giraffes grow in a quasi-weightless environment in utero, whereas adult animals reach 5-6 meters tall and tissues of their legs bear weights up to several hundred kg during upright standing posture. Tissue samples were obtained from four adolescent, two aged, and three newborn giraffes. After fixation in formalin; cell density, microcirculatory density, and tissue hypertrophy were quantified in menisci from knees, in arteries from the neck and legs, and in the heart. Newborn giraffes had higher densities of cells and microcirculatory vessels in their menisci than adult giraffes. The relative avascularity of adult menisci may be related to compression and functional loss of their microcirculation upon assumption of upright posture with increased weightbearing. In the cardiovascular system, pronounced arterial wall hypertrophy in dependent tissues was a prominent feature of the giraffe's adaptation to increased systemic and dependent blood pressures. Similarly, myocardial wall hypertrophy was also associated with giraffe maturity. In summary, the developing giraffe represents an excellent model for studying tissue adaptation to increasing load bearing in a normal gravitational field.

INTRODUCTION

Compared to our knowledge of the effects of weightlessness upon bone and muscle, relatively little is known about microgravity effects on other load bearing tissues (e.g., meniscus, intervertebral disc, cartilage, fascia and other connective tissues). Furthermore, more studies of developmental biology are needed to elucidate mechanisms by which various load-bearing tissues adapt to increasing weight bearing in a normal gravitational field on Earth. Such investigations may provide important information concerning readaptation of space travelers to normal gravity following prolonged exposure to weightlessness. Moreover, armed with this understanding, countermeasures against loss, atrophy and/or degeneration of load-bearing tissues may be developed more easily.

The giraffe, Giraffa camelopardalis, represents an important mammalian model for developmental studies of tissue adaptation to increasing weight bearing. Whereas fetal giraffes develop in a quasi-weightless milieu, postpartum giraffes must contend with increasing load bearing in their dependent tissues as they grow to heights over 5 meters and to weights over 1000 kilograms. Previous studies of the adult giraffe cardiovascular system (4,7,11) indicate that it is certainly a unique model for investigating the physiology of being tall and adaptations to large and variable gradients of blood pressure. These early studies of blood pressures document that arterial pressure near the giraffe heart is about twice that in humans in order to provide more normal blood pressure and perfusion to the brain. During our 1985 Giraffe Physiology Expedition to Africa, studies focused upon hemodynamic adaptations and edema prevention in legs of adult giraffes (5). Briefly, we found that the blood and tissue fluid pressures which determine transcapillary ultrafiltration are highly variable with exercise (Fig. 1).
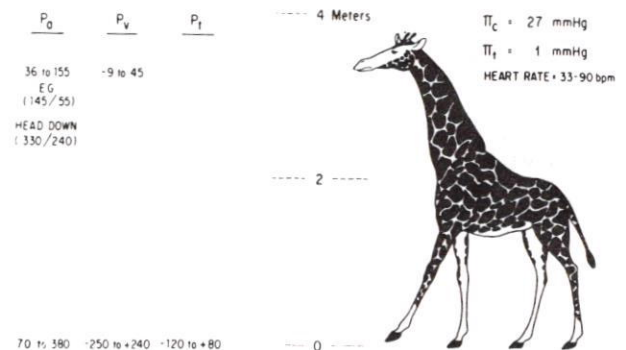
Figure 1. Range of meaned blood and tissue fluid pressures in neck and foot of walking giraffes. Negative venous and subcutaneous pressures during exercise help prevent dependent edema. With head upright, mean arterial pressure below the jaw ranged between 36 and 155 mm Hg (e.g., 145/55 mm Hg for systolic/diastolic pressures). Drinking water in head-down posture raised carotid pressures to 330/240 mm Hg. From Hargens et al. (6).

These pressures, combined with a tight skin and fascial "antigravity suit" (Fig. 2), move venous blood and tissue fluid upward against gravity, thus preventing pooling of blood and edema in dependent tissues. More proximally, an active skeletal-muscle pump aids venous return.
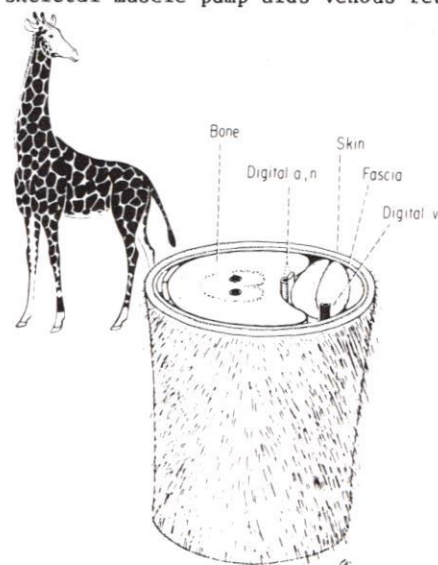
Figure 2. Anatomical evidence of "antigravity suit" in giraffe foot. Tight skin and fascia surround blood vessels and connective tissues. From Hargens et al. (6).

The nonhydrostatic pressure gradient down the giraffe's jugular vein indicates that blood cascades down from the head and that circulations above heart level do not depend upon a siphonlike principle as recently proposed (1). Other edema-preventing mechanisms include dependent precapillary vasoconstriction and very low capillary permeability to plasma proteins.

Our present study presents preliminary results concerning developmental alterations in load-bearing tissues of newborn and adult giraffes. In this paper emphasis is placed on vascular wall thickness in relation to local blood pressure and on meniscal adaptations to increased load bearing in the developing giraffe.

METHODS

Tissue samples were collected from four 5-6 year old, 3 1/2 - 4 meter, male and female giraffes during the 1985 Giraffe Physiology Expedition to Africa; three newborn and two 25 & 35 year old, 5 meter adult giraffes from the Cincinnati and Cheyenne Mountain Zoos. Arteries were obtained from the neck (carotid) and forelimbs (digital) and fixed in 10% buffered formalin prior to processing. Arterioles and other microcirculatory vessels were sampled from skin and muscle of the head, neck, thorax, legs, and feet. Medial and lateral menisci were collected from hindlimbs of two newborn and two aged giraffes (25 and 35 year old females). Cell and vascular densities were measured in 6 micron thick cross sections of each meniscus for superficial as well as central meniscal regions (Fig. 3).
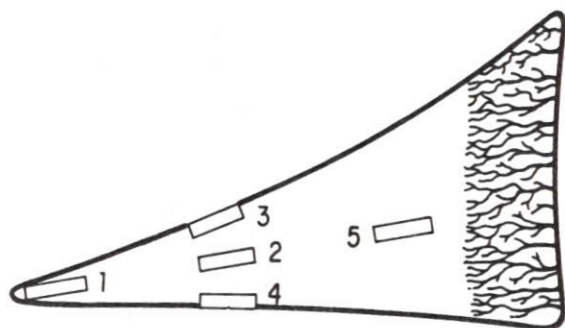
Figure 3. Cellular and vascular densities were measured in regions #1 through #5 of anterior, mid and posterior portions of each giraffe meniscus.

RESULTS

Although we never had the opportunity to measure blood pressures in newborn giraffes; based upon comparable studies of other species, arterial pressures of baby giraffes are probably significantly lower at heart level and in dependent tissues than those in adult giraffes. It was apparent that dependent arteries in adults had much thicker walls than those in newborn giraffes (Fig. 4).
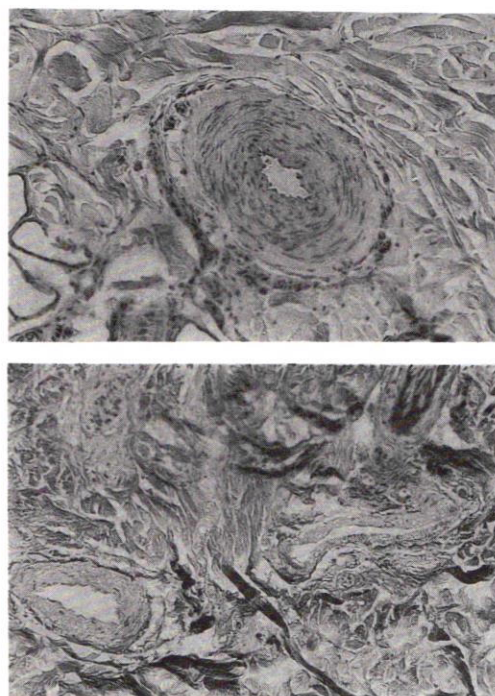
Figure 4. Four hundred micron diameter arteries from foot skin of adult (top) compared to newborn (bottom left) giraffe.

In adult animals it was evident that arterial wall hypertrophy correlated directly with the degree of tissue dependency (Table I). Thus, wall thickness to lumen ratios of large arteries increase from head to foot except for one "ankle" artery that didn't follow this pattern. In the tissues that we examined, arterial wall hypertrophy was apparently restricted to vessels with outer diameters greater than 400 microns.

Table I. Ratios of smooth-muscle wall thickness/lumen radius (w/r) for arteries from the neck to the feet of adult giraffes.

Artery	w/r	Outer Diameter (mm)
Carotid	0.15 - 0.20	9.5 - 1.0
Brachial	0.33 - 0.43	5.5 - 7.5
Femoral	0.65 - 0.68	4.0 - 4.2
Ulnar	0.70	7.2
Radial	0.70	5.4
"Ankle"	0.33	4.6
Metatarsal	0.51 - 0.81	3.1 - 4.8
Digital	0.56	4.0

In terms of cell density, our preliminary results suggest that menisci of newborn giraffes have higher cell densities than adult giraffes in all regions studied (Fig. 5).
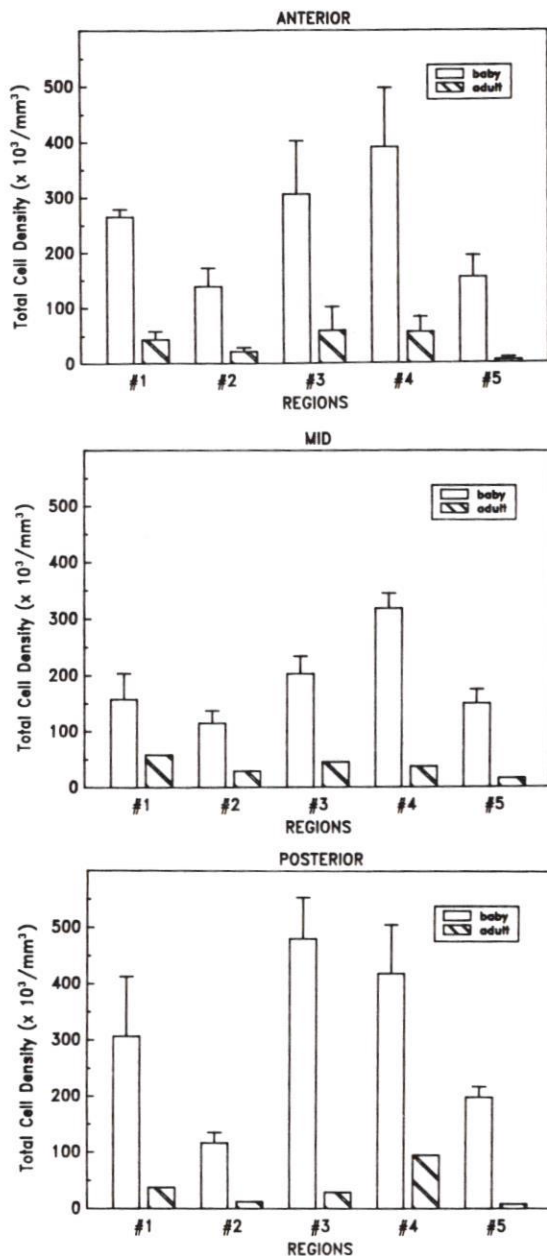
Figure 5. Meniscocyte density in anterior (top), mid (center), and posterior (bottom) portions of newborn versus adult specimens. Cell densities were lower in adult menisci than in baby menisci. Similar trends to lower vascular density were observed in menisci of newborns as compared to adults. Also, note that central regions #2 and #5 have fewer cells than superficial regions #1, #3, and #4.

DISCUSSION

We believe the developing giraffe provides an excellent model for investigations of adaptive mechanisms to increased weight bearing. Although this paper presents preliminary and intriguing results for the arterial system and menisci of newborn and adult giraffes, other load-bearing tissues such as bone, muscle, intervertebral disc, cartilage, ligament, tendon, fascia and veins should be investigated as well. Arteries of the feet are sometimes exposed to blood pressures greater than 400 mm Hg in adult giraffes (6). These vessels have developed pronounced smooth-muscle hypertrophy and narrowed lumens in order to accommodate their extraordinary blood pressures. Our previous report of a less than normal arterial

pressure gradient from heart to foot in the upright, stationary giraffe (5) suggests that the reduction in lumen cross-sectional area plays some role in blood pressure reduction to dependent tissues. It was interesting that the arterial wall hypertrophy was apparently confined to dependent vessels with diameters over 400 microns in adults and was not observed in newborn giraffes or in vessels near the head of adult giraffes.

The lower cell and vascular densities observed in adult giraffe menisci, as compared to those in newborns, may be related to increased load bearing and occlusion of the microcirculation during ambulation of the growing giraffe. In adult humans, blood perfusion of the meniscus is confined to its peripheral 10-25% (3) and human menisci lose vascularity during development from the fetus to adult (2). Also, in response to prolonged exercise, regions of the rat meniscus adapt differently in terms of both morphology and biochemistry (9). Other changes observed during meniscal development in cell culture include differentiation of cell types and proteoglycan-producing capacity (12). The lower cell density that we observed in adult specimens may represent an adaptation to decreased vascular density and poorer nutrition of central regions #2 and #5 as previously postulated by Smillie (8). As reported earlier in the rat (10), we also observed calcium deposits in menisci of aged giraffes.

In summary, our results concerning tissue adaptations to increased load bearing in the developing giraffe provide interesting and preliminary findings that deserve further investigation. More studies of the developmental biology of giraffes are needed to elucidate mechanisms by which tissues adapt to increased weight bearing. Such knowledge may provide useful information for understanding the effects of weightlessness on load-bearing tissues and for developing countermeasures to aid readaptation of these tissues to normal gravity after prolonged space flight.

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SPACE FLIGHT EFFECTS ON TISSUE LIPIDS IN GRAVID RATS AND IN THEIR OFFSPRING

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Serious changes of tissue lipids were detected in rats flown in average 20 days onboard biosatellites of "Cosmos" series. Shortly (several hours) after landing of satellites the analyses showed an increase in non-esterified fatty acid (NEFA) concentration in serum (plasma), liver, white and brown adipose tissues, an increase of triacylglycerols(TG) in serum, liver, thymus and bone marrow (femur), and an increase in total cholesterol (CH) concentration in serum and liver. Generally, hyperlipemia, hypermobilization of NEFAs with an biochemical patterns of fatty liver and TG accumulation in bone marrow appeared to be dominant among the changes manifested. In the readaptation period-approximately 25 days after landing- most of the changes have subsided, but the accumulation of TG in bone marrow and, inconstantly, the signs of enhanced lipolysis in adipose tissue persisted in the entire experiment. The use of artificial gravity onboard "Cosmos 936" prevented some changes. The microgravity and particulary the stress, originated in landing manoeuvre have participated in the pathogenesis of these changes (2,1).

The aim of recent experiment was to determine the effect of space factors on pregnant rats and on their offspring - first filial generation.

Methods.

Female SPF bred rats of Wistar strain in 13. day of pregnancy were orbited onboard "Cosmos 1514" biosatellite for 5 days and got through the flight in good general condition. The flight (F) mothers were divided into two groups. The first part was killed and analyzed several hours after landing, i.e. on day 18 of pregnancy. With some delay analogous animals from vivarial (V) conditions and from the so called synchronous (S) experiment - with all available space flight factors simulated in ground conditions-were analyzed. The second part of mothers gave birth to the offspring. The first filial generation (F1G, males only) was analyzed on postnatal days 15,30 and 100. The concentration of NEFAs in white (perirenal or epididymal) and brown (in-terscapular) adipose tissue; the concentration of TG and phospholipids (PL) in liver and thymus and the concentration of CH in liver were determined. Each group consisted of 6 animals. The significance of differences between F, V and S groups of animals was evaluated by t-test for non-paired values. The analysis of brown fat on postnatal day 100 and of thymus on postnatal day 15 was not realized.

Results.

A d i p o s e   t i s s u e. The concentration of NEFAs in white adipose tissue was enhanced in F mothers. On postnatal days 15 and 30 NEFA values increased in S offspring, compared with F and V animals; on postnatal day 100 the differences between groups disappeared (Fig.1). In the brown adipose tissue the NEFAs were higher in F mothers and also on postnatal day 15 (compared with V animals) in F and S offspring; later the differences disappeared (Fig.2).

L i v e r. Compared with V and S animals TG concentration was elevated in F mothers; no difference was found between individual groups of pups (Fig.3). No differences were seen in concentration of CH and PL between all groups analyzed (not shown).

T h y m u s. In F mothers there was a non-significant decrease in TG concentration. In S offspring (compared with F and V animals) TG increased on postnatal day 30 and the differences disappeared on postnatal day 100 (Fig.4). The concentration of PL was insignificantly higher in F mothers. On postnatal day 30 the concentration of PL decreased in S animals and the situation was normalized on postnatal day 100 (Fig.5).

Discussion.

The patterns of tissue lipid changes in pregnant rats flown onboard "Cosmos 1514" were similar to those seen in male rats after longer space flight (i.e.Cosmos 782, 936 and 1129) and after short-term flight (7 days- Cosmos 1667)-namely an increase in NEFAs in white and brown adipose tissue and an increase in TG concentration in liver. The increased lipolysis and lipomobilization seem to be responsible for TG accumulation in liver and for the appearance of biochemical picture of fatty liver (mild and reversible, verified histologically in previous experiments in Cosmos satellite series). The use of artificial gravity onboard"Cosmos 936" prevented the appearance of fatty liver, but did not markedly influence the increased mobilization of NEFAs (3). When comparing short-term with prolonged space flight (gravid female vs male rats) the former modified the lipid composition in thymus; after landing from longer flights there were regularly increased TG and decreased PL concentration-typical stress-effect caused probably by landing manoeuvre. Sex, biological modification (pregnancy) and duration of the flight might interact in the origin of those changes. The use of arteficial gravity on board "Cosmos 936" did not prevent changes in thymus lipid composition (3).
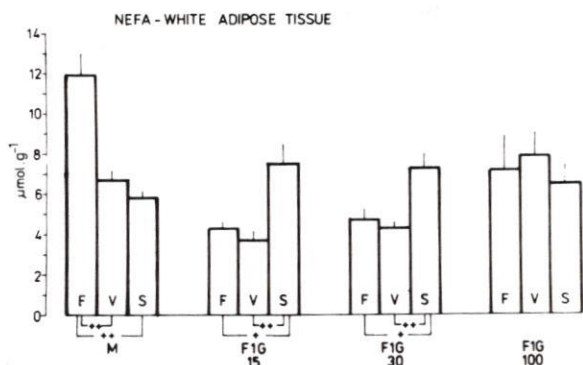
Fig.1. The concentration of non-esteri-
fied fatty acids in white adipose tissue
in flight (F), vivarial (V) and synchro-
nous (S) mothers (M) and in the offspring
(F1G) of F,V,S mothers on postnatal day
15,30 and 100. Values are given as M+SEM,
significances of differences between
groups depicted as+(P< 0,05) or ++
(P< 0,01).
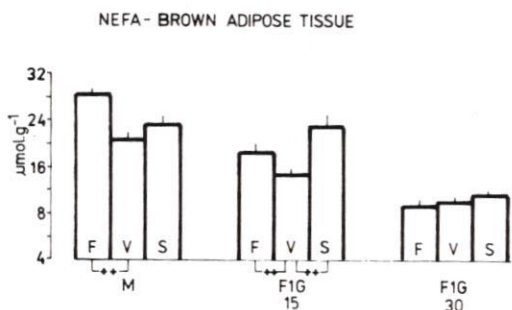
Fig.2. The concentration of non-esteri-
fied fatty acids in brown adipose tissue.
For details see text and Fig.1.
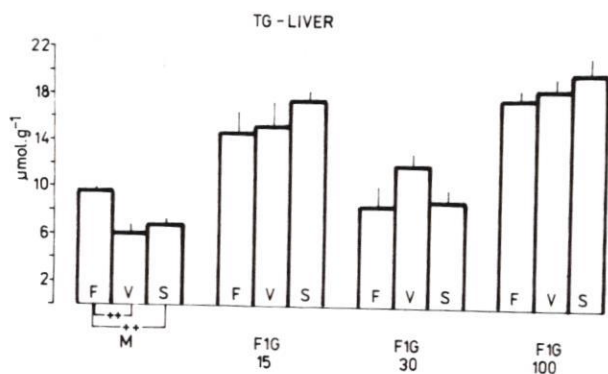
Fig.3. The concentration of liver tri-
acylglycerols. For details see text and
Fig.1.
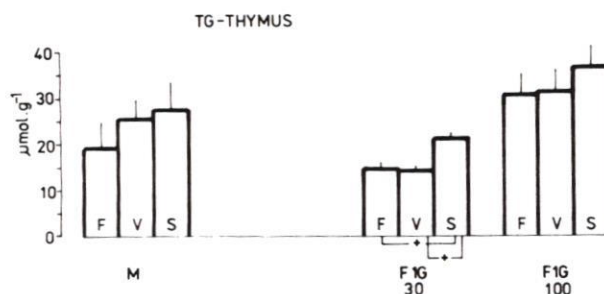
Fig.4. The concentration of thymus tri-
acylglycerols. For details see text and
Fig. 1.
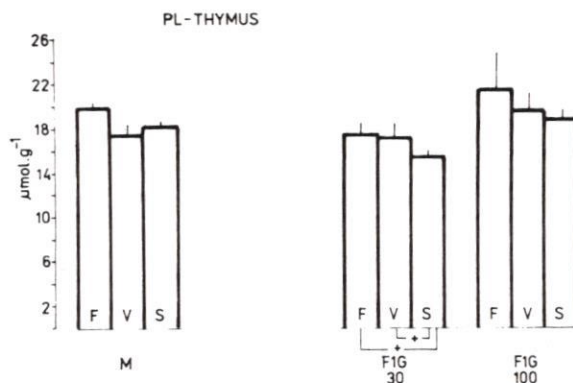
Fig.5. The concentration of thymus phos-
pholipids. For details see text and
Fig. 1.

Increase in NEFA concentration in
brown adipose tissue at postnatal day 15
was the only documented change in the
offspring of F and S animals. The reason
is not clear, but the greater thermogenic
effort in these animals may be considered
the basis for increased lipolysis. In S
offspring group some isolated changes
occured at postnatal day 15; these
stress-like effects may be attributed to
the different handling conditions in this
group. Minimal changes of tissue lipid
composition and its total recovery at
postnatal day 100 demonstrates-together
with other results in these animals- an
undamaged development of metabolic path-
ways in the offspring of mothers, subjec-
ted to the effect of space flight in the
last third of pregnancy. Further experi-
ments may bring more light into this in-
teresting problem of space biology.

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HEMOPOIETIC STEM CELL (CFUs) MEASUREMENTS IN PREGNANT RATS FLOWN ON COSMOS-1514 BIOSATELLITE

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Changes in the number of hemopoietic
stem cells (CFUs) were studied in pregnant
rats during the recovery day and in their
offspring during selected days after a
5-day flight on the biosatellite Cosmos-
-1514. A statistically significant de-
crease was found in the CFUs number in
bone marrow and spleens of pregnant rats.
Different changes in the number of CFUs/
/10^6 nucleated cells in the bone marrow
and spleen of F and SC rats indicate that
the extent of the decrease in CFUs pool
in the spleen was influenced by the action
of non-specific flight factors. In the
offspring no significant changes were
found in the bone marrow and spleen pools
of CFUs during their ontogenesis.

Introduction

One of the manifestations of the ef-
fect of space flight on living organisms
is the inhibition of erythropoiesis which
was observed in mice after the Apollo 11
mission (1), as well as the same depres-
sion observed in rats after their flight
onboard the biosatellites Cosmos-605 and
Cosmos-782 (2,3). The decrease in the CFUs
number in the bone marrow of rats on the
recovery day after flights on the bio-
satellites Cosmos-936 and Cosmos-1129 (4)
and changes in hematologic parameters of
astrorats flown on the shuttle mission
Spacelab 3 (5) indicate that factors as-
sociated with space flight may produce
changes in hemopoiesis.

The results of a short-term (5-day)
space flight on the hemopoietic stem cells
(CFUs) in bone marrow and spleens of preg-
nant rats flown on board the Cosmos-1514
biosatellite as well as those of their
offspring are presented in this report.
The experiment was part of a broader re-
search project (Embryogenesis) aimed at
determining the effects of weightlessness
on the prenatal development of foetuses
during active ontogenesis.

Material and Methods

Female rats of a Wistar strain were
4 months old, and on day 13 of their preg-
nancy at launch and on day 18 of their
pregnancy at landing. Pre-flight the rats
weighed from 280 to 310 g as well as con-
trol groups. The following groups of ani-
mals were used: (1), flight rats (F);
(2), a synchronous model control (SC)
- kept under the same conditions as the
F group, but on Earth; (3), vivarial con-
trol (VC) - placed in the conditions of
a terrestrial vivarium. For the hemopoie-
tic stem cell study a spleen - colony
assay method (6) was used, which was
adapted (7) for the application in rats.
The whole-body ^{60}Co-gamma irradiated
isogenic recipients were 21 - 28 days
of age.

Results

Changes in the CFUs number in femoral
bone marrow (BM) of pregnant rats on the
recovery day after the landing of the bio-
satellite exhibited a decrease in the
cellularity of the F group and in the num-
ber of CFUs/10^6 nucleated cells, and thus
a significant reduction of the total CFUs
pool. The SC control group displayed a
slight insignificant reduction of the pool
of CFUs in BM as a result of the decrease
in cellularity, because the CFUs number
in 10^6 nucleated cells was the same as
that in VC rats.
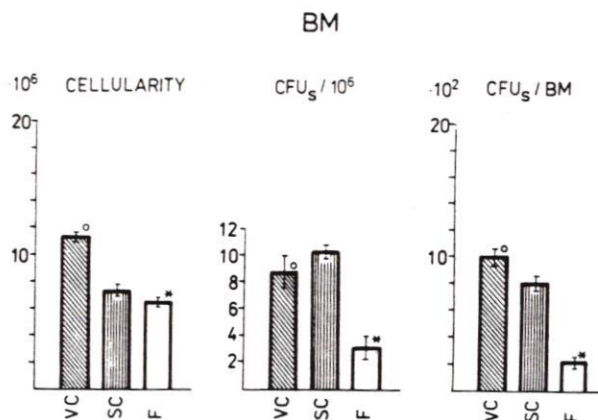
^{x}Significantly different from controls (o)
(p $\angle$ 0.01).

The different behaviour of the
spleens under the experimental conditions
was observed. Both the F and the SC rats
displayed a significant reduction of cel-
lularity and spleen weight as compared to
the VC rats. That total CFUs pool of F and
SC rats was not significantly different,
while it was so as compared with VC rats.

The results of the CFUs content in the BM and spleens in young rats - offspring of the main experimental groups - are shown in the following table:

CFUs number in 10^6 nucleated cells (offspring)

Age /days/	n	F	SC	VC
		B o n e m a r r o w s		
15	5	8.6+0.6	9.1+0.6	8.9+1.4
30	5	14.9+0.8	13.1+1.2	16.3+1.04
100	5	15.5+1.2	13.5+1.8	12.3+0.9
		S p l e e n s		
01	5	19.2+0.7[1]	8.5+0.8	10.0+0.8
15	5	5.4+0.9	6.0+0.4	10.0+0.6
30	5	9.6+0.7	7.8+0.4	8.0+0.6
100	5	3.6+1.1	1.2+0.3	2.1+0.8

n = number of young rats, each cell was inoculated into 15 recipients

[1]significantly different (p $\angle$ 0.01) from SC

Discussion

The results presented are consistent with a previous finding of a decrease in the CFUs number in the bone marrow in femurs and humeruses of rats on the recovery day after an 18 - 19 day flight on the biosatellites Cosmos-936 and Cosmos-1129 (4) and show that already a 5-day stay of animals in the state of weightlessness significantly changes the pool of marrow CFUs. It is of interest that there were no significant changes in the cellularity of bone marrow between F and SC rats, which is consistent with the observation of Lange et al. (5). The different changes of the CFUs pool of the spleens indicate a high influence of the stress reaction on the F as well as on SC rats. The course of the age changes in the pool of CFUs in BM and spleens of young rats document that the weightlessness had no significant influences on the ontogeny of foetuses of pregnant rats flown on a biosatellite.

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CHANGES OF DEOXYRIBONUCLEOPROTEIN AND NUCLEIC ACID CONTENT IN TISSUES OF PREGNANT RATS AND THEIR OFFSPRING AFTER 5 DAYS OF SPACE FLIGHT

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Exposure of pregnant rats to microgravity on board of "Cosmos 1514" biosatellite for 5 days caused significant decrease in nucleic acid content in spleen, thymus and blood. The extent of changes in nucleic acids was comparable with those in male rats after 18.5-21 days of flight in previous experiments. However, no signs of deoxyribonucleoprotein breakdown in spleen and thymus of pregnant rats were found. From these findings we suppose that the sensitive cells demaged at the start of satellite could not regenerate because of short flight duration. Therefore the sensitive cells were not present in the tissues during hypergravity at the satellite landing. In offspring of pregnant rats no significant changes were found, however, the tendency to retardation in nucleic acid increament during growth of weanlings is evident.

Introduction

In rats from the biosatellites Cosmos 782, 936 and 1129 we have found that the decrease in nucleic acid content in involuted thymus and spleen several hours after 18.5-21 day flights was accompanied with pronounced increase in the level of chromatin fragments determined in the form of polydeoxyribonucleotides (1). Recently it was shown that polydeoxyribonucleotide fraction represents deoxyribonucleic component of nucleosomes and their oligomers which are released at the breakdown of nuclear chromatin (2). Under various experimental conditions polydeoxyribonucleotide level is increased only temporarily with maximum at hours 4-8 after the treatment. In consequence, the increased level of these compounds is a good indicator of sensitive cell damage induced shortly before the examinations. Repeated stimulus leads to the same reaction only after reappearance of sensitive cells-namely lymphocytes and erythroblasts.

In this paper the effect of spaceflight onboard of Cosmos 1514 satellite on pregnant female rats and their offspring was studied.

Methods

Deoxyribonucleoprotein breakdown in spleen, thymus and liver was evaluated on the basis of polydeoxyribonucleotide level (3), quantitative changes in nucleic acids in the same organs and in the blood were examined by method of (4).

The pregnant rats were analysed several hours after 5-day spaceflight (i.e. on the day 18 of pregnancy) their offspring on the day 15, 30 and 100 were analysed. The results on flight group (F) were compared to those of model (synchronous) experiment (M) and control rats (C). The conditions of the experiment were described more in detail (5).

Results

In pregnant female rats polydeoxyribonucleotide level in the spleen, thymus and liver was the same or rather decreased as compared to the control rats (Fig. 1). Nucleic acid concentration in the lymphoid organs was in the range of control values; as a result in decrease in organ weight the total content of RNA and DNA in spleen (Fig. 2) of flight rats was reduced more than by one half and in thymus (Fig. 3) by one third compared with control rats. Concentration of RNA in the blood of flight rats was decreased to one half of normal values (Fig. 4). In model experiment the changes in polydeoxyribonucleotides and nucleic acids in spleen, thymus and blood in milder degree occured.

Fig.1 Pregnant female rats - concentration of polydeoxyribonucleotides in organs
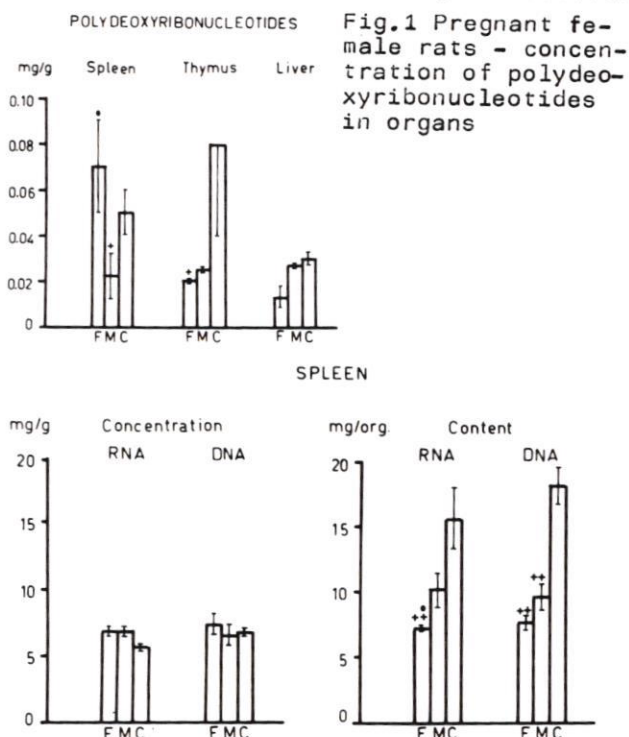
Fig.2 Pregnant female rats - concentration and content of RNA and DNA in spleen

In liver of pregnant rats no significant changes were found with the exception of mild increase in RNA concentration in flight group (non-demonstrated results).

In offspring the changes were less pronounced then in mothers. The level of
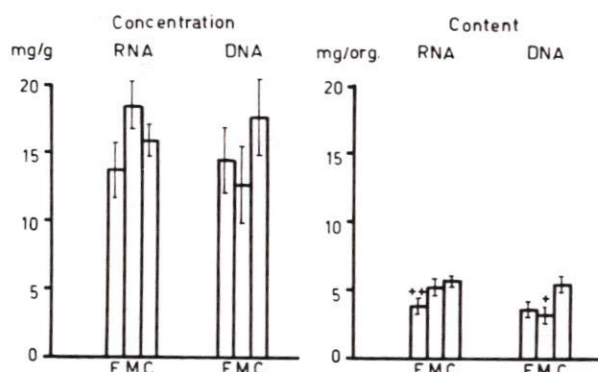
Fig.3 Pregnant female rats - concentration and content of RNA and DNA in thymus
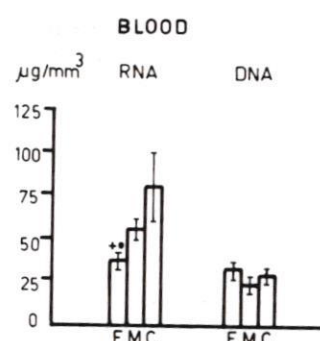
Fig.4 Pregnant female rats - concentration of RNA and DNA in blood

Fig.6 Offspring - concentration and content of RNA and DNA in spleen

Fig.7 Offspring - concentration and content of RNA and DNA in thymus

polydeoxyribonucleotides in spleen, thymus and liver was kept within the range of control values (Fig. 5). The quantitative changes in nucleic acids in the spleen (Fig. 6), thymus (Fig. 7), blood and liver (non-demonstrated results) although not statistically significant, are notable because of the apparent tendency toward reduction in RNA and DNA amount in flight group.

Fig.5 Offspring - concentration of polydeoxyribonucleotides in organs

Conclusion

1. The quantitative changes in spleen and thymus nucleic acids in this experiment on pregnant females after 5 day spaceflight are comparable to those in male rats after about 20 day spaceflights aboard previous biosatellites. This finding may be considered as manifestation of higher sensitivity of pregnant females to spaceflight conditions.

2. Contrary to the findings after 20 day spaceflights, in this experiment after 5 day flight and the another experiment on Cosmos 1667 after 7 day flight we have found no increase in polydeoxyribonucleotide level which would indicate the chro-

matin breakdown in spleen and thymus. The difference follows probably from the fact that during the short-term spaceflight there was not time enough to accomplish regeneration of sensitive cells damaged at the start of satellite. Then the effect of factors at landing didn't result in detectable breakdown of chromatin.
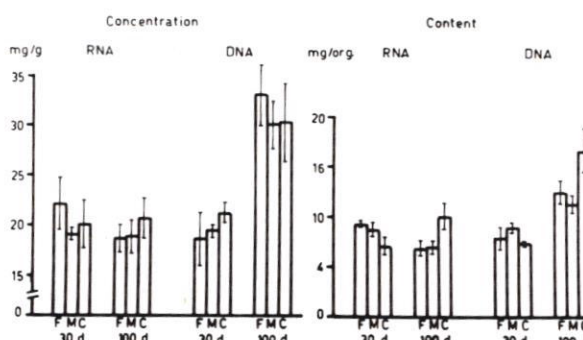
3. In offspring of pregnant female rats exposed to the spaceflight conditions no statistically significant changes were observed. Nevertheless, the tendency toward retardation in increament with the age of nucleic acid content in spleen, thymus and liver and to lower nucleic acid concentration in blood of offspring is notable.

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EFFECT OF CHRONIC CENTRIFUGATION ON MOUSE BREEDING PAIRS AND THEIR OFFSPRING.

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Fetuses of chronically centrifuged mice are smaller than 1g fetuses, and have reduced areas of long bone ossification (1). Fewer pregnancies occurred in centrifuged mice. In this study, exposure of chronically centrifuged females to male bedding 48 hours prior to mating induced estrus as determined by vaginal smears. Females were paired with males on the centrifuge or at 1g, and were sacrificed at 18 days gestation. Fetuses were weighed, measured, and stained with alizarin red amd methylene blue. This new mating method increased centrifuge pregnancies, but not to control levels. There were no pregnancies at 3.5g. Weights and crown rump lengths of centrifuged 18-day fetuses were less than those of controls, significantly so at 2.6 and 2.9g. There was no effect on litter size, number of resorptions per litter or male/female ratio. Bones of centrifuged fetuses were found to be smaller and shaped differently from controls. Effects were mitigated by matings with 1g males. Results confirm our previous reports of decreased fetal size and areas of ossification in hypergravity, and demonstrate a reliable method for providing timed-pregnant mice in hyper-g conditions.

Introduction

Previous studies have shown that fetuses of chronically centrifuged mice are smaller than fetuses of control mice and that areas of ossification in the long bones of these fetuses are reduced (1). There were also fewer pregnancies in centrifuged animals, which could have been due to changes in the estrus cycle resulting from centrifugation (2). In the current study, the possibility of such effects was obviated by using vaginal smears to confirm receptivity of the females prior to pairing with a male, either at the same g level, or at 1g. Again, centrifuged fetuses were found to be smaller than controls, and to have reduced areas of ossification. These effects were less in fetuses with 1g fathers.

Material and Methods

For in vivo centrifugation studies we use a small animal centrifuge based on the design of Walters, Wunder, and Smith (3) consisting of two cage holders with 4 cages which swingout at an angle determined by the r.p.m. For this experiment, cages were redesigned to allow division into 1 to 4 compartments, each with its own food and water supply.

Forty female and sixteen male mice (ICR; Harlan-Sprague-Dawley) were placed on the centrifuge at five weeks of age at g levels between 2.3 and 3.5g (rpm=45). There were 10 females and 4 males at each of the four g levels studied. Controls were housed in the same room. Mice on the centrifuge were allowed to adapt for 8 weeks prior to breeding to avoid effects of maternal stress on the embryos. Estrus was induced by replacing bedding in the female cages with used bedding from male cages. Two days later, vaginal smears were taken from females at either 2.3 and 2.9 or 2.6 and 3.5g levels, and those in pre-estrus or estrus (4) were paired overnight with a male at the same g level (CE fetuses), or with control (1g) males (CE-CN fetuses). Pregnant females were sacrificed by cervical dislocation on gestational day 18. Fetuses were fixed in 10% buffered neutral formalin, blotted and weighed, sexed, photographed, and stained with alizarin red and alcian blue (5). Measurements of area, form factor, longest dimension, and perimeter of the long bones were carried out on 5" x 7" photographs using the Bioquant Image Analysis system. In this system, form factor is defined as $4\pi(area)/perimeter^2$ and longest dimension (LD) as most distant point along the perimeter from the point of origin. Significance was determined using the Mann-Whitney rank test.

Results:

Results are shown in Table I. The new system of breeding reliably produced pregnancies in the centrifuged animals, but there were still fewer pregnancies in centrifuged females than in control females. There were no pregnancies at 3.5g. Number of resorptions per litter and percentage of male or female embryos was not affected. There was no significant effect on litter size except for 2.6g females mated with control males. Embryos exposed to excess gravity weighed significantly less than controls at 2.6 and 2.9g. Crown-rump lengths of centrifuged embryos at 2.6 and 2.9g were significantly less than those of controls (Fig. 1).

TABLE I: Centrifugation Effects on Reproduction

	#Pregnancies	$\bar{x}$ #Resorptions/ Litter	$\bar{x}$ Litter Size	% Male	% Female	$\bar{x}$ CRL(mm)	$\bar{x}$ Weight(gms)
CN	29	0.75	9.50	49.85	50.15	23.63	1.41
2.3g CE	6	1.00	8.25	48.62	51.39	22.59	1.27
2.3g CE-CN	1	0	5.00	40.00	60.00	23.31	1.57**
2.6g CE	4	0	10.67	53.33	46.67	21.40***	1.13**
2.6g CE-CN	3	1.00	7.00*	46.23	53.77	22.83+	1.36+
2.9g CE	4	0.50	10.00	43.75	56.25	21.61***	1.02***
2.9g CE-CN	1	0	9.00	66.70	33.30	19.65***+	0.99***

* p < .05
** p < .01
*** p < .001
+ differs sign. from CE

CONTROL   CENTRIFUGED
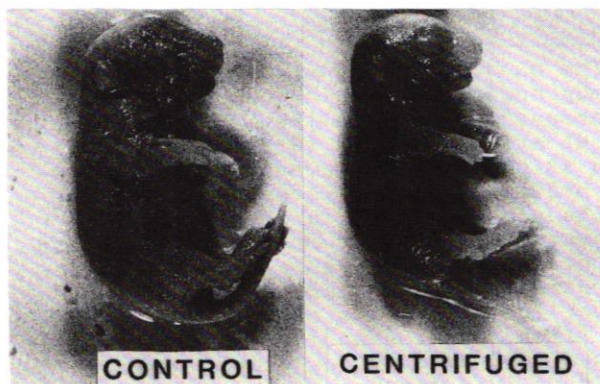
Figure 1: 18-day old fetuses.

Results from morphometric analyses are shown in Tables II, III, and IV and Fig. 2. At 2.3g, the primary effect was on bone shape, as shown by the increase in form factors (Table II). Significant changes were also seen in LD, which was increased except in left humerus. Perimeter was decreased in all cases, but the difference was significant only in ulna and tibias of CE-CN embryos.

MORPHOMETRIC ANALYSES OF LONG BONES
OF FETUSES CENTRIFUGED IN UTERO
EXPERIMENT IV
2.3 G

		LEFT HUMERUS	LEFT RADIUS	LEFT ULNA	LEFT FEMUR	LEFT TIBIA	LEFT FIBULA	RIGHT FEMUR	RIGHT TIBIA	RIGHT FIBULA
x̄ AREA (mm²)	CN	1.269	0.841	1.094	1.004	1.198	0.44	0.984	1.182	0.449
	CE	1.433*	0.85	1.164	1.072	1.153	0.418	1.05	1.152	0.421
	CE-CN	1.271	1.015**	1.31*	1.014	1.378	0.538*	0.935	1.426	0.481
x̄ FORM FACTOR	CN	0.239	0.207	0.2	0.281	0.285	0.182	0.356	0.291	0.185
	CE	0.364***	0.296***	0.289***	0.387***	0.348**	0.237**	0.379	0.331*	0.222*
	CE-CN	0.44**	0.381**	0.401***	0.526***	0.418**	0.346***+	0.51***	0.422***	0.327***
x̄ LONGEST DIMENSION (mm)	CN	2.35	2.109	2.281	1.831	2.342	1.805	1.825	2.284	1.593
	CE	2.277***	2.129**	2.426***	1.947*	2.415***	1.853**	1.952	2.309*	1.729*
	CE-CN	2.325**	2.252*	2.566***	1.888***	2.617*	1.67**	1.813*	2.521*	1.768**
x̄ PERIMETER (mm)	CN	8.616	7.579	8.63	6.878	7.414	5.647	5.953	7.208	5.67
	CE	6.568	6.047	7.086	5.93	6.46	4.771	5.937	6.601	4.886
	CE-CN	6.014	5.806	6.405***	5.014*	6.422*	4.45	4.78*	6.47*	4.3

*p< .05   **p< .01   ***p< .001
+differs sign. from CE

Table II: Morphometric Analyses-2.3g.

At 2.6g, area, longest dimension and perimeter were decreased, in most cases significantly (Table III). Areas of ossification in CE-CN fetuses were greater than those of CE fetuses with some of these differences being significant. There was no significant difference between CE-CN and CN. LD was significantly decreased in most CE bones, as was perimeter. Significant differences in LD were also found between CN and CE-CN fetuses (except for humeri), but perimeters, except for fibulae, did not differ. Form factor was significantly increased in both CN-CE and CE fetuses except for CE-CN humerus.

MORPHOMETRIC ANALYSES OF LONG BONES
OF FETUSES CENTRIFUGED IN UTERO
EXPERIMENT IV
2.6 G

		LEFT HUMERUS	LEFT RADIUS	LEFT ULNA	LEFT FEMUR	LEFT TIBIA	LEFT FIBULA	RIGHT FEMUR	RIGHT TIBIA	RIGHT FIBULA
x̄ AREA (mm²)	CN	1.269	0.841	1.094	1.004	1.198	0.44	0.984	1.182	0.449
	CE	1.221	0.673	0.858***	0.784***	0.963***	0.33***	0.805***	0.902***	0.33***
	CE-CN	1.424	0.863	1.339*	0.949	1.122*	0.419*	0.907	1.18	0.409*
x̄ FORM FACTOR	CN	0.239	0.207	0.2	0.281	0.285	0.182	0.356	0.291	0.185
	CE	0.448***	0.378***	0.353***	0.495***	0.416***	0.331***	0.511***	0.436***	0.319***
	CE-CN	0.36	0.378***	0.367***	0.536***	0.397*	0.316***	0.518*	0.444**	0.317***
x̄ LONGEST DIMENSION (mm)	CN	2.35	2.109	2.281	1.831	2.342	1.805	1.825	2.284	1.593
	CE	2.315***	1.88***	2.116***	1.682	2.155**	1.583**	1.667	1.949*	1.47*
	CE-CN	2.135	2.117**	2.483**	1.655**	2.372*	1.655***	1.708*	2.204**	1.708***
x̄ PERIMETER (mm)	CN	8.616	7.579	8.63	6.878	7.414	5.647	5.953	7.208	5.67
	CE	5.859	4.713***	5.541**	4.467*	5.39***	3.565***	4.456***	5.07***	3.61
	CE-CN	5.352	5.462	6.787*	4.87	6.325	4.112**	4.9	5.939	4.061***

*p< .05   **p< .01   ***p< .001
+differs sign. from CE

Table III: Morphometric Analyses-2.6g.

At 2.9g, area, LD, and perimeter were significantly decreased in all cases, and form factor was increased significantly (Table IV).

MORPHOMETRIC ANALYSES OF LONG BONES
OF FETUSES CENTRIFUGED IN UTERO
EXPERIMENT IV
2.9 G

		LEFT HUMERUS	LEFT RADIUS	LEFT ULNA	LEFT FEMUR	LEFT TIBIA	LEFT FIBULA	RIGHT FEMUR	RIGHT TIBIA	RIGHT FIBULA
x̄ AREA (mm²)	CN	1.269	0.841	1.094	1.004	1.198	0.44	0.984	1.182	0.449
	CE	1.013***	0.578***	0.796***	0.715***	0.823***	0.316***	0.69***	0.782***	0.31***
x̄ FORM FACTOR	CN	0.239	0.207	0.2	0.281	0.285	0.182	0.356	0.291	0.185
	CE	0.473***	0.38***	0.363***	0.491***	0.413***	0.316***	0.495***	0.417***	0.303***
x̄ LONGEST DIMENSION (mm)	CN	2.35	2.109	2.281	1.831	2.342	1.805	1.825	2.284	1.593
	CE	2.032***	1.783***	1.951***	1.588***	1.974***	1.446***	1.596***	1.865***	1.523***
x̄ PERIMETER (mm)	CN	8.616	7.579	8.63	6.878	7.414	5.647	5.953	7.208	5.67
	CE	5.187***	4.376***	5.266***	4.261***	5.003***	3.53***	4.171***	4.848***	3.586

*p< .05   **p< .01   ***p< .001

Table IV: Morphometric Analyses-2.9g.

Discussion

Mechanical and nutritional factors, both of which are altered by altered gravity, are important in skeletal development (6,7). Differences in bone
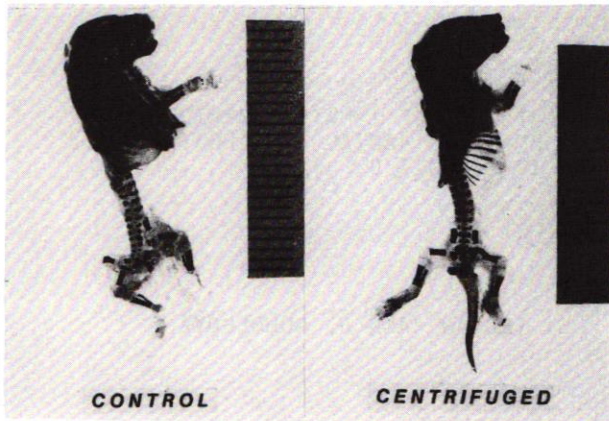
Figure 2: 18-day fetuses, alizarin stained.

shape seen in this study are likely to result from a direct effect on the fetus due to increased mechanical forces in utero. Differences in bone size may be indirect--related to smaller fetal and/ or maternal size. However, in vitro studies have shown direct effects on prenatal limb development: precocious chondrogenesis occurred in centrifuged limb buds (8), and in cultured fetal long bones, decreased formation and increased resorption resulted, unless the culture was pressurized (9).

Mechanical and nutritional factors, are largely known and can--and will--be dissected out. Less easily understood is the moderation of centrifugation effects by mating at 1g. This could be due to differences in sperm of 1g and CE males (10) or to the female being at 1g during the first few egg divisions (4). These and other results of altered g--effects of increased g on circadian rhythms in primates (chronic centrifugation) (11) and Neurospora (3g at liftoff) (12)--dictate that we increase our knowledge of centrifugation and of gravitational effects in general so that centrifuges on Space Station can be designed and used to their, and our, greatest advantage.

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ADAPTATION OF IMMATURE BRAIN TO POSITIVE RADIAL ACCELERATION OF 10 G AND 5 G

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Radial acceleration inhibits venous return and causes stagnant hypoxia (oligaemia) or anoxia (ischaemia) of the organism. Circulatory arrest, i.e. general stagnant anoxia, including the CNS, develops in rats at an acceleration of 10 g. It leads to changes in metabolism, structure and function of the CNS, the final outcome depending on the degree of developmental maturity of the CNS. Nervous tissue reacts to different degrees of oxygen deficiency in different ways. In young rats, positive acceleration of 5 g causes stagnant hypoxia of the CNS which is not lethal until it has acted a relatively long time. Resistance to this treatment diminishes with age.

One group of rats were exposed 4 times daily at 2-hour intervals, for 3 minutes less than the lethal exposure dose, to an overload of 10 g from birth to the age of 18 days.

None of the animals survived 4 daily exposures to stagnant anoxia at 2-hour intervals for longer that 6 days (Fig.1) i.e. the maximum duration af anoxia to which some of the animals were exposed was 5 hours and 3 minutes.

Another group of rats were exposed once a day to 5 g acceleration, either from birth to the age of 17 days, or from the 12th to 17th day. The duration of exposure was chosen according to LD_{50} for 5 g during ontogenesis (Fig.2) and was either 25 minutes or 50 minutes (Fig.3).

The indicator of adaptation of the exposed animals was survival under conditions of repeated oligaemia and the resistance of their CNS to ischaemia caused by 10 g acceleration on the 17th day, 6 hours after the last exposure to 5 g acceleration, and on the 19th day, 48 hours after the last exposure.

None of the animals survived repeated oligaemia of the CNS induced by 50 minutes exposure to 5 g acceleration once a day (Fig.4).
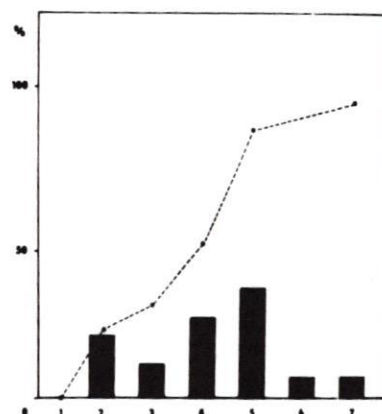
Figure 1. Adaptation to repeated stagnant anoxia (10 g acceleration) 4 times daily with 2-hour intervals.
Abscissa: age in days; ordinate:percentual survival.
Columns - percentage of deaths on individual days, broken line - dynamics of death in percentual form.
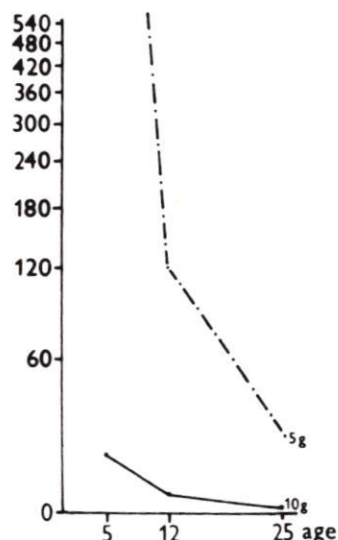
Figure 2. Development of resistance of rats to stagnant anoxia (10 g acceleration) and stagnant hypoxia (5 g acceleration).
Abscissa: age in days; ordinate; survival in minutes

Of the rats exposed to repeated oligaemia (5 g) for 25 minutes once a day from the age of 1 to 17 days, 90% survived. The resistance of the CNS of these animals to ischaemia (positive acceleration of 10 g) on the 17th and 19th day of life was 50% and 60% higher than normal (Fig.4).

Adapted animals were distinctly more resistant compared not only with intact rats, but also with animals exposed to a single dose of 10 g for 1 minute less than lethal exposure dose 4 or 24 hours before last. The increase in the resistance of adaptated rats was of a relatively

long-term character.
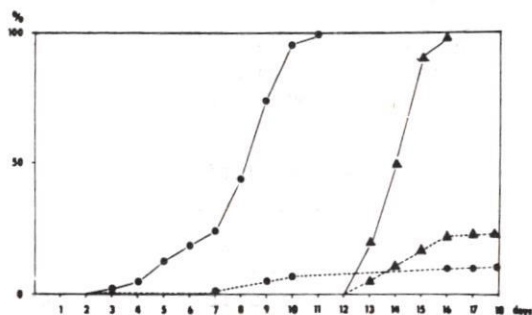
Figure 3. Dynamics of resistance to repea-
ted oligaemia (5 g acceleration) of rats
exposed once a day from the age of 1 (.)
and 12 ( ) days.
Abscissa: days of experiment (age in days);
ordinate: percentage of deaths on indivi-
dual days.
Bold line - 5 g once a day for 50 minutes,
broken line - 5 g once a day for 25 minu-
tes.
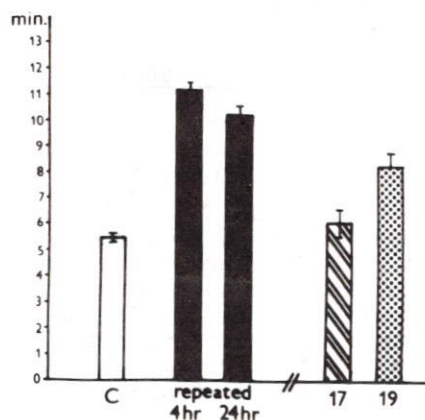
Figure 4. Resistance to ischaemia (10 g
acceleration) in rats exposed from birth
to age of 18 days twice a day with 4-hour
interval to repeated stress of 10 g.
C - resistance of 18-day-old controls;
black columns - resistance of adapted rats
4 or 24 hours after the last repeated ex-
posure; shaded columns - resistance to 10
g at 17th and 19th day.

From the biological and the clinical
aspect, therefore developmental immaturity
of the CNS has its pros and cons. On the
one hand it allows great resistance and
adaptability of the CNS to changes in the
interval environment, but on the other, it
limits the diagnostic possibilities. Since
hypoxic disturbances are manifested in such
unequivocal indicators as an increase in
the blood lactic acid level (Fig.5) and
plasma LDH activity (From Trojan S., Acta
Univ. Carol. Med. Monograph. LXXXV, 1978),
metabolic changes in the immature organism
seem at present to be the best prognostic
criteria. Large number of children are

still damaged by oxygen deficiency and
medical science is often able to save life
only at the cost of permanent impairment
in the motor and menthal sphere.
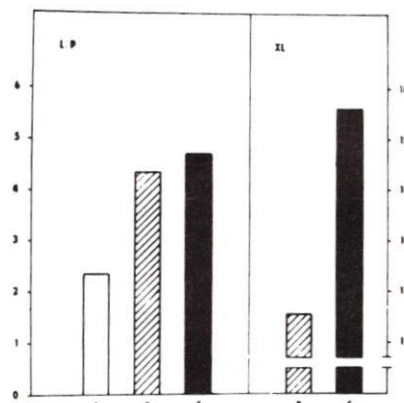
Figure 5. Lactate/pyruvate ratio (L/P) and
excess lactate (XL) in the prosencephalon
of 18-day-old rats.
Abscissa: A - L/P in the controls, B - L/P
and XL in rats exposed 1 minute less than
the lethal dose time to 10 g acceleration.
C - L/P and XL in rats adapted to repeated
exposure to 10 g acceleration twice a day
with a 4-hour interval, from birth to the
age of 17 days; ordinate - values in umol.

One of the chief interest of both ex-
perimenters and clinicians must therefore
to be look for new possibilities for the
prompt and objective diagnosis of imminent
hypoxic brain damage and for an objective
diagnosis (Fig.6).
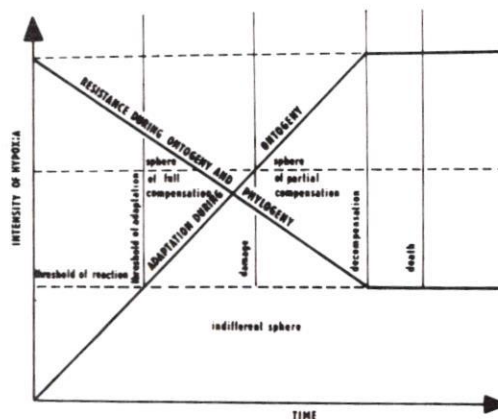
Figure 6. Scheme of reaction and adapta-
tion to oxygen deficiency during ontoge-
nesis in corellation to the intensity
of hypoxia and the exposure time.

DYNAMICS OF PROCESSES - A POSSIBILITY TO ANALYSE PHYSIOLOGICAL PARAMETERS

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Biological systems are influenced by geophysical and geochemical processes. These processes consist of periodical and aperiodical portions. In biological systems they are reflected by a hierarchy of biorhythms. By means of autocorrelation and Fouriertransformation a method to determine stable and instable conditions is offered. The chief component of this analys is the so-called double autocorrelation. The alternation of circaseptan rhythm in sleep distur - bed patients and its important desyn - chronical effects with following sta - bilisation influenced by drug abusus or placeboapplication is investigated. These presented method is a basis to analyse disturbed processes of biolo - gical systems both in physiological and biochemical parameters.

INTRODUCTION

Sleep in man is today a problem with many unanswered questions. It's well known, that the physiological basic of sleep is the existence of hierarchy of biorhythms. But these rhythms can be disturbed randomly or periodically. The problem of function diagnosis of sleep by man in earth and space must be:
- to recognize totality of biorhytmics processes of biological system "man",
- to recognize not only the inflow of inside but also of outside stochastic and periodic phenomenons,
- to determine the interaction of these phenomenons.

The sleep polygraphy is a objectiv method to result these problems. But this method is difficult, expendly and used only in 0,I % all of cases. Therefore it was necessary to use a special questionaire. In this the patient had to note careful daily following:
- duration of time in bed,
- duration of sleep,
- duration of awekenings,
- number of awekenings at night.

By helfs of a simple mathematical connection it's possible to determine the rest efficiency:

$$R_{eff.} = D\,e^{-x/k}$$

$R_{eff.}$ - rest efficiency
D - duration of sleep outcluding all times of awekenings
x - number of awekenings at night
k = IO.

The problem of determination of biorhythmics process

A biorhythmic process is characterized by a periodic chance by one or more biochemical or physiological parameters. It's necessary to provide a local and temporal measurement of this parameter to determine the periodic change. In this case the measuring time T_R should be essential bigger as the period of biorhythm T_p, which is verified.
It is possible to provide stochastic measurement too. In this case the chance was use a less number of measurements and false biorhythms were not generate. In any case it is necessary to consider, that not only regular but also irregular measurements takes an incluence of biorhythms, which is investigated and other biorhythms too. This fact had to be consider to select the parameters and methods of measuring.

Models of biorhythmic processes

In the most similar form of biorhythmic process can be described by y sinusfunction (here named: modelfunction):

$$A = A_o + \hat{A}\sin(\omega t + \varphi_o),\quad \omega = 2\pi/T_p$$

It is possible to get these parameters by different methods. Here the autocorrelationfunction was use to determine the period T_p. T_p are selected by first maximum of autocorrelatiofunction after providing a test of signification.
Using methods of Fourier-analysing are determine A_o, $\hat{A}$ and φ_o.
On this way the real-timefunction is represented by y sinusfunction as a first approximation. The normalized coefficient k of autocorrelation was understanded too as the probability of foundet biorhythm in the realtimefunction.
Biorhythmic process can't change erratic. Therefore it was necessary to look for other possibilities to determine dynamic change of biorhythms. The method of double autocorrelation may be a possibility. Usingthis method a window with a minimum breadth of N=25...30 (N=measuring points) is moved trough the real-time-function. In every case the coefficient of autocorrelation and his pertinent period T_p are determined. Two new functions was get and the result of first window are the first measuring points of new functions.
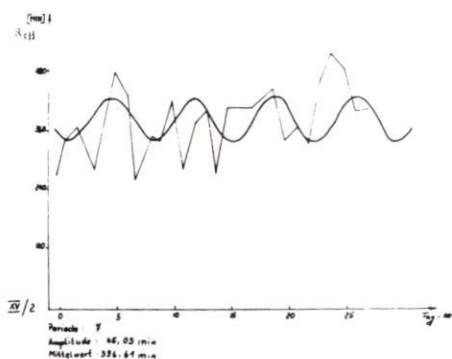
Figure I: Example for a real sleep-
timefunction and the determined model-
function. On the basic of special quest-
ionaire are selected a circaseptan rhythm
of undisturbed sleep
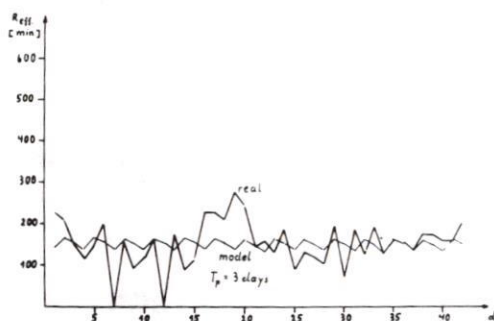
Figure 2: The real- and modelfunction
of disturbed sleep with a period of days
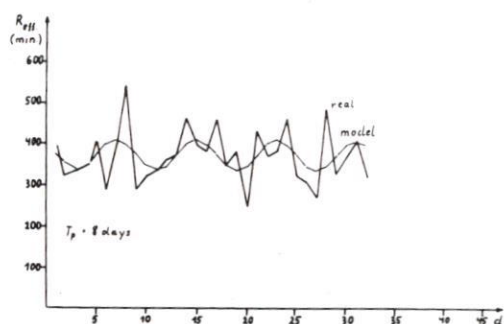
Figure 3: After administration of
5 µg/kg body weight in this patient the
circaseptan rhythm is completely restored

$$T_p = r_i | T_p \qquad i = I \ldots (N-N_{min})$$
$$r_i(\tau) = \frac{I}{(N_{min}-I)} \sum_{i=I}^{N_{min}} \left[(X(i)-\bar{X})(X(i+\tau)-\bar{X}) \right]$$

RESULTS

In this beginning investigation by
using these biorhythmic method in di-
sturbed and undisturbed sleep following
results can be found:
- for undisturbed volumtars appared a
  circaseptan rhythm,
- for patients with disturbed sleep the
  rhythm changed to 2 - 3 days,
- Phenomenons of detoxications treatment
  are characterized by desynchronoses of
  rhythms,
- the phase of stabilisation takes 2 -
  3 weeks,
- two significant peaks can be determi-
  ned;
  Firstly: the lowest sleep quality bet-
           ween sunday and monday
  Secondly: the highest sleep quality
            between friday and saturday
- substance P proved as a time regulator
  of circaseptan rhythms in sleep
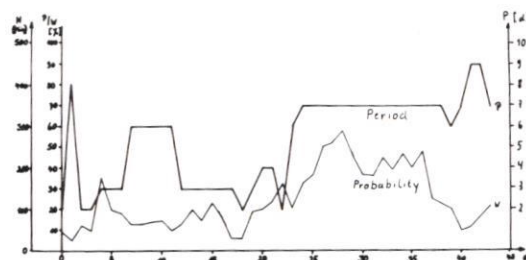
Figure 4: Change of biorhythm of a
sleep disturbed patient in case after de-
toxical treatment and simultanous appli-
cation of placebo

CONCLUSION

The represented dynamic of process
in Figure 4 is characterized by a swin-
ging reaction with followed stabilisation.
Using the parameters of this swinging
reaction may be verified and compared
difficult biological answering reac-
tions. The discribed method is applica-
ble to any physiological or biological
parameters.

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BIOLOGICAL MINUTE RHYTHMS OF SENSORIAL AND MOTORIAL FUNCTIONS OF PRIMATES IN THE ADAPTATION ON HYPOGRAVITATION

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The influence of hypogravitation on biological minute rhythms of sensorial and motorial functions of primates was explored on base of instrumental reflexes under the conditions of the experiment "COSMOS I667".

In preflight experiments the maximum of significant reaction time rhythms is located in the range from I to 2 minutes. Under hypogravitation minute rhythms show a considerable variability of frequencies and become stabilized in the period range from 2 to 4 minutes. After return to earth significant periods occured between I and 2 minutes.

Introduction

The gravitational physiology and space medicine need in relevant methods for describtion of stress reactions and mechanisms of adaptation on extremally environmental conditions. The physiological time organization represents an important criterion in the adaptation of an organism in the interaction with alternating environmental conditions. The organization of such time structures of organismic functions take place at many levels.

Biochemical reactions, cell cultures, organs and organisms display cyclic behaviours. There is a hierarchy of biorhythms with several length of periods in a living system. Biorhythms exists in ranges from microrhythms (msec to hour), macrorhythms (hour to week) and periods larger than week (longtime rhythms). Following specially characteristics of micro- and macrorhythms are known (Tab. I). The variability of frequencies of minute rhythms was used to investigate the adaptation on microgravitation in the soviet biosatellite "COSMOS I667".

Tab. I: Characteristics of micro- and macrorhythms.

Microrhythms	Macrorhythms
low frequency stability high amplitude stability	high frequency stability low amplitude stability
connected with a complex system	connected with conditioning of organism
determination (endogen) by metabalism processes	strong coupling with pace maker (exogen)

Methods

The investigations were carried out on rhesus-monkeys, higher nonhuman primates, whose mental functions are so complex that their emotional behavior and elementary operational acts were explored on base of continiously series of sensorial and motorial reaction times (Fig. I and 2). The alteration dynamics in minute rhythms of sensorial and motorial reaction times were investigated on 5 primates (2 in space, 3 control animals).
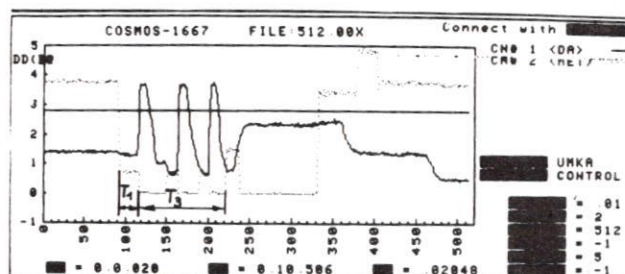
Figure I. Example of an operator action.
—— Leg movement – – – Signal channel
Definition of reaction times $\begin{cases} T_I\text{-sensoric} \\ T_3\text{-motoric} \end{cases}$
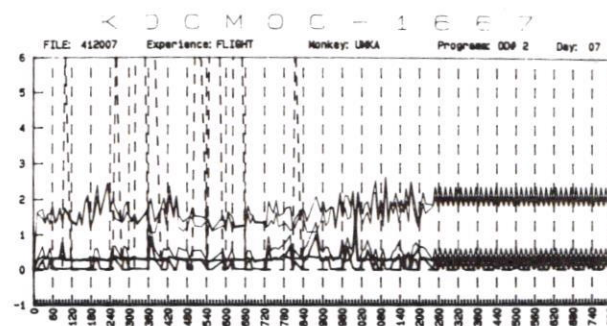
Figure 2. Continiously series of sensorial and motorial reaction times.

Using these instrumental reflexes which required stereotyped leg movements (pushing the stick of a leg actograf) in response to a light signal from a special panel we became sensorial and motorial time series in which we looked for minute rhythms by a special computer complex program (Spline-interpolation, Autocorrelation function, Cosinor-Rhythmometry, Crosscorelation function, Periodogram and Fourier-analysis).

Results

The verification of significant reaction time rhythms by complex spectral analysis showed periods with lenghts from 0,8 to 6 minutes under preflight conditions of the experiment "COSMOS I667".

The maximum of relative frequency (Fig. 3) in the histogram of minute rhythms periods is located in the interval from I to 2 minutes (57%). The evaluation of adaptive capabilities of the higher nervous activity and integrative functions of monkeys under flight conditions (hypogravitation) is demonstrated in the second histogram

Figure 3. Histograms of significant reaction time rhythms during operator actions in "Cosmos I667" $(N_0 = 7)$
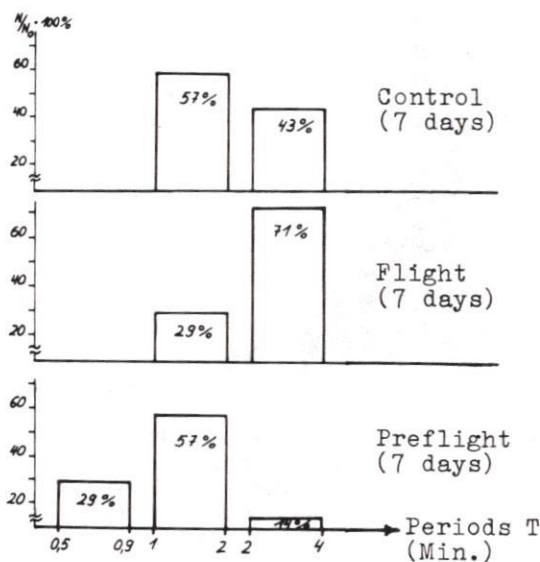
above the preflight columnes. While the first day of flight was characterized by a considerable variability of frequencies (acute desynchronisation) on following days up to the end of flight minute rhythms of both functions become stabilized in the period range from 2 to 4 minutes (7I%). That means that the minute rhythms of integrative functions are slowering in adaptation on space flight conditions. The monkeys also refused to perform this test throughout the flight (secondary desynchronisation). It can also be assumed that this functions should be restored completely as soon as adaptation has developed. These alterations of the

period length of minute rhythms in weightlessness were normalized after return to earth conditions. The maximum of relative frequency in the histogram of the control experiments occured to the range of periods from I to 2 minutes (57%).

Conclusion

Investigations of minute rhythms of rhesus-monkeys sensorial and motorial functions in biosatellite experiments demonstrate that the biorhythm models are adequate to the describtion of adaptation processes. Moreover, the results suggest the importance of psychophysiological minute rhythms as a criterion to describe stress reactions under extremally conditions and also to control the adaptation or readaptation in primates under gravitation-hypogravitation-gravitation.

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REM-CYCLES: A CRITERION FOR VERIFICATION OF SLEEP REGULATION, SLEEP DISTURBANCE AND THE EFFECTS OF SLEEP REGULATED PEPTIDES

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The reference of REM-cycles for diagnosis and therapia of sleep was examined in control and stressed rats with hyposomnia by polygraphical sleep recording.

The number of REM-cycles as well as their duration shown significantly differences in the mode of distribution between normal sleep and hyposomnia.

The treatment of hyposomnia with Substance-$P_{I,II}$ and Delta-Sleep-Inducing Peptide by i.p. administration induced nearly the same normalizing effects in the distribution and duration of REM-cycles. The results indicated that SP is more efficient than DSIP in the regulation of rhythms in stressed rats.

INTRODUCTION

The studies of sleep in the microgravity enviroment gain in importance with the development of long term orbital space flights. This problem is nowadays in early stage and included numerous unsettled questions.

The results of modell experiments have shown that hypokinesia with her reference to weightlessness is able to induce the disturbances of sleep (I,2): for example, the somnolence was observed in rats after 2-4 weeks intermittently immobilization; in opposite to this, hyposomnia was resulted after 4-6 weeks immobilization in the same rats.

It is remarkable that the opinion of the sleep quality as well as the administration of the sleep drugs take usually place without consideration to biorhythmical aspects of sleep. The sleep is a biorhythmical phenomenon:
- on the one side the sleep belong to circadian rhythms of vigilance;
- on the other side the sleep itself rhythmical organized into ultradian cycles of sleep with oscilatory changes of the central nervous activation referable to circadian low level of vigilancy.

. . . . . .

In the present paper the practical reference of REM-cycles for diagnosis and therapia of sleep in chronically stressed rats with substain hyposomnia was examined.

METHODS

REM-cycles of sleep in unstressed and stressed rats by somnopolygraphical recording during circadian minimum of activity on six following days were investigated. In stressed rats was induced the substain hyposomnia (2).

REM-cycles were determined as a period between ends of two neighbouring episodes of paradoxal-sleep stages. The first cycle was indeed refered to start the sleep registration.

Stressed rats were treated of hyposomnia by intraperitoneal administration either **Delta Sleep Inducing Peptide (DSIP:** 300 ug/kg/d) or **Substance-P (SP:** 250 ug/kg /d). The peptides were applicated one hour before the sleep recording.

RESULTS

In control rats REM-cycles in every somnogramm were presented. Altogether I40 those cycles (n=I40) were verificated. The numerical distribution of REM-cycles in relation to their duration is shown in

Figure I
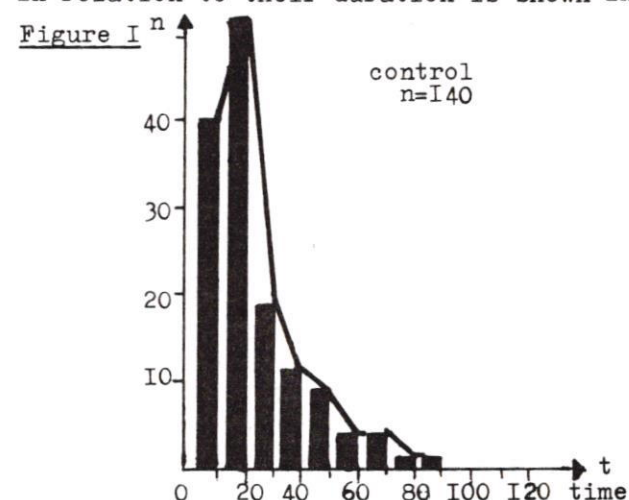
The significantly prioryty of REM-cycles between IO and 20 minutes duration is evident. Gradually decrease of REM-cycles frequency after maximum correlate with a increasing time periods. Durations of more than 50 minutes occured only sporadically. This results pointed out that the REM-cyclical organization of a normal sleep is rhythmical chronized.

In chronically stressed rats a number of REM-cycles (n=I08)was decreased. By 32% somnogramms those cycles were absent. The distribution of verificated cycles with regard to their duration did not underlie special regularities (Fig.2) and indicate a few peaks by various time periods. The time periods of REM-cycles demonsrated a trend to increase, if it all those cycles by chronically stressed rats were maked available. This results indicated that the diturbances of paradoxal - sleep during stress-induced hyposomnia take the first place. The reduction of REM- or paradoxal-sleep as well as

dissociation of REM-cycles chrakterized the development of desynchronosis.
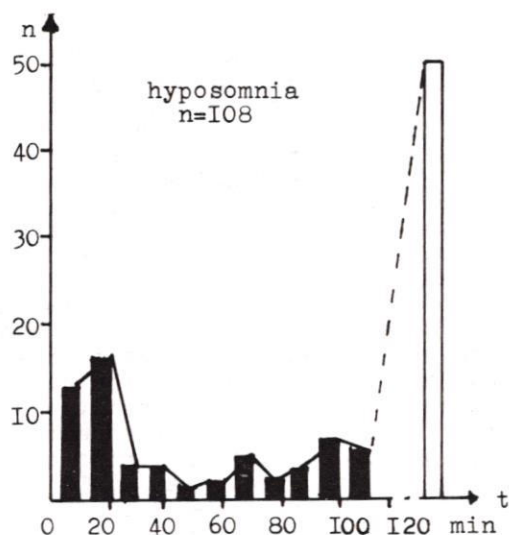
Figure 2: Frequency of REM-cycles with various time periods during hyposomnia in stressed rats

By treatment of hyposomnia with DSIP was occured a normalizing effect to regulate of the sleep rhythm. In Figure 3 is evidently shown that the numerical dominance of REM-cycles by duration between IO-20 minutes was restituted. By way of exception take place the maintained sleep disturbance with absence of REM-cycles. This results complemented and confirmed the pravious studies by other authors, for instance, Monnier et al(3,4), Schoenenberer et al (5), Graf et al (6).
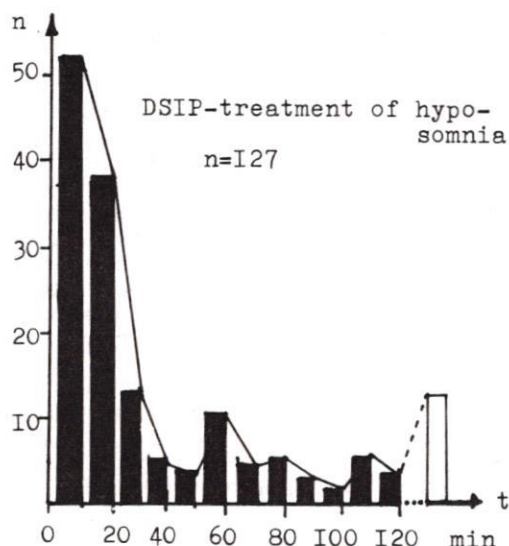
Figure 3: Frequency of REM-cycles with various time periods by DSIP treatment of stress-induced hyposomnia

After administration of Substance-P in chronically stressed rats with hypo-

somnia everywhere was occured generally normalization of rhythmical sleep structure. Figure 4 demostrate this normalizing effect.
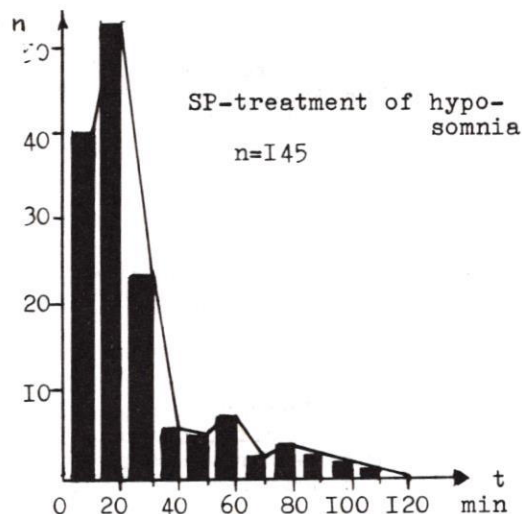
Figure 4: Frequency of REM-cycles with regard to their duration in stressed rats after SP-treatment

By comparison this results with the distribution of REM-cycles-frequencies in conrol rats take place significantly analogy Substance-P proved as a sleep programmer respectively as a regulator of biological time. This results confirmed the regulide conception for SP from Hecht and Oehme (7,8).

Based on the normalizing effects of SP and DSIP both common and differences in the mode of restituted distribution of REM-cycles indicated that SP is more efficient than DSIP in chronically stressed rats with stress induced hyposomnia.

CONCLUSION

The presented results have been proved that ultradian rhythm of REM-cycles is a subtle criterion for the opinion of sleep quality as well as for opinion of the efficience of sleep-medication.

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RELATIONS BETWEEN REM-CYCLES, SLEEP DISTURBANCES AND SUBSTANCE-P IN MAN

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Sleep alterations may be caused by stress. Ultradian rhythms of Rem-cycles are subtle criteria for the opinion of sleep quality. The distribution of frequencies of Rem-cycle durations was analysed. 72 polygraphical sleep recordings of healthy volunteers and 74 of patients with functional sleep distur - bances were studied. Following results have been obtained: Sleep disturbed patients were characterized by reduced number of Rem-cycles and a distribution of frequencies without peaks in opposite to healthy subjects with 95 min Rem-cycle duration in mean. Sleep disturbed neurotics treated by substance-P were characterized by an increased number of Rem-cycles per night and Rem-cycle durations nearly normal.

Introduction

The frequency of sleep disturbances is increased under hard or extreme conditions of life, for example under conditions of night-work, several kinds of psychic or physical stress and also under conditions of space flights. The development of new strategies against sleep disturbances is of great importance. Usual medications, that means the administration of hypnotics etc influence the mental and physical efficiency in man as well as the sleep cyclogram negatively. It seems to be necessary to look for substances without such negative effects. In animal experiments in our laboratory was demonstrated, that the peptide known as substance-P causes sleep regulating effects that means normalizing effects on Rem-cycles. It was the aim of these studies to verify the results obtained with animals in man in consideration of functional sleep disturbances, that means of primary hyposomnias.

Methods

12 patients with functional sleep disturbances were examined by mean of a single blind drug administration for eight consecutive nights. The sleep during the first, the second and third night was analyzed without any drug administration. Before the following four nights the patients were treated by 5 μg substance-P per kg body weight and night intranasal. The sleep of the eights night was recorded without drug administration again. 72 sleep recordings of healthy volunteers were analyzed. Seven channels per subject, consisting two for electro - oculogram (EOG), one for electromyogram (EMG), three for electroencephalogram (EEG),(FO-CO, CO-PO, FO-PO, bipolar) and one for electrocardiogram (ECG) were recorded at a paper speed of 15mm/sec to yield 60 sec epochs. The polygraphical recordings were analyzed visual according to Rechtschaffen and Kales(1) in the stages one to four, Rem and awake. The sleep cyclograms were analyzed mathematically and various standard parameters were determined: Total Recording Time (TRT), Total Sleep Time (TST), Total Time Awake (TTA), Sleep Latency (SL), Rem Latency (RL), Sleep Efficiency Index (SEI), Number of Awakenings (NA), Number of the shifts per hour from one sleep stage to the another (STS), Time balance with the shares of the stages awake, one to four, and Rem in % of Total Sleep Time was carried out. Furthermore the rate and duration of Rem-cycles and its content were analyzed. Another parameters were the Rem density (RD) and the NON-Rem-and Rem-phases and their comparison with the reference values known from literature (2) and from own measurements.

Results

1. In healthy volunteers the duration of Rem-cycles was found to be mostly between 70 and 120 minutes. Rem-cycles shorter than 70 minutes and longer than 120 minutes occured only sporadically.
2. Sleep disturbed patients were characterized by a reduced number of Rem-cycles (1.3 cycles per night), a Rem-cycle duration of 134 minutes (mean) and a decreased share of delta-sleep.
3. At our patients with functional sleep disturbances the administration of substance-P increases the number of Rem-cycles (2.1 cycles per night) and decreases the duration of these cycles (108 min in mean). In each case the duration of delta-sleep increases during the application of substance-P. The duration of NON-Rem and Rem periodes of the patients with sleep disturbances after administration of substance-P is comparable with values of healthy volunteers. The number of nearly undisturbed Rem-cycles especially during the last two administration nights is higher than in all treated and untreated nights before.

Table I. List of criteria measured I) before(first night) and II) after(seventh night) administration of substance-P at the same patient

		I)		II)	
TRT	min	446		478	
TST	min	333		4I4	
TTA	min	85		60	
SL	min	II2		48	
RL	min	39		45	
SEI	%	74.44		86.6I	
NA	-	7		8	
SSH	-	I4		I0	
RD	%	89.40		62.80	
awake	%	25.53		I4.49	
stage I	%	25.23		20.77	
stage 2	%	23.42		27.54	
stage 3	%	I4.II		I2.32	
stage 4	%	3.00		I8.60	
delta-sleep	%	I7.II		30.92	
stage Rem	%	8.7I		6.28	
Rem-cycles		a	b	a	b
		83	8.4	9I	0
		I48	52.7	89	0
		-	-	I09	5I.4

a duration (min)
b awake (% of duration)

NON-Rem and Rem periodes	a	b	a	b
NON-Rem I	39	58	45	67
Rem I	I	8	2	I6
NON-Rem 2	83	I05	89	II2
Rem 2	56	66	I7	8I
NON-Rem 3	I36	I82	67	97
Rem 3	I7	69	9	37

a duration (min)
b % of normal references

Table 2. Effect of substance-P on criteria of sleep (I2 patients with functional sleep disturbances)

parameter	control	+substance-P
number of Rem-cycles/ night	I.3	2.I
Rem-duration in mean	I34	I08
delta-sleep in mean	I7.3	22.5

4. The TST seems to be an unsuitable parameter for the valuating of the therapy efficiency at sleep disturbances in contrast to Rem-cycle number and-duration as appropriate criteria.

Conclusion

Further investigations are necessary to take notice of circadian and also circaseptan rhythms which modify remarkable the sleep cyclogram of each patient and furthermore the optimization of the mathematical procedure to analyze polysomnograpgical datas. The conclusion confirm that it was possible to reproduce the effects by substance-P reported from animal experiments in man.
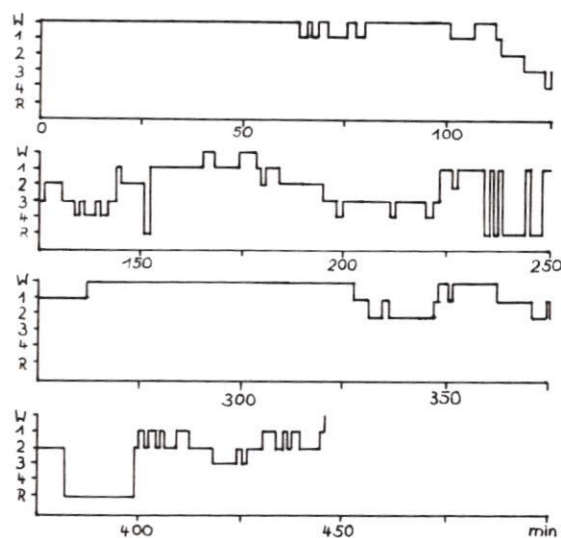
Figure Ia. Sleep cyclogram of the first night described in Table I
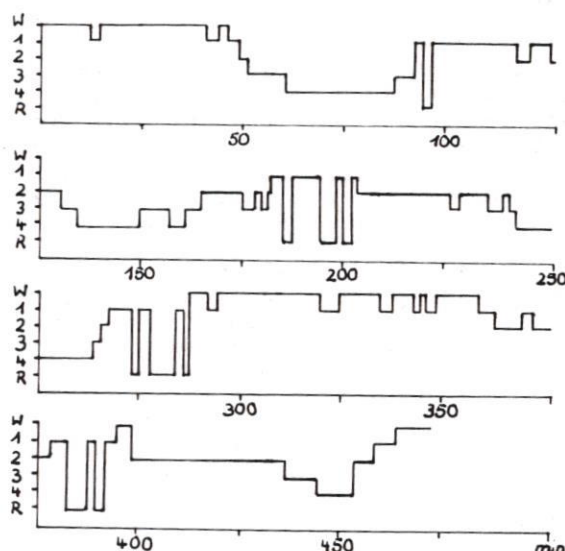
Figure Ib. Sleep cyclogram of the seventh night described in Table I

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METABOLIC AND HORMONAL CHANGES IN RATS IMMOBILIZED AT VARIOUS TIMES OF DAY

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Metabolic and hormonal changes caused by short-term immobilization are already quite well documented. We were interested in metabolic and hormonal responses to short-term immobilization at different times of day. Nyctohemeral arrangement of the experiment was chosen.

Methods.

After three week adaptation to standard condition (artificial illumination, L:D regimen of 12:12 h, cool light of 150 lux intensity per cage from 7 a.m. to 7 p.m., temperature 23± 2°C, relative humidity 70-80%, free access to standard diet and tap water) SPF bred male Wistar rats weighing about 240 g were immobilized for 24 h in special boxes. The so called morning group was restrained at 8 a.m., the evening group at 8 p.m. Both groups of restrained rats along with their analogous non-immobilized controls were deprived of food and water during the entire immobilization period. Immediately after ending of the immobilization the restrained and control rats were sacrificed by quick decapitation. The triacylglycerol (TG) and phospholipid (PL) concentration in serum, liver, bone marrow (femur) and thymus, the concentration of non-esterified fatty acids (NEFA) and of total cholesterol (CH) in serum and liver, the concentration of glucose in serum and of glycogen in liver was determined. Further, the concentration of corticosterone, thyroxine (T4) and triiodothyronine (T3) in serum was estimated. The experiment was carried out in winter, each group consisted of ten animals. The results were evaluated by t-test.

Results.

The aim of recent study was to analyse only the differences between restrained and non-restrained groups of animals at morning or at evening.
S e r u m. The values of NEFA were nonsignificantly lower in both parts of the day, TG concentration increased significantly in the evening group only. The levels of CH and PL remained unchanged (Fig.1).
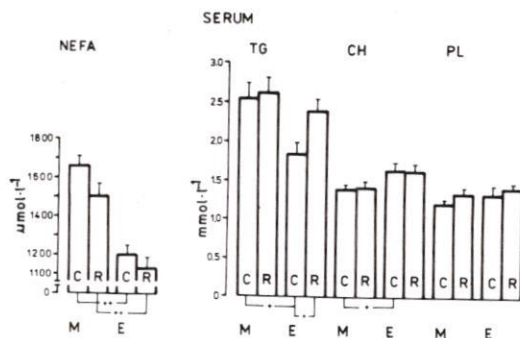
Fig.1. Concentration of non-esterified fatty acids (NEFA),triacylglycerols(TG), total cholesterol (CH) and phospholipids (PL) in serum of control (C) and restrained (R) rats at the morning (M) and the evening (E). Values are given as M+SEM, significance of differences between groups depicted as + (P < 0,05) or ++ (P < 0,01).

L i v e r. TG concentration rose in the evening group only, the decrease in CH concentration was observed in both parts of the day, PL increased in the morning group only. The levels of NEFA remained unchanged (Fig.2).
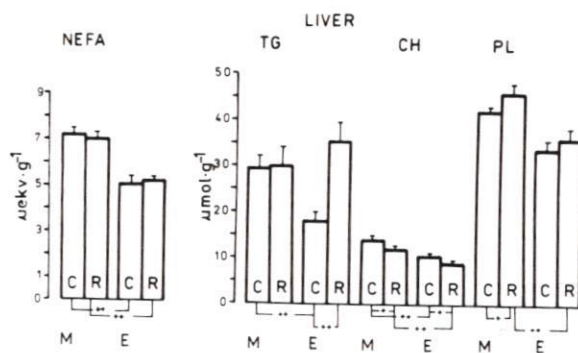
Fig.2. Concentration of NEFA,TG,CH and PL in liver. Other details as in Fig.1.

B o n e m a r r o w. TG concentration increased significantly only in the evening; no changes were seen in PL concentration. In t h y m u s PL content fell in both parts of the day equally but the TG content in the morning group only, when it was reduced nearly by one half as compared to controls (Fig.3).
Serum g l u c o s e rose equally in both parts of the day; a pronounced increase of liver g l y c o g e n was seen in the evening group only (Fig.4).
Only in the morning group a significant increase of serum c o r t i c o s t e - r o n e was noted. The concentration of t h y r o x i n e in the serum of restrained rats was similar to that in control group. The immobilization induced the rise in levels of t r i i o d o - t h y r o n i n e in serum, but significantly in the evening group only (Fig.5).

BONE MARROW
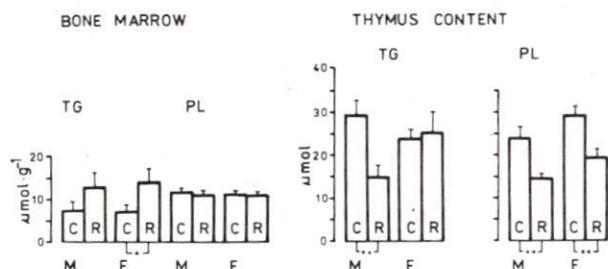
THYMUS CONTENT

Fig.3. Bone marrow concentration and thymus content of TG and PL. Other details as in Fig.1.

Discussion.

The studies on day-time dependence of the immobilization effect were concentrated namely on the activity of the hypothalamic-hypophyseal-adrenal axis. Increased serum corticosterone was observed in rats restrained in the morning, when basal concentration of hormone is low (2,4,5). Kant et al.(3) have found in serum of restrained rats more remarkable

Fig.4. Concentration of serum glucose and liver glycogen. Other details as in Fig.1.

increased levels of ACTH, prolactin and beta endorphin in the morning than in the evening.

Nyctohemeral arrangement of our experiment was chosen to elucidate whether or not the time of start and course of immobilization affects the metabolic and hormonal responses. Our results demonstrated the day-time dependence of such immobilization effects. Although the immobilization in both variants overshaded the circadian period, the majority of investigated parameters was changed in the evening variant only: the pronounced increase of TG in serum, liver and bone marrow, elevated T3 in serum and increase in liver glycogen was recorded at this time of day only. In evaluating the evening group it must be taken into consideration, that changed were namely indices connected closely with high level of food intake and locomotor activity in the dark part of day. On the other hand, the increase in serum corticosterone, liver PL and decrease in TG content
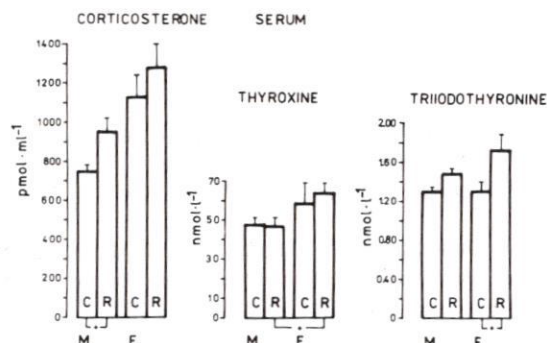
Fig.5. Concentration of serum corticosterone, thyroxine and triiodothyronine. Other details as in Fig.1.

in thymus in the morning (i.e. in the period of low activity) reflected better the response to stress reaction alone. The increase in glucose concentration in serum, the decrease of CH in liver and of PL content in thymus were observed in similar extent in both parts of the day. Contrary to one-peak circadian oscillation patterns in most of investigated parameters, the circadian oscillations of glucose in the serum of fed and 24 h starved rats had two peaks with 12 h period (1) and this fact could serve as a basis for equal response of glucose to the stressor in both parts of the day. Despite of the interaction of various factors as immobilization, circadian rhythms and starvation, our results demonstrated the dependence of metabolic and hormonal reaction of restrained animals on day-time, what should be taken into consideration in space biology.

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ORGAN SIZES AND BODY SIZE IN CHRONICALLY ACCELERATED GALLIFORM BIRDS

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The influence of chronic acceleration upon organ size in domestic fowl (Leghorn breed, body mass 2 kg) was reported previously [2]. Similar observations upon other galliform birds (Coturnix, body mass 0.1 kg) and a meat breed of domestic fowl (Arbor Acre, body mass 4-5 kg), are reported herein.

PROCEDURE:

Observations were conducted upon mature male birds. A carcass was prepared, by removal of the head, skin and shanks. Individual organs were removed, weighed, and the entire carcass comminuted. Samples of carcass material were used to determine fat, water and fat free dry matter contents. Relative organ sizes were calculated as gms per kg fat-free carcass mass (FFCM).

For Coturnix, observations were made on three groups of controls, one group adapted to 2 G, two groups at 2.25 G and one group at 2.5 G; and for Arbor Acre fowl, on one group of controls and one group adapted to 2 G. Data for Leghorn fowl were reported previously [2].

To be consistent with the previous report [2], relative organ sizes for experimental groups were standardized by presenting them as ratios to those from corresponding control groups. Regressions of standard organ masses on acceleration field strength (G) indicate arithmetic kinetics:

$$\text{Standard Organ} = \text{Intercept} \pm kG$$

RESULTS:

The principal gravitational effect is a decrease in body fat content (F, gms/kg FFCM) and a lesser decrease in the fat free carcass mass (FFCM, kg). The degree of these gravitational effects (k) increased with increasing body mass (BM, kg):

Carcass Fat (F):
Coturnix	$F = 1.17 -0.13\ G$ ($r = -0.458$, ns)
Leghorn	$F = 1.40 -0.49\ G$ ($r = -0.919$; $p < 0.001$)
Arbor Acre	$F = 1.61 -0.61\ G$ ($r-$)

Fat Free Carcass Mass (FFCM):
Coturnix	$FFCM = 1.00 + 0.01\ G$ ($r = 0.121$, ns)
Leghorn	$FFCM = 1.04 -0.06\ G$ ($r = -0.573$; $p < 0.04$)
Arbor Acre	$FFCM = 1.12 -0.12\ G$ ($r-$)

$$\text{Fat } k = 0.15 -0.15\ BM \ (r = -0.989,\ p < 0.02)$$
$$\text{FFCM } k = 0.0036 -0.027\ BM \ (r = -0.980,\ p < 0.02)$$

Since organ and tissue sizes are presented relative to the FFCM, which decreases with increasing field strength, indicated increases in organ/tissue sizes do not necessarily mean an absolute size increase.

Changes also were apparent in the sizes of some visceral organs, and generally these were related to field strength (G):

Kidney (K):
Coturnix	$K = 0.97 + 0.03\ G$ ($r = 0.150$, ns)
Leghorn	$K = 0.83 + 0.23\ G$ ($r = 0.817$; $p < 0.01$)

Liver (L)
Coturnix	$L = 0.01 -0.01\ G$ ($r = -0.737$, ns)
Leghorn	$L = 0.92 + 0.13\ G$ ($r = 0.650$; $p < 0.05$)
Arbor Acre	$L = 0.77 + 0.23\ G$ ($r-$)

G-I Tract (GI):
Coturnix	$GI = 1.02 -0.02\ G$ ($r = 0.518$, ns)
Leghorn	$GI = 1.18 -0.16\ G$ ($r = 0.732$; $p < 0.05$)
Arbor Acre	$GI = 1.13 -0.13\ G$ ($r-$)

The influence of body mass on the gravitationally induced changes in visceral organ size can be appreciated by comparing the gravitational coefficient (k) as a function of control body mass (Fig. 1).
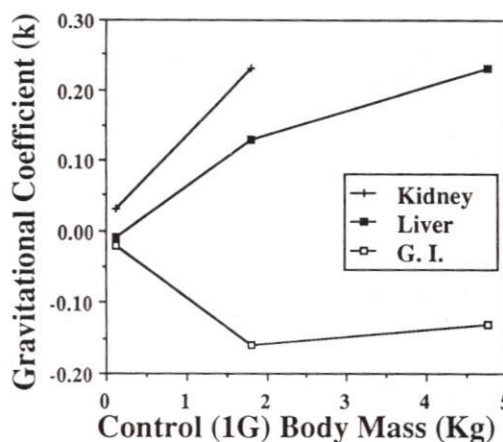
Figure 1. Influence of body mass on the gravitationally induced changes in organ size.

Little gravitational effect is apparent for Coturnix viscera, but similar gravitational effects occur in the two breeds of fowl. In the case of the G-I tract the principal gravitational effect appears at small body masses (less than 2 kg). Only for the liver is there an apparent proportionality between the gravitational coefficient (k) and body size (BM, kg):

$$\text{Liver } k = 0.006 + 0.05\ BM \ (r = 0.969;\ p < 0.04)$$

The presumed basis for liver enlargement is an increased fat metabolism associated with the gravitationally-enhanced fat mobility [3].

Dissections were made of leg bone and muscle (between hock and hip, corresponding to the drumstick and thigh), and these and the leg bone:muscle ratio were standardized as functions of the equivalent control values. Regressions of standard tissue mass upon acceleration field strength (G) indicated that arithmetic kinetics apply:

$$\text{Standard Tissue Mass} = \text{Intercept} \pm kG$$

Leg Muscle (LM):
Coturnix $LM = 0.91 + 0.12\ G$ ($r = 0.587$, ns)
Leghorn $LM = 1.10 - 0.10\ G$ ($r = -0.977$; $p < 0.001$)
Arbor Acre $LM = 1.08 - 0.10\ G$ (r-)

Leg Bone (LB):
Coturnix $LB = 0.98 + 0.02\ G$ ($r = 0.528$, ns)
Leghorn $LB = 0.93 + 0.10\ G$ ($r = 0.977$; $p < 0.001$)
Arbor Acre $LB = 0.89 + 0.11\ G$ (r-)

Leg Bone:Muscle Ratio (B/M):
Coturnix $B/M = 1.06 - 0.08\ G$ ($r = -0.616$, ns)
Leghorn $B/M = 0.76 + 0.26\ G$ ($r = 0.961$; $p < 0.001$)
Arbor Acre $B/M = 0.76 + 0.24\ G$ (r-)

The influence of body mass on the gravitationally induced changes in musculo-skeletal tissues can be appreciated by comparing gravitational coefficients (k) as functions of control body mass (Fig. 2).
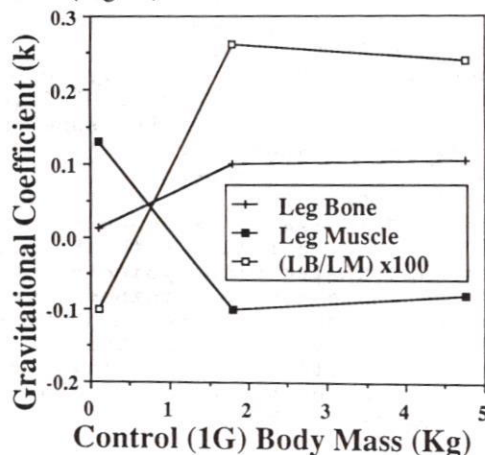
Figure 2. Influence of body mass on the gravitationally induced changes in leg bone and muscle sizes and the bone:muscle mass ratio.

These data indicate that the principal gravitational influence on antigravity tissues is a decrease in leg muscle mass, with proportional increase in bone mass. Changes in antigravity tissues in fowl are comparable for both breeds. As discussed previously [2], the increase in bone mass is considered to be a local response to gravitational loading. In one group of Leghorn

fowl (T, S_8) measurements were made of the dry masses of the humerus (H, a wing bone, not posturally loaded) and the femur (F, a leg bone). A regression of the standardized ratio of these bone masses (H/F) on the gravitational field strength (G) indicates that the gravitational effect is greater for the loaded bone:

$$H/F = 1.11 - 0.12\ G \quad (r = -0.986;\ p < 0.02)$$

The apparent decrease in leg muscle mass with gravitational loading has been interpreted as a selective involution of flexor elements. Direct comparisons between extensor (E, antigravity) and flexor (F) leg muscles indicate a large gravitationally-induced increase in the E:F muscle mass ratio [1]. In several experimental series of Leghorn fowl (not previously reported), the pectoral muscle mass (PM, a non-posturally loaded wing muscle) was compared with the leg muscle (LM) mass, and the ratios standardized as function of control muscle masses:

$$PM/LM = 0.96 + 0.05\ G \quad (r = 0.954;\ p < 0.001)$$

These data indicate that the gravitational effect upon muscle mass is greater for the loaded muscle.

DISCUSSION:

It is apparent that body size modifies the gravitational effect on organ and tissue size. However, among the animals reported herein, the principal body-size influence occurs among larger animals -- greater than 2 kg body mass. Little difference was apparent between the two breeds of fowl, in spite of a two-fold difference in body size. That both breeds are of the same species may be a factor.

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GASTROINTESTINAL TRANSIT AND LYSINE ABSORPTION IN THE JAPANESE QUAIL AT HYPERGRAVITY

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The transit of polyethylene glycol 4000 (PEG) as an inert intestinal marker as well as the absorption of lysine in the gastrointestinal tract (GIT) of cockerels of the Japanese quail at normal and elevated gravity was studied. Application of both ^{14}C-labelled PEG and ^{14}C-labelled lysine makes a chemical separation of isotopes necessary which however results in markedly improved sensitivity and reproducibility (C.V.2%) over double label approaches. Between the 19th and the 25th day post-hatching the experimental group was exposed to 2 g. The isotopes were applied in a starch gel into the crop. Exactly 30 min later the following segments of the GIT were analyzed: crop, stomachs, duodenum, jejunum (3 parts), ileum, colon + excreta. While at hypergravity PEG is significantly retained in the upper part of the GIT, total lysine absorption is unaffected. Analysis of the lysine:PEG ratios reveals a shift in the site of lysine absorption. Fractional growth rates in both groups remain essentially unchanged.

INTRODUCTION

The aim of the study was to test whether moderate hypergravity may cause changes in the transit and absorption of amino acids in the gastrointestinal tract of Japanese quails. In a SEM study Belák et al. (1) did not observe changes in the morphology of the small intestine of the same species after short-term hypergravity. Therefore, in the present study emphasis was put both on a long-term effect and on functional aspects of the GIT. For the measurements of intestinal amino acid absorption, lysine was chosen, as it represents an essential amino acid which is often used in its free form to supplement cereal-based feedstuffs. To follow the movement of the liquid phase of the digesta, polyethylene glycol 4000 was used as an inert marker.

MATERIAL AND METHODS

Animals: Growing cockerels of the Japanese quail (Coturnix coturnix japonica) were chosen for the experiment. Both the experimental and the control group were raised on a commercial diet containing 28 % N x 6.25.

Hypergravitation: At the 19th day post-hatching the experimental group was exposed for 6 days to a hypergravity of 2 g. Prior to the onset of hypergravity, the animals were adapted for 24 hours to the new environment. Hypergravity was produced by a large diameter centrifuge spinning at a rate of 23 rpm. Food and water was offered ad libitum. The light cycle was 24 hours per day.

Application of the dose: At the day of the main experiment, food was removed at 7.00 a.m. and the animals of both groups were maintained till 9.00 a.m. at the corresponding gravity. Immediately thereafter, a dosing gel was applied into the crop of animals of both groups. The gel contained 140 /umol of lysine labelled with 14 kBq of previously purified L-(U-^{14}C)-lysine (ÚVVVR Prague, Czechoslovakia) as well as 4 kBq of ^{14}C-polyethylene glycol 4000 (Amersham, Great Britain) in 4 % cooked maize starch. Six animals were used in each group.

Removal of GIT segments and sample processing: After an incubation time of 30 min the GIT was rapidly removed under halothane anesthesia and divided into the following segments: 1 crop, 2 stomachs, 3 duodenum, 4-6 proximal to distal third of jejunum, 7 ileum, 8 colon plus excreta. Immediately thereafter, the segments were flushed thoroughly with ice-cold saline. Trichloroacetic acid (TCA) was added to a final concentration of 5 % to precipitate proteins. TCA was removed from the supernatant by triple extraction with diethyl ether which had been previously saturated with unlabelled PEG.

Chemical separation of isotopes: The pH of the supernatant was adjusted to 5.5 by means of sodium acetate. The total radioactivity associated both with ^{14}C-labelled lysine and ^{14}C-labelled PEG was measured on a liquid scintillation counter (PACKARD TRICARB model 460 C). The chemical separation of both isotopes was achieved by the addition of 750 mg of a strongly acidic ion exchanger (DOWEX 50 W x 8, H^+ form, 200 - 400 mesh) to 5 ml of the supernatant. After mixing and short centrifugation, the radioactivity in the supernatant is exclusively associated with PEG, as the activity connected to lysine is bound to more than 99.7 % to the resin. Both the radioactivity associated with lysine and the lysine:PEG ratio can be obtained from the difference in the total and the PEG-associated radioactivities, respectively. In this study, a ratio of 3.45 : 1 was used. The coefficient of variation of the method outlined above is about 2 %.

RESULTS AND DISCUSSION

To detect changes in the transit and absorption of amino acids under the influence of hypergravity, a rapid and sensitive method for the simultaneous determination of the labelled amino acid and the inert intestinal marker is required. In view of the not too drastic differences to be expected between the experimental and the control group, chemical separation of the ^{14}C-labelled amino acid from the ^{14}C-labelled marker was preferred to an approach using tritium-labelled compounds. The former method is less sensitive to high quenching typical for samples of digesta.

Also, problems of channel spill-over are avoided.

Apart from this technical point, results on the gastrointestinal dynamics under hypergravity may critically depend on animal handling, feeding regimes, on the schedule of dosing and on the design of the experiment itself. Therefore, attention was paid to an identical animal handling and to a strict adherence to the schedule of dosing. It was tried to keep stress to the animals to a minimum. Taking the corticosterone level in blood plasma as a criterion of stress, long-term centrifugation itself does not seem to be a stressor for Japanese quails (2).

The distribution of the inert marker along the GIT is shown in fig. 1. The bar indicates the standard error of the mean.
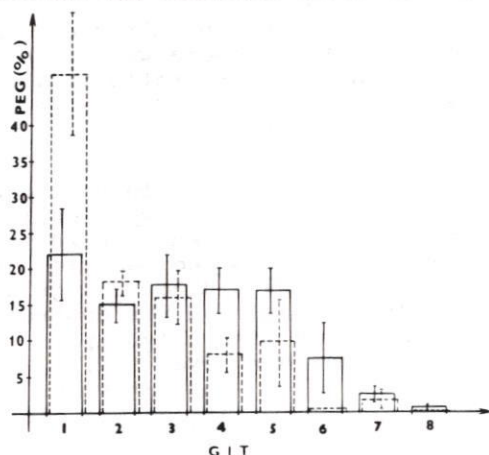
Fig.1: Distribution of PEG along segments 1 - 8 of the GIT. Full lines-control, broken lines-group exposed to hypergravity.

For the statistical evaluation, the F-test and the t-test according to Student were used. At p=0.05, the amount of inert marker being present in the crop of the animals exposed to hypergravity is significantly higher than that found in the **crop** of the control group. The inverse holds for the proximal jejunum. In spite of all effort to standardize the experimental conditions, the scatter in the data obtained from the other GIT segments is too large to observe significant differences. However, even in these segments there is a trend to a slow-down of the gastrointestinal transit at hypergravity. It should be mentioned, that all data were examined for the phenomenon of adaptation, i.e. it was checked, whether there are systematic changes in the gastrointestinal transit of animals exposed to long-term hypergravity with respect to the schedule of dosing. No such an effect could be observed. Another parameter to be analyzed is lysine absorption from the GIT. As outlined in the section MATERIAL AND METHODS, after chemical separation of isotopes the dimensionless lysine:PEG ratio can be calculated, which is a direct parameter of lysine absorption. The dependence of the lysine: PEG ratio on the position of the segment within the GIT is shown in fig.2. In both groups the ratio in the crop is practically identical to that of the dosing gel. A marked lysine absorption occurs in the stomachs. Here, the possibility of a certain experimental artifact due to a reflux from the duodenum cannot be completely ex-

cluded. In the duodenum and in the proximal as well as in the medial part of the jejunum there is a significant (p=0.025) decrease in the lysine:PEG ratio of the experimental group as compared to the control.
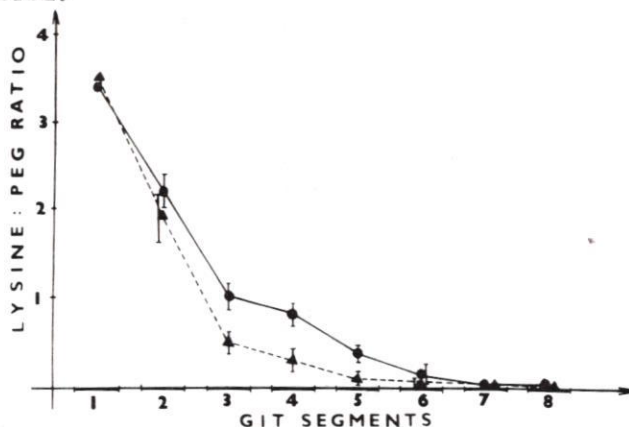
Fig.2: lysine:PEG ratio along the GIT

There are several possibilities to explain this increase in the absorption of lysine under the influence of hypergravity:
1. the transport parameters (maximal unidirectional flux, half saturation constant) remain unchanged. The slower passage of the digesta through the upper part of the GIT increases the probability of lysine absorption in that part of the GIT.
2. The passage rate is not directly affected. There is a change in the transport parameters of the upper part of the GIT towards a higher capacity.
3. combination of 1. and 2.
The tendency of a slower passage which can be seen from fig.1, would be in favour of the first alternative, though without additional kinetic studies the other alternatives cannot yet be excluded.
From the lysine:PEG ratio also total lysine absorption within 30 min from the whole GIT could be calculated. With respect to the control group, total lysine absorption was nonsignificantly lower in the group exposed to hypergravity. This is corroborated by the finding, that **the** fractional growth rates in both groups were quite similar (6.2 %/d in the experimental group versus 5.7 %/d in the control)

Thus, it may be concluded, that hypergravity does have an influence on the dynamics of marker transit and nutrient absorption in that sense, that while total amino acid absorption remains essentially unchanged, the site of intestinal absorption is shifted to the upper part of the GIT.

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INFLUENCE OF HYPERGRAVITATION, HYPODYNAMY AND THEIR COMBINATION ON JAPANESE QUAIL

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It is anticipated that Japanese Quail (Coturnix coturnix japonica)will provide animal proteins in a closed space ecosystem(2). Consequantly this species of birds is of research interest.Smith et al.(10, 11)have reported the effect of chronic acceleration on body composition of domestic fowls and Japanese Quail.

In our own previous work(5)the effect of hypergravitation,mechanical load and restrained movement on composition of some muscles in Japanese Quail was reported.From the results it was considered useful to verify and complete our knowledge with more extended experiment using the centrifuge constructed at our institute(9).

The objective of our experiment was study the effect of chronic acceleration at 2 G,hypodynamy and simultaneous effect of chronic acceleration and hypodynamy on the composition of the main muscles systems - thigh and breast muscles.We also examined plasma corticosterone as a stress marker, ultrastructure of the skeletal muscles and morphometry of the heart muscle in which an effect of a different load was anticipated.

MATERIALS AND METHODS

In our experiment 45 Japanese Quail cockerels,age of 48 days,were used.The animals were fed "ad libitum" a commercial mash prior to and during the experiment. They were divided in 5 groups.In group I, ten animals were kept under normal conditions,as control.Group II was placed in a cage of 50 by 50cm on the axis of the centrifuge,consisting of 5 animals,due to the limited space available into it.The other groups contained 10 animals each.Group III was subjected to hypodynamy,the birds being suspended in jackets without contact with floor as described by Jurání(7).Group IV was exposed to a chronic acceleration field of 2 G on the centrifuge.The animals of group V were simultaneously exposed to 2 G hypergravitation as well as to hypodynamy.The conditions of the experiment were the followings:radius of centrifuge - 320 cm; rotation 24 r.p.m.

The animals were rotated continously for 144 hours with only one interruption lasting for half an hour on the 4th day in order to fill the drinking water reservoir.

After 144 hours of treatment,the centrifuge was stopped and the 25 animals of groups II,IV and V were immediately exsanguinated by decapitation and samples of blood, heart,breast and thigh muscles were taken. The collection of these samples lasted only a few minutes.In skeletal muscles sarcoplasmic and myofibrillar protein fractions were separed by the method of Hellander(6);protein contents were determined by the method of Lowry(8);nucleic acid,expressed as phosphorus,were determined by the method of Canev(4).Plasma corticosterone was determined by the method of Beitins(1).Ultrastructure of left skeletal muscles was examined by electrone microscopy and the results will be reported by Dr.Kočišová.The morphometry of the heart muscle also was studied,but will not be reported at this time(3).

Analysis of variance and multiple comparisons among treatment means were made using the Duncans test.The statistical differences at the level of $P < 0.05$ were indicated in the figures.

RESULTS

No significant differences in food consumption were found between experimental groups.However,the experimental birds had approximately a 10% lower consumption than the controls.Body mass did not change in the experimental groups and the controls increased their body mass slightly.

Plasma corticosterone level(Fig.1),an indicator of stress was elevated in the hypodynamic animals (groupIII)and in the animals which were exposed to simultaneous effect of hypodynamy and hypergravitation (group V).However a significant increase of the plasma corticosterone values was also observed in

Fig.1: Plasma corticosterone (nmol.l⁻¹)

group II,rotated controls,which were located on the axis of rotation of the centrifuge.This high value of plasma corticosterone is difficult to explain,since hypergravitation(group IV)had no apparent stress effect.

Fig.2: RNA concentration in mg P-RNA.g⁻¹tissue
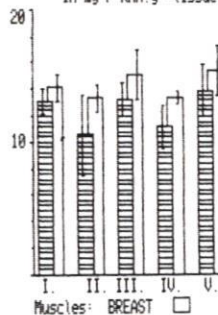
Fig.3: DNA concentration in mg P-DNA.g⁻¹tissue

Muscles: BREAST □   ⟨groups⟩   THIGH ≡
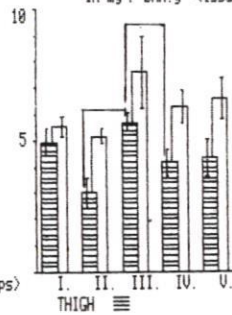
The ribonucleic acid(RNA)content in the muscles(Fig.2)was not significantly influenced by the various treatments.In breast muscles there were no significant differences in the desoxyribonucleic acid (DNA)concentration(Fig.3).In thigh muscles

slight significant differences were observed among the groups. The values in rotated controls(group II)were the lowest and the values in hypodynamic birds(group III)were the highest. Essentially the same DNA values were observed in the two hypergravitational groups(IV and V).

Fig.4: Sarcoplasmic proteins in mg protein.g⁻¹tissue

Fig.5: Sarcoplasmic proteins in mg protein.mg⁻¹P-DNA

Muscles: BREAST □   THIGH ≡   (groups)
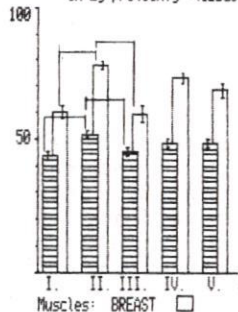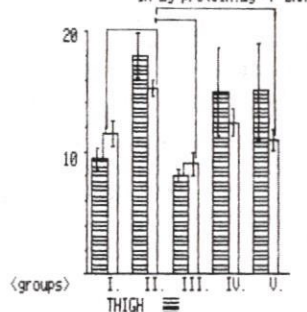
The concentration of the sarcoplasmic proteins(Fig.4)in breast muscle was increased in the centrifuged animals(group II, IV and V).The highest values for sarcoplasmic proteins were obtained in the animals placed at the axis of rotation and these significantly different from controls(group I)or the hypodynamic animals (group III).The sarcoplasmic fraction in the thigh muscles was influenced in approximately the same manner. The hypergravitation animals showed a tendency for increased sarcoplasmic proteins values in both muscles.

Expression of the sarcoplasmic protein content in breast muscles as mg per mg P-DNA(Fig.5)changed these relationships. On this basis hypergravitational animal(group IV and V)did not differ from those of the control(group I).However in thigh muscles the hypergravitational groups(IV and V)became enhanced. The hypodynamic animals (group III)had the same values as the controls. The highest values were observed in rotated control(group II).

Fig.6: Myofibrillar proteins in mg protein.g⁻¹tissue

Fig.7: Myofibrillar proteins in mg protein.mg⁻¹P-DNA

Muscles: BREAST □   THIGH ≡   (groups)
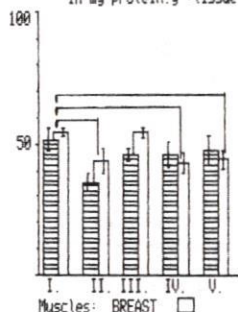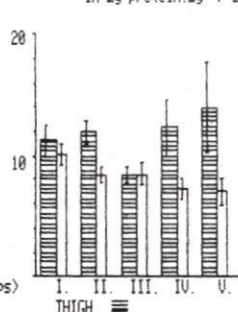
The content of the myofibrillar fraction (Fig.6)in the breast muscles was significantly lower in the animals exposed to centrifugation(groups IV and V)and the rotated controls(group II).The values in the breast muscles of the hypodynamic(group III)did not differ from those of the control group.In the thigh muscles there were no significant differences among the various groups,although the rotated controls (group II)showed a tendency to decreased myofibrillar fractions of breast muscles.

The presentation of the myofibrillar protein content as a function of DNA content (Fig.7)generally diminished the sta-

tistical significance of the differences and changed the tendency in the thigh muscles. The hypodynamy decreased the myofibrillar protein content but only without hypergravitation(group III).Both effects (group V)increased the content of this fraction.

DISCUSSION AND CONCLUSION

Six days exposure of Japanese Quail cockerels to hypodynamy with and without hypergravitation appeared to be stressfull as indicated by the concentration of plasma corticosterone level(7).Chronic acceleration at 2 G had no effect on this physiological parameter. The apparent stress in rotated controls(group II),placed at the axis of rotation of the centrifuge,was unanticipated.Since the number of animals in this group was small,only five animals, the results must be appraised with caution.In this group were found the highest plasma corticosterone levels,indicating a strong stress,and the highest content of the sarcompasmic fraction in both muscles.

The hypodynamy showed a tendency to increase the DNA content in thigh muscles but had no effect on the content of myofibrillar proteins in breast muscles.In breast muscles myofibrillar proteins were influenced negatively by hypergravitation. No convincing differences in any of the studied parameters could be found between the breast and thigh muscles,although the sarcoplasmic and myofibrillar protein content calculated as function of DNA were differentially influenced in the thigh and breast muscles. The findings of Dr. Kočišová in the ultrastructure of the muscles could not be correlated with our findings on the level of protein composition. This experiment which was enabled by our new centrifuge,is considered to be the starting point of futher studies on problems of chronic accelerations and hypodynamy in Japanese Quail.

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SELECTION OF JAPANESE QUAIL LINE RESISTANT TO HYPODYNAMY

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INTRODUCTION

The idea to incorporate Japanese quail as one of the alimentary links in closed space-ship ecosystem has resulted from its performance properties one of which is also the production of high-quality protein in egg form at effective fodder conversion. Gravitation is absenting in conditions of space flights. Taking into account the fact that weightlessness is relatively abnormal situation in comparison with 1-G terrestrial environment we can consider biologically microgravitation in cosmic space as stress-forming situation or perturbation of normal state. It is difficult to simulate microgravitation trustworthy in terrestrial conditions. In this case experimental models of weightlessness one of which is also hypodynamy are helpful. In our previous experiments with hypodynamy (1,3) we have proved that Japanese quail has good adaptive abilities and it is able to adjust to this unphysiological state and that it is possible to strengthen these properties by genetically suitably applied selective pressure (2).

MATERIAL AND METHODS

Parental population of Japanese quail was formed by hybrids of randombred and imbred lines of quails. The first part of population - the control line - was left at randombred breeding in every further filial generation. The second part of quails - selected line - was exposed to the two weeks' hypodynamy in the age of 8 weeks in every filial generation. Quails were tightly harnessed in jackets and suspended in individual cages without any possibility to contact the bottom of the cage. Food and water were provided ad libitum. Animals were held under 16:8 hours light:dark cycle. Cumulative egg-laying during hypodynamy was the criterion of further selection. 50 % of quails with the highest egg production from 80 quails were selected in every generation. Quails were mated with males from the same filial generation of selected line what formed the basis of the subsequent generation. 8th filial generations were formed in such a way up to now. Intensity of growth before and during hypodynamy was the further observed indicator. Physical condition of quails in 2nd, 5th and 8th filial genera-

tion was classified by 10 minutes' test immediately after completing the hypodynamy. In these tests four possible variations of postures (lying, sitting, getting up on shanks and standing) were evaluated. The results were processed by mathematical and statistical methods.

RESULTS AND DISCUSSION

In the 8th filial generation the effect of selection was manifested in criterion of selection, it means in increase of total egg-laying (Fig.1). In comparison with the control line the period of initial egg-laying decline was shortened in selected line. While control line was whithout eggs on the 4th day of hypodynamy, selected line reached on this day about 50 % of initial egg-laying. Higher rate of egg-laying raise in selected line contributes to the increase of total egg-laying, too.
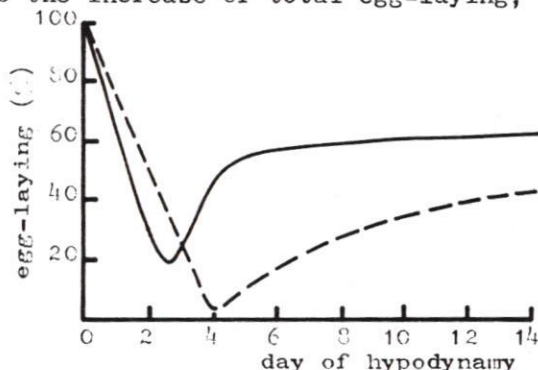
Fig.1 Egg-laying dynamics during hypodynamy in females of the 8th filial generation;---control line,——selected line

Comparison of divergent straight-lines characterizing control and selected quails lines during 8 filial generations (Fig.2) shows that cumulative egg-laying remains in range from 20 to 25 % while marked progress in egg-laying is visible in selected line. It reached 52 % in the last filial generation.
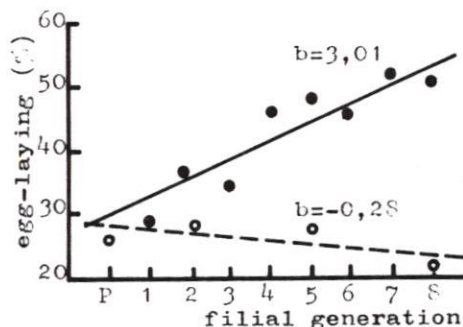
Fig.2 Mean egg-laying during hypodynamy in course of selection process; o control line, ● selected line

Despite the fact that global selection caused an increase in intensity of egg-laying during hypodynamy, there did not occur marked selection response in all generations. This is expressed as value of realised heritability for single filial generations (Tab.1). Value of coefficient points out to marked selection response in 2nd, 5th and 7th generations. It was not recorded in 3rd, 6th and 8th generations. The results of analysis of genetic and overall variability contribution in egg-laying during hypodynamy are expressed

Table 1 Responses to selection

Generation	Realised heritability (h^2_R)
F 2	0,431
F 3	< 0
F 4	0,343
F 5	0,142
F 6	< 0
F 7	0,340
F 8	< 0

h^2_R=selection response/selection differencial

by coefficient of heritability in table 2. The estimation of heritability for control line is 0,254, for selected line 0,091. It refers to the fact that we gained population geneticaly homogenous from the point of view of resistance to hypodynamy.

Table 2 Heritability (h^2) of total egg-laying during hypodynamy and body weight estimated from mother-daughter relationship in the 8th filial generation

Line	egg laying during hypodynamy	body weight after hatching	in adult females
Control	0,254	0,126	0,327
Selection	0,091	0,575	0,684

We observed also other physiological characters in connection with changes in growth curves (Fig.3). The growth of selected line females in the 8th filial generation is characterized by higher intensity and by lower decrease of body weight during hypodynamy in comparison with control line. The growth of the selected line males does not differ significantly from control line.

Because mentioned course of curves is typical also for proceeding generations starting from the 2nd filial generation, we were interested in possibility to consider changed growth of quails as correlated response to selection. From the comparison of the coefficient of heritability values of the body weight on day of hatching and in the 5th week of age (Tab.2) results that contribution of genetic variability of growth is increasing as consequence of selection. It means that changes in growth are not correlated response to the selection criterion.

Immediately after 14 days hypodynamy we classified the physical condition of quails because we were interested whether the selection affects the behaviour, too. Results from the 5th filial generation (Fig.4) indicate that the ratio of individuals reaching posture standing, it means better physical condition, was higher in both sexes of the selected line in comparison with control line. In case of males it was 100 % of individuals. In the 8th filial generation we have not found so impressive differences, probably it was concerned with absence of selection response in this generation.
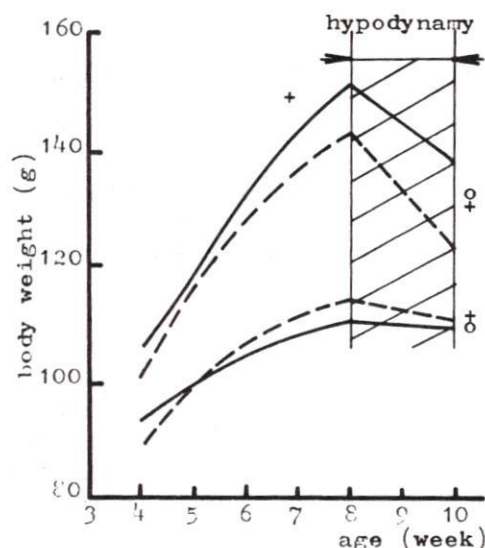
Fig.3 Growth in Japanese quail selected to resistance to hypodynamy in the 8th filial generation; ---control line, —selected line; + P < 0,05
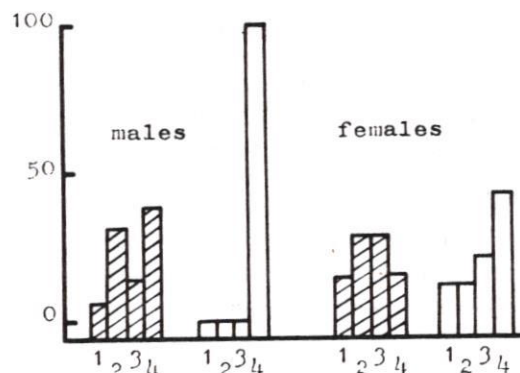
Fig.4 Percentual representation of quails in the 5th filial generation reached immediately after hypodynamy posture:1-lying,2-sitting,3-getting up on the shanks,4-standing,▨-control line, □-selected line

CONCLUSION

Summarizing the results of selection we can state that the selection has still positive effect on the parameters of egg-laying and it improves some other physiological and behavioral parameters as well.

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The FRANCO-AMERICAN MACAQUE EXPERIMENT

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In 1981, the National Aeronautics and Space Administration and the Centre National d'Etudes Spatiales initiated discussions about undertaking life science space-related projects of mutual interest. In 1985, the agencies signed a joint agreement formalizing their cooperation to develop a facility capable of supporting two rhesus monkeys (*Macacca mulata*) during spaceflight. These two male monkeys, each weighing 8-10 kg, will be restrained during the planned 7-10 day missions on board the space shuttle.

France will provide the cages, animal restraint system, and subsystems that include the feeder system, drinking valves, urine collection, fecal collection and electronic controls. The United States will provide the Research Animal Holding Facility, environmental control system, water tanks and water delivery manifold, overall system electronics and the monkeys.

Seven science discipline teams have been formed; each team is comprised of investigators from the United States and France. These seven science teams will test hypotheses on bone and calcium metabolism, behavior and performance measurements, cardiovascular dynamics, body fluids, electrolyte changes, muscle metabolism, neurovestibular responses, biorhythms and sleep.

In the bone experiment, the science team will investigate the effects of microgravity on bone formation and resorption, examining the phenomenon at the cell and tissue levels. The primary question will address the underlying mechanisms of calcium loss that is associated with spaceflight. Is it due to a sustained loss through the urine or feces or is there a reduced calcium uptake through the intestine? The investigators will examine the calcium balance by using labelled calcium in the diet and measuring its distribution in the bony tissues as well as the urine and fecal samples that are collected. One of their objectives will be to determine the quality of bone formed during flight by quantifying the spongy and dense bone. The relationship between muscle atrophy and bone density will be examined by computer tomography preflight and postflight. Bone biopsy samples will help the investigators determine the distribution of osteoclasts and osteoblasts. Major hormones such as calcitonin, osteocalcin and parathyroid hormones will be measured in the preflight and postflight blood samples.

What began as an idea for the environmental enrichment of the animals during flight has turned into a major experimental focus that involves several areas, including behavior neurovestibular, sleep, biorhythms, muscle and bone disciplines. In planning the missions, we began to recognize that by training the animals to respond to behavioral tasks and measuring a variety of parameters, the entire team would benefit from the behavioral training. For instance, they will be able to determine eye/hand coordination, the response time to target tracking, visual discrimination, muscle forces used by the animal, and so forth.

The immediate objectives of the behavioral science team will compare the behavioral responses inflight to those obtained during the preflight and postflight measurement periods. Use of the behavioral performance system will provide a continuous assessment of the animals' wakefulness and alertness during spaceflight. More importantly, the device will offer a counter
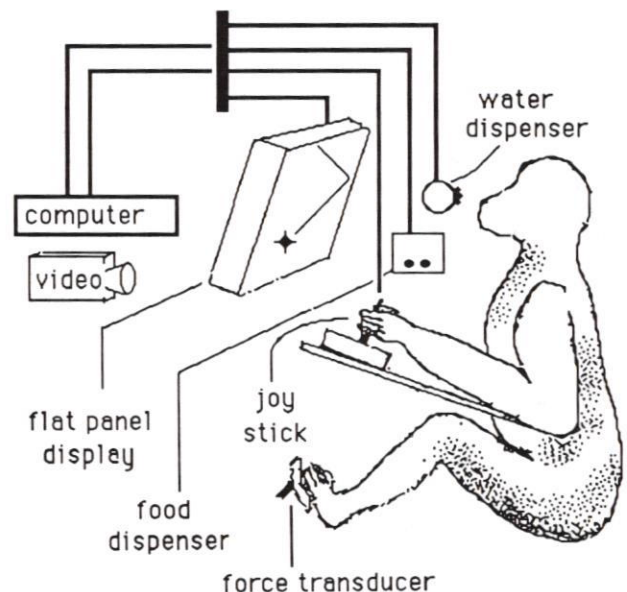
Major elements in the large primate cage

measure to the restraint and isolation endured during the flight. We plan to provide the monkeys a video display that presents moving targets. The monkey will use a lever (e.g.,

joystick) to electronically direct the screen cursor to the target. Proper interception of the target will provide access to nonnutritive food rewards and/or sips of fruit juice.

Preliminary results from the new training techniques have exceeded our expectations. Instead of taking months, the monkeys have proven to be rapid learners and the target tracking familiarization was reduced to a span of weeks. With the targets speed proportionally controlled by the joystick deflection distance, the monkeys are entirely capable of following the target and moving with it for a minimum of ten seconds. It may be possible to incorporate a force transducer into the lever handle permitting the performance comparisons of fine motor movements by the hand with the gross motor movements of the lower limb on a similar force transducer.

The cardiovascular group will address the effects of spaceflight on cardiac output, dimensions, rhythms, pressures and rates. The team is also interested in cardiovascular reflexes and adrenergic control of the heart tissue before and after spaceflight. They will examine the telemetered ECG, and follow arterial and venous pressure changes by using fluid filled catheters. If possible, we will implant doppler flow transducers in the descending aorta, the primary site, and use the renal, iliac, mesenteric and carotid arteries as secondary sites. The cardiovascular team is also considering preflight and postflight provocative tests such as challenges by sypathomimetic drugs or nonadrenergic agents like angiotensin, vasopressin and nitroglycerin.

Another group is planning a study of the fluid balance and electrolytes. Their study will include the nervous and humoral responses that result from fluid balance shifts during spaceflight. Inflight, we plan to collect the urine in four hour aliquots and preserve representative samples by freezing. After flight, blood plasma and urine will be analyzed for aldosterone, vasopressin, electrolytes (Na^+, K^+, Ca^{++},) and atrial natriuretic factor. Other hormones under consideration are catecholamines and prostoglandin. The team is also considering a provocative salt challenge by substituting a salt-laden pellet for a food pellet.

The spaceflight evidence suggests that microgravity exposure causes atrophy of the leg extensor muscles as well as other antigravity muscles. Our muscle team will address the question of atrophy. The team's scientific objectives will include the assessment of biochemical and anatomical changes. By taking postflight muscle biopsies under local anaesthesia, they will perform analyses for myosin ATPase and other enzymes, as well as provide a characterization of the muscle fiber types. Muscles of choice will include the soleus, gastrocnemius, tibialis anterior, biceps and medial triceps. The use of EMG electrodes and tendon force transducers will enable the muscle team to compare inflight muscle activity and strength with preflight and postflight measurements.

The neurovestibular team is keenly interested in examining the effects of microgravity on the eye, hand and head movement interactions that influence the vestibular apparatus. Using Helmholtz magnetic coils attached to both the eyes and the head, they will be able to follow eye movements during controlled pitch and roll movements of the head. Monitoring any performance decrements in target tracking, as well as signs of anorexia or emesis, will provide clues about the onset and recovery of space motion sickness.

The sleep/biorhythm team plans to examine a variety of biorhythyms that include the animal's temperature cycle, food and water consumption, sleep and wake cycles and the variations caused by neuroendocrine responses. They plan to obtain data from skin and core temperature sensors and movement information from EOG and EMG signals. The team anticipates using provocative tests of the diurnal cycles by adjusting the light intensity during flight.

Completion of the large data set will be obtained from different animals on multiple flights. We anticipate that it will take two to three flights on the space shuttle to address all of the scientific questions indicated. In addition, we plan to apply the hardware technologies developed for this large primate facility to those needed for primate experimentation on the space station. The use of rhesus monkeys, as human surrogates, will significantly aid our understanding of the microgravity effects on humans.

GRAVITATIONAL EFFECTS ON MAMMALIAN CELLS

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Abstract

In this paper we present first the re-
sults of our most recent investigations on
gravitational effects on the activation of
human lymphocytes: By immunoenzymatic
staining and by using concanavalin A
(Con A) coated to red blood cells (RBC)
we demonstrate that the increase of
activation measured at 10xg is due to a
simultaneous activation of T- and B-
lymphocytes whereas at 1xg only T-cells
are stimulated. Conversely, activation of
T-cells by chemical modification of the
membrane with sodium periodate is
depressed at 10xg.
Secondly, experiments performed in the
centrifuge as well as in the clinostat
with Friend, K-562, and hybridoma cells
show that each cell line develops its own
adaptation reaction to gravitational
stress.

1. Introduction

Since ten years we are investigating the
behavior of single cells cultured under
altered g-conditions (g stands for gravity
throughout the text). Our studies comprise
investigations under true micro-g in
space, simulated low-g in the clinostat,
and hyper-g in the centrifuge.
The first and most extensively system
investigated was the activation of lympho-
cytes in-vitro by mitogens. Later we ex-
tended our studies to other cell lines in
order to gain a complete picture of the
adaptation of mammalian cells to altered
g-conditions.
The most striking discovery was that acti-
vation of human lymphocytes is almost to-
tally depressed at zero-g (1,2) whereas it
is enhanced at 10xg (3).
The purpose of this paper is, first, to
present some new findings which contribute
to understand the effect of hyper-g on
lymphocyte activation, secondly, to clar-
ify certain aspects of our methods, and
thirdly, to show that the reaction of the
cell to a change of the g-environment does
not follow general rules, but rather de-
pends on the type of cells investigated.

2. Gravitational Effects on Lymphocytes

2.1 Methodology

In this section, we discuss only those as-
pects of the experimental approach which
are important to understand the meaning
of our results. A detailed description of
the methods is given elsewhere (2,3).

2.1.1. Cultures. We use two types of cul-
tures: (i) Purified lmyphocytes are ob-
tained from human peripheral blood by gra-
dient centrifugation on Ficoll and resus-
pended in culture medium. The cultures
contain approximately 80% lymphocytes,
other leucocytes and variable amounts of
RBC depending on the donor. (ii) Periph-
eral blood is diluted 1:10 with culture
medium (whole-blood cultures).

2.1.2. Measurement of Activation. We use
three different methods depending on the
experimental conditions and on the amount
of material available. In most cases acti-
vation is measured by incorporation of
tritiated thymidine into DNA. This method
is widely used by other authors in the
study of lymphocyte activation by mitogens
(4). When we compare the data obtained by
^{3}H-thymidine incorporation with those from
counts of metaphase preparations of mi-
totic cells incubated with colcemid (5) a
fair correlation is observed (see also
Fig.3). The third method consists of
counting the cells labeled with ^{3}H-thymi-
dine by autoradiography.

2.2 Activation Pathways

We describe here three different ways by
which T- and B-lymphocytes are polyclon-
ally activated. We use these approaches in
order to gain a deeper understanding of
gravitational effects on lymphocyte acti-
vation in hyper- and low-g.

2.2.1 Concanavalin A. Mitogens are known
to activate polyclonally T- and/or B-lym-
phocytes (4). Con a is a typical T-cell
mitogen. It is a protein (MW 100'000) ex-
tracted from the jack bean (canavalia en-
siformis) consisting of four subunits car-
rying each one binding site specific for
α-glucosides. When Con A is added to cul-
tures of lymphocytes it binds to membrane
glycoproteins causing intra- and intercel-
lular cross-linking (Fig. 1A). Activation
of T-lymphocytes follows. Although Con A
interacts also with B-lymphocytes, they
are not activated. However, when Con A is
covalently bound to a substratum, only the
stimulation of B-lymphocytes is triggered
(6). It has been suggested that substra-
tum-coated Con A is "presented" to B-lym-
phocytes in a different and more favorable
configuration than free Con A dissolved in
the culture medium (Fig. 1B). It is impor-
tant to point out this difference in order
to understand hyper-g effects on lympho-
cytes (see below).

2.2.2. Periodate. Novogrodsky et al. (7)
discovered that mild oxidation of the
sugar moiety of membrane proteins by peri-
odate specifically activates T-lympho-

ACTIVATION OF LYMPHOCYTES BY CON A

A)

B)

ACTIVATION OF LYMPHOCYTES BY PERIODATE

C)

$$\text{CH-CH-R} \overset{OH \ OH}{} + IO_4^- \rightarrow \text{CHO} \rightarrow \text{CH}$$
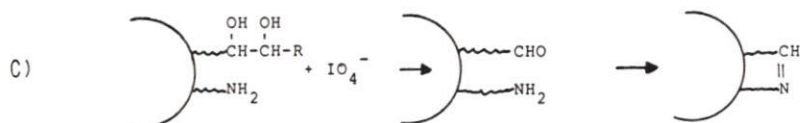
FIG. 1 PATHWAYS OF ACTIVATION OF LYMPHOCYTES IN VITRO

A: T-lymphocytes are activated by Con A dissolved in the medium,
B: B-lymphocytes are activated by Con A bound to a substrate,
C: T-lymphocytes are activated after oxidation of sugar residues
   with sodium periodate (30 min at 0 °C).

ERYTHROCYTE COATED CON A
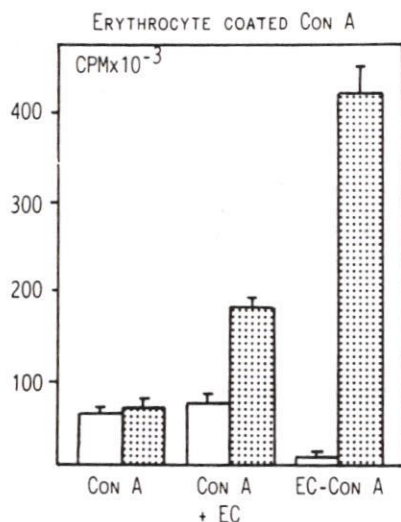
FIG. 2 HYPER-g EFFECT OF RBC-Con A ON CULTURES OF
       PURIFIED LYMPHOCYTES

Lymphocytes were incubated at 1xg (void bars) and at
10xg (shaded bars) respectively in the presence of
dissolved Con A (Con A), dissolved Con A + autologous
erythrocytes (Con A + EC), and red blood cells-coated
Con A (EC-Con A). Activation is given as cpm of tritiated
thymidine incorporated into DNA.

A. CLINOSTAT (0.02xG)

B. CENTRIFUGE (10xG)
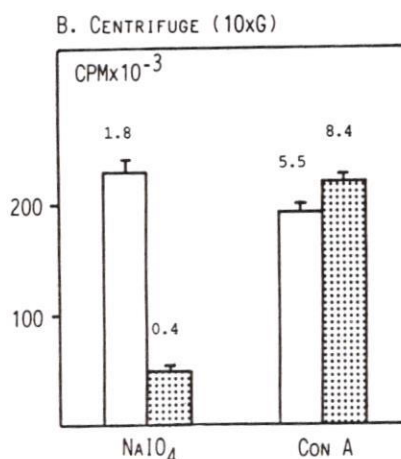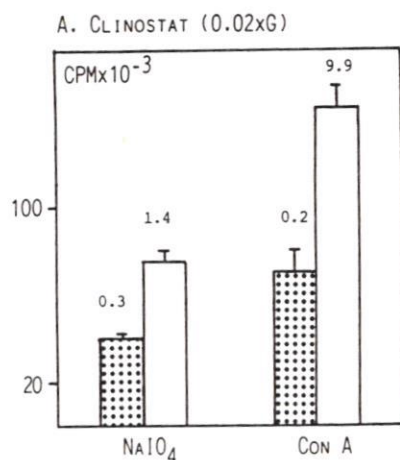
FIG. 3 HYPER- AND HYPO-g EFFECTS ON THE ACTIVATION OF PURIFIED LYMPHOCYTES BY PERIODATE

Cultures of purified lymphocytes were activated either by treatment with periodate (NaIO$_4$)
or by exposure to Con A (Con A) and cultured in the clinostat (panel A) or in the
centrifuge (panel B) respectively. Void bars correspond to the control at 1xg, shaded bars
to the experiment. Activation is measured either by incorporation of tritiated thymidine
into DNA (cpmx10^{-3}) or by counting the percent of mitotic cells in metaphase preparations
(numbers above bars).

cytes. Periodate splits vicinal alcohols generating aldehyde groups (Fig. 1C). The last react with free amino groups, of lysine and N-terminals, and via formation of Shiff's bases, inter- and intracellular cross-linking of membrane proteins is achieved. In analogy to what occurs with Con A, cross-linking is followed by lymphocyte activation. The essential difference between periodate and Con A is that Con A has to be present in culture for at least 16 hours (8) in order to trigger activation, whereas periodate oxidizes irreversibly sugar residues, and is removed after a 30 minutes treatement at 0 °C from the resuspended lymphocytes.

2.3. Results

2.3.1 Activation by Con A.
These experiments were performed in order to understand the cause of the striking difference of the hyper-g effect between cultures of purified lymphoyctes and whole-blood cultures (3). In fact, while in purified cultures at 10xg the increase of activation is 20-30% with respect to the control at 1xg, in whole-blood cultures the increase is as high as 400%. The work of Lorenzi et al. (3) indicated that certain components present in significant amount in whole-blood and only in minor amounts in purified cultures are responsible of the hyper-g effect.

The results reported in Fig.2 were obtained by exposing purified lymphocytes to Con A coated to autologous erythrocytes.The magnitude of the hyper-g effect is comparable to that of whole-blood cultures. RBC-Con A was obtained by preincubation of RBC with Con A. The binding originates from the interaction between one or more binding sites of Con A and α-glucosides on the RBC membrane. Therefore RBC-Con A is in thermodynamic equilibrium with free RBC and Con A. Immunoenzymatic staining (specific for B-cells (9)) shows that in whole blood cultures as well as in cultures of purified lymphocytes supplemented with RBC-Con A, B-lymphocytes are activated in addition to T-cells at 10xg, whereas at 1xg almost no B-cells are stimulated. Hence, in whole-blood cultures part of Con A first interacts with the sugar moiety on the erythrocytes membrane forming erythrocyte-coated Con A. Then the RBC-Con A complex "presents" Con A to B-lymphocytes in a configuration capable of triggering their activation. On the other hand, T-cells are activated by free Con A. In cultures exposed to RBC-Con A, activation of T-lymphocytes is achieved by free Con-A always present in equilibrium with the RBC-Con A.

In cultures of purified lymphocytes, erythrocytes are present in variable amounts, depending on the donor and on the quality of the preparation. Only a small quantity of Con A is coated to erythrocytes and therefore only a few B-lymphocytes are activated.

We conclude, therefore, that the hyper-g effect is due mainly to the simultaneous activation of T- and B-cells. However, we are not yet able to explain why at 1xg B-lymphocytes are not activated by RBC-ConA.

TABLE 1 — GRAVITATIONAL EFFECTS ON MAMMALIAN CELLS

	CENTRIFUGE	CLINOSTAT	BALLOON	SPACE
Lymphocytes				
Activation by Con A¥ (1-3)	+++*	--	+	----
Activation by IO₄	--	--		
Friend cells + DMSO				
Proliferation	--	++		
Glucose consumpt.	--	--		
HG production @	O	--		
% HG producing cells		O		
K-562 cells + Hemin (10)				
Proliferation	O	O	O	
Glucose consumpt.	O	-	+	
HG production #	-	--	--	
% HG producing cells	O	O		
Hybridoma (11)				
Proliferation	O	O		
IgG production	O			

+ : Increased, - : Decreased, O : Unchanged
HG: Hemoglobin, IgG: Immunoglobulin
¥ Only T-lymphocytes are activated at 1xg by Con A and Periodate
* T- and B-lymphocytes are simultaneously activated at 10xg
@ Induced by DMSO
\# Induced by Hemin

2.3.2. Activation by periodate. Fig. 3 shows the hyper- and hypo-gravity effects on the activation of lymphocytes either by periodate or by Con A.. In the clinostat, the inhibition is of the same magnitude as that observed with free Con A. When the incubation is carried out at 10xg, activation is also inhibited, at difference from what occurs with free Con A. We interpret our results as follows: The depression observed at low-g either with Con A or with periodate depends on changes of the interaction between the cell membrane and intracellular structures rather than on an extracellular receptor-ligand interaction. Therefore the depression obtained with Con A and periodate at low-g have probably similar g-dependent mechanisms. The inhibition at high-g is probably due to mechanisms similar to those operating in other cells as shown in Table 1.

3. Gravitational effects on other cells

Table 1 summarizes the behavior of all cell types hitherto tested in our laboratory in different g-conditions. Depending on the properties of the cell, several parameters were determined. Friend leukemia virus-transformed cells (Friend cells) and K-562 cells have the peculiar property of expressing hemoglobin genes upon induction with dimethylsulfoxide (DMSO) and hemin respectively. The fact that the hybridoma line tested here did not show changes in the behavior either in the centrifuge or in the clinostat does not mean that this applies to all kinds of hybridoma cells lines since these are known to have different properties between each other.
The experiments on the stratospheric balloon were carried out in order to discriminate between effect of microgravity and of cosmic radiation in space. As Table 1 shows lypmhocyte activation is not affected by cosmic rays.
In conclusion the experiments presented in Table 1 clearly show that magnitude and direction of the g-response depend on the cell type and that no general rules on g-sensing of mammalian cells can be applied.

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ARTIFACT-FREE RECORDING OF THE ECG SIGNAL IN THE COURSE OF ACCELERATION STRESS

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The principles of artifact-free ECG recording, giving the high-quality electrocardiographic curve and HR values in the course of acceleration stress up to +20 G_z on an experimental centrifuge are explained.

Electrocardiography belongs to the obligate monitoring techniques in human and animal objects, subjected to centrifugation experiments. It's information is often altered by a number of artifacts, rised by the stressfull environment. In this connection the ECG signal's monitoring without artifacts and disturbances can be held as one of the key problems of an experimenter.

For experiments with rats the animal centrifuge with the turning radius of 25 centimeters was manufactured in the Institute of Aviation Medicine, Prague. It is equipped with the stepless speed control of acceleration up to 20 g and with a reliable system for continuous registration and monitoring of the ECG and the heart rate (HR) data.

An electrical signal from the standard bipolar lead II is picked-up by needle electrodes from an animal, fixed to the bottom of a novodure cage. The signal is then amplified by the selective amplifier, placed on a small platform in the centrifuge's rotation axis. A constituent part of the facility is the heart rate amplifier. After processing, both signals are transfered from the rotating part to the stationary part of the device through the collector rings. They consist of mutually insulated brass rings 40 mm in diameter, attached to the rotor. A pair of brush holders fit to each ring. At the same time the system serves for the power supply ± 10 V of the amplifying unit. For reliable transmission of a single signal always two parallelly connected rings are used.

The ECG signal is monitored on an oscilloscope, and if need be, it can be registered on a recorder. The HR signal is displayed on a digital cardiotachometer. There is a possibility to monitor both physiological signals in the on-line mode or to store them on an analog FM tape recorder. The time synchronisation of the instrument chain is provided by a timer in second's and minute's intervals.

Input followers provide high output impedance for the amplifier. The differential stage rejects the undesirable common AC signal. The low-pass filter rejects the noise higher than 80 Hz. The input RC circuit of the final output stage has a time constant = 2,5 seconds.

It proved useful to process the ECG signal for a cardiotachometer already in the amplifier, at the rotating part of the centrifuge. Rectangular pulses, generated at the pulse-shaper's output, are derived from the QRS complex of ECG. The pulse shaper contains a self-acting comparator, initiated by peak voltage of the QRS complex. The interference with voltage level, lower than the peak amplitude of the R wave at the selective amplifiers output is automatically suppressed. Rectangular +10 V pulses are fed through the collector rings to the cardiotachometer's input. In most cases the conversion into HR pulses is safe, only under occasional situations, like in the course of clonic convulsions, reading of the HR from a recorder is necessary.

The ECG signal processing already at the rotating part calls for a major number of transmission rings, than the processing at the stationary part. However, in this way the undesirable outages of the cardiotachometer's function, caused by the ECG's signal limitation, are reduced. Optimal filtering with suppression of disturbing effects towards the useful signal improves the reliability of the HR data evaluations.

Following ECG surves demonstrate technical aspects of the problem only. The load applied was sufficiently high and stressfull to check the possibilities of artifact-free recording of the ECG signal in extreme conditions. The records, obtained with the paper speed of 100 mm . s^{-1} are evaluable both from the morphological as from the timing characteristics of ECG at rest, in the course of the acceleration load, after it's termination and even throughout the terminal state. Thus all the +G-stress related ECG changes, described by various authorities, could be successfully reproduced.

We are aware of the fact, that the fixation of the animal's limb affects the metabolic and hemodynamic response, the well-being and the resistance of experi-

mental animals against the acceleration stress. Therefore more suitable methods, enabling the ragistration of physiological signals from the relatively unrestra-ined animals, as well as better fixation of electrodes, remain under further investigation.
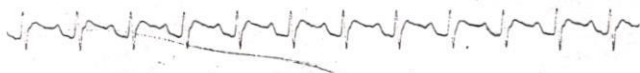
Figure 1. White rat N° 28. Gravitational stress +10 G$_z$, 55.-60th second.
ECG: heart rate 539.s^{-1}, PQ depression

Figure 2. White rat N° 18. Gravitational stress +10 G$_z$, 175-180th second.
ECG: sinus rhythm with 2nd degree A - V block (2:1), ventricular rate 245.s^{-1}, ST elevation (Pardee), isolated ventricular extrasystole

Figure 3. White rat N° 18. Gravitational stress +10 G$_z$, 235-240th second.
ECG: 3rd degree A - V block, atrial rate 276.s^{-1}, ventricular rate 131.s^{-1}

Figure 4. White rat N° 18. 295-300th second after interruption of gravitational stress (+10 G$_z$, 240 s.).
ECG: ventricular fibrilation

ELECTROPHOTOGRAPHY AS A TOOL FOR EVALUATION OF CHANGES INDUCED IN HUMAN ORGANISM BY HYPERGRAVITY

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Electrophotograms were recorded with eight persons exposed twice for 1.5 min to 3 and 5 G. The electrophotographs were evaluated by a Leitz Image Analyzer. The shapes of the electrophotographs recorded before and after centrifugation are different. Thus, in combination with physiological and biochemical methods electrophotography can be used as a tool for evaluation of integral and complex changes induced in the human organism by hypergravity.

During the last few years an increasing amount of publications appeared in which electrophotography was used for evaluation of physical loads as well as of various pathological processes (see Leach, A.B.,Brit.J.Photogr. 127 (16):304-305,1981, Chudáček,I.,Matoušek,L., The Royal Photographic Soc. of Great Britain, Cambridge 1984, pp.41-45).

The principle of the electrophotographic technique is based on the observation that objects in contact with a photographic emulsion exposed to a high-voltage electric field, give a typical pattern on the developed film (see Loeb,L.B.,Electrical Coronas, Berkeley, University of California Press 1965).

A typical electrophotogram of a human fingertip is demonstrated in Fig.1A, using a subject at case, under balanced conditions. After exposure to physical and psychic load the electrophotogram is changed (Fig. 1B).
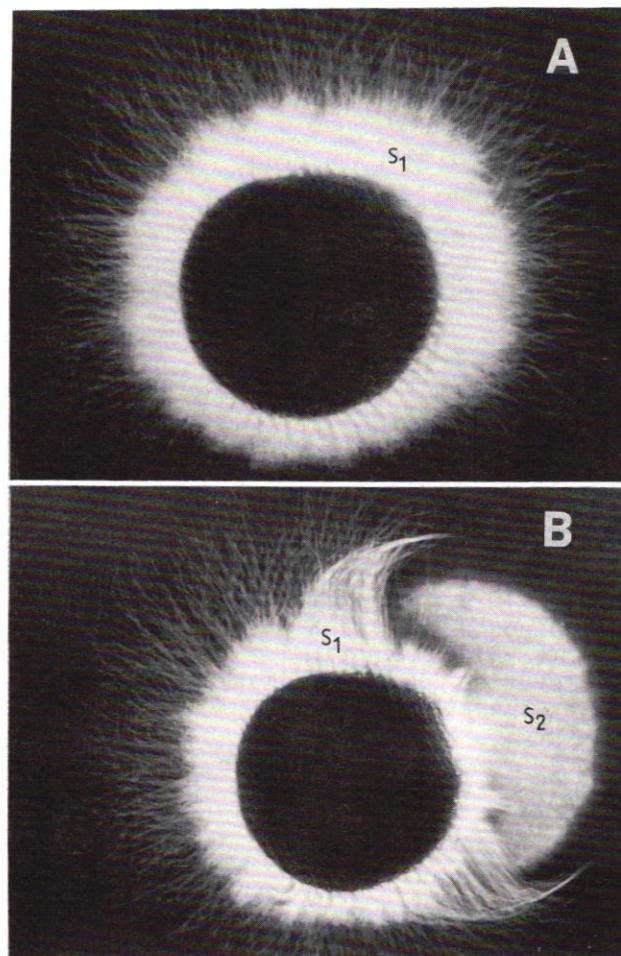
Fig. 1. Electrophotogram of a human fingertip exposed to a high-voltage electric field (200 kV; 200 Hz; 0.5 s). A-subject under balance conditions. B-subject under stress conditions. S_1 - area typical of balanced conditions, S_2 - area typical of stress conditions. Electrophotograms were recorded by an apparatus constructed locally.

Changes in electrophotograms were evaluated in such a way that the area typical of balanced conditions was marked with S_1 (Fig 1A) and the area typical of stress conditions was marked with S_2. Using a Leitz Image Analyzing system the S_1 and S_2 areas of the electrophotographic corona were measured and the percentage of the S_2 area was calculated.

Electrophotograms were recorded with 35 male subjects under balanced conditions. The results are summarized in Table 1. Under balanced conditions 80% of the subjects displayed only the S_1 area. With 20% persons, the S_2 area, which reflects stress or pathological conditions, was detected. The S_2 area in this group represented 27% of $S_1 + S_2$ ranging from 14 to 40%. We may thus conclude that on the basis of the electrophotographic data the group of persons evaluated can be divided into two subgroups (see Table 1).

Number of subjects	Age (years)	Area (mm^2) S_1	S_2	%S_2
35 (100%)	22-50	152	10	6.2
		±10.3	±3.5	±5.3
27 (77%)	22-52	162	1	0.6
8 (23%)	26-59	117	42	26.6

Table 1. Evaluation of electrophotographs of human fingertip with persons under balanced conditions.

In further experiments we applied the above procedure to evaluating the effect of hypergravity on the human organism. Eight persons were exposed twice for a short period of time (1.5 min) to 3 and 5 G with a 10 min pause between the two centrifugations. Physiological data were monitored continuously while electrophotographic data were recorded before and after centrifugation. The results obtained are presented in Fig. 2.

From this data it is evident that the character of the changes obtained on electrophotographs is quite different from that of, e.g. the heart rate or blood pressure (Fig. 2).

The data of individual subjects showed, in aggreement with our previous observations, that our experimental group can be divided into two subgroups. Six persons

have no measurable S_2 area before centrifugation whereas with two persons an S_2 area was found. Individual evaluation of these data also indicates that with seven persons out of eight the S_2 area significantly increased after centrifugation whereas with one person no change was found (Table 2).
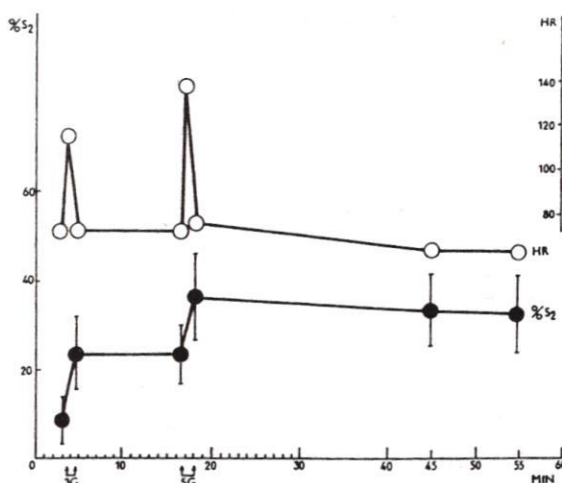
Fig. 2. Changes of heart rate and S_2 area of the electrophotograph after centrifugation.

Subject number	S_2 (mm^2) control	3+5G	S_2 increase (mm^2)
3	0	0	0
2,4,6,7,8	0	29	+29
1.5	37	80	+43

Table 2. Individual differences in changes of the S_2 area induced by hypergravity.

It may be concluded that electrophotography in combination with an Image Analyzer which permits digitalization of electrophotographic pictures can be used as a noninvasive technique for evaluating integral and complex changes occurring in human organisms under physical and psychical loads. According to our data such a method can also be used for evaluating complex changes occurring in a human organism after exposure to hypergravity.

CHANGES IN METABOLIC ACTIVITY OF THE CENTRAL NERVOUS STRUCTURES DURING HYPODYNAMIC EXPOSURE (BODY SUSPENSION)

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INTRODUCTION

It is well known that a hypodynamic condition induces many physiological responses, i.e., circulatory and metabolic. It is also considered that, in the development of such responses, hypogravic reception is integrated in the central nervous system (CNS), and eventually activate many controllers in the CNS which organize various effector-systems. Recently, studies on the central mechanism of gravireception gradually started to investigate the contributions of several brain regions involved in development of several responses during hyper- or hypo-gravic condition. In the 8th annual meeting IUPS commission on gravitational physiology in Tokyo (1986), the symposium on "Mammalian gravitation and brain function" was also held and many results were reported in this text. However, because of limited techniques, such as lesion-, stimulation, or unitary recording techniques, these reports could not represent the spacial relation of the activated CNS as whole, but suggested the characteristic contributions of each region.

Sokoloff and his colleagues (4,5) have developed a new autoradiographic method with 2-deoxy-D- [^{14}C]glucose (2-DG). This method utilizes 2-DG as a tracer for glucose utilization in the brain regions. The higher the grain density of 2-DG on the autoradiograph, the greater is the 2-DG incorporation into that region. Subsequently, this method enables us to provide a pictorial representation of changes in metabolic activity of brain regions. It has already proved to be a valuable tool in a number of sophisticated neurophysiological investigations (1,2,3), because this method easily enables us to investigate the functional activities of many brain regions of conscious subjects. Using the 2-DG technique, the present study was carried out to investigate changes in functional activities of various brain regions in conscious rats during hypodynamic condition, which was induced by body suspension.

METHODS

Ten male albino rats (Wistar strain) weighing 250-300 g were used. In each rat Silastic tubing for injection of 2-DG was inserted in the superior caval vein under Nembutal anesthesia (25 mg/kg, ip). After implantation of the catheter into the caval vein, at least 1 week was allowed for recovery in a temperature-controlled room at 26 ± 1^{O}C with natural illumination. The animals were not fed during 12 h before the experiment but had continuous access to water. Experiments were conducted at the same time (1200-1400 h) and under the same lighting condition for all animals.

Ten animals were divided into the two groups: 1) suspension group (n=5), where animals were horizontally suspended with surgical bandage netting, and 2) control group (n=5), where animals were not suspended. Ninety min after biginning the suspension, each rat was given a single injection of 0.5 ml saline containing 10 μCi/100g of 2-DG (specific activity 60 mCi/mol, Amersham) into the caval vein through the catheter previously implanted. Forty-five min after injection of 2-DG, the animals were decapitated and their brains were rapidly removed, frozen in Freon chilled with liquid nitrogen and cut into sections (28 μm) in a cryostat maintained at -20 ^{O}C. For autoradiography, the sections were then exposed to single emulsion X-ray film (Kodak, SB-5) in X-ray film cassetes for 10 days, and then the films were developed. Adjacent sections were stained with hematoxylin and eosin and served for histological identification of parts in the autoradiographs, using rat brain atlas. For quantitative comparison, optical densities (OD) of the brain regions and polymer reference sources for standardizing (set for autoradiography CFR-10, Amersham) in autoradiographs were measured with a microscanning photometer (Nikon, Vickers, M85) with an aperture setting of 0.15 mm. Before measurement of ODs in selected brain regions, the photometer had been previously adjusted so that ODs of the [^{14}C]-DG standards have an almost linear relationship to the ^{14}C content of those standards. Five readings of the OD were averaged for the selected brain regions, and the OD ratios of the various brain regions to the corpus callosum were calculated. The data were analyzed for statistical significance by Student's t-test for unpaired data.

RESULTS AND DISCUSSION

In Table 1, optical densities for 27 selected brain regions are expressed as ratios to opitical density of the corpus callosum. The data in Table 1 show the relative changes of the 2-DG uptake of each brain region under the present experimental conditions. Therefore the data do not represent quantitative derminations of the actual rate of each brain regions glucose consumption. Compared with respondent regions in control group, significantly higher ratios of the 2-DG incorporation were observed in suspension group in the following brain regions: anterior cingulate cortex, septal nuclei, lateral preoptic area, medial and lateral habenula. While ratios lower than those in the control group were observed in the sensory-motor cortex and the olivary nuclei. 2-DG autoradiographs at the level of habenula are shown in Fig. 1. It is noted that habenula in the suspension group showed a greater increase in 2-DG incorporation than that in the control group. Moreover significant differences in pattern of activation in the parietal cortex between control and suspension groups are observed.

The present study has shown the changes in the

metabolic activity in the several brain regions during hypodynamic exposure. As for these brain regions, it is possible to consider that these regions will more or less take part in the development of responses induced by hypodynamic condition. At this time, it is difficult to analyze these brain regions with regard to precise localization in the complicated chain of central mechanism to induce these responses. However it is strongly expected that the present results provide a useful starting point for precise studies in the near future.

[^{14}C]deoxyglucose incorporation in brain regions during suspension

	Control (n=5)	Suspension (n=5)
Cortex		
Frontal	1.95 ± 0.04	1.90 ± 0.05
Anterior cingulate	1.93 ± 0.05	2.10 ± 0.04*(↑)
Sensory-motor	2.10 ± 0.04	1.91 ± 0.04*(↓)
Visual	2.09 ± 0.05	2.12 ± 0.05
Limbic nuclei		
Hippocampus	2.10 ± 0.04	2.05 ± 0.07
Septal nuclei	1.99 ± 0.05	2.11 ± 0.05*(↑)
Amygdala	1.75 ± 0.05	1.72 ± 0.04
Basal ganglia		
Caudate putamen	2.04 ± 0.03	2.10 ± 0.05
Globus pallidua	1.68 ± 0.05	1.75 ± 0.06
Diencephalon		
Medial preoptic area	1.43 ± 0.04	1.55 ± 0.05
Lateral preoptic area	1.84 ± 0.03	2.15 ± 0.04*(↑)
Lateral hypothalamus	1.50 ± 0.04	1.55 ± 0.06
Posterior hypothalamus	1.68 ± 0.06	1.75 ± 0.05
Thalamus		
Ventrolateral	2.10 ± 0.04	2.06 ± 0.05
Ventroposterior	2.12 ± 0.05	2.08 ± 0.07
Habenula		
Medial	2.05 ± 0.04	2.38 ± 0.04*(↑)
Lateral	2.18 ± 0.05	2.40 ± 0.04*(↑)
Zona incerta	2.12 ± 0.06	2.19 ± 0.05
Mammillary body	2.26 ± 0.05	2.33 ± 0.04
Substantia nigra		
pars compacta	1.70 ± 0.04	1.72 ± 0.05
pars reticulata	1.61 ± 0.06	1.70 ± 0.04
Red nucleus	2.04 ± 0.04	2.10 ± 0.05
Reticular formation	1.99 ± 0.05	2.06 ± 0.06
Olivary nuclei	2.16 ± 0.04	1.95 ± 0.05*(↓)
White matter		
Anterior commisure	1.06 ± 0.04	1.10 ± 0.03
Fornix	1.03 ± 0.05	0.99 ± 0.04
Internal capsule	1.05 ± 0.03	1.08 ± 0.06

Values are mean ± S.E. expressed as a ratio of optical density of selected region to that of corpus callosum
* p < 0.05
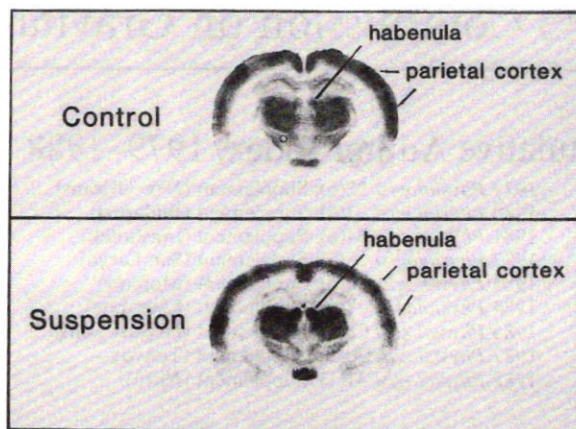
Fig. 1 [^{14}C]deoxyglucose autoradiographs of coronal section at the level of habenula and parietal cortex.

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IUPS Commission on Gravitational Physiology

Cumulative Author Index, 1979–1988

Volume no. given in parentheses, followed by page no.

American Physiological Society
Centennial Collection

The Centennial Founders Set

The Centennial Founders Set is a limited production of ceramicware commemorating the 100th anniversary of the founding of the American Physiological Society. Each piece—plate, cup, and tile—is fired in a radiant cobalt blue porcelain and etched in 23-carat gold. The face of the 10-inch plate features a reproduction in gold of the Centennial portraits of the five founders and inscribed on the back is a brief history of APS. The tile features a gold reproduction of the Centennial Seal and the cup has both the founders' portraits and Centennial Seal embossed on the sides. A Founders Plate is to be donated to the White House Collection of Commemorative Plates in Washington, D.C.

The cost of the Centennial Founders Set is $45.00. Individual pieces are priced as follows: $35.00 for the plate; $10.00 for the cup; and $6.00 for the tile.

The Centennial Coffee Mug

Centennial Coffee Mug is a replica of the Founders Cup with the Centennial Seal imprinted in white on a radiant cobalt blue mug. The cost for the coffee mug is $7.50.

The Centennial Medallion

Centennial Medallion is a 2.5-inch bronze commemorative medallion that features the sculptured faces of the five founders on the front side and the Centennial Seal on the reverse side. The cost is $25.00 for each medallion.

NASA Space Biology Program

The advent of the space age provided the first access to the "gravity-free" state and an opportunity to manipulate gravity from its norm of one down to zero. Therefore NASA has assumed the responsibility to investigate the biological significance of gravity and thereby expand biological knowledge.

Objectives

The objectives of NASA's Space Biology research program are *1*) to investigate the biological significance of gravity; *2*) to use gravity to solve relevant biological questions; and *3*) to enhance our capability to use and explore space.

Goals

The goals of the program are *1*) to enhance our knowledge of normal physiological adaptive mechanisms in both plants and animals and thereby provide new insight into both normal and pathological mechanisms; *2*) to provide for the multiple generation survival of plants and animals in space through an understanding—and ultimately control—of the affects of gravity on development, adaptation, and evolution; and *3*) to enhance plant productivity through an understanding and control of gravitational and related environmental stimuli and the manipulation of response mechanisms.

The achievement of such goals depends on answers to basic scientific questions that include the following.

1) Does gravity influence fertilization and early development and can fertilization and early development proceed normally in a near 0–G environment? If gravity does affect fertilization and early development, what are the sensitive physiological systems and how are they affected? If early development is affected by gravity, is it a result of an effect on the parent or the direct effect on the embryo itself?

2) What is the role of gravity in the formation of structural elements, such as lignin, cellulose, chitin, and bone calcium, at the molecular as well as at the more complex organizational levels?

3) What role does gravity play in calcium-mediated physiological mechanisms and in calcium metabolism?

4) What is the gravity-sensing mechanism? How does it perceive information? How is the information transmitted to evoke a response?

5) How does gravity as an environmental factor in-
teract with other environmental factors to control the physiology, morphology, and behavior of organisms? *Or* how do gravitational and other environmental stimuli interact in their control and direction of living forms? Can the action of gravity be replaced by different stimuli?

Strategy

The strategy so far has been to manipulate gravity on earth and develop weightless simulation models to develop and test gravitational hypotheses; to identify gravity-sensitive biological systems and interacting environmental response mechanisms; to address valid gravitational biological questions on earth when possible; and to plan and design future space experiments. As spaceflight opportunities, either manned or unmanned, become more prevalent, increasing emphasis will be placed on flight experiments. Similarly, as longer flight missions become available, emphasis will be directed toward biological questions that require longer periods of microgravity for adequate experimentation.
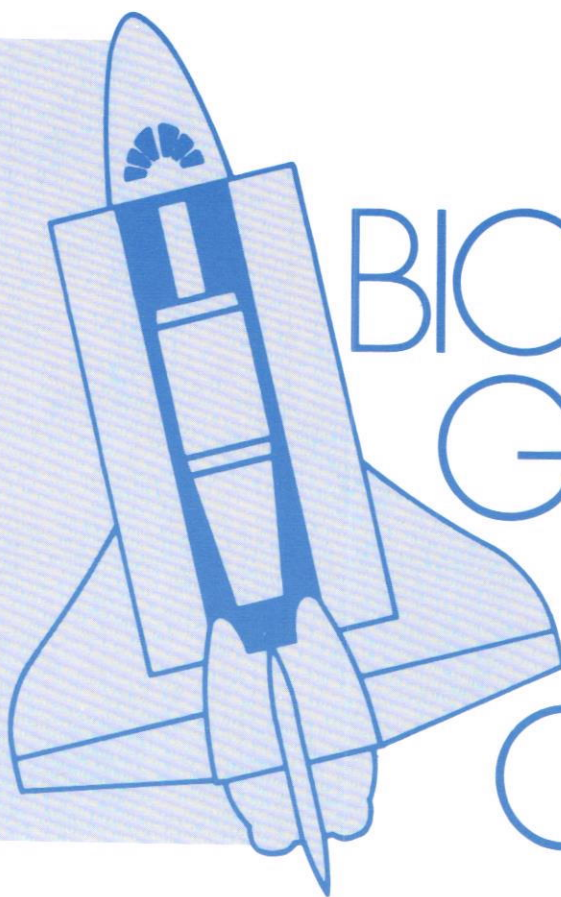
Program Content

The program has been divided into the following three broad areas: *1*) the role of gravity in reproduction, development, maturation, and evolution; *2*) gravity receptor mechanisms (these include the identification of the organ or site of gravity reception and the biological systems and mechanisms that transmit the information to a responsive site); and *3*) the physiological effects of gravity (this includes the biological mechanisms by which living systems respond and adapt to altered gravity, particularly that of the space environment, as well as the interactive affects of gravity and other stimuli and stresses on the physiology, morphology, and behavior of organisms).

This NASA program in space biology is carried out intramurally by the NASA Research Centers and by a system of extramural grants. Qualified scientists interested in learning more about the program and the development of research proposals should contact

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