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PROCEEDINGS OF THE SECOND ANNUAL MEETING

OF THE IUPS COMMISSION ON GRAVITATIONAL PHYSIOLOGY

JULY 13-19, 1980

BUDAPEST, HUNGARY

DEDICATION

These Proceedings are dedicated to Rodolfo Margaria, who has retired from the Commission during the past year. He is Professor Emeritus of the Istituto di Fisiologia Umana, University of Milano, Italy.

Professor Margaria is memorable for his many contributions to the world both in science and in international cooperation toward the preservation and expansion of human values.

A cosmopolitan in science, Margaria travelled widely, and made scientific contributions in many of the major physiology laboratories of the world. Each place he visited produced a shower of valuable contributions, with Margaria frequently a co-author.

A patriot of his native Italy, Margaria served in the resistance movement in World War II, entered politics briefly thereafter, and has continued to work for the broad development of Italian science.

A non-conformist in personal behavior, Margaria has continued to stimulate those around him by his refreshing approach to life. Oggi magazine, in which Margaria authored several popular articles on scientific subjects including gravitational physiology, published a biographical vignette "Margaria il non-conformista."

Gravitational physiology, along with other branches of physiology and science in general, has been a beneficiary of the genius of Rodolfo Margaria. On the basis of a masterly analysis of the mechanics of human locomotion, he and his colleague G. A. Cavagna were able to predict with dramatic success the difficulties of normal walking and running, as well as the efficacy of jump progression, in lunar subgravity five years before the first Astronaut landing on the Moon (R. Margaria and G. A. Cavagna, Human locomotion in subgravity, Aerospace Medicine, 35: 1140-1146, 1964).

Margaria served with distinction as an IUPS Council member and as IUPS representative to COSPAR from 1965 to 1974. He was instrumental in the formation of this Commission in 1974.

On behalf of gravitational physiologists, Grazie, Professor Margaria.

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The Second Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences was held during the XXVIII International Congress of Physiological Sciences in Budapest, Hungary, July 13-19, 1980. The activities included a two-day symposium of 24 invited papers which dealt largely with findings from experiments performed during the Joint USSR/USA Biosatellite Mission on Kosmos 1129, flown September 25-October 14, 1979. Two days of open sessions were also held in which 34 contributed papers were presented.

The following pages are presented as the Proceedings of the meeting by arrangement with the American Physiological Society. In the interests of timeliness and economy they are published without editorial review. Financial support for the preparation of the Proceedings was generously provided by the USA National Aeronautics and Space Administration. We also should like to express appreciation to the Hungarian Organizing Committee of the Congress for their kind cooperation and provision of excellent facilities for the meeting.

The Third Annual Meeting of the IUPS Commission on Gravitational Physiology will be held in Innsbruck, Austria on September 30-October 2, 1981 in conjunction with a regional meeting of the German and Austrian Physiological Societies, at their gracious invitation. A one-day symposium of invited papers is being planned, as well as open sessions for contributed papers dealing with the effects on physiological systems of humans, animals and plants of changes in magnitude or direction of the force environment. Included are the effects of the weightlessness of space flight, of acute and chronic acceleration, of vibration, and of the various forms of simulated weightlessness. Also included is consideration of the role of gravity in the manifestation of scale effects in animals and plants. A welcome to participate is extended to all interested scientists.

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REMARKS ON PRESENT STATUS OF GRAVITATIONAL PHYSIOLOGY

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This Symposium is organized as part of the Second Annual Meeting of the Commission on Gravitational Physiology. It deals with the physiological significance of gravity, perhaps the most familiar of all environmental forces. The main theme of the Symposium, which is organized in three parts is the results from the recent Cosmos 1129 joint USSR/USA spaceflight experiments.

Before turning to the problems to be discussed here, it may be of interest to give some brief historical notes concerning the role of gravitational physiology as a science and its position within the scientific activities of IUPS. Speculation on the significance of gravitation has a long history, but it was not until the nineteenth century that the appreciation of its biological effects began to develop. Early examples of recognized overt effects of gravity on organisms were the geotropism of plants, and man's circulatory responses to changes in gravitational vectors. During the first decades of our century, an impetus to research was given by the advent of high-performance aircraft. Interest in the physiological effects of increased gravitational stress developed rapidly, and human and animal centrifuge experimentation started in earnest about 50 years ago in order to investigate the physiology and pathophysiology produced by extreme force environments.

The constancy of the force of gravity on earth, and our long-standing inability to adequately simulate or produce a gravity-free environment for any significant duration, not only prevented systematic studies of gravity's influence on living matter; in fact, questions concerning the biological role of gravity in life processes rarely entered the conceptual world of biologists and medical scientists. The situation was, of course, radically changed with the advent of weightless space flight, and the newly found access to the 'gravity-free' state and the possibility of manipulation of the G factor from its norm of 1 down to zero suddenly offered rich opportunities for basic research into vital functions in organisms of varying complexity. Thus, with the new and significant extension of the G spectrum available for experimental study, gravitational physiology as a science was given a proper foundation.

Our current state of knowledge concerning the biological effects of the removal of effective gravity derives largely from experiments carried out in space over the last two decades, notably those conducted in the American Skylab and the Soviet Cosmos Biosatellite and Salyut series. With the recent development of the Shuttle/Spacelab system, Western Europe is now entering a phase in the peaceful use of space that calls for greater readiness on the part of its bioscience community to reap the potential benefits that space technology is soon to offer. Involvement of investigators not previously committed to work in the space environment is welcomed as of vital importance. New hypotheses and methods in gravitational physiology will be advanced and tested effectively only as long as the connection with the mainstream of bioscience research is preserved and developed.

As we moved into the Space Age, it seemed most important that international cooperation was maintained in related areas of the life sciences. This was the basic philosophy in the establishment of the ICSU Committee on Space Research (COSPAR). It is only natural that IUPS played a most important role in maintaining the international character of gravitational physiology. During the 1950's and 1960's, when new frontiers in space were in the offing, several symposia in space physiology were organized within the framework of IUPS congresses, and at this time weightlessness was the great unknown factor as it still continues to be in many important respects. IUPS became one of the original adhering organizations in COSPAR from its start. At this time W.O. Fenn, later Secretary-General and President of IUPS, became a great spokesman for space biology and medicine, and was the IUPS representative for the first COSPAR meetings. In the early 1970's, the emergence of gravitational physiology as a viable major discipline had proceeded sufficiently to merit consideration of the formation of a Commission by IUPS. Accord-

ingly, at the instigation of R. Margaria, N. Pace, and H. Rahn, the Commission on Gravitational Physiology was established in 1974 with H. Bjurstedt as Chairman, and O. Gauer, O. Gazenko, R. Margaria, N. Pace, H. Saiki, and A.H. Smith as Members.

The Commission now consists of 10 members representing human and mammalian physiology and disciplines concerned with the physiology of plants and with cell and developmental biology. A brief review of the areas of research opportunity encompassed by the activities of the Commission shows that of the problems that have so far aroused interest, many involve fundamental issues in physiology. Relevant topics include the effects on physiological systems of humans, animals, plants and cells of changes in magnitude or direction of the force environment, such as in acute and chronic centrifugal acceleration, in the weightlessness of space flight, and in vibration. Important basic problems are concerned with the manifestation of gravitational scale effects in animals and plants with respect to weight, shape and energy requirement. Simulation techniques are widely employed in gravitational physiology to study certain responses to earth's gravity, on one hand, and to the absence of effective gravity on the other. Thus, in the case of man's physiology, the use of lower body negative pressure often replaces the tilt table in ground-based research. Other examples are the use of long-term bed rest and immobilization, water immersion, and lower body positive pressure to study certain cardiovascular and metabolic responses to weightlessness by sophisticated methods not readily available or applicable in current space laboratories.

In the following, I will restrict myself to just a few basic questions concerning the physiological role of gravity which may aid in evaluating the papers of this Symposium and place them in perspective. It should be made clear, that the selection and order of these questions have no bearing on their relative scientific significance.

Can man truly adapt to a change in the force of gravity? Chronic exposure of man to the weightless environment makes it possible to search for and analyze factors which are normally masked in earth's gravitational field. In the case of the circulation, the observation of a massive shift of blood volume into the upper regions of the low-pressure system raises the question, not yet satisfactorily answered, whether chronic engorgement of the thorax and heart may, perhaps also by reflex actions on renal, electrolyte and endocrine functions, eventually lead to irreversible pathophysiological changes. The suspicion that a true adaptation to long-term weightlessness cannot be attained is reflected in futuristic notions of revolving space stations in which centrifugal force replaces earth's gravity, wholly or in part. Continued studies are needed to reveal the nature, sequence and interdependence of changes in cardiovascular, renal and electrolyte, endocrine and sensory functions. Although ground-based simulation models must be fully exploited, essential aspects require sequential measurements with sophisticated techniques in the space environment. The results of such studies in man as well as in carefully selected animals will enhance our understanding of the physiology of health and disease on earth.

What is the nature of the disturbances in bone and mineral metabolism that occur in prolonged exposure to weightlessness? It has long been recognized that such exposure produced continuous loss of bone calcium; thus, demineralization and associated bone brittleness represents a threat to the conduction of extended space missions. Attempts to alleviate the problem by dietary measures have so far not been successful. How does gravity intervene in the development of bone, tendon and muscle tissue? Mechanical stress is known to strongly affect the orientation of bone tissue which gains or loses substance according to direction of the stress. In every-day life on earth, gravity normally provides the major portion of the mechanical stimulus responsible for the development of bone tissue. In the weightless environment, loss of calcium, reduced rate of bone growth and increased resorption may be related to bone atrophy due to disuse; on the other hand, even a rigorous schedule of muscular exercise does not retard calcium excretion significantly. Furthermore, mineral deficit has been observed in mammalian skeletal elements that are not of the weight-bearing type. Continued research is required to study the extent to which disturbances in intestinal calcium absorption affects the overall picture of calcium metabolism in weightlessness. On the cellular level, the availability of in vitro cultures of isolated bone and skeletal muscle cells will continue to provide a possibility for analyzing in detail how bone and muscle formation, destruction and regeneration are affected by the weightless environment.

How does the absence of effective gravity affect the development of gravity-sensing organs and mechanisms? These are at the apex of a hierarchy of neurones that control balance, posture and locomotion. The problem requires continued study in e.g. small mammals reared in the zero-G environment from the time of conception. If disturbances are observed on return to earth, will these be mainly functional or will there also be defects in the neuro-

anatomical development? If postnatal malfunction should occur, the animal may, or may not, be able to learn by experience the appropriate movement patterns, locomotor orientation, etc., which in turn might shed some new light on the old issue of heredity versus learning.

Gravity influences the design (weight, shape and energy requirement) of terrestrial animals, organic form and metabolism being adapted to body size. What is the relative importance of gravity and genetic factors in such adaptations? By removing animals and plants from the gravitational stimulus, it has now become possible to study the role of gravity in the manifestation of scale effects, e.g. in the relative mass of various body components.

To what extent and how does gravity affect the development, function and growth of cells? A presumed gravity-sensitive property is reflected in the degree of polarity of the cell. The spatial organization of constituents in plant cells is a classical example of gravity-sensitive polarity. Recent developments in methodology with regard to the maintenance and growth of embryonic and fetal tissues in artificial environments have made the very early stages of mammalian development accessible to direct observation and experimentation. Of special interest is the possibility that gravitational forces might modify the morphogenetic pattern in its earliest and most fundamental manifestations, such as polarity and bilateral symmetry. Gravity may be necessary for normal function in certain specialized cells. An example is the lymphocytes which are responsible for the immune response. There are indications that lymphocytes show lower reactivity toward mitogens after exposure to weightlessness, or other space-flight factors, and continued in vitro experiments in space are therefore required to study this problem and to evaluate its significance for man in space.

ASPECTS OF CARDIOVASCULAR ADAPTATION TO GRAVITATIONAL STRESSES

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INTRODUCTION

Most of the literature concerning gravitational stresses in man describes the conditions during rest. In our laboratory we, therefore, thought it of interest to investigate the conditions of gravitational stresses during exercise, both dynamic and static.

MATERIAL AND METHODS

Only healthy young male subjects were investigated. In a preliminary study we compared the use of freon and acetylene simultaneously as the soluble inert gas in a rebreathing technique for measuring cardiac output (CO) during rest and exercise (Triebwasser et al 1977, Bonde-Petersen et al 1980) Argon (Ar) was used in the rebreathing system to track incomplete mixing and system volume (lung-plus rebreathing bag volume). We concluded that freon can replace acetylene as the soluble gas, and that the rebreathing method gives reliable results provided that the subject is not hyperventilating. Especially during low values for CO, as during rest and light exercise, it was demonstrated that hyperventilation during the rebreathing period caused a mean increase in CO of up to 50%. We recommend that a spontaneous rebreathing rate be used. Another aspect of this study was that it makes possible studies of CO during space flights, because freon, unlike acetylene, is non-explosive and non-toxic. Further, freon is already applied in space crafts as a cooling liquid.

The following parameters were measured in the studies reported below: CO using either acetylene or freon-22 as the inert soluble gas and Ar as the inert non-soluble gas. The rebreathing bag was continually analyzed for gas fractions on a mass spectrometer (Centronic MGA200). Further we measured mean arterial blood pressure (MAP, arm cuff), heart rate (HR, ECG), total peripheral resistance (TPR=MAP/CO), forearm blood flow (FBF, venous occlusion plethysmography), forearm vascular resistance (FVR=MAP/FBF), skin blood flow (133-Xe clearance), systolic time intervals (STI), and blood volume (RHISA 131-J). Blood samples were further drawn for analyses of Hct, Hb, proteins and colloid osmotic pressure. Inulin space was measured during 3-H-inulin infusion and muscle biopsies obtained for measurements of intra-and extra cellular water space according to Sjøgaard (1979). Dynamic exercise was performed on either of two frictionally braked bicycle ergometers (Monarch, Sweden) one of which was adapted to under water exercise. Oxygen consumption rate was measured during steady state exercise to make possible a comparison between the different conditions under which the subjects exercised. A level of 2 litres of O_2/min was chosen. When isometric exercise was performed a strain-gauge dynamometer for handgrip was used, and the strength of contraction was held by the subject by visual feed back on a level of 40% maximal voluntary contraction (MVC).

PROCEDURES

A tilt table was used for short term (2h) and a tilt bed for long term (6h) investigations during either head up (+5 and +25°) horizontal (0°) or head down (-5 and -25°) bedrest (BR). Increased above 1-g conditions were simulated by lower body negative pressure (LBNP), and low gravity by -5° BR. For comparison also 0° and +5° BR were performed as well as the steeper inclinations of 25°. Low gravity was also simulated by lower body positive pressure (LBPP, pressure suit) or water immersion. Heat load was induced in an environmental chamber. During water immersion the temperature was kept thermoneutral at 35°C, and for comparison exercise was also performed having the subjects waring a water perfused garment kept at 35°C.

In the present review of the different activities in our laboratory over the past two years some of the results from 5 protocols (I-V) shall be mentioned briefly followed by a short discussion.

Protocol I: Six hours head out water immersion (HOWI), +5° BR and -5° BR were compared in 4 subjects. After an overnight's sleep at the laboratory basal values were measured between 7 and 9 AM. After a short period of ambulation and a light breakfast the subjects were submitted to either of the three experimental conditions.

Protocol II: Local regulation of subcutaneous blood flow in the forearm was studied at the wrist by the local 133-Xe wash

-out technique (Kety 1949, Lassen et al 1964). The local response to increased or decreased transmural pressure (arm elevated above or lowered below heart level in the supine position) was studied in 7 subjects with and without LBNP.

Protocol III: Bicycle exercise in the upright position was studied in 8 subjects working at 50% of their maximal oxygen consumption rate or at 2-2.2 l/min. The conditions were: 25°C air, 45°C air, 35°C water perfused garment (suit) and 35°C water immersion to the xiphoid level (XLWI).

Protocol IV: Isometric exercise (hand grip for 2 min at 40% MVC) was performed after 2 h adaptation to XLWI, and after 20 min adaptation to either 0°, +25° or -25° BR in 5 subjects.

Protocol V: Intermittent bicycle exercise (12 min exercise, 8 min rest periods) was performed by 3 subjects in the reclining position at 55% $\dot{V}_{O_2}$ max. The experiment was continued for 120 min either with the circulation free (control) or with the circulation intermittently occluded during the 8 min rest periods. These results were compared with the condition when a pressure suit inflated to 40 mmHg was decreasing the pressure head in both legs during the entire 120 min experimental run.

RESULTS

Protocol I: During the first hour there was a tendency for CO during HOWI and -5° BR to be increased above that at +5° and the basal values (Fig. 1) but after 2 h basal values were reached. Lung tissue volume (LTV) calculated by extrapolation to time zero for the clearance curves during rebreathing was at first increased during -5° BR and HOWI but also recovered during the first 2 hours. However, as expected, HOWI had the greater effect on LTV (Fig. 2). No significant differences were found in MAP between the three conditions but a slight increase from ca 95 to 100-105 mmHg was seen. The most striking difference was observed in FBF and FVR. During HOWI FBF immediately went up from 20 to 40-60 ml/min /l paralleled by a decrease in FVR. During

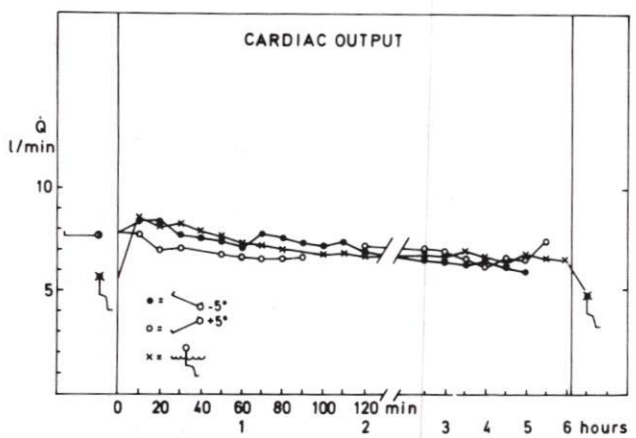

Fig.1. Cardiac output from protocol I

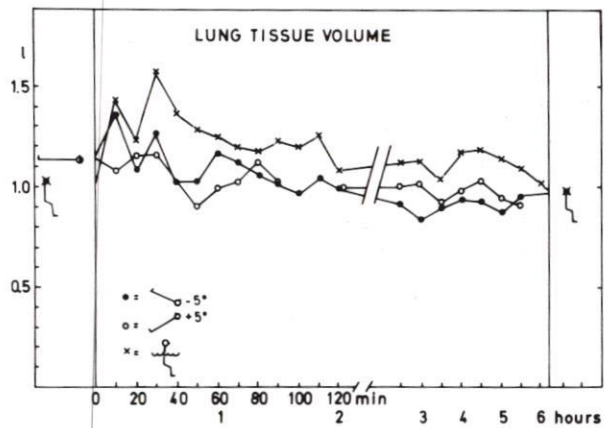

Fig. 2. Lung tissue volumes, protocol I

-5° BR the same values were gradually obtained after 6 hours, while +5° had no effect upon FBF or FVR. Volume of legs (8.1 litre below the knees) decreased by 3.6% and 0.7% after HOWI and -5° BR respectively and increased by 0.8% after +5° BR. These volume changes were probably due to changes in skin volume, because no changes in intra-or extra cellular muscle water was seen (Sjøgaard and Bonde-Petersen 1980)

Protocol II: LBNP induced a decrease in skin blood flow which could be blocked by proximal nervous blockade (Skagen et al 1980). Lowering the arm decreased flow and increased resistance (Fig. 3 first columns) but this reflex (Henriksen 1977) was absent during LBNP (Fig 3 second columns) but reappeared if a proximal conduction anaesthesia was applied (Fig. 3 third set of columns). Even if LBNP lowered skin blood flow

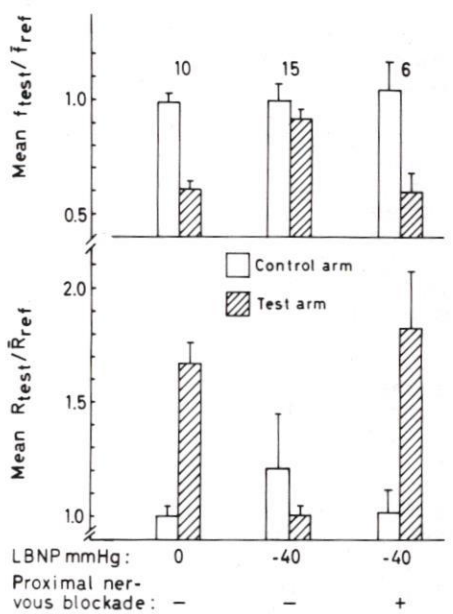

Fig. 3. Protocol II, relative changes of mean skin flows (f) and resistance (r) during lowering of test arm

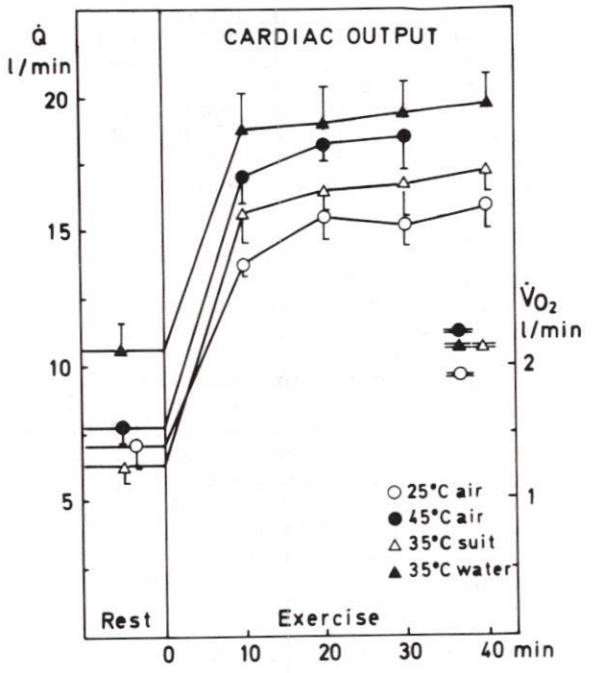

Fig 4. Cardiac output and $\dot{V}_{O_2}$, protocol III

it was still possible to demonstrate that autoregulation existed during arm elevation.

Protocol III: During exercise CO was highest in XLWI and even higher than 45°C air (Fig. 4). Increasing temperature from 25 to 45°C in air, and increasing central venous filling pressure by water immersion at 35°C skin temperature produced similar increments. Stroke volume was highest in XLWI and lowest during heat stress (Fig. 5). FBF increased to higher levels at end of exercise during XLWI (from 65 at rest to 250 ml/min/l at 40 min) while FBF levelled off during heat stress (at 190 ml/min/l).

Protocol IV: Isometric sustained handgrip for 2 min has a profound effect upon the cardiovascular system: CO, HR, TPR and MAP increase while SV decreases. Locally in the forearm, however, FBF increases due to a decreased FVR. Only the magnitude and not the direction of these changes were affected by the tilt and immersion conditions applied. If anything, the changes during XLWI were the most pronounced in absolute terms, but expressed in per cent changes the greatest increments in CO, FBF and FVR were seen during isometric exercise during +25°BR.

Protocol V: Increments in MAP were highest and in FBF lowest during exercise with LBPP, while occluded recovery induced the reverse effects (peripheral vasodilatation enhanced during exercise). During the recovery periods MAP was increased in face of a pronounced decrease in FVR probably because the dilated vascular beds in the legs were excluded from the circulation. CO tended to recover more slowly during occluded recovery. $\dot{V}_{O_2}$ was higher du-

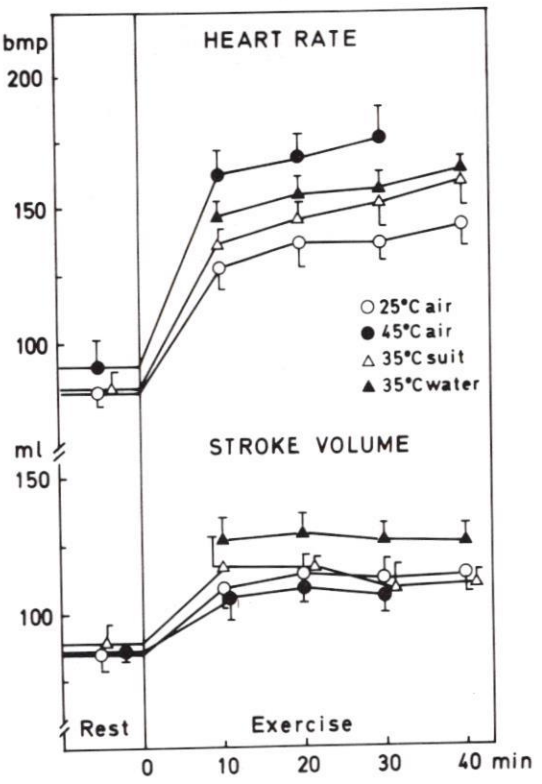

Fig 5. Heart rates and stroke volumes in protocol III

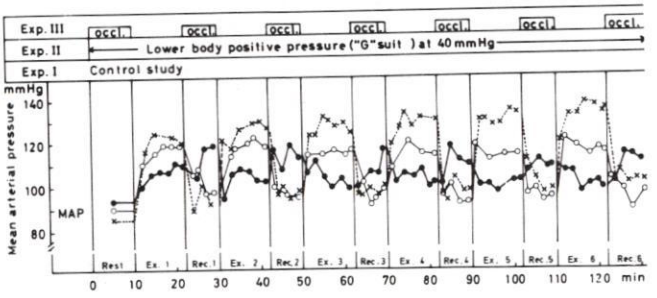

Fig. 6. MAP during intermittent exercise. o=Experiment I, control study; x=exp. II, LBPP; ●=exp. III, occluded recovery.

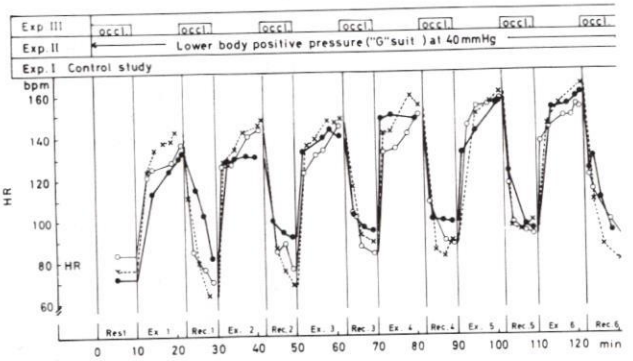

Fig 7. Heart rate in protocol V. See Fig 6

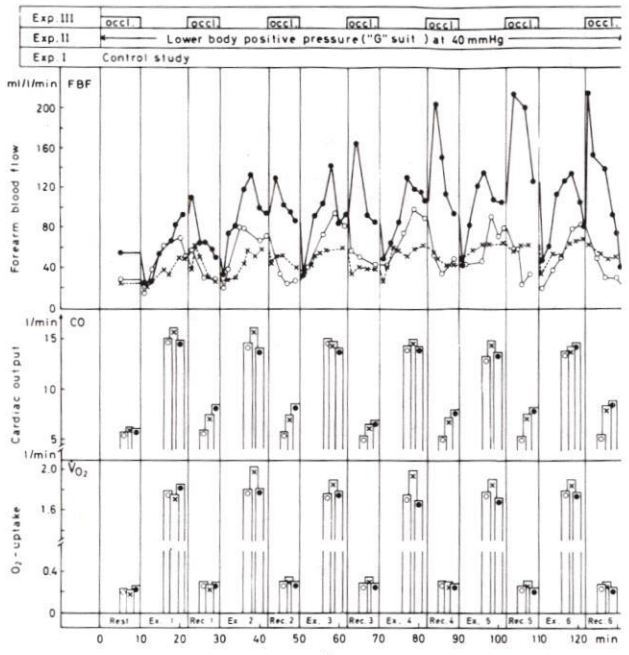

Fig. 8. FBF, CO and $\dot{V}_{O_2}$, protocol V
Results from one subject as in Fig 6-7.

ring LBPP, explained by the resistance to leg movements produced by the pressure suit (Fig. 6-8). HR was similar during exercise but higher during occluded recovery (Fig 7) and showed an over all increase of 20 beats with time. The changes seen in STI followed the changes in HR, and no systematic difference was seen with time nor experimental condition indicating that the contractility of the heart was unaffected by fatigue or LBPP.

DISCUSSION

As pointed out by Gauer (cf. Gauer and Henry 1977) the increased central venous pressure (CVP) during water immersion elicits a blood volume decreasing reflex via ADH. Our blood analyses of Hct and Hb supported that a decrease in plasma volume took place during the 6 h studies. The adaptation of CO, which first was increased by up to 60%, was more rapid as CO was partly recovering during the first 2 h. However, the decrease in FVR, which was present immediately at the onset of HOWI, was persistent. Is this due to a reflex action from the low pressure baroreceptors (Bevegård et al 1980) or would the increased pulse pressure (CO and SV increased) affect aortic baroreceptors to the same effect?

During exercise, static and dynamic XLWI produced greater changes than during the control situation. This might be a pure mechanical effect of the increased CO and SV and thus explained on a purely passive basis, but also here baroreceptor activity (high-or low pressure) might play a part. It was interesting that the levelling off of FBF during heat stress with increasing oesophageal temperature (which was also measured in experiment III)was not seen during XLWI, indicating that pooling of blood in the lower parts of the body is responsible for the circulatory collaps seen during heat stress. This is prevented by the counteraction of hydrostatic forces during water immersion and the increased CVP, and would be expected also to occur during low gravity conditions.

Also local hydrostatic forces can elicit vascular reflexes, which, however, can be dominated by the general sympathetic nervous activity, when this is increased during LBNP (protocol II).

Gravity increases the pressure head to exercising legs. When this pressure head is decreased (protocol V) muscle blood flow is also decreased as indicated by an increase in blood lactate concentration, an effect also to be expected in low gravity.

At present the relative importance of these factors acting in concert during low gravity is not clear but constitute an obvious field for future investigations in this laboratory.

For a more detailled discussion of the present results the reader is referred to articles to be published from this laboratory. Reprints will be mailed on request as they appear.

Acknowledgement.
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REFERENCES

Bonde-Petersen, F., P. Norsk and Y. Suzuki, Abstr. Scand Physiol Soc April 1980, D30.
Bevegård, S. et al. Abstr. ICPS,July,1980.
Gauer, O.H. and J.P. Henry, Internat Rev Cardiovasc Physiol (Guyton and Cowley eds) II,Vol 9, 1976.
Henriksen, O., Acta Physiol Scand Suppl 450, 1977.
Kety, S.S., Amer Heart J 38:321.1949.
Lassen et al. Lancet i.686.1964.
Sjøgaard, G., Thesis. University of Copenhagen 1979.
Sjøgaard, G. and F. Bonde-Petersen, Abstr. ICPS, 1980.
Skagen, K., O. Henriksen and F. Bonde-Petersen, Acta Physiol Scand (in print).1980.
Triebwasser et al, Aviat Space Environ Med 48:203.1977.

ADAPTATION TO WEIGHTLESSNESS AND ITS PHYSIOLOGICAL MECHANISMS (RESULTS OF ANIMAL EXPERIMENTS ABOARD BIOSATELLITES)

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The aims of experiments on mammals aboard Soviet biological satellites Cosmos-605, 690, 782, 936 and 1129 were to investigate patterns of adaptation of higher animals to the weightless state, to study physiological and morphobiochemical characteristics of organs and tissues of weightless animals, and to explore their readaptation to Earth gravity after exposure to zero-g.

The experiments were carried out on male Wistar rats from the Stolbovaya colony (Cosmos-605 and 690) and male specific pathogen free (SPF) Wistar rats from the colony of the Institute of Endocrinology, Slovak Academy of Sciences (Cosmos-782, 936 and 1129).

The animals flown aboard Cosmos-605 and 690 were examined 1-2 days post-flight, and those flown aboard Cosmos-782, 936 and 1129 as soon as possible after recovery (5-11 hours), in order to discriminate the changes that developed inflight from those that could occur upon return to Earth (10, 15).

The results of these examinations gave evidence that various adaptive reactions developed during space flights of 18-22 days. Concurrently, the exposure caused a number of distinct structural and metabolic changes, including specific changes associated in a different degree with weightlessness effects and nonspecific changes developing as a stress-reaction. Those latter involved changes in the hypothalamus-hypophysis-adrenal system, lymphoid organs and some others.

Histological, histochemical and biochemical examinations of the hypothalamus, hypophysis and adrenals demonstrated changes suggesting moderate activation of their function (13, 21, 28, 35, 36, 41). The studies also revealed weight losses of lymphoid organs (14) and inhibition of protein synthesis in the thymus and spleen (23, 29).

In addition, manifestations of a stress-reaction included biochemical and morphological changes in blood (increase in the corticosterone and glucose concentration in plasma, change in the lipid pattern, lymphopenia, eosinopenia, neutrophilia), myocardium (increase in the concentration of catecholamines and activity of phosphorylases A and B), and gastrointestinal tract (alteration in the content and localization of mucopolysaccharides in the mucosa) (1, 20, 22, 27, 37, 39).

However, macroscopic and histological examinations of stomachs of weightless animals did not reveal ulcerations or erosions of the mucosa (3). This strengthens the contention that the space flight-induced stress-reaction remains at a moderate level.

Specific manifestations of weightlessness effects embraced, first of all, changes in the musculo-skeletal system. Histological and biochemical investigations of skeletal muscles and studies of their contractile properties showed that muscles developed both atrophic and adaptive changes. The pattern of muscle reaction depended at large on functional specialization; the greatest changes occurred, therefore, in the muscles involved most actively in antigravitational activity. For instance, histological examinations of the soleus muscle showed changes in practically all structural elements of myofibers suggestive of atrophic developments (2, 9, 18). Biochemical studies demonstrated a decrease in the content of myofibrillar and sarcoplasmatic proteins in the soleus and a lack of such changes in other muscles, where a few atrophic shifts were, however, seen (5, 7). No changes in the glycogen content or in the activity of glycogen phosphorylase, an important enzyme involved in glycogenolysis, took place in skeletal muscles, including the soleus. Activities of adenylate cyclase and phosphodiesterase, mediating hormonal control of glycogenolysis, also remained unaltered (30).

Of great interest was the cardiac-to-intermediate (or muscular) type transformation in the ratio of lactate dehydrogenase isoenzymes of the soleus muscle, shown histochemically and biochemically (7, 32).

The dependence of the level of changes upon the antigravitational role of a given

muscle was also demonstrated by the study of contractile properties of whole muscles in vitro and glycerinated myofibers.

Whole muscles displayed a selective acceleration of the soleus as well as a decrease in the strength, elasticity and tolerance to fatigue of the soleus and the extensor digitorum longus, although the degree of changes in the latter case was significantly lower. Glycerinated myofibers of the soleus muscle also exhibited a decline in the contraction strength and work capacity. In the extensor digitorum longus muscle these changes were statistically insignificant (45).

A more marked decline of the strength of the soleus muscle and an acceleration of its contraction were most likely associated not only with tissue losses but also with a reduction of mechanical efficiency of individual myofibers that could be induced by changes in the composition and properties of muscle proteins under the influence of weightlessness (31).

These data are in good agreement with the study of muscular activity of intact rats: postflight the animals showed a lowered tolerance to static loads measured as the time lapse during which they could hold on to the pole (26).

The structural, functional and metabolic changes that developed in the muscle system under the influence of space flight factors were adaptive, being manifestations of animal adaptation to weightlessness. The changes proved reversible in flights of 22 days in duration. On R + 25 the weight of the soleus muscle of flight animals was identical to the control. Histological examinations of muscles on R + 25 did not reveal any changes either, except for small foci of reparation which evolved in the sites of muscle fiber death. It cannot however be ruled out that after longer-term flights readaptation of the muscle system to 1 g would not proceed uneventfully.

In bones, as in muscles, the most appreciable changes developed in those of them that carry the largest loads on the Earth, particularly in femoral bones.

The inflight study of bones with the aid of a pre-injected label (declomycin) showed a decrease in periosteal bone formation in the diaphysial area, a reduction of apposition growth, and a delay in osteoid maturation and bone mineralization (42).

Postflight study of skeletal bones by histological methods showed osteoporosis of spongy compartments, noticeable loosening of the spongiosa of metaphyses of the femur, and reduction of primary spongiosa in the zone adjacent to the cartilage growth plate of the metaphysial area (43). The studies also revealed a decline in bone mechanical strength (38). Biochemical investigations demonstrated redistribution of calcium in bones of extremities and the skull and of other minerals in epiphyses and diaphyses of limb bones (34).

It should be noted that certain changes in the musculo-skeletal system of rats induced by weightlessness are similar to those observed in hypokinetic and hypodynamic experiments. The similarity of these effects seems to be dictated by a common component involved in the above exposures, i.e. functional underloading of the musculo-skeletal system.

The above signs of adaptive transformation of contractile properties and metabolism of skeletal muscles as well as changes in limb bones in the weightless state appear to reflect changes occurring in certain compartments of the central nervous system associated with "excitation deficiency" and alteration of reflex-trophic relations in the neuro-muscular system (33). For instance, biochemical manifestations of a diminished function of motoneurons and large neurons of spinal cord ganglia at the level of the lumbar enlargement were noted (12). A prolonged exposure of animals to space flight caused a slight inhibition of metabolism in the brain, especially in those brain compartments whose function is associated with motor activity, i.e. in the cerebellum and sensorimotor zone of the cortex of large hemispheres. A 15-20% decrease in the absolute content of RNA and a normal content of proteins were seen in Purkinje cells; this suggests a decline of the rate of protein metabolism in the above structures (8).

Among weightlessness-sensitive organs those of equilibrium should be mentioned. Both manned and animal space flights furnished information on changes in the vestibular function and deterioration of the equilibrium function (26).

Histological and histochemical examinations of the rat organ of equilibrium showed changes that can be related, on the one hand, to disturbances calcium metabolism within the otolith - otolith membrane system and, on the other, to local changes in circulation and outflow of endo- and perilymph (4).

Functional underloading in the weightless state may be responsible not only for the above changes in the musculo-skeletal system but also for disorders in the erythrocyte system and the myocardium.

The rats flown aboard biosatellites displayed a decrease in erythropoiesis in bone marrow and spleen, a reduction of red

cell survival time, and a significant increase of spontaneous hemolysis in vivo. However, hemoglobin, erythrocyte, reticulocyte counts and hematokrits postflight did not essentially differ from the controls (14, 37, 44, 47). It is very likely that the mechanism of these alterations is not associated with a decrease of energy requirements, as was assumed previously (25), since biosatellite experiments revealed a trend for increased oxygen consumption and diet utilization (11, 24).

Histological examinations of the myocardium did not show any structural changes in the contractile and mitochondrial systems of muscle cells. Cytochemical studies demonstrated an increase in the activity of phosphorylases A and B (20). Biochemical investigations carried out after every biosatellite flight indicated a reduction -44% on the average- of ATPase activity of myosin and essentially no changes in the fractional composition of proteins (6, 40).

The decrease in myosin ATPase activity may be a result of the underloading of the cardiac muscle - an adaptive reaction to a "facilitated" workload. Since half-life of myocardial myosin is 6-8 days, it can be assumed that due to the underloading a new myosin with a lower ATPase activity is synthesized in weightlessness. In this situation return to Earth may provoke disparities between the required force of myocardial contraction and the potential to meet this need, provided that myosin ATPase activity is lowered (6).

The changes noted in fluid-electrolyte metabolism postflight included a slight reduction of water and electrolytes. However, in spite of potassium deficiency, its retention during potassium loading tests lowered as a consequence of a reduced potassium pool potential, probably due to a decrease of muscle mass (16, 17, 19). Postflight the weight of kidneys increased (46); they showed signs of tissue hydration and no important changes in the structure of nephrons (17).

Thus, biosatellite experiments demonstrated certain patterns of adaptation of higher animals to the weightless state. It was found, among other things, that a relatively prolonged exposure to weightlessness (making up approximately 1/50 of the animal lifetime) did not cause pathological changes in the structure of any of the organs tested. The abovementioned changes in the hypothalamus-hypophysis-adrenal system, muscles, bones, myocardium and other organs proved reversible. The parameters measured almost returned to normal 25 days postflight.

As known, the biosatellite Cosmos-936 afforded a unique opportunity to perform a comparative study of effects of weightlessness and artificial gravity of 1 g upon mammals. The study showed, first of all, the normalizing effect of artificial gravity on the function of the myocardium, musculo-skeletal system and excretory system; second, it gave support to our notion that the absence of gravity plays the major role in the genesis of the above changes in various bodily systems (10). At the same time, deleterious effects of artificial gravity, particularly on the functions performed through interactions of sensory systems (optic, vestibular, motor) may be associated with engineering inadequacies of the onboard centrifuge (a short arm and a high velocity of rotation). This is undoubtedly very important for the development of countermeasures to be used in future prolonged space missions.

The animal studies aboard biosatellite furnished detailed information about structural and metabolic changes in muscles and bones. These changes were similar to those seen in ground-based simulation experiments (hypokinesia and hypodynamia); this holds good promise that the changes can be reversed with the aid of specially developed exercises. From the practical point of view, it is important that the level of changes in various muscles and bones was different and closely correlated with the degree of their involvement in the antigravitational function on the Earth. This gives impetus to differential approaches to the prevention of changes occurring in different compartments of the musculo-skeletal system.

In conclusion, the studies aboard biosatellites are of great importance. They can be considered as a necessary foundation for the physiological knowledge formed through our joint efforts in new fields of science - gravitational biology and space medicine. On the basis of the data obtained we are already able to develop new criteria and methods of functional diagnostics for medical support of manned space flights and to formulate perspective lines of research in the future. Thus, we may hope that with further accumulation of our knowledge yielded by studies aboard biological satellites their theoretical potential and practical efficiency will be increasing.

REFERENCES

1. Alers I., Tigranyan R.A., Alersova E., Paulikova E., Prslicka M. 1976. IX Symp. Space Biol. Med., Budapest, 96.

2. Baranski S., Baranska W., Rimasewska-Karsakowska T., Sliwinski A. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 85.

3. Brown P., Vernikos-Danellis J. 1979. In: Effect of Dynamic Factors of Spaceflight on the Human Body. Moscow, Nauka, 217.

4. Vinnikov Ya.A., Gazenko O.G., Titova L.K., Bronstein A.A., Gowardovsky V.I., Pevzner R.A., Gribakin F.G., Aronova M.Z., Kharkevich T.A., Tsirulis T.P., Pyatkina G.A., Lychakov D.V., Palmbach L.R., Anichin V.F. 1978. Arch. Anat. Histol. Embryol., 74, 1,22.

5. Gaevskaya M.S., Ushakov A.S., Belitskaya R.A., Veresotskaya N.A., Vlasova T.P., Kolchina E.V., Kolganova N.S., Kurkina L.M., Nosova E.A., Rassolova N.P. 1976. Kosm. Biol. Aviakosm. Med., 10, 4, 25.

6. Gaevskaya M.S., Kolchina E.V., Nosova E.A., Kolganova N.S., Versotskaya N.A. 1978. Kosm. Biol. Aviakosm. Med., 12, 5, 12.

7. Gaevskaya M.S., Veresotskaya N.A., Kolganova N.S., Kolchina E.V., Kurkina L.M., Nosova E.A. 1979. Kosm. Biol. Aviakosm Med., 13, 1, 16.

8. Gazenko O.G., Dyomin N.N., Panov A.N., Rubinskaya N.L., Tigranyan R.A. 1976. Kosm Biol. Aviakosm. Med., 10, 4, 14.

9. Gazenko O.G., Genin A.M., Ilyin E.A., Portugalov V.V., Serova L.V., Tigranyan R.A. 1978. Kosm. Biol. Aviakosm. Med., 12, 6, 43.

10. Gazenko O.G., Ilyin E.A., Genin A.M., Kotovskaya A.R., Korolkov V.I., Tigranyan R.A., Portugalov V.V. 1980. Kosm. Biol. Aviakosm. Med., 2,22.

11. Golov V.K., Ilyin E.A. 1977. X Symp. Space Biol. Med., Sukhumi, 15.

12. Gorbunova A.V., Portugalov V.V. 1977. Bull. Exp. Biol. Med., 84, 8, 168.

13. Grindeland R., Keil L., Ellis S., Parlow A., Kendall J., Gaudette M., Geschwind I. 1979. In: Effect of Dynamic Factors of Spaceflight in the Animal Body, Moscow, Nauka, 74.

14. Durnova G.N., Kaplansky A.S., Portugalov V.V. 1977. Kosm. Biol. Aviakosm. Med., 11, 2, 53.

15. Ilyin E.A., Serova L.V., Noskin A.D. 1976. Kosm. Biol. Aviakosm. Med., 10, 3, 9.

16. Ilyin E.A., Korolkov V.I., Ilyushko N.A., Kondratyev Yu. I., Kondratyeva V.A., Zenin V.F. 1978. XI Symp. Space Biol. Med., Prague, 55.

17. Ilyin E.A., Natochin Yu. V., Ilyushko N.A., Kondratyev Yu.I., Bakteeva V.T., Gazhala E.M., Goncharevskaya O.A., Lavrova E.A., Shakhmatova E.I. 1980. Kosm. Biol. Aviakosm. Med., 3, 21.

18. Inlyina-Kakueva E.I., Portugalov V.V., Krivenkova N.P. 1977. Kosm. Biol. Aviakosm. Med., 11, 1, 20.

19. Ilyushko N.A., Kondratyev Yu.I., Besedina E.G. 1977. X Symp. Space Biol. Med., Sukhumi, 28.

20. Kaplansky A.S., Rokhlenko K.D., Mulkiyarov P.E., Petrova N.V. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 126.

21. Kvetnansky R., Tigranyan R.A., Torda T., Rapcackova L., Jagnova E., Babusikova D. 1976. IX Symp. Space Med., Budapest, 100.

22. Kvetnansky R., Tigranyan R.A., Torda T., Genin A.M., Macho L. 1978. XI Symp. Space Biol. Med., Prague, 59.

23. Komolova G.S., Guseinov F.T., Makeeva V.F., Egorov I.A., Tigranyan R.A., Serova L.Vl. 1977. Kosm. Biol. Aviakosm. Med., 11, 4, 17.

24. Kondratyev Yu.I., Ilyushko N.A., Besedina E.G. 1979. In: Effect of Dynamic Spaceflight on the Animal Body, Moscow, Nauka, 21.

25. Korzhuev P.A. 1971. Evolution, Gravity, Weightlessness. Moscow.

26. Kotovskaya A.R., Aizikov G.S., Shipov A.A., Markin A.S., Mokrousova A.V. 1978. XI Symp. Space Biol. Med., Prague, 68.

27. Loginov A.S., Aruin L.I., Smotrova I.A., Ulyanova V.V., Gorodinskaya V.S., Yakovleva V.I. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 208.

28. Macho L., Tigranyan R.A., Kalita N.F., Knopp J., Strbak W. 1978. IX Symp. Space Biol. Med., Prague, 81.

29. Misurova E., Tigranyan R.A., Kropacheva K., Praslicka M. 1976. IX Symp. Space Biol. Med., Budapest, 103.

30. Nesterov V.P., Zheludkova Z.P., Kuznetsova L.A. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 114.

31. Oganesyan S.S. 1978. Biol. J. Armenia, 31, 7, 661.

32. Petrova N.V., Portugalov V.V. 1977. Kosm. Biol. Aviakosm. Med., 11, 5, 66.

33. Portugalov V.V. 1978. Izv. AN SSSR, Ser. Biol., 4, 501.

34. Prodhonchukov A.A., Tigranyan R.A., Kolesnik N.G., Novikov L.L., Timofeeva N.G., Zhizhina N.A., Volozhin A.I., Neustroev G.V., Matvienko V.P. 1977. Kosm. Biol. Aviakosm. Med., 11, 1, 26.

35. Savina E.A. 1975. VIII Symp. Space Biol. Med., Varna, 101; 1977. X Symp. Space Biol. Med., Sukhumi, 58.

36. Savina E.A., Pankova E.S., Alekseev E.I. 1977. Kosm. Biol. Aviakosm. Med., 11, 5, 72.

37. Serova L.V., Chelnaya N.A., Britvan I.I., Chivkunov Yu. P. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 17.

38. Stupakov G.P., Korolev V.V. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 174.

39. Tigranyan R.A., Popova I.A., Belyakova M.I., Kalita N.F., Sochilina L.B., Tuzova E.G. 1976. IX Symp. Space Biol. Med., Budapest, 83.

40. Tigranyan R.A., Nosova E.A., Belyakova M.I., Veresotskaya N.A., Kolganova N.S., Kolchina E.V. 1978. XI Symp. Space Biol. Med., Prague, 132.

41. Torda T., Kvetnansky R., Chulman J., Murgas K., Tigranyan R.A. 1978. XI Symp. Space Biol. Med., Prague, 135.

42. Holton E., Baylink D. 1979. In: Effect of Dynamic Factors of Spaceflight on the Animal Body, Moscow, Nauka, 148.

43. Yagodovsky V.S., Triftanidi L.A., Gorokhova G.P. 1977. Kosm. Biol. Aviakosm. Med., 11, 1, 14.

44. Leon H.A., Serova L.V., Cummins J., Landaw S.A. 1978. Aviat. Space Environ. Med., 49, 65.

45. Oganov V.S., Potapov An.N. 1976. In: Life Sci. Space Res., XIV, Berlin, Akademie Verlag, 137.

46. Savina E.A., Pankova A.S., Alekseev E.I., Podymov V.K. 1976. Aviat. Space Environ. Med., 47, 853.

47. Shvets V.N., Portugalov V.V. 1976. Aviat. Space Environ. Med., 47, 746.

PHYSIOLOGICAL MECHANISMS OF ADAPTATION OF RAT SKELETAL MUSCLES TO WEIGHTLESSNESS AND SIMILAR FUNCTIONAL REQUIREMENTS

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INTRODUCTION

Physiological activities of mammals in the Newtonian World (33) manifest primarily as movement, spatial displacement or postural maintenance in the gravity field. Therefore, it can be postulated that the Earth field of gravity has played a part in the evolutionary development of neuromuscular and motor systems of living beings, particularly of mammals (26,34). In view of this, it can be expected that in the weightless state the system of movement control and its effector apparatus, i.e. skeletal muscles, undergo certain changes.

Long-term manned space missions have demonstrated that, despite the use of various countermeasures, skeletal muscles show changes which can be defined on the whole as "functional atrophy". They include muscle mass loss, and decline of muscle tone, strength and endurance, primarily of leg and torso muscles (2,31); these changes cause, in turn, deterioration of the upright posture regulation, disturbances of locomotions, and disorders of more complex movements (2,3).

In the present investigation an attempt was made to penetrate into mechanisms of the above phenomena (their nature, depth, etc.) on the basis of animal experiments carried out aboard biosatellites and in ground-bound weightlessness simulation studies.

MATERIALS AND METHODS

In all experiments, animals were sacrificed by decapitation: after recovery of Cosmos-605 and Cosmos-690 at R + 1 and R + 25, after recovery of Cosmos-936 at R + 0, R + 25.

Duration of simulation studies varied and is indicated in the text. Experiments were performed on skeletal muscles of forelimbs (medial head of the triceps brachii muscle - TRIC and the brachialis muscle - BRACH) and the hindlimbs (the soleus muscle - SOL and the extensor digitorum longus muscle - EDL).

Contractile properties of intact muscles in vitro were examined with respect to the amplitude of twitch (A_o) and tetanic (A) tension and to the time of development of tetanic tension (T_A) to reach 1/2 A (18). Contractile properties of glycerinated muscle fibers were evaluated in relation to the maximum amplitude of isometric tension (P_m) (quantitative analysis) and time (T_p) of an empirically isolated linear segment of the curve of P_m development (normalized index). Glycerinated muscle fibers were obtained and their contractile properties examined by the method of Szent-Gyorgyi (28) modified according to (1,23).

In every experimental series the results obtained in flight animals (F) were compared with the data of synchronous controls (S) and vivarium controls (V).

RESULTS AND DISCUSSION

Studies of SOL and EDL intact muscle preparations performed as part of Cosmos-605 (22 days) and Cosmos-690 (20.5 days) programs showed a decrease of muscle contraction strength (A) upon their tetanic stimulation, loss of elasticity and decline of tolerance to fatigue (18,19) (Fig. 1). The SOL also displayed a selective acceleration of tetanus development (T_A decrease, Fig. 1, 2) and a decrease of the A/A_o ratio (19). The above changes proved adaptive and reversible: they disappeared by R + 25. It was suggested that in the SOL they were induced by weightlessness effects and could be associated with a partial rearrangement of the structure and function of muscle fibers that made them similar to fast fibers (18); in the EDL these changes were more likely bound with the combined effect of experimental and maintenance factors.

It was also hypothesised that manifestations of adaptive transformation of contractile properties of the anitgravity SOL not only reflected its structural (11) and metabolic (4,21) changes but were also related to alterations of contractile properties of myofibrillar proteins as such.

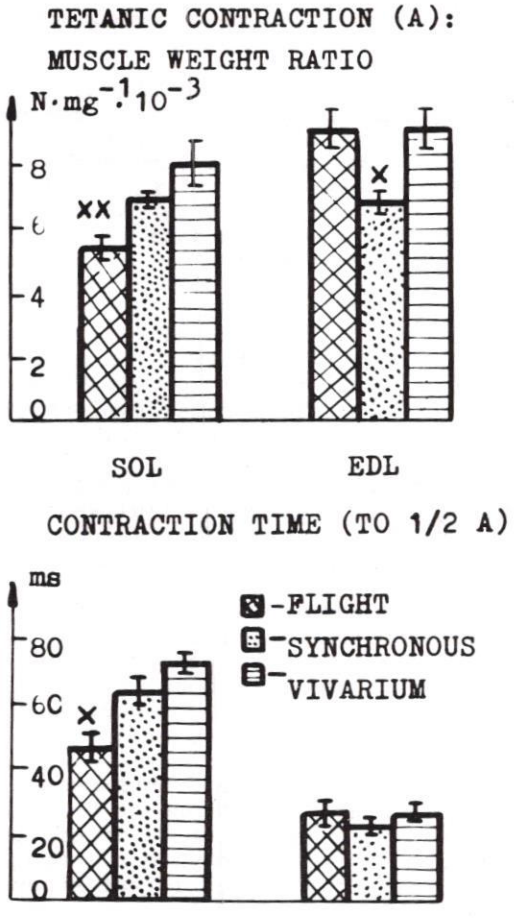


Fig. 1. Contractile properties of rat skeletal muscles after the experiment on Cosmos-690. EDL - m. extensor digitorum longus, SOL - m. soleus. Changes in F rats were statistically significant at $^{xx}P_{(S,V)}<0.05$ and $^{x}P_{(S \text{ or } V)}<0.05$.

In order to verify this hypothesis, the Cosmos-936 (18.5 days) program included a study of contractile properties of glycerinated muscle fibers. The study has been suggested by the knowledge that pre-glycerination, partially degrading the sarcolemma and washing metabolic substrates out of muscle fibers, maintains the native organization of myofibrillar proteins and their ability to form actomyosin complex and to hydrolyze ATP, which in this case, are similar to the processes occurring during intact muscle contraction (1,15).

It should be noted that, unlike strength parameters, the time-course of contraction development in this model is influenced by ATP concentration, and rate of ATP diffusion towards active myosin sites; this is in turn dependent on the preparation size, number of fibers and other things (1,30). All this makes it difficult to quantitate and compare time

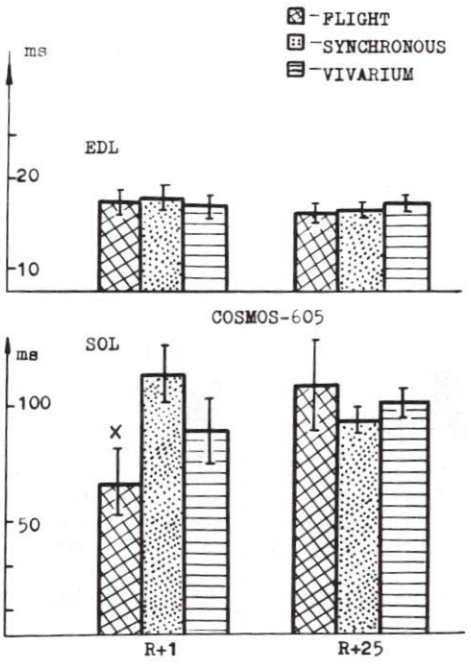


Fig. 2. Time of tetanic contraction development (to reach 1/2 max, T_A) of rat skeletal muscles at R + 1 and R + 25 after Cosmos-605 flight. Designations see in Fig. 1.

parameters characterizing reduction of muscle circumferences; therefore, the above-mentioned time index T_p has been used in qualitative evaluations.

As it follows from Fig. 3 and (29), a decrease of P_m of the slow SOL (Cosmos-936, 1129) and a lesser decrease of P_m of TRIC have been revealed as compared to S - Cosmos-936 and to S and V - Cosmos-1129 (29). A tendency toward shortening of time of SOL tension development (T_p decrease) has been found. A trend for an increase in T_p of the EDL and lack of significant changes of P_m (Cosmos-936) and, on the contrary, lack of changes in time parameters and a statistically significant increase of P_m of the EDL (Cosmos-1129) from the light muscle portion (29) have been demonstrated. In preparations of another fast muscle - BRACH - a significantly increased P_m (Cosmos-936) in both F and S groups (Fig. 3) or a slightly decreased P_m (Cosmos-1129) (29) have been observed in the absence of noticeable changes in T_p in either case.

In accordance with histological (12) and physiological (13) data, the TRIC is similar to the fast EDL in its fiber composition. However, it is believed (12) that due to its anatomical, topographical and biomechanical characteristics the TRIC (an extensor muscle) is involved in maintaining posture to a greater extent than the EDL

(a flexor muscle). This may have a bearing on the similarity between SOL and TRIC reactions to space flight effects shown in the present investigation.

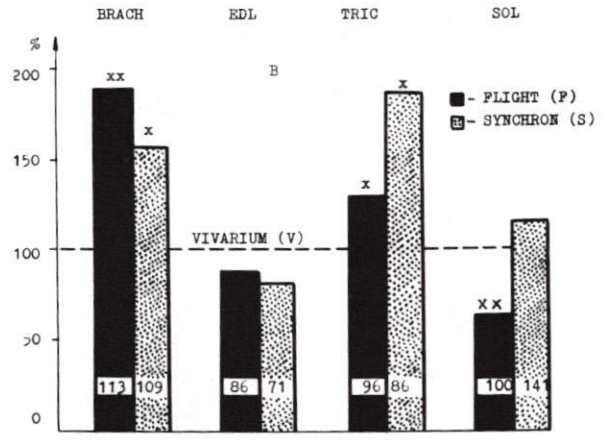


Fig. 3. Changes in maximal isometric tension of glycerinated muscle fibers after Cosmos-936 flight. BRACH - m. brachialis, TRIC - m. triceps (caput medialis). The number of preparations (bundles) is shown in diagrams. Other designations see in Fig. 1.

The data on an increased P_m of BRACH and TRIC in S rats (Cosmos-936) as well as EDL in F rats (Cosmos-1129) needs further study. Theoretically, this can be attributed to 1) the environmental effects of a small enclosure, and 2) an increased contribution of the postural load caused by body stabilization in the support - free space. This activity may first of all involve forelimb muscles which are well adjusted to differentiated movements in rats. This concept is indirectly supported by the evidence indicating an increase of total motor activity of rats in space flight (14).

The biomechanical study of factors that could explain the above observations was conducted in experiments simulating in a different manner altered loads upon skeletal musculature (hypokinesia, hypodynamia) in comparison with the biosatellite findings.

Hypokinetic experiments in which animals were kept in small cages limiting primarily their physical movements demonstrated a decline in the strength (A) of intact SOL and EDL, which was most pronounced in the latter muscle, as well as a selective delay of tetanus development (an increase in T_A) of intact SOL ($p < 0.02$). This muscle also showed a reduced tolerance to fatigue. These changes were significant only after 3-month exposure to hypokinesia (17).

Similar hypokinetic experiments of shorter duration (22 days) did not reveal significant changes of P_m in glycerinated fibers of any fore- or hind-limb muscle tested (Fig. 5). At the same time a distinct trend for an increase in T_p in SOL preparations and for a decrease in T_p in EDL preparations was seen.

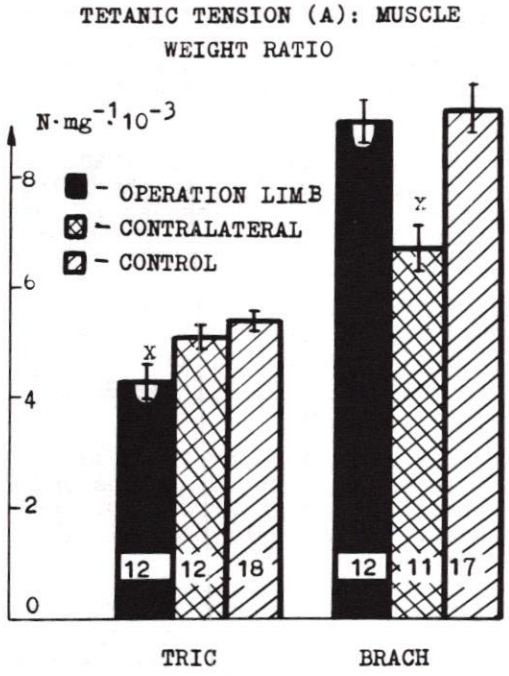
Hypodynamic studies (13) in which strength unloading of brachial muscles was achieved by forearm amputation demonstrated a decrease of strength (A) ($p < 0.01$) and shortening of time of tetanus development ($p < 0.05$) in intact TRIC (Fig. 4).

In other hypodynamic experiments rats were kept in special devices (10) where animal hind- and, in part, fore-limbs had no support ("suspension"). Due to the suspended two-coordinate system animals could move (within an area of 400 mm x 400 mm) in two planes with the aid of their forelimbs, thus having access to food and water. These studies showed a decrease of P_m of glycerinated fibers of SOL ($p < 0.01$) and TRIC ($p < 0.02$) (Fig. 5).

An analysis of the data obtained allows the following conclusions to be made. The phenomenological similarity of effects of weightlessness and hypodynamia (amputation and suspension) suggests that strength unloading is the major factor causing the above changes in strength and time parameters of antigravity muscles in the weightless state. The hypokinetic effect, particularly, on time parameters of SOL contraction is often opposite to the weightlessness effect, resulting in an increased time of isometric tetanus development (13, 17). In analogy to the delay of a fast muscle in response to a prolonged low-frequency stimulation of the appropriate nerve (22,27), it can be thought that the SOL of hypokinetic animals (in our model) may have an inflow of reflectively enhanced efferent impulses, for instance, due to its extension with the ankle flexed.

Among mechanisms, that may be involved in the fairly complex changes noted, at least two groups can be distinguished: myogenic and neurogenic. There are findings that can be discussed in the light of modern concepts of the neurous control over the structure and function of muscles.

It can be assumed that various deviations from the normal postural activity as simulated in the present investigation (weightlessness, hypokinesia, hypodynamia) are accompanied by corresponding changes in the pattern and type of total afferent impulsation from various proprioreceptors (5,25). This may result in certain changes



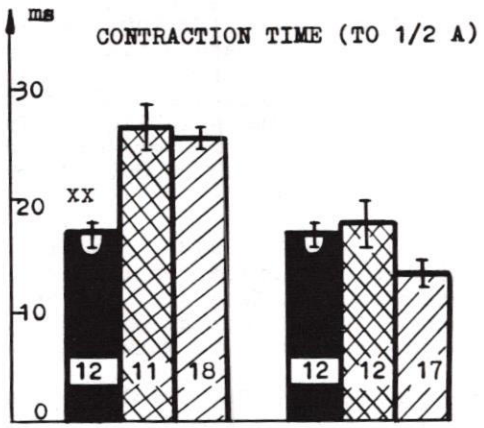


Fig. 4. Contractile properties of shoulder skeletal muscles of rats after their unloading using amputation of the forearm distal third (amputation model). The diagrams show the number of preparations (intact muscles) tested. Significance of differences between amputated limbs and contralateral and control animals ($^{xx}P < 0.01$), contralateral limbs or control animals ($^{x}P < 0.05$)

in the functional state of nerve centers involved in the regulation of movements and contractile properties of skeletal muscles as well as in an adequate rearrangement of reflex-trophic relations between the nerve and the muscle. This may be illustrated by

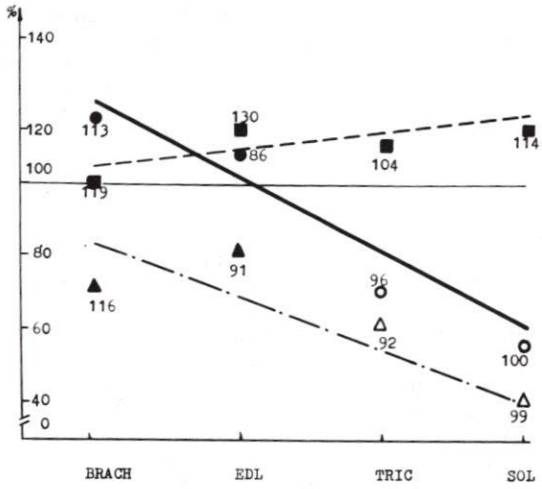


Fig. 5. Weightlessness and simulation studies: pattern of dependence of changes in contractile properties of glycerinated muscle fibers (P_m) of different skeletal muscles upon their involvement in antigravitational activity:
● Cosmos-936 (F/S·100%),
■ hypokinesia (small enclosures) and
▲ hypodynamia (suspension) of duration equal to that of space flight.
Unshaded symbols $P < 0.05$. Other designations see in Figs. 1 and 3.

electrophysiological (24) and biochemical (7) evidence of changes in the functional state of spinal cord motoneurons of hypokinetic rats as well as by postflight decrease in the rate of protein synthesis in spinal cord motoneurons and cerebellar Purkinje cells (6,8) which may give rise to alterations in their function and regulatory influences on skeletal musculature. At the myon level this may lead to a modification of various, possibly including autoregulatory mechanisms controlling synthesis of muscle proteins and, finally, to an appropriate rearrangement of the phenotype of muscle fibers as judged by the isozyme pattern of myosin (29) and composition of regulatory proteins (16).

Thus, the results of our examinations of contractile properties of skeletal muscles in real weightlessness and simulation studies as well as in increased gravity experiments (20) may be regarded as a specific case of realization of general mechanisms underlying dynamic nature of muscle specialization (9,27,32). In this event all the above data may be of theoretical interest as another illustration of functional plasticity of muscles and their

capacity to adapt adequately to an unusual environment, to an allowed motor activity, and to new biomechanical conditions of contraction. From the practical point of view this holds good promise as a potential tool of controlling adaptation of skeletal muscles to the weightless state.

REFERENCES

1. Bendall J.R. 1970. Muscles, Molecules and Movement. Mir, Moscow.

2. Bryanov I.I., Emelyanov M.D., Matveev A.D., Matsnev E.I., Tarasov I.K., Yakovleva I.Ya., Kakurin L.I., Kozerenko O.P., Myasnikov V.I., Eryomin A.V., Pervushin V.I., Purakhin Yu.N., Rudomyotkin N.M., Chekirda I.F. 1976. Peculiar features of stato-kinetic reactions. In: Manned Space Missions on Soyuz Spacecraft. Biomedical Investigations, Moscow, Nauka, 195-229.

3. Chekirda I.F., Bogdashevsky R.B., Eryomin A.V., Kolosov I.A. 1971. Coordination structure of the step of Soyuz-9 crew-members before and after flight. Kosm. Biol. Aviakosm. Med., 5, 6, 48-52.

4. Gaevskaya M.S. Veresotskaya N.A., Kolganova E.V., Kurkina L.M., Nosova E.A. 1979. Metabolic changes in the soleus muscle of rats after flight of Cosmos-690. Kosm. Biol. Aviakosm. Med., 13, 1, 16-19.

5. Gazenko O.G., Gurjian A.A. 1967. Physiological effects of gravity. In: Problems of Space Biology. Moscow, Nauka, 6, 22-42.

6. Gazenko O.G., Dyomin N.N., Panov A.N., Rubinskaya N.L., Tigranyan R.A. 1976. Some characteristics of metabolism of proteins and nucleic acids in the central nervous system of rats flown aboard Cosmos-605. Kosm. Biol. Aviakosm. Med., 10, 4, 14-17.

7. Gorbunova A.V. 1971. Effect of hypokinesia on the RNA content and nucleotide composition and protein content in motoneurons of anterior horns of spinal cord of rats. Dokl. AN SSSR, Ser. Biol., 199, 4, 976-978.

8. Gorbunova A.V., Portugalov V.V. 1977. Cytochemical examination of proteins and RNA in individual neurons of spinal cord and neurons of intervertebral ganglia of rats postflight. Kosm. Biol. Aviakosm. Med., 11, 4, 24-28.

9. Guth L. 1972. Regulation of metabolic and functional properties of muscle. In: Regulation of Organ and Tissue Growth. New York - London, 61-75.

10. Ilyin E.A., Novikov V.E. 1980. A test bench to simulate physiological effects of weightlessness in laboratory rat experiments. Kosm. Biol. Aviakosm. Med., 14, 3, 79-80.

11. Ilyina-Kakueva E.I., Portugalov V.V., Krivenkova N.P. 1977. Effect of Spaceflight factors aboard Cosmos-690. Kosm. Biol. Aviakosm. Med., 11, 1, 20-25.

12. Katinas G.S., Potapov A.N. 1971. Changes in skeletal muscles under conditions of lowered load. Arch. Anat. Histol. Embryol., 61, 11, 74-82.

13. Katinas G.S., Oganov V.S., Potapov A.N. 1974. Hypodynamic and hypokinetic state of muscles. Fiziol. J. SSSR, 60, 10, 1606-1608.

14. Klimovitsky V.Ya., Oganov V.S., Ilyin E.A., Noskin A.D., Verigo V.V., Magedov V.S., Murashko L.M., Rostopshin Yu.A., Skuratova S.A. 1979. Temperature homeostasis and motor activity of animals inflight. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Moscow, Nauka, 13-16.

15. Lyudkovskaya R.G. 1977. Functional and physico-chemical characteristics of muscle fibers of different types. In: Molecular and Cellular Biophysics. Moscow, Nauka, 217-226.

16. Oganesyan S.S. 1978. Biological effects of gravity field. Biol. J. Armenii, 31, 7, 661-771.

17. Oganov V.S., Potapov A.N. 1973. On the functional state of skeletal muscles of hypokinetic rats. Kosm. Biol. Aviakosm. Med., 7, 2, 22-26.

18. Oganov V.S., Potapov A.N. 1976. On the mechanisms of changes in skeletal muscles in the weightless environment. In: Life Sci. Space Res., 15, 136-143.

19. Oganov V.S., Potapov A.N. 1979. Study of functions of skeletal muscles in biosatellite experiments. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Moscow, Nauka, 118-123.

20. Oganov V.S., Potapov A.N., Skuratova S.A., Shirvinskaya M.K. 1980. Variability of physiological properties of rat skeletal muscles at different gravity levels. Physiologist.

21. Portugalov V.V., Petrova N.V. 1976. LDH-isoenzymes of skeletal muscles of rats after space flight and hypokinesia. Aviat. Space, 47, 8 834-838.

22. Salmons S., Vrbova G. 1969. The influence of activity of some contractile characteristics of mammalian fast and slow muscles. J. Physiol. (Lond.), 201, 535-549.

23. Sexton A.W. 1967. Isometric tension of glycerinated muscle fibers following adrenalectomy. Amer. J. Physiol., 212, 2, 313-316.

24. Shirvinskaya M.A., Skuratova S.A. 1977. Effect of prolonged deficiency of motor activity on the functional state of spinal cord motoneurons. In: Important Problems of Space Biology and Medicine, Moscow, I, 154-155.

25. Skuratova S.A., Oganov V.S., Shirvinskaya M.A., Magedov V.S. 1978. Dynamics of afferent impulsation in posterior roots of the spinal cord of hypokinetic dogs. Biol. Aviakosm. Med., 12, 4, 88-89.

26. Smith A.T. 1975. Foundations of Gravitational Biology. In: Foundations of Space Biology and Medicine. Moscow, Nauka, II, Pt. 1, 141-176.

27. Sreter E.A., Gergely J., Salmons S., Romanul F. 1973. Synthesis by fast muscles of myosin light chains characteristic of slow muscles in response to long-term stimulation. Nature-New Biology, 241, 105, 17-19.

28. Szent-Gyorgyi A. 1949. Free energy relations and contraction of actomyosin. Biol. Bull., 96, 140-160.

29. Szilagyi T., Szoor A., Rapcsak M., Takacs O., Oganov V.S., Skuratova S.A., Murashko L.M., Oganesyan S.S. 1980. Contractile properties and composition of myofibrillar proteins of skeletal muscles in Cosmos-1129 experiments: preliminary results. Physiologist.

30. Szoor A., Boross A. 1979. Glycerines psoas keszitese uj eljarassal. Kiserletes Orvostudomany, 31, 452-456.

31. Thornton W.E., Rummel J.A. 1974. Muscular deconditioning and its prevention in space flight. In: Proc. Skylab Life Symp., NASA, Houston, I, 407-414.

32. Van der Meulen J.P., Peckham P.H., Mortimer J.T. 1974. Use and disuse of muscles. Ann. N.Y. Acad. Sci., 228, 177-189.

33. Went F.W. 1968. The size of man. Amer. Sci., 56, 400-413.

34. Zenkevich L.A. 1944. Essay on Evolution of the Motor System of Animals. J. Obshch. Biol., 5, 3, 129-145.

WEIGHTLESSNESS EFFECTS ON RESISTANCE AND REACTIVITY OF ANIMALS

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In the literature there are numerous indications that exposure to weightlessness is accompanied by a decline in bodily resistance, even with countermeasures applied. Different signs of diminished resistance, e.g. decrease of work capacity, rapidly developing fatigue, orthostatic intolerance, altered immunoreactivity, have been seen both during and after manned space flights (8).

Since resistance and reactivity are integrative parameters characterizing viability of the human body and its ability to adapt to a new environment, it is important to discuss this problem in detail, having in view relevant data from animal experiments flown aboard biosatellites of the Cosmos series.

The flight experiments were performed on male Wistarline rats, which were 60-85 days of age and weighed 200-350 g, from the Stolbovaya breeding colony (Moscow) or from the breeding colony of the Institute of Experimental Endocrinology (Bratislava, Czechoslovakia).

The flight time varied from 18.5 to 22 days, thus making approximately 1/50 of the rat life-time. No countermeasures against adverse effects of weightlessness were used either during or after flights.

The flight data were compared with the results obtained from vivarium and synchronous ground-based controls kept in the biosatellite mock-up, where the flight profile, except for weightlessness, was simulated. A more comprehensive description of the experimental details was reported elsewhere (9).

Postflight state of rats was in all cases satisfactory. However, their motor activity was lowered during the first days post-recovery. It looked as if the animals adhered to a sort of gentle regimen, i.e. functional hypokinesia, to alleviate their readaptation to Earth gravity after prolonged weightlessness. Interestingly, a similar phenomenon - "physiological reduction of motor activity" - was observed by Bengele (1) in rats during their transition from 1 g to hypergravity generated by chronic centrifugation.

For rats - long growing mammals - one of the basic parameters of viability may be the rate of growth, reflecting accurately enough the proportion of anabolic and catabolic processes. Biosatellite studies demonstrated weight losses of flight animals as compared to the controls. In Cosmos-605 rats, the delay in weight gain was 60 g, in Cosmos-782 rats it was much lower amounting to 18 g. In both flights food consumption was normal. Inflight changes in body weight seemed to depend on the preflight weight and metabolism. For instance, Cosmos-1129 rats, larger animals with a more marked daily weight gain, showed only a trend for growth delay inflight: the difference from the controls was statistically insignificant.

It should be noted that in flights, the duration of which was 1/50 of the animal life time, there were signs of activation of both catabolic and anabolic processes. Enhancement of catabolism was indicated by activation of proteolytic enzymes in all compartments of the digestive tract, decrease of muscle weight and other changes (4, 10). Activation of anabolism was indirectly suggested by a better assimilation of diet, slightly increased oxygen consumption and other variations (3, 5). It is likely that during space flights of the above duration the animal body is capable to make for, either partially or completely, enhanced catabolism developing in response to the underload of the musculoskeletal system. However, in spite of phylogenetically determined conservatism of anabolism activation, it can be hypothesized that with an increase in flight time this reaction, without an adequate reinforcement, may extinguish to result in a noncompensated progressive activation of catabolic processes.

It is known that reactivity of the animal body depends largely on the state of its lymphoid tissue which is very labile during stress effects and adaptation. Flight animals showed involution of lymph organs: a decrease in the thymus and

spleen weight and a reduction of the thymocyte and splenocyte count. They also exhibited lymphopenia in blood and a decrease of the percentage content of lymphocytes in bone marrow.

Following Cosmos-1129 flight, some animals were exposed to an additional stress - 2.5 hour immobilization at R + 0, 3, 4, 5 and 6 days. In spite of a distinct involution of the thymus in flight animals at R + 0 (252 mg versus 394 mg in the control, $p < 0.001$), their reaction to additional stress-effects was very close to that in the control - 38% and 30%, respectively. Similar changes were seen in the spleen: postflight its weight was 636 mg on the average versus 893 mg in the control ($p < 0.001$), and the reaction to additional stress effects was even greater than in the control (29% versus 14%) regardless of the initial (postflight - pretest) involution.

Blood analysis performed 7 hours postflight showed neutrophilia and lymphopenia (Table 1). This was suggestive of the development of an acute stress-reaction by that point in time.

However, the depth of responses to the stress-test was identical in both flight and control rats. Fig. 1 illustrates the degree of changes (as a percentage of the pretest level) in the lymphocyte/neutrophil ratio at the end of the immobilization test carried out at R + 0, 3 and 5. In all cases responses of flight and control animals were identical, although absolute values before and after test differed significantly: for instance, the lymphocyte/neutrophil ratio in the flight group before and after test was lower than in the controls.

Fig. 2 shows the lymphocyte/neutrophil ratio 30 min after the immobilization test. At R + 0 and 5 there was no difference between flight and control animals and at R + 3 vivarium rats reached the pretest level within this time period, in contrast to the flight and synchronous rats.

Thus, the animals exposed to space flights effects aboard the biosatellite retained largely their ability for adequate reactions of blood cells to additional stressors, in spite of the fact that the

TABLE 1. Blood Picture of Animals 7-10 Hours Postflight

Rat group	Hemoglobin (g%)	Erythrocytes (mln/mm^3)	Leukocytes (thous/mm^3)	Lymphocytes (thous/mm^3)	Neutrophils (thous/mm^3)
Vivarium (V)	7.1 ± 0.24	15.9 ± 0.16	8.2 ± 0.51	5.18 ± 0.51	2.43 ± 0.29
Synchronous (S)	7.4 ± 0.16 $p_V > 0.1$	15.3 ± 0.13 $p_V > 0.05$	6.8 ± 0.41 $p_V = 0.05$	4.0 ± 0.23 $p_V = 0.05$	2.43 ± 0.2 $p_V > 0.1$
Flight (F)	8.9 ± 0.22 $p_{V,S} < 0.001$	17.4 ± 0.17 $p_{V,S} < 0.001$	11.22± 1.07 $p_V = 0.02$ $p_S < 0.002$	2.91 ± 0.27 $p_V < 0.001$ $p_S < 0.01$	7.16 ± 1.01 $p_{V,S} < 0.001$

Nevertheless, the 2.5 hour immobilization test caused similar reactions in both flight and control animals: further increase in the neutrophil count, decrease in the lymphocyte count, and decline in the lymphocyte/neutrophil ratio. Prior to the test, the ratio was 0.64 in the flight group, 2.37 in the vivarium group and 1.83 in the synchronous group ($p < 0.001$); by the end of the test it was 0.21, 0.51, and 0.44, respectively ($p < 0.002$) and 30 min after the test it was 0.11, 0.24, and 0.22, respectively ($p < 0.001$).

initial (postflight - pretest) blood of flight animals differed significantly from that of controls.

Weight variations during immobilization tests were also similar in the flight and control animals (Fig. 3).

Mention should be made of individual variations in animal responses to space flight factors. They can be seen in weights of body and organs, blood picture, reactions to the stress-test postflight,

reaching sometimes significant values. Obviously, there is good reason to speak not only about an average reaction to the space flight but also about a gradient of reactions associated, in all likelihood, with differences in individual resistance and reactivity of animals.

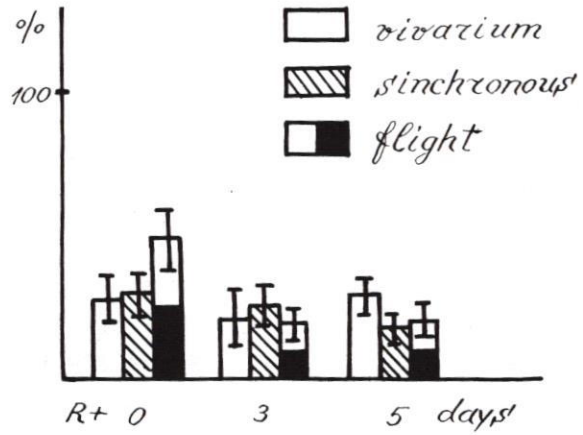

Fig. 1. Lymphocyte/neutrophil ratio in peripheral blood. Level of reaction at the end of the stress-test at R + 0, 3 and 5.

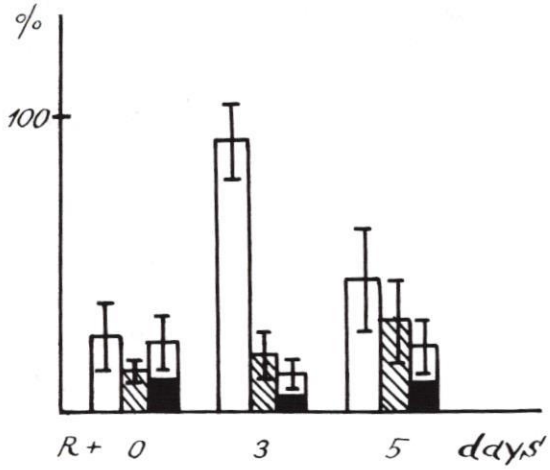

Fig. 2. Lymphocyte/neutrophil ratio during recovery after immobilization test.

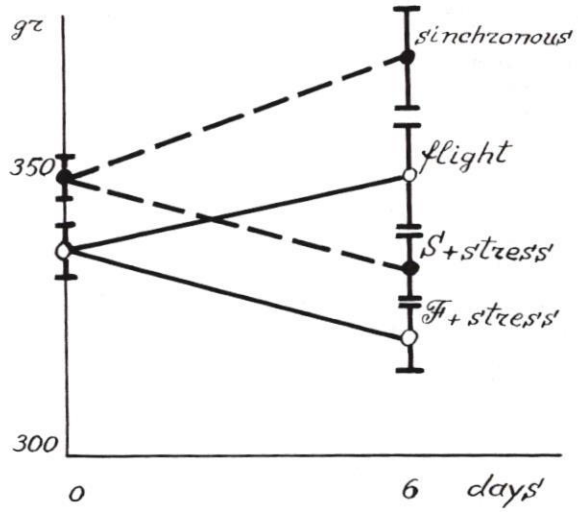

Fig. 3. Weight variations during the first postflight days - at rest and during immobilization tests.

It is interesting to note that similar observations were made by Burton and Smith (2) in their chronic acceleration studies of birds. The authors distinguished five groups with a varying pattern of responses - from a group with very acute responses to the symptom-free group.

The very fact of distinct individual reactions in the population of normal animals is important for preflight selection of animals. At the present time the standard practice is to select most resistant animals. It cannot be ruled out that doing this we deprive ourselves of an opportunity to study extreme reactions to weightlessness.

No matter what parameters are used to assess resistance at different stages of individual development, its basic integrative parameter is the rate of aging. The exposure to weightlessness did not shorten the life-time of animals. Out of 4 rats allowed to live after Cosmos-605 flight 2 rats died at the age of 29-30 months, one rat at the age of 35 months, whereas all control animals (6 rats) died at the age of 23-25 months. There was only one flight rat that died earlier, at the age of 13 months.

The offspring obtained from mating male rats flown for 22 days aboard Cosmos-605 and intact females 2 months postflight did not differ from the controls in the total number of newborns, weight at birth, weight variations during the first month of life, weights of organs, blood picture, and tolerance to hypoxic hypoxia (Table 2).

TABLE 2. Characteristics of the Offspring Produced by Flight and Control Male Rats. (The animals were 30 days of age at the time of examination).

Rat group	Number of rats	Body weight (gms)	Weight of organs (mg)						Hemoglobin (g%)	Erythrocytes (mln/mm³)	Femoral karyocytes (mln)
			Adrenals	Thymus	Spleen	Myocardium	Kidneys (2)	Liver			
Control	12	76±5	24±1.09	245±15.4	351±62.9	325±20.3	723±41.7	3750±341	10.6±0.5	6.5±0.4	80.8±7.4
Flight	18	79±3	21±1.2	223±15.2	287±18.5	324±12.4	788±30	3660±174	10.4±0.4	6.0±0.3	76.6±7.9
		p>0.1	p=0.1	p>0.1	p>0.1	p>0.1	p>0.1	p>0.1	p>0.1	p>0.1	p>0.1

It cannot be excluded that animal mating at earlier postflight stages may yield different results.

These findings allow the conclusion that an 18-22 day exposure to weightlessness reduces resistance of the animal body. However, this reduction is not great, exerting no significant effect on animal viability.

Obviously, an exposure to weightlessness of the above duration forms the situation in which reserve capabilities of the animal body prove adequate to maintain homeostasis at rest and upon certain loads and enhanced catabolism is to a large extent compensated by enhanced anabolism. In spite of involution of lymphoid organs occurring at the end of the flight, they show a reaction to an additional immobilization stress similar to that in the control. The same can be said about blood cells. Nevertheless, according to the data obtained by Macho et al. (6) from the same Cosmos-1129 rats, repeated postflight immobilization decreased substantially the content of epinephrine and norepinephrine and increased tyrosinehydroxylase activity in the adrenals as compared to the control animals exposed to a similar test. Natochin et al. (7) described an inadequate reaction of rats to the potassium load at R + 1 which was manifested as diminished capacity to retain potassium despite its deficiency at the end of the flight.

Thus, the animals exposed to weightlessness for 1/50 of their life-time show reactions to additional loads that appear adequate in some cases and inadequate in others. It can be assumed that in missions with a longer-term exposure to weightlessness, on the one hand, or with more complicated provocative situations, on the other, a progressive decline of resistance of the animal body and an increase in the number of inadequately responding systems may be observed. Therefore, further investigations should concentrate on limits of adaptive capabilities of the animal body during adaptation to weightlessness and readaptation to 1 g upon return to Earth.

REFERENCES

1. Bengele H.H. 1969. Water intake and urine output during chronic centrifugation. Amer. J. Physiol. 216, 659-665.

2. Burton R.R., Smith A.H. 1965. Chronic acceleration sickness. Aerosp. Med., 36, 1, 39-43.

3. Golov V.K., Ilyin E.A. 1977. Study of gas exchange of animals flown aboard biosatellite. X Symp. Space Biol. Med., Sukhumi, Abstr., 15.

4. Ilyina-Kakueva E.I., Portugalov V.V., Krivenkova N.P. 1977. Space flight effects on skeletal musculature of rats. Kosm. Biol. Aviakosm. Med., 11, 1, 20-25.

5. Kondratyev Yu.I., Ilyushko N.A., Besedina E.G. 1979. Animal feeding in-flight and metabolic studies. In: Effect of Dynamic Factors of Space-flight on the Animal Body. Ed. Genin A.M. Moscow, Nauka, 21-24.

6. Macho L., Kvetnansky R., Nemeth S., Palkovic M., Tigranyan R.A., Serova L.V. 1980. Changes in animal responses to the immobilization stress-test after space flight. XIII Symp. Space Biol. Med., Dresden, Abstr., 73.

7. Natochin Yu.I., Ilyusho N.A., Kondratyev Yu.I., Lavrova E.A., Shakhmatova E.I. 1979. Ion regulatory function of rat kidneys after flight aboard Cosmos-936. V USSR Conf. Space Biol. Aerosp. Med., Moscow-Kaluga, Abstr., 64-65.

8. Pestov I.D., Gerathewohl Z.G. 1975. Weightlessness. In: Foundations of Space Biology and Medicine. Eds. Marbarger J.P. & Vasilyev P.V. Moscow, Nauka, II, b. 1, 324-369.

9. Serova L.V., Ilyin E.A., Noskin A.D., Milyavsky V.I., Chelnaya N.A., Golov V.K., Pishshik V.B. 1979. Experimental conditions, animal selection and training. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Moscow, Nauka, 7-12.

10. Smirnov K.V., Ugolev A.M., Goland L.G., Medkova I.L., Permyakov N.K., Pechyonkina R.A., Sokolova T.A. 1979. Digestive and resorptive functions of the gastrointestinal tract. Ibid, 202-204.

ARTIFICIAL GRAVITY IN SPACE FLIGHT

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Great achievements have been recently scored in the prophylaxis of adverse effects of weightlessness. This is evidenced by the successful 175-day mission of Soviet cosmonauts. However, we are in no position to assert that the counter-measures available (exercise, lower body negative pressure, drugs, etc.) can entirely prevent detrimental effects of weightlessness on the human body. Besides, application of various countermeasures may distract crewmembers from their duties and take too much of their time required for work and rest. It should also be borne in mind that current biomedical studies do not provide reliable data to guarantee good health condition in flights much longer than those performed by the present time.

In view of this, one of the basic goals of space medicine is to develop such countermeasures that would be, on the one hand, efficient and safe and, on the other, not encumbersome for crewmembers.

The best solution of the problem may be afforded by artificial gravity generated by spacecraft rotation (5).

From the biomedical point of view advantages and disadvantages of artificial gravity can well be understood only on the basis of the results of long-term space flights. Assuming that in future artificial gravity may in principle find practical application in astronautics, it seems important to summarize our knowledge concerning the life under artificial gravity conditions.

In 1975 studies on plants and lower vertebrates flown aboard the Soviet biosatellite Cosmos-782 showed that biological effects of artificial gravity of 1 g generated by a centrifuge in space flight were similar to those of Earth gravity (2). (Ilyin, Parfenov, 1980).

Comparative studies of effects of weightlessness and artificial gravity of 1 g on the vital processes in the animal body were continued in Cosmos-936 experiments on mammals, viz. rats (1).

The 19.5-day space flight influenced the general state and posture-motor reactions of animals. The rats flown in the weightless state showed the largest changes during recovery. They were inactive, lay flat most of the time, and moved either crawling or with altered step. They did not exhibit typical movements and postures associated with orientation reflexes in a new environment. The rats exposed to artificial gravity showed less distinct changes. They were more active, readily overcame obstacles, stood on hindlimbs, and moved with normal step.

Static endurance diminished postflight in all rats returned to the normal in centrifuged rats mcuh faster, reaching the preflight level by R + 5. The weight gain of weightless and centrifuges rats did not differ significantly.

Weightless rats showed moderate manifestations of a stress-reaction: increase in the functional activity of the adrenal cortex, hypoplasia of the thymus and spleen, increase in the percentage content of segmented neutrophils in the peripheral blood, lympho- and eosinopenia. Centrifuged rats displayed insignificant, if any, changes of this kind (peripheral blood).

The water load test (5 ml per 100 g body weight) applied at R + 1, in order to evaluate the osmoregulatory function and possible fluid deficiency, did not reveal any differences in fluid excretion - dynamics and maximum - between the two rat groups. Nevertheless, sodium excretion of weightless rats following the water load test was significantly higher than that of centrifuged rats and ground-based controls.

The potassium load test (5 ml - 1.25% KCl per 100 g body weight) applied at R + 2 resulted in an almost identical fluid excretion of both weightless and centrifuged rats. However, potassium excretion of weightless rats was higher (849 ± 24.6 uequiv per 100 g) than that of centrifuged rats (262 ± 43.9 uequiv per 100 g) and synchronous rats (228 ± 27.5 uequiv per 100 g). Thus, artificial gravity exerted a normalizing effect on the renal excretion of sodium and potassium.

An exposure of animals to weightlessness brought about distinct signs of atrophy, metabolic disorders (alteration in the pattern of lactate dehydrogenase isozymes, accumulation of glycogen, increase in the phospholipid content and enhancement of ALT activity) in hindlimb muscles, especially the soleus muscle. Centrifuged rats displayed moderate atrophic changes of the same muscles and essentially no metabolic alterations.

Weightlessness caused an increase in the content of sarcoplasmatic proteins and a noticeable decrease in the activity of myosin ATPase. Centrifuged animals did not show changes in the content of sarcoplasmatic proteins of the myocardium and displayed a much less expressed decrease of myosin ATPase.

The investigation of femurs and tibia of weightless rats revealed a delay in their growth, development of osteoporosis, decrease of density and mineral content as well as a 30% decrease of bending strength. Artificial gravity prevented changes in the calcium and phosphorus content in tubular bones.

The equilibration function assessed in relation to the ability of animals to hold on to a narrow horizontal rail deteriorated immediately postflight, although to a lesser degree in the centrifuged rats. It was characteristic of weightless rats to behave passively while on the rail: equilibration lost, they did not try to restore it. Centrifuged rats were active in maintaining balance and, having lost it, they caught at the rail with their paws in the back downward position (4).

Vestibular studies demonstrated that a prolonged exposure to weightlessness did not alter functions of semicircular canals and otoliths or did not affect central components of vestibulo-spinal and vestibulo-oculomotor reflexes. It is probable that these effects were so weak and transient that they could not be detected on the 1st or 2nd day postflight. A long-term centrifugation of animals resulted in a decline of reactivity and sensitivity of semicircular canals; this may be one of the factors responsible for the decline in the equilibration function noted immediately postflight.

Four-five hours postflight, weightless rats demonstrated an almost normal reflex of turning around when falling down from the back downwards position; the only deviation from the normal was the fact that it was difficult for them to stabilize their body after turning around and, therefore, to land in the proper way, especially with eyes closed. Centrifuged rats turned around in a similar manner but with eyes closed they simply did not try to turn around and fell down on their back. The animals that were centrifuged on the ground in a short-arm centrifuge behaved similarly. It can therefore be inferred that in weightlessness and, to a greater extent, in a rotating system the role of vision in spatial orientation acquires far greater importance.

Studies of the higher nervous activity (the ability for orientation in the maze) showed that the functional changes of centrifuged rats were more marked than of weightless animals. This was particularly evident when the rats were offered complicated tests.

Biochemical examinations of brain structures responsible for the higher motor control demonstrated that centrifuged rats developed a more significant and prolonged decrease of RNA in cerebellar Purkinje cells and of the content of SH-groups in the frontal (motor) zone of the cortex. Besides, they showed a decline in the RNA content in glial cells-satellites of the cerebellum.

Thus, artificial gravity of 1 g generated inflight helped to prevent in a large measure the development of weightlessness-induced adverse changes in the animal body. The normalizing effects of artificial gravity on the function of the myocardium, musculo-skeletal system and excretory system need special mention. At the same time specific effects of rotation concomitant with artificial gravity were detected; they involved the functions realized through a combined action of optic, vestibular and motor sensors (equilibration function, turning reflex, orientation in the maze, higher motor control). Since ground-based manned studies have demonstrated that, from the biomedical point of view, rotational factors cannot be an obstacle for the manufacture of spinning spacecraft (3), it can be concluded the Cosmos-936 experiment was the first study to demonstrate practical feasibility of artificial gravity as a countermeasure against adverse effects of prolonged space flights on mammals.

The final goal of pertinent biomedical investigations is to identify the minimum value of the centripetal force required to maintain good health condition and high performance of space travellers in long-term space missions as well as to determine the optimum ratio of the arm and angular velocity of rotation of a spacecraft carrying an artificial gravity system.

REFERENCES

1. Gazenko O.G., Ilyin E.A., Genin A.M., Kotovskaya A.R., Korolkov V.I., Tigranyan R.A., Porugalov V.V. 1980. Main results of physiological experiments on mammals aboard biosatellite Cosmos-936. Kosm. Biol. Aviakosm. Med., 14, 2, 22-25.

2. Ilyin E.A., Parfenov G.P., Ed. 1980. Biological Investigations aboard Cosmos Biosatellites. Nauka, Moscow, 239.

3. Kotovskaya A.R., Galle R.R., Shipov A.A. 1977. Biomedical investigations into the problem of artificial gravity. Kosm. Biol. Aviakosm. Med., 11, 2, 12-19.

4. Kotovskaya A.R., Shipov A.A., Aizikov G.S., Markin A.S., Mokrousova A.V., Sarkisov I.Yu., Tabakova L.A. 1980. Vestibular and vestibul-motor reactions of rats after flights aboard biosatellites Cosmos-782, 936 and 1129. XIII Symp. Space Biol. Med., Dresden, Abstr. 1,82-83.

5. Tsiolkovsky E.K. 1895. Dreams about the Earth and Heaven, and Effects of Gravity. Moscow, Goncharov Publ.

BIORHYTHMS OF RATS DURING AND AFTER SPACE FLIGHT

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Scientists responsible for medical support of manned missions are concerned with potential effects of space flight on biorhythms (1,2,8,9). In this context, it is very important to determine whether biorhythms are influenced by weightlessness, confinement, and remoteness of natural ground-bound time cues as opposed to other space flight factors associated with work-rest cycles of crewmembers. In order to discriminate these effects and to eliminate the influence of social and motivational factors and countermeasures, it was reasonable to carry out biorhythmological experiments on animals. The experiment of this kind performed previously on a non-human primate (3) showed biorhythm disorders; however, its reliability was questioned in view of the poor health condition of the animal who died at R + 1.

Cosmos-782 (6) and Cosmos-936 studies demonstrated that circadian rhythms of motor activity and body temperatures of animals remained stable throughout the flight, and distribution of their activity and rest patterns within the circadian cycle was normal.

The aims of the Cosmos-1129 experiment were 1) to investigate phasic rearrangements of the circadian cycle of motor activity (MA) and body temperature (BT) of rats inflight, once the light-dark cycle was inverted, and 2) to assess the state of circadian and minute rhythms of animals during their readaptation on return to Earth.

Preflight, SPF Wistar male rats were exposed to artificial illumination with the lighting schedule 12 hours light:12 hours dark.

Minute rhythms of motor behavior were examined, using the conditioned avoidance reaction procedure. The animals were trained to press a bar after a sound signal followed by electric painful stimulation (4) was applied. The trained rats used to work without reinforcement. The following parameters were measured: time interval between signal application and onset of movement (central reaction time, ct), and time interval between onset of movement and bar pressing (motor reaction time, mt). The sound signal was applied every 30 sec for each pair of simultaneously examined animals during 32 min. In the course of this experiment the rats were housed in a chamber consisting of two identical parts. In this manner the animals were taken under study twice a day during time periods that corresponded to their maximum and minimum activities within circadian cycles 19 days before flight and 5, 10, 15, 20 and 29 days after flight.

Circadian variations in the excretion of fluid, Ca, Na, K, phosphates and hydroxyproline were determined within 36 hours on postflight days 3.5-5, 8.5-10, 13.5-15 (7). To do this, animals were housed one per cage, and urine samples were collected every 4 hours (7).

Preflight, animals were trained to live one per cage in routinely used flight qualified 1000 ml cages combined in five to form a unit with automated air ventilation, feeding and watering devices, a waste management system, and MA and BT recorders attached.

In the course of training, during flight and postflight examinations, the animals were kept on a 12 hour:12 hours light:dark cycle (10 and 0 lux). The caged animals were fed every 6 hours with one fourth of the daily diet which remained accessible until the next portion was supplied.

Postflight on days 2, 7, 12 and 17, MA and BT were recorded for 36 hours. During flight and synchronous experiments BT of 5 animals housed in one unit was obtained on the even days, and MA of the same animals on the odd days. In addition, MA was registered in 20 more rats located in 4 other units; these animals remained intact: they were not implanted with BT recorders or used in biorhythm studies pre- and post-test. MA and BT data were recorded with an interval of 2 hours. The informational signal in the BT channel was

the value at the actual time of recording, and that in the MA channel was the sum total of movements performed within 2 hours.

In order to investigate phasic rearrangement of circadian rhythms of MA and BT in weightlessness, the light-dark cycle was inverted on flight day 10 (DD, $\Delta\varphi = 180°$).

In total the flight continued for 18.5 days. During postflight examination the rats were exposed to inverted light-dark cycle (Fig. 1).

MA and BT data were treated by the Kosinor method. In the preflight period all animals showed a distinct circadian rhythm of MA and BT with a normal distribution of activity and rest within the circadian cycle.

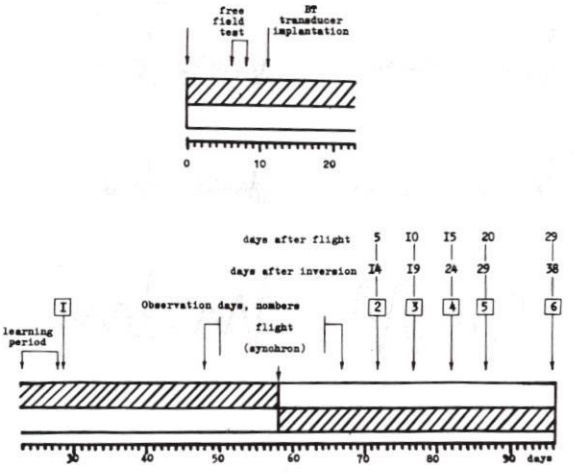

Fig. 1. Scheme of the experiment. Figures in squares indicate days of observation of minute rhythms.

During flight days 1 through 9 the position of the phase of MA and BT rhythms was relatively stable or at least did not show a trend for continuous drift (Fig. 2).

The rearrangement of rhythm phases started immediately after light-dark cycle inversion and developed at a similar rate both in flight and synchronous experiments. Six-seven days later the shift of MA and BT phases reached 180° and by the end of the flight the drift still continued in the same direction both in flight and synchronous animals.

The synchronous rats with implanted BT recorders showed a monotonous drift of the MA phase from the very beginning of the experiment (Fig. 2B). After day-night inversion the process continued at the same rate and in the same direction. This may be attributed to incidental uncontrolled influ-

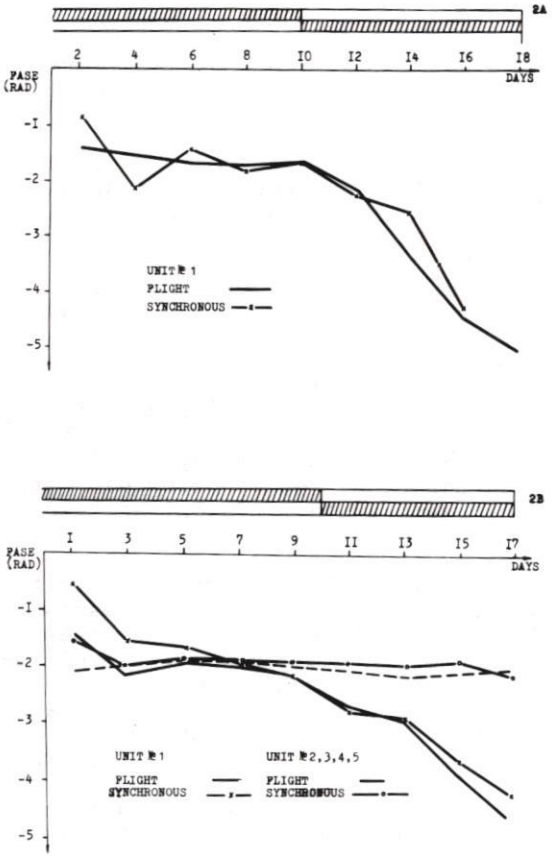

Fig. 2. Phase of circadian cycle of animals during flight and synchronous experiments

2A - Phase of changes in mean body temperature of 5 rats with implanted body temperature transmitters (from rat cage unit 2)

2B - Phase of changes in mean level of motor activity of 5 animals with implanted body temperature transmitters (from rat cage unit 2) and 20 intact rats (from rat cage units 2 through 5)

ences on these very rats, because in other animal groups the position of the MA phase remained unaltered throughout the experiment.

In the recovery period the circadian rhythm of MA and BT of flight and synchronous animals was hardly discernible in the first two and restored only during the third-fourth observation days.

Similar results were obtained with respect to the circadian variations in the urine, hydroxyproline and electrolyte excretion.

The study of circadian variations in the excretion of fluid, Ca, Na, K, phosphates and hydroxyproline postflight are discussed in the paper by Kwarecki et al (7).

As minute rhythms are concerned, the following results have been obtained. Preflight, at the time of maximum activity the amplitude was lower and the period of minute rhythms was shorter than at the time of minimum activity (ct = 3.6 min, mt = 3.7 min at the maximum, and ct = 5.3 min, mt - 5.3 min at the minimum).

From the minute rhythms of vivarium animals it followed that parameters of rhythms of the range corresponded to the inverted day-night cycle on day 14 after inversion (this time period coincided with the day of examination of flight animals on the 5th day postflight).

Further examinations carried out with a 5 day interval demonstrated progressive stabilization of minute rhythm processes and a trend for an emergence of multiplicative waves. The maximum-minimum differences persisted, thus indicating a normal relationship between minute rhythms and appropriate phases of the circadian rhythm.

On the 5th post-test day, flight and synchronous rats showed significant disorders in the minute rhythms of both phases of the circadian cycle. At the minimum activity the values ct and mt were very similar to those typical of the maximum activity phase. A similar situation may occur (5) after transition from alternating day-night cycle to constant darkness (disappearance of periodicity, phase inversion, demultiplication of wave length). However, 15 days post-test minute rhythms in the synchronous animals returned to the normal and did not differ from those in vivarium controls. On the 15th postflight day the flight animals only started to get tuned to the day-night cycle which was a continuation of the inverted (on flight day 10) light-dark cycle. Nevertheless, this involved only the phase which corresponded to the minimum activity, whereas at the maximum stage periodicity was still lacking.

Figures 3A-C demonstrate changes in minute rhythms of flight, synchronous and vivarium rats shown by the autocorrelation function.

It can be inferred from the experimental data that weightlessness did not exert an adverse effect on the formation and stability of circadian rhythms of rats inflight. The rate with which the phase of the circadian rhythm of motor activity and body temperature drifted after the day-night inversion was in fact normal. It is

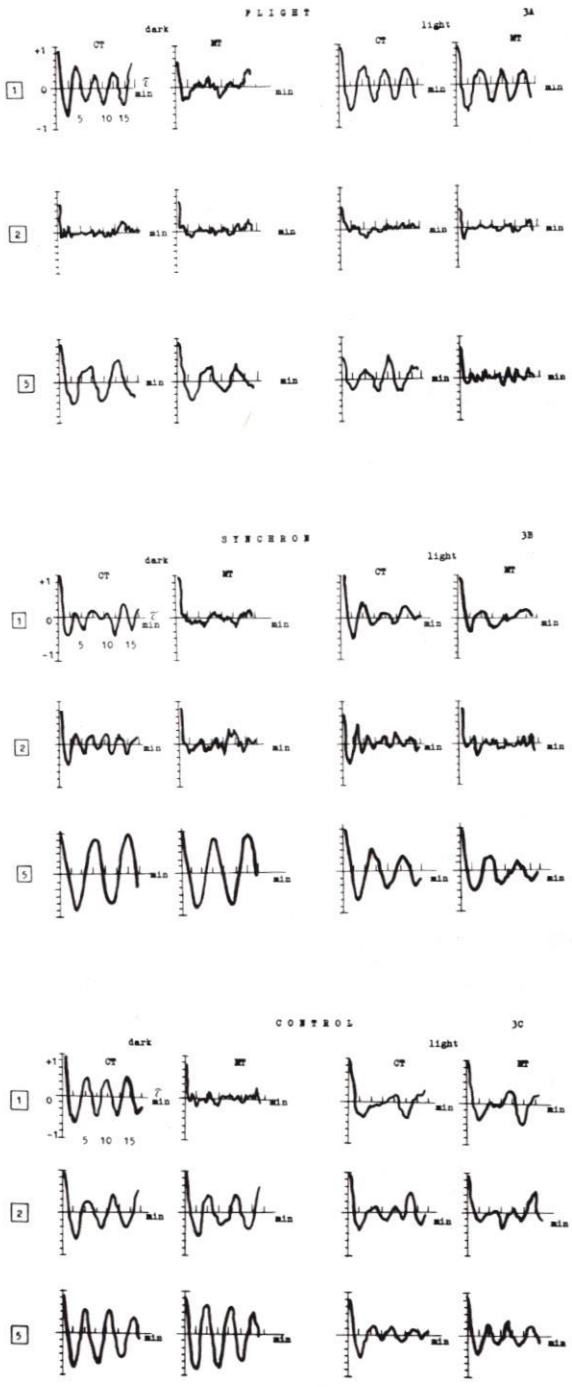

Fig. 3. Autocorrelation function of motor behavior parameters (ct and mt, minute rhythms) for one of the animals in flight (3A), synchronous (3B) and vivarium (3C) groups

probable that weightlessness, being an additional stressor that was absent in the synchronous animals, somehow helped to accentuate circadian rhythmicity.

After flight and synchronous experiments circadian and minute rhythms changed in both animal groups. In this context, the fact that both experiments were completed during the phase drift which continued after day-night inversion should not be ignored. However, physiological loads associated with adaptation to weightlessness and readaptation to 1 g upon return to Earth of flight animals were responsible for more stable changes in their biorhythms as compared to synchronous rats.

REFERENCES

1. Alyakrinsky B.S. 1977. Modern state of space biorhythmology. Kosm. Biol. Aviakosm. Med., 11, 2-12.

2. Aschoff J. 1971. Circadian rhythms in modern medicine. In: IV Intern. Man-in-Space Symp., Erevan, 26-28.

3. Hahn P.M., Hoshizaki T., Adey W.R. 1971. Circadian rhythms of the Macaca nemestrina monkey in Biosatellite III. Aerosp. Med., 42, 3, 295-304.

4. Hecht K., Treptow K., Choinowski S., Peschel M. 1972. Die raumzeitliche Organizations - der Reiz reactions-Beziehungen bedingt - reflektosicher Prozesse. Brain and Behav. Res. Monograph Ser., 5, 21-32.

5. Hecht K., Treptow K., Poppei M., Hecht T., Choinowski S., Peschel M. 1976. Uber die Rolle zyklischer Verlaufe von Korperfunktioned im Organismus. In: R. Glaser, K. Unger und M. Koch. Umweltbiophysik, Berlin, 247-261.

6. Klimovitsky V.Ya., Verigo V.V., Ilyin E.A., Magedov V.S., Oganov V.S., Rostopshin Yu.A., Skuratova S.A. 1978. Biosatellite Cosmos-782. Temperature homeostasis and biorhythms as derived from rat experiments. Chronobiology and Chronomedicine Symp., USSR-GDR, Halle, GDR.

7. Kwarecki K., Debiec H., Koter Z. 1980. Rhythms of electrolytes and hydroxyproline excretion in urine of rats after 3 weeks of weightlessness (biosputnik Kosmos-1129). XXVIII IUPS. XIV. Abstr. Budapest, 171.

8. Litsov A.N. 1972. Study of work and sleep cycles of Soyuz 3-9 crewmembers before, during and after space flights. Izv. AN SSSR. Ser. Biol., 6, 836-845.

9. Stepanova S.I. 1977. Important problems of space biorhythmology. In: Problems of Space Biology, Moscow, Nauka, 23.

RHYTHMS OF ELECTROLYTES AND HYDROXYPROLINE EXCRETION IN URINE OF RATS AFTER THREE WEEKS OF WEIGHTLESSNESS / BIOSATELLITE COSMOS-1129

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ABSTRACT: Investigations were carried out on rats subjected to a 180° light-dark phase shift within 10 days of spaceflight.On the third,eighth and thirteenth day after landing the following rhythms were determined:urine volume,excretion of sodium,potassium, calcium,phosphate and hydroxyproline with urine.All rhythms studied in the urine in control groups were synchronized within 13 days after being subjected to the inverted lighting schedule.In postflight animals the circadian rhythms of diuresis,sodium and potassium excretion with urine were synchronized to the new lighting regimen whereas the others variable were not phase-shifted even within 23 days period. Our results suggest that the synchronizing effect of the light-dark cycle under space conditions was weekened.

INTRODUCTION

Some earlier chronobiological investigations have demonstrated that some degree of dissotiation of circadian rhythms take place during spaceflight / 2,4 /.

Since direct measurments of many biochemical parameters during a spaceflight is technically very difficult,some indirect data on the state of synchronization can be obtained from studies of inversion rate an organism to a new phase of the internal synchronizer.

The purpose of this study was to describe ability of organism to inversion in the excretory rhythms of electrolytes and hydroxyproline in response to a 180°change in photoperiod during spaceflight.

MATERIAL AND METHODS

The experiments were carried out in two stages.

I. In the first stage the rate of resynchronization of electrolytes and hydroxyproline excretion were determined after changing the light-dark phase.These investigations were carried out on 12 male sexually mature SPF rats. The animals were kept under 12/12 light-dark conditions /L 6.00-18.00/ food and water were available ad libitum.Urine samples were collected at 4-h intervals during 24-h period every other day. After 2 weeks the light phase was delayed by 12 hr and during the following 2 weeks urine samples were collected as before. In urine samples following parameters were determined: volume, sodium,potassium,calcium,phosphate and hydroxyproline. The urine sodium and potassium concentrations were measured using a flame photometer.Calcium concentrations were determined by Corning analyser,phosphate by turbidymetry /kit made by Serva Heidelberg /,hydroxyproline by the method of Woessner / 14 /.

II. In the second stage of the experiment 15 male sexually mature SPF rats were used. Animals were divided into three groups: control groups vivarium and synchronic and postflight group,with 5 rats in each group.For 3 weeks before experiment animals were kept under light-dark cycle 12/12 /L 09.00-21.00 /.The same light-dark regimen were maintained during the first 10 days of spaceflight and in both control group. Then the light phase was delayed by 12 hr on the Earth and in the biosatellite / L 21.00-09.00 /. In the synchronic experiment the animals remained in a dummy spacecraft under conditions identical as during spaceflight with the exception of weightlessness.After 19 days of flight the animals returned to the Earth.On the 3rd, 8th and 13th day of the postflight period urine samples were collected from all animals starting from 21.00 at 4-h intervals for 36-h period. The conditions of experiments have been described in detail by Klimovitsky et al / 7 /.The urine samples were kept in plastic vials at -10°C.Urine analysis were as in the first stage of the experiment.

The results odtained in both experiments were subjected to statistical analysis: to each series of results a best-fitting curve was found with the least square method.For each animal mean 24-h value,amplitude and acrophase were calculated.These parameters were evaluated in different groups in the cosinor circle/5/. When determination of the confidence in-

terval was impossible /error ellipse overlap the centre of the circle/ the amplitude and the acrophase was calculated for individual animals and on summary graphs the time range for individual acrophases was shown.

RESULTS

In the first stage of the experiment statistically significant /$p<0.05$/ group circadian rhythms of electrolytes and hydroxyproline excretion were detected by mean cosinor. All rhythms studied in the urine were relatively stable therefore only data from one 24-h period are summarized in Fig 1 and 2. After 2 weeks of lighting shift acrophases of urinary electrolytes and hydroxyproline excretion return to their normal position which indicate a nearly complete rephasing to a 180° change in photoperiod. Circadian timing of acrophases are shown in Fig 1 and 2.

In the second stage of the experiment statistically significant /$p<0.05$/24-h individual rhythms of electrolytes and hydroxyproline excretion were detected in all rats. However statistically significant group rhythms were found by mean cosinor only for sodium excretion in synchronic control and for potassium excretion in postflight on the 8th day. Circadian acrophases of urinary constituents have been found to be stable throughtout all period of the experiment in the vivarium and synchronic control groups. Aware of what only the results of the first investigation are shown in Fig 1 and 2.

In the postflight group circadian pattern changed during succesive days. Fig. 1 demonstrates that during postflight period the rhythms of urine volume, excretion of sodium and potassium adjusted their phase to light-dark cycle. Only on the 8th day after recovery slight movement of the acrophases to light period can be seen. But finally on the 13th day they shifted back into the normal position and rhythms became identical with those observed in control groups.

The acrophases for urinary calcium occuring in normal position i.e. near the light-dark transition was observed only on the 8th day. On other experimental days the acrophases appeared in the wrong time: on the 3rd day in the middle of light period and on the 13th day in the second part of dark period / Fig.2 /.

Reverse data were found for urinary phosphate rhythm. The acrophases properly adjusted to the light-dark cycle on the 3rd and on the 13th day i.e. they occured near the beginning of light phase. On the 8th day the acrophases lost its coupling with light regimen and occured in the other part of light phase / Fig.2 /.

The acrophases for urine hydroxyproline were found in the normal position i.e. at the end of dark phase during the first postflight experiment, but after more prolonged observation / on the 13th day / it shifted to the beginning of the dark phase./ Fig.2 /. On the 8th day the acrophases for hydroxyproline were dispersed on the whole night-day period so no time range for acrophases were demonstrated in Fig. 2.

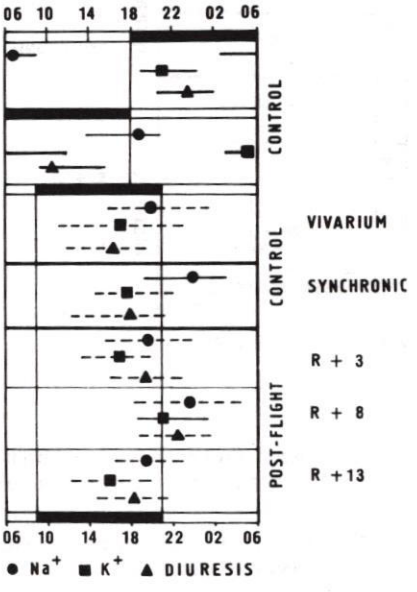

Figure 1. Urinary volume, sodium and potassium acrophases in rats.

Upper part-the first stage of the experiment.
The rhythmic pattern was nearly inverted 180° within 14 days after subjecting the animals to the new light-dark cacle.

Lower part- the second stage of the experiment.
Both control groups-data from one 24-h period after 13 days of the lighting inversion.
The timing of acrophases in postflight group is comparable with vivarium and synchronic control groups.

Solid line-confidence interval for group acrophases

Dotted line- time range for individual acrophases.

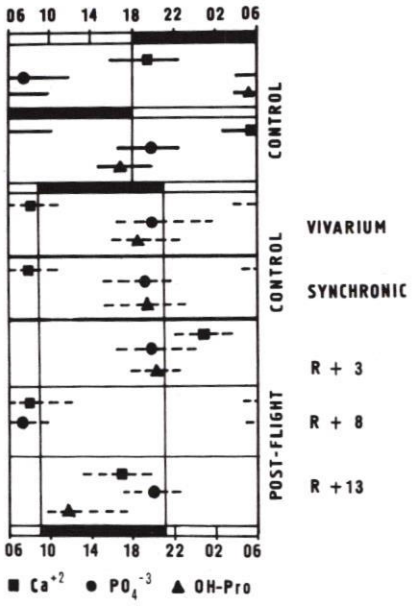

Figure 2. Urinary calcium, phosphate and hydroxyproline acrophases in rats.

Upper part-the first stage of the experiment.
The rhythmic pattern was nearly inverted 180° within 14 days after subjecting the animals to the new light-dark cycle.

Lower part-the second stage of the experiment.
Both control groups-data from one 24h period after 13 days of the lighting inversion.
Note that the timing of acrophases in postflight groups are unstable.

Solid line-confidence interval for group acrophases
Dotted line-time range for individual acrophases

DISCUSSION

Our results suggest that spaceflight may strongly modify the synchronizing effect of phase-shifted light-dark cycle on investigated variables of the rat circadian system. It is accepted that the rhythmic pattern of normal functions in the rodent is effectively synchronized by the light-dark cycle / 6,12 /. In the first stage of the experiment we found that the electrolyte and hydroxyproline excretory rhythms were already synchronized to 180° change in photoperiod after 2 weeks of acclimatization. Therefore the second stage of our experiment began on the 13th day after reversal light-dark cycle, that is at the time when resynchronization should have been completed. In fact, in both control groups the rat's system were completely phase shifted by the new lighting schedule, whereas in postflight they were not and still 10 days later the postflight group showed disorganization of the normal renal rhythms pattern. Of all rhythms studied in the urine duresis and excretion of sodium and potassium were synchronized to the new lighting regimen apart from slight disturbances in the middle period. The excretory rhythms of calcium, phosphate and hydroxyproline appeard to be the most unstable: from one investigation to another we observed remarkable changes in the phasing of its circadian pattern. Furthermore, the mutual coupling among these urinary rhythms were disturbed. The usual timing of acrophases for calcium excretion is about 180° out of phase to the excretion of phosphate and hydroxyproline however the rhythms of calcium and phosphate excretion in the postflight rats were always parallel without a phase shift. The rhythms of phosphate and hydroxyproline excretion which were coupled to each other during initial part of postflight period were decoupled in the end. This might be an evidence of persisting disturbances in the functions of the bone tissue, both of its mineral and organic components. Detailed investigations carried out during spaceflight demonstrated that due to the reduced rate of tissue formation the excretion of calcium and hydroxyproline increased. Increased excretion of nitrogen and phosphate on the other hand, reflects muscular tissue atrophy. The loss of mineral substances never exceeded the tolerance level and all changes were reversible after return to normal conditions / 11 /. Therefore it seems that the observed disturbances in the rhythms of calcium, phosphate and hydroxyproline may be due to delay in resynchronization.

Now, it is accepted at least for some rhythmic variables of rodents that differences in the synchronizing effects of an inverted light-dark cycle are strongly dependent on season, sex and strain / 8,9 /. On the other hand it is well known that some rhythms are modified in their phasing, amplitude and 24-h mean value by season, strain and sex / 8,9 /. Other authors observed the quicker return prereversal circadian pattern of locomotor activity in rats treated with antidepresant drug. These data were discussed in connection with observations of disturbed circadian rhythms in depressed patients / 1 /. These findings indicate that the time required to adjust the biological clock of animals correlates with the state of rhythmic integration required for the obtainment of relevant experimental results.

Hormonal rhythms are usually assumed to be a major pacemaker for circadian rhythms of renal excretion / 10 /.Some data documented that bedrest simulation of weightlessness caused marked internal hormonal asynchrony / 13 /. Also environmental stressors are known to influence the rates of secretion of hormones / 3 /. It is possible that loss of normal hormonal circadian periodicities in mediating system may account for the failure of the adjustment rate. Therefore it may be concluded that under spaceflight conditions the couplings between different oscillators were weakened to such a amount that the synchronizing effect of light was very weak too.

REFERENCES

1. Baltzer,V.,L.Weiskrantz.1975.Antidepresant agents and reversal of diurnal activity cycles in the rat.Biol.Psychiat.10:199-209.
2. Berry,C.A.1970.Medical experience in the Apollo manned spaceflights. Aerospace Med. 41:500-519.
3. Brown,G.M.,D.S.Schalch,S.Reichlin. 1971.Patterns of growth hormone and cortisol response to psychological stress in the squirrel monkey.Endocrinology. 88:956-963.
4. Hahn,P.,T.Hoshizaki,W.R.Adew.1971. Circadian rhythms of the Macaca nemestrina monkey in Biosatellite III.Aerospace Med.42:295-304.
5. Halberg,F.,Y.L.Tong,E.A.Johnson.1967. Circadian system phase,an aspect of temporal morphology;procedures and illustrative examples.Proc. International Congress of Anatomist.In:The cellular aspects of biorhythmus.Symposium on Biorhythms.Springer-Verlag pp 20-48.
6. Hilfenhaus,M.,T.Hertig.1979.Effect of inverting the light-dark cycle on the circadian rhythm of excretion of aldosterone,corticosterone and electrolytes in the rat.In:Chronopharmacology.Eds.A.Reinberg,F.Halberg.Pergamon Press New York pp 49 55.
7. Klimovitsky,V.Ya.,E.A.Ilyin,V.S.Oganov,V.S.Magedov,G.G.Shlyk,L.M.Murashko,K.Hecht,M.Poppai,T.Schlegel K.Kwarecki,Z.Koter,H.Dębiec.1980. Studies of biorhythms in biosatellite experiments.Proc.of IUPS Congress,Budapest.
8. Mayersbach,H.v.,K.M.M.Philippens, L.E.Scheving,1977.Light a synchronizer of circadian rhythms.XII Int. Soc.Chronobiol.Washington 1975. Proceedings.The Publishing House Il Ponte Milano,pp 503-510.
9. Mayersbach,H.v.,K.M.H.Philippens, W.Poesche.1977.Seasonal influences a reason for nonreproducibility of circadian rhythms in highly standarized rats.XII Int.Conf.Int.Soc. Chronobiol.Washington.1975.Proceeding.The Publishing House.Il Ponte Milano,pp 511-524.
10. Moore Ede,M.C.;1975. Internal synchronization of spontaneous circadian oscillators:The identification of the hormonal mediator synchronizing a renal oscillator. Presented at the Symposium on Physiological and Biochemical Aspects of Circadian Rhythms at the 59th Annual Meeting of the Federation of American Societies for Experimental Biology.Atlantic City,New Jersay.
11. Morey,E.R.,D.J.Baylink.1978.Inhibition of bone formation during spaceflight.Science 201:1138-1141.
12. Scheving,L.E.,H.v.Mayersbach,J.E.Pauly 1974.An overviev of chronopharmacology.Eur.J.Toxicol.7:203-227.
13. Winget,C.M.,J.Vernikos-Danellis,C.W. De Roshia,S.Cronin.1974.Rhythms during hypokinesis.In:Biorhythms and human reproduction.Ed.M.Ferin New York,John Wiley,pp 575-587.
14. Woessner,J.F.1961.The determination of hydroxyproline in tissue and in samples containing small proportions of this imino acid.Arch.Biochem. Biophys. 93:440-447.

ULTRASTRUCTURAL QUALITATIVE AND QUANTITATIVE EVALUATION OF CYTOPLASMATIC STRUCTURES OF HEART MUSCLE OF RATS LIVING ABOARD BIOSPUTNIK KOSMOS 936

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ABSTRACT

Ultrastructural morphometrical examinations of some cytoplasmatic structures from heart muscle of rats flight on board of biosputnik Kosmos 936 were carried out. Two groups of animals were examined; one living in weightlessness conditions and the second in weightlessness with artificial, centrifuge induced gravity.
In both groups of animals the following was observed; changes in the structure of mitochondria, smooth endoplasmatic reticulum and miofilaments as well as in glycogen content. Artificial gravity on space craft does not prevent changes in rat heart muscle.

INTRODUCTION

In earlier studies performed on animals completing space flights, disturbed functions and changes in the structure of some organs /1,2,3,4,5,6,7/ were stated. Some authors attribute the said changes to weightlessness. Histological examinations on animals completing space flights did not reveal substantial morphological changes in heart muscle, although hemodynamic disturbances are known to occur in weightlessness.Therefore it seemed advisable to perform ultrastructural qualitative and quantitative examinations of heart muscle cells in animals completing space flight in weightlessness conditions and find out whether artificially induced gravity prevents conceivable changes.

MATERIAL AND METHOD

The studies were performed on SPF rats completing 21-day space flight aboard biosputnik Kosmos 936. Experimental animals were divided into two groups staying in the following conditions:
a/ weightlessness
b/ weightlessness with artificial, centrifuge induced gravity.
Control constituted:
a/ group of animals staying on earth in limited mobility. They were twice exposed to accelerations, vibrations and noise of the same parameters as those occurring during the take off and landing of spacecraft /synchronous experiment/,
b/ group of animals staying in proper laboratory conditions /control/.

There were five animals in each experimental and control group. Immediately on landing, heart tip's samples were taken for study from each experimental and control rat. Material for electron microscopy was prepared by standard method.
Morphometric analysis was carried out on electronograms basing on stereologic methods adapted to electron microscopy studies /8/.
The following parameters of heart muscle cells cytoplasmatic structures were evaluated;

1. Relative volume of mitochondria and smooth endoplasmatic reticulum.
2. Average number of mitochondria and glycogen grains profiles in 100 μm^2 of heart muscle cells section area.

In each group a random sample of 75 electron microscopic pictures were selected and analysed. Thus obtained results were statistically processed basing on single factor variancy analysis.

RESULTS AND DISCUSSION

Stereologic evaluation of relative volume of mitochondria revealed decrease of its value /in relation to control and synchronous experiment/ in both groups of animals completing space flight /Table I/. The difference between the two experimental groups was not statistically important.

In both groups of animals completing space flight also statistically important decrease of relative volume of smooth endoplasmatic reticulum as related to control and synchronous experiment was stated. The difference between the two groups of animals following space flight was not statistically important.
Evaluation of average number of mitochondria profiles in 100 μm^2 of heart muscle cells section area /Table II/ showed their decrease /in relation to control and synchronous experiment/ in both groups of

animals completing space flight. Statistically important differences between the two groups of experimental animals were not found.

TABLE 1

Relative volume /%/ ± SE of mitochondria and smooth endoplasmatic reticulum in heart muscle cells in rats completing space flight aboard biosputnic Kosmos 936.

	Control	Synchronous experiment	Space flight	
			weightlessness	weightlessness with centrifuge
Mitochondria	43.2 ± 1.76	44.7 ± 1.51	34.5 ± 1.51 x	37.05 ± 1.66 x
Smooth endoplasmatic reticulum	1.76 ± 0.09	1.84 ± 0,15	1.45 ± 0.09 x	1.45 ± 0.11 x

x/ Statistically important difference from control at $p < 0.05$

TABLE 2

Average number of ± SE mitochondria and glycogen grains profiles in 200 μm^2 of heart muscle cells section area in rats after space flight aboard biosputnik Kosmos 936.

	Control	Synchronous experiment	Space flight	
			weightlessness	weightlessness with centrifuge
Mitochondria	53.83 ± 2.17	55.86 ± 2.08	34.26 ± 1.62 x	38.66 ± 1.72 x
Glycogen grains	1441 ± 175	1128 ± 158	1688 ± 162	2512 ± 422 x

x/ Statistically important difference from control at $p < 0.05$

Analysing average number of glycogen grains in 100 um^2 of heart muscle cells section area, a statistically important increase in rats completing space flight in artificially induced gravity was observed. In qualitative studies, in both groups after space flight, along with normal ultrastructural picture, sometomes were observed changes consisting in: damaged mitochindria abnormal setting and miofilaments atrophy. On the basis of ultrastructural qualitative and quantitative analysis of cell organelles, conclusions concerning functional state of the organ can be made.

Decrease of the mitochondrial fraction and the number of their profiles, as well as qualitative changes of these organelles found in the cells in both groups of animals completing space flight, can be the result of degeneration processes leading to decay of the discussed structures. It can disturb oxydoreduction processes in heart muscle cells of the studied animals.

In animals experiencing space flight in artificially created gravity, along with changes in mitochondria, accumulation of glycogen was observed. It seems to indicate additional glycogenolisis disturbances in this group of animals.

Changes in transmission and transport of ions through cell membrane as well as hemodynamic disturbances may result from decreased relative volume of smooth endoplasmatic reticulum noticed in rats completing space flight both in weightlessness and in artificially obtained gravity. In this study, also qualitative changes in the structure of miofilaments i.e. contraction apparatus in cells, were observed. These changes were of focal nature. It is worth noting that in earlier histological examinations /6/ obvious changes in the structure of heart muscle cells of animals in weightlessness conditions during space flight were not observed. It must be emphasided that artificially induced gravity /with centrifuge/ did not prevent changes observed in animals in the state of weightlessness during space flight.

REFERENCES

1. Barański S., Barańska W., Godlewska-Jędrzejczyk J., Marciniak M., Rymaszewska-Kossakowska T., Śliwowski A. 1976. Morphological investigations of muscles and neuromuscular junctions in animals staying in weightlessness conditions for 21 days. Interkosmos. IX Symposium of Biology and Space Medicine. Budapest.
2. Barański S., W.Barańska, M.Marciniak, E.J.Ilyina-Kakueva.1979. Morphometric ultrastructural investigations of muscle fibres and neuromuscular junctions in the solues muscle of rats after space flight on the Biosputnik 936.

Aviat.Space.Environ.Med. 50,14
3. Durnowa G.N., A.S. Kaplansky, V.V. Portugalov, N.P.Krivenkova. 1976. Aviat. Space.Environ.Med.47, 588.
4. Ilyina-Kakueva E.J., V.V.Portugalov, N.P.Krivenkova. 1976. Aviat. Space. Environ.Med. 47, 700.
5. Portugalov V.V., N.V.Petrova. 1976. Aviat,Space.Environ.Med. 47, 834.
6. Portugalov V.V., E.A.Savina, A.S.Kaplansky, V.I.Yakovleva,G.I.Plakhuta-Plakutina, A.S.Pankova, P.I.Katunyan, M.G.Shubich, S.A.Buvailo. 1976.Aviat. Space.Environ.Med. 47, 813
7. Savina E.A., A.S.Pankova, E.I.Alekseyev, V.K.Podymov. 1976. Aviat.Space.Environ. Med. 47, 853.
8. Weibel E.R. 1969. Int.Rev.Cytol. 19, 235.

BODY COMPOSITION OF RATS FLOWN ABOARD COSMOS-1129

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Studies of body composition of certain animal species exposed to various chronic effects (G.C. Pitts, 1956, 1973, 1977, 1978; G.C. Pitts, L.S. Bull and J.A. Oyama, 1972, 1975; G.C. Pitts, L.S. Bull, 1977; J.P. Hannon, G.B. Roger, 1975; N. Pace, D.F. Rahlmann, A.M. Kodama and A.H. Smith, 1977) have demonstrated that in adult animals the composition of lean body mass remains unaltered whereas that of adipose tissue varies. There are data indicating gravity dependence of changes of total body mass and individual components (lean body mass, fat, water) (G.C. Pitts, L.S. Bull and J.A. Oyama, 1972).

Body composition was studied in animals after an 18.5-day flight on Cosmos-1129 (Flight Group - F), after a synchronous ground experiment for which the mission was duplicated as closely as feasible but at terrestrial gravity (Synchronous Control Group - S), and in animals which were housed and maintained under standard vivarium conditions (Vivarium Controls - V). The three groups consisted of five rats each. These were male, Wistar derived specific pathogen free, approximately 84 days of age and 270-320 g body mass; they were obtained from the nursery of the Institute of Experimental Endocrinology of the Slovakian Academy of Sciences, Bratislava, Czechoslovakia.

During flight and synchronous experiment the rats were individually housed in cylindrical cages ~ 20.8 cm long and ~ 9.5 cm in diameter. Each cage had feeding and watering devices, a lighting system, and a waste management system attached. Five cages were operationally combined to form a unit. The light period lasted 12 hours - from 8 a.m. to 8 p.m. Moscow time. Every day the rats were fed 40 g of a balanced paste diet of a caloric value of 68.7 Ccal given in 4 equal portions at 6-hour intervals.

All rats were started on the flight 10 days prior to launch (L). At L+6 flight simulation for the S Group was initiated on the ground.

The Vivarium Control rats were housed three per cage. V Group contained 100 animals. They were kept on a 12 hours light: 12 hours dark cycle. The rats received the same paste diet as F and S Groups but in one portion (at 9 a.m.).

At L+18.5 days and 32 hours after recovery the rats were killed by ethyl ether inhalation and dissected following the procedure developed at the Environmental Physiology Laboratory, Berkeley, California, USA. The animals were dissected into: body mass, net body mass (live mass less fur and gastro-intestinal and bladder contents), skin mass, musculo-skeletal system mass (eviscerated carcass + tail) and all viscera.

In skin, musculo-skeletal system and sum of viscera, water content was measured by means of lyophilization and fat content by means of continuous extraction by petroleum ether in a Soxhlet apparatus. All body parts, in dried and fat-free form were reduced to fragments with the aid of Wiley mill and were carefully mixed. In dry fat-free mass, nitrogen was measured by Kjeldahl-Convey procedure; phosphorus was assayed colorimetrically; potassium and sodium by means of emission spectrometry of flame; calcium and magnesium by the atomic absorption spectrometry method; creatine according to the Folin procedure in a modification of Grunbaum and Pace.

Prior to launch, the weight of rats in all groups was approximately equal. During 18.5 days the flight animals gained on an average 43 g, S rats - 62 g, and V rats - 60 g. Body mass of F rats was smaller than that of S rats by 15.8 g and of V rats by 14.0 g.

Studies performed 32-37 hours after recovery showed changes in masses of body and organs. In F animals, there was a significant decrease in net body mass (body mass less masses of fur, gastro-intestinal and bladder contents), masses of skin, spleen and an increase of mass of kidneys as compared to the parameters in S rats expressed in grams (Table 1).

As compared to V animals, F rats showed a significant decrease of skin mass and an increase of mass of viscera due to a significant increase of masses of abdominal fat, GI tract, liver, neck glands and kidneys.

Synchronous Controls exhibited an appreciable increase of viscera mass due to increases of abdominal fat, GI tract, genitalia, and neck glands as compared to the Vivarium Controls.

Both Flight and Synchronous rats showed a slight decrease of mass of the musculo-skeletal system (eviscerated carcass + tail). This decrease did not achieve statistical significance but was more distinct in F rats (Table 1).

The mass of body components expressed as a percentage of total body mass gives a better idea of the changes observed.

Both flight and synchronous experiments resulted in a decrease in mass of musculo-skeletal system, and an increase of masses of abdominal fat, GI tract, and neck glands. Flight conditions as compared to those of synchronous and vivarium controls were responsible for a decrease of skin and spleen masses, and an increase of liver mass. The increase of kidney mass in Flight Group animals was significant only in comparison with Synchronous Controls.

As mentioned above, masses of net and skin in the F rats were less than in the S rats. This was due to a significant decrease of the fat-free (lean) mass and water in skin and eviscerated carcass of the F rats. Percentage of water in total lean mass of the body, skin and carcass of the F rats was also significantly lower than in the S rats (Table 1).

As compared to Vivarium Controls, F rats showed still larger losses of total body water and, accordingly, lean mass both in the skin and in the carcass. In this case fresh body mass did not decrease due to a two-fold increase of fat content and, consequently, a significant increase of dry mass. Fat content increased significantly in skin, carcass, and viscera, the rise being the greatest (2 times) in carcass and viscera.

The rats of Synchronous Controls also exhibited a significant accumulation of fat and, accordingly, dry mass as compared to the Vivarium Controls. However, fat-free mass (lean) decreased since water content in the body lowered. Water losses occurred only in the musculo-skeletal system. The increase of fat content was significant for all components - skin, viscera, and eviscerated carcass.

Bioassays of the homogenate of fat-free dry mass of the whole animal body for nitrogen, creatine, phosphorus, calcium, magnesium, potassium, and sodium revealed some changes. For instance, the content of tissue creatine, phosphorus, and calcium in F rats decreased as compared to that in Synchronous Controls (Table 1).

In comparison to the Vivarium Control Group, these parameters of F rats varied in a similar manner but to a lesser extent.

F rats versus S rats showed a significant decline of the corrected live mass (body mass less fur, GI and bladder contents), lean body mass, water, creatine, phosphorus and calcium.

In F rats as compared to the Vivarium Controls the decrease of lean mass and water was more significant and fall of creatine content in the lean mass was less marked. The fat content in the body almost doubled, and the level of phosphorus and calcium decreased. Due to a significant increase of fat, losses of corrected live mass were less pronounced.

As in the case of F rats, in S rats the content of lean body mass decreased, although to a lesser extent, and fat content increased. Because of a less distinct decrease of lean mass and a significant increase of fat, the corrected live mass of these animals increased slightly. Creatine content in lean body mass was larger than in V rats, and phosphorus and calcium level was lower.

The comparison of F rats with V group makes it possible to evaluate the influence of a combined effect of weightlessness and non-gravitational factors involved in the mission on the body composition. The animals differed markedly in high content of fat in all components of the body; in increased wet and dry masses of the viscera, especially of abdominal fat, GI tract, liver, kidneys, and neck glands; in decreased total body, skin and musculo-skeletal system masses, their fat-free dry mass and water; in decreased quantities of creatine, phosphorus and calcium in the lean body mass. Thus, the combined effects of weightlessness and non-gravitational factors associated with space flight resulted in more significant changes of body composition in the animals than the effect of these factors taken separately.

The comparison of S and V groups emphasizes the effect of non-gravitational factors involved in the mission on the body composition. The content of fat in the skin, musculo-skeletal system and especially in the viscera in S rats was significantly higher than in V. In S rats masses of the viscera, their dry mass, and

TABLE 1. Summary of Cosmos-1129 K-316 Rat Body Composition Results

	Flight Rats n = 5		Synchronous Rats n = 5		Flight Minus Synchronous Mean	% Mean	
	Mean	C.V.	Mean	C.V.	Difference	Difference	P
Total Body Mass, g	349.06	3.8	358.86	1.7	-9.80	-2.7	0.17
Net Body Mass, g	331.38	3.0	344.68	1.0	-13.30	-3.9	0.024
Carcass Mass, g	182.64	5.0	189.91	0.7	-7.27	-3.8	0.12
Viscera Mass, g	72.60	5.1	70.50	5.3	+2.10	+3.0	0.40
Skin Mass, g	52.94	5.0	60.14	4.7	-7.20	-12.0	0.003
Body Fat Mass, g	59.74	6.7	56.33	13.4	+3.41	+6.0	0.40
Carcass Fat Mass, g	19.67	7.1	17.70	20.6	+1.97	+11.1	0.29
Viscera Fat Mass, g	25.71	9.5	23.70	13.3	+2.01	+8.4	0.29
Skin Fat Mass, g	14.36	23.3	14.93	9.6	-0.57	-3.9	0.73
Fat-Free Body Mass, g	271.64	4.5	288.35	2.9	-16.71	-5.8	0.036
Fat-Free Carcass Mass, g	162.97	5.6	172.21	2.6	-9.24	-5.4	0.074
Fat-Free Viscera Mass, g	46.89	6.8	46.80	2.9	+0.09	+0.2	0.96
Fat-Free Skin Mass, g	38.58	4.4	45.21	8.1	-6.63	-4.6	0.006
Dry Fat-Free Body Mass, g	76.11	4.4	78.80	3.5	-2.69	-3.4	0.21
Dry Fat-Free Carcass Mass, g	51.22	5.4	52.61	3.4	-1.39	-2.6	0.37
Dry Fat-Free Viscera Mass, g	11.95	4.3	11.96	2.0	-0.01	-0.1	0.95
Dry Fat-Free Skin Mass, g	12.94	5.4	14.23	8.2	-1.29	-9.0	0.068
Body Water Mass, g	195.53	4.6	209.55	2.7	-14.02	-6.7	0.018
Carcass Water Mass, g	111.75	5.7	119.60	2.3	-7.85	-6.6	0.035
Viscera Water Mass, g	34.94	7.9	34.84	3.4	+0.10	+0.3	0.94
Skin Water Mass, g	25.64	4.3	30.98	8.2	-5.34	-17.2	0.003
Carcass Water Fraction	0.5713	1.6	0.5708	1.5	+0.0005	+0.1	0.93
Viscera Water Fraction	0.1787	5.9	0.1663	2.8	+0.0124	+7.4	0.044
Skin Water Fraction	0.1313	5.3	0.1477	5.5	-0.0164	-11.1	0.009
Body Protein (Nitrogen) Mass,g	58.94	4.9	60.21	3.6	-1.27	-2.1	0.45
Body Cell (Potassium) Mass, g	206.25	5.4	190.52	6.6	+15.73	+8.3	0.069
Body Magnesium Mass, g	0.117	3.1	0.119	11.5	-0.002	-2.0	0.72
Intracellular Water Mass, g	150.56	5.4	139.08	6.6	+11.48	+8.3	0.069
Extracellular Water Mass, g	44.97	17.4	70.47	6.8	-25.50	-36.2	0.001
Body Sodium Mass, g	0.290	4.8	0.327	3.1	-0.037	-11.3	0.001
Body Bone Mineral(Calcium) Mass g	8.37	7.7	10.75	10.5	-2.38	-22.1	0.003
Body Phosphorus Mass, g	1.98	7.5	2.29	12.9	-0.31	-13.5	0.071

fat-free dry mass were larger than in V rats. This was associated with an increase of abdominal fat, GI tract, and neck glands. Also, values of lean body mass and musculoskeletal system in S rats were smaller than in V rats. This was due to a reduced content of water and fat-free dry mass. The content of creatine in fat-free dry mass was higher and that of phosphorus and calcium was lower.

Based on the data obtained an inference can be made that the comparison of body composition in F and V groups is of particular interest because it allows differentiation of weightlessness effects. As a result of an 18.5-day exposure to weightlessness the weight gain in rats was significantly lower. F rats had smaller lean body mass, smaller skin, musculo-skeletal system (eviscerated carcass), and spleen masses. There was a significant increase of liver and kidney masses. F rats and S rats differed mainly in the lower content of water, especially in skin and musculo-skeletal system. Mention should be made of larger kidneys and smaller spleens in F rats, since these organs can be regarded as fat-free components. In fat-free dry mass there was a decreased content of creatine, phosphorus and calcium. These are preliminary data which need further study.

REFERENCES

1. Hannon, J.P., G.B. Rogers. 1975. Body composition of mice following exposure to 4300 and 6100 meters. Aviat. Space Environ. Med., 46, 1232-1235.

2. Pace, N., D.F. Rahlmann, A.M. Kodama and A.H. Smith. 1977. Body composition changes in monkeys during long-term exposure to high acceleration fields. COSPAR XX, Tel. Aviv. v. 2, 2.

3. Pitts, G.C. 1956. Body fat accumulation in the guinea pig. Am. J. Physiol., 185, 41-48.

4. Pitts, G.C. 1973. Effects of gravity on ontogeny in animals. Life Sci. Space Res., 11, 171-176.

5. Pitts, G.C. 1977. Body size and chronic acceleration. Life Sci. Space Res., 15, 251-255.

6. Pitts, G.C. 1978. Physiologic regulation of body energy storage. In press. Metabolism.

7. Pitts, G.C. and L.S. Bull. 1977. Exercise, dietary obesity, and growth in the rat. Am. J. Physiol., 232, 38-44.

8. Pitts, G.C., L.S. Bull and J. Oyama. 1972. Effect of chronic centrifugation on body composition in the rat. Am. J. Physiol., 223, 1044-1048.

9. Pitts, G.C., L.S. Bull and J. Oyama. 1975. Regulation of body mass in rats exposed to chronic acceleration. Am. J. Physiol., 228, 714-717.

STRESS IN SPACE FLIGHT: METABOLIC ASPECTS

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INTRODUCTION

Long-term space flight involves a number of stress-effects affecting the endocrine system and biochemical processes in tissues. Studies of the major physiological systems of rats flown aboard biosatellites of the Cosmos series demonstrated their adaptive capabilities to prolonged weightlessness.

At the same time the studies showed structural and functional signs of activation of the hypothalamic-hypophyseal adrenocortical system induced by space flight factors and rapid return to 1 g.

In order to identify the stress reaction associated with a prolonged exposure to weightlessness, various stress indicators (concentration of catecholamines and activity of enzymes involved in their metabolism in adrenals, hypothalamus, myocardium and blood, hormone content in blood and enzyme activity in the liver) were investigated in different organs and tissues of rats flown for 18.5 days on Cosmos-936. The peculiar feature of the flight program was an experiment to study biological effects of artificial gravity generated by a centrifuge mounted aboard the biosatellite.

METHODS

Studies were carried out on male Wistar-SPF rats weighing 215 ± 5 g. Rats were housed in small cages - rat per cage - at 20-22°C, kept on a 12 hr:12 hr day:night cycle, and fed four times a day. A comprehensive description of flight details was reported in (8). Rats were sacrificed by decapitation, blood withdrawn, and then as soon as possible myocardium, adrenals, hypothalamus and liver were removed, frozen in liquid nitrogen and shipped to the laboratory for further analysis. Blood was analyzed for the content of corticosterone (B) (1), epinephrine (E) and norepinephrine (NE) (20), ACTH and testosterone (RIA); myocardium was examined for the total content of catecholamines (CA) (3), activities of dopamine-β-hydroxylase (DβH) (16), monoamine oxidase (MAO) (24) and catechol-o-methyl transferase (COMT) (25); adrenals analyzed for the CA content (5), B production (22), activities of DβH (16) and tyrosine hydroxylase (TH) (17); hypothalamus was examined for NE content (3), activities of DβH (21), TH (21) and MAO (24); and liver was studied for activities of tyrosine aminotransferase (TAT) (4), tryptophan pyrolase (TP) (7), fructose-1,6-diphosphatase (FDP) (23), glucose-6-phosphatase (G6P) (6), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) (2). Measurements were done in rats flown in the weightless state (F), and rotated in the onboard centrifuge (FC); used in the synchronous experiment as such (S) and during centrifugation (SC), and exposed to angular velocities (A) and compared with vivarium controls (C).

RESULTS

The NE content in plasma in F and FC rats increased significantly 6 hours postflight as compared to vivarium and both synchronous groups. It is interesting to note that the NE content in plasma of flight weightless and centrifuged rats grew by the same value. The E concentration in plasma of flight rats remained unchanged as compared to vivarium controls; the only significant difference was found between F and S rats. 25 days postflight the CA level in flight and control rats was similar but surprisingly increased in all control groups (Fig. 1).

The ACTH level was similar in flight rats as compared to the controls both at R + 0 and R + 25. Accordingly, F rats did not show changes in B production in vitro from the controls. The adrenal reaction to ACTH was also similar in flight and control animals.

At R + 0 the B content in plasma of F rats increased in comparison to that in FC and control rats. At R + 25 this parameter was identical in both flight and control animals and was increased in A rats (Fig. 2).

The testosterone level in plasma of flight animals did not differ from the controls at R + 0 or R + 25.

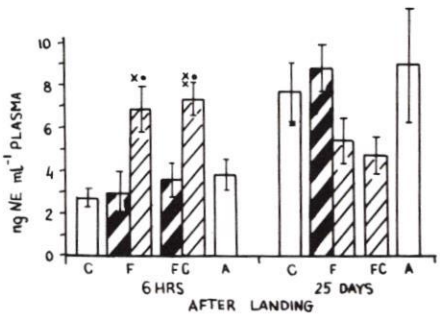

Fig. 1. Norepinephrine (NE) concentration in plasma of Cosmos-936 rats
F - flight weightless rats;
FC - flight centrifuged rats;
S - synchronous rats;
C - vivarium controls;
A - rats exposed to angular acceleration
- synchronous data;
- flight data
$^{x}p < 0.01$ and $^{xx}p < 0.001$ versus C o $p < 0.05$ versus S

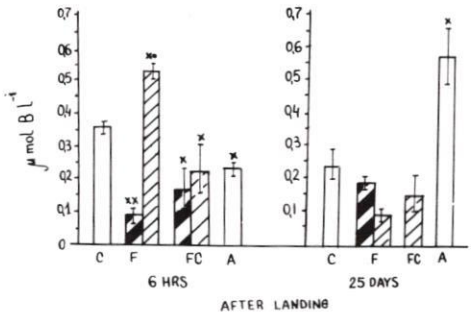

Fig. 2. Corticosterone (B) concentration in plasma of Cosmos-936 rats. For designations see Fig. 1.

Both flight groups - weightless and centrifuged - did not show any changes in the E content in the adrenals as compared to the controls immediately and 25 days postflight (Fig. 3). Measurements of NE in the adrenals of flight and control groups yielded similar results. Activities of CA-synthesizing enzymes (TH and DβH) in the adrenals of flight rats at R + 0 and R + 25 postflight remained unaltered in comparison to the controls.

The NE concentrations in the hypothalamus of weightless and centrifuged flight rats tended to decrease immediately postflight; the decrease being, however, insignificant as compared to S, SC and C controls. The NE concentration lowered significantly only in A group rats. The rats sacrificed 25 days postflight did not show any changes in this parameter (Fig. 4). Activities of CA-synthesizing enzymes (TH and DβH) as well as those of the degrading enzyme MAO in the hypothalamus of flight animals also remained unchanged.

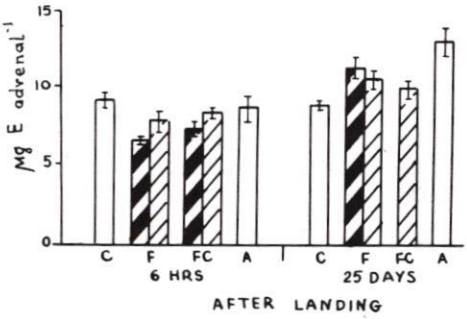

Fig. 3. Epinephrine (E) content in adrenals of Cosmos-936 rats. For designations see Fig. 1.

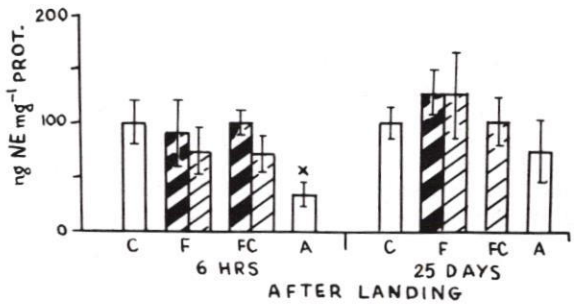

Fig. 4. Norepinephrine (NE) content in the hypothalamus of Cosmos-936 rats. For designations see Fig. 1.

Immediately postflight the CA concentration in the myocardium of F and FC groups was significantly increased as compared to that of C and A rats and insignificantly increased as compared to that of synchronous S and SC groups. It should be noted that CA concentration in the myocardium of synchronous controls did not differ from that of vivarium rats. 25 days postflight this parameter of both flight groups returned to the control level (Fig. 5). Activities of the CA-synthesizing enzyme (DβH) and CA-degrading enzymes (MAO and COMT) in the myocardium of flight rats at R + 0 and R + 25 did not differ from the controls.

Immediately postflight hepatic activities of TAT, TP, ASAT, ALAT, FDP and G6P of flight weightless rats increased as

compared to the vivarium controls and those of flight centrifuged rats remained unchanged. It should be mentioned that both synchronous groups also showed increased activities of ALAT and G6P probably induced by manipulations to which the animals were exposed. At R + 25 enzyme activities in the liver did not differ from the control level (Fig. 6).

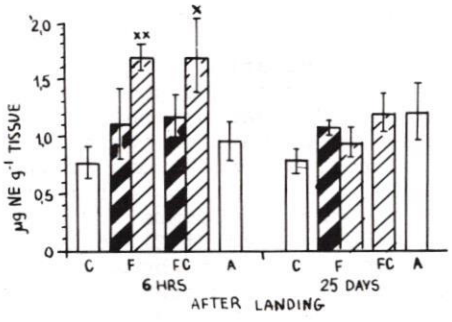

Fig. 5. Content of catecholamines in the heart muscle of Cosmos-936 rats. For designations see Fig. 1.

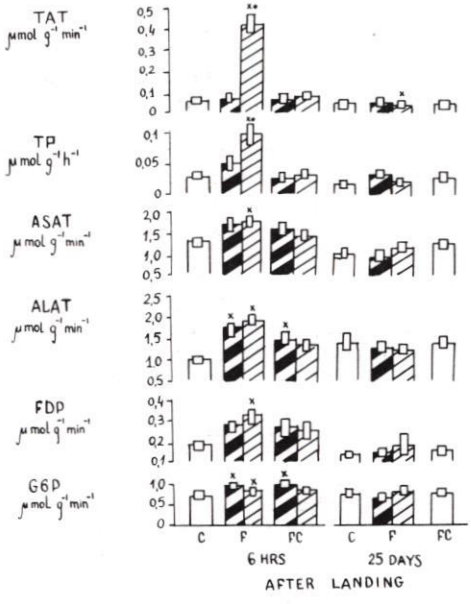

Fig. 6. Activity of hepatic enzymes per g body weight. For designations see Fig. 1.

DISCUSSION

Stress effects of space flight can be reliably assessed through comparison of flight results with the data obtained from animals exposed to an acute or a repeated stress, i.e. immobilization.

The major symptom of a stress reaction of the animal body is activation of the sympatho-adrenal system. During an acute stress the E and NE content in plasma increases; however, the level of this increase depends on the blood withdrawal technique: in immobilized rats killed by decapitation the CA increase was 2-3-fold, whereas in immobilized rats from which blood was withdrawn by means of a special artery catheter the increase was 40-80-fold (15). The NE level in plasma of flight rats increased 2-3 times as a maximum. Bearing in mind the fact that Cosmos-936 rats were sacrificed by decapitation, more significant changes in the CA content of flight animals than actually seen could not have been expected. The notion that decapitation per se is a strong stress agent is supported by the following finding: the content of E in blood of decapitated animals is several times higher than that of NE, whereas in the catheterized blood the proportion is just the opposite (15). To discriminate effects of weightlessness per se, a group of rats was exposed to artificial gravity throughout the flight of Cosmos-936. However, NE levels in plasma of both - weightless and centrifuged - groups did not differ and exceeded the control values approximately 2.5 times. This data gives evidence that the factor responsible for an increased NE content in plasma was not weightlessness as such but another agent that influenced both weightless and centrifuged rats or recovery manoeuvres (the centrifuge generating artificial gravity inflight was stopped several hours before reentry and landing and, therefore, recovery manoeuvres were identical for both groups). At the same time the manoeuvres, being an acute stressor, should have activated the adrenal medulla; however, the E content in plasma of flight rats increased insignificantly. In repeatedly immobilized and later decapitated rats the CA, mostly E level in plasma grew significantly (14). Nevertheless, weightless rats did not show a greater level of E as compared to the centrifuged animals. This data suggests that a prolonged exposure to weightlessness does not act as a stress agent for the sympatho-adrenal system.

TH activity in the adrenals as a sensitive indicator of a repeated stress in flight rats did not alter in any way. This points to the fact that an 18.5 day space flight produces no chronic stress effect. The inference is supported by the finding that TH activity in the adrenals increases

several times after chronic or repeated stress effects (9,10,12): repeated immobilization may result in a 3-4-fold increase of TH and DβH activities in rat adrenals (10,11). It can be obviously postulated that at early flight stages TH and DβH activities increased and at later flight stages they returned to the normal, i.e. developed adaptation. This, however, seems very unlikely since the stress-induced enhancement of the enzyme activity brings about an increase in the protein moiety of enzymes; besides, the thus changed TH activity in the rats immobilized 7 times returned to the normal 14 days post-test; this time period has a half-life of 3 days (10). It should be indicated that TH and DβH activities in the adrenals of weightless rats did not differ from those of centrifuged animals. It can therefore be asserted that weightlessness did not induce changes in the adrenomedullary system, and artificial gravity did not have a target to be restored. Simultaneously, this data gives evidence that in the course of space flight there was no other long-term stressor because enzyme activity in the adrenals remained unaltered postflight. The CA content in the adrenals of flight rats did not change either, thus suggesting that prolonged weightlessness does not act as a powerful stressor for the adrenomedullary system.

Activation of the adrenocortical system is another important indicator of a stress-reaction of the animal body. However, the 18.5 day space flight did not reveal any signs of adrenocortical activation in flight rats. In view of the fact that the adrenal production of B remained unchanged postflight, it can be thought that the increase in the B content in plasma of weightless rats occurred also due to a changed degradation of the enzyme.

Our findings demonstrated that a prolonged space flight, both in weightlessness and artificial gravity, did not cause any significant changes in the NE concentration in the hypothalamus. It is known that an acute stress results in a loss of CA in the hypothalamus (13), whereas a repeated or chronic stress leads to adaptation with no further decline of CA in the organ (13). Therefore, the invariability of the CA concentration in the hypothalamus of flight rats can be interpreted in a dual way: a) an 18.5 day flight is no stressor, and b) by the end of an 18.5 day flight animals develop adaptation which prevents detection of any changes in the NE content in the hypothalamus. Repeatedly immobilized rats in which the NE concentration was already similar to that in controls showed a significantly enhanced activity of TH (13), thus suggesting an increased synthesis of CA in the hypothalamus of the adapted animals. This gave impetus to our measurements of TH and DβH activities in the hypothalamus of flight rats which, however, remained unaltered. Our data gives evidence that the NE content did not develop any changes due to the fact that space flight was no chronic stressor rather than due to enhanced synthesis of CA. This concept also finds support in the lack of changes in MAO activity in flight rats, because invariability of the CA concentration in the hypothalamus is not a manifestation of CA altered degradation.

The CA concentration in the myocardium of flight rats increased significantly in comparison to the vivarium control and insignificantly in comparison to the synchronous control, probably, due to the limited number of rats used. It is interesting to note that in Cosmos-782 rats the increase in the myocardium CA content was singificant as compared to both controls. The increase in the CA content cannot be ascribed to weightlessness effects because it also occurred in centrifuged rats. Therefore, the factor responsible for this increase seems to be not weightlessness per se but another space flight factor which affected both weightless and centrifuged rats in a similar fashion before and during reentry. The increased myocardium CA content could have been attributed to changes in CA metabolism but, in view of the invariability of activities of DβH (CA-synthesizing enzyme) and MAO and COMT (CA-degrading enzymes) in the myocardium, it can be postulated that the increase was not induced by an altered synthesis or degradation of CA in the myocardium; it was presumably brought about by an augmented uptake or a reduced excretion of CA, or altered properties of receptors, etc. In any case, a change in the CA content in the myocardium of rats postflight is indicative of alterations in the function of the sympathetic nerve and, probably, myocardium itself in space flight. Our unpublished data on rat immobilization has demonstrated that an acute stress leads to NE losses, while a repeated stress does not reduce NE content in the myocardium. This invariability of NE content in the myocardium of rats repeatedly exposed to stress effects seems to be a result of an increased CA synthesis because the activity of CA-synthesizing enzymes in the myocardium of these animals was also enhanced. Comparison of this and flight data does not allow the conclusion that prolonged space flight can be considered as a well documented stressor for the sympatho-adrenal system.

Our previous findings give evidence that the hepatic enzymes whose activity may rapidly increase (TAT and TP) are indicators of an acute stress, whereas ASAT and ALAT which need long-term glucocorticoid effects to be activated are indicators of a chronic stress (18,19).

Immediately postflight increased activities of TAT and TP, i.e. the enzymes requiring short-term glucocorticoid effects, were seen; this is suggestive of an acute stress associated, presumably, with the biosatellite recovery. At the same time increased activities of ASAT, ALAT, FDP and G6P in the liver of flight animals are indicative of a chronic stress. This chronic stress seems to be induced by the weightless state because the centrifuged rats did not show an increase in activities of any of the hepatic enzymes tested.

In summary, it can be concluded that the space flight of the above duration was not a powerful stressor for the sympathoadrenal system; the data available speaks in favor of an acute stress associated, in all probability, with the biosatellite recovery, while an increase in activities of ASAT, ALAT, FDP and G6P is the only convincing evidence of weightlessness-induced chronic stress to which rats were exposed throughout the flight. The Cosmos-936 study has demonstrated a good adaptation of animals to prolonged weightlessness alone and to space flight effects on the whole.

REFERENCES

1. Beitins J.Z., Shaw M.H., Kowarski A., Migeon C.J. 1970. Comparison of competitive protein binding radioassay of cortisol to double isotope dilution and Porter-Silber methods. Steroids, 15, 765-776.

2. Bergmeyer H.U. und Bernt E. 1974. Glutamat-Oxalacetat-Transaminase. UV-Test, manuelle Methode. Methoden der enzymatischen Analyse, 3 Auflage 1974, Bd. 1, Verlag Chemie, 769-775.

3. Coyle J.T., Henry D. 1973. Catecholamines in fetal and newborn rat brain. J. Neurochem., 21, 61-67.

4. Diamondstone T.J. 1966. Assay of Tyrosine transaminase activity by conversion of p-hydroxyphenyl pyruvate to p-hydroxybenzaldehyde. Analyt. Biochem., 16, 395-399.

5. Euler U.S. von and Lishajko F. 1961. Improved technique for the fluorimetric estimation of catecholamines. Acta Physiol. Scand., 51, 348-356.

6. Harper A.E., Bergmayer M.W. 1962. Methoden der chemischen Analyse, 3 Verlag Chemie, Weinheim a. Bergstrasse, 788-794.

7. Knox W.E., and Auerbach V.H. 1965. Hormonal control of tryptophane peroxidase in rat. J. Biol. Chem., 214, 307-313.

8. Ilyin E.A., Korolkov V.I., Kotovskaya A.R., Noskin A.D., Kondratyeva V.A., Shipov A.A., Britvan I.I. 1979. Goals and conditions of physiological experiments on rats flown aboard the biosatellite Cosmos-936. Kosm. Biol. Aviakosm. Med., 13, 6, 18-22.

9. Kvetnansky R. 1973. Transsynaptic and humoral regulation of adrenal catecholamine synthesis in stress. In: Frontiers in Catecholamine Research. Eds. E. Usdin and S. Snyder, Pergamon Press, New York, 223-229.

10. Kvetnansky R., Weise V.K., Kopin I.J. 1970. Elevation of adrenal tyrosine hydroxylase and phenylethanol amine-n-methyl-transferase by repeated immobilization of rats. Endocrinology, 87, 744-749.

11. Kvetnansky R., Gewirtz Y.P., Weise V.K., Kopin I.J. 1971. Enhanced synthesis of adrenal dopamine beta-hydroxylase induced by repeated immobilization in rats. Mol. Pharmacol., 7, 81-86.

12. Kvetnansky R., Gewirtz Y.P., Weise V.K., Kopin I.J. 1971. Catecholamine-synthesizing enzymes in the rat adrenal gland during exposure to cold. Amer. J. Physiol., 220, 928-931.

13. Kvetnansky R., Mitro A., Palkovits M., Brownstein M., Torda T., Vigas M., Mikulag L. 1976. Catecholamines in individual hypothalamic nuclei in stressed rats. In: Catecholamines and stress. Eds. E. Usdin, R. Kvetnansky, I.J. Kopin, Pergamon Press, Oxford, 39-50.

14. Kvetnansky R., Weise V.K., Kopin I.J. 1979. The origins of plasma epinephrine, norepinephrine and dopamine levels in stress rats. In: Catecholamines: Basic and Clinical Frontiers. Eds. E. Usdin, I.J. Kopin, J. Barchas, Pergamon Press, New York, I. 684-686.

15. Kvetnansky R., Sun C.L., Lake C.R. Thoa N., Torda T., Kopin I.J. 1978. Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine and dopamine-beta-hydroxylase. Endocrinology, 103, 5, 1868-1874.

16. Molinoff P.B., Weinshilboum R., Axelrod J. 1971. A sensitive enzymatic assay for dopamine-β-hydroxylase. J. Pharmacol. Exp. Ther., 178, 425-431.

17. Nagatsu T., Levitt M., Udenfriend S. 1964. A rapid and simple radioassay for tyrosine hydroxylase activity. Anal. Biochem., 9, 122-126.

18. Nemeth S., Vigas M. 1975. Adrenal hormones and increase of liver tyrosine aminotransferase and tryptophan pyrolase activity after immobilization in rats. Endocrinol. Exp., 9, 2, 100-104.

19. Nemeth S., Vigas M., Kvetnansky R., Orlicky J., Miculaj L. 1977. The effect of repeated immobilization on the level of plasma corticosterone and on the activity of several liver enzymes in rats. Endocrinologie, 69, 1, 87-93.

20. Peuler J.D., Johnson J.A. 1977. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci., 21, 5, 625-636.

21. Saavedra J.M., Brownstein M., Palkovits M., Kozer S., Axelrod J. 1974. Tyrosine hydroxylase and dopamine-beta-hydroxylase: distribution in the individual rat hypothalamic nuclei. J. Neurochem., 23, 4, 869-871.

22. Saffran M., Schally A.V. 1955. In vitro bioassay of corticotropin: modification and statistical treatment. Endocrinol., 56, 523-532.

23. Taketa K., Pogell B.M. 1965. Allosteric inhibition of rat liver fructose 1,6-diphosphatase by adenosine 5'-monophosphate. J. Biol. Chem., 240, 651-662.

24. Wurtman R.J., Axelrod J. 1963. A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol., 12, 1439-1441.

25. Wurtman R.J., Axelrod J. 1966. Control of enzymatic synthesis of adrenal cortical steroids. J. Biol. Chem., 241, 2301-2305.

RESULTS OF MORPHOLOGICAL INVESTIGATIONS ABOARD BIOSATELLITES COSMOS

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Prior to the flights of Soviet biological satellites of the Cosmos series, the question of weightlessness effects on different levels of mammalian structural organization remained open. Nevertheless, it was very important to identify the "substrate" of biological effects of weightlessness in order to advance theories explaining them and to resolve pertinent medical problems. The aim of morphological studies involved was to identify systems and organs showing reactions to weightlessness and to determine the pattern, specificity and reversibility of the changes induced.

Using morphological techniques, important conclusions of biomedical significance have been drawn. First of all, it has been demonstrated that weightlessness does not induce any specific, in the strict sense of the word, changes previously unknown to "ground-based" pathology. Second, an exposure to weightlessness for 18.5-22 days does not cause severe or irreversible structural changes. However, this exposure is not indifferent to the animal body.

All morphological changes detected immediately postflight can be subdivided into two categories: changes occurring inflight and those developing after recovery. Flight changes include, in their turn, manifestations of a weightlessness-associated stress-reaction and shifts caused by weightlessness per se. Postflight changes are a direct result of weightlessness effects, reflecting essentially the "gravity" stress.

This paper concentrates on the changes developing inflight in response to direct effects of weightlessness. Weightlessness-induced changes involve, as an important part, functional rearrangements in the musculo-skeletal system. Lack of static and drastic decrease of dynamic loads in the musculo-skeletal system result in its hypofunction and, as a consequence, in the development of changes typical of "functional atrophy".

Summarizing the results of bone studies, it can be asserted that weightlessness brings about inhibition of periosteal neoformation and long growth of tubular bones, development of osteoporosis of the spongy and, to a lesser extent, of compact substance of tubular bones, and bone demineralization. These changes are more pronounced in weight-bearing tubular bones, metaphyses and epiphyses being damaged in the largest and diaphyses in the smallest degree (1,8,20). The development of osteoposis and reduction of bone density and mineralization lead naturally to a decline in bone strength; due to this, some of flight rats had bone fractures caused by impact acceleration at touch-down.

The question of involvement of regulatory systems in bone rearrangement still remains unanswered. It should be noted that immediately postflight hypophyseal somatotrophs and thyroid C-cells showed morphological signs suggesting their diminished activity inflight. Light microscopy of parathyroid glands did not show significant structural changes although it revealed single sites of activation of parathyreocytes.

The diminished load upon the musculoskeletal system gave rise to atrophic changes in muscles which involved reductions in muscle mass and cross-section of myofibers; in this respect, muscles of hindlimbs were damaged to a greater extent than those of forelimbs; among hindlimb muscles, the most marked changes were noted in antigravity leg muscles and, especially, in the soleus (9). Muscle atrophy was accompanied by metabolic changes. As shown histochemically and electromicroscopically (2,16), postflight skeletal muscles had glycogen and lipid aggregations which may, in our opinion, be associated with decreased energy expenditures due to deterioration of the muscle function. This may cause a dysbalance between the synthesis and utilization of energy substrates: utilization of glycogen and lipids decreased whereas their synthesis remained unaltered or only slightly lowered.

Thus, it can be inferred that during an 18.5-day exposure to weightlessness rearrangement of muscle metabolism at a new

(lower) level is not yet completed.

From our point of view, glycogen accumulation in muscles speaks against intensification of glycolytic processes in weightlessness (5,12); changes in the pattern of lactate dehydrogenase isozymes (from the cardiac type in the norm to the intermediate type after recovery) as well as dystrophic changes of myofibers seem to develop within the first hours after landing due to tissue hypoxia resulting from the dysbalance between drastically increased loads on muscles and their deficient blood supply. Quantitative evaluation of the capillary bed of individual muscles has shown that muscle disuse in weightlessness leads to depletion of almost 30% of capillaries (10).

Musculo-skeletal changes are of dual importance: as such and as contributing to other pathogenetic developments in the animal body in weightlessness. The reduction of total load upon the musculo-skeletal system (and, consequently, lowered oxygen requirements of muscles) and depletion of part of capillaries seem to cause, directly or indirectly, suppression of erythropoiesis and perturbation of thrombocytopoiesis. Morphological signs of inhibition of erythropoiesis were seen in both bone marrow and spleen (3,19). Suppression of erythropoiesis was accompanied by enhanced degradation of erythrocytes. This was indicated by an increase in the spleen content of hemosiderin (4). Increase in hemolysis and decrease in erythrocyte survival time inflight were documented by a specific study of red blood cell survival parameters (11).

Shifts in thrombocytopoiesis in weightlessness were indicated by the appearance in bone marrow of a large number of abnormal megakaryocytes which amounted to 20-30% of their total count (19).

If blood redistribution and partial fluid losses are assumed to take place inflight, then increase in erythrocyte hemolysis, decrease in red blood cell survival (11), inhibition of erythropoiesis, and disturbances in thrombocytopoiesis can be regarded as adaptive reactions aimed at normalizing the proportion of blood formed elements and plasma.

During previous discussions of animal experiments flown aboard biosatellites Cosmos the hypothesis of heart functional unloading in zero-g was often proposed (6). Histological and visual examinations of myocardiocytes did not reveal any structural rearrangements of the myocardium. However, electron microscopic studies (15) indicated a decreased number of mitochondria in myocardiocytes.

Morphological studies of the neuroendocrine system pursued mainly two purposes: to investigate the hypothalamic-hypophyseal-neurosecretory system involved in the regulation of fluid-electrolyte metabolism, and the adenohypophysis-target glands system demonstrating the level of stress-effects and adaptive capabilities of the animal body.

A reservation should be made here that it was extremely difficult to make differential diagnoses of the inflight changes because 5 hours post-recovery was a time period sufficient for alterations induced by return to 1 g to develop.

However, variations in morphological changes in neurosecretory neurons of the supraoptic and paraventricular nuclei of the hypothalamus immediately postflight give grounds to believe that their function inflight was not enhanced.

A significant decrease in the number of Herring bodies in the posterior lobe of the hypophysis and a marked reduction of the volume of nuclei of pituicytes were indicative of possible reduction of the basal level of secretion in the weightless state (17).

After 18.5-22 day flights the adenohypophysis did not show any morphological signs of its dysfunction at the cellular level. This conclusion has been drawn from the lack of pathological cellular forms, that develop after prolonged disorders in hormone formation, and from the status of endocrine glands, whose function is controlled by adenohypophyseal hormones. The absence of important structural changes is consistent with biochemical data suggesting no changes in the hormone content in the adenohypophyseal tissue (7). Nevertheless, the study of the adenohypophyseal state furnished direct and indirect evidence indicating potential decline in the activity of cell elements, particularly somatotrophs and thyreotrophs, in weightlessness.

This inference was based on examinations of the adenohypophysis and the thyroid gland of weightless and centrifuged rats. The weightless rats sacrificed immediately (5-8 hours) after recovery showed decreases in the nuclear volume (23%) and somatotroph size (14%) as compared to the vivarium controls. The diminished functional activity of thyreotrophs in weightless rats was indicated by the state of the thyroid gland characterized by an increase in the size of follicles, a decrease in the height of thyreocytes, colloid densification, lack of resorption vacuoles, and a change of colloid tinctorial properties indicating its diminished content of thyreoglobulins. Alongside with that, it was found that the number, size and volume of

nuclei of C-cells producing thyro-calcitonin involved in the regulation of calcium metabolism decreased (13). The lack of such changes in somatotrophs and thyreotrophs of centrifuged rats gave evidence that the above perturbations could be attributed to weightlessness effects.

As it can be inferred from the state of gonadotrophs and testes, weightlessness did not disturb the gonadotropic function of the adenohypophysis (14).

Assessment of the adrenocorticotropic function of the adenohypophysis and, accordingly, the adrenal cortex in weightlessness presented the largest difficulties because even the earliest examination of sacrificed animals at the recovery site (5-8 hours after touch-down) showed morphological signs of an acute stress-reaction in these organs. However, a consistent decrease of the mass of lymphoid organs is indicative of an increased functional activity of the adrenal cortex inflight and, consequently, of a stress-reaction developing in weightlessness. Today it is beyond doubt that involution of the thymus and hypoplasia of the lymphoid tissue of the spleen and lymph nodes took place inflight, being induced by the stress-effect of weightlessness (3).

This contention is confirmed by the absence of hypoplasia of lymphoid organs of centrifuged rats (4). If hypoplasia of lymph organs persisted throughout the flight, then the total structure of the adrenal cortex essentially recovered by the end of the flight. Normalization of the cortical structure (architectonics of its components) is an indication of animal adaptation to the space environment; this suggests that weightlessness exerts a stress effect which, however, is not strong. This point of view finds support in the fact that adaptation to weightlessness could be accompanied by an increase in the adrenal mass, which, however, was no more than 20% or in some cases insignificant (18). Thus, the direct effect of weightlessness causes inhibition of bone growth, resorption and demineralization of bone tissue, functional atrophy of muscles, depletion of a portion of capillaries in muscles, inhibition of erythropoiesis and thrombocytopoiesis, and appearance of morphological signs of a decreased function of certain components of the neuroendocrine system. A stress-reaction in weightlessness results in involution of lymphoid organs.

All morphological effects of weightlessness per se are directly or indirectly associated with a diminished function of different organs and systems, reflecting adaptation of the animal body to a new environment. The ensuing consequence of weightlessness effect and adaptation is the development of a "gravitational" stress and morphological changes developing at an acute stage of readaptation to Earth gravity. The level of these changes may be a measure of deconditioning and adaptation of the animal body to weightlessness.

REFERENCES

1. Asling C.W. 1979. Histological examinations of the tibia. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Nauka, Moscow, 157-165.

2. Baranski S., Baranska W., Marciniak, M., Ilyina-Kakueva E.I. 1978. Ultrasonic investigations of the soleus muscle after space flight aboard Cosmos-936. Aviat. Space Environ. Med., 50, 9, 930-934.

3. Durnova G.N., Kaplansky A.S., Portugalov V.V. 1977. Changes in lymphoid organs of rats in space flight. Arch. Anat. Histol. Embryol. 72, 5, 14-20.

4. Durnova G.N. 1978. Comparative study of lymphoid organs of rats exposed to weightlessness and artificial gravity in space flight. Ibid. 75, 11, 41-46.

5. Gaevskaya M.S., Nosova E.A., Beletskaya R.A., Veresotskaya N.A., Kolganova N.S., Kolchina E.V., Kurkina L.M. 1979. Effect of spaceflight on metabolism of skeletal muscles. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Nauka, Moscow, 104-109.

6. Gazenko O.G., Genin A.M., Ilyin E.A., Serova L.V., Tigranyan R.A., Oganov V.S. 1980. Weightlessness adaptation and its physiological mechanisms (Results of animal experiments aboard Earth biological satellites). Izvestia AN SSSR, Ser. Biol., 1, 5-18.

7. Grindeland R.E., Keil L.S., Ellis S., Parlow A.F., Kendall J.W., Geschwind I.I. 1979. Concentration of hypophyseal hormones in plasma and hypophysis. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Nauka, Moscow, 74-79.

8. Holton E.M., Baylink D.G. 1979. Quantitative assessment of selected bone parameters. Ibid, 148-157.

9. Ilyina-Kakueva E.I., Portugalov V.V. 1979. Effect of artificial gravity on skeletal musculature of rats in

space flight. Arch. Anat. Histol. Embryol., 76, 3, 22-27.

10. Kaplansky A.S. 1978. Peculiar features of microcirculation in the gastrocnemius muscle of rats exposed to weightlessness. Ibid, 75, 8, 27-30.

11. Leon H.A., Serova L.V., Landaw S.A. 1979. Changes in the parameters of red blood cell survival. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Nauka, Moscow, 183-186.

12. Petrova N.V., Portugalov V.V. 1977. Lactate dehydrogenase isoenzymes of rat skeletal muscles after space flight and hypokinesia. Kosm. Biol. Aviakosm. Med., 5, 66-72.

13. Plakhuta-Plakutina G.I. 1980. The state of C-cells of the thyroid gland of rats after flight aboard biosatellites of the Cosmos series (morphological data). Ibid, 14, 3, 29-33.

14. Plakhuta-Plakutina G.I., Serova L.V., Dreval V.A., Tarabrin S.B. 1976. Effect of 22-day space flight on the state of sex glands and reproductive capacity of rats. Ibid., 10, 5, 40-47.

15. Rokhlenko K.D., Muldiyarov P.J. 1979. The ultrastructure of the myocardium of rats flown aboard Cosmos-936. Proceed. XI Soviet Conf. Electron Microscopy, Moscow, Abstr., 221.

16. Savik Z.F., Rokhlenko K.D. Effect of space flight factors on the ultrastructure of skeletal muscles. Proceed. XI Soviet Conf. Electron Microscopy, Moscow, Abstr., 223.

17. Savina E.A., Alekseev E.J. 1980. On the functional state of the posterior lobe of the neurohypophysis of rats flown on Cosmos-936 (morphological data). Arch. Anat. Histol. Embryol., 78, 1, 62-68.

18. Savina E.A. 1978. Morphological examinations of the adrenals of rats flown on Cosmos-605 and Cosmos-782. Ibid, 75, 9, 30-36.

19. Shvets V.N., Portugalov V.V. 1976. Space flight effects on the hemopoietic function of bone marrow of the rat. Aviat. Space Environ. Med., 47, 7, 746-749.

20. Yagodovsky V.S., Gorokhova G.P. 1979. Changes in skeletal bones. In: Effect of Dynamic Factors of Space-flight on the Animal Body. Ed. Genin A.M. Nauka, Moscow, 165-174.

STUDIES OF SPECIFIC HEPATIC ENZYMES INVOLVED IN THE CONVERSION OF CARBOHYDRATES TO LIPIDS IN RATS EXPOSED TO PROLONGED SPACEFLIGHT ABOARD COSMOS 1129

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Examination of liver, blood, muscle and skeletal tissues from rats aboard the earlier Cosmos flights, indicated changes in the lipid and carbohydrate levels of these tissues in response to space flight (1-5).

After the 936 space mission, specific alterations in hepatic enzyme activities (diglyceride acyl transferase, palmitoyl-CoA desaturase and glycogen phosphorylase) as well as changes in liver glycogen and the levels of specific fatty acids were noted in flight animals but not in comparable animals subjected to continuous 1G centrifugation during the mission (6).

The present study (Cosmos 1129) was designed to reinvestigate some of the 936 observations and to extend the range of inquiry to include additional hepatic microsomal and mitochondrial enzymes, as well as other liver constituents (triglycerides, phospholipids and sterols) not included in our Cosmos 936 protocol.

The experiment called for 25 rats to be caged individually within the spacecraft. Seven rats were sacrificed at recovery, (R+0) and 5 animals after 29 days, (R+29). Of the remaining flight rats, 7 were stressed in a "backupward" position on days 3, 4, 5 and 6 after recovery, and were sacrificed after the final stress on day 6 (R+6S). The other 6 rats served as unstressed flight controls and were also sacrificed on day 6 (R+6). Synchronous controls, housed and fed in the same manner as the (R+0) and (R+29) flight animals were included in the experimental design.

Sacrifice of the animals and preparation of liver homogenates and mitochondria were done in the Soviet Union. Pieces of the excised livers (4 grams) were individually homogenized in a Potter-Elvehjem tissue grinder at 2°C. The nuclei and cell debris were sedimented at 800 x g for 10 min. The pellet was discarded and the supernatant fraction containing cytosol, mitochondria and microsomes was centrifuged at 4500 x g for 30 min. which served to pellet the mitochondria. The supernatant fraction (cytosol plus microsomes) from this centrifugation was removed and immediately frozen to -80° for shipment to our laboratories in the U.S.A. The crude mitochondrial fractions were first washed by resuspension in sucrose and recentrifugated at 4500 x g for 30 min. for isolation. They were then frozen to -80°C for shipment to the U.S.A. Additional samples of each liver (approx. 100 mg) were frozen to -80°C prior to shipment to the U.S.A. for subsequent analysis of specific liver constituents.

Upon arrival, the homogenate fractions were slowly thawed, and centrifuged at 100,000 x g for one hour at 2°C to separate the cytosols and microsomal particles. The cytosol and resuspended microsomes were divided into small aliquots which were individually frozen and stored at -80° until used for analysis.

Table 1 lists the liver constituents and enzyme activities that were examined in this study and indicates which of these were significantly affected by the 18.5 day flight. The negative sign denotes a decrease and the positive sign, an increase.

Caution must be used in interpreting these results of the Cosmos 1129 hepatic tissues, since the cytosol + microsomal homogenate samples which were sent to the United States were not completely devoid of mitochondrial elements. The presence of mitochondria was confirmed by measuring cytochrome oxidase activity in pellets obtained by centrifugation of these homogenate fractions at 8,000 x g for 10 min. at 2°C. We have previously noted that such contamination by mitochondria of the cytosol + microsomal fractions can adversely affect certain enzyme activities. For example, some microsomal enzymes showed one tenth to one-twentieth the activity normally found in rat liver. Some doubt also exists about the similarity of treatment used for the rats in each group in that the levels of hepatic glycogen differed significantly from one control group to another.

Given these constraints, the data were compiled and statistically evaluated. The activities of most of the enzymes and of the liver constituents appeared to be unaffected by the weightless condition, confirming our observations in Cosmos 936. A significant difference was seen between the flight and synchronous control animals at (R+0) with respect to their contents of liver glycogen. The weightless group contained 30% more glycogen than that of the synchronous control animals. This finding also confirms our previous observations aboard Cosmos 936. However, in the present study we observed no significant decrease in glycogen phosphorylase activity nor was there an increase in glycogen synthetase activity (Table 1).

Once again a very significant difference was found in the ability of the flight animals to

TABLE 1

Values and Differences Found in Livers of Flight and Synchronous Control Rats at (R+0) and (R+29)

Measurement	Units[1]	(R+0) Flight	(R+0) Synch.	(R+0) Flight vs. Synchronous	(R+29) Flight	(R+29) Synch.	(R+29) Flight vs. Synchronous
Liver Constit.							
Glycogen	a	4.7±1.0	3.3±0.8	+30% < 0.02	0.2±0.1	2.9±1.0	-94% < 0.001
Total lipids	a	3.8±0.7	3.7±0.8	none	4.3±0.5	4.4±0.3	none
Phospholipids	a	2.3±0.2	2.4±0.4	none	3.0±0.3	2.8±0.2	none
Total cholesterol	a	0.4±0.1	0.4±0.1	none	0.6±0.1	0.5±0.1	none
Triglycerides	a	1.1±0.5	0.9±0.3	none	0.7±0.1	1.0±0.2	-31% < 0.05
Fatty acids[2]							
16/16:1		8.9±1.5	9.6±4.9	none	15.7±5.9	20.3±7.4	none
18/18:1		1.0±0.3	0.9±0.3	none	2.8±0.8	2.0±0.6	none
18:2/20:4		1.2±0.4	1.2±0.3	none	1.0±0.3	1.1±0.3	none
Enzyme activity							
1. Cytosolic							
GS[2]	b	9.2±1.7	9.1±2.9	none	9.2±2.4	11.0±2.4	none
GP	b	27.2±3.9	28.4±4.9	none	50.8±1.9	21.4±2.9	+58% < 0.001
GK	b	29.4±8.8	36.5±9.9	none	6.3±3.4	6.0±3.7	none
HK	b	4.8±1.2	5.2±1.4	none	3.7±1.4	8.4±3.8	-56% < 0.05
G6PDH	b	27.5±11.5	20.5±8.3	none	53.0±15	62.7±18	none
6PGDH	b	16.3±2.3	16.1±2.6	none	15.5±1.8	17.3±4.5	none
AC	b	61.5±14.4	61.6±12.6	none	53.5±11.3	69.9±27.0	none
GPT	b	632±86	533±66	+16% < 0.05	610±50	551±130	none
GOT	b	464±75	399±51	none	444±59	302±57	none
ICDH	b	330±56	378±56	none	387±28	420±45	none
LDH	b	6547±1384	6408±457	none	6779±1053	6339±866	none
MDH	b	5452±697	5229±352	none	5965±567	6678±457	none
2. Mitochondrial							
GDH	b	603±369	822±250	none	-	-	-
ICDH	b	45±18	62±7	-27% < 0.05	-	-	-
AC	b	6±4	13±5	-52% < 0.01	-	-	-
MDH	b	1282±636	1546±243	none	-	-	-
3. Microsomal							
αGP acyl trans.	c	12.8±6.2	21.6±9.7	none	17±13	16±15	none
DG acyl trans.	c	39.3±23.9	133±72	-70% < 0.01	43±26	35±24	none
PC phosphotrans.	b	0.9±0.2	1.2±0.2	-22% < 0.05	0.9±0.3	0.9±0.1	none
PE phosphotrans.	b	0.5±0.1	0.6±0.1	none	0.5±0.1	0.4±0.1	none
Pal-CoA Desat.	d	2.1±1.2	3.3±2.4	none	0.9±0.5	1.8±0.8	none
Stear CoA Desat.	d	3.7±2.0	4.7±3.6	none	1.6±1.2	3.1±0.9	none
HMG-CoA Red.	c	24±15	27±15	none	15±5	72±48	-79% < 0.05

[1] Units used are: a for % tissue wt; b for nmoles/min/mg protein; c for picomoles/min/mg protein; d for nmoles unsaturated acyl CoA produced per 5 min per mg protein.

[2] Abbreviations used are: 16 for palmitate; 16:1 for palmitoleate; 18 for stearate; 18:1 for oleate; 18:2 for linoleate and 20:4 for arachidonate; GS for glycogen synthetase; GP for glycogen phosphorylase; GK for glucokinase; HK for hexokinase; G6PDH for glucose-6-phosphate dehydrogenase; 6PGDH for 6-phosphogluconate dehydrogenase; AC for Aconitase; GPT for glutamate pyruvate transaminase; GOT for glutamate oxaloacetate transaminase; ICDH for isocitrate dehydrogenase; LDH for lactate dehydrogenase; MDH for malate dehydrogenase; GDH for glutamate dehydrogenase; αGP for α-glycerol phosphate; DG for diglyceride; PC for phosphatidyl choline; PE for phosphatidyl ethanolamine; Pal-CoA for palmitoyl-CoA; Stear CoA for stearoyl-CoA and HMG-CoA Red. for β-hydroxy-β-methyl glutaryl-CoA reductase.

complex long-chain fatty acids. Both the diglyceride acyl transferase and the PC phosphoglyceride acyl transferase of the flight rats showed severely reduced activities when compared with synchronous controls at R+0. However, these decreased activities did not appear to result in a change in hepatic lipid values. The triglyceride and phospholipid contents were similar for the flight and synchronous control rats at (R+0). As noted in Cosmos 936, all of the affected enzyme activities were similar to control values 29 days post-flight (Table 1).

Stressing the flight rats, after spaceflight, produced changes in the levels of liver constituents not seen in the R+0 flight animals. Total lipids and phospholipids increased in the flight rats after stress while the control groups showed no differences and several enzyme activities were changed (Table 2). It would appear from our data that rats exposed to weightlessness show changes in hepatic constituents and in the activities of certain liver enzymes and that these controls are less capable of tolerating stress than their synchronous controls.

TABLE 2

Values and Differences Found in Livers of Stressed (R+6S) and Non-Stressed (R+6) Flight and Synchronous Control Rats Sacrificed 6 Days After Recovery

		Values For:					
		Stressed, (R+6S)		Non-Stressed, (R+6)		Comparison Between:	
Measurement	Units[1]	Flight	Synch.	Flight	Synch.	Flight (R+6S) vs. (R+6)	Synchronous (R+6S) vs. (R+6)
Liver Constit.							
Glycogen	a	0.4±0.2	1.1±0.3	3.4±0.9	2.9±0.8	-87% < 0.001	-53% < 0.01
Total lipids	a	4.4±0.6	5.0±1.4	3.4±0.8	3.9±0.6	+23% < 0.05	none
Phospholipids	a	2.6±0.3	2.6±0.8	2.1±0.4	2.7±0.4	+19% < 0.05	none
Total cholesterol	a	0.5±0.1	0.5±0.1	0.3±0.1	0.4±0.1	none	none
Triglycerides	a	1.3±0.7	1.9±0.9	0.9±0.3	0.8±0.3	none	+61% < 0.02
Fatty acids[2]							
16/16:1		12.8±5.2	16.1±2.0	9.4±3.6	9.6±2.2	none	+40% < 0.001
18/18:1		1.2±0.5	1.4±0.5	1.1±0.3	1.3±0.3	none	none
18:2/20:4		1.5±0.6	1.3±0.4	1.0±0.3	1.0±0.3	none	none
Enzyme activity							
1. Cytosolic							
GS	b	10.9±2.2	7.3±2.8	8.7±1.5	10.8±1.6	none	-32% < 0.05
GP	b	36.5±10.2	41.4±6.8	32.2±7.0	33.3±3.5	none	+20% < 0.05
GK	b	3.8±1.3	16.4±10.8	25.8±10.8	31.9±5.6	-85% < 0.001	-49% < 0.01
HK	b	2.6±1.2	4.1±1.3	5.3±1.5	6.3±1.4	-51% < 0.01	-35% < 0.02
G6PDH	b	39.1±23.8	42.2±30.1	48.3±21.9	27.8±6.1	none	none
6PGDH	b	25.0±9.6	17.1±3.9	23.8±5.1	16.6±3.3	none	none
AC	b	75±16	66±20	63±18	53±22	none	none
GPT	b	698±163	573±169	596±185	557±78	none	none
GOT	b	617±101	388±84	377±94	422±94	+39% < 0.001	none
ICDH	b	430±79	425±34	396±33	430±46	none	none
2. Microsomal							
αGP acyl trans.	c	29.9±22.3	35.4±11.9	22.2±4.1	28.8±15.2	none	none
DG acyl trans	c	11.1±4.0	227±181	194±138	217±179	-94% < 0.01	none
PC phosphotrans.	b	0.5±0.3	1.2±0.2	1.0±0.3	1.3±0.3	-54% < 0.02	none
PE phosphotrans.	b	0.2±0.1	0.5±0.1	0.4±0.2	0.6±0.1	-58% < 0.01	-23% < 0.05
Pal-CoA Desat.	d	0.4±0.3	1.4±1.2	4.6±1.5	2.8±1.2	-92% < 0.001	none
Stear CoA Desat.	d	1.4±2.4	1.8±1.3	6.4±4.0	4.1±2.4	-78% < 0.02	-73% < 0.05
HMG-CoA Red.	c	14±5	17±5	15±3	23±14	none	none

[1] and [2] - See Table 1 for footnotes.

REFERENCES

1. Berry, C. A. 1973. Weightlessness. In Bioastronautic Data Book, 2nd ed., Chap. 8, pp. 349-415. Washington, D.C., NASA, (NASA SP-3006).
2. Belitskaya, R. A. 1977. Carbohydrate and lipid content of liver tissue following a 22-day space flight. Space Biol. Aerospace Med. 4:97-99.
3. Yakovleva, V. I. 1977. Arkhiv. Anat. Gisto logii i Embriologii 73:39.
4. Preliminary reports from KOSMOS 690.
5. Tigranyan, R. KOSMOS 782 Post-flight bio chemical studies of various organs and tissues of rats.
6. Abranam, S., H. P. Klein, C. Y. Lin and C. Volkmann. 1978. The effects of space flight on some liver enzymes concerned with carbohydrate and lipid metabolism in the rat. In Final Reports of U.S. Experiments Flown on the Soviet Satellite COSMOS 936, ed. by S. N. Rosenzweig and K. A. Souza, NASA Tech. Memorandum 78526, pp. 78-134.

EFFECT OF PROLONGED WEIGHTLESSNESS ON CERTAIN ASPECTS OF BRAIN METABOLISM OF THE RAT

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INTRODUCTION

Uniqueness of factors affecting living organisms in space flight determines the lack of their adaptive capabilities developed in the course of evolution. This concerns, first of all, weightlessness. It could be assumed a priori that an exposure to weightlessness would give rise to both direct responses and complex processes of adaptation to the extreme environment, including metabolic changes. After recovery readaptation to 1 g would also affect metabolic conversions - both specific and nonspecific changes in various organs. We focused in particular on metabolic changes in the central nervous system. The present investigation was devoted to the study of certain aspects of brain metabolism of rats, highly organized mammals with a well developed higher nervous activity, exposed to space flight for a relatively long period of time.

METHODS

Experiments were carried out on male Wistar-line rats flown aboard biosatellites Cosmos-605, 782, and 936. The experimental conditions, rat selection and training were previously described (3). Some of the Cosmos-605 rats were decapitated a day after recovery and transportation to Moscow. Part of Cosmos-782 and Cosmos-936 rats were sacrificed immediately after landing at the recovery site. Other flight rats were examined 25-26 days postflight, during which they were kept in a vivarium. As controls two groups of rats were used: a) rats kept in a vivarium - vivarium controls, and b) rats kept in a biosatellite mock-up and exposed to the flight profile, except for weightlessness, within the flight time period - ground-based synchronous controls.

During the Cosmos-936 flight some rats were exposed to artificial gravity of 1 g in order to eliminate weightlessness effects (effects of all other space flight factors being maintained). With respect to this flight, two additional control experiments were conducted: c) as in group b) but with centrifugation at 1 g, and d) exposure to angular acceleration. Some of the control animals were sacrificed simultaneously with flight rats immediately after recovery, and the remaining rats were sacrificed 25 days after their transportation to the vivarium.

Cosmos-605 flight and control rats were analyzed cytospectrophotometrically for a relative concentration and absolute content (per cell) of proteins and RNA in the cytoplasm of cerebellar Purkinje cells and in cells of their perineuronal glia in sections in situ as well as in neurons and gliacytes of the supraoptic nucleus of the hypothalamus and anterior horns of the lumbar segment of the spinal cord (2); in addition, activity of neural protamine-peptidehydrolase was measured in homogenates of the cerebellum, lamina quadrigemina, frontal and occipital parts of large hemispheres (2). The Cosmos-782 experiment also involved measurements of proteins and RNA in cerebellar Purkinje cells and their glial cells-satellites; besides, the content of thiol groups in homogenates of the cerebellum, midbrain and different parts of the cortex of large hemispheres (4), as well as activity of acetyl cholinesterase and nonspecific cholinesterase in homogenates of the midbrain, cerebellum, frontal and occipital parts of the cortex of large hemispheres (1) were measured. Cosmos-936 rats were used for similar measurements of proteins and RNA in cerebellar Purkinje cells and adjacent gliacytes, as well as the content of thiol groups in homogenates of certain parts of the cortex of large hemispheres.

Due to various circumstances we were unable to use identical objects and to measure identical biochemical components; however, the data available are unique and worthy of discussion.

RESULTS

First of all, let us dwell upon the content of proteins and RNA in the cytoplasm of cerebellar Purkinje cells and their perineuronal glia. This parameter was studied in three space-borne

experiments, the results of which are summarized below.

It can be seen from the data obtained (as a percentage of the vivarium control values) that at R + 1 (Cosmos-605) and at R + 0 (Cosmos-782 and Cosmos-936) the absolute content (per cell) of RNA in Purkinje cells of flight animals was significantly reduced and in synchronous rats did not differ from that in vivarium animals (Fig. 1, Table 1). Nevertheless, there were differences between various experimental series. For instance, Cosmos-605 and Cosmos-782 rats did not show changes in the content of proteins in the cytoplasm of cerebellar neurons whereas Cosmos-936 rats displayed a reduced content of both proteins and RNA due to an unknown reason. As to cerebellar gliacytes, their content of RNA remained unchanged in all three cases; the content of proteins was unaltered in Cosmos-605 and Cosmos-782 rats and in Cosmos-936 rats it decreased not only in Purkinje cells but also in their perineuronal glia.

An exposure of rats to artificial gravity aboard Cosmos-936 (Table 1) did not reverse the above changes in the content of proteins and RNA in cerebellar cells noted immediately after recovery. Similar (although less expressed) changes were observed (especially in case of proteins) in synchronous rats exposed to centrifugation at 1 g and angular acceleration. It should be noted that an exposure of flight animals to artificial gravity resulted in a significant decline of RNA content but did not lead to a decrease of the protein content in the glia.

After 25-26 day readaptation of rats to Earth gravity no differences in the protein or RNA content in cerebellar cells were found: the changes proved completely reversible.

An examination of the protein and RNA content in cells of the neurosecretory (stress-sensitive) hypothalamic supraoptic nucleus of Cosmos-605 rats (Fig. 2) at R + 1 revealed a decrease of both variables in neurons and a decrease of proteins in gliacytes; the changes in synchronous rats were insignificant. However, mention should be made of the observation, according to which the decline of the RNA content in neurons of the supraoptic nucleus persisted after protein content already disappeared.

Different variations occurred in cells of the anterior horns of the spinal cord (Fig. 3). At R + 1 the protein and RNA content in the cytoplasm of motoneurons of their perineuronal gliacytes was unchanged whereas at R + 25 the protein content was significantly lowered and the RNA content remained unaltered.

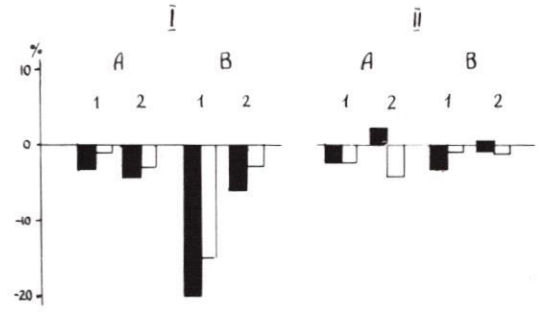

Fig. 1. Absolute content (per cell) of proteins and RNA in the cytoplasm of cerebellar Purkinje cells and their glial cells-satellites. A - proteins, B - RNA; 1 - Purkinje cells, 2 - gliacytes; Cosmos-782 rats, Cosmos-605 rats; I - immediately after recovery, II - 26 days after recovery. Ordinate - changes as a percentage from vivarium controls.

The study of the content of thiol groups in the frontal lobe of the cortex of large hemispheres demonstrated reproducible changes (Figs. 4 and 5). For instance, Cosmos-782 (Fig. 4) and Cosmos-936 (Fig. 5) flight rats showed on the average an almost equal decrease in the amount of thiol groups; synchronous animals exhibited a twice less but significant reduction. It should be added here that centrifugation of flight animals did not reverse this change (Fig. 5); ground-based exposure of control rats to centrifugation and angular acceleration induced changes that did not differ from those regularly observed in synchronous experiments (Fig. 5). At R + 25 the content of thiol groups in the frontal lobe returned to normal, and in the temporal and occipital lobes, mesencephalon and cerebellum this parameter was never changed.

Activities of protamine-peptide-hydrolase in the above segments of the brain of Cosmos-605 flight rats did not differ from those in vivarium controls either at R + 1 or at a later stage.

Immediately after recovery activity of acetyl cholinesterase of Cosmos-782 flight rats was significantly diminished in the frontal lobe and the cerebellum (33% and 10%, respectively); activity of nonspecific cholinesterase was lowered in the frontal and occipital lobes (30% and 31%, respectively) and in the cerebellum (27%). In synchronous rats acetyl cholinesterase activity decreased only in the frontal lobe (12%), and activity of nonspecific cholinesterase in the mesencephalon slightly

TABLE 1. Protein and RNA content in the cytoplasm of Purkinje cells and their glial satellites of Cosmos-936 flight and control rats immediately after recovery (as a percentage of vivarium values)

Experiment	Proteins	RNA
Neurons		
Space flight at 0 g	- 22[x]	- 17[x]
Synchronous control	- 7	- 2
Space flight at 1 g	- 20[x]	- 18[x]
Synchronous control with centrifugation	- 19[x]	- 12[x]
Angular acceleration	- 10[x]	- 16[x]
Glia		
Space flight at 0 g	- 14[x]	- 6
Synchronous control	- 3	± 0
Space flight at 1 g	+ 5	- 16[x]
Synchronous control with centrifugation	- 3	- 2
Angular acceleration	- 1	- 2

Note: [x] - indicates statistically significant changes.

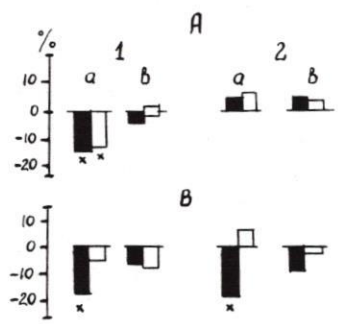

Fig. 2. Absolute content (per cell) of proteins (A) and RNA (B) in the cytoplasm of neurons (■) and their glial cells-satellites (▭) of the supraoptic nucleus.
1 - R + 1, 2 - R + 25; a - flight rats, b - synchronous rats.
Ordinate - changes as a percentage from vivarium controls.
x shows statistically significant changes.

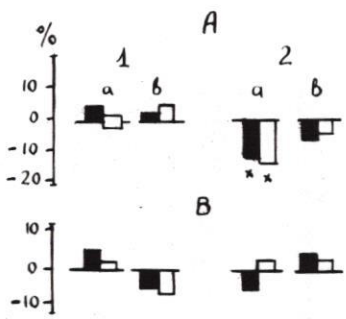

Fig. 3. Absolute content (per cell) of proteins and RNA in the cytoplasm of motoneurons and their glial cells-satellites of anterior horns of the spinal cord. Designations see Fig. 2.

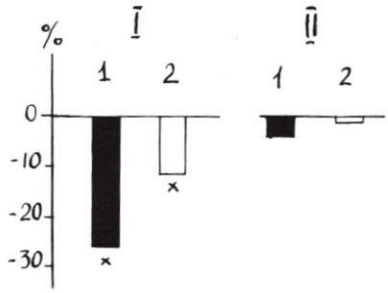

Fig. 4. Content of thiol groups in homogenates of the frontal lobe of the cortex of large hemispheres of the brain of rats flown on Cosmos-782. I - immediately after recovery, II - 26 days after recovery; 1 - flight rats, 2 - synchronous rats. Ordinate - changes as a percentage from vivarium controls. x shows statistically significant changes.

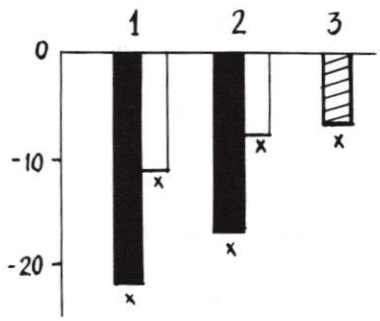

Fig. 5. Content of thiol groups in homogenates of the frontal lobe of the cortex of large hemispheres of the brain of rats flown on Cosmos-936 (immediately after recovery of flight rats). 1 - weightlessness per se, 2 - weightlessness + centrifugation, 3 - angular acceleration; a - flight rats, b - synchronous rats. Ordinate - changes as a percentage from vivarium controls. x shows statistically significant changes.

increased (10%), remaining unaltered in other brain segments.

DISCUSSION

We still do not know potential neurochemical results of metabolic reactions of the central nervous system to acceleration at launch and to a rapid transition to weightlessness during the first days upon insertion into orbit aggravated by specific rat-per-cage housing aboard the biosatellite (3). It can be inferred from our observations that during stable adaptation to space flight factors of prolonged action cerebral metabolism was slightly inhibited; this was well expressed in the brain segments associated with motor activity, i.e. cerebellum and motor (frontal) lobe of the cortex of large hemispheres. The decline in the absolute content of RNA and the normal content of proteins in Purkinje cells may give evidence that the level of protein metabolism in these neurons was lowered, i.e. their function was inhibited. This is also indicated by a decrease in activities of acetyl cholinesterase and cholinesterase. In the motor lobe the content of thiol groups as well as cholinesterase activity decreased; this may also reflect inhibition of central structures. In general, the changes we have identified may be suggestive of passive suppression of the brain functional activity (in the case of deficient external stimuli) rather than be manifestations of stress-reactions. It is obvious that artificial gravity aboard Cosmos-936 exerted an adverse effect on the brain, probably, due to vestibular stimulation induced by large angular acceleration.

REFERENCES

1. Ellman G.L. 1959. Tissue sylfhydryl groups. Arch. Biochem., 82, 70-77.

2. Gazenko O.G., Dyomin N.N., Panov A.N., Rubinskaya N.L., Tigranyan R.A. 1976. Some characteristics of metabolism of proteins and nucleic acids in the central nervous system of rats flown aboard Cosmos-605. Kosm. Biol. Aviakosm. Med., 10, 4, 14-19.

3. Serova L.V., Ilyin E.A., Noskin A.D., Milayavsky V.I., Chelnaya N.A., Golov V.K., Pishchik V.B. 1979. Experimental conditions, animal selection and training. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Moscow, Nauka, 7-12.

4. Sokolovsky V.V. 1962. Measurement of the content of sulfhydryl groups in blood by means of amperometric titration. Laboratornoye delo, 8, 3-6.

EFFECT OF SPACEFLIGHT ON LYMPHOCYTE STIMULATION

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INTRODUCTION

The purpose of this paper is to review experiments on human lymphocytes performed either during space missions or at high-g and simulated low-g in order to test the efficiency of the specific immune system during and after spaceflight.
Past findings on lymphocytes from crew members of spaceships and recent work in our laboratory suggest the hypothesis that low -g depress and high-g enhance the activation of lymphocytes by mitogens in vitro. Since part of the data are contradictory and difficult to interpret further investigations in space are needed. Two experiments on lymphocytes to be performed on Spacelab are described here.

Weakening of the immune resistance against infections is a hazard encountered during and after long duration spaceflight.Therefore the effect of space environment on the immune system has been studied on past missions: The efficiency of specific and non-specific immunity was tested on crew members after flight. However, a clear conclusion cannot be withdrawn from the data available.
This problem is becoming more and more actual since the advent of the Space Shuttle and probably of new large space stations will offer the opportunity to work and stay in space to a broader community of scientists and technicians than so far. This is a valid justification to perform a systematic study on the effect of spaceflight on the immune system on future missions.
Here we will discuss the effect of spaceflight on the specific immune system, i.e. on the capacity of lymphocytes to react specifically against antigens. The interaction between antigen and receptors on the cell surface stimulates lymphocytes to proliferate and to produce antigen-specific antibodies (the last limited to cells of the B-type). Optimal activation is achieved by cooperation between T and B-lymphocytes and probably also via interaction with macrophages.
A similar reaction can be triggered in vitro when lymphocytes are exposed to a number of substances from different origins called mitogens. At difference from antigens, mitogens are able to activate a whole lymphocyte population like all B- or all T-cells or even all B- and T-cells together. The transition from the status of resting to that of activated lymphocytes is an example of cell differentiation (1). Therefore, the in vitro activation of lymphocytes by mitogens at 0-g can be regarded as a suitable model for the study of a) cell survival, b) the triggering of the immune response, and c) the mechanism of cell differentiation in space.
The reactivity of lymphocytes in the presence of mitogens has been widely used as a test of the efficiency of the specific immune system after spaceflight in man and rat (2-6). Most of the work described here was performed with the T-cell specific mitogens concanavalin A (Con A) and phytohaemagglutinin (PHA). However, the tests with Con A and PHA can be considered as an assay of the functional fitness of the whole specific immune system since T-cells play a key role in the regulation of B-lymphocyte response, i.e. in the synthesis of antibodies.
Lymphocytes are easily purified from human peripheral blood with the Ficoll/Hypaque method (7). Activation is routinely measured by incorporation of labeled thymidine or uridine into DNA or RNA respectively (1).

EXPERIMENTS PERFORMED ON SPACE MISSIONS

The reactivity of lymphocytes toward mitogens was tested in crew members before and after spaceflight.
Cells from 5 cosmonauts of the Soyuz 6,7, and 8 missions were exposed to PHA (2): Lymphocytes were purified from blood samples withdrawn 20-30 days before launch and 3-4 days after landing. The flight lasted 5 days. Cell activation was measured by incorporation of tritiated uridine into RNA. It was found that in 4 of 5 cosmonauts the reactivity was significantly diminished after flight.
In the Apollo flights 7 through 17 (duration 10-12 days) the lymphocytes from all 33 astronauts were tested 30, 15, and 5 days before launch, immediately after recovery, and various days later (3). DNA and RNA syntheses were measured in PHA activated cells after 24 and 72 h of incubation. No significant effect was observed, although for various reasons the data from flights 14 to 17 were less consistent.

Analogous tests were performed on the 9 astronauts of the Skylab 2,3, and 4 missions which lasted 29, 59, and 84 days respectively. Lymphocytes showed a markedly decreased response toward PHA when RNA-synthesis was measured after 24 h of incubation. The effect was less evident on DNA-synthesis after 72 h incubation (4). Lymphocytes recovered to normal preflight levels 7-13 days after landing. Lymphocytes from 3 astronauts of the 9-days flight of the Apollo-Soyuz Test Project showed depression of RNA-synthesis after incubation with PHA. However, the data may be affected by the exposure of the crew to toxic gases during splashdown (5).
A summary of these results is given on Table 1.
A slight increase of the blastogenic response over the ground controls was detected in rats flown for 20 days on the biosatellite Cosmos 782 (6).
In conclusion, the data collected so far indicate that, in spite of contradictions and discrepancies, spaceflight has an influence on the reactivity of lymphocytes. The problem is complicated by the fact that the behavior of the immune system is individually different. Probably only studies in vitro under standardized conditions will give a demonstration of a direct effect, if any, of the space environment on lymphocyte activation.

GROUND SIMULATION OF HIGH- AND LOW-g

The adaptation of lymphocytes to spaceflight will be investigated by us on the first Spacelab mission (8) and probably on a NASA Life Sciences dedicated mission.
As a complement to the Spacelab experiments we started a study on the effect of high-g and low-g on the activation of lymphocytes by Con A in vitro. Stimulation was measured by incorporation of ^{3}H-thymidine into DNA, cell ultrastructure was analysed by electron microscopy. High-g were generated in a centrifuge. Low-g were simulated in a fast rotating clinostat (9).We must point out that the clinostat is not generating true 0-g conditions, however, it provides useful indications on possible effects of microgravity on living systems.
We found that rat (10) and human (11) lymphocytes react remarcably faster at 2-g and 4-g than at 1-g. Maximum activation is observed on day 2 of culture at high-g instead of day 3 as usually found at 1-g. In addition, maximum activation is often higher at high-g than in 1-g controls. When lymphocytes were cultured in the clinostat we found that activation by Con A was reduced by 50% (12). Ultrastructural analysis suggests that the gravitational environment may have an influence on the cell cycle: High-g appear to accelerate the activation process, on the other hand cell death also is observed earlier than at 1-g as indicated by the appearance of large vacuoles (10,11). Hypogravity seems to stop cell development in a portion of the lymphocytes (50%) at an early stage observed 12-24 h after exposure to Con A in 1-g controls. This stage is characterized by the presence of a high number of mitochondria in a small cytoplasmic volume (B. Humbel, unpublished observations).
When lymphocytes are cultured with Con A in a flask subjected to vibrations at 500 Hz, activation is remarcably higher than in the control (U. Bay, unpublished observation). This too could be due to a g-effect generated by the vibration.
Irradiation by X-rays (50 rad, 200 kV) depresses activation by 30%. When the combined effect of radiation and hypogravity or of radiation and hypergravity are investigated on lymphocytes, no synergism is observed (11).
The effects described in this section are summarized on table 2.

Table 2. Effect of physical stress on Lymphocyte activation

Conditions	Depression	Enhancement	Ref.
High-g	-	+	10,11
Simulated low-g	+	-	12
Vibration	-	+	
Radiation	+	-	11
Radiation+high-g	+*	-	11
Radiation+low-g	+*	-	11

* No synergism observed

HYPOTHESES AND SPECULATIONS

Although we are comparing results derived from considerably different experiments, our findings and those from previous spaceflights suggest the hypothesis that low-g depress the activity of lymphocytes, whereas high-g have a stimulating effect.
At present we cannot interpret the pheno-

Table 1. Effect of spaceflight on human lymphocyte reactivity

Flight	Duration (days)	Test Subjects	Depression	No Effect	Ref.
Soyuz 6,7,8	5	5	4	1	(2)
Apollo 7-17	10-12	33	-	33	(3)
Skylab 2,3,4	28,59,84	9	9	-	(4)
Apollo-Soyuz Test Project	9	3	3	-	(5)

mena observed in terms of mechanism of activation, however, a number of effects can be envisaged (10) Gravity or weightlessness may change the intracellular distribution of organelles as shown by calculations of Kessler (13). As a consequence the altered distribution of organelles induced by different g-levels may change the concentration of molecules (metabolites, nucleic acids or proteins) or ions (e.g. Ca) involved in lymphocyte activation.
A typical consequence of exposure to mitogens in vitro is lymphocyte aggregation with strong cell-cell interaction. Aggregation could be impaired in the clinostat with consequent weakening of the interaction, an important factor for activation. Folkman and Moscona (14) described the correlation between cell shape and growth: Cells of various lines in suspension are spherical, whereas cells adhering to the walls of a culture flask are rather flat. It was found that the rate of cell growth decreases as cell shape becames more spheroidal. Similarly slow-growing lymphocytes at low-g may tend to assume a spheroidal conformation which is different from that of fast-growing cells at high-g which sink to the bottom of the culture flask.
Finally, one can speculate that each living organism has its own biological clock. Our observations on the effect of gravity on the rate of lymphocyte activation suggest that a not yet identified biological clock in lymphocytes (cytoplasmic streaming?) could be regulated by gravity. Indeed, cytoplamic streaming was observed in our clinostat experiments (12).

EXPERIMENTS ON SPACELAB

This section gives a short outline of two experiments which we are presently preparing for the Spacelab. One will fly on the first Spacelab mission (actual launch date May 26 1983), the other has been selected by NASA as a candidate for a Life Sciences dedicated mission.
The primary objective of our programme is to study the effect of spaceflight on lymphocyte activation in order to establish possible alterations of the specific immune system during prolonged space missions. The experimental approach consists of three main lines of investigations:
1. Discriminate between the effect of stress on the whole organism and the effect of O-g per se on lymphocyte activation. Lymphocytes withdrawn from crew members on mission day 6 will be tested in vitro during flight. The response to mitogens will be compared to that of the same subjects before and after flight.
2. Effect of a gravitational environment between 0 and 4-g. Cells will be cultured during the mission in a multi-g centrifuge providing 0.5, 1, and 4-g environments.
3. Kinetic of lymphocyte activation in space. Stimulation will be measured 1,2,3,and 4 days after incubation with Con A in order to clarify the effect of O-g on the biological clock of the cell.
Three parameters will be determined: DNA and protein synthesis, and cell ultrastructure by electron microscopy. The data collected should allow a prediction on the efficiency of the immunocompetent cells during spaceflight and to test the hypothesis that low-g depress lymphocyte activity and high-g have a stimulatory effect.
Fig. 1 shows the flight hardware to be used on Spacelab 1. One of the major problems encountered was the manufacture of culture flasks satisfying the safety requirements of ESA/NASA and being compatible with cell viability. Forty materials, metals and plastics, were tested. The most satisfactory results were obtained with vessels made of Teflon reinforced by 25% glass fiber. The carry-on incubator has a working temperature of 37°C. It can be powered either by batteries or by direct current 28V available on Spacelab.

CONCLUSIONS

Investigations hitherto performed in space or ground simulations at high and low.g indicate that only studies performed in vitro during flight will provide conclusive information on the effect of spaceflight on lymphocyte activation. Our experiments on Spacelab should answer at least part of the open questions. Man in Space is needed as test subject and operator of the experiment. Several actual aspects of cell biology and biochemistry like growth, differentiation, ultrastructure, and biosynthesis of macromolecules are involved in this study.

ACKNOWLEDGEMENT

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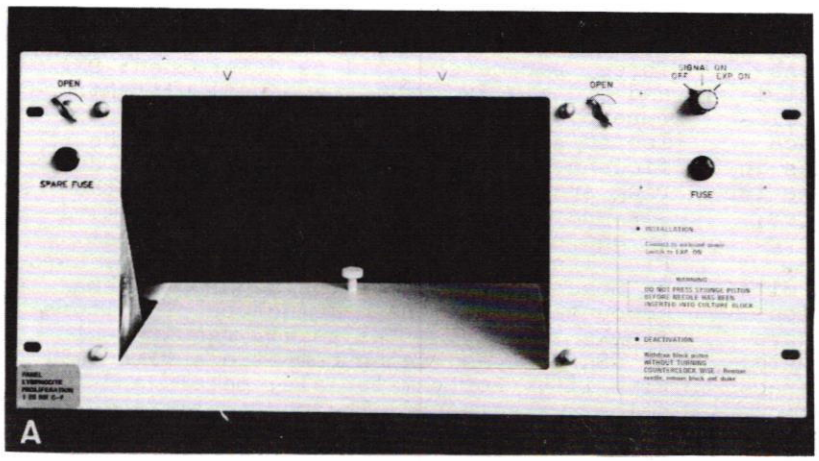
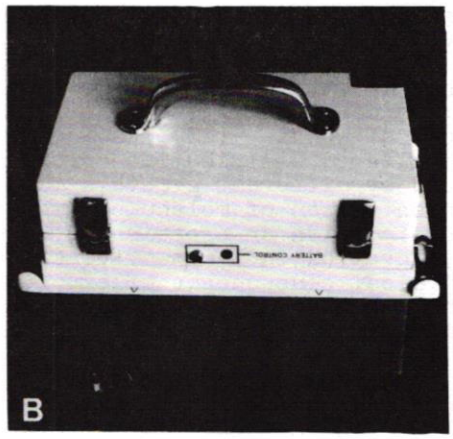
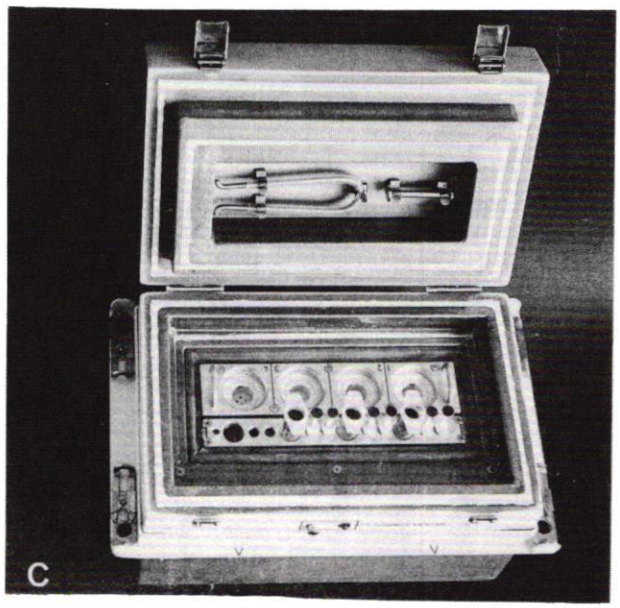
REFERENCES

1. Ling,N.R., and J.E. Kay. 1975. Lymphocyte stimulation. Amsterdam: North-Holland Publ. Co.
2. Konstantinova,I.V., Ye.N. Antropova,V.I. Legen'kov, and V.D. Zazhirey. 1973. Space Biology and Medicine 7: 48-55.
3 Kimzey,S.L., C.L. Fischer, P.C. Johnson, S.E. Ritzmann, and C.E. Mengel. 1975. Biomedical results of Apollo. NASA SP-368: 197-226.
4. Kimzey, S.L. 1977. Biomedical results from Skylab. NASA SP-377: 249-282.
5. Criswell, B.S. 1979. Biospex: Biological space experiments. NASA TM 58217: 14.
6. Mandel,A.D., and E. Balish. 1977. Aviat. Space Environ. Med. 48: 1051-1057.
7. Boyum,A. 1976. Scand. J. Immunol. Suppl. 5: 9-15.
8. Cogoli, A. 1978. Spacelab Mission 1, Experiment description. NASA TM-78173: V, 17-19.
9. Briegleb,W., A. Schatz, and J. Neubert.







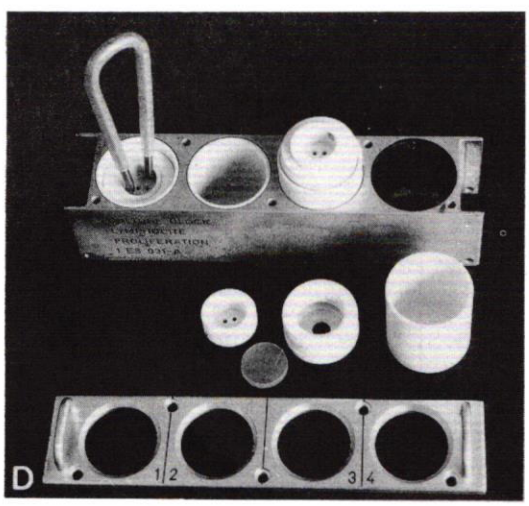


Figure 1. Flight hardware for Spacelab-1.
A) Front panel to be fixed to single rack in SL-Module,
B) Carry-on incubator,
C) Incubator with cell culture block, syringes and tools,
D) Chamber block and cell culture chambers.

1976. Umschau Wiss. Techn. 76:621-623.
10. Cogoli,A., M. Valluchi-Morf, H.R. Böhringer, M.R. Vanni, and M. Müller. 1979. Life Science and space research XVII, COSPAR, Oxford: Pergamon Press, 219-224.
11. Cogoli,A., M. Valluchi, J. Reck, M. Müller, W. Briegleb, I. Cordt, and Ch. Michel. 1979. The physiologist 22: S, 29-30.
12. Cogoli,a., M. Valluchi-Morf, M. Müller, and W. Briegleb. 1980. Aviat. Space Environ. Med. 51: 29-34.
13. Kessler,J.O. 1979. The Physiologist 22: S, 47.
14. Folkman,J., and A. Moscona. 1978. Nature 273: 345-349.

STUDY OF CONTRACTILE PROPERTIES AND COMPOSITION OF MYOFIBRILLAR PROTEINS OF SKELETAL MUSCLES IN THE COSMOS-1129 EXPERIMENT

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ABSTRACT

We examined m. soleus, EDL, m. triceps /caput mediale/, m. brachialis of rats flown for 18.5 days aboard the biosatellite "Cosmos-1129" /F/. There were two control groups: animals living free /V/ and rats living under conditions synchronous with biosatellite /S/. The greatest decrease in weight and contractility occured in group F and in the case of m. soleus. On the effect of weightlessness the quantity of LC-3 fast myosin subunit increased in both soleus and EDL muscles. It was found in the recovery experiments that soleus and brachial muscles showed a significant change on the 6th day, whereas on the 29th day readaptation was complete.

INTRODUCTION

The morphological and biochemical properties of the organs are significantly influenced by their functions. Consequently, hypofunction leads to well-observable modifications of metabolism. Incapacitating in function results in the atrophy of the skeletal musculature and considerable changes in its metabolism and contractile properties.

In previous studies we examined the atrophy of the hind extremities of rabbits and rats due to plaster-cast immobilization /9, 11, 12, 13/. Our main conclusion was that plaster-cast immobilization primarily affects the tonic soleus muscle. This muscle displayed greater changes in weight, in the subcellular components and sarcoplasmic proteins and in the decrease of contractile properties than the tetanic extensor digitorum longus /EDL/. Similar observations were reported by Brooks /1/, Summers et al. /7/ and a number of other authors.

In further experiments some characteristics of muscle contraction and mechanical properties of two muscles /M. soleus and EDL in Wistar rats after 22 days of weightlessness have been investigated. On second day after return to earth, the following changes were evident: slowing of twitch responses of the muscles studied; shortening half tetanic contraction time /defined by point of interactions of the increasing curve with 50% level of the peak value/ in soleus; a rise of tension in both muscles as shown by the curve "length-force"; an increase of twitch/tetanus ratio and fatigability in both muscles. During repeated study of muscle properties, in the 26th day after return to earth, there were not any significant changes in values of most the above mentioned indices except the diminished strength of soleus /2, 3, 4, 5, 6/.

Shortening of contraction time in slow antigravitary soleus muscle is believed to be a sign of adaptive change in its characteristics resulting from unloading under the conditioning of weightlessness. Increased stiffness and diminished strength of muscles are considered as functional signs of atrophic processes, developing in the experiment. Dynamics of functional changes, conditioned, as we believe, mainly by weightlessness and correlated with morphological and biochemical data obtained on analogous material, allows us to postulate a reversible character of the changes described.

The purpose of the present investigations was to verify this concept in experiments on skeletal muscles of animals flown for 18.5 days aboard the biological satellite Cosmos-1129. The paper summarizes preliminary results of the investigations.

METHODS

In our earlier experiments /10/ we also had the objective to elaborate methods suitable for the accurate examination of samples taken from the small quantity of muscles of rats exposed to weightlessness during space-biological experiments. The contractile properties of muscles were first studied by a modified procedure of the glycerinated

muscle fibre preparation method first applied by Szent-Györgyi /8/.

The essence of the preparation of glycerinated muscle fibres is that freshly excised muscles are treated alternately with hypo- and hypertonic saline, thereby destroying the membrane of the muscle fibre. Our procedure allowed us to perform experiments as early as in 48 hours, instead of the earlier lag of 3-4 weeks. Furthermore, stored at -20°C in 50% glycerine these preparations preserve their contractile properties for months. Under appropriate conditions the preparations give easily measurable contractile response to ATP-Ca^{++}.

The subcellular components of myofibrils were studied by Weber and Osborn's SDS PAG electrophoretic method /14/.

In the biosputnik "Cosmos-1129" male rats of 300-360 g participated in a space journey of 18 and half days. In consistence with our earlier experiments we studied the soleus muscle as a representative of the slow muscle and the EDL muscle which is built up almost entirely of fast muscle fibres. Investigations were also performed on the brachial muscle, composed mainly of fast fibres, and the significantly mixed triceps muscle /medial head/. The rats that participated in the space journey were called the flight /F/ groups; in addition there were two control groups: one made up of animals living free in the vivarium /V/ and another containing rats living under conditions synchronous with the biosatellite /S/.

The examination of the rats that had participated in the space journey took in three periods of time. Part of them were sacrificed just after their return to earth /0 group/, others on the subsequent 6th and 29th days. The muscles were exposed to glycerine treatment.

RESULTS

First of all we examined the muscular weight changes of the 0 group. The results showed that in groups S significant muscle weight decrease was observed. in group F decrease in weight was highly significant in all muscles. The greatest decrease in weight occured in both group of the soleus muscle.

Our equipment used in the glycerinated muscle experiments is shown in Fig. 1. and Fig. 2.

Myofibril preparations of 0.25-0.4 mm in diameter are fixed with microclamps attached to the transducer head and the isometric tension produced by ATP-Ca^{++} treatment is recorded through

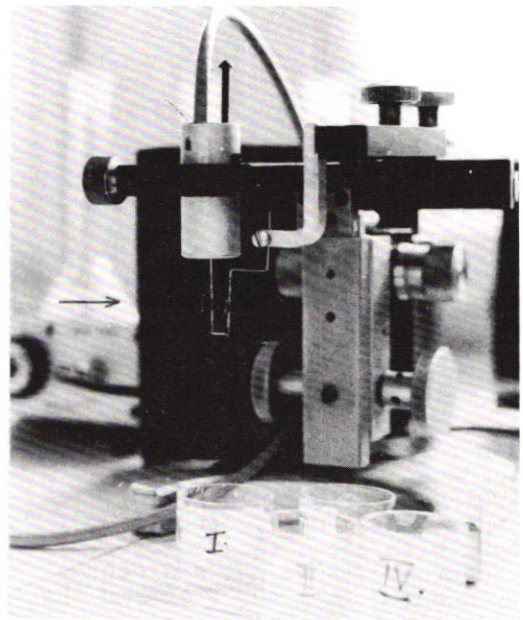


Fig. 1.
Clamps for fixation of glycerinated myofibrils

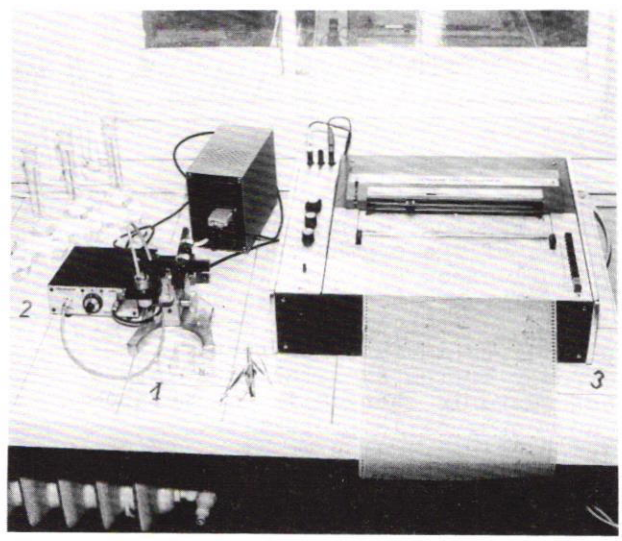
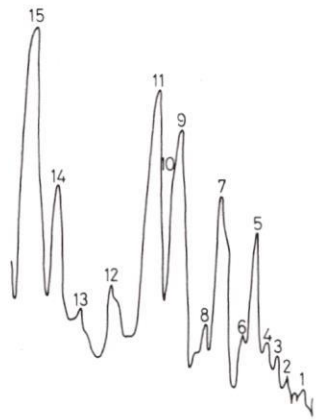


Fig. 2.
Equipment for registration of muscle contractility
1. Clamps. 2. Transducer. 3. Recorder

a signal transformer on a potentiometric recorder. In the knowledge of the diameter of the fibre it is possible to calculate the value of maximum tension and contraction referred to 1 cm of fibre circumference, i.e. the time

needed for maximum contraction. These parameters permit us to determine the differences in the contractile properties of the individual muscles. Several bundles were prepared from each muscle, which made it possible for us to perform a greater number of experiments. The results suggest that in group S significant and in group F a highly significant decrease in contractility can be observed for all muscles. In group F this decrease in contractility is much greater than in group S. The greatest decrease was observed in both groups of the soleus muscle.



1. Myosin LC-3F
2. Troponin-C
3. Troponin-I
4. Myosin LC-2F
5. Myosin LC-2S
6. Myosin LC-1F
7. Myosin LC-1S
8. Unknown
9. Tropomyosin
10. Troponin-T
11. Actin
12. Unknown
13. ∝-actinin
14. C-M-protein
15. Myosin heavy chain

Fig. 3.
SDS PAG densitogram of normal soleus muscle

In Fig. 3. the SDS PAG densitogram of the normal soleus muscle is seen. The 15 components can be well distinguished in the figure. The numbering of the peaks representing the protein components begins with the peptides of greater mobility. Significant quantitative changes were found in the composition of components with smaller molecular weight, primarily in that of the myosin light chains. On the effect of weightlessness the quantity of LC-3 fast myosin subunit increase in both soleus and EDL muscle, while they decrease in triceps and brachial muscle.

It has already been mentioned that not only the changes due to weightlessness were investigated, but also the readaptation of the muscles. In both groups the muscle weight decrease for the soleus muscle showed a significant change on the 6th day, whereas on the 29th day readaptation was complete. The contractility of the soleus muscle showed similar recovery. We had same results in the case of the brachial muscle. The recovery of EDL muscle containing almost entirely fast muscle fibres was complete on the 6th day in all three parameters. In the case of triceps muscle readaptation took place as early as the 6th day in contractile properties but recovery in weight occured only on the 29th day.

The results of our investigations allow us to draw the conclusion that the changes brought about by the condition of weightlessness depends on the functional properties of muscles. Under the influence of weightlessness for 18.5 days the weight and contractile properties of muscles show a great decrease, however, regeneration takes place in a short time. In the muscles under study the most sensitive reaction was displayed by the soleus muscle which predominantly consists of slow fibres. On the basis of gel electrophoretic experiments we can say that as a result of weightlessness transformation of the muscles may take place. This is in agreement with the results of our plaster cast model experiments. On the basis of all these it can be established that the plaster cast immobilization serves as a good model for the simulation of the effects of weightlessness.

REFERENCES

1. Brooks, J.E. 1970. Disuse atrophy of muscle. Arch Neurol. 22: 27-30.
2. Gaevskaya, N.S., Veresotskaya, N.A., Kolganova, E.V., Kurkina, L.M., Nosova, E.A. 1979. Changes in metabolism of the soleus muscle of rats after flight aboard Cosmos-690. Kosm. Biol. Aviakosm. Med.13: 16-19.
3. Gazenko, O.G., Genin, A.M., Ilyin, E.A., Serova, L.V., Tigranyan, R.A., Oganov, V.S. 1980. Adaptation to weigthlessness and its physiological mechanismus. /The results of animal experiments aboard Earth biological satellites/. Izv. AN SSSR. Biol. 1: 5-8.
4. Ilyina-Kakueva, E.I., Portugalov, V. V., Krivenkova, N.P. 1977. Effect of spaceflight factors on skeletal musculature of rats. Kosm. Biol. Aviakosm. Med. 11: 20-25.
5. Oganov, V.S., Potapov, A.N. 1976. On the mechanisms of changes in skeletal muscles in the weightless environment. Life Sci. Space Res. 14: 136-143.

6. Portugalov, V.V., Petrova, N.N. 1976. LDH isoenzymes of skeletal muscles of rats after space flight and hypokinesis. Aviat. Space 47: 834-838.
7. Summers, I.B., Hines, M.M. 1951. Effect of immobilization in various positions upon the weight and strength of skeletal muscle. Arch. Phys. Med. Rehab. 32: 142-154.
8. Szent-Györgyi, A. 1949. Free energy relations and contraction of actomyosin. Biol. Bull. 96: 140-147.
9. Szöőr, Á., Boross, A., Hollósi, G., Szilágyi, T., Kesztyüs, L. 1977. Experimental investigations on hypokinesis of skeletal muscles with different functions I. Changes in muscle weight, protein and contractile properties. Acta biol. Acad. Sci. hung. 28: 195-204.
10. Szöőr, A., Rapcsák, M., Boross, A. 1980. Short procedure for preparation of glycerol treated muscle fibres. Acta Biochim. Biophys. Acad. Sci. hung. /in press/
11. Szöőr, A., Rapcsák, M., Hollósy, G. 1980. VIII. Effect of plaster cast immobilization on the contractile properties of skeletal muscles of different functions. Acta biol. Acad. Sci. hung. /in press/.
12. Takács, Ö., Sohár, I., Pelle, T., Guba, F., Szilágyi, T. 1977. Experimental investigations on hypokinesis of skeletal muscles with different functions. III. Changes in the subcellular components. Acta biol. Acad. Sci. hung. 28: 213-219.
13. Takács, Ö., Sohár, I., Szilágyi, T., Guba, F. 1977. Experimental investigations on hypokinesis of skeletal muscles with different function IV. Changes in sarcoplasmic proteins. Acta biol. Acad. Sci. hung. 28: 221-230.
14. Weber, K., Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulphate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406-4410.

VARIABILITY OF PHYSIOLOGICAL PROPERTIES OF RAT SKELETAL MUSCLES AT DIFFERENT GRAVITY LEVELS

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INTRODUCTION

The principle of complementation of studies of weightlessness and acceleration effects discussed by Gazenko and Gurjian (6) has been recognized by gravitational physiology. In the light of Tsiolkovsky's concepts (24) an exposure to acceleration is also regarded as a potential countermeasure against adverse effects of weightlessness (15). This hypothesis has been for the first time verified in the mammalian experiment aboard the biosatellite Cosmos-936 (7).

The purpose of the present investigation was to study on a comparative basis physiological parameters of skeletal muscles of rats exposed to weightlessness per se (Cosmos-605, 690, 936) and to artificial gravity in the ground-based laboratory and in space flight (Cosmos-936).

MATERIALS AND METHODS

The design and protocol of experiments carried out aboard biosatellites of the Cosmos series were reported previously (10, 11). Artificial gravity was generated in a 320 mm arm centrifuge mounted aboard Cosmos-936. The environmental parameters for the centrifuged rats were identical to those for weightless animals. The velocity of centrifuge rotation was programmed in such a way as to generate artificial gravity of 1 g (i.e. to equal Earth's gravity) (10). Some synchronous rats kept in a biosatellite mock-up were housed in an identical centrifuge to provide an acceleration of 1 G (10).

In the course of preflight investigations the effect of artificial gravity of 1 G was studies with the aid of a laboratory centrifuge (with a 560 mm arm) where the animals were kept in an environment similar to that described elsewhere (10, 11). In the laboratory centrifuge rats were continuously rotated for 22 days with a 20 min stop once a day to do the cleaning.

In all experiments rats were killed by decapitation. Skeletal muscles of forelimbs - medial head of the triceps brachii muscle (TRIC) and the brachialis muscle (BRACH) - and hindlimbs - the soleus muscle (SOL) and the extensor digitorum longus muscle (EDL) - were examined. The animals from Cosmos-605 and 690 and the laboratory centrifuge were used to study contractile properties of intact muscles (SOL and EDL) in the course of isometric contractions in vitro in response to single and rhythmical electric stimuli (17). Cosmos-936 rats were employed in the study of isometric contractions of glycerinated preparations of myofibers of the above four skeletal muscles. Preparations were obtained and analyzed for contractile activity according to the method of Szent-Gyorgyi (22) in our own modification based on (1,2,20).

Throughout the paper the following abbreviations are used. The experimental animals are designated as F - flight weightless, FC - flight centrifuged, LC - centrifuged in the ground-based laboratory; synchronous ground-bound control animals are designated as S, SC and S - LC, respectively; vivarium animals from all groups are designated as V. The difference (P) between flight and synchronous data is shown as P_s and between the flight and vivarium results as P_v.

RESULTS AND DISCUSSION

Previous (Cosmos-605) experiments on preparations of intact muscles of the slow (SOL) and fast (EDL) types showed a reduction of maximal tetanic tension (A), elasticity decrease and lowered tolerance to fatigue. These changes were mostly distinct in SOL, which also exhibited a selective acceleration of tetanic contraction development ($P_s < 0.05$). These changes were adaptive and disappeared 25 days postflight (17).

Cosmos-690 experiments also demonstrated a decrease of maximal tetanic tension and acceleration of tetanic contraction development of SOL (Fig. 1).

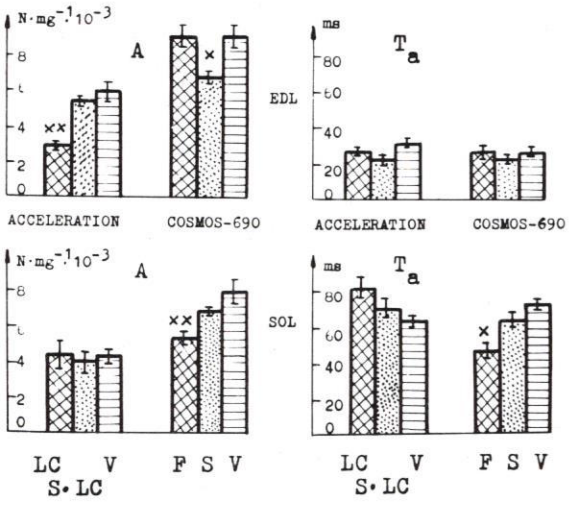

Fig. 1. Contractile properties of skeletal muscles of rats following flight on Cosmos-690 and chronic exposure to 1 G.
A - tetanus per unit muscle weight;
T_a - time of tetanus development to reach 1/2 A
The differences are statistically significant:
xx - $P_{(S,V)} < 0.05$
x - $P_{(S \text{ or } V)} < 0.05$

In contrast to this, a chronic exposure to acceleration of 1 G for as long as the space flight continued did not alter the strength and delayed the process of tetanic contraction development of SOL; this exposure resulted in a decrease of maximal tetanic tension and did not bring about significant changes in time parameters of EDL contraction (Fig. 1).

It is assumed that weightlessness-induced changes in contractile parameters of SOL may be a result of partial rearrangement of the structure and function of muscle fibers of the antigravity SOL, as a consequence of which they acquire a certain similarity with fast myofibers. This is supported by the morphological (9), biochemical (5,16) and histochemical findings (19).

In order to answer the question as to how this rearrangement may influence contractile muscle proteins, studies of contractile properties of glycerinated myofibers were carried out as one of the Cosmos-936 experiments. The design of the experiment was based on the well known fact that glycerination, which leads to partial decomposition of the sarcolemma and washout of metabolic substrates from muscle fibers, maintains the native organization of myofibrillar proteins and their ability for ATP hydrolysis and contraction (1).

As follows from Table 1, space flight effects on hindlimb muscles manifest, particularly, in a significant decrease of maximal amplitude of isometric tension (P_m) of SOL (as compared to both control groups) in the absence of significant changes in contraction strength of EDL.

The data of space flight effects on forelimb muscles are shown in Fig. 2, which also illustrates the effect of artificial gravity on glycerinated preparations of these muscles.

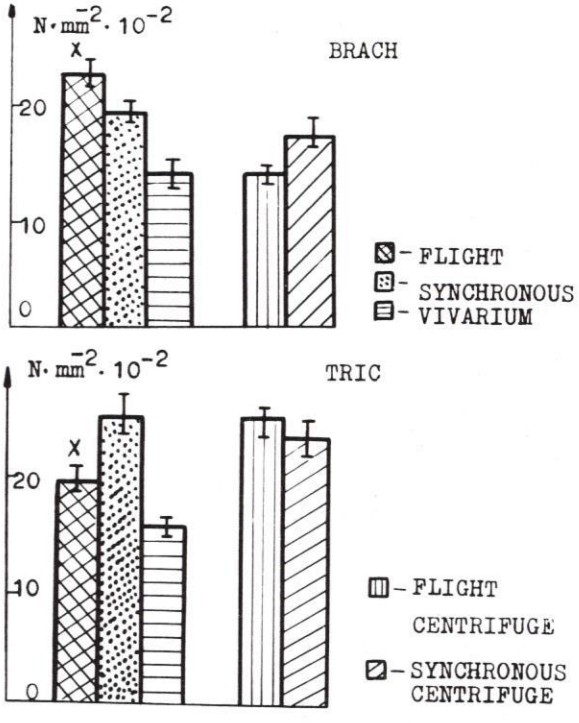

Fig. 2. Changes in maximal isometric tension of glycerinated muscle fibers of skeletal muscles of rats flown aboard Cosmos-936.
Designations - see in Fig. 1.

These studies suggest that in the synchronous experiment TRIC is to perform a new, most likely postural function, whereas in space flight this effect is reversed; therefore, the final result of weightlessness effect on TRIC is very similar to the reaction of the antigravity SOL. It should be noted that, according to histological examinations, TRIC was close to EDL in the composition of muscle fibers; however, due to its anatomical, topographical and biomechanical characteristics TRIC is much more important in maintaining posture than EDL (13).

The response of BRACH to space flight includes a more marked "training" effect manifesting as increased P_m. Mention should be made of an equal sign of P_m changes in F and S rats which also seems

TABLE 1. Amplitude of isometric tension (P_m) of glycerinated muscle fibers of rats after Cosmos-936 flight ($N/mm^2 \cdot 10^{-2}$)

	F	S (1)	V (2)
SOL	$15.43 \pm 3.18^{x1,2}$	27.88 ± 3.17	24.24 ± 2.29
N:n	4 : 100	5 : 141	5 : 126
EDL	14.44 ± 2.92	13.95 ± 1.41	16.55 ± 1.98
N:n	5 : 36	5 : 71	5 : 80

Note: Mean $\pm$ SE; ^{x1,2} - statistically significant differences ($p < 0.05$) between flight and control data;

N:n = number of animals: number of preparations.

to be a result of a combined effect of weightlessness and confinement of animals in a small enclosure.

As it can be seen from Fig. 2, artificial gravity applied in spaceflight neutralizes the deprivation effect of weightlessness on the strength gain of the mixed muscle TRIC in animals housed in small enclosures (S rats). Therefore, TRIC reaction to artificial gravity in spaceflight and on the Earth (S) resembles the response of the extensor muscle (SOL) to acceleration (Fig. 1). At the same time artificial gravity in spaceflight essentially reverses the training effect noticed in the fast muscle - flexor BRACH (Fig. 2).

The experimental findings give evidence that reactions of the contraction system of different muscles to an altered gravity are strongly dependent on their functional specialization and biomechanics of their contraction.

Changes in contraction properties of muscles similar to those found in weightlessness were seen previously in various simulation studies where the major factor of relative disuse of muscles was their strength unloading, i.e. hypodynamia. An acceleration of slow muscle contraction combined with a decrease of strength and tolerance to fatigue was observed in experiments with spinal segment isolation (4, 14), tentomy (26), immobilization (23), and amputation of the part of distal segment of a forelimb (12).

Previous studies furnished data on the specific and selective effect of an increased gravity upon the antigravitational musculature and, particularly, on the predominant growth of extensor muscles in various animals under the influence of prolonged centrifugation (21). There is evidence indicating centrifugation-induced increase of strength (3) and tolerance to fatigue (3,21) of slow muscles of animals.

In the light of the above data in the literature the results of our study are of interest when discussed from the point of view of Smith (21) who has postulated the principle of continuity of biological effects of acceleration, assuming a continuum of physiological reactions of the animal body in the gravitational field varying from zero g to acceleration values approximating limits of biological tolerance. This theory finds support in our findings. Fig. 3 illustrates the scale of changes of selected physiological parameters of different skeletal muscles as related to the gravity field.

Another theoretically important aspect of the present study is that the data obtained can be regarded as evidencing the dynamic pattern of specilization of skeletal muscles (8,25) and their high functional plasticity, for instance, under the conditions of varying gravitational field (16) within the limits studied. From the practical point of view this seems very promising for potential control of adaptation of skeletal muscles to altered gravity.

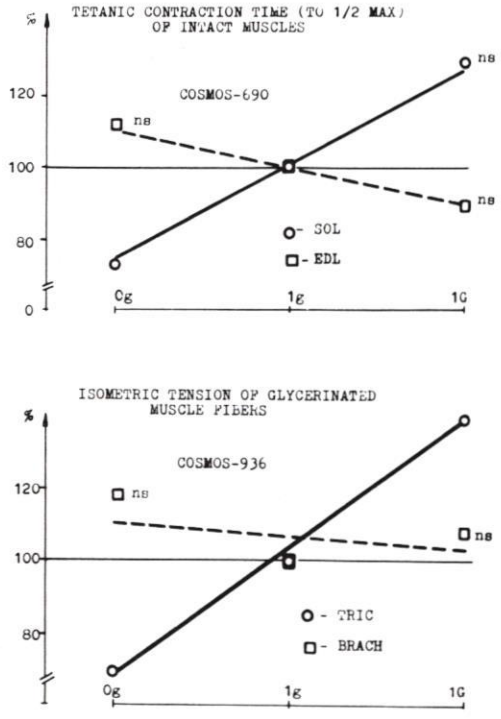

Fig. 3. Contractile properties of rat skeletal muscles at different gravity levels.
0_g - weightlessness on Cosmos-690 and Cosmos-936 (F/S x 100%)
1 g -vivarium control
1 G -laboratory centrifuge (intact muscles) and centrifuge on the synchronous experiment (glycerinated muscle fibers). In all cases, except for ns, $p < 0.05$.

REFERENCES

1. Bendall J.R. 1970. Muscles, Molecules and Movement. Mir Publ., Moscow.

2. Bukatina A.E. 1971. On possible causes of self-relaxation of glycerinated muscle fibers. Biophysics, 16, 1, 52-59.

3. Canonica. 1966. cited after Smith A.H. 1975.

4. Eccles J.C. 1944. Investigations on muscle atrophies arising from disuse and tenotomy. J. Physiol., 103, 252-266.

5. Gaevskaya M.S., Veresotskaya N.A., Kolganova E.V., Kurkina L.M., Nosova E.A. 1979. Metabolic changes in the soleus muscle of rats after flight Cosmos-690. Kosm. Biol. Aviakosm. Med., 13, 1, 16-19.

6. Gazenko O.G., Gurjian A.A. 1967. On the biological note of gravity. In: Life Sci. Space Res., 3, 241-257.

7. Gazenko O.G., Ilyin E.A., Genin A.M., Kotovskaya A.R., Korolkov V.I., Tigranyan R.A., Portugalov V.V. 1980. Results of physiological experiments on mammals aboard the biosatellite Cosmos-936. Kosm. Biol. Aviakosm. Med., 14, 2, 22-25.

8. Guth L. 1972. Regulation of metabolic and functional properties of muscle. In: Regulation of Organ and Tissue Growth. New York-London, 61-75.

9. Ilyina-Kakueva E.I., Portugalov V.V., Krivenkova N.P. 1977. Effect of spaceflight factors on skeletal musculature of rats. Kosm. Biol. Aviakosm. Med., 11, 1, 20-25.

10. Ilyin E.A., Korolkov V.I., Kotovskaya A.R., Noskin A.D., Kondratyeva V.A., Shipov A.A., Britvan I.I. 1979. Goals and conditions of physiological experiments on rats flown aboard the biosatellite Cosmos-936. Kosm. Biol. Aviakosm. Med., 13, 6, 18-22.

11. Ilyin E.A., Serova L.V., Noskin A.D. 1976. The rat experiment in a 22-day flight aboard the biosatellite Cosmos-605 (aims and methods). Kosm. Biol. Aviakosm Med., 10, 2, 9.

12. Katinas G.S., Oganov V.S., Potapov A.N. 1974. Hypodynamic and hypokinetic state of skeletal muscles. Fiziol. J. USSR, 60, 10, 1606-1608.

13. Katinas G.S., Potapov A.N. 1971. Changes of skeletal muscles in hypokinetic conditions. Arch. Anat. Histol. Embryol. 61, 11, 74-82.

14. Klinkerfuss G.H., Hugh M.G. 1970. Disuse atrophy of muscle. Arch. Neurol., 22, 309-320.

15. Kotovskaya R.R., Galle R.R., Shipov A.A. 1977. Biomedical investigations into artificial gravity. Kosm. Biol. Aviakosm. Med., 11, 2, 12-18.

16. Oganesyan S.S. 1978. Biological effects of gravity field. Biol. J. Armenii, 31, 7, 661-671.

17. Oganov V.S., Potapov A.N. 1976. On the mechanisms of changes in skeletal muscles in the weightless environment. In: Life Sci. Space Res., 15, 136-143.

18. Oganov V.S., Potapov A.N. 1979. A study of functions of skeletal muscles in biosatellite experiments. In: Effect of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M. Moscow, Nauka, 118-123.

19. Portugalov V.V., Petrova N.V. 1976. LDH isoenzymes of skeletal muscles of rats after spaceflight and hypokinesia. Aviat. Space, 47, 8, 834-838.

20. Sexton A.W. 1967. Isometric tension of glycerinated muscle fibers following adrenalectomy. Amer. J. Physiol., 212, 2, 313-316.

21. Smith A.H. 1975. Foundations of gravitational biology. In: Foundations of Space Biology and Medicine. Moscow, Nauka. II, b.1, 141-176.

22. Szent-Gyorgyi A. 1949. Free energy relations and contraction of actomysin. Biol. Bull., 96, 140-141.

23. Szoor A., Boross A.A., Hollosi G., Szylagyi T., Kestyus L. 1977. Experimental investigations on hypokinesis of skeletal muscles with different functions. 1. Changes in muscle weight, protein and contractile properties. Acta biol. Acad. Sci. Hung., 28, 2, 195-204.

24. Tsiolkovsky K.E. 1964. Collected Works. USSR Acad. Sci. Publ.

25. Van der Meulen J.P., Pechman P.H., Mortimer J.T. 1974. Use and disuse of muscle. Ann. N.Y. Acad. Sci., 228, 177-188.

26. Vrbova G. 1963. Changes in the motor reflexes produced by tenotomy. J. Physiol. 166, 241-250.

MORPHOMETRIC ANALYSIS OF RAT MUSCLE FIBERS FOLLOWING SPACE FLIGHT AND HYPOGRAVITY

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ABSTRACT

The effect of hypogravity in striate muscle was studied in two space flights: Aboard Cosmos 936, five Wistar rats exposed to hypogravity for 18.5 days were studied. Using computer-assisted image analysis of extensor digitorum muscles processed for alkaline ATPase reaction, they showed a reduction of the mean fiber diameter (41.32 ± 0.55um), compared to synchronous (46.32 ± 0.55um) and vivarium (49 ± 0.5um) controls. On Cosmos 1129 mission, 25 Wistar rats under 18.5 days of hypogravity were sacrificed in four groups at various intervals after recovery. Additional 50 animals were included as synchronous and vivarium controls. Using the same technique described previously, the gastrocnemius muscle showed a reduction of total muscle fiber area in square micron and percentage of slow fibers of flight animals as compared to controls.

INTRODUCTION

Space flight and hypogravity are known to produce systemic and metabolic changes in animals and human (7). Even though the effects of weightlessness in various organ systems are well described, the pathophysiological mechanism is largely unknown. During adaptation to decreased gravity aboard space flight, the musculoskeletal system is dynamically responsive to changes in insufficient loading mechanism, leading to hypokinesia and hypodynamia. As a result of this, decreased in body mass and leg volume has been described in manned space flights, resulting in loss of muscle bulk (1).

Skeletal muscle is capable of two basic types of contraction: Some fibers utilize a glucolytic anaerobic energy mechanism. These fibers (fast twitch glycolytic) contract rapidly, but fatigue easily. Other fibers require an available oxigen supply, since they utilize oxidative of metabolism for their energy (slow twitch oxidative), they contract more slowly and are resistant to fatigue. These fibers are important in maintaining posture against gravity, while fast twitch glycolytic fibers are required for quick, forceful movement (2). Upon close analysis, it becomes apparent that the above scheme outlined is an oversimplification, and that all muscle fibers are not readily classified into only two distinct groups. In fact, there are fast twitch fibers having high level of enzymes for both glycolitic and oxidative metabolism. These fibers exhibit both fast contraction and fatigue-resistant characteristics. It has also become apparent that for any given fiber, the type of energy metabolism employed is not immutably fixed throughout its life. During fetal development for example, the fiber types undergo changes depending upon the type of innervation they receive; and more importantly, they change with exercise and the demmands put upon the muscle by the environment. Electrical stimulation of muscle can also change the contracting mechanism (4, 8, 10). These observations have far reaching implications for the effects of space flight on human and animal neuromuscular system.

MATERIAL AND METHOD

A total of seventy-five pathogen-free adult Wistar rats recovered from 18.5 days of orbital flight were included in the present study. They were divided into three groups:

Group I = Flight (f), 25 animals.
Group II = Synchronous Control (S), 25 animals.
Group III = Vivarium Control (V), 25 animals.

Seven animals of the flight groups were sacrificed at the recovery site within six hours of landing (R+0). Six were sacrificed six days later (R+6). Seven additional flight animals were also sacrificed six days later, but after immobilization stress. Finally, five flight animals were sacrificed twenty-five days after recovery. Similar number of animals were obtained from Vivarium and Synchronous Control Groups.

The left gastrocnemius and plantaris muscles were carefully removed immediately after sacrifice by decapitation. Specimen were placed in pre-labeled and pre-chilled scintilation vials and immersed in liquid

nitrogen for a period of approximately ten minutes. They were stored in insulated containers, packed with dry ice and shipped to the University of Southern California, Neuromuscular Research Laboratory for histochemistry processing. Whole gastrocnemius and plantaris muscles were cryostat sectioned at 10um thick. Three consecutive serial sections were obtained for every 2000um interval. Routine histochemical reaction was prepared as previously described (3).
Muscle histochemistry reaction for ATPase ph 4.6 from all three groups of animals were analized in the Medical Image Analysis Facility at the Jet Propulsion Laboratory using computer assisted morphometric analysis (3).

RESULTS

Results obtained from our previous experiment (Cosmos 936-K208) clearly shoed a reduction of the mean fiber diameter of the extensor digitorum muscle from flight animals ($41,34 \pm 0.55$) compared to synchronous (46.32 ± 0.55) and vivarium (49 ± 0.5) controls. Unfortunately, only fast twitch glycolytic fibers were measured, because no significant number of slow twitch oxidative fibers were observed (3). Morphometric analysis of muscle fibers from present experiment was obtained from gastrocnemius only, since this was the only muscle containing enough number of mixed fibers for comparison of both fiber types. Cross section of gastrocnemius muscle showed that slow twitch oxidative fibers were distributed into three distinct regions: Region I (11%), region II (43%) and region III (24%).

TABLE 1

GROUP	REGION	RATS	FIBERS	SLOW FIBER AREA (μ^2)	FAST FIBER AREA (μ^2)	% SLOW	SLOW/FAST AREA RATIO
1F	I	3	1491	1644	2973	20.6	0.553
1V	I	3	981	2797	3967	19.4	0.705
1F	II	3	894	2480	2602	39.2	0.953
4F	II	3	838	3278	3721	43.8	0.881
1V	II	4	1621	3876	3271	44.7	1.185
4V	II	3	873	4153	3947	36.8	1.052
1F	III	4	2000	2365	2946	19.15	0.803
4F	III	3	658	3230	4989	24.5	0.647
1V	III	2	1844	3465	3379	22.3	1.025
4V	III	4	1425	3445	3627	21.1	0.950

The result of fiber area measurement in all three regions of the gastrocnemius muscle is shown in table 1. Space flight animals sacrificed immediately after recovery (IF) showed a significant reduction in total area of both fiber types. However, the slow fibers were more affected as evidenced by the decrease in slow to fast-area ratio. No significant changes were observed in 25 days post flight animals (4F).

TABLE 2

PERCENT CHANGE IN FIBERS

GROUPS COMPARED	NUMBER OF RATS	No. FIBERS MEASURED	% SLOW FIBERS	SLOW FIBER AREA	FAST FIBER AREA	SLOW/FAST AREA RATIO
1F vs 1V (I)	5	1833	+35	-44	-32	-16
1F vs 1V (II)	7	2950	-9	-35	-20	-16
1F vs 1V (III)	6	3844	-20	-32	-26	-20
1,2,3F vs 1,2,3V (I)	12	3647	+28	-25	-13	-13
1,2,3F vs 1,2,3V (II)	17	4291	-2	-33	-13	-23
1,2,3F vs 1,2,3V (III)	16	7437	-20	-25	-30	-5

Table 2 indicates the percent of fiber changes compared by groups (Flight vs vivarium control). This also clearly indicates a significant reduction in percentage of slow and fast fiber areas.

DISCUSSION

Previous study on the 22-day Cosmos 605 flight showed that the weight of the extensor digitorum muscle was 12% lower in flight than control animals, and fiber area was decreased by 13%. Changes in the soleus muscle were more pronounced, showing 22% reduction in fiber size (5). Aboard Cosmos 690, which included a 24 hour exposure to 800 rad of radiation, the soleus showed 25% and the gastrocnemius 19% weight deficit (6). On Cosmos 782, the weight of the soleus was 38% lower in flight animals and 17% in synchronous control (9). The observations derived from these experiments are such that hypogravity certainly induces changes consisting in reduction of weight and fiber size. More profound changes were observed in antigravity muscles, like the soleus. Our present study is in agreement with previous results, that slow oxidative fibers are mostly affected, even in a mixed, non-antigravity muscle like the gastrocnemius muscle.
The mechanism of these changes are not clear and it can only be postulated. Hypogravity or weightlessness produces insufficient loading of muscle, leading to hypokinesia (motion) and hypodynamia (force). This in turn produces trophic changes in antigravity muscles, altered protein metabolism, negative nitrogen balance, etc., producing muscle atrophy as final result, with possible consequence of decreased muscle tone, strength and decreased tolerance to physical work capacity. These observation have far reaching importance in prolonged manned space fligh ts, where preventive measures could be achieved by designing appropiate exercise programs to improve functional performance

of the neuromuscular system.

REFERENCES

1. Berry, C.A. (1971): Medical Results of Apollo 14 - Implications for Longer Duration of Space Flights. XXII Int. Astronaut. Congress, Brussels, Belgium.

2. Burke, R.E. and Edgerton, V.R. (1975): Motor Unit Properties and Selective Involvement in Movement. Exercise and Sport Sciences Revies, 3:31-81.

3. Castleman, K.R. et al (1978): Spaceflight Effects on Muscle Fibers: Final Reports of U.S. Experiments Flown on Soviet Satellite Cosmos 936. Rosenzweig, S.N. and Souza, K.A., Editors: NASA Technical Memorandum 78526 - pp. 224-289.

4. Close, R.I. (1972): Dynamic Properties of Mammalian Skeletal Muscles. Physiological Reviews, 52:129-197.

5. Ilyina-Kakueva, E.I. et al. (1976): Space Flight Effects on the Skeletal Muscle of Rats: Aviation, Space and Environmental Medicine, 47:700-703.

6. Ilyina-Kakueva, E.I. and Portugalov, V.V. (1977): Combined Effect of Space Flight and Radiation on Skeletal Muscle of Rats: Aviation, Space and Environmental Medicine, 48:115-119.

7. Pace, N. (1977): Weightlessness: A Matter of Gravity, New England Jour. Med. 297:32-37.

8. Peckham, P.H. et al. (1970): Electrical Activation of Skeletal Muscle by Sequential Stimulation: The Nervous System and Electric Currents. Ed. Wulfsohn, N.L. and Sances, A., Plenium Press, N.Y., Londonn pp. 45-50.

9. Portugalov, V.V. (1976): Morphological and Cystochemical Study of the Organs and Tissues of Animals on Cosmos 782. Academy of Sciences of the USSR Report, Moscow, NASA Technical Translation F-17257.

10. Van Der Meulen, J.P. et al (1974): Use and Disuse of Muscle, Annals N.Y. Academy of Sciences, 228:177-189.

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COSMOS 1129: SPACEFLIGHT AND BONE CHANGES

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ABSTRACT

Male Wistar rats were placed in orbit for an 18.5 day period aboard the Soviet Cosmos 1129 biological satellite. The skeletal changes which occurred during spaceflight were determined to be a reduced rate of periosteal bone formation in the tibial and humeral diaphyses, and a decreased trabecular bone volume and an increased fat content of the bone marrow in the proximal tibial metaphysis.

INTRODUCTION

The weightless environment and hypodynamic conditions associated with space flight have produced alterations in calcium homeostasis. Studies of mineral metabolism in Gemini and Apollo crewmembers suggested that increased urinary excretion of calcium was a consequence of orbital flight (1,2). Skylab astronauts also exhibited a marked hypercalciuria (3). Since the skeleton is the major reservoir of calcium in the body, abnormalities in calcium homeostasis may reflect skeletal changes. A significant decrease in the bone mineral density of the calcaneus has been reported for crewmembers of the Skylab flights (4). Skeletal changes which occurred in rats during previous Cosmos experiments include a decreased mass of trabecular bone in the tibial and femoral metaphyses (5) and a reduced rate of periosteal bone formation in the tibial diaphysis (6). The purpose of the current study is to investigate the skeletal alterations which occurred in rats during orbital flight aboard the Soviet Cosmos 1129 biological satellite.

MATERIALS AND METHODS

Specific pathogen-free, male Wistar rats from the Institute of Experimental Endocrinology of the Slovakian Academy of Sciences were approximately 83 days of age and weighed an average of 290 grams at the beginning of the experimental period. The rats were divided into three groups. The flight animals were placed in orbit in individual cylindrical cages aboard a modified Soviet Vostok space craft for a period of 18.5 days. There were two groups of ground-based controls. The flight control rats were also housed individually in a modified Vostok space craft and subjected to the conditions associated with launch and reentry. An attempt was made to simulate as closely as possible the space craft environment experienced by the flight animals. The vivarium control rats were housed in animal quarters and were not subjected to flight conditions. The first group of flight, flight control, and the vivarium control rats was sacrificed at the end of the 18.5 day flight period. The second and third groups were sacrificed at 6 and 29 days postflight, respectively.

All rats were injected intraperitoneally with 1 mg/kg body weight of Declomycin three days prior to launch. Declomycin is a tetracycline derivative which labels areas of bone formation (7). A second Declomycin injection was administered to the postflight group 5 days after flight. The rats were decapitated at the end of the experimental periods and the left tibia and humerus were placed in a fixative composed of paraformaldehyde, glutaraldehyde, and diflourodinitrobenzene. The vials containing the bones were refrigerated and shipped to the United States.

The bones were sawed into three parts corresponding to the proximal, middle, and distal thirds. The proximal tibia was dehydrated in increasing concentrations of acetone and embedded undecalcified in methyl methacrylate. Multiple sections of ∿5 μm thickness were cut parallel to the longitudinal axis of the bone with a Jung Model K microtome. Following removal of the plastic with xylene, the sections were stained according to the von Kossa method (8).

Portions of the tibial and humeral diaphyses were processed for ultraviolet microscopy of flourescent tetracycline labels. The bone specimens were dehydrated in a series of acetone and ether changes and embedded undecalcified in a styrene monomer which polymerizes into a polyester resin (Tap Plastic Inc., San Jose, CA). The portion of the tibial diaphysis immediately proximal to the tibiofibular junction and the portion of the humeral diaphysis immediately distal to the deltoid tuberosity were sawed into 50 μm thick cross-sections with a Gillings-Hamco thin sectioning machine.

The fractional area of trabecular bone and the fractional area of fat in the bone marrow were quantified in a 2 by 2.5 mm area of the proximal tibial metaphysis with the aid of a Merz grid (9). This grid consists of 6 semicircular lines and 36 points within a square. The number of points

1 The author was supported by the National Research Council as a post-doctoral research associate.

superimposed over bone and bone marrow were counted at a magnification of 160 X. The fractional area of trabecular bone was calculated by dividing the number of points lying over the bone by the total number of points lying over bone and bone marrow. The fractional area of fat in the bone marrow was quantified in a similar manner.

The Merz grid was also used to quantify the rate of periosteal bone formation in the tibial and humeral diaphyses. In a growing rat, the periosteal surface is forming new bone and will therefore be labeled with tetracycline. The number of points superimposed over the newly-formed bone between the flourescent tetracycline label and the periosteal surface was counted at a magnification of 160 X under ultraviolet illumination. The area was calculated by multiplying the number of points by the constant d^2, with d equal to the distance between points. The rate of periosteal bone formation was calculated by dividing the volume of newly-formed bone (assuming that the cross-sections were 1 mm thick) by the time interval between administration of the tetracycline label and sacrifice. The medullary area in cross-sections of the tibial and humeral diaphyses was also quantified by point counting with a Merz grid.

RESULTS

The values for periosteal bone formation rate in the tibial and humeral diaphyses during the flight period are listed in Table 1. The flight rats exhibited a reduced rate of periosteal bone formation in comparison to both flight control and vivarium control rats. These differences were evaluated by means of Student's t-test and found to be highly significant. The inhibition of periosteal bone formation in the humerus was not as marked as in the tibia.

Table 1. Periosteal bone formation rate in the tibial and humeral diaphyses during the flight period

	Periosteal Bone Formation Rate* (10^{-3} MM^3/Day)		
	Flight	Flight Control	Vivarium Control
Tibia	10.0 ±2.1	17.9** ±2.7	22.6** ±4.7
Humerus	10.9 ±2.2	14.2*** ±2.8	17.9** ±5.3

*Each value is the mean of 11 animals ± the standard deviation.
**Significantly different from flight values at the level of $P<0.001$.
***Significantly different from flight value at the level of $P<0.01$.

Table 2 lists values for periosteal bone formation rate in the tibial and humeral diaphyses during the postflight period. In the tibia, the flight rats exhibited a significantly increased rate of periosteal bone formation, in contrast to the reduced rate which was observed during the flight period. A similar increase was not observed in the humerus. At this skeletal site, there were no significant differences among the three groups in periosteal bone formation rate during the post-flight period.

Table 2. Periosteal bone formation rate in the tibial and humeral diaphyses during the postflight period

	Periosteal Bone Formation Rate* (10^{-3} MM^3/Day)		
	Flight	Flight Control	Vivarium Control
Tibia	18.4 ±2.1	12.6** ±2.4	14.5*** ±2.3
Humerus	11.3 ±1.4	10.7 ±2.4	12.3 ±3.0

*Values for the flight group are the mean of 4 animals, and the values for both control groups are the mean of 5 animals.
**Significantly different from flight value at the level of $P<0.025$.
***Significantly different from flight value at the level of $P<0.05$.

The mean medullary area in cross-sections of the tibial and humeral diaphyses was approximately 1 mm^2 for each of the three experimental groups.

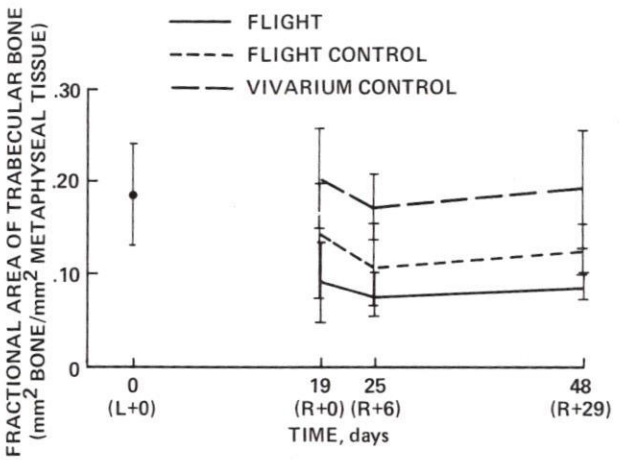

Figure 1. The fractional area of trabecular bone (trabecular bone volume) in the proximal tibial metaphysis vs. time. The spacecraft was launched on day 0, and recovered on day 19. Day 25 is equivalent to recovery +6 days, and day 48, the end of the postflight period, is equivalent to recovery +29 days. Each point is the mean of 7 animals at day 19, 6 animals at day 25, and 5 animals at day 48. The vertical lines represent

the standard deviations. The point at day 0 is the mean of 10 basal control rats ± the standard deviation. These rats were housed in animal quarters and sacrificed at the beginning of the flight period.

The fractional area of trabecular bone, commonly referred to as trabecular bone volume, is plotted vs. time in Figure 1. There appears to be a trend for a reduced trabecular bone volume in the proximal tibial metaphysis in flight rats. This parameter was significantly lower in the flight group relative to the vivarium control group at all time periods. The flight rats also had a consistently lower trabecular bone volume in comparison to the flight control rats. This difference was not significant ($P<0.10$) at days 19 and 25, but there was a significant difference ($P<0.05$) at day 48. The trabecular bone volume remained relatively constant in each of the three groups throughout the experimental period. If the data from all three time periods were combined, the trabecular bone volume in the flight group was significantly different from both the flight control ($P<0.025$) and the vivarium control ($P<0.001$) groups.

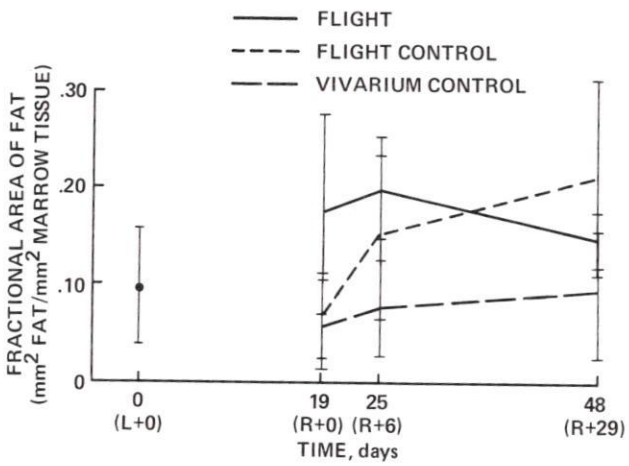

Figure 2. The fractional area of fat in the bone marrow in the proximal tibial metaphysis vs. time. See the legend for Figure 1 for a detailed description.

Figure 2 is a similar plot of the fractional area of fat in the bone marrow vs. time. The data suggest that the fat content of the bone marrow in the proximal tibial metaphysis increased during spaceflight. The difference in fat content at day 19 between the flight group and both control groups was significant at the level of $P<0.05$. By the end of the postflight period (day 48), there were no significant differences among the three groups.

DISCUSSION

This study demonstrates that periosteal bone formation in the tibial and humeral diaphyses was inhibited during orbital flight aboard the Soviet Cosmos 1129 biological satellite. A similar effect was observed in the tibia during previous Cosmos experiments (6), but the humerus was not included in prior investigations. The inhibition of periosteal bone formation in the humerus was not as dramatic as in the tibia. This may be due to the lower rate of periosteal bone formation in the humerus relative to the tibia. The rebound in periosteal bone formation rate in the tibia during the postflight period has also been previously observed. The humerus did not exhibit a similar rebound, but periosteal bone formation did return to normal during the postflight period.

There were no significant differences among the three experimental groups in cross-sectional medullary area in the tibial and humeral diaphyses. The predominant activity along the endosteal surface of the medullary canal in growing rats is bone resorption. An alteration in bone resorption during spaceflight would presumably be accompanied by a change in the dimensions of the medullary canal. Since this did not occur, these data suggest that no gross changes in endosteal bone resorption occurred during spaceflight. However, subtle changes in bone resorption may not be revealed by this technique.

A decreased trabecular bone volume and an increased fat content of the bone marrow in the proximal tibial metaphysis appear to be a consequence of spaceflight. However, these parameters are difficult to evaluate due to a great deal of variability within groups. A decreased mass of trabecular bone in the femoral and tibial metaphysis has been reported in half of the rats subjected to spaceflight aboard the Soviet Cosmos 605 biosatellite (5). A marked elevation of triglycerides in the bone marrow of flight rats during the Cosmos 936 experiment (10) supports our finding of an increased fat content in the bone marrow.

In summary, we have shown that the skeletal changes induced by spaceflight aboard the Soviet Cosmos 1129 biological satellite were an inhibition of periosteal bone formation in the tibial and humeral diaphyses, and a decreased trabecular bone volume and an increased fat content of the bone marrow in the proximal tibial metaphysis. Endosteal bone resorption was not affected markedly. Identification of the mechanisms responsible for these skeletal alterations must await additional studies.

ACKNOWLEDGMENT

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REFERENCES

1. Lutwak, L., G. D. Whedon, P. A. LaChance, J. M. Reid, and H. S. Lipscomb. 1969. Mineral, electrolyte, and nitrogen balance studies of the Gemini-VII fourteen-day orbital space flight. J. Clin. Endocr. 29:1140-1156.

2. Rambaut, P. C., C. S. Leach, and P. C. Johnson. 1975. Calcium and phosphorus changes of the Apollo 17 crewmembers. Nutr. Metab. 18:62-69.

3. Whedon, G. D., L. Lutwak, P. C. Rambaut, M. W. Whittle, M. C. Smith, J. Reid, C. Leach, C. R. Stadler, and D. D. Sanford. 1977. Mineral and nitrogen metabolic studies, experiment M071. Pages 164-174 in R. S. Johnston and L. F. Dietlein, eds. Biomedical Results from Skylab. NASA Document SP-377, Washington, D. C.

4. Vogel, J. M., M. W. Whittle, M. C. Smith, and P. C. Rambaut. Bone mineral measurement-experiment M078. Ibid, pages 183-190.

5. Yagodovsky, V. S., L. A. Triftanidi, and G. P. Gorokhova. 1976. Spaceflight effects on skeletal bones of rats (light and electron microscope examinations). Aviat. Space Environ. Med. 47:734-738,

6. Morey, E. R., and D. J. Baylink. 1978. Inhibition of bone formation during spaceflight. Science 201:1138-1141.

7. Milch, R. A., D. P. Rall, and J. E. Tobie. 1957. Bone localization of the tetracyclines. J. Nat. Cancer Inst. 19:87-93.

8. McManus, J. F. A., and R. W. Mowry. 1960. Page 201 in Staining Methods, Histological and Histochemical. Paul B. Hoeber, Inc., New York, N.Y.

9. Merz, W. A., and R. K. Schenk. 1970. Quantitative structural analysis of human cancellous bone. Acta Anat. 75:54-66.

10. Ahlers, I., R. A. Tigranyan, and M. Praslicka. 1980. The effect of artificial gravitation on rat plasma and tissue lipids: The Cosmos 936 experiment. Page 493 in Abstracts for the Twenty-Third Plenary COSPAR Meeting. Budapest, Hungary.

BONE RESORPTION AND CALCIUM ABSORPTION IN RATS DURING SPACEFLIGHT

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INTRODUCTION

Calcium metabolism is altered in weightlessness. Bone loss occurs and urinary calcium output is increased in humans (1) and there is a significant decrease of tibial bone formation rate in young rats (2,3). These changes which occur during spaceflight are similar to changes observed in immobilized humans (4,5) and monkeys (6), but the underlying causes of these changes are not known. A primary detect in the development of immobilization osteoporosis in adults appears to be an unexplained increase in bone resorption coupled with a mineralizaton defect, leading to a rapid loss of bone. The sequence of metabolic changes which occurs after this postulated increase in bone resorption includes a slight increase in serum calcium and phosphorus. The homeostatic response to this change in serum calcium involves changes in circulating levels of parathyroid hormone and the active metabolites of vitamin D. Calcium turnover and urinary calcium output increase. This increased calcium excretion coupled with a decline in intestinal calcium absorption produces a strongly negative calcium balance. In paralyzed patients and bed-rested subjects, this process appears to be self-limiting. Following the initial rapid loss of bone, calcium turnover and urinary output decrease to below-normal values. This chronic phase of immobilization osteoporosis appears to be a state of rarefied bone with a low turnover rate. Remobilization in adults does not appear to completely correct the bone mass defect, so that adult bone loss due to immobilization or weightlessness may be irreversible.

The characteristics of immobilization osteoporosis are quite different in young, growing patients or animals. Bone turnover in a juvenile skeleton is qualitatively different from that in an adult skeleton. Adult bone is characterized primarily by remodeling activity, in which existing bone is resorbed by osteoclasts and then replaced by osteoblasts, with no net change in bone mass or spatial orientation of the bone. In contrast, juvenile bone changes are composed of two processes: 1) growth and modeling, and 2) remodeling. The growth and modeling component includes periosteal apposition, endosteal resorption and apposition, and growth in the epiphyseal-metaphyseal regions of the bones, and dominates bone turnover until the skeleton matures. During this time of growth, bone formation exceeds bone resorption by a significant amount so that net body calcium balance is positive. little data exist on the quantitative values of bone formation and resorption in juveniles, and even less on the effects of immobilization or weightlessness on these parameters. Experiments on previous Cosmos flights has shown that bone formation in the tibia is depressed in young, growing rats (2), but no direct information has been obtained about bone resorption or any of the other calcium metabolic parameters such as excretion, absorption or net calcium balance. The basis for the present study was to determine the response of calcium homeostasis and bone to weightlessness.

Natural calcium is a mixture of the stable isotopes $^{40,42,43,44,46,48}Ca$ in varying percentages, with ^{40}Ca making up the largest fraction (96.94%). The other stable calcium isotopes are present in relatively small amounts, such as ^{46}Ca at 0.0033% and ^{48}Ca at 0.185%. Natural calcium can therefore be considered a bulk isotope (^{40}Ca) labeled with small quantities of stable isotopic tracers (^{46}Ca,^{48}Ca). In this way, one can consider also that all the skeletal calcium of man or any other animal is labeled with these stable isotopic tracers, and bone breakdown or resorption can be measured directly if one measures the rate of release of one of these tracers from bone into the serum/extracellular fluid pool. The only continuous sources of calcium into the serum pool are bone and the diet (Fig. 1). In the normal situation, both bone and dietary calcium are made up of natural calcium, and thus both are labeled with stable isotopic tracers such as ^{48}Ca. If one removes ^{48}Ca from the diet, however, then it is distinguished from bone calcium by this lack of tracer. This is done by replacing natural dietary calcium with isotopically-separated ~100% ^{40}Ca. As calcium is excreted from the serum, it is replaced by calcium coming from both bone and the diet, but the only source of ^{48}Ca is the bone. Therefore, the amount of ^{48}Ca in the serum or muscle will fall to a value which represents the fraction of calcium turnover coming directly from bone (Fig. 2).

The primary measurements made in these tracer studies were the ratio of ^{48}Ca to total calcium in muscle (or serum) and the excreta. Continuous tracer administration calcium kinetic methods were used. Bone resorption was measured directly as the release from the skeleton of the stable calcium isotope ^{48}Ca. Endogenous calcium excretion was also measured. When data on calcium

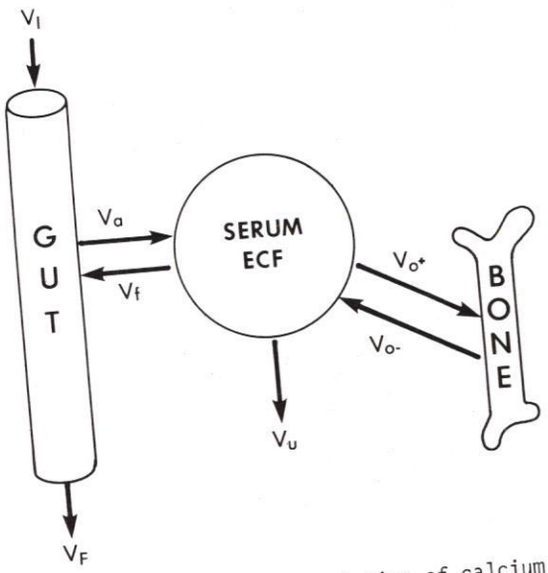

Figure 1. Schematic representation of calcium movement in the body. Calcium enters the serum pool from bone and intestine and leaves via the urine, feces, and bone formation.

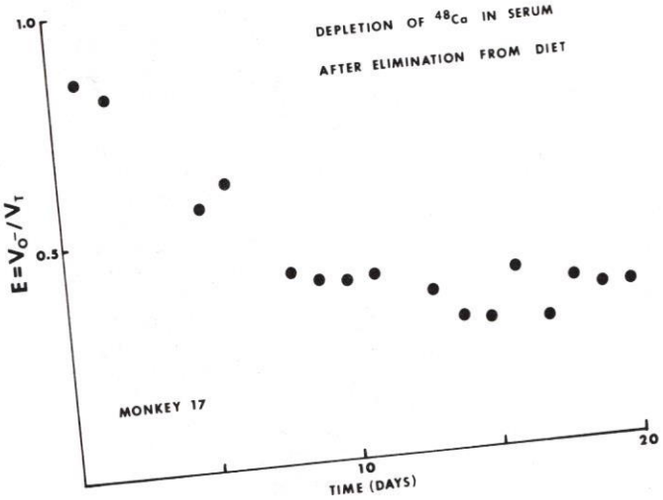

Figure 2. Disappearance of ^{48}Ca from the serum with time after elimination from the diet. Asymptotic value represents the fraction of exchangeable calcium coming from bone.

intake is provided then intestinal calcium absorption whole body bone formation will be calculated. The excretion of sodium, potassium, magnesium and zinc was determined to compare with the calcium results as an indicator of overall mineral homeostasis.

METHODS AND PROCEDURES

Subject material

A total of 10 rats were used in this study: 5 rats from the flight group killed immediately postflight (1F1-5) and their 5 synchronous controls (1S1-5). Specimens received from the Soviets following flight were the rib cage (left and right sides) from each animal and approximately 50% of each 2-day excreta collection from each animal. A total of 10 rib cages and 102 fecal specimens were received. The muscle from each rib cage was used as an indicator of tracer activity in the serum because it has been shown that muscle calcium equilibrates rapidly with serum calcium in tracer studies (7).

Diet preparation

In order to use the continuous calcium tracer methods outlined in the introduction, natural dietary calcium had to be replaced with stable, isotopically-separated ^{40}Ca. The major source of calcium in the Soviet flight paste diet was calcium carbonate ($CaCO_3$). When the diet was prepared for the 1F and 1S groups of rats, the natural $CaCO_3$ was replaced with the chemically identical $^{40}CaCO_3$ (99.991% ^{40}Ca, < 0.001% ^{48}Ca). This diet was indistinguishable from the normal paste diet except in its ^{48}Ca content which was approximately zero. Animals were started on this diet at the time of loading into the flight hardware.

Sample preparation

Approximately 50% of each 2-day pooled excreta collection was received dry in a polyethylene vial. These specimens represented pooled urine and feces due to the manner in which excreta collection was done. Virtually all calcium excretion in the rat is through the feces, however, with less than 1 mg/day in the urine. For this reason separation of urine from feces in calcium metabolic studies is not critical and for the purposes of this study the pooled excreta will be referred to as "feces". Each sample was weighed, ground in a mortar and pestel, and dried at 110°C. An aliquot of the dried powder was accurately weighed (±0.1 mg) into a crucible for ashing. 0.1-0.2 gm of feces was used depending upon amount received for each sample. Feces were ashed at 600°C for 48 hours and the ash was weighed (±0.1 mg). The ash was dissolved in 12N HNO_3, taken to dryness, and the residue dissolved in HNO_3 and diluted to either 25 ml or 50 ml depending on sample size. This was the stock solution on which mineral and calcium tracer measurements were made.

The rib cage (left and right sides) of each animal was received frozen. The specimens were

thawed and the intercostal muscles were dissected out from between the third and eleventh ribs. Extreme care was taken in the dissection to be sure that no cartilage or bone was included in the muscle samples. To do this only 60-70% of the total musculature was used. Rib cages from left and right sides of each animal were treated as separate specimens, providing a total of 20 samples. Each muscle specimen was placed into a crucible, dried, weighed, ashed at 600°C and weighed again to determine ash content. The ashed samples were then dissolved in HNO_3 for tracer analysis.

Tracer and chemical analysis

Total calcium, magnesium, sodium, postassium and zinc were determined in each fecal sample solution using atomic absorption spectrophotometry. Results were expressed in terms of mg of each mineral per gram of dried fecal material and total mg per 2-day collection period.

A 10 ml aliquot of each stock fecal solution was taken for calcium tracer measurements. The sample was adjusted to pH 4-5 with 8N NH_4OH. 3 ml of saturated ammonium oxalate ($(NH_4)_2C_2O_4$) was added to each solution to precipitate calcium as CaC_2O_4. Solutions were centrifuged and supernate discarded. The precipitate was washed, redissolved and reprecipitated, washed again and finally dissolved in 4N HNO_3. 1.00 ml of this solution was used for neutron activation analysis determination of ^{48}Ca content and total calcium was determined by atomic absorption on an aliquot of the remaining solution.

Neutron activation analysis for ^{48}Ca was done at the Berkeley Research Reactor, University of California, Berkeley. Each prepared sample was paired with a standard containing a known quality of ^{48}Ca and irradiated in the Flexorabbit facility of the BRR for 10 minutes at a thermal neutron flux of 1.0×10^{13} $cm^{-2}sec^{-1}$. Two minutes after irradiation, the samples were counted for 5 minutes (real time) with a high efficiency Ge(Li) detector coupled to a 4096-channel pulse height analyzer. Standards were counted in the same geometry immediately after the sample count was completed. Live counting time of the system was determined using pulser electronics. The intensity of the 3084 keV photopeak from ^{49}Ca was determined in each spectrum using the computer code SAMPO. The quantity of ^{48}Ca in each specimen was determined from sample and standard photopeak intensities, decay times and counting times, and the known standard mass of ^{48}Ca.

RESULTS

The results of the mineral analyses of the excreta calcium, magnesium, sodium, potassium, zinc and ash are presented graphically in Figure 3. Each data point is the mean of the values obtained for the 5 rats in the flight and control groups. The points are plotted in 2-day collection periods without correcting for actual endogenous excretion periods. Results are expressed in mg per gram of dried feces for the minerals and percent of dry feces for ash. Total fecal material excreted by flight and control groups was not significantly different when averaged over the whole flight period (10 collections). When averaged over collection periods 2-9, however, the synchronous control animals excreted approximately 14% more feces than flight animals (1.24 g/day vs. 1.09 g/day). It is not known whether this difference is due to increased food utilization in the flight animals or to a decreased food intake because food consumption data for 1F and 1S rats has not yet been provided.

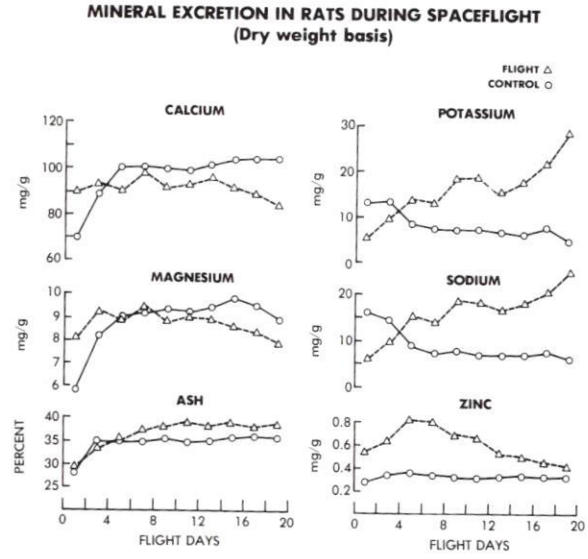

Figure 3. Excretion of Ca, Mg, K, Na, and Zn by flight and control rats. All values are expressed on a dry weight basis.

Bone resorption expressed as the fraction of the exchangeable calcium pool coming from bone was 0.690 ± 0.089 in flight animals vs. 0.675 ± 0.085 in controls, measured at the end of the flight period. Fecal excretion of ^{48}Ca/total Ca was 0.159 ± 0.011 for the flight rats and 0.157 ± 0.006 for control rats at the end of the flight period. Endogenous excretion of calcium, based on the measured ^{48}Ca/total Ca ratio and measured total calcium excretion was $29.0 \pm 3.1\mu g$ 48Ca/day in flight animals and $37.4 \pm 3.2\mu g$ ^{48}Ca/day in control animals. Bone resorption rate at the end of the flight period was 15.7 mg Ca/day in the flight rats and 20.2 mg Ca/day in the controls.

DISCUSSION

Bone formation in rats is known to be decreased during spaceflight. In normal mineral homeostasis, a decrease in bone formation will lead to a decrease in bone resorption as well, so that bone mass will be maintained. If bone resorption either proceeds at its normal rate or increases, then bone mass will be lost at a rate which is proportional to the difference between formation rate

and resorption rate. Estimates of the kinetics of the decrease in bone formation in the rat during spaceflight (3) suggest that formation decreases linearly with time, finally virtually ceasing at 11-12 days of flight. In contrast, the kinetics of bone resorption measured during this experiment suggest that the breakdown of bone in flight rats is maintained at the same level as in control rats until 10-12 days into flight, then starts to decrease, reaching a level which is 20-25% below that for synchronous controls at the end of the flight period. It is significant that resorption normalized by calcium turnover does not decrease during flight, so that the decrease seen in the bone resorption rate is probably secondary to a decrease in total body calcium turnover. These results indicate that in rats during spaceflight, as in immobilized humans, bone formation and bone resorption are uncoupled, and the difference in their rates should lead to significantly less bone mass for flight animals compared to controls. Of particular interest may be the fact that while the bone resorption rate decreases during flight, it is still 75-80% of normal at the end of flight. This may indicate that bone loss on even longer flights will continue unless some method can be found to either turn off resorption completely or turn on formation again.

The other parameters of calcium metabolism such as calcium balance, bone formation, and intestinal calcium absorption have not been calculated due to lack of food intake data for the flight and synchronous control groups. The decrease in bone resorption apparently secondary to decreased calcium turnover, however, indicates a probable decrease in intestinal calcium absorption as well. This absorption has been found to be decreased ~40% in hypokinetic rats (7).

The excretion of minerals other than calcium show some interesting patterns. Sodium and potassium are virtually identical in their excretion with a consistent rise even until the end of flight when levels were 4-5 times normal. Whether this is decreased absorption or increased endogenous excretion is not known, although a rise of this magnitude would be expected to be due to increased excretion. Zinc excretion is the most consistent of all the elements in the control rats, and in the flight rats shows a rapid rise followed by a gradual fall back to near-normal values. This pattern has not yet been analyzed on the basis of zinc physiology.

SUMMARY

Bone resorption was measured directly in flight and synchronous control rats during Cosmos 1129. Continuous tracer amdinistration techniques were used, with replacement of dietary calcium with isotopically enriched ^{40}Ca and measurement of the ^{48}Ca released by the skeleton by neutron activation analysis. There is no large change in bone resorption in rats at the end of 20 days of spaceflight as has been found for bone formation. Based on the time course of changes, the measured 20-25% decrease in resorption is probably secondary to a decrease in total body calcium turnover. Total calcium excretion starts to decrease after 10-12 days of flight, again probably due to decreased turnover but possibly due to decreased intake. The excretion of sodium, potassium and zinc all increase during flight, sodium and potassium to a level 4-5 times control values. The continual imbalance of bone formation and breakdown and the large excretion of other minerals from the body during spaceflight indicate that mineral homeostasis does not adapt to weightlessness at least within the time frame studied in this experiment, and that the long-term consequences of weightlessness are not yet known.

REFERENCES

1. Whedon GD, L Lutwak, PC Rambaut, MW Whittle, MC Smith, J Reid, C Leach, CR Stadler, DD Sanford. 1977. Mineral and nitrogen metabolic studies, experiment M071. In Biomedical Results of Skylab, NASA SP-377, p 164-174.
2. Morey ER and DJ Baylink. 1978. Inhibition of Bone Formation During Space Flight. Science 201:1138-1141.
3. Holton EM, RT Turner, DJ Baylink. 1978. Quantitative Analysis of Selected Bone Parameters. In Final Reports of US Experiements Flown on the Soviet Satellite Cosmos 936, NASA TM-78526, p 135-178.
4. Heaney RP. 1962. Radiocalcium metabolism in disuse osteoporosis in man. Amer. J. Med. 33:188-200.
5. Minaire P, P Meunier, C Edouard, J Bernard, P Courpron, J Bourret. 1974. Quantitative histological data on disuse osteoporosis. Calcif. Tiss. Res. 17:57-73.
6. Cann CE and DR Young. 1979. Calcium metabolism in disuse osteoporosis in monkeys: continuous tracer and pulse tracer kinetics (abst). Calcif. Tiss. Internat. 28:162.
7. Cann CE, unpublished results.

BONE GROWTH IN THE RAT MANDIBLE DURING SPACE FLIGHT

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Rats flown for 18.5 days in the COSMOS 1129 biosatellite exhibited normal rates of dentinogenesis (lower incisors) and osteogenesis in the body of the mandible during 18.5 days at 0-G. The rate of migration of the molar teeth owing to bone remodeling around the roots was slightly but not significantly reduced. The total calcium, phosphorus and hydroxyproline levels in the jaws and incisors of the flight rats were normal. Gravity density fractionation studies suggested however, that spaceflight caused a delay in the normal maturation of bone mineral and matrix; the teeth were spared, and exhibited a normal circadian and ultradian pattern of enamel-dentin calcification. We conclude that the rat mandible did not suffer the deficits of bone formation common to weight-bearing parts of the skeleton of animals during spaceflight.

INTRODUCTION

Efforts to understand how prolonged spaceflight affects changes in calcium homeostasis and bone formation-resorption have been pursued in laboratory rats during three joint NASA-Soviet Biosatellite Flights of 18.5 - 22 days duration (COSMOS 605, 782, 936) (1-4). The appendicular weight-bearing bones (femurs and tibias) suffered reduction in the rate of cortical and trabecular bone growth and in their ash content, but certain of the forelimb elements (ash content of ulna and radius) were spared. Quite unexplained was the fact that periosteal growth was diminished while endosteal growth and resorption remained unchanged. The COSMOS 936 biosatellite also included some animals maintained in a centrifuge which provided a 1G environment, and their skeletons were largely spared the deleterious effects of null gravity. Healing of the periosteal growth deficit and restoration of long bone mechanical strength was noted in the 0-G group after a 25 day post-flight recovery period in a Moscow vivarium. In the most recent COSMOS 1129 flight (September-October, 1979) there was an opportunity to examine the effect of null gravity on the integrated growth and remodeling of a non-weight-bearing bone-the mandible, and its teeth. How might 0-G affect tissues in a skeletal element which is supplied with a large antigravity muscle?

MATERIALS AND METHODS

Three groups of 5-7 SPF male rats (270-320 grams) were injected with 1.0 mg/kg Declomycin 3 days prior to being loaded into a block modules of 5 cages (singly housed) mounted in a modified Soyez spacecraft. The animals were then launched into orbit for a period of 18.5 days. The particular details of the flight have been described by Wronski et al, (5). During flight, the rats were exposed to a 12 hour/12 hour light-dark cycle. Water was supplied ad lib, and they were fed 10g alliquots of a nutritionally adequate paste diet four times per day. Control animals were maintained in a landbound mock-up of the biosatellite under nearly identical conditions, and these were subjected to simulated stresses of launch and recovery (acceleration and vibration). Additional groups of (unstressed) control animals were maintained in a Moscow vivarium. A group of 5 flight rats were injected with Declomycin (1.0 mg/kg) shortly after recovery of the spacecraft, and they were sacrificed 38h later. The control groups and one group of flight rats received a second and/or third injection of Declomycin on the 6th and 27th days following recovery, and they were sacrificed 48h later. The total number of groups of animals were as follows:

Groups

I Preflight Vivarium Controls

II Flight Basal

III Synchronous Basal

IV Synchronous Controls

V Flight Rats

At autopsy the mandibles were recovered, cleaned of soft tissues and fixed in either 70% or 95% ethyl alcohol, for analysis of the growth (tetracycline data) and matrix-mineral maturation.

Analysis of Growth: The left jaw was divided into three regions - the premolar, molar and post-molar regions. The premolar and molar areas were embedded, undecalcified in methyl methacrylate, and sectioned transversely to the long axis of the jaw on a high speed rotary saw at 50-60 µm. The molar region was sectioned in the frontal plane at 10 µm on a Jung Microtome to reveal the roots of the molar teeth, and these sections were stained with the Goldner method to reveal mineralized bone and osteoid. All the sections were examined by UV microscopy, to reveal the distribution of the tetracycline time markers. The mineralization rate (= appositional bone growth and dentinogenesis) was estimated by measuring the distance between the tetracycline bands, and dividing that

value by the time interval (days) between injections. These measurements around the roots of the molar teeth, based on Vignery and Baron's model (6), provided information about the rate of tooth migration via resorption (anterior surface) and formation (posterior surface).

Dentinogenesis was estimated in the region of the diastema, a region providing a period of growth of 19-21d. When the tetracycline labeling intervals were longer than 21d, we used sections of the erupted portions of the teeth where the dentin was formed almost exclusively during the flight period.

Matrix-Mineralization Maturation: The incisor was removed from the right jaw and it was divided into 3 regions representing the distal region of tooth formation, the middle region of tooth maturation, and the coronal or erupted portion. The teeth sectors and jaw bones were individually separated by a bromoform-touluene gradient density separation into 3 specific gravity fractions (1.3-1.9, 2.0-2.1, 2.2-2.9) and these were analyzed for calcium (Ca), inorganic pohsphorus (Pi) and hydroxyproline (HO-Pr). In the normal growing rat, most of the mineral and HO-Pr in bone and teeth are concentrated in the highest gradient density fractions (2.2-2.9) - the less mature bone and tooth mineral being distributed in fractions 1.3-1.9 and 2.0-2.1. Thus, these analyses recorded a maturational profile for bone matrix and mineral.

RESULTS

Dentinogenesis: At the mandibular diastema, the average preflight rate of dentinogenesis was 17-18 µm/d, and this was unchanged during the flight and post-flight recovery periods (Figure 1). In the vivarium and synchronous control groups, however, the rates of dentinogenesis increased slightly during the recovery period.

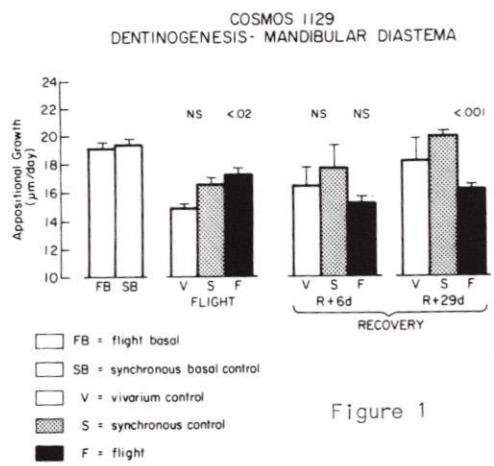

Figure 1

Osteogenesis: A. Body of the Mandible. No changes from the normal rate of osteogenesis was noted in the area of the mandibular diastema during spaceflight. The normal rate of growth of about 3-4 µm/d was also maintained during the postflight recovery period (Figure 2). Growth in

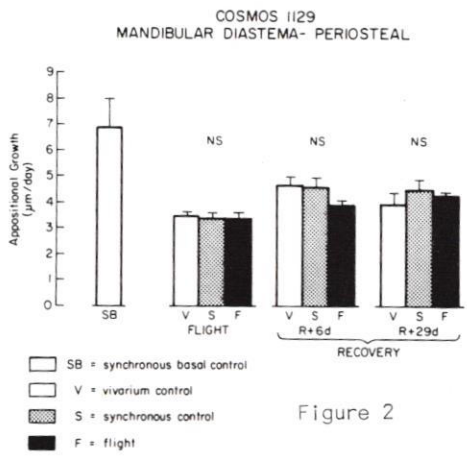

Figure 2

the groups of control animals proved to be the more variant. Intergroup variations were also absent in the ossification of the post-molar mandibular ramus. Periosteal growth along the superior and inferior rami averaged 2-3 µm/d both during the flight and postflight recovery periods.

These data suggested that spaceflight had no effect upon the appositional growth of the body of the rat mandible - a non-weight-bearing bone. These results stand in sharp contrast to the data presented by Wronski (1980) that appositional growth rates in the weight-bearing bones (appendicular skeleton) were depressed 30-60%. In fact, however, the jaw bones from the animals flown in space were not entirely normal. There were distinct changes in the remodeling of the alveolar bone around the roots of the molar teeth and in the maturational status of the bone mineral and collagen of the mandibles.

B. Alveolar Bone. Changes in the rate of osteogenesis in the jaw were obvious only around the roots of the molar teeth. The rates of formation in the control were 8.6 µm/d versus 6.8 µm/d for the flight animals. There was no change in the volume of the periodontal ligament, but the extent of specific surfaces of formation and resorption were reduced. The most conservative interpretation of the data is that the tipping of the molar teeth which is a normal consequence of age was reduced in the flight animals.

Bone Maturation: When the jaws and each of the 3 regions of the incisors were analyzed for total Ca, Pi and HO-Pr, we could not distinguish the flight from the control animals by any changes in their hard tissue chemistry (Figure 3). The illustrations also include, for comparison, data from animals of the same age and sex fed Purina Laboratory Block Chow and tap water. The density gradient fractionation studies indicated, however, that rat alveolar bone was distinctly abnormal after spaceflight (Figure 4). In all flight rats, there was a highly significant diminution of bone collagen and mineral moieties in the most dense, mature fractions (2.2-2.9), with a corresponding increment in the least dense, most immature

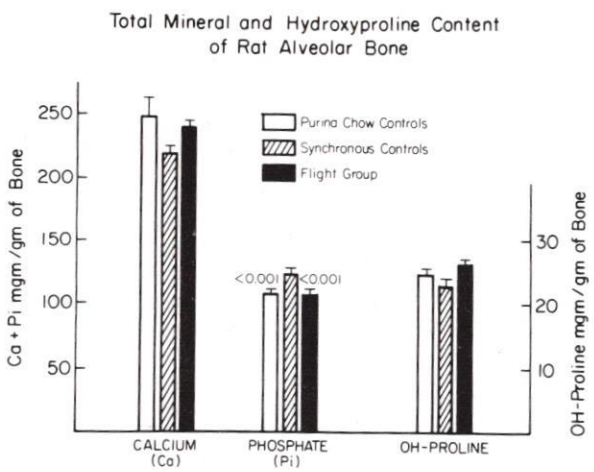

Figure 3

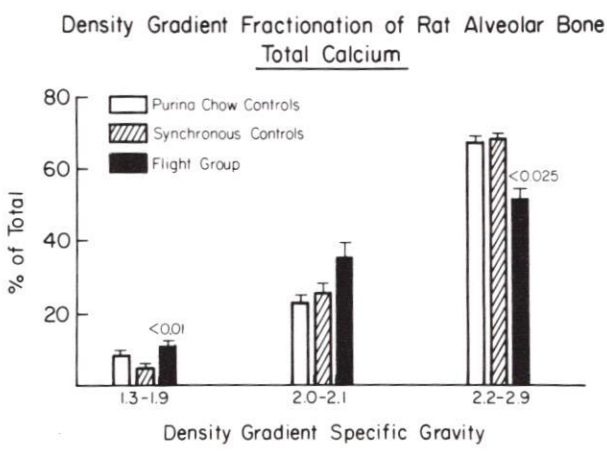

Figure 4

fractions (1.3-1.9, 2.0-2.1). The highest density fractions of the flight rat bones had 30% less mineral and collagen (HO-Pr) than the corresponding fractions of the control rat bone. These changes suggested that there was a distinct deficit in the flight animals-that there was a delay in the maturation of the collagen (lack of intramolecular cross linds) and apatite mineral. Importantly, these deficiencies were fully corrected during the post-flight recovry period of 29 days.

Similar changes were not apparent when the 3 regions of the teeth were analyzed (Figures 5ab), suggesting that the teeth were highly conserved elements, i.e., vitally essential to life itself.

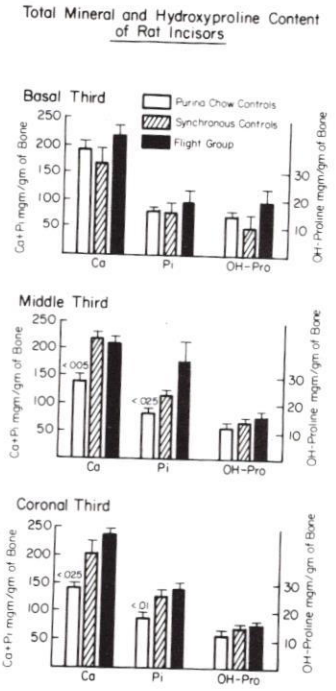

Figure 5a

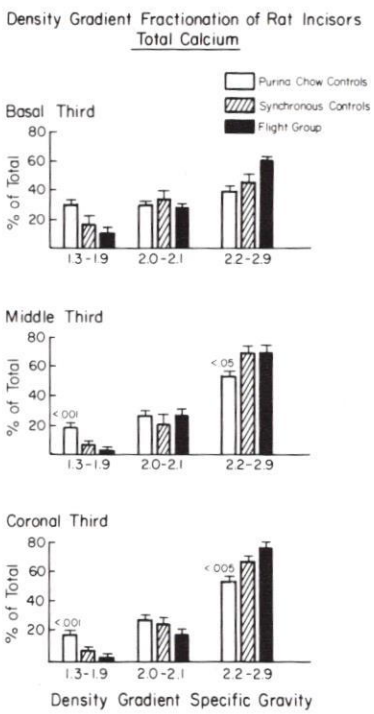

Figure 5b

Microradiographs of the growing surfaces of the bones did not detect the mineral deficit associated with the period of null gravity, but they did reveal alternating bands of high and low density in the dentin of the rat incisor. There were no obvious differences between the flight and control rats in this regard. An attempt was made to define the biorhythmical components of growth in the incisor dentin since it was possible that spaceflight interfered with the normal rhythmic behavior of the odontoblasts.

Biorhythmicity (Electron Microprobe): Continuous 1 µm traces with the electron microprobe (Monsanto Corp., St. Louis, MO) from the pulp to the labial surface of the enamel revealed that there were repeatable Ca and Pi peaks (Fourier Analysis) at intervals of 5 µm. Since labial dentin was deposited at a rate of 20 µm/d, these 5 µm intervals obviously represented periods of formation at times shorter than 24 hrs, and multiples of this rhythm represented a circadian time period (= 24 hours).

COMMENT

There were few direct measurable effects of spaceflight in the mandible of the rat. This suggests that as opposed to the long bones which are weight-bearing parts, the skeletal elements that are non-weight-bearing- even when supplied with an anti-gravity muscle- would tend to be unaffected. This was not surprising since the growth of the entire body of the rat mandible must be coordinated with the production of the incisor teeth which grow along a spiral axis. The lower incisors must appose after eruption if the animal is to continue eating and assure its survival. While the appositional growth of the body of the mandible is relatively normal, we did detect changes in the maturation of bone collagen and mineral. Such changes involving a delay in the maturation of the collagen and mineral fractions is typical of some other skeletal problems such as those which have been documented in uremic rats (7). It was also notable that the rates of bone formation and resorption around the roots of the molar teeth were reduced. This might occur if there were a flight-related reduction in the maturation or the osteoprogenitor cell population to the osteoblast class. The reduction in bone turnover and tooth migration may have been due to, in part, the soft diet which did not provide sufficient resistance to chewing. Such a change has been reported in the tissue surrounding the maxillary molar teeth. Roberts (7) noted that, on the basis of nuclear: cytoplasmic volume ratios (large ratios being typical of the mature osteoblast), there was a predominence of cells with a low volume ratio, i.e., more than the normal number of osteoprogenitor cells. The retardation of cell maturation may have been due to alterations in blood corticosteriod levels and/or parathyroid gland function. In toto, the data suggests that even the non-weight-bearing bones of the skeleton will not escape the effects of spaceflight and that it is rather the quality of the bone which is impaired rather than the actual volume of the tissue or its mineralization status.

REFERENCES

1. Holton, E.M. and D.J. Baylink. 1978. Final reports of U.S. experiments flown on the Soviet Satellite COSMOS 782 (NASA TM-78525, p. 321.
2. Holton, E.M., R.T. Turner and D.J. Baylink. 1978. Final reports of experiments flown on the Soviet Satellite COSMOS 936 (NASA TM-78536, p. 135.
3. Spengler, D.M., E.R. Morey, D.R. Carter, R.T. Turner and D.J. Baylink. 1979. Effect of spaceflight on bone strength. The Physiologist 22:5-75.
4. Morey, E.R. and D.J. Baylink. 1978. Inhibition of bone formation during space flight. Science 201:1138.
5. Wronski, T.M., E.R. Morey, and W.S.S. Jee. 1980. The Physiologist (IN PRESS).
6. Vignery, A. and R. Baron. 1980. Dynamic Histomorphometry of alveolar bone remodeling in the adult rat. Anat. Rec. 196:191-202.
7. Russell, J.E. and L.V. Avioli. 1972. Effect of experimental chronic renal insufficiency on bone mineral and collagen maturation. J. Clin. Invest. 51:3072-3079.
8. Roberts, W.E., W.C. Goodwin, and S.R. Heiner. 1980. Dental Clin. North America, (IN PRESS).

A NEW RAT MODEL FOR STUDIES OF HYPOKINESIA AND ANTIORTHOSTASIS

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A new rat model (suspension and immobilization) is described for induction of hypokinesia and orthostatic manipulations. Hypokinetic responses were comparable to those in prolonged bed rest and weightlessness in humans, body or limb casted and small cage restrained animals. Responses to antiorthostasis (15 to 20° head down tilt) in rats were similar to those in neutral bouyancy tests in humans and animals and to those in prolonged bed rest in humans. During 7 days of hypokinesia there was an atrophy of the gastrocnemius and increased excretion of urinary nitrogenous end products. The antiorthostatic (AOH) 15 to 20° head down tilt resulted in diuresis, natriuresis and kaliuresis. No comparable responses were observed in orthostatic hypokinetic (OH) rats. Readaptation from AOH and OH occurred during one week recovery in metabolic cage conditions.

APPROACHES TO HYPOKINESIA AND ANTIORTHOSTASIS

Physiological responses to hypokinesia have been studied in animals and man. In animals, cast immobilization (1), small animal cage restraint (2) and suspension (7,8,9,10) have been used. Human subjects have been tested with prolonged bed rest (4,5), weightlessness (12) and neutral bouyancy (5).

During bed rest, body casting and weightlessness, the human subject is often both hypokinetic and antiorthostatic. Thus, the causitive factors in physiological responses are often not separable. This led to our development of an animal model which can be used for studies of hypokinesia and antiorthostasis either separately or concurrently.

Sprague Dawley rats were suspended in a harness such that the hind legs were never in contact with a supportive surface. To simulate antiorthostasis, the rat was tilted 15° to 20° head downward, for details see Musacchia et al. (9) and Deavers et al. (3). Our model differs from previous experimental approaches in that the animal is suspended in a cloth-velcro harness without sutures or adhesives. The animal retains the ability to utilize the front legs for feeding and grooming.

Since bed rest and weightlessness result in loss of muscle mass, increased excretion of urinary nitrogenous end products and negative calcium balance, our study using a suspension harnessed subject was designed to test the animal model for comparable physiological responses. Furthermore, since human subjects in a horizontal posture or in neutral bouyancy conditions respond with diuresis, natriuresis and often kaliuresis, the rat model was adapted to produce comparable physiological changes.

RESULTS

Rats were suspended for seven days and permitted to recover for seven days. After seven days of hypokinesia, there was a marked increase in kidney papillary urea levels, from control levels of 698 to 1234 mg/100 ml in suspended animals. Also, there was a twofold increase in urine urea excretion from 4.5 to 8.0 m moles/day. This occurred as early as the second day of hypokinesia and remained elevated throughout the hypokinetic suspension. During recovery, with animals in metabolism cages, urinary excretion of urea returned to control rates within the first day.

There were parallel changes in ammonia excretion. From the first day of hypokinesia, through the seventh day, there was a twofold or greater rate of excretion, from 0.1 - 0.25 to 0.45 - 0.6 m mole/day.

Urinary excretion of 3 methylhistidine (3MH), an amino acid contained only actin and myosin, increased significantly from about 0.42 (controls) to 0.55 (hypokinetic) μ moles/100 gm·day and remained elevated throughout the seven days. After rats were removed from the suspension harness and placed in metabolism cages, 3MH excretion showed a return to control rates, i.e. 0.42 μ moles/100 gm·day.

The gastrocnemius, an antigravity extensor muscle, of the hind leg showed a significant atrophy. Following one week of hypokinesia the weight had fallen to 604 mg wet wt. compared to weight matched control values of 734 mgs wet wt. During a week of recovery, they regained most of

their muscle mass. Current studies using soleus muscle also show evidence of considerable atrophy.

Antiorthostatic responses are seen in terms of progressively increased urine volume, from 8 to 16 cc/day during seven days of head-down tilt position; these are compared to orthostatic hypokinetic rats (OH) or metabolism cage controls. After removal from suspension, during the first day in the normal posture in metabolism cages, there was a dramatic reduction in urine volume. During the next seven days there was a progressive return to control rates of urine production.

Simultaneous measures of water intake of OH and AOH rats showed no differences throughout the week, thus the diuresis cannot be explained by differences in water consumption. Also, during the first day of recovery, there was a significant increase in water intake. Our deduction was that significant water retnetion during the first day of removal from the anti-orthostatic position resulted from both increased intake and decreased output. It is noteworthy that water retention is one of the more consistent responses to recovery from bedrest and weightlessness.

Alteration of Na^+ and water excretion tend to parallel each other in order to prevent changes in plasma osmotic pressure. The AOH rats showed a progressive increase of urinary Na^+ excretion during the week of head-down tilt suspension, from 1.2 to 2.8 mEq/day, and a marked reduction of urinary Na^+ loss during the first day of recovery, followed by a return of excretion rates to control levels. Also, after seven days of suspension, serum osmolarity was similar in AOH and OH rats. Additional testing of control rats in metabolism cages showed similar values. These results support the effectiveness of parallel alterations in excretion of water and Na^+ in controlling plasma osmolarity.

Measurements of K^+ showed progressive increases in AOH rats during seven days of head-down tilt suspension, and a marked reduction during the first day of recovery, paralleling the changes in the Na^+ loss. However, interpretation for K^+ changes are not as easy. One view is that with muscle atrophy and reduction in muscle fiber size, there is subsequent release of intracellular fluid high in K^+ which contributes to the high K^+ output. However, kaliuresis has been observed in short term water immersion and antiorthostasis studies which were probably not of sufficient duration to cause atrophy (5,11). Thus other mechanisms for increased K^+ loss should not be overlooked.

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REFERENCES

1. Booth, F. W. 1978. Regrowth of atrophied skeletal muscle in adult rats after ending immobilization. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 44:225-230.

2. Bykov, G. P. and V. P. Smirnov. 1970. Morphological changes in bone and muscle tissue during hypokinesia. Kosm. Biol. Med. 4:46-41.

3. Deavers, D. R., X. J. Musacchia and G. A. Meininger. 1980. A model for antiorthostatic hypokinesia: head-down tilt effects on water and salt excretion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. (in press).

4. Donaldson, C. L., S. B. Hulley, J. M. Vogel, R. S. Hattner, J. H. Bayers, and D. E. McMillan. 1970. Effect of prolonged bed rest on bone mineral. Metab. Clin. Exp. 19:1071-1084.

5. Epstein, M. 1978. Renal effects head-out water immersion in man: implications for understanding volume homeostasis. Physiol. Rev. 58:529-581.

6. Hyatt, K. H. 1971. Hemodynamic and body fluid alterations induced by bedrest. In: Hypogravic and Hypodynamic Environments. Edited by R. H. Murray and M. McCally. NASA SP 269, Washington D.C., p. 187-209.

7. Morey, E. R. 1979. Spaceflight and bone turnover: correlation with a new rat model of weightlessness. Bio Science 29:168-179.

8. Musacchia, X. J., D. R. Deavers and G. A. Meininger. 1977. Water balance and renal corticomedullary electrolyte distribution in hypokinesia. Physiologist 20:67.

9. Musacchia, X. J., D. R. Deavers, G. A. Meininger and T. P. Davis. 1980. A model for hypokinesia: effects on muscle atrophy in the rat. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 48:479-486.

10. Saiki, H., M. Nakaya, Y. Sugita and M. Kamachi. 1976. Metabolic and hormonal mechanisms of mineral metabolic adaptation to induced hypokinesia in rats. Aviat. Space Environ. Med. 47:846-852.

11. Stahl, W. M. 1965. Renal hemodynamics: the effect of gravity on sodium and water excretion. Aerosp. Med. 36:917-922.

12. Whedon, G. D., L. Lutwak, P. C. Rambaut, M. W. Whittle, M. C. Smith, J. Reid, C. Leach, C. R. Stadler, and D. D. Sanford. 1977. Mineral and nitrogen metabolic studies, experiment M071. In: Biomedical Results from Skylab. Edited by R. S. Johnston and L. F. Dietlein. NASA SP-377, Washington, D.C., p. 164-174.

Na^+-K^+ DEPENDENT ATP-ase MODIFICATIONS OF SKELETAL MUSCLE AND MYOCARDIUM OF HYPOKINETIC RATS

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INTRODUCTION

Hypokinesia, by restraint of rats or mice, produced in skeletal muscle and myocardium different functional and structural changes (2, 4, 5).
The present work was devoted to the study of membrane ionic transfer in skeletal and cardiac muscle in rats submitted to 15, 30 and 60 days hypokinesia, by determining the Na^+-K^+ dependent ATP-ase activity.

METHODS

Investigations were carried out on a total number of 70 male rats (180-200 gr) submitted to hypokinesia by including them in narrow cages for 15, 30 and 56 days. Na and K content was determined by flame-photometer in muscular tissues and also in plasma. The Na^+ and K^+ dependent ATP-ase membrane activity was determined in muscle sections incubed with ^3H-ouabaine. After washing the free, unbound fractions, marker was extracted with 5 % trichlor-aceto-acid and was read in a Tricarb-Packard spectrophotometer. The results were expressed in percentage of control (1).

RESULTS

<u>The Na^+ content</u> after 15 days of hypokinesia was not significantly changed in soleus and increased slightly but significantly in the left ventricle. After 30 days the Na of soleus and myocardium were both increased.

<u>The K^+ content</u> does not change in both muscles after 15 days and decreased after 30 days (Fig.1). In these intervals Na^+ and K^+ plasma concentrations do not significantly change.

<u>The Na^+ and K^+ dependent ATP-ase</u> of both muscles were investigated after 15, 30 and 56 days of hypokinesia. In soleus it did not change after the first interval, but was decreased by 55 % after the second, and by 60 % after 56 days. In myocardium the decreased activity was with

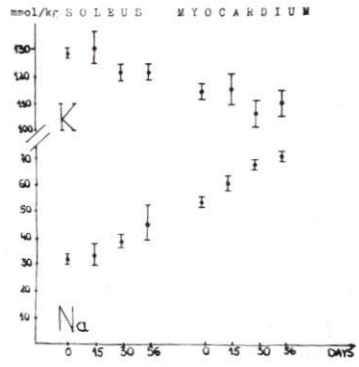

Fig.1 Na^+ and K^+ content of soleus and myocardium

19 %, 60 % and respectively with 70 %, demonstrating thus a more important lability (Fig.2). The recovering capacity was also investigated. Thus after 30 and 56 days of hypokinesia, the rats were kept

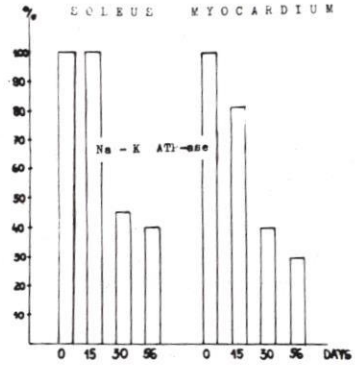

Fig.2 Na^+-K^+ ATP-ase

another 6 days in free movement conditions and then sacrificed and the Na^+ and K^+ dependent ATP-ase determined. There was

an important recovery in soleus and more important in myocardium, in which, after the first interval was complete (Fig.3).

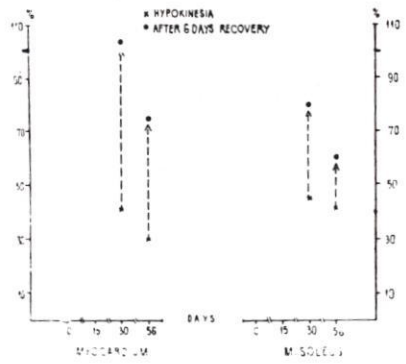

Fig.3 Na^+-K^+ ATP-ase recovery

DISCUSSION

After hypokinesia were found muscular and myocardial electron microscopic modifications, mitochondrial alterations and atrophy of myofibrils (2, 4, 5). Those observed in myocardium were more marked expressing the decreased metabolic rate due to diminished oxygen needs and use and a functional deficiency. Partially, the changes that occured in myocardium may be due to neuro-humoral reactions induced by "stress", for which it is a special target (7).
An expression for the functional alterations are the changes found in Na^+-K^+ dependent ATP-ase, that appeared diminished, in relation with the hypokinetic time interval. In myocardium the enzyme decrease is more important and may appear sooner, demonstrating a greater functional lability.
The modifications of Na and K content of both muscles produced in these conditions are the consequence of structural modification and of alteration of the complex ATP-ase membrane system, that ensures the Na^+ and K^+ extra- and intracellular distribution and polarisation (3, 6, 8). This way are explained the augmented intracellular Na^+ and the diminished K content. The minimal changes observed in electrolitic plasma concentration are due probably by compensatory hormonal reactions.

REFERENCES

1. Baker P.F., Willis J.S., 1969, The Number of Sodium Pumping Sites in Cell Membranes, Biochim.Biophys.Acta, 183,646-649
2. Bykov G.P., Smirnov V.P., 1970, Morphological Changes in Bone and Muscle Tissue unde Hypokinetic Conditions, Kosm. Biol.Med.,1970, 4, 2, 46-51
3. Glynn I.M., 1968, Membrane Adenosine Triphosphatase and Cation Transport, Brit.Med.Bull., 24, 2, 165-169
4. Portugalov V.V., Gazenko O.G., Ilina-Kakueva E.I., Malkin V.B., Artiuhina T.V., Bukaeva I.A., Gotlieb V.I., Rohlenko K.D., Roscina N.A.,Starostin V.I., 1967, On Some Effects Developing during Hypokinesia, Kosm.Biol.Med., 1, 6, 18-24
5. Romanov V.S., 1976, Quantitative Assesment of Ultrastructural Changes in the Rat Myocardium in Prolonged Hypokinesia, Kosm.Biol.Med., 10, 3, 50-54
6. Rowell M., 1970, Membrane and Ion Transport, Ed. Edward Bittar, Wiley Interscience, London-New York, 52-66
7. Selye H., 1975, Stress and Chemical Prevention of Myocardial Necrosis, in Cardiovascular Therapy, Ed.H.J.Russek, Williams and Willkins Comp.Baltimore, 135-141
8. Skou J.C., 1965, Enzymatic Basis for Active Transport of Na^+ and K^+ Across Cell Membrane, Physiological Rev.,45, 3, 596-617

EFFECT OF IMMOBILIZATION ON THE ATPase ACTIVITIES AND Ca-UPTAKE OF SARCOPLASMIC RETICULUM IN DIFFERENT TYPES OF MUSCLES.

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Isolated sarcoplasmic reticulum /SR/ vesicles rapidly accumulate Ca^{++} from the medium and contain a Ca^{++} stimulated ATPase activity. The splitting of ATP occurs through covalantly linked phosphoprotein intermediate. Relative pure SR vesicles were isolated by sucrose gradient fractionation from normal and immobilized rabbit muscles. Experiments were made on m.gastrocnemius and m.soleus. The changes in the "extra" /Ca^{++}/ ATPase activity of normal and immobilized muscles were examined in order to study the Ca transport. It was found that "extra" /Ca^{++}/ ATPase increased /about 2-fold/ in m.gastrocnemius and /about 5-fold/ in m.soleus if comparing to the control values after two weeks. Ca uptake was measured under similar conditions to the determination of the "extra" /Ca^{++}/ ATPase activities. In the case of Ca uptake, labelled Ca-45 was used. Significant changes have been found in the Ca uptake on the effect of immobilization.

INTRODUCTION

Sarcoplasmic reticulum /SR/ is an intracellular network system surrounding myofibrils. It regulates the concentration-relaxation cycle in muscle by accumulation and release of calcium ions.

When isolated as microsomes, SR retains the capacity to accumulate calcium in an ATP dependent process. Since calcium accumulation and ATPase activity are readily measured in SR, these preparations are good models for representing the ion transport system and the Ca uptake.

METHODS

Adult, male, New Zealand, white rabbits were used. The right hind legs of animals were immobilized by plaster casts for 1-2-4-6 weeks. After immobilization m.gastrocnemius and m.soleus were excised and the sarcoplasmic reticulum membrane fraction was prepared by a modified method /1/.

The total and basic ATPase activities were measured according to /2/, and the extra Ca^{++}-ATPase activity was calculated from the difference between the values of total and basic activities. The concentration of proteins were 0.03-0.1 mg. Protein content was determined according to /3/ with bovine serum albumin as standard. The temperature was 23°C in the experiments. The amount of the split inorganic phosphorous was measured by turbidimetric method /4/. Ca-45 uptake was determined according to /5/.

The incubation mixture of the calcium uptake was the same as that used for the determination of total ATPase activity except that labelled Ca-45 was present /0.1 mM/. The measurement was carried out by millipore membrane filter method, applying $^{45}CaCl_2$. Samples of filtrate /0.2 ml/ were counted in scintillation fluid /Tri Carb Spectrometer, Packard/.

RESULTS

The aim of this investigation was to study the effect of immobilization on the activity of ATPase ^{45}Ca-labelled uptake. Purified SR fractions were obtained by differential centrifugation from rabbit skeletal muscle. Contamination with other organelles was monitored by using outer membrane 5' nucleotidase activity /6/ and Na^+-K^+ ATPase for sarcolemma /7/ and acid phosphatase activity for lysosomes /8/. These activity values were not detectable.

The total, basic and "extra" /Ca^{++}/ ATPase activity values of SR vesicles from m.gastrocnemius and m.soleus showed changes. Figs. 1-2 show the changes of the ATPase activities of SR membranes from m.gastrocnemius and m.soleus.

The total ATPase activities went through a maximum at the second week of immobilization and approached the control values after six weeks. The basic ATPase activity values were nearly equal in both control and immobilized muscles. The extra ATPase activity values were twice higher than the control ones. After the maximum level the enzyme activity values tended towards that of the control.

In the case of m.soleus /Fig. 2/, the total ATPase activities also went through a maximum at the second week of immobilization.

The basic ATPase activity values increased two-fold after two weeks of immobilization.

The "extra" /Ca^{++}/ ATPase activities were twice higher in comparison with the control. After the maximum level, the enzyme activities decreased to the half of the control values in the case of m.soleus.

We investigated the labelled ^{45}Ca uptake capacity of SR reticulum vesicles /0.03-0.1 mg/. The labelled Ca-45 uptake of m.gastrocnemius and m.soleus SR vesicles changed after immobilization.

Figs. 3-4 show the changes of the Ca uptake capacity of SR reticulum vesicles from m.gastrocnemius and m.soleus.

Ca-45 uptake values were the highest after two weeks of immobilization in both types of muscles. In m.gastrocnemius the levels of the uptake in-

creased to three-fold and in m.soleus to four-fold.

It seems that the immobilization has a strong effect on the Ca metabolism of the fibres. The muscle adapts itself to the new situation establishing a new steady state.

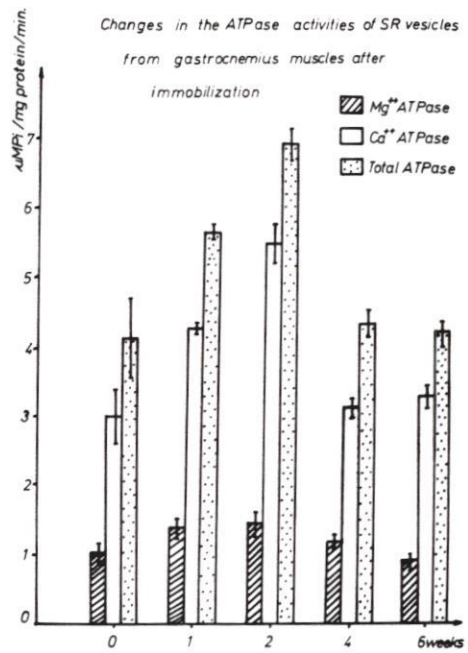

Fig. 1.

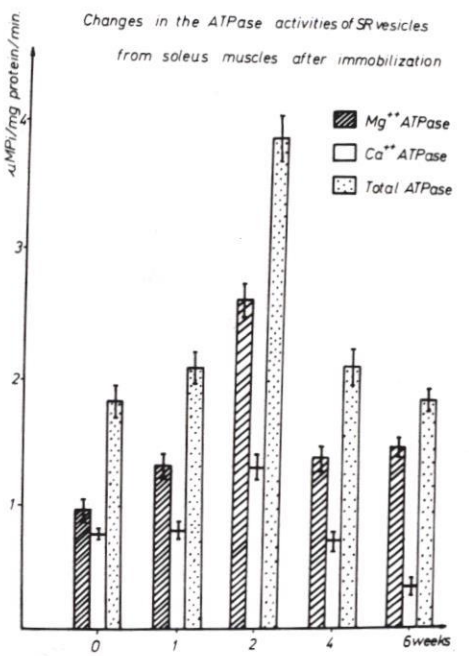

Fig. 2.

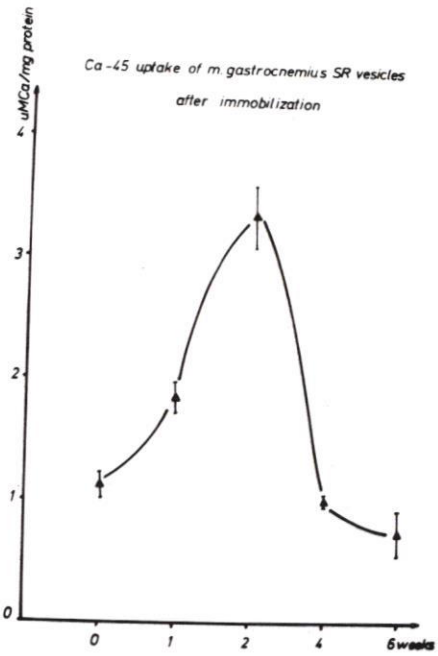

Fig. 3.

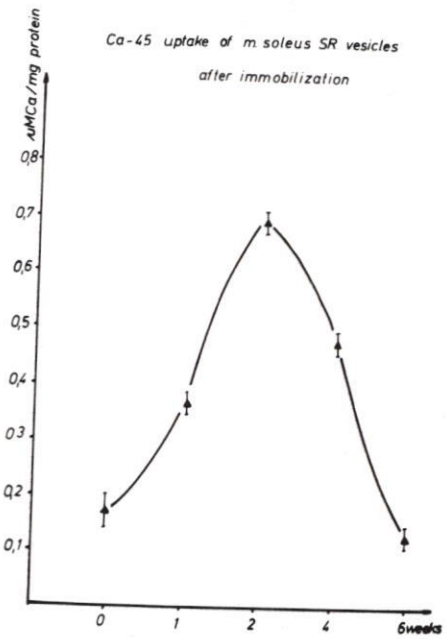

Fig. 4.

REFERENCES

1/ Meissner, G. et al. /1971/ BBA, 241:356-378.
2/ Sarzala, M. G. et al. /1975/ Eur.J.Biochem. 57:25-34.
3/ Lowry, O. H. et al. /1951/ J.Biol.Chem. 193: 265-275.
4/ Eibl, H. /1969/ Anal.Biochem. 30:51-55.
5/ Drabikowski, W. /1972/ BBA, 274:158-170.
6/ Bodansky, A. /1963/ J.Biol.Chem. 238:3420-3423.
7/ Sulakhe, P. V. M. /1971/ Biochem.Biophys.Res. Comm. 42:793-810.

PLASTICITY OF FAST AND SLOW MUSCLE MYOFIBRILLAR PROTEINS IN MODEL EXPERIMENTS SIMULATING WEIGHTLESSNESS

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Plaster cast immobilization seems to be an adequate method for simulating the effect of weightlessness on muscles under terrestrial conditions.

Therefore the protein composition of myofibrils from fast and slow muscles of rabbit hind limbs immobilized by plaster cast was analyzed using one and two dimensional gel-electrophoretic techniques. It was demonstrated that the response of structural elements from functionally different muscles on the altered demands manifests itself in a fading of isomyosin pattern characteristic of fast and/or slow contractile properties. In the fast muscle the quantity of homodimeric LC-3 isomyosin decreases, while increases in the slow one. This results a transformation in the myosin population, consequently a dedifferentiation of muscle cells. The changes will be discussed in the term of plasticity of vertebrate striated muscles.

INTRODUCTION

It was shown in our previous paper /1/ that various forms of inactivity, such as hypokinesis and akinesis induce muscle atrophy, and changes in the composition of structural proteins. From the data presented here some conclusions can be drawn concerning the pathophysiological mechanism of loss of muscle mass. One of them seems to be the reduced oxygen supply taking place as a consequence of altered physiological activity, furthermore the addition of both effects, i.e. hypokinesis and hypoxia. Therefore, it seemed reasonable to investigate the biochemical changes occuring on the effects of reduced activity, akinesis and the combination of both.

METHODS

The following experiments were performed:
I. A group of rats was kept in normal cages, but exposed to hypobaric conditions corresponding to 7000 m altitude above the sea level for 8 hours/day in a special baro-chamber.
II. Combined treatment. This group was subjected to both hypokinesis and hypoxia, i.e. the rats put in small cages were kept in baro-chamber for 8 hours/day. Two experimental series were performed: the first group was exposed to the experimental conditions for one month, the second for two months. Each group consisted of 20 rats. The control animals were carefully selected from the same breed.
III. Immobilization. Right hind legs of adult rabbits were fixed in extended position with plaster of Paris as reported previously /3/.

RESULTS

The changes observed in the mass of soleus are moderate but significant after four weeks of hypoxia and combined treatment. The EDL shows about 25-30 % loss of mass on the influence of experimental conditions. The highest degree of atrophy has been found in the gastrocnemius muscles. After one month the hypoxia and hypokinesis damages this muscle in equal degree, the combined treatment to some more degree. In the two months duration of experiments the loss of mass was more pronounced in the groups exposed to both conditions. The analysis of the composition of myofibrils gave the following results.

MUSCLE	MONTH	CONTROL LC-1:2:3	HYPOKINESIS LC-1:2:3	HYPOXIA AND HYPOKINESIS LC-1:2:3	HYPOXIA LC-1:2:3
GASTROCNEMIUS	1	1,0 : 1,5 : 0,75	1,0 : 2,0 : 0,5	1,0 : 2,0 : 0,25	1,0 : 2,0 : 0,8
	2	1,0 : 1,5 : 0,75	2,0 : 1,5 : 0,5	2,0 : 1,5 : 0,2	1,0 : 0,8 : 0,4
EXT DIG LONG	1	1,0 : 1,0 : 0,5	1,0 : 1,0 : 0,5	1,0 : 1,0 : 0,5	1,0 : 1,5 : 0,5
	2	1,0 : 1,0 : 0,5	1,0 : 2,0 : 0,5	1,0 : 1,5 : 0,25	1,0 : 1,0 : 0,25
SOLEUS	1	5 %	15 %	22 %	6 %
	2	2 %	19 %	16 %	10 %

Table 1: The ratio of myosin light chains in the experimental groups

	M Soleus				M Gastrocnemius			
	Rabbit		Rat		Rabbit		Rat	
	Norm.	Immob.	Norm.	Immob.	Norm.	Immob.	Norm.	Immob.
LC-1F	0	0	11,12	15,8	25,8	28,9	22,9	18,7
LC-2F	2,3	4,51	4,41	9,13	50,6	57,2	36,8	28,7
LC-3F	2,14	14,5	2,01	7,9	23,5	13,9	16,4	9,4
LC-1S	41,2	32,4	49,9	39,6	0	0	10,9	19,4
LC-2S	54,8	48,6	32,6	27,5	0	0	12,9	24,5
ΣF %	44,5	18,95	17,5	25	100	100	76,2	56,5
ΣS %	95,6	81	82,5	75	0	0	23,8	43,8

Relative distribution of myosin light chains taking the of slow and fast light chains as 100%

Table 2

Table 1 demonstrates the light chain pattern of myosin population measured densitometrically after SDS polyacrylamide gel electrophoresis. As it can be seen the response of skeletal muscles to hypoxic conditions differ from the changes observed on the influence of hypokinesis. While hypokinesis leads to a shift in the LC-1 to LC-2 ratio, the hypoxia alone affects the quantities of LC-2 and LC-3 subunits . The light chain pattern of EDL seems to be relatively constant. The distribution alters only after two months. The transformation of soleus can be characterized by the increase of fast myosin light chains. In this respect the alteration is more pronounced on the effect of combined treatment. The relative content of fast myosin light chains increases from 2-5 % to 22 %.

The relative distribution of slow and fast myosin light chains from immobilized rabbit and rat muscles is demonstrated in Table 2. It can be observed that the analogous muscles of rats and rabbits response with different changes. The rabbit soleus contains about 95 % slow light chains. On the effect of four weeks disuse this decreases to 81 %, and at the same time the quantity of fast light chains increases from 4.5 % to 19 %. In the rat soleus the percent of slow light chains decreases from 82 % to 75 %, and the fast ones increase from 17 % to 25 %. We have to stress that the highest increase has been found in LC-3F components.

As regards the composition of gastrocnemius myosin population, table 2 shows that in rabbits the LC-3F and LC-2F do not alter significantly, but the LC-3F underwent a 50 % decrease. The total fast light chain decreases from 76 % to 56 %. From the fast light chains the LC-3 shows a 45 % decrease.

The light chain pattern of myosins prepared from normal /N/ and immobilized /I/ gastrocnemius /G/ and soleus /S/ muscles from rats is shown in Fig. 1. In the two dimensional pictures it is possible to compare the normal and disused myosin light chains. On the effect of four weeks of disuse the soleus shows a "fast" character, having LC-3F, which is absent in the normal slow myosin. In the gastrocnemius two unknown /U/ spots could be detected as a result of immobilization. These could be assumed as proteolytic fragments originating from LC-1 or LC-2 peptides.

From a comparison of submolecular changes very interesting conclusions can be drawn. As it has already been pointed out, the subunit structure of the fast muscle myosin changes characteristically due to experimental immobilization. The change is manifested in the shift of the ratio of the light chains from 1:2:1 to 1:2:0.5. In the slow muscle the relative amount higher molecular weight peptides decreases, and the LC-3 peptide characteristic of the fast muscle appears and displays an increasing trend as a function of time. According to recent investigations /2/ the light chain formula of fast fibres means that in these there exist three types of myosin isoenzymes: homodimeric LC-1, homodimeric LC-3, heterodimeric LC-1.

The light chain pattern of the myosin of the slow muscle cells, LC-1/LC-2, is equal to 0.75. Immobilization results in a gradual decrease in the amount of myosin without any essential change in the relative distribution of the light chains.

Our present results prove that de-differentiation induced by the disuse of various types of muscles is manifested in the transformation of the subunit structure of the structural elements. In other words it can be expressed that there is a switch-over in the distribution of the myosin isoenzymes, i.e. in the phenotype of the muscle. Of necessity, this also means that the genetic expression of the muscle cells becomes adapted to the changed conditions.

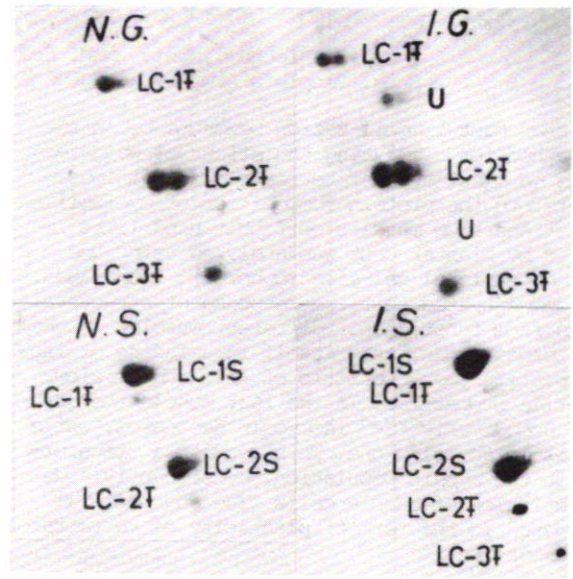

Fig. 1: Two-dimensional pictures of myosin light chains.

This may allow us to draw the conclusion that disuse impairs the re-synthesis of the slow LC-1 and LC-2 isoenzymes, and at the same time the rate of synthesis of the homodimeric fast LC-3 is enhanced. Consequently, the slow muscle takes up to a certain degree the properties of the fast one, and vica versa.

REFERENCES

1/ Hideg, J., Takács, Ö., Berényi, E., Rapcsák, M., Guba, F. Physiologist /This vol./
2/ Hoh, J., McGrath, P., White, R. /1976/ Biochem.J. 157, 87-95.
3/ Takács, Ö., Sohár, I., Pelle, T., Guba, F., Szilágyi, T. /1977/ Acta biol.Acad. Sci.hung. 28, 213-219.

EFFECT OF IMMOBILIZATION ON THE EXCITATORY PARAMETERS OF DIFFERENT TYPE SKELETAL MUSCLE

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There is now some evidence on the changes of ultrastructure and biochemical processes in immobilized skeletal muscle and their relation to its physiological function.We were wonder how the immobilized muscle's biochemical changes presented on the level of excitability and contraction force in skeletal muscles with different metabolic type. The increase of the latency-time which may be regarded as one of the excitation parameters $-\Delta t-$ may be in relation to the effect of disuse.Beside the change of the membrane-conductancy this may be in connection with the quantitative decrease in the regulatory proteins - tropomyosin and troponin.The degradation of troponin-C which had an important role in the connection of contraction within the overall troponines was higher in both muscles /gastrocnemius and soleus/.

INTRODUCTION

Our present paper is in close connection with the earlier ones /Takács et al.1977 and Guba et al. 198o./ in which have published some data on experimental study of skeletal muscles with different functions. There had been demonstrated that longer disuse periods in different muscles have caused different quantitative changes in the regulatory proteins having role in the tension and relaxation.Obviously these submolecular changes can be attributed to the disuse of muscles so it seemed reasonable to examine the influence immobilization to the contractile parameters of skeletal muscles with different types.It was proved from the experiments mentioned that disuse causes quantitative decrease in the contractile proteins of the muscle fibre.The biochemical examinations showed changes in the myosine subunit pattern of the immobilized fast,glycolitic muscle: first of all the quantity of the myosin light chain with molecular weight of 15,ooo daltons /LC3/ changes which is mainly characteristic for the fast muscle. On the other hand the same peptid showed an increase in the slow muscle after disuse.

MATERIALS AND METHODS

Hind limbs of adult New Zealand white rabbits were immobilzed by plaster cast.The animals were anaesthetized by pentobarbital /Nembutal 4o mg/kg/,then we have firstly explored the soleus muscle and then the white head of the gastrocnemius muscle. /SM. and GM./

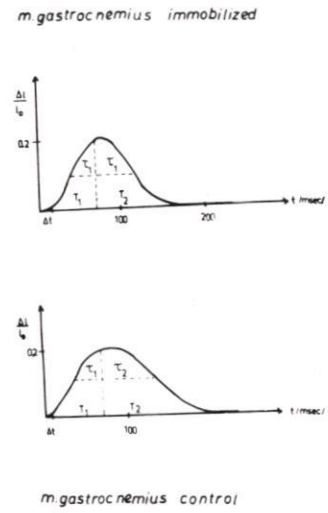

Fig.1: The average curve of 25 twitches obtained from normal and immobilized GM. stimulated by direct electrical pulses.

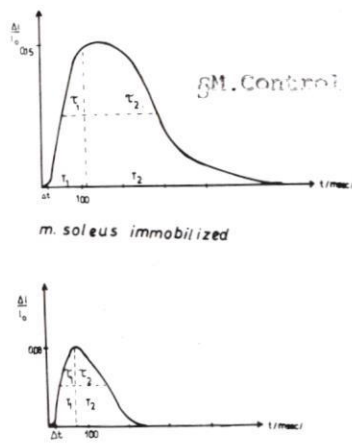

Fig. 2.
The average curve of 25 twitches obtained from normal and immobilized SM. stimulated by direct electrical pulses.

The longitudinal changes of isotonic twitches were converted into an electrical sign by means of an induction-method;these were brought into a storage-unit of Philips ANALOG 7 type with magnetic band,level DC. These contractional curves were digitalized and averaged,respectively using an analyzer of 512 channel.The analog-digital conversion was performed by sampling time of o,1 msec.Before the evaluation of the results we must note that the parameters both of loading and stimulus were the same at the control and disuse muscles: in case of GM: 12o Newtons, 5 V, 1 pps; in case of SM: 6o Newtons, 5 V, o,5 pps.

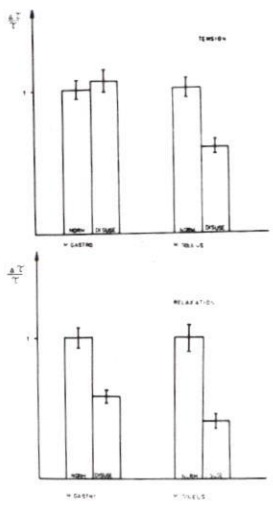

Fig. 3.

The Fig.3. shows: The "relativ-halftime" changes of the immobilized GM. and SM.compared with control values.

Δt: time of latency. The time between the stimulus and the initiation of twitch.

τ_1:the half-time of tension development.

τ_2:the half-time of relaxation.

$\tau_1 + \tau_2 = \tau$:the half-time of twitch.The width of the twitching curve's amplitude at its 5o % high.

T_1: the time required for development of tension - the time of running in.

T_2: the time of relaxation.The time required for the "damping" of curve.

$T_1+T_2=T$: the whole time twitch.

time /msec/ / muscle	Δt	T_1	T_2	T	τ_1	τ_2	τ
m gastrocnemius 14days of disuse	18,46	61,86	63,27	125,13	34,44	40,70	75,14
m gastrocnemius control	8,43	63,20	109,60	172,80	32,30	70,25	102,55
m soleus 20days of disuse	16,86	56,20	168,60	224,80	33,72	70,25	103,97
m soleus control	11,24	101,60	432,70	533,86	56,20	174,20	230,40

Table 1.
The numerical values of the time-parameters in Figures 1. and 2.

It can be established that in the case of GM.the amplitude of contraction /the relative shortening of the muscle/ did not change significantly. On the other hand a decrease of approximatively 5o % can be observed in case of SM.The most explicit change was that on the effect of immobilization the twitch-time - T - decreased in both muscles. The decrease was 28 % in case of GM and 58 % in case of SM. At the same time the half-time of tension at the SM. practically could reach the same time-parameters of the control GM. twitch on the effect of immobilization and the half-time values of relaxation became the same. It can be established that the half-times of the development of tension and the half-times of relaxation decreased significantly excepting the τ_1 change of GM.

DISCUSSION

Compared our experimental data with the results of the biochemical analyses the following conclusion may be drawn.
The increase of the latency-time /Δt/ which may be regarded as one of the excitation parameters, may be in relation to the effect of disuse.
In fact the twitching amplitude of GM. did not change on the effect of disuse;at the same time decrease was about 5o% in case of SM.Nevertheless,this phenomenon is parallel with the decrease of myofibrillar protein content /Takács et al.1977/ according to which the myofibrillar protein content of GM. is only 2o %,while that of SM.shows a decrease of 5o%.
The most important conclusion may be drawn from the comparison of changes in τ_1 and τ_2 according to which the tensional and relaxational half-time of SM. are the same as the similar characteristics of the control GM. as a results of disuse. This means that the most characteristic time-values of the slow muscles became the same as those of the fast muscle.
After all the results of the present series of experiments support the above-mentioned biochemical establishments and allow this conclusion: the disuse leads to the dedifferentiation of muscles with different functions.

REFERENCES

Guba,F., Takács,Ö., Kiss, Z.: /198o/ The effect of disuse on protein patterns in fast and slow twitch muscles.In: "Plasticity of Muscle". Ed:Drink Peete. Walter de Gruyter, Berlin,New York. 5o7 - 523.

Takács,Ö.,Sohár,I.,Pelle,T.,Guba,F., Szilágyi,T.:/1977/. Experimental investigations of hypokinesis of skeletal muscles with different functions III. Changes in protein fractions of subcellular components. Acta biol.Acad.Sci.hung. 28. 213 - 219.

EFFECT OF IMMOBILIZATION ON THE NONHISTONE PROTEIN COMPOSITION IN DIFFERENT TYPES OF SKELETAL MUSCLES

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ABSTRACT

Nonhistone proteins of fast-twitch glycolytic head of the gastrocnemius muscle and slow-twitch oxydative soleus muscle of rabbit were extracted and analysed by two-dimensional gel electrophoresis in normal stages and after 2 weeks of disuse. The ratio of the quantities of nonhistone chromosomal proteins / NHCP / to DNA was nearly standard in soleus muscle: 1.21 / normal /, 1.29 / disused / ; and decreased in gastrocnemius muscle : 2.66 / normal / , 2.26 / disused / . After disuse, besides the basically unchanged nonhistone patterns, there were disappearance of the low mol. weight spots in the soleus muscle while new polypeptides emerged in the same range in the gastrocnemius muscle.

INTRODUCTION

Recently, it has become evident that a part of nuclear proteins play a marked regulatory role in the control of the gene expression. The interesting contribution which muscle could make to our better understanding of the gene expression, is the studies on its specific properties. These are connected with the transformation of myosin isoenzymes, metabolic profile of the different fibre types of the skeletal muscles in changing from one type to another during disuse, weightlessness, cross innervation. We have examined the fast-twitch glycolytic head of the gastrocnemius muscle and the slow-twitch oxydative soleus muscle, wether the nonhistone protein patterns would change during 2 wks of disuse, concerning to the two-dimensional electropherograms.

METHODS

Adult New Zealand rabbits were used for the experiments. The hind limbs were immobilized by plaster for fortnight. The excised muscles were cut into about 1 mm^2 pieces, washed for 2 x 10 min and homogenized in the following solution: 0.25 M sucrose, 0.1 M KCl, 5 ug/ml soybean trypsin inhibitor, 1 mM p-Cl-mercurybenzoate, 0.2% Triton X-100, 5 mM aethyleneglycol-bis-tetraacetic acid, 1 mM sodium pyruvate 5 mM $MgCl_2$, 0.1 TRIS-HCl / pH7.4 /. The suspension was filtered through 1-10 ascending gauze layers. The pellet of the filtrate was suspended in 2.2 M sucrose and layered over the same sucrose pillow, to ultracentrifuge it for one hour, with 100,000 x g. The sedimented nuclear fraction was osmotically shocked then washed three times with o.14 M NaCl solution. After dehistonization with 0.25 M HCl, the nonhistone proteins were extracted from the final sediment with 2 M NaCl, 5 M urea 10 mM sodium phosphate / pH 7 /. The lyophilised extracts were analysed by O'Farrell's two-dimensional gel electrophoresis technique/ 3 /. Protein determinations were carried out according to Dorsey et al./ 1 /. DNA content of the samples was measured by the method of Karsten et al. / 2 /.

RESULTS

Following a two weeks immobilization period, parallel with the relative increase of the DNA content, a decrease in the protein content is obsevable in both types

Table I. Average Protein and DNA Yields

FRACTIONS	M. SOLEUS				M. GASTROCNEMIUS			
	NORMAL		IMMOBILIZED		NORMAL		IMMOBILIZED	
	Prot. mg wet.wt.g	DNA mg wet.wt.g	Prot. mg wet wt. g	DNA mg wet wt.g	Prot. mg wet wt.g	DNA mg wet wt.g	Prot.mg wet wt.g	DNA mg wet wt.g
HOMOGENATE	276	0.368	125	0.797	262	0.333	127	0.621
FILTRATE	253	0.344	72.9	0.606	237	0.316	85	0.195
PELLET AFTER U.CENTRIFUGE	0.340	0.090	0.402	0.102	0.325	0.060	0.53	0.121
NONHISTONE	0.109		0.132		0.160		0.274	
NONHISTONE mg / DNA mg	1.21		1.29		2.66		2.26	

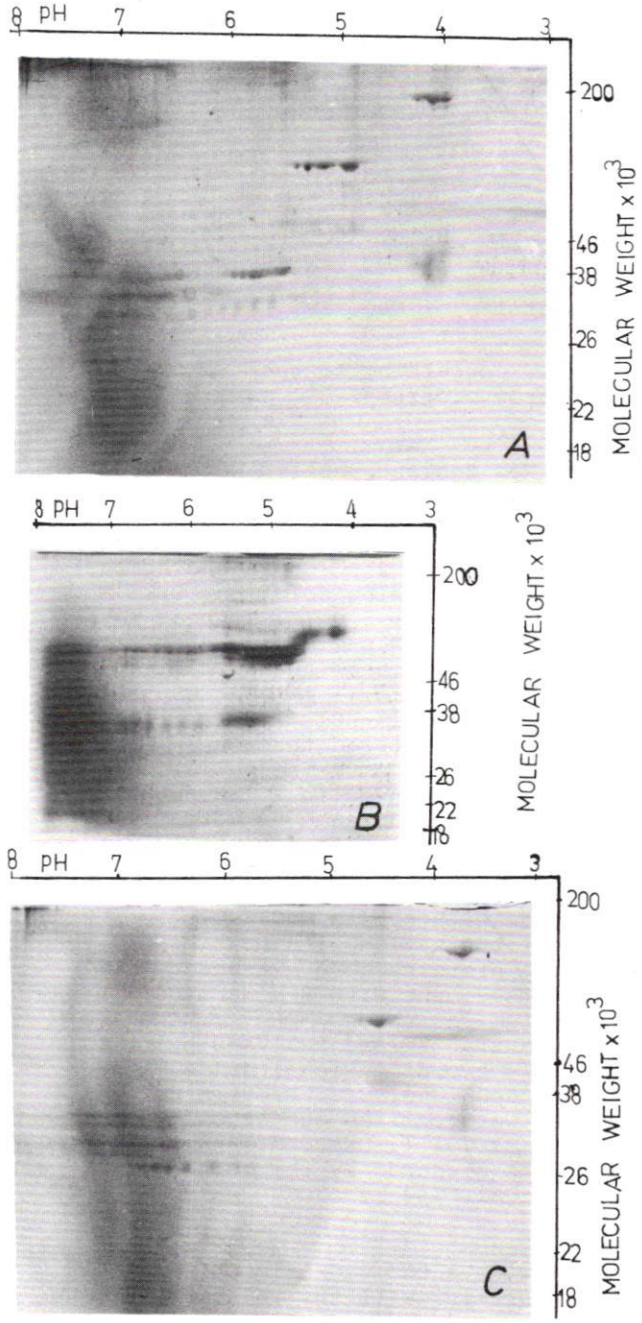
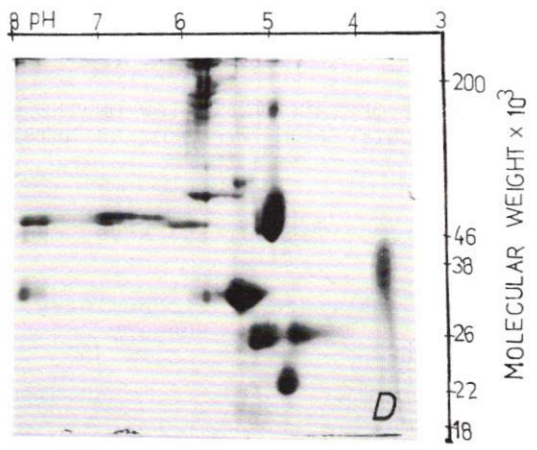


Fig.1. NHCP composition of muscles, 150 ug proteins to load to the gels. A and B: normal and immobil. soleus muscle; C and D: normal and immobil. gastrocn. muscle respect.

of muscles, probably because of suffering a serious loss of amount, what on the one hand increases the density of the nuclei, and the connective tissue elements relatively overweigh the structural proteins on the other / Table /. The two-dimensional NHCP patterns are basically similars,though there are detectable differences in the pI 4.2-4.8, mol. weight 2o-26 kd range in the normal muscles / Fig. 1. A, C /.



Upon the effect of disuse, the main trends of the changes were disappearing of low mol. weight acidic NHCPs in the soleus and appearing nearly same peptides in the gastrocnemius muscle/ Fig.1.B, D and Fig.2/

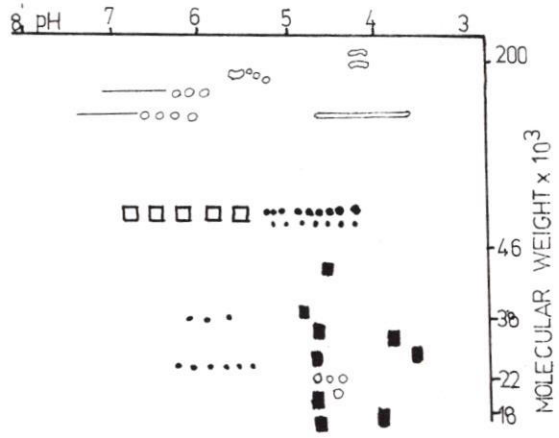


Fig.2.Schematic diagram of the differences between the two-dimensional NHCP patterns of the immobilized muscles and their normal counterparts.Empty and dark circles: disappearing and appearing , empty and dark quadrangels: disappearing and appearing NHCP spots after disuse in soleus and gastrocnemius muscles respectively.

Although our investigations have not clarified the biological functions of the disappearing and appearing proteins / enzymes, chromatine structure stabilisers, distinguished receptors of regulativ macromolecules, having control function in gene regulation /, it can be said that during disuse there are changes as related to nuclear proteins as well.

REFERENCES

1.Dorsey,T.E.,McDonald,P.W.,Roels,O.A. /1977/Anal. Biochem. 78:156-164
2.Karsten, U. and Wollenberger, A. / 1972/ Anal. Biochem. 46: 135-148
3.O'Farrell,P.H./1975/J.Biol.Chem.25o:4007-21

IMMOBILIZATION EFFECTS UPON AEROBIC AND ANAEROBIC METABOLISM OF THE SKELETAL MUSCLES

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The effect of immobilization for different periods /1, 2, 4 and 6 weeks on the aerobic and anaerobic metabolism of rabbit muscles with different functions was studied by measuring the total activity of lactic dehydrogenase /LDH EC 1.1.1.27/, malic dehydrogenase /MDH EC 1.1.1.37/, aldolase /ALD EC 4.1.2.7/, isocitrate dehydrogenase /ICDH EC 1.1.1.42/ as well as the distribution of LDH izoenzymes. On immobilization the metabolism of m. soleus and that of m. gastrocnemius approached each other, a certain dedifferentiation was observed.

INTRODUCTION

The atrophy of actively working muscles resulting from different damaging effects has been a problem of clinical practice for a long time. Of the damaging effects, primarily genetic, toxic myopathies, tenotomic and neurogenic atrophies have been investigated morphologically and enzymatically /2, 4/. In the present paper we give an account of our experiments studying the changes in the aerobic and anaerobic metabolism of muscles of various functions upon the effect of plaster-fixation.

METHODS

The experiments were performed with male New Zealand rabbits. The right hind limbs of the rabbits were fixed in extension with circular plaster casting. After 1, 2, 4 and 6 weeks of fixing the plaster was removed, the soleus and gastrocnemius muscles of the prepared limb were excised. M. soleus, containing mainly slow oxidative fibres was regarded as muscle with aerobic metabolism and the m. gastrocnemius as fast glycolytic muscle. To characterize the metabolism the following enzymes were used: LDH, MDH, ALD and ICDH.

RESULTS

With increasing time of immobilization the total activities of all investigated enzymes in both muscles gradually decreased /Fig. 1, 2/.

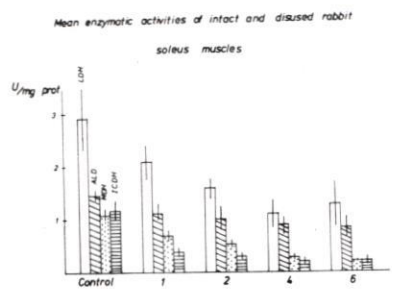

Fig. 1.

In m. soleus the decrease in activity of enzymes taking part in the oxidative metabolism /ICDH, MDH/ was largely over the rate of glycolytic enzymes. Changes in m. gastrocnemius, in turn showed an opposite tendency. Here, the extent of decrease of glycolytic enzyme activities /LDH, ALD/ considerably exceeded that of ICDH, MDH.

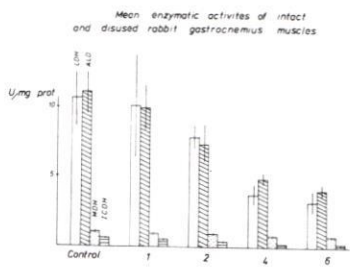

Fig. 2.
In both figures the numbers indicate the time of immobilization in weeks.

The study of LDH izoenzymes gave a similar results both in the case of m. soleus and m. gastrocnemius. With increasing time of immobilization in both muscles izoenzyme types, characteristic for anaerob metabolism, containing much M subunits /LDH-3, LDH-4, LDH-5/, accumulated. The ratios of the subunits /H and M/ constituting the LDH izoenzymes were also calculated. Table 1. shows that upon immobilization this ratio greatly decreased for both m. soleus and m. gastrocnemius.

Time of immobilization /in weeks/	m. soleus H/M ratio	m. gastrocnemius H/M ratio
0	3,2	1,3
1	3,o	o,9
2	2,o	o,9
4	1,9	o,4
6	1,7	o,5

Table 1.

DISCUSSION

The distribution of enzymes in different types of skeletal muscle varies according to the function of the muscle /1, 4/ and undergoes characteristic alterations on training or following muscle damage /2/.

Changes in the activity of enzymes in slow oxidative m. soleus unequivocally indicate the decrease of the aerobic processes and the relative preponderance of glycolytic metabolism. This is indicated by the changes in the ratio of glycolytic and oxidative enzymes as well as the increasing quantity in the LDH izoenzyme spectrum izoenzyme variables containing a great number of M subunits /LDH-3, LDH-4/. In the gastrocnemius muscle the glycolytic processes slowed down following plastering while the oxidative metabolism was less influenced by immobilization.

In the pathogenesis of muscle atrophy, brought about by immobilization, presumably several important factors are involved. In our opinion diminished tissue blood circulation due to the deletion of active muscle function is an important inducing factor. The resulting tissue hypoxia, subsequent metabolic switches and shifts in substrate concentrations may give rise to regulatory changes in the rates of synthesis of individual enzymes. On the other hand, the more intensive catabolism induced by immobilization /3/ may alter the catabolic rate of the different enzymes and shift their half life. Presumably as a results of both processes, the metabolic difference between m. soleus and gastrocnemius decreased and a certain dedifferentation takes place.

REFERENCES

1. Bass, A., Brdiczka, P., Hofer, S., Pette, D.: Eur. J. Biochem. 1o, 198 /1969/.
2. Dolan, L., Chew, L., Morgan, G., Kidman, A.D.: Exp. Neurology 47, 1o5 /1975/.
3. Goldspink, D.F.: J. Physiol. 264, 267 /1977/.
4. Pette, D., Dölken, G.: Advances in Enzyme Regulation 13, 355 /1975/.

THE EFFECT OF HYPOXIC AND HYPOBARIC EXERCISES ON THE BLOOD-BRAIN BARRIER IN RATS

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We have studied the effects of intermittent hypoxic and hypobaric environment on the ultrastructure of the blood-brain barrier of adult male and pregnant female rats and on the prostacyclin and prostaglandin (PG) synthesizing ability of the isolated brain capillaries in adult male rats and in the pups of the exercised female ones. In pregnant rats, the hypoxic exercises resulted in the enhancement of the transport process through the endothelial cells, as shown by the enhanced number of pinocytotic vesicles and the contraction of the nuclei of the endothelial cells. The prolonged progesterone treatment prevented the alterations. The PG synthesizing ability was effected by the increased synthesis of $PGF_{2\alpha}$ and PGE_2 in the pups of hypoxic exercised rats. The normoxic condition and the progesterone treatment prevented these changes. In adult male rats, no ultrastructural changes and milder alterations of the PG synthesis were detectable.

INTRODUCTION

Pathological changes in the blood-brain barrier can be induced experimentally by different procedures and can be detected in certain human diseases. The actual state of the barrier is important in the regulation of the neuronal activity through the blood supply. Besides the relevance of these experiments to the physiology of flying and astronautics, our investigation can also give new information about genetical malformations and other pathological alterations which often afflict people living in high mountain regions (1, 2).

In this study we wanted to investigate the effect of the hypoxic and hypobaric environment on the ultrastructure of the blood-brain barrier in rats. Additionally, using biochemical techniques, we also determined the ability of the isolated brain capillaries from the pups of the previously exercised rats and the adult male ones to synthesize prostacyclin and prostaglandin.

METHODS

Adult pregnant Wistar rats were separated into four groups. The first one was subjected to a hypobaric-hypoxic state in a low pressure chamber equivalent to 7000 m above sea level. The second group was exercised in a hypobaric-normoxic state; the oxygen tension was raised to the normal atmospheric value while maintaining low pressure. In the third group hypobaric-hypoxic rats were treated with 50 mg progesterone capronate (Richter Gedeon Ltd.) divided in four equal doses and administered between the exercise periods. The fourth group was the control. The exercise periods were six hours in length and successive periods were separated by an interval of two days.

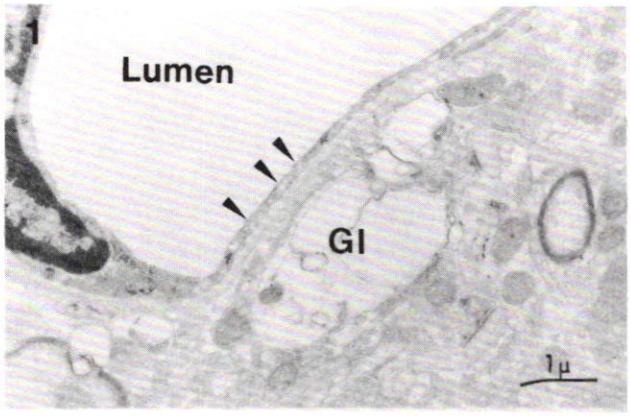
For the electron microscopic investigations one day before the expected time of birth the one-half of animals were perfused with Krebs-Ringer solution and then with Karnovsky solution containing 5% glucose. After decapitation small pieces of the parietal cortex were fixed in the latter solution for 4 hours at 4°C. After postfixation and dehydration the samples were embedded in Durcupan (Fluka). Sections of gold and silver interference colours were cut on a Porter-Blum ultramicrotome. After staining with uranyl acetate and lead citrate the sections were viewed under a JEOL 100B electron microscope.

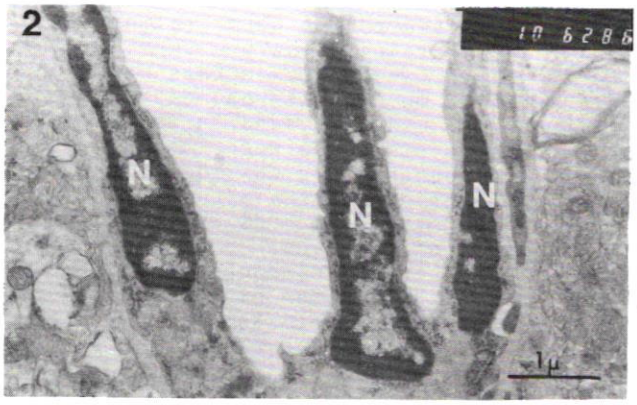
The remaining exercised rats were allowed to give birth to their pups. The newborn animals were kept under normal laboratory conditions for 8 weeks, which is the time required for the sexual maturation (3). Capillary-rich fractions were prepared from all groups of animals according to the method of Joó and Karnushina (4). The prostacyclin and prostaglandin synthesis by the capillaries was assayed with 1-^{14}C-arachidonic acid as substrate using 2 mM reduced glutathion and 1 mM norepinephrine as cofactors. The incubation and determination were made according to our earlier determinations (5).

Adult male Wistar rats were exercised in the same ways. The fine structure of the blood-brain barrier and the PG synthesizing ability of the brain capillaries were investigated as mentioned above.

RESULTS

Fig. 1 shows the ultrastructure of the blood-brain barrier of a rat after hypobaric-hypoxic exercises. The transendothelial





Figs 1-2. The fine structural appearance of brain capillaries in rats subjected to hypoxic-hypobaric exercises. Signs of increased transendothelial transport are labelled by arrowheads. Gl : swollen glial process, N : contracted endothelial nucleus.

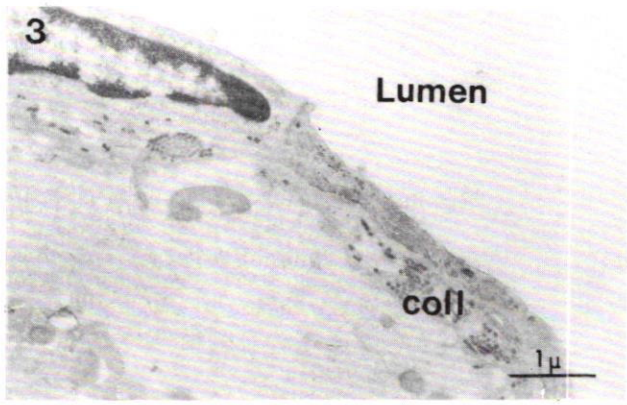


Fig. 3. Collagen-like fibers (coll) have appeared in the basal lamina in rats after hypobaric-normoxic exercises.

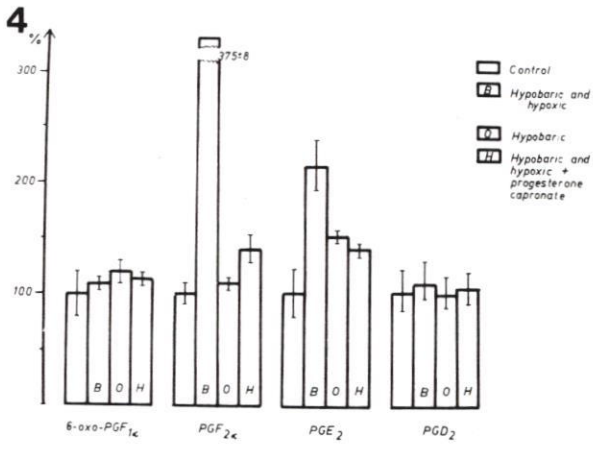


Fig. 4. The prostacyclin and prostaglandin synthesis in isolated brain capillaries of rat.

transport seems to have been enhanced when compared to the controls. Actually, some signs of the oedematous swelling of the glial end-feet system could be seen. The majority of capillary endothelial cells were found more or less contracted, which is undoubtedly represented by the well pronounced contraction of the nucleus (Fig. 2). In rats subjected to the hypobaric-normoxic environment only milder alterations were observed in the endothelium, while formation of collagen-like fibers was seen in the basal lamina (Fig. 3). A similar kind of fiber-formation has been observed after ATPase inhibition (6). The prolonged progesterone treatment prevented the changes in the fine structure of the brain capillaries of pregnant rats kept in hypobaric-hypoxic atmosphere.

Fig. 4 shows the distribution of synthesized products from arachidonic acid in the brain capillaries of rats from different groups. The synthesis of prostacyclin, which is represented by its metabolite, 6-oxo-$PGF_{1\alpha}$ as well as PGD_2 remained unchanged in all four groups. The most striking effect of hypoxia was expressed in the enhanced synthesis of $PGF_{2\alpha}$ and PGE_2. The increase was undoubtedly due to the hypoxic conditions, because the synthesis in animals kept in hypobaric-normoxic atmosphere was similar to the control values. Progesterone treatment of the mothers normalized the changes in the synthesizing ability of their pups. The ultrastructure of brain capillaries of the pups was without any oedematous changes.

Another experiment investigated the effect of hypobaric-hypoxic exercise on adult male rats. No oedematous ultrastructural alterations were seen even after 92 hours of exercise. Preparing capillary-rich fractions from the brain, similar but milder alterations of the PG synthesizing

ability could be seen when compared to the young animals. The stimulation of synthesis of $PGF_{2\alpha}$ was 40%, and that of the PGE_2 was 80% of the stimulations in the capillaries of the pups.

The hypobaric and hypoxic exercises seem to damage the ultrastructure of the blood-brain barrier of pregnant rats but the alterations are reversible and can be prevented by prolonged administration of progesterone. This protective effect is probably due to the well-known anti-oedematous effect of steroids (7). In our experiments, the PG synthesizing ability concerning only $PGF_{2\alpha}$ and PGE_2 were stimulated even two months after the exercises. It is very probable that the oxygen-dependent fatty acid cyclo-oxygenase enzyme is responsible for the increased synthesis (8). In embryos the enzyme is probably adapted to the lower oxygen supply in those animals which are kept in hypoxic atmosphere. After birth the normal partial pressure of oxygen is too high for the adapted enzyme and that is why the two fractions are synthesized in higher quantity than in normoxic cases. The fact that $PGF_{2\alpha}$ and PGE_2 were involved in both pups and adults suggests that these components of arachidonate cascade are responsible for the changes of vascular tone after such hypoxic exercises.

Since the $PGF_{2\alpha}$ is known to have a vasoconstrictor effect and the PGE_2 has a vasodilatator influence on the brain capillaries (9, 10), the enhanced synthesis of both fractions represents a balance at a higher level. The observed ultrastructural changes suggest that examination of the contractile proteins should be necessary.

REFERENCES

1. Alzamora, V., Rotta, A., Battilana, G., Abugattas, R., Rubio, C., Bourancle, J., Zapata, C., Santa-Maria, E., Binder, T., Subiria, R., Paredes, D., Pando, B. and Graham, G.G. 1953. On the possible influence of great altitudes on the determination of certain cardiovascular anomalies. Pediatrics 12: 259-262.
2. Ingalls, T.H. 1960. Environmental factors in causation of congenital anomalies. In: CIBA Found. Symp. on congenital malformations. Eds: Wolstenholme, G.E.W. and O'Connor, M.C. Churchill Ltd. London, 51-67.
3. Piacsek, B.E. and Goodspeed, M.P. 1978. Maturation of the pituitary-gonadal system in the male rat. J. Reprod. Fert. 52: 29-35.
4. Joó, F. and Karnushina, I. 1973. A procedure for the isolation of capillaries from rat brain. Cytobios 8: 41-48.
5. Gecse, Á., Ottlecz, A., Mezei, Zs., Telegdy, G., Joó, F., Dux, E. and Karnushina, I. 1980. Prostacyclin and prostaglandin synthesis in isolated brain capillaries. Prostaglandins (submitted for publication)
6. Joo, F. 1979. The role of adenosine triphosphatase in the maintenance of molecular organization of the basal lamina in the brain capillaries. In: Frontiers of matrix biology. Vol. 7. Eds: Robert, A.M., Boniface, R. and Robert, L.S. Karger, Basel, 166-182.
7. Reulen, H.J. and Schürmann, K. 1972. Steroids and brain edema. Springer Verl. Berlin-Heidelberg-New York.
8. Samuelsson, B., Granström, E., Green, K., Hamberg, M. and Hammarström, S. 1975. Prostaglandins. Ann. Rev. Biochem. 44: 669-695.
9. White, R.P., Heaton, J.A. and Denton, I.C. 1971. Pharmacological comparison of prostaglandin $F_{2\alpha}$, serotonine and norepinephrine on cerebrovascular tone of monkeys. Eur. J. Pharmacol. 15: 300-309.
10. Denton, I.C. Jr., White, R.P. and Robertson, J.T. 1972. The effect of prostaglandin E_1, A_1 and $F_{2\alpha}$ on the cerebral circulation of dogs and monkeys. J. Neurosurg. 36: 34-42.

RELATIONS BETWEEN RESPIRATORY AND CIRCULATORY CONTROL DURING GRAVITATIONAL LOAD IN MAN

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Abstract

The respiratory CO_2-response (0, 2, 4 % CO_2 in air) of 5 healthy males were investigated in a horizontal (0^0), a 30^0 and 70^0 upright position. The response curves were shifted to the left dependent on the gravitational load. As the cardiac output diminishes under these conditions it is concluded, that the influence of circulatory control on respiratory regulation is not based on the magnitude of bloodflow. The ratio of relative change in heart rate and respiratory minute-volume was 1:2,35 compared to 1:3 or 1:4 during workload (values from lit.). Furthermore increasing CO_2-concentrations provoked chronotropic effects with their direction and extent depending on the initial state. Going from horizontal to vertical positions the changes in the inclinations of CO_2-heartrate and CO_2-respiratory response curves were always correlated.

INTRODUCTION

If the so called CO_2-response-curve (a curve which relates alveolar or arterial CO_2-partial pressures with respiratory minute volumes) is used to describe the homeostatic performance of a certain organism's respiration, every influence acting in a definite situation on the ventilatory regulation can be expressed by displacement of that curve.

The influence of graded gravitational loads on the displacement of CO_2-response curves was investigated in order to find a relation between circulatory and respiratory reactions under these conditions. The following hypothesis was tested: The maintenance of homeostasis requires the magnitude of ventilation to depend on the flow of CO_2 (and/or O_2) through the lungs during each interval of time. This flow equals the blood-flow multiplied by the respective blood-gas concentration. As had been shown by arterio-venous shunt experiments /1,2/ both values are able to alter the magnitude of ventilation, so that CO_2-response curve displacements (as during muscular exercise) seem to be explainable by blood-flow-related influences on respiratory regulation. Whereas blood-gas concentrations can be measured directly by known chemoreceptors, a biological flowmeter does not exist within the circulatory system; but there is a theoretical possibility to obtain the flow by measurements of the baroreceptors indirectly. (The latter are known to affect the respiratory regulation /3,4/.) The circulatory performance depends on the pressures 'before' and 'behind' the ventricles and on the heart's 'nervous drive', which is regulated by the blood pressure control system. If a test-person's position is changed passively from horizontal to vertical with head up, the heart's minute volume drops down (about 20%); the mean pressure 'before' the ventricle falls, the mean pressure 'behind' remains constant and the heart's 'nervous drive' increases /5,6/. Therefore during gravitational load

- if the body's pressure measuring mechanism detects the magnitude of blood flow and influences respiration depending on blood flow the CO_2-response curve shifts to the right;
- if the respiratory nervous center is excited more unspecific in parallel to the circulatory center, the CO_2-response curve shifts to the left;
- if the respiratory regulation is not influenced by the circulation's pressure control, the CO_2-response is equal in both horizontal and vertical positions.

MATERIALS AND METHODS

Five healthy males aged between 25 and 45 were investigated. During procedure they lied on a table, which was tilted from a horizontal (0^0) to 30^0 and 70^0 positions. The CO_2-response of the test-person was evoked by breathing mixtures of 2% and 4% CO_2 in air. The duration of each CO_2-load was 15'. Values obtained during the last 10' had been rated. After each load the testpersons were in a normal state for 15' in a horizontal position. The expiratory minute volume was

measured by an integrating pneumotachograph, the endexpiratory CO_2-concentration by an infrared analyser, the heartrate by ECG. The blood pressure was checked in 2-3' intervals by Riva Rocci.

The respiratory CO_2-response was estimated as the linear regression-function with endexpiratory CO_2-concentration as independent and expiratory minute volume as dependent variable for each of the different positions. For a complex description of every testperson's relation between ventilatory minute volume, heartrate and endexpiratory CO_2-concentration, partial correlation coefficients and linear multiple regression functions were computed on the basis of the data collected during all positions and inspiratory CO_2-loads.

RESULTS AND DISCUSSION

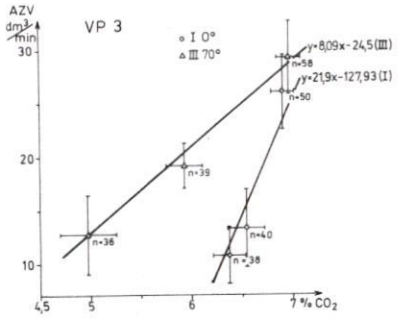

Fig.1: Displacement of CO_2-response, Values of testperson, 3' in horizontal (0^0) and upright (70^0) position.
Ord.: respir.vol., Absc.: endexpir. CO_2-concentration

- Gravitational load provokes a significant (tested by analysis of variance with $p \leq 0,05$) elevation of heart rate.
- The arterial blood-pressure proved to be constant or diastolic pressure raised negligibly.
- The CO_2-response curves shifted to the left depending on the changes in heart rate. (Fig. 1).
(The partial correlation coefficients between heart rates and respiratory minute volumes are significant (Tab. 1)

Tab. 1: Partial correlation coefficients (PCC) between expiratory minute volume (V) and heart rate (HR)

Designation of testperson	PCC V/HR	number of coupled values
3	0,6013+	302
7	0,4209+	364
10	0,4539+	360
11	0,7783+	352
15	0,7092+	440

+ significant (p 0,05)

These results correspond qualitatively with the findings of /6,7,8/ and confirm the 2nd of the hypotheses framed above: the circulatory influence on respiratory regulation being based on the degree of excitement in the circulatory nervous center, not on a precise calculation of the blood flow.

To compare the extent of CO_2-response displacement during gravitational load and workload, the ratios of relative changes in heart rate (HR_2:HR_1) and in respiratory minute volume (V_2 : V_1) were calculated for both these conditions. (For the calculation of the workload ratio we used data from /9,10/ for the gravitational load ratio we computed the minute volumes of the horizontal and vertical position at a constant CO_2-concentration by the multiple regression functions).
The ratios are:

	gravitational load	workload
$HR_2/HR_1 : V_2/V_1$	1:2,35	1:3 /9/ 1:4 /10/

Although comparison of data gathered in a small number of different testpersons under different conditions is not very reliable, the greater relative respiratory change during workload seems probably, because chemical and motor variations accompany the circulatory alterations during muscular exercise.

Furthermore the CO_2-response curves not only showed a shift during gravitational load, but also an altered inclination. For an explanation one should regard this modification being correlated to CO_2-dependent changes of heart rate (Fig. 2).

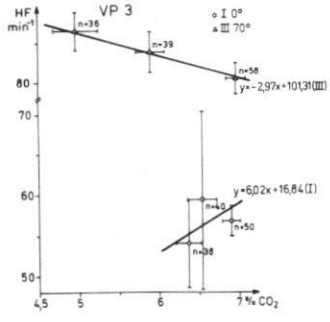

Fig.2: Heart rate CO_2-response. Testperson and conditions as in Fig.1.
Ord.: heart rate;
Absc.: endexp. CO_2-concentration

In other words: a certain testperson produces in a definite position respiratory as well as circulatory (heart rate) CO_2-responses. Represented in curves, both change their inclinations in the same direction. Starting with a low heart rate, CO_2 provokes in the majority of cases (but not always) a positive chronotropic effect and with a higher rate the

effect becomes less positive or negative (as described by /11/).

The cause of individual different heart rate reactions on CO_2 loads is unknown; but the similarity of circulatory and respiratory reaction gives further evidence on the circulatory and respiratory control coupling. In consequence the CO_2-respiratory response would not be the stationary characteristic of an isolated ventilation control's controller only, but also an expression of how circulation responds with increasing CO_2 load.

REFERENCES

/ 1/ Ponte, J. and M.J. Purves (1978): Carbon dioxide and venous return and their interaction as stimuli to ventilation in the cat. J.Physiol. London 27: 455-475

/ 2/ Wasserman, Kl, J.T. Castagna and M.L. Trombetta (1973): Cardiodynamic hyperpnoe: hyperpnoe secondary to cardiac output increase. Fed. Proc. Vol. 33:3, 936

/ 3/ Heymans, C. and J.J. Bouckaert (1930): Sinus caroticus and respiratory reflexes. J.Physiol. London 69: 254-266

/ 4/ Grundstein, M.M., J.P. Derenne and J. Milic-Emili (1975): Control of depth and frequency of breathing during baroreceptor stimulation in cats. J.Appl.Physiol. 39: 395-404

/ 5/ Tyden, G. (1977): Aspects of cardiovascular reflex control in man - an experimental study. Acta physiol. scand.Suppl. 448

/ 6/ Matalon, S.V. and L.E. Farhi (1979): Cardiopulmonary readjustments in passive tilt. J.Appl.Physiol.: Respirat. Environ. Exercise Physiol. 47: 503-507

/ 7/ Herberg, D., K. Mechelke, E. Nusser und P. Schenck (1962): Über den Einfluß der Barorezeptoren auf das Atemzentrum des Menschen. Kliwo. 40: 276-280

/ 8/ Anthonisen, N.R., D. Bartlett and S.M. Tenney (1965): Postural effect on ventilatory control. J.Appl. Physiol. 20: 191-196

/ 9/ Asmussen, E. (1964): Muscular exercise. In: Handbook of Physiology Section 3: Respiration Vol. II: 939-978, Washington

/10/ Bainbridge, F.A. in: Rein, H. und M. Schneider (1960): Einführung in die Physiologie des Menschen, Springer Verlag Berlin-Göttingen-Heidelberg, p.187

/11/ Mithoefer, J.C. and H. Kazemi (1964): Effect of carbon dioxide on heart rate. J.Appl.Physiol. 19: 1151-1156

THE EFFECTS OF HUMAN GROWTH HORMONE ADMINISTRATION ON THE FUNCTIONAL STATUS OF RAT ATROPHIED MUSCLE FOLLOWING IMMOBILIZATION

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Abstract

In 40 adult rats the right hind limb was immobilized by insertion of a metal pin.Fifteen days later the pin was removed and the animals divided into two equal groups:Group A-untreated and group B-treated by 0,06IU hGH/daily.After two weeks the following parameters of both gastrocnemius muscles in all the animals were obtained:Total EMG activity,contractile properties and work capacity.From our results it can be concluded that hGH administration a) increased the EMG activity of atrophied muscle in group B by 73% , b) increased the isometric tension of the same muscles by 58% and 65% (twitch and tetanic tension respectively)and c) restored to a great extent (by 44%) the work capacity of the atrophied muscles in group B as compared to the atrophied muscles of group A.

INTRODUCTION

In recent years there has been an increased interest in the adaptation of muscles to immobilization and in particular in the changes that occur in their morphological and contractile properties. Previous investigations (2,5,6) showed significant decreases in the functional capacity and weight of disused skeletal muscles under controlled experimental conditions. However little is known concerning the course of inprovement of the atrophy with different methods of treatment under experimental and/or clinical conditions.The purpose of this study was to investigate the effects of human growth hormone(hGH)administration on muscle weight, EMG activity, contractile capacity and work performance of the rat gastrocnemious muscle following immobilization atrophy.

MATERIALS AND METHODS

A total of 40 adult male Wistar rats weighing 200-220 g were employed. The right limb of each animal was immobilized by inserting a sterile metal pin through femur and tibia under pentobarbital sodium (6.0 mg ip) anaesthesia. During the operation,the limb was complete extended. Fifteen days after the immobilization the pin was removed and the animals were divided into two equal groups A and B. Group A animals were injected with normal saline daily and were used as controls; Group B animals received 0.06 I.U. hGH (Crescormone, AB, Kabi,Stockholm) per day, subcutaneously, for 15 days. Fifteen days after pin removal the following parameters in both the normal (left) and the atrophied (right) gastrocnemius muscles were evaluated in all animals:1.Total muscle weight. 2. Total evoked EMG activity as quantitated by means of the integration (I) and the continuous definite integral (CD) procedures (5). 3. Muscle contractile capacity, as measured by twitch and tetanic tension, during isometric contraction. 4. Maximum muscle work performance , as measured by means of a "Phipps and Bird" linear motion transducer, the muscle being loaded progressively with 10, 15,20 and 25 gram weights. The electrical stimulus on the corresponding sciatic nerve, which was maximal and standard for every functional parameter, was applied by a "Disa"Electromyograph stimulator, while the EMG tracings and the contraction curves were registered on an "Electronics for Medicine" Physiograph.

RESULTS

The results obtained (see table) demonstrate that:

1. In group A the gastrocnemius muscle weight on the atrophied (right) side was reduced to 62% of normal ($p<0.001$);in groupB GH administration increased the weight of both atrophied (AM) and normal muscles (NM) by 47% and 19% respectively, as compared to control animals ($p<0.001$)
2. Total EMG activity of atrophied muscle in group A was reduced significantly (by 63%) as evidenced by both EMG quantitation methods (I and CD) , in comparison to normal muscle ($p<0.001$);the administration of hGH increased the EMG activity of atrophied muscle in group B by 73% (I method) and 67% (CD method) as compared to atrophied muscle of group A ($p<0.001$). No effect is evident in normal muscle.
3. Twitch and tetanic tension were reduced by 66% and 50% respectively in the atrophied muscle of group A in comparison with the normal gastrocnemius muscle ($p<0.001$); hGH administration increased both twitch(by58%) and tetanic tension (by 65%) of atrophied muscle in group B as compared to the corresponding muscle of the group A ($p<0.001$); no hGH effect was evident in normal muscle.
4. The mean value of maximum work capacity of the atrophied gastrocnemius muscle in group A was 43% of that of the normal muscle; the corresponding value for the atrophied muscle following GH administration (group B) was 62% of that of the normal muscle ($p<0.001$).

DISCUSSION

It has been shown (1,2,5,6) that disuse of skeletal muscles by immobilization produces severe muscular atrophy with significant reduction of their functional capacity. The main histochemical changes wich were observed in the muscle fiber following this

TABLE

Muscle weight, EMG activity, work capacity and contractile properties of the gastrocnemious muscles *

Experimental groups	Muscle weight (g)		EMG activity**				Work capacity ratio	Contractile properties			
			"I" method		"CD"method			Twitch tension (g)		Tetanic tension (g)	
	NM	AM	NM	AM	NM	AM	AM/NM	NM	AM	NM	AM
A (Controls) n:20	1,55 ± 0,03	0,96 ± 0,04	2,08 ± 0,07	0,77 ± 0,06	1,56 ± 0,06	0,57 ± 0,05	0,43 ± 0,03	189,17 ± 20,32	63,66 ± 7,20	565,49 ± 27,12	283,80 ± 27,99
B (hGH-treated) n:20	1,85 ± 0,04	1,41 ± 0,03	2,07 ± 0,09	1,33 ± 0,07	1,47 ± 0,05	0,95 ± 0,04	0,62 ± 0,02	182,25 ± 10,15	100,44 ± 3,70	630,08 ± 27,69	467,73 ± 33,93

* Mean values $\pm S_E$

** EMG activity expressed in arbitraty units.

condition were a decrease in contractile proteins (mainly actomyosin) and an increase in collagen (5). The morphological changes of the atrophied muscle, i.e. reduction in muscle fiber diameter accompanied by the reduction of the contractile protein level, lead to a considerable decrease of muscle EMG activity and of its contractile capacity (5,6).However, these changes are more significant in muscular atrophy due to denervation because of the interruption of the "trophic" effect of the nerve on the muscle (4).

The main treatment methods applied to improve the function of atrophied muscle tissue are planned exercise and anabolic agents administration although it has not yet been possible to evaluate fully the relative merits of each (1,5). The use of growth hormone, the most potent anabolic agent, in experimental muscle atrophy following denervation or tenotomy obtained a beneficial increase in muscle size (3); however, in this paper the functional changes occurring under the influence of this treatment were not investigated. Our data show that growth hormone administration caused a significant restoration of muscle weight as well as of muscle EMG activity, contractile capacity and work performance. These changes are due probably to the beneficial effects of this hormone on muscle contractile proteins; no effects are evident on functional status of normal muscle.

REFERENCES

1. Apostolakis ,M.,K. Madena - Pyrgaki, A. Deligiannis, and C.Pappas.1977.The effects of exercise and of the anabolic steroid nandrolone decanoate on the EMG of atrophied rat gastrocnemius muscle. IRCS Med. Sci. 5:226.
2. FudemaJ,J.Fizzell, and E. Nelson.1961.Electromyography of experimentally immobilized skeletal muscles in cats. Am.J.Physiol.200:963-967.
3. Goldberg, A.L., and M. Goodman.1969.Relationship between growth hormone and muscular work indetermining muscle size. J.Physiol.(Lond.)200:655-666.
4. Lewis D.M., I.M. Pardoe, and N.S. Webb. 1978. The effect of denervation on the mechanical and electrical properties of cat skeletal muscle. J. Physiol.(Lond.) 277:48-49P.
5. Madena-Pyrgaki, A.,C. Pappas, A. Deligiannis, and M. Apostolakis. 1979. Work capacity, contractile protein and quantitative EMG changes following exercise or nandrolone decanoate treatment in experimentally induced muscle disuse atropy in rats. Acta Endocrinol. 90:568-576.
6. Maier, A., J. L. Crockett, D.R. Simpson, C.W.Saubert, and V.R. Edgerton. 1976. Properties of immobilized guinea pig hindlimb muscles. Am.J. Physiol. 231: 1520-1526.

GRAVITATIONAL ADAPTATION OF ANIMALS

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All terrestrial animals evolved under the Earth's gravity which imposes a load upon the various organ systems. This has required an adaptation, which is readily apparent between animals of increasing size. Load bearing systems become relatively greater in larger species. This scale effect was described by Galileo in 1638 who recognized it as a response to gravity since it was not shared by aquatic animals. The circulatory system also is susceptible to gravity, hydrostatic pressures tending to affect the distribution of body fluids. This was studied by Piorry in 1826, noting the influence of human posture upon blood distribution -- and experimentally, in centrifuging dogs, by Salathé in 1877.

Recently, the influence of artificial dynamic environments has been studied by summing gravitational and inertial fields -- as in Earth orbiting satellites and in centrifuges [7]. Several chronic acceleration research programs are currently being pursued, and their results have been reported elsewhere [4,6,9].

In hyperdynamic fields, the treatment becomes stressful above some threshold (about 1.5 G for kg-size animals), and the classical changes of physiological stress [1,5] are observed. This stress is readily reversed by transferring affected animals to Earth-gravity -- but the stressed condition returns rapidly, if such animals are reintroduced to the hyperdynamic field. However, surviving animals maintained in this environment become physiologically adapted with the indications of stress becoming ameliorated. This may require 30-60 days for kg-size animals exposed to 2-2.5 G -- and in them adaptation does not occur in fields above 3.5-4 G. The stress-adaptation sequence in birds can be followed by measuring lymphocyte frequencies [2], since their lymphocyte production is inhibited by adreno-corticoids. As the lymphocyte frequency recovers, animals resume a normal appearance and mortality becomes uncommon.

Such gravitational adaptation also exhibits an exhaustion phenomenon [5] -- animals which have successfully adapted suddenly developing the signs of chronic acceleration sickness. This is similar to Monge's disease, the sudden and irreversible development of acute altitude sickness in Andean natives [3]. However, in the absence of the stressor (residence under Earth gravity) the adaptation is retained for a long period of time. This was tested in a group of five Leghorn cocks which had become physiologically adapted to 2 G after 162 days of centrifugation. They were removed from the centrifuge, and then periodically exposed to a 2 G field (G), accompanied by five previously unexposed hatch-mates (C). The degree of retained acceleration adaptation was estimated from the decrease in lymphocyte frequency (L, lymphocytes per 100 white blood cells) after 24 hours at 2 G. After 24 hours at 2 G, lymphocyte frequency in the previously adapted birds had decreased about 20% -- but it did not decrease below the level found at the end of 162 days at 2 G (Fig. 1). The previously adapted birds exhibited an 84% greater lymphopenia ($p < 0.01$) which persisted to 206 days, when the experiment was terminated (Fig. 2).

Figure 1. Mean lymphocyte frequencies in previously G-adapted (G) and control (C) Leghorn cocks before and after 24 hours exposure to a 2 G field.

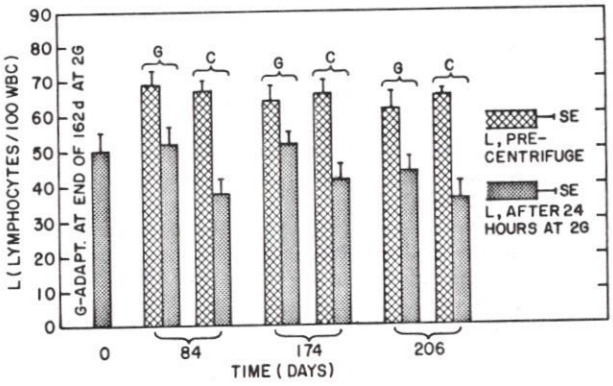
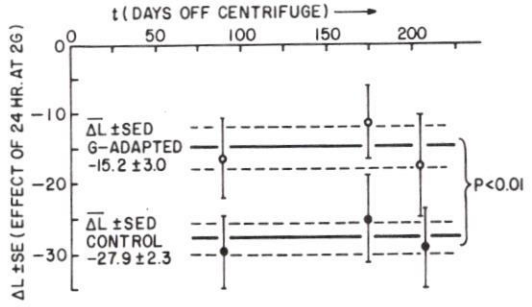


Figure 2. Decrease in lymphocyte frequency resulting from 24 hours exposure to a 2 G field in previously G-adapted (G) and in control (C) Leghorn cocks.



This pattern of change in lymphocyte frequency in response to 24 hours exposure to a 2 G field indicates that the previously adapted animals had retained much of their acceleration adaptation during 7-months residence at Earth-gravity. This was found to be sufficient to permit their continued exposure to a 2 G field without exhibiting signs of acceleration sickness. This implies that Earth-return of astronauts, after protracted weightlessness, will not lead to a systemic hyperdynamic stress, although it is well understood that such weightlessness leads to musculo-skeletal and circulatory decompensation.

The capacity for adaptation to chronic acceleration was found to be highly heritable. We have developed an acceleration tolerant strain by reproducing survivors of chronic acceleration experiments, which is now in the 22nd selected generation (S_{22}). These animals have a lesser mortality during chronic acceleration -- and particularly in intermediate fields. The lesser improvement in a 3 G field may result from its closeness to the tolerance limit, 3.5-4 G. So it does not appear that this treatment particularly improved the G-tolerance limit.

	Mid-lethal Exposure at:	
	2.5 G	3.0 G
P (parent stock)	25 days	20 days
S_4	--	35 days
S_{10}	--	70 days
S_{17}	175 days	80 days

Such rapid selection progress is considered by geneticists to indicate a metabolic basis [8] -- in this case for acceleration tolerance.

The result of the acceleration selection is largely quantitative -- with merely more of a treatment group surviving. Qualitatively both acceleration tolerant or susceptible animals are behaviorally and physiologically similar, and irrespective of their being from unselected or from acceleration-
selected stock. However, some differences are becoming apparent in the selected strain. Mature body size (of un-centrifuged controls) of the selected line is becoming smaller. This appears to have exponential kinetics -- the body mass of untreated cocks decreasing from 2.27 kg for the parent stock towards a limit of 1.85 kg:

$$\text{Mature size (kg)} = 1.85 + 0.42e^{-0.162n \text{ selections}} \quad [r = -0.785;\ p < 0.001]$$

This change is reasonable since there is generally an inverse relationship between body size and acceleration tolerance.

There also is an increased capacity for hens of the selected strain to produce eggs during chronic acceleration. In unselected stock, hens tend to suffer an oviduct prolapse after laying a few eggs -- and this is frequently encountered in fields of 2 G. However, chronically accelerated hens of the S_{21} generation maintain a good egg production (about 50 eggs per 100 hens per day) at 2.5 G, with only infrequent oviduct prolapse.

Although our selected strain of fowl is showing some differences from the barnyard variety, it provides a very useful model for chronic acceleration studies. It is a biped, and more similar in gravitationally susceptible characteristics (e.g., musculo-skeletal, circulatory) to humans than are the usual laboratory quadrupeds.

ACKNOWLEDGEMENT

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REFERENCES

1. Burton, R. R., and A. H. Smith, 1965. Chronic acceleration sickness. Aerospace Med. 36(1):39-44.
2. Burton, R. R., and A. H. Smith, 1972. Stress and adaptation responses to repeated acute acceleration. Am. J. Physiol. 222(6):1501-10.
3. Monge M., C., and C. Monge C., 1966. High Altitude Diseases. C. C. Thomas (Springfield).
4. Oyama, J., 1976. Effects of altered gravitational fields on laboratory animals. Ch. 8 in: Progress in Biometeorology. Vol. I. S. W. Tromp, J. J. Bouma, H. D. Johnson, Eds. Swets and Zeitlinger (Amsterdam).
5. Selye, H., 1950. Stress. Montreal Acta, Inc.
6. Smith, A. H., 1976. Physiological changes associated with long-term increases in acceleration. COSPAR Life Sci. and Space Res. XIV:91-100.
7. Smith, A. H., 1978. Gravitational Physiology. The Physiology Teacher 7(1):4-13.
8. Wagner, R. P., and H. K. Mitchell, 1955. Genetics and Metabolism. Wiley (New York).
9. Wunder, C. C., and L. O. Lutherer, 1964. Influence of chronic exposure to increased gravity upon growth and form of animals. Internat. Rev. Genl. Exptl. Zool. 1:334-416.

SCALING OF METABOLIC RATE ON BODY MASS IN SMALL LABORATORY MAMMALS

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INTRODUCTION

Metabolic heat production rate (MR) is related empirically to total body mass (TBM) among mature mammals from the mouse to the elephant by the well-established (1,3) allometric equation

$$MR, \text{ kcal/hr} = 2.92\ (TBM, kg)^{0.75}$$

which, when logarized, conveniently yields a straight line. Kleiber (3) has postulated that the 3/4 power relationship may represent an evolutionary compromise between the 1/2 power of body mass constraint on heat transfer through the body surface dictated by Fourier's Law of Heat Flow, and the 1st power of body mass requirement for energy production to counteract the loading of the organism by gravity.

A consequence of Kleiber's hypothesis is that in the absence of gravitational loading, as during the weightlessness of space flight, the scale relationship of metabolic rate to total body mass should tend to shift from the 3/4 power toward the 1/2 power of body mass. In contrast, the scale relationship should shift toward the 1st power of body mass if gravitational loading is increased, as in chronic centrifugation of animals. Some evidence for such an effect has been adduced by Smith (4) from changes in daily maintenance feed requirements of animals subjected to chronic centrifugation. Supportive evidence also comes from the studies of Hemmingsen (2) on the effects of narcosis on standard metabolic rate in rats of differing body size.

The NASA Shuttle/Spacelab Program is expected to provide a practical opportunity to examine the effect of several days of weightlessness on the scaling of metabolic rate on body mass in a series of mammalian species of differing body size. However, in order to carry out a meaningful flight experiment, it is necessary to identify a series of species of size range sufficient to define scaling parameters of the allometric equation with adequate precision to detect the expected changes, yet of small enough size to be compatible with Spacelab dimensions. It is also important to assess carefully the effects of age and sex difference within species on the scaling parameters, so as to arrive at an optimal experimental animal model.

PROCEDURES

Five species of common laboratory animals were selected for study: the Swiss-Webster mouse, the Syrian hamster, the Simonsen albino rat, the Hartley guinea pig, and the New Zealand white rabbit. This species series ranges from 0.05 kg mature body size for the mouse to 5 kg for the rabbit, providing 2 orders of magnitude of body mass range.

Arrangements were made with commercial suppliers to provide 6 animals of a particular species, sex and age at weekly intervals. Eight age cohorts, 1, 2, 3, 5, 8, 12, 18 and 24 months, were examined for each sex and species making a total of 80 groups of 6 animals each to form the study matrix. The animals were housed in individual cages under standard temperature and lighting conditions for a week after receipt, and fed *ad libitum* the same diet used by the supplier in rearing the animals.

After the week of stabilization in our laboratory the 6 animals were transferred to individual metabolic chambers at about 0830 on the day of measurement of metabolic rate. Each chamber was provided with a constant, measured flow of ventilating air, and the exhaust gas from each chamber was monitored for PO_2 and PCO_2 to provide mean hourly values for O_2 consumption and CO_2 production rates for 5 hours. The mean of the 2 lowest hourly values for O_2 consumption rate was multiplied by 4.85 to provide a resting metabolic heat production rate for each animal. Total body mass was also measured.

RESULTS AND DISCUSSION

Although both Brody (1) and Kleiber (3) emphasized that the 3/4 power relationship of metabolic rate to body mass applies only to mature animals, neither provided clear-cut criteria for describing maturity even though both cited extensive data describing the substantial changes that occur in metabolic rate during growth and development in a variety of mammalian species. They examined a number of units for comparative expression of metabolic rate, such as body surface area and various fractional powers of body mass, but did not arrive at a definitive result.

We chose to employ metabolic intensity, defined as kilocalories of metabolic heat produced per hour per kilogram of total body mass, as the unit of comparison among the various groups of animals studied, on grounds of simplicity and least number of assumptions. The results are given in Fig. 1,

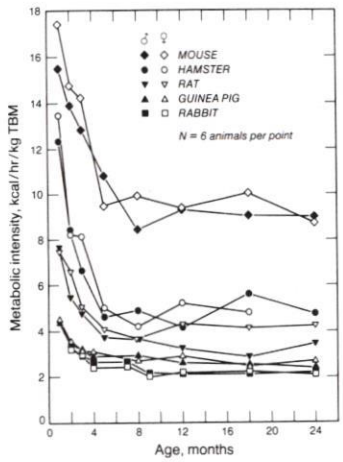

Fig. 1. Metabolic intensity as a function of age in both sexes of 5 species of small mammals.

which shows the mean metabolic intensity of 6 animals of each species, sex and age cohort examined,

plotted as a function of animal age. The coefficient of variation ranged from 3.7% to 18.7% for the individual mean values in seemingly random fashion, and overall averaged 9.2% among the groups.

A general pattern emerges for all 5 species which indicates that metabolic intensity is very high in young animals and decreases dramatically as the animals become older until an age of 6 to 8 months is attained, at which point the metabolic intensity stabilizes while both metabolic rate and body mass continue to increase with age. Also, there seems to be little or no consistent difference in metabolic intensity between sexes within each species. The phenomenon is shown even more clearly by normalizing the data for each species to the mean of the values from 8 to 24 months of age.

From these results it may be concluded that metabolic intensity represents a valid and useful comparative criterion of metabolic activity among the species represented, and that metabolic maturity is not attained until these animals reach an age of 6 to 8 months. It is also evident that younger animals are in a highly transitory metabolic state, making interspecies comparisons difficult for animals in this age range. In particular, the examination of scaling of metabolic rate on body size would seem to be best carried out in animals of at least 8 months of age for these species.

The allometric relationship of metabolic rate to total body mass for the animal groups 8 to 24 months of age is shown in Fig. 2.

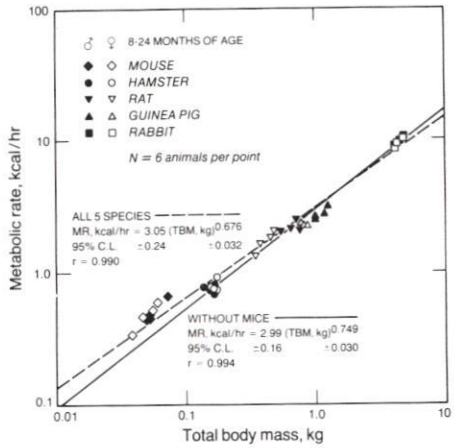

Fig. 2. Allometry of 4 species versus 5 species.

For all 5 species, the relationship is given by

$$MR, kcal/hr = 3.05\ (TBM,kg)^{0.676}$$

the power function of which is significantly different from the classic Kleiber/Brody equation. However, it may be seen that the values for the strain of mouse used in the present study were out of line with respect to the other 4 species. It has been shown by Yousef and Johnson (5) that levels of metabolic rate among different species of mice can depart substantially from predictions by the 3/4 power relationship, so that mice as a group are probably not good candidates for inclusion in a limited species series where their weighting becomes appreciable.

On the other hand, the allometric relationship for the 4 species, exclusive of the mouse, was found to be

$$MR, kcal/hr = 2.99\ (TBM,kg)^{0.749},$$

and the parameters are statistically indistinguishable from those of the Kleiber/Brody equation.

As a final test, the allometric relationship between metabolic rate and total body mass for a 24-animal group of 6 males each of the 4 species, hamster, rat, guinea pig and rabbit, 8 months of age is shown in Fig. 3 and was determined to be

$$MR, kcal/hr = 3.06\ (TBM,kg)^{0.741}.$$

A closely similar allometric relationship was found for a comparable 24-animal group of 6 females each of the 4 species of the same age.

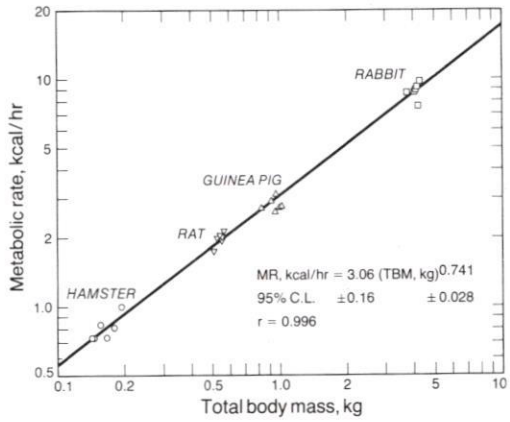

Fig. 3. Allometry of proposed animal model.

It is apparent that the 4-species series of either sex yields allometric parameters with sufficient accuracy, i.e. ± 5%, to define the relationship between metabolic rate and total body mass for terrestrial mammals in an acceptable and useful fashion. Therefore, it is proposed that 6 male animals, 8 months of age, of each of the 4 species, hamster, rat, guinea pig and rabbit, can be used experimentally as a suitable model to study the scale effects of alteration of the magnitude of gravitational loading on the metabolic energy requirements of terrestrial mammals.

ACKNOWLEDGMENT

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REFERENCES

1. BRODY, S. *Bioenergetics and Growth*. New York: Reinhold, 1945.
2. HEMMINGSEN, A.M. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Reports of the Steno Memorial Hospital and the Nordisk Insulinlaboratorium, vol. 9, part 2, p. 1-110, 1960.
3. KLEIBER, M. *The Fire of Life*. New York: Wiley, 1961.
4. SMITH, A.H. The roles of body mass and gravity in determining the energy requirements of homoiotherms. In: *COSPAR Life Sciences and Space Research XV*. Edited by R. Holmquist and A.C. Stickland. Oxford: Pergamon, 1978, p. 83-88.
5. YOUSEF, M.K. and H.D. JOHNSON. Thyroid activity in desert rodents: a mechanism for lowered metabolic rate. *Am. J. Physiol.* 229: 427-431, 1975.

SOME OF BIOCHEMICAL PARAMETERS IN RAT BRAIN DURING +GZ ACCELERATION

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ABSTRACT

Stability of lysosomes and mitochondria were tested to determine the influence of acceleration on the brain injuries. Degree of enzymatic adaptation to hypoxic conditions were also evaluated. After 1 hr of +5Gz acceleration cytoplasmic activity of intralysosomal and intramitochondrial enzymes increased what indicates damage of these cell structures. A raise in isoenzyme LDH_5 activity suggests a change of cell metabolism to anaerobic way.

INTRODUCTION

In earlier experiments we have shown that animals could survive 1 hr of +5Gz acceleration but about 50% animals died within the next days after exposure /5a/. It may suggest that some irreversible injuries occur during exposure to acceleration. This damages gradually spread leading to cellular destruction and finnally to death of organism.

To test the posibility that acceleration has deletorious effect on organism we determined injuries of lysosomes and mitochondria as well as enzymatic adaptation to anaerobic metabolism

MATERIALS AND METHODS

The investigations were performed on 60 male Wistar rats. Thirty animals were subjected to centrifugation for 1 hr at +5Gz. The remaining 30 rats were killed by decapitation and the brain was taken for further investigations. To obtain lysosomal stability homogenates were analysed for free and total activity B-galactosidase / B-gal /and acid phosphatase / AP / by the method described by Barret /1/. Mitochondrial stability was assessed on the basis of changes in percent free activity of glutamate dehydrogenase / GLDH /. Total and free activity was determined according to Bergmeyer /2/. Total activity of lactate dehydrogenase / LDH / and activity of isoenzymes were made as described by Bergmeyer /2/. Protein was determined by Lowry's method /7/. Statistical significance of experimental data was estimated with Student two-tailed t-test for unpaired samples.

RESULTS

Statistically significant fall was observed in the total LDH activity. Because activity of isoenzymes LDH_5 remained unchanged its percent contribution in the total LDH activity increased / Tab 1 /.

TABLE 1. Effect of acceleration /1hr; +5Gz/ on lactate dehydrogenase activity in rat brain

	U/mg protein ± SE	
	Control	Acceleration
Total activity	2.15 ± 0.08	1.71 ± 0.09^{xx}
LDH_5	0.87 ± 0.05	0.90 ± 0.06
% LDH_5	40.5 ± 2.52	52.6 ± 2.40^{xx}

TABLE 2. Effect of acceleration /1hr; +5Gz/ on lysosomes and mitochondria stability in rat brain

	mU/mg protein ± SE	
	Control	Acceleration
B-Gal		
Total activity	24.3 ± 0.92	18.4 ± 0.59^{xx}
Free activity	4.4 ± 0.45	4.6 ± 0.51
% Free	18.2 ± 0.75	25.2 ± 0.63^{xxx}
AP		
Total activity	22.4 ± 0.90	17.5 ± 0.69^{xx}
Free activity	10.2 ± 0.46	9.6 ± 0.48
% Free	45.7 ± 2.14	54.8 ± 2.73^{xx}
GLDH		
Total activity	121.5 ± 10.5	114.8 ± 6.4
Free activity	22.3 ± 1.3	38.1 ± 2.2^{xx}
% Free	18.4 ± 1.9	33.3 ± 2.8^{xxx}

xx $p<0.01$ xxx $p<0.001$

Acceleration reduced also the total activity of B-gal and AP /$p<0.001$/ while free activity was not decreased. The calculated percent free activity was significantly raised /$p<0.01$/ /Tab 2/. Acceleration failed to change the total activity of GLDH but the extramitochondrial activity of the enzyme was increased thus the calculated percent of free activity increased too /$p<0.001$/ /Tab 2/.

DISCUSSION

During +Gz acceleration the delivery of oxygen to brain cells is reduced due to the fall in cerebral blood flow. Increased contribution of LDH_5 activity in the total LDH activity indicates change of metabolism to anaerobic way as a compensatory response to such oxygen deprivation. However reduced total activity was found after exposure to acceleration. It is well known that under stress conditions increase release of cytoplasmic enzymes outside of cell /3/. It suggests that loss of LDH from cell rather than reduced rate of glycolysis is responsible for decrease of total LDH activity. Increased contribution of LDH_5 activity in the total LDH activity when lowering of this isoenzyme content occurs may be explained by higher specific activity. This activation could be induced by raised level of pyruvate and $NADH_2$.

In previous experiments 6.5°C of deep body temperature after 1 hr of +5 Gz acceleration was found /5/. The drop in deep body temperature correlates with the decrease in rate of brain metabolism so the needs for oxygen and energy demands are lower. The lowering of metabolism during acceleration may protect cells against irreversible injury.

When level of ATP falls below a critical value the cell is no longer able to maintain its morphological integrity and irreversible ultrastructural damage occurs /4/. In the present study marked changes are observed in stability of lysosomes and mitochondria /Tab 2/. They suggest that during 1 hr period of +5 Gz acceleration the lowering of metabolism and elevated glycolytic production of ATP is not able to meet all cell requirements. The intracellular release of lysosomal enzymes and their subsequent extralysosomal activity can participate significantly in the damage of cell membranes and cellular organelles. The decrease in the total activity of lysosomal hydrolases /Tab 2/ suggest that this enzymes are released into plasma too, where they are involved in degradation of other non-injured cells.

Stimulation of adrenergic system by isometric contraction of muscle during acceleration was also observed /6/. It seems therefore plausible that this play an important role in the regional impairement of neural tissues. Increase of afferent neuronal imput is related to the activation of metabolic rate. Enhanced oxygen consumption and requirement of ATP could lead to local imbalance of energetical homeostasis. It can suggest that during acceleration cerebral degree of hypoxia develops heterogenously. Areas where both ATP production and utilization is conterbalanced border with area where ATP level is too low.

REFERENCES

1. Barrett, A.J. 1972. Lysosomal enzymes. In Lysosomes: A Laboratory Handbook. J.T.Dingle, editor. North-Holland Publishing Co. Amsterdam 1 st edition 46-135.
2. Bergmeyer, H.U. 1974. Methods of Enzymatic Analysis. Verlag Chemie . Academic Press 2 ed. 2:574-656.
3. Cole, W.G., J.S.W. Steward, T.N. Palmer. 1977. The protective effects of purine nucleotides on the release of intracellular enzymes in hypoxia. Biochem. soc. trans. 5:1732-34.
4. Hearse, D.J., S.M. Humphrey, 1976. Myocardial enzyme loss: factor leading to reduction or exacerbation of release. In Recent Advences on Cardiac Structure and Metabolism. P. Haris, R.J. Bing, A. Fleckenstein, editors. University Park Press, Baltimore 7:327-334.

5a. Kwarecki, K., H. Dębiec, S. Wróblewski. 1979. Unpublished data.

5. Kwarecki, K., H. Dębiec, S. Wróblewski, W. Mydel, W. Kowalski. 1980. Influence of hypoxia and acceleration on morphological and biochemical changes in central nervous system. Med. Lot. 66:40-52.
6. Kozłowski, S., Z. Brzezińska, K. Nowar, W. Kowalski, M. Fronczyk. 1973. Plasma catecholamines during sustained isometric exercise. Clin. Sci. and Molecular Med. 45:723-731.
7. Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall. 1951. Protein measurment with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

ALTERATIONS IN HEAT LOSS AND HEAT PRODUCTION MECHANISMS IN RAT EXPOSED TO HYPERGRAVIC FIELDS

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INTRODUCTION

This paper traces a series of studies that began with the observation that rats exposed to 2.5G, a gravitational field well below maximal tolerance levels, exhibit an inappropriate thermoregulatory response. The thesis is developed that, in light of recent experiments, this response can be related to the altered behavior of specific physiological control mechanisms.

In classifying responses as inappropriate, we imply that effectors are not precisely controlled to maintain homeostatic conditions. At 1G, rats can maintain core temperature, T_c, within close limits despite a variety of environmental challenges. Thus, at an ambient temperature, T_a, of 22°C, T_c is normally held near 37°C, with a circadian variation of about 1°C. Moreover, rats must be severely stressed before T_c will deviate by more than a few degrees -- e.g., although rats become hyperthermic after 4 hrs at 40°C, T_c rises only about 2.5°C.

Where rats have remained in a hypergravic field of 2.5G for several days, with T_a controlled at 22 ± 2°C, the value of T_c after 100 hrs was similar to that prior to hypergravic exposure (5). These rats were able to move freely about their cages (having been chronically implanted with telemetry units); and the ambient temperature of 22 ± 2°C appeared to impose no insurmountable stress upon them. Based on the "normal" values of T_c observed after 100 hrs at 2.5G, one might conclude that thermoregulatory mechanisms had functioned appropriately. That such was not the case, however, is indicated by the fact that during the first hour on the centrifuge, T_c continuously fell. This drop was prompt and marked, with temperatures falling in some cases as much as 6°C after 1 hr at 2.5G (5). Such large decreases have been independently confirmed (2) and have evoked studies aimed at elucidating the mechanisms underlying the initial rapid fall and subsequent slow recovery of T_c (2-4).

COMBINED STRESSORS

In view of the thermal responses of rats exposed to a very mild cold stressor at hypergravity (i.e., T_a=22°C while thermoneutrality is near 28°C), subsequent experiments have utilized more severe cold exposures in combination with 1 to 4G fields (2-4). Lowering T_a to 12°C (a temperature at which rats at 1G are still able to maintain T_c near 37°C) provided a means of applying a stressor (lowered T_a) that specifically challenged the thermoregulatory system. On the other hand, the ongoing hypergravitational field served as a more general stressor affecting a number of physiological systems (1,7).

Using a delayed decrease in T_a as a challenge to the thermoregulatory system, it was found that even after several hrs in 1.5 to 4G fields, when T_c had ceased falling and, in fact, was slowly moving back toward 37°C, the thermoregulatory responses of the rats were impaired (2,3). Moreover, when step decreases in T_a were applied at the onset of hypergravity, the observed fall in T_c was significantly greater than that occurring at 22°C. This decrease in T_c during the first hour in a hypergravic field thus appears to be proportional to the field amplitude (a general stressor) as well as to the amplitude of the step decrease in T_a (a specific stressor) (2-5).

Recent experiments (Oyama, unpublished observations) demonstrate that when T_a is within the rat's thermoneutral zone, there is no drop in T_c. This observation implies that the lower T_c occurring when T_a is at 22°C or at 12°C results from the inability of the rat to activate thermoregulatory mechanisms that, at 1G conditions, are brought into play to maintain T_c at 37-38°C. For cold-exposed rats, these mechanisms include pathways that may be passive (vasoconstriction to conserve heat) and/or active (heat production via shivering and non-shivering modes). These effector mechanisms are under the influence of a central controller for temperature (Fig. 1).

INTERLOCKING CONTROL SYSTEMS AND PASSIVE HEAT LOSS

When placed in a 1G environment at 22°C or at 12°C, rats first alter blood flow, vasoconstricting so that warm blood is shunted away from the periphery. This passive conservation requires minimal expenditure of energy and serves to retain heat in the core. In man, blood flow to the skin is reduced; in rabbits, flow to the ears is reduced; and in rats, flow to the tail is reduced. A factor emphasized in Fig. 1 is that peripheral blood flow is not controlled solely by the thermoregulatory system, although changes in T_a do induce the thermocontroller to alter blood flow to the periphery.

The possibility that altered vasomotor control, as manifested by inappropriate shunting of blood to the rat's tail, is partly responsible for the fall in T_c occurring upon exposure to hypergravic fields has been evaluated in restrained and loosely-restrained rats using thermistors to measure tail temperatures (2,3). In these experiments, a rise in tail temperature was observed during the first hour of hypergravic exposure -- the same period during which T_c fell several degrees. It was thus concluded that one mechanism contributing to the initial drop in T_c is a transient shunting of warm blood to the tail of the rat -- a response

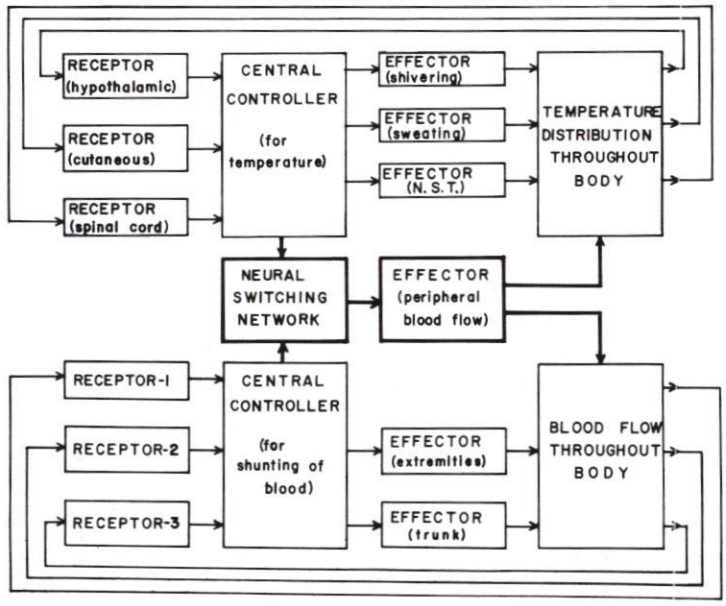

Figure 1. Interlocking control systems for temperature regulation (upper half of the diagram) and cardiovascular channeling of blood flow (lower half of the diagram). Both control systems are shown as sharing control of peripheral blood flow to the rat's tail via a neural switching network. During exercise, the central controller for shunting of blood augments flow to the muscles in the limbs (extremities) and in the trunk. In cold-exposed rats, receptors in the hypothalamus, skin and spinal cord relay signals to the central controller for temperature that in turn signals effectors.

radically inappropriate, from a homeostatic viewpoint, in light of the marked fall in T_c.

HEAT PRODUCTION

When rats are cold exposed at 1G, they can bring into play heat production pathways that utilize much greater amounts of metabolic energy than that required to control peripheral vasoconstriction and vasodilation. Heat production mechanisms draw upon stored chemical energy reserves, converting them to heat either through shivering or nonshivering processes. Since this conversion of chemical energy to heat requires oxygen, rates of oxygen consumption indirectly measure rates of heat production. Recent measurements of oxygen consumption, T_c, and tail temperature during the early periods of hypergravic exposure indicate no consistent alterations in oxygen consumption accompanying the decrease in T_c and increase in tail temperature (Oyama, unpublished observations). These results imply that altered rates of heat loss, rather than heat production, are responsible for the observed temperature changes. In addition, the core temperatures seen after the first hour of hypergravity (following recovery of peripheral blood flow control) fail to show the increase that would be expected if shivering and nonshivering thermogenesis were fully activated (Horowitz and Horwitz, unpublished observations).

CONCLUSION

There now appear to be several lines of evidence indicating that the neural switching network shown in Fig. 1 is transiently affected during the first hour a rat is exposed to hypergravity. Moreover, even after the first hour, when core temperature has fallen several degrees, shivering and nonshivering thermogenesis are not fully activated. Only after prolonged exposure to hypergravic fields do heat production mechanisms recover sufficiently to bring core temperature back to 37-38°C. Experiments thus point to a more rapid recovery of effector mechanisms for heat loss than for heat production. This sequential recovery is consistent with the hierarchical arrangement of parallel thermocontrollers proposed by Satinoff (6). The use of hypergravic fields to test neural models based on data obtained at 1G has the advantage that hypergravic effects are reversible and, after several days at hypergravity, the rat recovers homeostatic control of body temperature.

REFERENCES

1. Daligcon, B. C. and J. Oyama. Increased uptake and utilization of glucose by diaphragms of rats exposed to chronic centrifugation. Am. J. Physiol. 228:742-746, 1975.
2. Fuller, C. A., J. M. Horowitz, and B. A. Horwitz. Effects of acceleration on the thermoregulatory response of unanesthetized rats. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 42:74-77, 1977.
3. Giacchino, J., B. A. Horwitz, and J. M. Horowitz. Thermoregulation in unrestrained rats during and after exposure to 1.5-4G. J. Appl. Physiol.:Respirat. Environ. Exercise Physiol. 46:1049-1053, 1979.
4. Horowitz, J. M. and B. A. Horwitz. Thermoregulatory responses of unanesthetized rats exposed to gravitational fields of 1 to 4G. In: COSPAR: Life Science and Space Research XVI, ed. by R. Holmquist and A. C. Strickland. New York: Pergamon, 1978, pp. 77-82.
5. Oyama, J., W. T. Platt, and V. Holland. Deep-body temperature changes in rats exposed to chronic centrifugation. Am. J. Physiol. 221: 1271-1277, 1971.
6. Satinoff, E. Neural organization and evolution of thermal regulation in mammals. Science 201: 16-22, 1978.
7. Smith, A. H. Gravitational biology. Physiol. Teacher 7:4-13, 1978.

ACKNOWLEDGEMENT

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SYMPATHETIC-ADRENOMEDULLARY ACTIVITY IN RATS AFTER SPACE FLIGHT ON THE BIOSATELLITES COSMOS

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ABSTRACT

The indicators of the adrenomedullary activity - catecholamine (CA) content and the activity of the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH)- were measured in the adrenals of rats living in space on board the biosatellites COSMOS in the state of weightlessness during 18.5 - 19.5 days. These indicators were not significantly changed by space flight but the animals exposed after space flight to repeated immobilization stress on Earth showed a significant decrease of adrenal epinephrine and an expressive increase of adrenal TH activity compared to stressed animals which were not in space. Our results corroborate that prolonged state of weightlessness during space flight does not represent by itself an intensive stressful stimulus for the adrenomedullary system but potentiates the response of rats to stress exposure after return to Earth.

The state of weightlessness, which is a highly nonphysiological situation for the organism of human beings as well as animals, has become one of the greatest medical problems of prolonged space flights. Is the state of weightlessness a stressful stimulus for the mammalian organism? If so, is then adaptation of the organism to this situation conceivable?

These questions were studied in rats which experienced space flight on the biosatellites COSMOS. On board the biosatellite COSMOS 782 all rats spent 19.5 days in space in the state of weightlessness. The results obtained from this experiment have already been published (1) and they suggest that the sympathetic-adrenomedullary system (SAS) is not significantly activated by prolonged space flight. On board the biosatellite COSMOS 936, some rats were caged in a centrifuge (1 g) to distinguish the effect of weightlessness from other stressful factors connected with space flight. Not even this experiment did clearly answer the question whether a prolonged state of weightlessness acted as a stressor for SAS. This was to be answered by analyzing the material from the biosatellite COSMOS 1129. This experiment was built on our previous results obtained in rats exposed to single or repeated immobilization stress on Earth (2,3). On the basis of our experience in the field of stress we argued that if rats are during space flight chronically exposed to intensive stressors, after return to Earth, they should react to repeated immobilization stress as chronically stressed rats. If however, space flight is not connected with prolonged intensive stressful stimulation of SAS, then the rats should react to immobilization stress as animals exposed to stress for the first time.

The biosatellite COSMOS 1129 spent in space 18.5 days. The animals were decapitated immediately after landing (7-10hrs) and 6 days after landing. The third flying group was after landing exposed to immobilization stress 5 times, daily for 150 minutes, and the animals were killed immediately after the last exposure. The first immobilization was performed directly at the place of landing but the following day (transport of rats to Moscow) this procedure was omitted. The control and synchronous groups were also exposed to repeated immobilization stress. Adrenal catecholamines (CA) were measured by the fluorometric method (4) and the activity of TH according to Nagatsu (5).

Adrenal epinephrine (EPI) content was not changed in the group of rats killed immediately after landing of COSMOS 1129, in comparison with the control and synchronous groups (Fig.1). Six days after landing these indicators were as well not changed compared to the control group, and EPI was a little higher compared to the synchronous group. However, in the rats that were during these 6 days after space flight exposed 5 times to forced immobilization, the adrenal EPI content was significantly decreased compared to both the control and synchronous repeatedly immobilized groups.

No significant changes of adrenal tyrosine hydroxylase activity in rats killed immediately after landing of COSMOS 1129 were demonstrated (Fig.1). However, in rats which were during 6 days after landing exposed

5 times to forced immobilization, the adrenal TH activity was significantly increased compared to control as well as synchronous repeatedly immobilized rats.

The experiment COSMOS 1129 as well as both previous experiments, COSMOS 782 and 936, showed that the adrenal CA content and TH activity were not substantially changed in the rats decapitated immediately after landing suggesting that most probably the activity of SAS was not influenced during space flight by any chronic stressor.

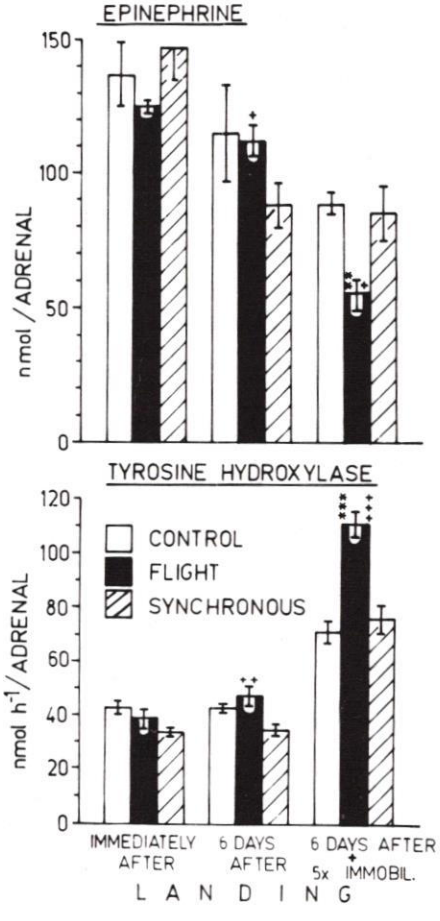

Fig.1. Adrenal epinephrine and tyrosine hydroxylase in rats after space flight on COSMOS 1129. Animals were exposed to immobilization stress for 150 min. daily and killed after the last interval still mounted on the board. Mean ± SEM of 6-7 rats. Statistical significance:
+ $p<0.05$; ++ $p<0.01$; +++ $p<0.001$ compared to adequate synchronous group
** $p<0.01$; *** $p<0.001$ compared to control group

Repeated exposition to stress, e. g. forced immobilization, is responsible for a 3 to 4 - fold elevation of adrenal TH activity in rats (3). On the other hand, a three-week stay of rats in space failed to affect TH activity in the adrenals of flight rats. The results from COSMOS 1129 (Fig.1), however, clearly demonstrate that rats which after 18.5 days of space flight were exposed to stress on Earth, showed a significantly increased adrenomedullary activity compared to the control and synchronous groups. These results, interpreted in the light of our previous findings in immobilized rats (2,3) indicate that during space flight no intensive long-acting stressor could have affected the SAS activity. The fact that after return to Earth, the animals responded to stress more intensively suggests that during space flight some factor (maybe weightlessness) must have been effective in sensitizing SAS to an enhanced response to intensive stress on Earth. This might have been brought about by diminished requirements of the organism upon the activity of SAS due to the state of weightlessness during space flight.

Our finding of the pronounced reactivity of the organism to stress after prolonged space flight is a significant phenomenon which should be cosidered particularly in the readaptation phase.

REFERENCES

1. Kvetňanský, R., R. A. Tigranjan, T. Torda, D. Repčeková, E. Jahnová, and K. Murgaš. 1980. Effect of a prolonged space flight on the responses of adrenal cortical and medullary layers. Kosmič. Biologija Aviakosmič. Medicina 14 (N° 1): 24-27 (in Russian).
2. Kvetňanský, R. and L. Mikulaj. 1970. Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. Endocrinology 87: 738-743.
3. Kvetňanský, R., V. K. Weise, and I. J. Kopin. 1970. Elevation of adrenal tyrosine hydroxylase and phenylethanolamine-N-methyltransferase by repeated immobilization of rats. Endocrinology 87: 744-749.
4. Euler, U. S. v. and F. Lishajko. 1961. Improved technique for the fluorimetric estimation of catecholamines. Acta Physiol. Scand., 51: 348-355.
5. Nagatsu, T., M. Levitt, and S. Udenfriend. 1964. A rapid and simple radioassay for tyrosine hydroxylase activity. Analyt. Biochem., 9: 122-126

CHRONIC CENTRAL VASCULAR EXPANSION INDUCES HYPOKALEMIA IN CONSCIOUS PRIMATES

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The shift of fluid from the lower limbs to the thoracic central vascular space during weightlessness can be mimicked by head-out water immersion or bedrest in man, or atrial balloon distension in animals (2,3). These studies induced comparable changes in central venous pressure and demonstrated a reflex diuresis and natriuresis, decrease in ADH secretion (Henry-Gauer reflex), and decreases in plasma renin and aldosterone (2,3).

Certain important adaptations to spaceflight, however, were not predicted from ground-based simulations. Instead of the predicted decrease in plasma aldosterone, normal plasma levels were maintained and urinary aldosterone excretion was markedly elevated. Furthermore, in the early Apollo missions there was a decrease in both total exchangeable potassium and plasma potassium concentration which, in some astronauts, precipitated cardiac arrhythmias (1). We report here that central vascular expansion maintained for 4 days in conscious squirrel monkeys (*Saimiri sciureus*) reconciles the apparently conflicting short-term fluid and electrolyte responses to water immersion and atrial balloon distension, with those described for prolonged weightlessness during spaceflight. Potassium is lost in the urine during the initial diuresis and plasma potassium concentration then remains low during a persistent high sodium and fluid output state. Changes in plasma aldosterone do not appear to mediate this response.

Six monkeys, weighing 800 to 1200 gms, were trained to sit in a metabolism chair restrained only at the waist, with an airtight compartment below the waist in which they could be subjected to an increased lower body positive air pressure (LBPP) of 20 torr which produced a 3 cm H_2O increase in central venous pressure (5). The experiments were conducted within an isolation chamber with lights-on from 08^{00} hrs to 20^{00} hrs (LD 12:12) each day. Food and water were available continuously *ad lib.*. Urine was continuously collected from a padded funnel between the animal's legs. After 2 days of adjustment to the chair, the monkeys were studied for a 30 hr control period, followed by 96 hrs of continuous LBPP and then 24 hrs of recovery. The animals were previously prepared with chronic indwelling arterial and venous catheters which were led outside the isolation chamber and enabled plasma aldosterone and potassium determinations without disturbance to the animal.

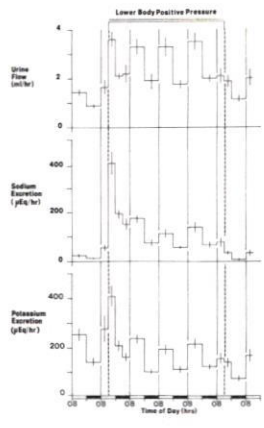

FIG. 1: Urinary responses ($\bar{x} \pm$ SEM) of squirrel monkeys (n=6) before, during and after exposure to 4 days of continuous lower body positive air pressure (indicated by the bar on top of the graph). Vertical lines are placed at 08^{00} hr on each day of the experiment. At 14^{00} hr on day 2, LBPP was introduced and the pressure was maintained at 20 torr until 14^{00} hr on day 6. The isolation chamber illumination (LD 12:12 cycle) is indicated along the time axis.

There was a marked increase in the urinary excretion of sodium, potassium and water during the first 6 hrs of LBPP (Fig. 1). The diuresis was maintained throughout the 4 days of LBPP, but sodium excretion fell after the first 24 hrs to an intermediate level, still considerably elevated over pre-LBPP levels. In both control days and during the LBPP stimulus, there was a circadian modulation of renal electrolyte and water excretion with maximal excretion during lights-on each day and nocturnal suppression of the response (5). By day 2 of LBPP, drinking by the animals had increased from 71.2 ± 10.6 to 107.6 ± 8.9 ml/day ($p<0.05$), and a stable rate of high volume input and output was thereafter maintained.

Potassium excretion (Fig. 1) was increased during the first 12 hrs of LBPP to 310 ± 28 μEq/hr from a 12 hr control rate at the same circadian phase of 231 ± 25 μEq/hr ($p<0.05$). During this time, plasma potassium concentration (Fig. 2) decreased from 3.6 ± 0.1 to 3.1 ± 0.1 mEq/L ($p<0.02$). Thereafter, daily potassium excretion returned to control levels while plasma potassium concentration remained low. Termination of LBPP was accompanied by reductions in potassium, sodium and volume excretion; plasma potassium concentration reattained normal levels within 24 hrs.

The role of aldosterone in the electrolyte response to central volume expansion has been a matter of particular debate. Water immersion and

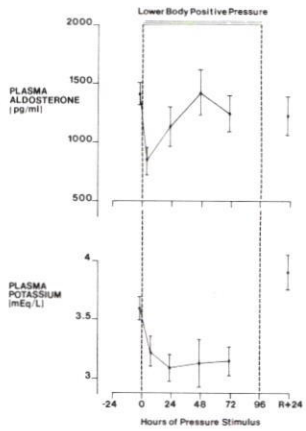

FIG. 2: Responses of plasma aldosterone and potassium concentration ($\bar{x} \pm$ S.E.) to prolonged LBPP (n=5) in comparison to 1 hr pre-, and 24 hr post-, control values.

atrial balloon distension experiments showed that plasma renin and aldosterone fall after volume receptor stimulation (2,3), whereas studies in space (with no data collected for the first 24 hrs) show plasma aldosterone was maintained within the normal range and urinary aldosterone increased (1). Fig. 2 reconciles these two observations showing that plasma aldosterone transiently drops within the first 3 hrs of LBPP ($p<0.01$ compared with controls), and then regains normal levels within 24 hrs. These levels are maintained despite the continued LBPP stimulus.

Aldosterone would appear to play little role in the acute response to central vascular expansion. The natriuresis commencing within the first hrs of LBPP was too rapid to be explained by the fall in plasma aldosterone concentration. Furthermore, this initial fall in plasma aldosterone was accompanied by an increase rather than a decrease in potassium excretion. The most probable explanation for the kaliuresis was that the potassium excretion was promoted by the high fluid flows and enhanced sodium delivery to the distal renal tubules, induced by LBPP (4). The potassium loss of 2.4 ± 0.9 mEq/kg during the first day of LBPP apparently resulted in the observed hypokalemia.

Over the subsequent days of the LBPP-induced central vascular expansion, two conflicting stimuli would appear to account for the observed levels of plasma aldosterone. Hypokalemia induced by the initial kaliuresis and sustained by the continuing high urine output state would tend to suppress aldosterone, whereas the hypovolemia associated with the continuing diuresis and natriuresis would tend to stimulate secretion. Thus the normal plasma aldosterone levels observed in our experiments, as well as during spaceflight, might represent a relative hyposecretion in terms of volume homeostasis and a relative hypersecretion with respect to plasma potassium regulation.

We have shown that the kaliuresis and marked natriuresis is confined primarily to the first 24 hrs of central volume expansion. The lack of such data from space missions to date may explain some of the differences between studies during weightlessness and those utilizing ground-based analogs. Further resolution of this question, however, must await the opportunity offered by NASA's Space Shuttle Program, to examine in detail the early fluid and electrolyte responses to weightlessness.

We thank Drs. Frank M. Sulzman and Charles A. Fuller, Ms. Christine Harling, Ms. Beverly Tepper, Dr. Carolyn S. Leach, Mrs. Vernell Sesperman and Ms. Louise Kilham for their assistance. Supported by NASA Grant NSG 9054.

REFERENCES

1. Berry, C.A. Medical legacy of Apollo. *Aerospace Med. 45:* 1046-1057, 1974.
2. Epstein, M. Renal effects of head-out water immersion in man: implications for an understanding of volume homeostasis. *Physiol. Rev. 58:* 529-581, 1978.
3. Gauer, O., J. Henry and C. Behn. The regulation of extracellular fluid volume. *Ann. Rev. Physiol. 32:* 547-595, 1970.
4. Good, D. and F. Wright. Luminal influences on potassium secretion: sodium concentration and fluid flow rate. *Am. J. Physiol. 236:* F192-F205, 1979.
5. Kass, D.A., F.M. Sulzman, C.A. Fuller and M.C. Moore-Ede. Renal responses to central vascular expansion are suppressed at night in conscious primates. *Am. J. Physiol.*, In Press, 1980.

AUTONOMIC REACTIONS IN LABYRINTHECTOMIZED RABBITS DURING CENTRIFUGE ACCELERATION

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ABSTRACT The effects of acceleration produced by centrifugal force upon cardiopulmonary and somatosensory system were investigated using 23 adult rabbits. The experiments were included to compare with intact and labyrinthectomized animals, and also with awake and light anaesthetized ones. The centrifugal force (G) was applied up to 5G making a load paradigm of staircase-type or trapezoid-type, in which each plateau level of G continued over 45 sec. The respiratory rate and heart rate were not in proportion to magnitude of acceleration. Responses by the staircase acceleration drew a hysteresis loop and it seems that the responses of respiratory and heart rates produced by acceleration took either of those two values on the hysteresis curve. And it was observed in some cases that the nystagmus induced by acceleration was correlated inversely to EMG response of hind limbs.

Abundant results on the physiological effects of the acceleration have been presented, and among others relations of cardiopulmonary system to the acceleration have come into the light. They have mainly discussed and proved these physical factors because of the centrifugal force affects directly on the cardiopulmonary system (2,3,4,5). On the other hand, it is well known fact that the autonomic nervous system responds to the vestibular and the somatic sensation produced by the acceleration (3,5,6). The aim of this paper is to ascertain the effects of acceleration as to the afferent information and to verify that the cardiopulmonary system constitutes a non-linear system, forming a hysteresis loop, in this experimental conditions. Moreover we wish to describe that the effects of the labyrinthectomy and light anaesthesia have the diminishing effect on the afferent information.

MATERIALS AND METHODS

We used 23 adult rabbits weighing from 1.8 Kg to 3.0 Kg. Experimental procedures were conducted in comparing the two groups of the intact and labyrinthectomized animals, and each groupe was prepared to study the anaesthetic effects of the barbiturate (Nembutal®) after the control run. Surgical procedures of the labyrinthectomy used were the modification of Cohen and Suzuki's method (1).

All experiments were conducted abord a small animal centrifuge. The machine had a 120 cm radius and the cabin attitude was controlled to maintain the resultant acceleration normal to the cabin floor. The animal was fixed on the frame provided in the cabin. The centrifugal force (G) was determined by an acceleration meter. The respiration was monitored by a thermister which was put into the nasal cavity of the animal. The EMG was obtained from the right quadriceps muscle. The ECG, EOG and all above mentioned parameters were transmitted from the cabin by a 5 channel telemeter and recorded on a date-recorder.

The 1G experiments were conducted with the postural changes, which were composed of two positions, one position was the body normal to the ground (R1) and this position was used for the 30 min resting period. The other was the body laid lateral to the ground (R2) which was used for the 1G level calibration before each centrifuge run. By the centrifuge run, animal was started from the R2 state, and the acceleration was applied always to the lateral side of the body, due to the swing-bucket type.

The centrifuge run was programed in two types: one was a staircase-type which had a each step of 1G with raising rate of 1G/15 sec and plateau of 45 or 60 sec, and raised up to 5G followed by falling phase with the same steps down to 1G. Another was a trapezoid-type, ascended by the rate of 1G/15 sec to the goal of G and maintained it, then descended by the same rate down to 1G. The total running period in each trial was 3 min, so that the larger acceleration trial had the shorter plateau. Between each trial the animals were rested more than 30 min.

RESULTS AND DISCUSSION

1. General feature of the G-y acceleration

The case of the staircase-type acceleration was shown in Fig.1. The 2nd trace is EOG, in which sometimes large eye movements and blinking were observed and at 5G a vigorous nystagmus was noted. There was no definite correlation between the magnitude of acceleration and the occurence of nystagmus, but in some cases by increasing the acceleration to 5G, obvious nystagmus appeared and continued till the acceleration was lowered to 3G. On the other hand, the muscle activity in the 3rd trace was also correlated inversely to the nystagmus, so that when nystagmus occured at 5G, the muscle activity conversely decreased, and after a short time of the ceassation of nystagmus the muscle activity recovered.

The respiratory and heart rate were increased or decreased accompany with the increase or decrease of the acceleration respectively. However in most cases these responses were delayed from the change of acceleration, namely they have not a linear relation to the acceleration change. These relationship were shown that the respiratory and heart rates formed loops (hysteresis loop) upon the increase of acceleration. By decreasing of acceleration they took always the upper route of the loops.

2. Effects of labyrinthectomy and barbiturate anaesthesia on the hysteresis

From a point of view that the reflex action of the autonomic nervous system to the centrifuge acceleration is produced mainly by the vestibular informations, the labyrinthectomized animals were compared with the intact ones. On the other hand the acceleration affects not only the vestibular

organs, but also to the somatosensory organs, therefore the anaesthesia was employed in order to minimize the reaction to the sensory stimuli.
In Fig. 2 and 3, the effects of acceleration applied by the staircase-type on the respiratory and heart rate are shown. All of the intact and labyrinthectomized rabbits with or without anaesthesia showed the hysteresis loops in the respiration and heart rate upon the increase and decrease of the acceleration.

In comparing the loop-slope of each hysteresis loop, the steepest change was found in the intact animals, and the slope became lower in the order of labyrinthectomized ones without anaesthesia, intact ones with anaesthesia and labyrinthectomized ones with anaesthesia. The decrease of the slope in the Nembutal anaesthetized animals was more intense than in the labyrinthectomized ones. This finding implies that the deafferentiation, especially labyrinthectomy, is highly effective to the respiratory and heart rate changes, but Nembutal anaesthesia is more effective than the labyrinthectomy alone.

A question is arised whether the staircase acceleration, which is applied in an ascending order from 1G to 5G produces the same tendency as a randomly applied acceleration by means of the trapezoid-type or not. A schedule of the application of the acceleration in the trapezoid-type was taken at random, such as at first 4G and in turn 3G, 5G and 2G, and between each run a pause of 30 min was inserted. In 2G, a transient increase of respiratory rate appeared only at an ascending phase of acceleration, but in 3G, 4G and 5G the time lag of responses was observed at ascending, plateau and descending phase of the acceleration. When the amount of the increase of respiratory rate in plateau level was compared in each G run, it was well correlated to the increase of G. This relation curve of the increase of the respiratory rate and magnitude of G was well fitted on the hysteresis loop of the staircase-type acceleration (Fig. 4). The above mentioned results demonstrate that the hysteresis phenomenon of the respiratory rate change produced by the acceleration has a stable system, in which the respiratory response taken either of two values of the hysteresis loop, even if the acceleration was applied randomly.

REFERENCES

1. Cohen,B. et al. 1962. Eye movements induced by ampullary nerve stimulation. Am. J. Physiol. 204(2). 347-351
2. Glaister,d.h., 1970. Distribution of pulmonary blood and ventilation during forward(+Gx) acceleration. J. Appl. Physiol. 29. 432-439
3. Howard,P., 1965. The physiology of positive acceleration. In: A Textbook of Aviation Physiology, edited by J.A.Gillies New York Pergamon
4. Nieding,G. Von and K.Krekler, 1973. Effect of Acceleration on Distribution of Lung Perfusion and on Respiratory Gas exchange. Pflugers Arch. 342. 159-176
5. Shubrooks,S.J.,Jr. and S.D.Leverett,Jr. 1973. Effect of the Valsalva maneuver on tolerance to +Gz acceleration. J. Appl. Physiol. 34. 460-466
6. Smith,A.H. et al. 1979. Effects of Brief Exposure of Domestic Fowl To Very Intense Acceleration Fields. Aviat. Spase Environ. Med. 50(2). 126-133

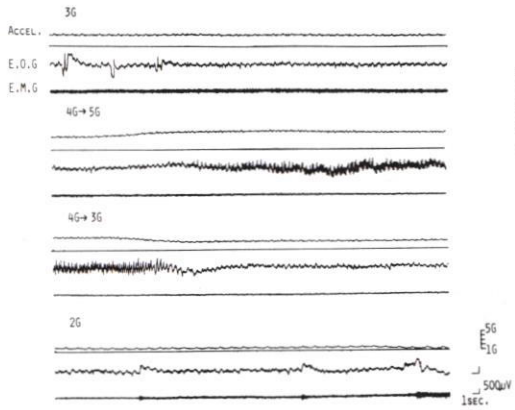

Fig.1 Relations of the EOG and EMG to the G.

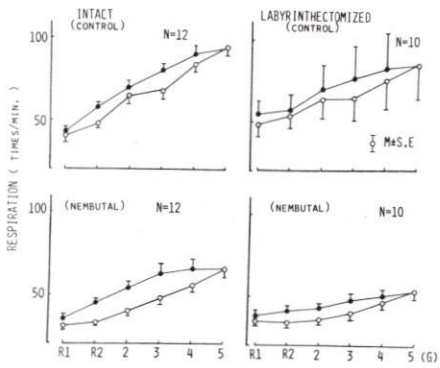

Fig.2 Responses of respiratory rates to the G. The open and closed circles are for the ascending and descending courses of the acceleration.

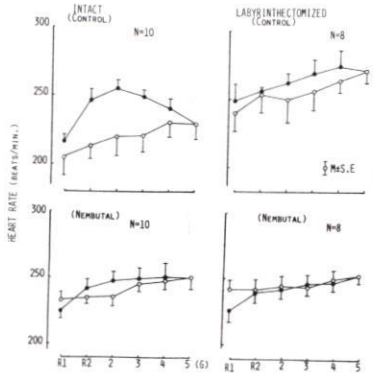

Fig.3 Responses of heart rates to the G. The open and closed circles are for the ascending and descending courses of the acceleration.

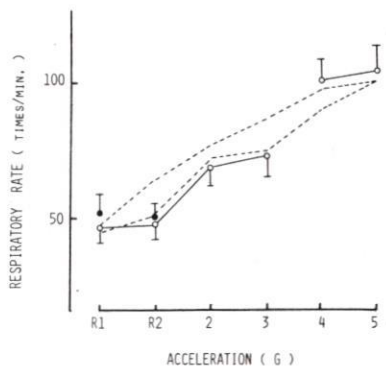

Fig.4

Responses of the respiratory rates during staircase-type and randamly applied trapezoid-type acceleration.
solid line: trapezoid-type, broken line: staircase-type

LABYRINTH PLUGGING AS A MODEL OF SUSPENDED VESTIBULAR SENSORY INPUT

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INTRODUCTION

Experimental locking of the labyrinthine functions serves as a model for studying the vestibular adaptation in normogravity and also gives the possibility to differentiate in the physiological state of animals during orbital flight depending on whether they are controls or previously adapted to reduced or blocked vestibular input.

The current method for the suspension of the vestibular functions - labyrinthectomy - is a rather drastic process with the handicap of the occasional completeness and also the incontrollable extension of the intended abolition.

For example, even being best executed, it is accompanied by a complete acoustic impairment, not negligable particularly in chronic experiments relying on behavioral parameters. Although cross-cutting the branches of the vestibular nerve is much more clear idea, being convenient expecially in acute experiments, but resulting in a zero-level input, instead of the aimed nonresponsive receptor activity.

This problem resembles the development of the experimental procedures for blocking the visual input - it started with enucleation as an undoubtedly complete and standard method - nowadays overcome by the acute and chronic versions of the more physiological type of visual input blocking or deprivation, e.g. by keeping the animals in dark, closing the eyelids or allowing only a patternless glooming light.

As an approach to that idea, applied to the vestibular blockade, a relatively simple, clearly reproducible method, the labyrinth-plugging was elaborated on the rat (Simon - report at XII. Intercosmos Symposion, Krakow, 1979).

It means the gentle filling the vestibulum with a plastic, indifferent mass, aiming to prevent

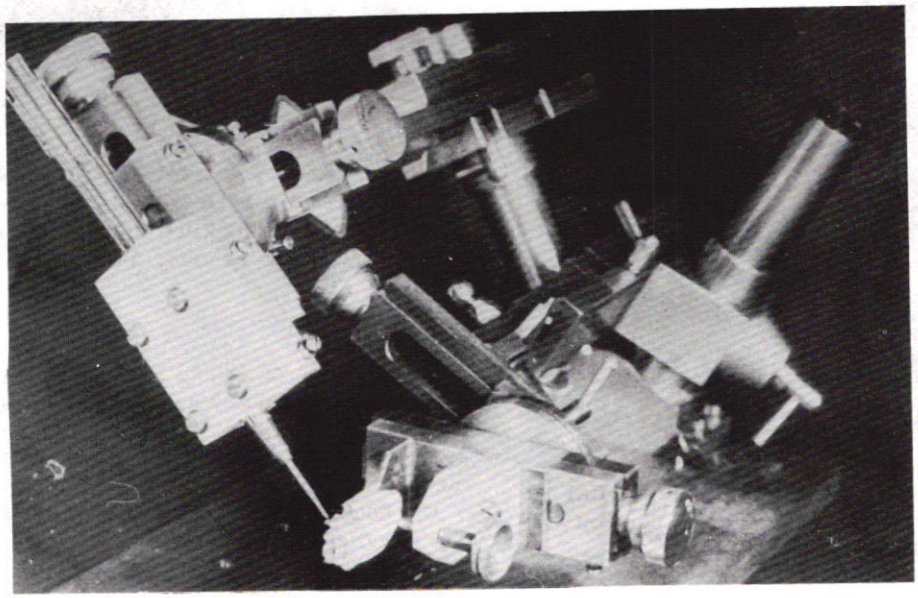

Fig. 1. Stereotaxic apparatus for labyrinth-plugging in the rat. It allows the independent orientation of the skull and the dental drill or the pressure cartridge.

as well the movements of the endolymph in the semicircular ducts, as the deformation of otolithic receptor organs at future accelerational or gravitational stimulation.

METHODS

For the surgical procedure the anaesthetized pretreated animal (35 mg/kg Nembutal, 1 mg/kg Atropin, 200 000 I.U. Penicillin) is kept by an oral type of skull fixation (see Fig.1.), in a position, the head turned on side by 60 degr. A dental drill and later on a filling needle is guided by stereotaxic means at an angle downward by 30 degrees and anterior by 15 degrees from the interauricular axis. After opening by a dental drill of 2 mm in diameter the upper anterior wall of the bulla, the mucosa of the tympanic cavity is pushed down until the upper surface of the promontorium has been cleaned. Now a hole of 0.6 mm in diameter should be made by a fine drill under the control of an operational microscope just in front of the oval window. Than a surgical needle of 0.5 mm is inserted into the hole, and a steril mixture of beeswax and paraffin (7:2, melting point at 50 C^{o}) is pressed in by a simple mechanic pressure-cartridge, the wax forming a plastic thread, which completely and gently fill the entire cavity, pressing also endolymphatic sacks to the faceing wall and closing the openings of the semicircular ducts. During the 3-4 minutes long priod of the filling, a complementary amount of liquid flows out, followed by a wax stream, which indicates the complete plugging of the vestibulum (see Fig. 2.).

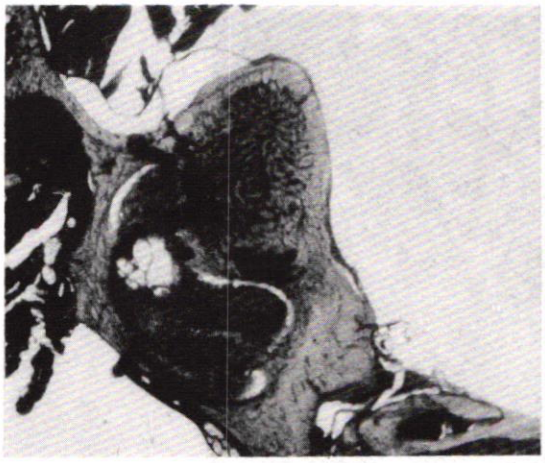

Fig. 2. The plugged left labyrinth at the frontal plane of crus simplex. From a section of the decalcinated skull.

After completing the operation in one side, with awaking the animal, a considerable assimmetry of postural tone is appearing, later on exaggerated into a continuous rolling towards the plogged side. The alert animal can keep posture, but a manage-circling can be observed.

TESTING

For the quantitative evaluation of the resulted assymmetry in the postural and motor behavior, as well as to follow up the time curve of adaptation we use the nystagmography, the biaxial stabilography and the lift reaction (see Fig. 3.).

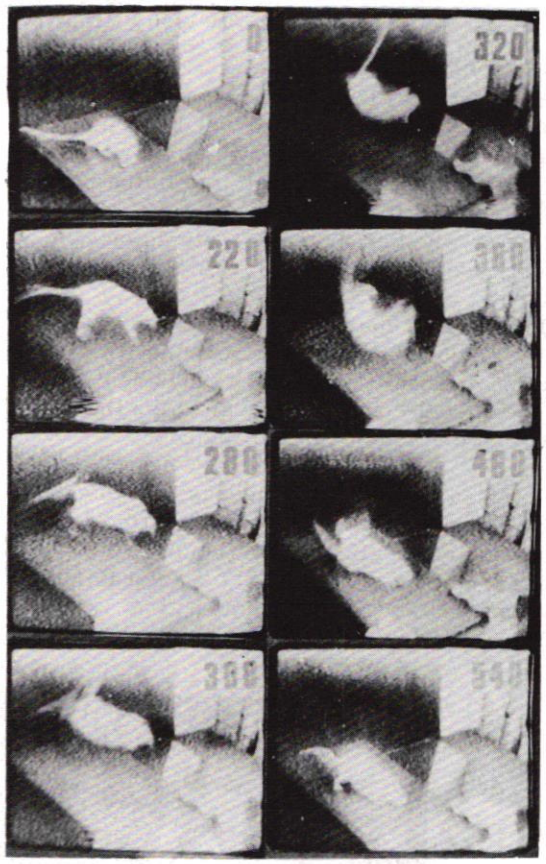

Fig. 3. Preliminary test of lift reaction after labyrint-plugging on the right side. Videotape record. Time scale in msec.

For being proposed to replace the labyrinthectomy, this method should be supported by complete morphological and physiological controls, which prove, that after plugging the vestibulum, besides the partial (bone-conduction-type) maintenance of the hearing the labyrinthine receptor apparatus can survive and deliver its basic activity, modified only by possible efferent control, but not responding to specific stimulation, as angular and linear acceleration, or any change of the gravity vector.

IN VITRO $^{45}Ca^{++}$ UPTAKE AND EXCHANGE BY OTOCONIAL COMPLEXES IN HIGH AND LOW K^+/Na^+ FLUIDS

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Recently, data have been accumulating to indicate that saccular and utricular otoconial complexes of the gravity receptor organs are dynamic and interact constantly with their environment. This study investigates the possibility that the ionic composition of the surrounding fluid influences calcium ion binding and release, and explores the importance of the K^+/Na^+ ratio. Two in vitro methods were developed, the first of which employed artificial endolymph and perilymph while ionically balanced fluids in which only the K^+/Na^+ was altered were used in the second. The ability of rat complexes to take up $^{45}Ca^{++}$ during incubation with these fluids was assessed using liquid scintillation spectrometry. In vitro uptake of $^{45}Ca^{++}$ was greater in fluids with a high K^+/Na^+ ratio than in fluids in which the ratio was low. The ability of the complexes to take up $^{45}Ca^{++}$ appeared to decline with age.

INTRODUCTION

Otoconial complexes of the mammalian saccular and utricular gravity receptors consist of an organic otoconial membrane and a multitude of minute crystals of calcium carbonate in the form of calcite. The crystals (otoconia) add mass to the complexes to increase gravity receptor sensitivity to forces of linear acceleration. Recently, however, evidence has been accumulating to indicate that otoconial complexes are dynamic mineral deposits constantly interacting with their environment. Their mineral stores are often depleted with age and disease, particularly in the saccule (Johnsson, '71; Ross et al.,'76); and complexes of the adult rat take up and exchange $^{45}Ca^{++}$ on a time scale comparable to bone mineral, although to a lesser degree (Ross and Williams, '79). Saccular uptake is the more rapid and greater. These results suggest that the complexes at the two sites are not identical, or that they exist under slightly different environmental conditions.

In order to shed more light on these issues, and to learn more about how calcium binding and release is regulated in the complexes, we have taken up in vitro work in which the ionic environment can be controlled. Endolymph normally has a high K^+/Na^+ ratio. Such an ionic ratio appears to be necessary for calcium binding to occur in other biological systems that sequester Ca^{++}, and a decline in the ratio favors Ca^{++} release (for bone mineral, Canas et al., '69; for heart mitochondria, Carafoli et al. '69; and for squid axoplasm, Baker, '76). In our work we first tried artificial endolymph (high K^+/Na^+) and artificial perilymph (low K^+/Na^+) formulated after Rauch ('64) and Bosher and Warren ('78), then switched to fluids balanced ionically, for reversal of $[K^+]$ and $[Na^+]$.

METHODS

Method 1: Adult male Sprague-Dawley rats (300 g) were decapitated. Saccular and utricular otoconial complexes were dissected under artificial endolymph, AE (1 mM NaCl; 138 mM KCl; 10 mM $KHCO_3$; 1mM $MgCl_2$; 1.5 mM $CaCl_2$), or artificial perilymph, AP (130 mM NaCl; 10 mM $NaHCO_3$; 1mM $NaHPO_4$; 4mM KCl; 1mM $MgCl_2$; 1.5mM $CaCl_2$), both buffered to pH 7.4 with Hepes. Complexes were incubated in 0.5 ml of AE or AP to which $^{45}Ca^{++}$ was added to yield 1000 cpm and agitated in a water bath shaker at 37°C, 50 rev/min for 30 min, beakers covered. Complexes were removed to a millipore filter over a Hirsch funnel for vacuum filtration, 4 pooled/sample, and then washed with 8 µl of "cold" fluid. Otoconia were demineralized in 1 N HCl. Blank filters prepared similarly were used as controls. All samples were prepared for liquid scintillation spectrometry. Results were expressed in counts/min/patch (cpm/pa).

Method 2: Two groups of rats were used: 150-200 g and 300 g. AE and AP were balanced ionically. AE: 1 mM NaCl; 1 mM $MgCl_2$; 1.5 mM $CaCl_2$, 10 mM $KHCO_3$; 138 mM KCl. AP: 138 NaCl, 10 mM $NaHCO_3$; 1 mM KCl; 1 mM $MgCl_2$; 1.5 mM $CaCl_2$. Buffer was omitted. Fluids were gassed with 20% O_2, 30% N_2 and 50% CO_2. This maintained pH at 7.4 for up to 24 hrs. Complexes were incubated in a water bath shaker as above in 0.5 ml of AE or AP to which 1.3×10^{-3} mM $^{45}CaCl_2$ had been added for 5,10, or 15 min, beakers uncovered. Complexes were picked up on preweighed 300 mesh copper grids and excess fluid was removed with wicks. Six samples were pooled, dried, reweighed and vortexed in scintillant in preparation for liquid scintillation spectrometry. Blank grids were used as controls. Total Ca^{++} uptake was calculated as picomoles/microgram dry weight (pMoles/µg).

Scanning electron microscopy: Complexes were examined after incubation in AE or AP for 30 or more min in a JSM U3, JEOL scanning electron microscope.

RESULTS

Method 1: $^{45}Ca^{++}$ uptake was high in AE but virtually absent in AP; some standard deviations (SD) were very high. Means of cpm/pa for (n) experiments were as follows: AE: saccular patches, 417 ±232 (19); utricular patches, 305±245 (18). AP saccular patches, 45±69 (7); utricular patches, 29 ±48 (7). (In AP, some counts were zero).

Method 2: Results of the initial experiments were encouraging but our inability to reduce the data to uptake/unit weight was troubling. Further issues were the use of Hepes buffer, which added

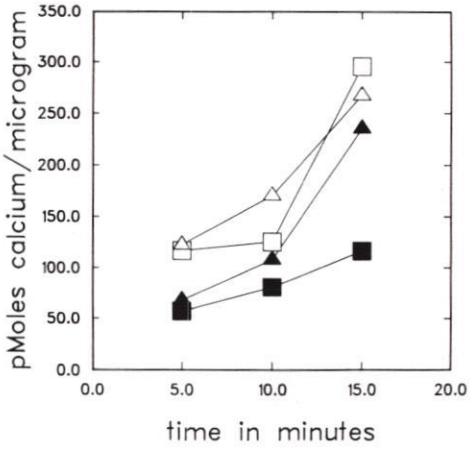

Figure 1. Ca^{++} uptake into otoconial complexes incubated in ionically balanced AE (open symbols) and AP (solid symbols) is shown here. Utricular complexes are represented by squares, saccular by triangles. 150-200 g rats; (n) = 6 at 5 and 10 min, 4 at 15 min.

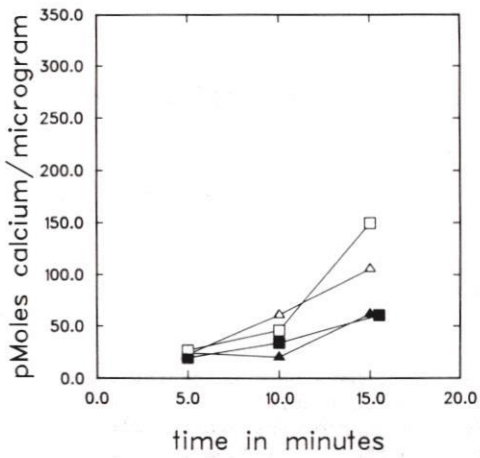

Figure 2. Symbols used are the same as those for Figure 1. 300 g rats, (n) = 2 at each time point.

ions to the system; and the presence of phosphate in AP but not in AE. Use of copper grids, omission of buffer, balancing of ions and gassing with CO_2 resolved these problems and refined the technique.

Under the new experimental conditions the trend for greater Ca^{++} uptake to occur in AE was apparent in 150-200 g rats (Figure 1). SD were lower than before but still high (SD pooled: in AE, utricles ±94, saccules ±137; in AP, utricles ±35, saccules ±90). Uptake increased over time. Levels of Ca^{++} uptake in saccular and utricular complexes were more similar than in the previous experiment.

A few experiments run on 300 g rats are included here for their interest (Figure 2). The trend for higher uptake in AE was present, but total uptake was lower at all time points compared to younger animals (compare Figures 1 and 2).

Scanning electron microscopy: Complexes incubated in fluids used here for 30 or more min showed no obvious demineralization or structural damage.

DISCUSSION

The only prior study of in vitro uptake of $^{45}Ca^{++}$ of which we are aware is an autoradiographic one by Belanger ('53), in which he found uptake to be greater in vitro than in vivo. Our results are in agreement on this point (see Ross, '79).

It would be unwise at this time to put undue emphasis on our finding that, with either in vitro method, calcium uptake and/or exchange (the two cannot be differentiated here) is favored when the K^+/Na^+ ratio is high, but seems to be inhibited when that ratio is low. Our experiments require further documentation and procedural refinements. Nevertheless, the trend is apparent. It is noteworthy, also, that Ca^{++} uptake was higher in the younger animals for the times investigated.

What is not readily apparent from the data presented is that, based upon mathematical calculations using the activity coefficients of all the ions in the fluids, pure calcite would have gone into solution. This means that the organic fraction of the otoconial complexes protects the crystals against demineralization. The organic material likely plays a role also in calcium binding and release because of the rapidity of both in vitro and in vivo $^{45}Ca^{++}$ uptake. The next important question to resolve would appear to be whether or not Na^+ competes for the calcium binding sites.

REFERENCES

1. Baker, P.F. 1976. The regulation of intracellular calcium. In Calcium in Biological Systems, Duncan, C.J. (ed.). Cambridge University Press, Cambridge, pp. 67-88.
2. Belanger, L.F. 1953. Autoradiographic visualization of in vitro exchange in teeth, bones, and other tissues, under various conditions. J. Dent. Res. 32: 168-176.
3. Bosher, S.K. and R. L. Warren. 1978. Very low calcium content of cochlear endolymph, an extracellular fluid. Nature 273: 377-378.
4. Canas, F., Terepka, A.R. and W.F. Neuman. 1969. Potassium and milieu interior of bone. Amer. J. Physiol. 271: 117-120.
5. Carafoli, E., Tiozzo, R., Lugli, G., Crovetti, F. and Kratzing. C. 1974. The release of Ca^{++} from heart mitochondria by sodium. J. molec. cell. Cardiol. 6: 361-371.
6. Johnsson, L.G. 1971. Degenerative changes and anomalies of the vestibular system in man. Laryngoscope 81: 1682-1694.
7. Rauch, S. 1964. Biochemie des Hörorgans. Georg Thieme Verlag, Stuttgart.
8. Ross, M.D., Peacor, D., Johnsson, L.G., and Allard, L.F. 1976. Observations on normal and degenerating human otoconia. Ann. Otol. 85: 310-326.
9. Ross, M.D. 1979. Calcium ion uptake and exchange in otoconia. Adv. Oto-Rhino-Laryng. 25: 26-33.
10. Ross, M.D. and Williams, T.J. 1979. Otoconial complexes as ion reservoirs in endolymph. The Physiologist 22(6): S63-S64.

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SPACE FLIGHT EFFECTS UPON PLASMA AND TISSUE LIPIDS IN RATS

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Plasma and tissue lipids were determined in rats flown in average 20 days aboard four /690,782,936,1129/ Cosmos biosatellites. Immediately after landing there was an increase in lipomobilization, in plasma lipids, in liver, thymus and bone marrow triglycerides, The creation and use of artificial gravity /Cosmos 936/ prevented the appearance of "fatty liver", the increase of phospholipids in serum and the accumulation of triglycerides in bone marrow. The additional stress /repeated short-termed immobilization/ on days 1-6 postflight acted more profoundly on space flight group, as compared with animals from terrestrial modelling experiment or with intact control ones. On day 25 postflight all changes disappeared but accumulation of bone marrow triglycerides persisted, irregulary also with some indices of increased lipomobilization.

INTRODUCTION

The scope of experiments with laboratory animals aboard space vehicles is to obtain data on living system adaptation to zero gravity situation, follow the sequelae of some extra-ordinary stimuli /e.g. irradiation/ and demonstrate changes in various tissues /whole organs/. Preliminary experiment with rats aboard the Cosmos 605 biosatellite was followed by next four ones /Cosmos 690, 782, 936, 1129/ in 1974-1979. Several problems were investigated in this program, namely the effect of irradiation, simulating large solar eruptions /Cosmos 690/, the effect of individual space flight factors, especially that of weightlessness /Cosmos 782/; artificial gravity was created by the use of a centrifuge aboard the Cosmos 936 biosatellite; the problem of additional stress action in different groups of animals was studied /Cosmos 1129/.

This short report summarizes data on lipid analyses of plasma and tissues in rats, flown aboard the Cosmos biosatellites.

MATERIALS AND METHODS

Male Wistar rats /200-300 g/ were placed in special boxes aboard the Cosmos biosatellites. Space flight lasted in average 20 days; a part of animals was analyzed several hours after landing, the next one 6 days /only Cosmos 1129/ or 25 days postflight. On day 11 a group of animals was irradiated aboard the Cosmos 690 biosatellite 24 h from 137-Cs source with total dose of 2.2 and 8.0 Gy of gamma rays, one group was placed on centrifuge aboard the Cosmos 936 biosatellite and subjected to gravity of approximately 1 G; rats from the Cosmos 1129 biosatellite were analyzed after an additional postflight stress /repeated 2.5 h restraint/ on day 6 after landing. To each space an terrestrial experiment was build, modelling all available space flight factors. Intact animals served as controls.

Intact, model and flight animals without any other influence were analyzed. In plasma, liver, white /epididymal/ and brown /interscapular/ adipose tissue nonesterified fatty acids /NEFA/ according Dole, Meinertz /1960/, in plasma, liver, thymus and bone marrow triglycerides /TG/ according Eggstein, Kreutz /1966/ and phospholipids /PL/ according Bartlett /1959/, in plasma and liver total cholesterol /CH/ according Zlatkis et al./1953/ were determined.

RESULTS

The results are presented as summarizing data.

NEFA in plasma, adipose tissue and liver/ plasma lipids

Immediately after landing NEFA values were elevated especially in plasma and brown adipose tissue; the use of artificial gravity did not essentially change the situation. 6 days postflight there were normal or slightly elevated values, which rose after the additional stress. At the end of the experiment normal or /irregulary/ slightly elevated values were found.

Plasma lipids were elevated after landing, the use of artificial gravity inhibited the increase of PL and depressed the TG concentration. Normal values were found at the end of the experimental period.

Liver lipids

An elevation of TG and CH concentration was observed immediately after landing; TG increase was inhibited by the creation of artificial gravity. Only minimal changes were seen at day 6 postflight and no changes at the end of the experiment.

Thymus lipids

An increase of TG occured as a rule after landing, disappeared at day 6 postflight and the repeated immobilization increased its level only insignificant. At the end of the observation period lipids in the thymus were unchanged as compared with intact animal values.

Bone marrow lipids

Gross accumulation of TG occured as analyzed several hours after landing, complete inhibition of these changes was noted in rats subjected to artificial gravity aboard the Cosmos 936 biosatellite. Additional stress /immobilization/ even markedly enhanced TH increase and these patterns remained to the end of the observation period.

The changes in irradiated rats from the Cosmos 690 biosatellite were described in details elswhere /Ahlers et al. 1976/.

The extent of changes in modelling terrestrial experiments was substantially smaller and non-regular.

DISCUSSION

Some of tissue lipid changes registrated by first-step analysis may be accepted as a result of acute stress, resulting from the complex of landing manoeuvre /Ahlers et al. 1976, 1980/. This is the case of increased lipomobilization, with corresponding increase of plasma and liver TG. The patterns of postflight "fatty liver" probably of multifactorial origin could be prevented by use of artificial gravity. The nature of postflight hyperlipemia is still unclear, namely the explication of cholesterol and phospholipid elevation. Involution of thymus with increased TG concentration is a well-known effect of acute stress or glucocorticoid administration and was found as a regular change soon after landing of rats from all biosatellites. All changes attributed to acute stress changes disappeared on day 6 postflight. Of great interest and significance are changes of lipid composition in bone marrow /femur, humerus/, caused primarily by weightlessness, or by combination of weightlessness and immobilization of rats. Decreased blood and oxygen supply to bone marrow in zero gravity situation may favour decrease in fatty acid oxidation and enhancement of new adipocyte formation, which all resulted in gross accumulation of TG. The use of artificial gravity inhibited completely this phenomenon. Repeated immobilization on days 1-6 postflight accentuated lipid changes namely in flight animals, as compared with rats from terrestrial /model/ experiment and with intact animals. All changes disappeared on day 25 postflight, with exception of TG accumulation in bone marrow.

REFERENCES

1. Ahlers, I., E. Mišúrová, M. Praslička, R. A. Tigranyan, 1976. Biochemical changes in rats flown on board the Cosmos 690 biosatellite. Life Sciences and Space Research P.H.A. Sneath, Ed., Akademie-Verlag Berlin, XIV:185-188.
2. Ahlers, I., R. A. Tigranyan, E. Ahlersová, E. Paulíková, M. Praslička, 1980. Plasma and tissue lipids in rats after long--termed space flight /in Russian/. Kosm. biol. aviakosm. med. 14:76-78.
3. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234:466-468.
4. Dole, V. P., H. Meinertz. 1960. Microdetermination of long-chain fatty acids in plasma and tissues. J. Biol. Chem. 235:2595-2599.
5. Eggstein, M., F. H. Kreutz. 1966. Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe. Klin. Wschr. 44:262-273.
6. Zlatkis, A., B. Zak., A. J. Boyle. 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41:486-490.

EFFECTS OF SPACE FLIGHT FACTORS AND ARTIFICIAL GRAVITY ON DEOXYRIBONUCLEOPROTEIN IN LYMPHOID ORGANS OF RATS

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Changes in the deoxyribonucleoprotein /DNP/ and nucleic acids in the spleen and thymus of rats after approximately 20 day flight onboard the biosatellites Cosmos 782, 936 and 1129 have been studied. The changes in DNP were evaluated on the basis of soluble polydeoxyribonucleotide level /e.i. fraction of oligonucleosomes/, which increases in the course of DNP breakdown. Several hours after landing the indication of partial DNP breakdown in spleen and thymus was found and the content of nucleic acids was reduced to about 60 %. Artificial gravity onboard the biosatellite apears to have a positive effect namely on the thymus. Partial recovery of the changes induced by factors of cosmic flight took place during the first 7 days after landing. Stress from repeated immobilization during this period of readaptation resulted in more profound changes as compared with those in model experiment. Majority of changes recovered till the 26-th day.

INTRODUCTION

Deoxyribonucleoprotein /DNP/ breakdown-evoked in lymphoid and haematopoietic tissues by some physical and chemical agents-might be detected on the basis of polydeoxyribonucleotide level increase, occuring several hours after treatment, with the maximum at the 4th-8th hour /3, 5/. The increase in the polydeoxyribonucleotide level is associated with pyknosis of sensitive cells and is accompanied by the decrease in the content of both DNP and nucleic acids in organs.

In this paper we present the partial rewiev from our results obtained on rats from three biosatellites in which the influence of space flight factors and of artificial gravity have been studied.

METHODS

The experiments have been carried out with rats of the Wistar strain, exposed during their approximately 20 day flight onboard the Cosmos /C-/ biosatellites to:

1/ the influence of space flight factors including weightlessness /C-782, C-936, C-1129/ - group F
2/ or artificial gravity of 1g /C-936/ - group FG

The rats from the C-782 and C-936 biosatellites were examined several hours after landing and after 25 days of readaptation. In rats from the biosatellite C-1129 the first examination was carried out approximately in the same time as in former ones, but the second examination was already done after 6 days of readaptation. Some of these rats were subjected to five times repeated immobilization stress - group FS.

Results were compared with the values in control rats from vivarium /group C, eventually CS i.e. control+stress/ and those from model experiments in which conditions of space flight were simulated with the exception of weightlessness /group M/ or with the addition of gravity +1g /group MG/ or immobilization stress /group MS/.

Deoxyribonucleoprotein changes were evaluated on the basis of the polydeoxyribonucleotide level and amount of DNA /bound in DNP complex/ by the method elaborated by Cole and Ellis /1/. RNA amount was estimated by the method of Schmidt, Thannhauser modified by Tsanev and Markov /4/.

RESULTS

The findings in rats exposed to weightlessness /gr.F/ onboard of all three biosatellites were similar, we will, therefore, demonstrate only our results on rats from C-936 and partially C-1129 biosatellites.

The level of polydeoxyribonucleotides in the spleen of group F was increased as compared with groups C and M more than two times several hours after the landing /Fig. 1/. The artificial gravity /gr.FG/ did not prevent from the DNP breakdown in the spleen as the polydeoxyribonucleotide level was increased in the same extent as in the group F. This change was evoked also by additional gravity of + 1g /gr.MG/. In following intervals the level of polydeoxyribonucleotides in these groups was within the range of control values. Immobilization stress, however, caused the increase in polydeoxyri-

bonucleotide level in flight rats /gr.FS/ only.

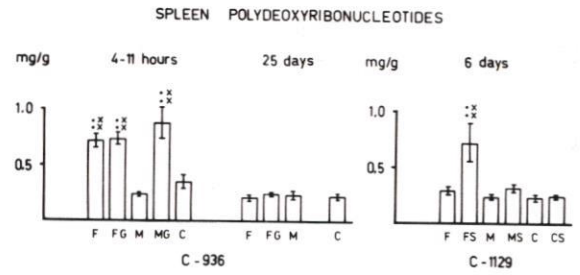

Fig.1. Polydeoxyribonucleotide level in spleen

DNA content was decreased at first examination in rats of both flight groups and model experiments /Fig.2/. Fast recovery took place during the first 6 days. Immobilization resulted in decreased DNA content in flight rats /gr.FS/ only.

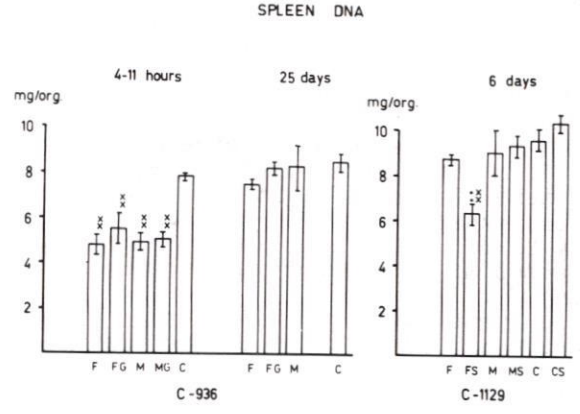

Fig.2. DNA content in spleen

The changes of RNA content at first examination were more moderate than those of DNA; the RNA content decrease was significant only in the group F in that time /Fig.3/.

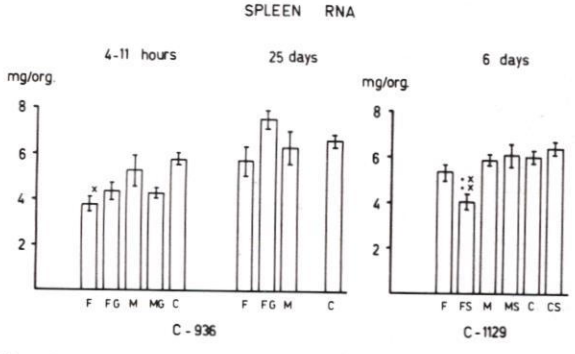

Fig.3. RNA content in spleen

The changes in thymus were similar to those in spleen with some exceptions: 1/ at first examination the polydeoxyribonucleotide level was increased in flight rats /gr.F/ only, 2/ repeated immobilization stress resulted in polydeoxyribonucleotide level increase and nucleic acid content decrease in all three groups /FS, MS, CS/.

DISCUSSION

The increase in the polydeoxyribonucleotide level in flight rats several hours after landing gives evidence of the DNP breakdown in the sensitive cells which took place during the landing. This finding was discussed in details elsewhere /2/. The increase in the polydeoxyribonucleotide level at the first examination indicates, moreover, that the factors of the cosmic flight /including the weightlessness/did not evoke substantial changes in DNP as tissues after more extensive injury loose temporarily their ability to react this way on the next treatment.

The artificial gravity prevented DNP from breakdown in thymus but not in spleen indicating the mechanism of this injury may not be the same in both organs. As predominately the DNP of lymphocytes and of immature erythroid cells is the source of polydeoxyribonucleotides and the DNP breakdown can also be evoked by glucocorticoids /5/, we presume, the artificial gravity prevented DNP from breakdown mediated mainly by glucocorticoids, what is more expressed in thymus.

The changes were in general recovered during 25 days. The quick recovery took place already during the first 6 days, in flight rats, however, the reaction to immobilization stress was more pronounced than those in rats from model experiment or control ones.

REFERENCES

1. Cole, L.J., Ellis, M.E. 1957. Radiat.Res. 7:508-517
2. Mišurová, E., Tigranyan, R.A., Kropáčová, K., Prasličkа, M. 1979. Kosm. biol. med. 13:32-35
3. Skalka, M., Matyášová, J., Chlumecká, V. 1965. Folia biol./Praha/ 11: 113-122
4. Tsanev, R.G., Markov, G.G. 1960. Biokhimiya. 25:151-159
5. Yermolayeva, N.V., Vodolazskaya, N.A. 1970. Biokhimiya. 35:1039-1045

INITIATION OF NUTATION IN SUNFLOWER HYPOCOTYLS

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INTRODUCTION-Circumnutation, when first studied intensively by Charles and Francis Darwin [1], was thought to be regulated by some kind of oscillating mechanism within the plant. Later the driving force was postulated to lie outside the plant. A biophysical model developed by Anders Johnsson and coworkers simulates circumnutation of the sunflower hypocotyl and for the most part it agrees well with experimental observations. The theory by which the model was derived "was based on the existence of a time lag between the gravitational stimulation and the corresponding response, i.e., the curvature" [2]. The initial oscillation cycle identifiable as a very young seedling's first circumnutation should be proceeded, in theory, by only small "spontaneous curvatures" as they were referred to by Larsen [3] and which seem adequately described as a "random walk process" [4]. Until displacement of the shoot away from the vertical becomes large enough and sufficiently prolonged to create an adequate gravitropic response (with overshoot leading to a sustained oscillation) circumnutation could not occur.

For a rough estimate of the conditions of shoot curvature that would constitute an adequate gravitropic stimulus we may take for granted the Reciprocity Rule and define as K the smallest $g \times t$ product needed to induce a detectable gravitropic response. K has been measured by various workers using the same or different plant materials [5,6, 7,8]. A representative value is about 300 g sec. We assumed the g-stimulus intensity is given by the expression, $g \sin \alpha$, where α is the displacement angle from the plumb line. We then calculate T, defined as the minimally adequate stimulus duration where $T = K/\sin \alpha$.

As an example, for K = 300 g sec the shoot would have to be curved away from the plumb line by 10° for nearly a half-hour in order for it to accumulate an adequate stimulation that could initiate circumnutation. Such values of angular displacement and duration are large enough to be observed easily. If, prior to the onset of nutating activity, we could see random walk type movements by the epicotyl which in time grow large enough to elicit a gravitropic response with overshoot, then we should interpret this as additional support for the validity of Johnsson's model for circumnutation. We attempted in three different ways to obtain such confirmation.

EXPERIMENTAL-All observations were made by IR sensitive video cameras operating in time lapse mode. One frame was recorded every 10 min. Each camera viewed one plant from above (morphologically). Illumination was from an array of IR emitting diodes which produced a narrow band centered at 890 nm. Test plant material was the dwarf sunflower, Helianthus annuus L., cv. Teddy Bear.

Our initial attempt was to observe young seedlings as the shoots emerged from the soil. Data were collected on 5 plants. In each case we were unable to detect the time of onset of nutation. It appeared that the oscillating growth movements began even before the epicotyl (covered by the seed coat) had emerged from the soil. We discontinued using this method.

Our second method was to begin with older seedlings that were rotating on horizontal clinostats. Although it had been reported [9] that clinostatting abolished nutational oscillations in the sunflower hypocotyl, our own observations [10] showed that this was not always the case. Under socalled gravity compensation on the clinostat we found that a plant's nutational cycles were much reduced but on average they persisted at about 20% of the normal excursion and at an increased oscillation frequency. However, some plants on the clinostat would exhibit little or no circumnutation. Often the oscillations would cease for a time and then be resumed. Sometimes a plant would show no identifiable cyclic activity for many hours. Although we could not identify such cases in advance we could do so after analysis of the video records. By turning the clinostat axis to the vertical position we would impose a 1 g force in the normal (axial) direction as the plant rotated now vertically. After studying data from 13 plants handled in that way we found that circumnutation always began fairly soon after the plants were moved into an upright position. We were unable to observe large random walk type excursions of the tops of the plants as a prelude to the onset of circumnutation.

Although the method just described yielded acceptable data we preferred for several reasons to expend most of our effort on a third method of applying the axially directed g-stimulus that seemed to be responsible for initiating oscillatory behavior. We started with clinostatted plants but, instead of turning them upright, we applied a 1 g centripetal force in the axial direction while clinostatting continued. The clinostats were mounted horizontally on the arm of a centrifuge oriented so as to align each plant-clinostat axis along a radius of the centrifuge. Each test plant was observed during clinostatting with the centrifuge at rest for about 5 hr; then by starting up the centrifuge a 1 g force was applied (centrip-

etal) in line with the plant's morphological axis. Uninterrupted time lapse observations continued for an additional 18 hr. When the video data were analyzed we found that out of 42 test plants 17 had shown no cyclic oscillations during the 5 hr prior to the beginning of centrifugation. In each of those 17 cases we observed that clearly distinquishable circumnutation began after the 1 g, axially directed, centripetal force had been applied. There had been random walk movements but not of large excursion and nothing that could be confidently identified as a nutational cycle until the centrifuge was started. After that the initiation of the first nutational cycle was seen on average at 29 ± 5 min (Fig. 1). Thereafter, until about the fifth cycle, the nutational amplitude increased (Fig. 2) progressively about 80%, the oscillation frequency decreased by about 80%, (Fig. 3) and the ellipticity, (long axis of the nutational ellipse)/(short axis), decreased also by about 80% (Fig. 4). Thus it required at least 4 or 5 cycles for an average seedling to establish a more or less constant pattern of circumnutational oscillation but the initiation of the cyclic motion, at least in clinostatted plants, did occur without it being preceeded by very large, wandering, growth movements. Since there was no statistically significant difference between the average behavior of those 17 plants that had not nutated prior to the start of centrifugation and of 25 that had done so, the data from both groups were combined. All data plotted in figures 1-4 represent results from 42 plants.

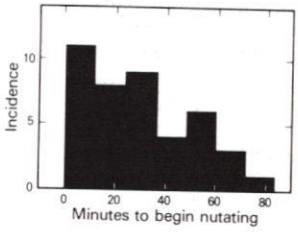

Fig. 1 Incidence of initiation of nutational oscillation at various times after imposition of axial force of 1 g.

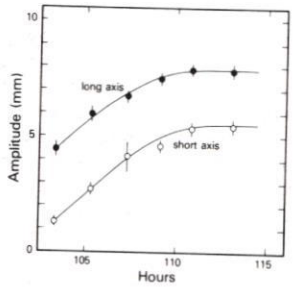

Fig. 2 Growth in size of nutational ellipse (seen from above). Ordinate, amplitude in mm. Abscissa, mean age of seedlings. Centripetal force of 1 g imposed at 102 hr.

CONCLUSION-Our observations are not decisively incompatible with the model of circumnutation as a gravitropic hunting process. They are, nevertheless, more easily reconciled with the unfortunately less definitive concept of an endogenous oscillator which can initiate nutational motion when triggered by an axially imposed g-force without a significant prelude in the form of patently inductive wandering growth movements.

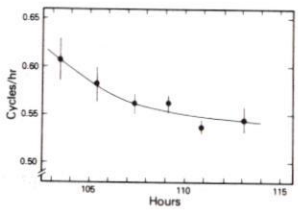

Fig. 3 Frequency of nutational oscillation subsequent to initiation by application of axial force of 1 g at 102 hr. Abscissa, mean seedling age for six successive cycles.

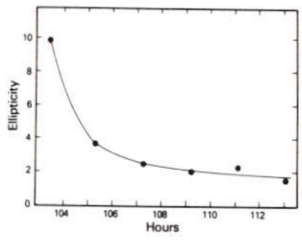

Fig. 4 Ellipticity of nutational cycle as viewed from above the seedlings. Ordinate, ratio of long axis amplitude to short axis amplitude. Abscissa: mean seedling age at successive cycles after oscillations began.

REFERENCES

1. Darwin CR 1880 The Power of Movement in Plants. John Murray, London
2. Johnsson A 1968 Introduction of noise in the gravity compensating system in Helianthus annuus. Studia Biophysica, Berlin 11:149-154
3. Larsen P 1957 The development of geotropic and spontaneous curvatures in roots. Physiol Plantarum 10:127-163
4. Johnsson A 1966 Spontaneous movements in plants studied as a random walk process. Physiol Plantarum 19:1125-1137
5. Rutten-Pekelharing CJ 1910 Untersuchungen über die Perzeption des Schwerkraftreizes. Rec Trav bot Neerl 7:241-335
6. Shen-Miller J, R Hinchman, SA Gordon 1968 Thresholds for georesponse to acceleration in gravity-compensated Avena seedlings. Plant Physiol 43:338-344
7. Shen-Miller J 1970 Reciprocity in the activation of geotropism in oat coleoptiles grown on clinostats. Planta 92:152-163
8. Johnsson A 1965 Investigation of the reciprocity rule by means of geotropic and geoelectric measurements. Physiol Plantarum 18:945-967
9. Israelsson D and A Johnsson 1967 A theory for circumnutation in Helianthus annuus. Physiol Plantarum 20:957-976
10. Chapman DK and AH Brown 1979 Residual nutational activity of the sunflower hypocoytl in simulated weightlessness. Plant Cell Physiol 20:473-478

CHANGES OF PERIODIC PROTOPLASMIC MOVEMENTS ON THE FAST CLINOSTAT

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Abstract

The question of cell differentiation is still open. For example, calculations of Oosava and Asakura demonstrate the necessity of an additional cybernetic principle and an input of energy for the thermodynamic self-assembly of G-actin into larger molecules. This indicates that the macromolecules in question are both primarily structures in the light microscopic range and that they are influenced by gravity, this last point being demonstrated by calculations of Schatz and Silver and by direct observation. As a thesis, cybernetic mechanisms for synthesis of the larger cell structures have to compensate gravity. Simulating O-g using a fast rotating clinostat microscope, one may recognize disturbed or changed differentiations or movements. - The cytoplasmic shuttle stream within the plasmodial veins of a slime mold or the translational movements of a plant cell nucleus are systems with slow periodic movements ideally suited to initial testing. - Certain variations in the character of the shuttle streaming of Physarum polycephalum and of the translational activity of nuclei in Elodea canadensis cells have been found.

Introduction

During daily jogging my body is subjected for several minutes to increasing g and weightlessness. If there was no counterregulation within the cells of our body, the order of the different parts of cells would be disintegrated. Within a horizontal microscope, using turns of 180 degrees we could demonstrate the influence of normal gravity on heavy free particles (organic crystals) within a living cell [1] . It could be shown that the threshold of interference of gravity on the behavior (sedimentation) of such particles in respect to their diameters is on the order of 0.1 to 0.5 μm. This threshold is caused by Brownian motion which is an absolut barrier for effective sedimentation in certain systems. The observed threshold of sedimentation for organic crystals (calcium oxalate) is in agreement with calculations of Schatz and Silver [5,6]. Yet, density differences between common cell organelles and the hyalo-plasma are far lower and, along with another parameter which is involved, namely viscosity of hyalo-plasma, are difficult to establish. Therefore we can only suppose that gravity influences essential cell functions; especially the assembly of all larger structures and their active transport within the cell. There is no doubt that besides regulation of the first order, that of the second order, namely feedback regulation, also takes place in the cell. This field lacks information in respect to the rated valuedonors and their changeability. For example Oosawa and Asakura [4] established the necessity of a still unknown special regulation for the assembly of larger actin or myosin molecules (polymeres). They found that with increasing size of the polymeres, the difference of free energy is no longer sufficient for spontaneous polymerisation (self assembly). It is this step of cell differentiation where a hypothetical compensation of the sedimentation forces should take place. Besides a rated value donor, such a system would need an acceleration receptor system too. Depending on the adaptability of a topologic rated value donor under weightlessness, the behavior or even morphology of the cell should be transiently or constantly changed. Real-time observation of rhythmic movements within cells under weightlessness should give first hints about the existence of such a mechanism.

Small specimens of single cells are ideally apted for testing on a fast clinostat, a platform which rotates relatively fast about a horizontal axis. Under favorable conditions particles within a rotating suspension on a fast clinostat show no relative movement; remaining movements add to the Brownian motion; that means a functional zero g state is achieved [2]. We constructed a clinostat microscope; with it we looked for changes of periodic innercell movements of two different organisms.

Materials and Methods

Test organisms were leaf-cells of the water plant Elodea canadensis and the slime mold Physarum polycephalum. Both were observed in the submersed condition between an object slide and a cover slide (microchamber). For the technique of preparation of the Elodea leaves see [2]. About 0.5μ l of a piece of a young "macroplasmodium" of the slime mold was inoculated on a thin film of agar on a cover glass. Some hours later, the newly differentiated plasmodium is included "head-down" in water on a concaved object slide. Because of linear extension (in this case 2 - 5 mm) of the "veins" of the slime mold, forced protoplasmic streaming within the veins has to be avoided. It is induced by hydrostatic pressure and by the resulting deformation of the cover glass in inproperly arranged microchambers, during vertical positioning in the turning clinostat. The clinostat consitsts from a horizontal microscope rotatable about its optical axis. (see also [2]).

The measured parameters were the speed of motion of the nucleus within cells of Elodea, and the half-period length of the "shuttle stream" of the endoplasma in veins of Physarum; in addition, quantitative judgement of the speed maxima during each half-period of the shuttle stream was made.

Results

In an earlier paper we made a partial analysis of the speed of motion of the nucleus in three Elodea cells, each

standing and being clinostated (100 RPM) alternately for four days [2].

Table 1. Significance-test for difference of motion of the nucleus of standing and clinostated Elodea cells

		period length in hours	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
standing	cell III A			98.2*						98.3					91.3		95.1					
	cell II B					95.5	98.6												90.1		98.5	
	cell I B										90.2											
rotating	cell I A		94.2							96.7			96.7					98.8				
	cell II A							99.2													92.0	100
	cell III B											92.8								90.7		

* significance of frequencies in % by double-variance analysis

From this experiment we made a frequency analysis (see tab. 1) which emphasizes the significance of individual differences of behavior during the two environmental conditions only; none the less, the optic impression of the summation of the Fourier power spectra of the three measurements gave a general difference [2]. A similar result was obtained from Physarum; no systematic variance could be detected. We did establish a list of variations of the protoplasmic shuttle streaming of Physarum; one or several of these points are always valid during clinostating. The following may happen after onset of clinostating:

- o Lengthening of the period of shuttle stream (see fig. 1,);
- o induction, reduction, twisting or even oscillation of "asymmetric" half-period lengths (see fig. 1,2);
- o increase of resting times of the shuttlestream (see fig. 2);
- o increase of speed of the stream up to half an hour (only short duration but marked increase in fig. 1).

Discussion

Assuming the effects are really attributed to weightlessness, then different physiological states of the tested systems react in a different way. In future it will be necessary to define such states to get reproducible results. In the case of Physarum we see the first indications for this. We assume the amount of change of periodic length to be correlated with the degree of the detrimental effect of enclosuring the plasmodium into the microchamber; and this seems to depend on the state of differentiation of the plasmodium before enclosure.

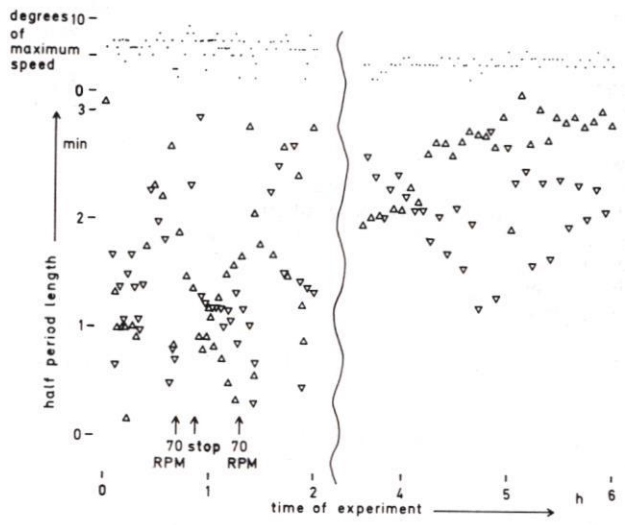

Figure 1. Speed maximum and halfperiod length of the shuttle stream of Physarum polycephalum on the clinostat; triangles indicate direction of protoplasm stream; temperature 20.5 (± 0.3)°C.

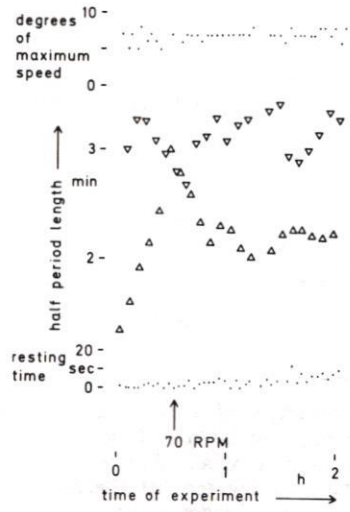

Figure 2 as fig. 1. Temperature 16.0 (± 0.3)°C; in addition resting time of the shuttle stream.

Furthermore, the results are compatible with a general g-sensitivity of the cell and can be interpreted as an adaptation process of a rated value donor which cybernates the cell matrix. Two different reactions of Physarum are of special interest: 1) The temporary increase of speed of protoplasmic motion (see also[2]) which, if no O-g effect, may be attributed to the in part pressure regulated shuttle mechanism [7] being influenced by hydrostatic side effects. 2) In contrast, the often lasting induction of asymmetric half-period lengths may be more directly attributed to growth differentiation of the ectoplasm. - General g-sensitivity of cells may be intrinsic for life. We would propose that during ontogeny of vertebrates (amphibia) an active gravity orientation takes place even before the vestibulum comes to function[3].

Moreover the changes of period lenghts, especially in Elodea, indicate correlations between endogenous clocks, especially those of circadian nature, and gravity (discussion see [2]).

REFERENCES

1. Briegleb, W., Neubert, J., Schatz, A., 1973. DGLRM-Prep.: XXI. Int. Congr. Aviat. and Space Med., München 1973: 113-114.
2. Briegleb, W., Schatz, A., Neubert, J. 1977. J. interdiscipl. Cycle Res. 8: 387-391.
3. Neubert, J., Briegleb, W., 1977. ESA SP-130: 165-168.
4. Oosawa, F., Asakura, S. 1975. Thermodynamics of the Polymerization of Protein, London: Academic Press.
5. Schatz, A., Briegleb, W., Neubert, J. 1973. DGLRM-Prep.: XXI. Int. Congr. Aviat. and Space Med., München 1973: 111-112.
6. Silver, I.L. 1976. J. theor. Biol. 61: 353-362.
7. Wohlfarth-Bottermann, K.E. 1979. J. exp. Biol. 81: 15-32.

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SKIN TEMPERATURE AND THERMAL COMFORT IN WEIGHTLESSNESS

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INTRODUCTION

A variety of articles were devoted to the questions of treating microclimate in cosmic objects (1, 2), the latest knowledge in this field was reviewed by Webb (6). As yet no systematic experimental studies have been done, however, dealing with the influence of weightlessness on the very process of heat transfer between the human body and outer environment.

In the previous work Novák (3) presented the possibility of experimental testing of the effect of weightlessness on the changes in heat output of a homoiothermic system by means of an Electrical Dynamic Katathermometer (EDK). The experimental proof aboard the biosatellite Kosmos 936 that the weightlessness interfers with the heat output of the warm body (5) indicated the importance of this phenomenon for the physiology of heat balance and man´s thermal comfort in the weightless state.

The objective in the present experiment Heat exchange-2 was to verify the principle and reliability of the developed method for the assesement of man´s thermal comfort in weightlessness.

METHODS

The principle of the method used is a simultaneous measurement of the microclimate cooling effect by means of the EDK sensor (in Wm^{-2}) together with the skin temperature (in ^{o}C) measured by thermistors with a constant pressure on the skin surface. At once with this objective data about the thermal conditions of the body and its surroundings the subjective feeling of thermal comfort of the cosmonaut was recorded. All instrumentation needed for the experiment was concentrated in one instrument Electric Dynamic Katathermometer with a Skin Thermometer (EDK-IK) as illustrated in (Fig. 1).

The experiments were done on board of the soviet orbital laboratory Salyut-6 within the framework of the Interkosmos international programme of scientific cooperation by the cosmonauts V. Remek (ČSSR), A. Gubarev (USSR) and M. Hermaszewski (PRL), P. Klimuk (USSR), the members

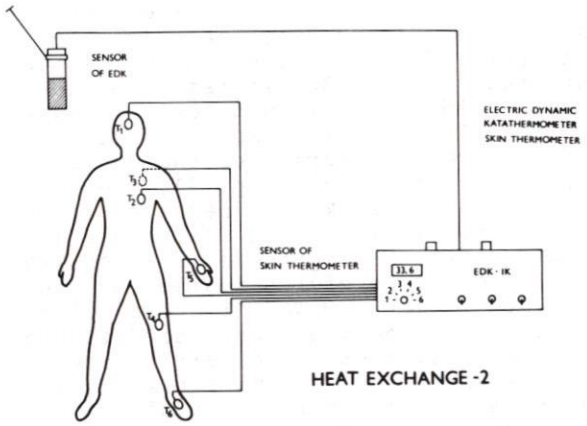

Fig. 1. Scheme of measurement of the skin temperature and the cooling power of the microclimate.

of the Interkosmos crews. In each expedition the experiment was acomplished in three independent series on the fifth day of the mission. Control measurements were performed in the laboratory on Earth before and after the mission.

RESULT AND DISCUSSION

As demonstrated in (Fig. 2) the skin temperature measured by the cosmonaut V. Remek exhibits significant differences

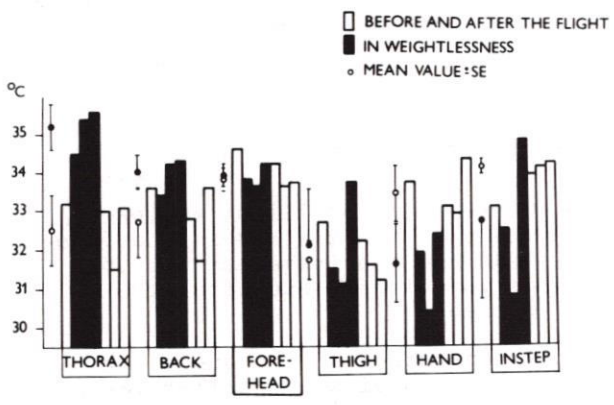

Fig. 2. The change of the skin temperature.

between the values obtained under the terrestrial conditions and in the weightlessness. These differences are marked by the increase of the skin temperature in the chest and the simultaneous fall of the skin temperature in extremities. The increased value of the skin temperature of the third measurement in the instep declines, because taken after the woolen socks were dressed as a measure against the previous feeling of the thermal discomfort (4).

In the second experiment the cosmonauts M. Hermaszewski and P. Klimuk confirmed the findings of V. Remek and have extended the observations for the changes of the skin temperature after the workload on veloergometer. The results from the first and the second experiment are plotted in (Fig. 3), the values of skin temperature taken 15 and 30 minutes after the workload (115 W for six minutes) are marked P and IP. The skin temperature in thermal comfort is marked by the dashed lines. As follows from (Fig. 3), the values of the bare skin temperature in the forehead and namely in the instep fairly correlate with the regression line marked in the temperature of the instep by the slope -2.6 °C per 100 Wm^{-2} increase of the microclimate´s cooling power, conditioned as by the variations of the air movement so by the variations in the air temperature. From the regression line of the skin temperature in the instep significantly deviates the value of V. Remek taken in the dressed woolen socks and the values of P. Klimuk taken before and after the workload on veloergometer. The skin temperatures in the forehead are higher, however with the milder slope (-1.5 °C per 100 Wm^{-2}) and without extreme deviations.

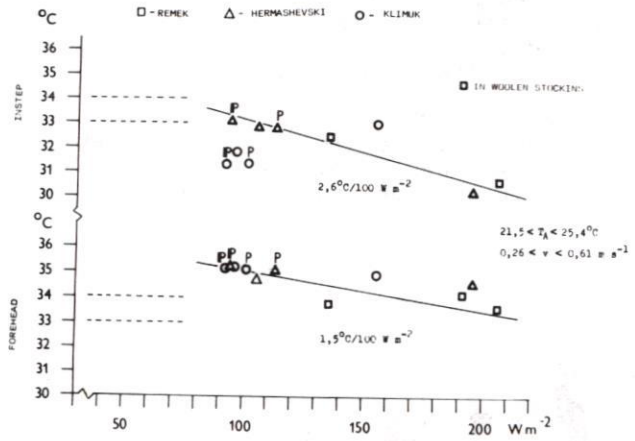

Fig. 3. Correlation of flight values of the skin temperature in the forehead and the instep during the period of rest and after the workload.

The temperature in the space cabin during flight exhibited a similar variation (21.5 to 25.4 °C) as in the laboratory on Earth (23.0 to 27.0 °C). However their absolute values are shifted. This shift together with at once greater air velocities in the space cabin during the flight are responsible for the higher values and greater variability of the space cabin microclimate´s cooling power as demonstrated by means of the EDK sensor with the surface made of polished dural. The cooling power (katavalue) varied during the flight from 92 to 206 Wm^{-2} while in the laboratory on Earth it moved from 71 to 112 Wm^{-2}. This fairly great difference in the estimated cooling power of the space cabin microclimate as compared to the laboratory conditions on Earth was reflected not only by greater variations in the skin temperature in the extremities (see Fig. 2 and Fig. 3), but also in the subjective feeling of cold. In all cosmonauts taking part in the experiment Heat exchange-2 the subjective feeling of cold correlated with values of the environmental cooling power higher than 170 to 180 Wm^{-2}.

It is apparent that by means of the instrument EDK-IK it was possible to estimate quantitatively the differencies between the cooling power of the microclimate in the space cabin during the flight and in the laboratory on Earth, although both had almost the same air temperature and to assess the limit of thermal discomfort to cold for a man in weightlessness.

REFERENCES

1. Genin A.M., Gazenko O.G., Sergeev N.P., 1962. Nekotorie itogi metodiko-biologicheskikh issledovanee na vtorom a tretiem kosmicheskikh korablyakh - sputnikakh. In: Probl. kosm. biol. med. T 1. Ed.: Sisakyahn N.M., Moskva, p.267-284
2. Hardy J.D., 1964. Temperature problems in space travel. In: Physiological Problems inSpace Exploration. Ed. Hardy J.D., Charles C. Thomas Publ., Springfield, Illinois, p. 3-48
3. Novák L., 1976. Heat exchange between the organism and environment under conditions of weightlessness; methodical approach. In: Life Sciences and Space Research XIV. Ed. Sneath P.H.A., Academie Verlag Berlin, p. 329-333
4. Novák L., Remek V., Genin A.M., Poleshchuk A.T., Bárta A., 1979. The microclimate in the space cabin and skin temperature of man in weightlessness. Physiol. bohemoslov. 28(5), p. 459
5. Novák L., Genin A.M., Prokopová L., Golov V.K., 1980. Results of the Experiment "Teploobmyen-1"(Heat exchange-1) Made on Board the "Kosmos 936" Biosatellite. Scripta medica 53(2), p. 67-78
6. Webb P., 1975. Thermal exchanges and temperature stress. In: Osnovi kosmicheskoi biologii i medicini. Ed. Gazenko O.G., Melvin Calvin, Izd. Nauka, Moskva, II/1, p. 105-138

AORTIC AND TIBIAL BLOODFLOW RESPONSE TO LOWER BODY NEGATIVE PRESSURE (LBNP)

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ABSTRACT

A 3.0 MHz pulse Doppler velocity meter (PD) was used to determine blood velocities in the ascending aorta from the suprasternal notch before, during and after progressive 5-min stages of LBNP in 7 subjects. Changes in stroke volume (SV) were calculated from the systolic velocity integrals. A unique 20 MHz PD was used to estimate bloodflow in the posterior tibial artery. With -20 Torr mean SV fell 11% and then continued to decline by 48% before LBNP was terminated. Mean tibial flow fell progressively with LBNP stress, due to an increase in reverse flow component and a reduction in peak forward flow and diameter. SV increased and HR fell dramatically during the first 15 sec of recovery. LBNP was terminated early in 2 subjects because of vasovagal symptoms (V). During V the SV rose 86% which more than compensated for the drop in HR. This implies that V is accompanied by a paradoxical increase in venous return and that the reduction in HR is the primary cardiovascular event. During the first 15 sec of recovery these 2 subjects had a distinctive marked rise in HR reminiscent of the Bainbridge reflex.

INTRODUCTION

The redistribution and alteration of central and peripheral bloodflow in man during gravitational stress has been extensively studied. The problems with measuring these transients during or after the removal of gravitational stress have been that the techniques were often invasive or required respiratory maneuvers which altered pulmonary bloodflow or contained assumptions of respiratory and circulatory steady states which are not applicable to transients. The recent development of noninvasive techniques to estimate cardiac output ($\dot{Q}$) and left ventricular stroke volume (SV) are potentially superior. A suitable method is that of pulse Doppler echocardiography (PD) whereby centerline blood velocities in the ascending aorta can be continuously monitored beat-by-beat. Simultaneous bloodflow changes in specific vessels in the lower extremities during LBNP have not been directly measured because they are inaccessable to precise placement of measuring devices. These bloodflows have been inferred from forearm or hand measurements. Appropriate pulse Doppler velocity meters with transcutaneous transducers are now also available to obtain these bloodflows. The purpose of this study was to noninvasively determine the relative changes in SV, $\dot{Q}$, and bloodflow in the posterior tibial artery during progressive LBNP stress and after termination of the stress (T) and to determine whether vasovagal presyncope (V) produced any unique variations in these responses.

METHODS

Seven males served as subjects. The means (±SD) for age, body surface area and $\dot{V}_{O_2}$max were 31(3)yr, 1.88(.09)m^2, and 50(8)ml/min/kg respectively. The LBNP apparatus and procedures have been described previously (8). LBNP was applied in 5-min stages, increasing from -20 to -60 Torr in 10-Torr increments. The subjects' response to LBNP was monitored for imminent V with continuous recordings of leg volume (LV) by Hg strain gauge, heart rate (HR) and arm blood pressure (BP) each min. Two subjects developed clear signs of V after 3 min at -60 Torr as BP and HR fell before T (Group S). One subject was terminated after 2 min at -50 Torr due to complaints of nausea with no bradycardia. The remaining 4 subjects completed the protocol, with 2 of them going an additional 5 min at -60 Torr without V. The latter 5 were grouped together (N) since their central circulatory responses were similar during LBNP and after T.

A 3.0 MHz PD (ATL, Model 500A, Mark IV) was used to determine aortic blood velocities at selected times (1, 10). PDE allows a specific flow region (sample volume) to be chosen by varying the sample gate or depth control to prevent the reception of extraneous flow information (Fig 1). The transducer was an ATL medium-focus crystal with a focal beam width of approximately 4.0mm. It was manually placed and held in the suprasternal notch with the beam angled toward the heart, co-axial to the flow stream at a beam angle of 0 ± 15°. The Doppler frequency shift is converted by the calibrated audio spectrum decoder (zero-crosser) to a voltage proportional to the spatially-averaged velocity. A typical waveform is shown in Fig 1. The systolic velocity integral (SI) is proportional to SV (3). The true representation of SV by SI is critically dependent upon maintaining the sample volume in the vessel center by careful transducer manipulation to ensure an optimal audio signal and maximal SI on the visual display. Relative SV and Q were obtained from SI and SI x HR for the same beat. Ten to 15 consecutive beats were averaged for each subject after 4 min at each LBNP stage, with continuous recordings from 10 sec before to 30 sec after T.

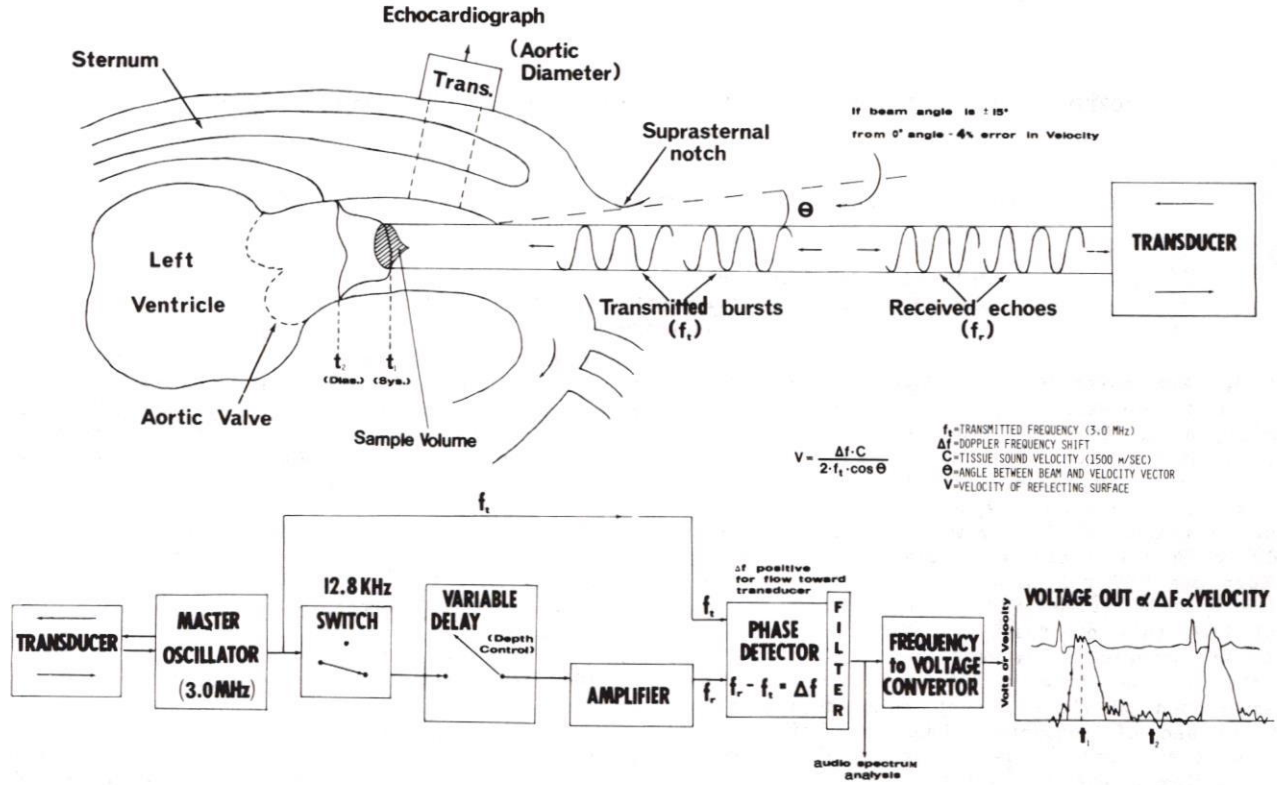

Fig. 1 Simplified Diagram of Pulse Doppler Echocardiograph to Obtain Aortic Bloodflow.

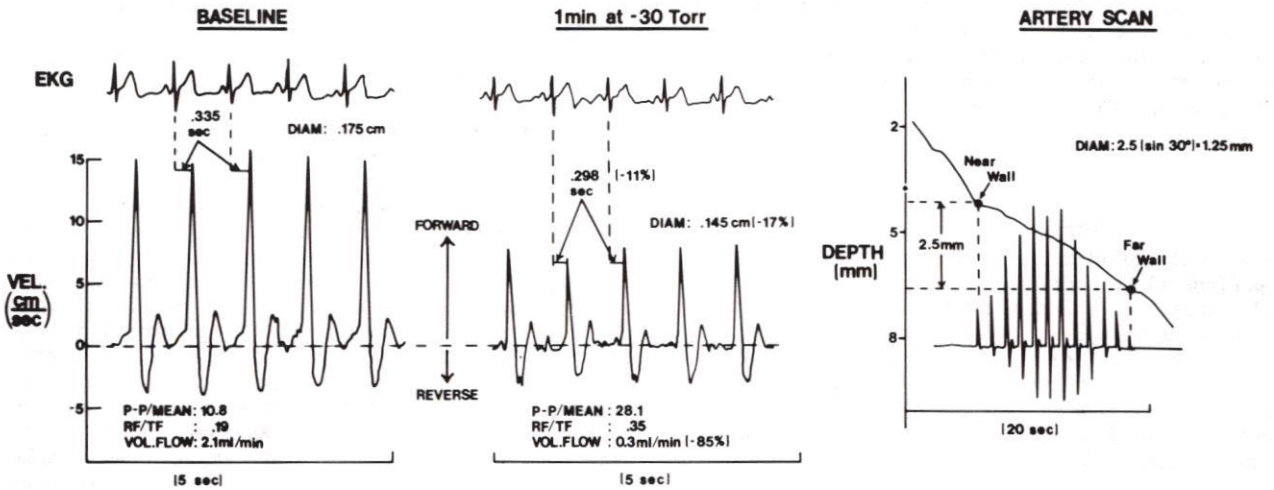

Fig. 2 Velocity Waveforms in Posterior Tibial Artery and Depth Scan for Determining Diameter. P-P/Mean: Peak-to-Peak Height/Mean Height, RF/TF: Reverse Flow/Total Reverse and Forward Flow (By Integration), Vol. Flow: Perfusion.

Tibial artery blood velocities were obtained with a unique 20 MHz pulse Doppler velocity meter (5) which is similar in principle to the PD. A PZT-5A 1.0mm^2 piezoelectric crystal was mounted to a diaphoretic EKG electrode at approximately 30° to the electrode plane, which was attached to the foot prior to entry into the LBNP box. A typical recording is shown in Fig 2 along with a vessel scan obtained by altering the range-gate to determine the diameter and confirm the sample volume being in the centerline flow stream. Small foot movements could displace the beam from centerline and since the angle could not be determined precisely (but remained constant), only relative changes in velocity characteristics are described which were deemed to be valid based on visual and auditory screenings of the signals. Two flow recordings and one scan were attempted at each LBNP stage. Hardcopy aortic and tibial velocity signals were processed with the aid of a digitizer linked to a microcomputer.

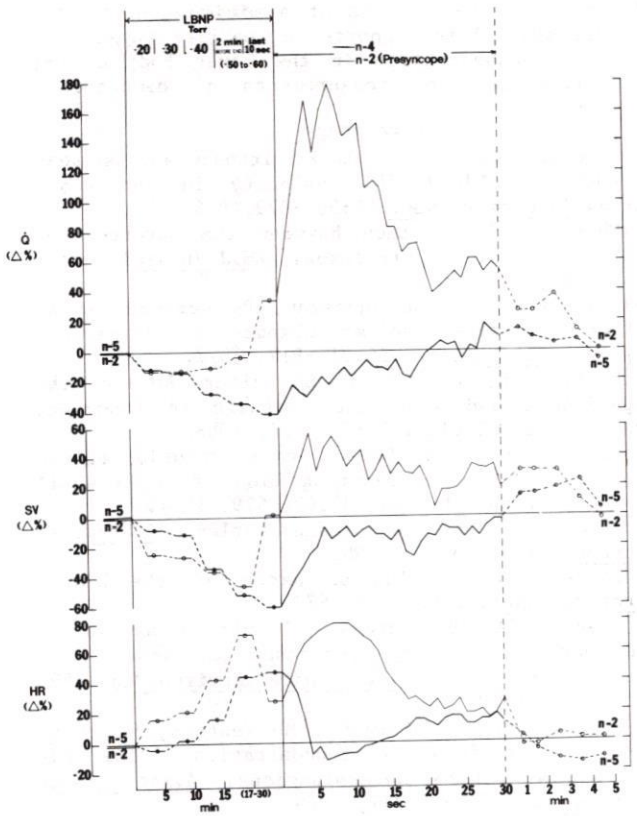

Fig. 3 HR, SV and $\dot{Q}$ During and After LBNP (Beat-by-Beat for 30 sec) as Percent of Baseline. N=2: Subjects who Developed Clear Vasovagal Symptoms, N=5 or 4: No Symptoms.

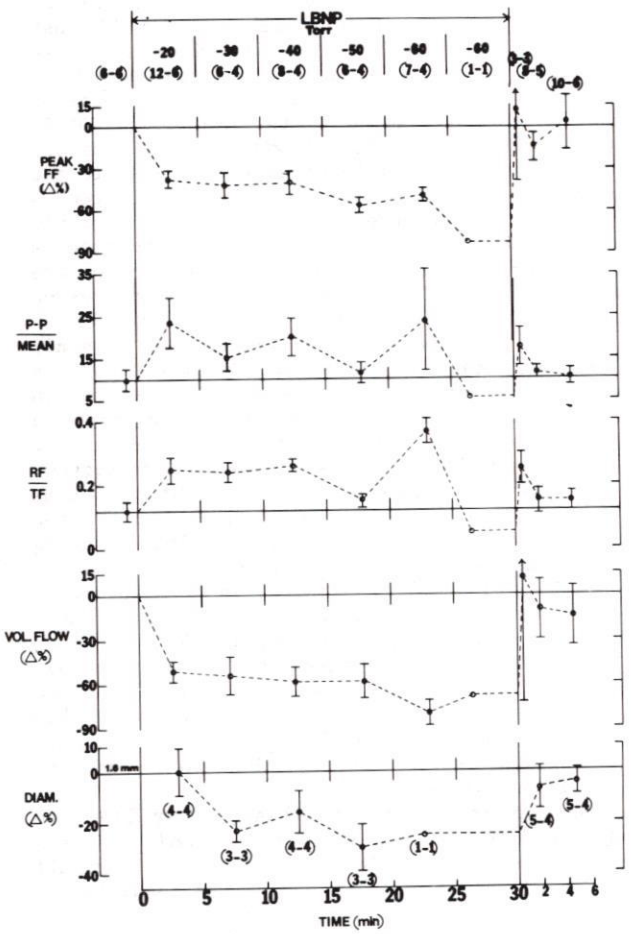

Fig. 4 Tibial Artery Bloodflow Characteristics with LBNP. Parentheses: No. of Observations-No. of Subjects, FF: Forward Flow Velocity, See Fig. 2.

RESULTS AND DISCUSSION

Relative changes in Fig. 3 were computed from mean values of HR, SV and $\dot{Q}$. S responded to the first 3 stages of LBNP with a significantly higher HR than N ($p<.02$) and a larger percentage fall in SV at -20 and -30 Torr, although S had lower baseline values for both. The higher HR served to return $\dot{Q}$ almost to baseline prior to V whereas N continued to decline to -41%. The divergence in $\dot{Q}$ was statistically significant after -30 Torr ($p<.02$). Pulse pressure fell linearly and equally in both groups to 53% of baseline in S prior to V and in N before T with no change in mean BP. This suggests that S had a more responsive baroreceptor or cardiopulmonary reflex to maintain $\dot{Q}$ which may have contributed to V onset. Although the relative fall in SV was about 50% for both groups prior to V or T, group S had a significantly smaller absolute SV prior to V than N (30% lower) since baseline was also lower. A smaller ventricular volume can augment the Bezold-Jarisch reflex whereby vagal fibers in the ventricular wall are stimulated by mechanical distortion to produce a dramatic fall in HR and loss in arterial tone (11). When V occurred (Fig 3) there was a rapid drop in HR of 20 bpm without loss of consciousness before T, with SV increasing and $\dot{Q}$ actually rising 34% above the pre-LBNP baseline as BP fell precipitiously. This points to a marked increase in venous return with LBNP still applied. This blood must have been moblized from the venous capacitance system by venoconstriction which has been shown to occur during V (4). Since LV rose about equally until T in both groups by about 5%, and no decline was noted during V, it would suggest that the additional aortic flow during V came from the splanchnic region which maintains its volume during LBNP (12). SV and $\dot{Q}$ are thought to decline with V, but that occurs only after the splanchnic region is depleted.

After T, markedly divergent response patterns were noted in S and N. The HR in S showed a rise and fall in the first 15 sec, while in N the usual sudden bradycardia occurred, presumably due to baroreceptor stimulation (10). LV showed the usual rapid decline after T in all subjects (8, 10). The HR rise in S is reminiscent of the controversial Bainbridge reflex whereby intravenous volume loading produced an increase in HR (6). Since venous return rose in all subjects after T with the return of pooled blood from the legs it suggests that V produced an "unloading" or

blocking effect on the normal bradycardia seen in N (2). The reduction in HR for the first 15 sec of recovery correlated inversely with the HR just prior to T ($r = -.92$, $p < .005$), which has also been noted in animals (6). The response of HR thus serves as a mechanism to maintain or increase SV during volume loading of the heart. If HR is initially low as in S, SV will not be reduced because filling is completed early in diastole and when HR is high, further tachycardia would reduce Q because of incomplete filling. This optimization of HR during a rise in venous return seems evident from the nearly identical pattern in the rise of SV after T in both groups above the value prior to T. This feedback loop of HR and SV probably originates in the atria (7). As a result of the equal response of SV and divergent courses in HR, Q was markedly increased above baseline in S during the first 30 sec after T, but rose in linear fashion to baseline after 20 sec in N. This must mean that venous return and consequent aortic flow were greater in S than N at this time as well. The rise in Q for the first 30 sec accounted for 1.7 and 0.8 L of blood passing through the aorta in S and N respectively, assuming 5 L/min for baseline Q in all subjects (Integration of Q curves). Since LV contributed equally in both groups, the extra 0.9 L of blood in S could have originated from the splanchnic region which can hold about 25% of the blood volume. Splanchnic venoconstriction presumably began with the onset of V (which preceeded T by only 10 to 15 sec), but contributed the majority of the volume after T.

The characteristics of tibial artery flow are summarized in Fig 4 with means and standard errors, whereby the times were averaged for all subjects since N and S did not differ consistently and no measurements were obtained during V. Flow dropped acutely by 51% at -20 Torr without any change in diameter, but the latter decreased by 23% at -30 Torr reflecting vasoconstriction with minor fluctuations during the remainder of the test, while flow declined progressively to -80% at -60 Torr. Thus vasoconstriction was only partly responsible for the reduction in flow and other factors such as impedance of venous outflow by reversal of the venous pressure gradients must be involved. This is supported by characteristic changes in the velocity patterns. Peak forward flow dropped by 50% during the test, while the reverse component became a larger fraction of the total, further reducing net flow (Fig 2 and 4). This is typical of augmented downstream impedance, as is the increase in pulsatility index (P-P/Mean). As a result, with progressive LBNP bloodflow in the legs is at a minimum until LBNP is released. This supports our earlier suggestion of blood sequestration in the lower body during LBNP from indirect measurements of hemoconcentration (9).

REFERENCES

[1]Angelsen BAJ, AO Brubakk. Transcutaneous measurement of blood flow velocity in the human aorta. Cardiovasc.Res. 10:368-379,1976.

[2]Chen HI. Interaction between the baroreceptor and Bezold-Jarisch reflexes. Am.J.Physiol. 237: H655-H661, 1979.

[3]Colocousis JS, LL Huntsman, PW Curreri. Estimation of stroke volume changes by ultrasonic Doppler. Circulation. 56:914-917, 1977.

[4]Epstein SE, M Stampfer, GD Beiser. Role of the capacitance and resistance vessels in vasovagal syncope. Circulation. 37:524-533, 1968.

[5]Hartley CJ, JS Cole. An ultrasonic pulsed Doppler system for measuring blood flow in small vessels. J.Appl.Physiol. 37:626-629, 1974.

[6]Jones JJ. The Bainbridge reflex. J.Physiol. (London). 160:298-305, 1962.

[7]Linden RJ. Function of nerves of the heart. Cardiovasc.Res. 6:605-626,1972.

[8]Loeppky JA, MD Venters, UC Luft. Blood volume and cardiorespiratory responses to lower body negative pressure. Aviat.Space Environ.Med. 49: 1297-1307, 1978.

[9]Loeppky JA, Y Kobayashi, MD Venters, UC Luft. Effects of regional hemoconcentration during LBNP on plasma volume determinations. Aviat. Space Environ. Med. 50:763-767, 1979.

[10]Loeppky JA, KL Richards, ER Greene, MW Eldridge, DE Hoekenga, MD Venters, UC Luft. Instantaneous stroke volume in man during lower body negative pressure (LBNP). Physiologist. 22(6): S81-S82, 1979.

[11]Pelletier CL, JT Shepherd. Circulatory reflexes from mechanoreceptors in the cardio-aortic area. Circ.Res. 33:131-138, 1973.

[12]Wolthuis RA, A LeBlanc, WA Carpentier, SA Bergman Jr. Response of local vascular volumes to lower body negative pressure stress. Aviat. Space Environ. Med. 46:697-702, 1975.

EFFECTS OF LOWER BODY NEGATIVE PRESSURE ON THE RELIABILITY OF CARDIOVASCULAR SYSTEM USING X-RAY KYMOGRAMS

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ABSTRACT

The effects of lower body negative pressure (LBNP) on man's cardiovascular system reliability (functional capacity) was investigated by the X-ray kymography method. 26 experiments were conducted on 16 physically healthy males in the age group from 18 to 22 years. X-ray kymograms were recorded before and during the exposure to LBNP of -40 and -80 mm Hg. Diastolic, systolic and stroke volumes of the heart were computed.

A decrease in the diastolic, systolic and stroke volumes and cardiac output, and contractile activity and an elevation in tilting of myocardium axis were determined. Under the influnce of LBNP the reliability of cardiovascular system exhibits an appreciable impairment.

INTRODUCTION

LBNP with respect to its physical properties can be considered as a means for simulating gravitational effects on the human body. The changes which the cardiovascular system exhibits under such conditions are extremely similar to the alterations manifested under possitive accelerations and orthostatic tests (1-3). Under the influnce of LBNP there is a reduction of circulation in the forearm (4, 5), an increase in circumfirance of the lower leg (4-6), a reduction in the heart size (7), blood mass redistribution (5, 8), and a reduction in the volume of actively circulating blood (9, 10).

It is known that the X-ray kymography method makes possible to objectivelly determine the contractability of the myocardium and also directly assess the blood stroke volume by computing the size of the heart on the photographs in diastolic and systolic regimes. Furthermore, one can evaluate the functional state of individual chambers in the heart, alterations in the size, shape and tilt of the heart.

METHODS

A total of 26 experiments were conducted on 16 physically healthy males in the age group from 18-22 years. For the production of LBNP they were placed in a rigid container with an elastic belt which held them tightly around the waist at the level of the upper protuberance of the flak bones. Within the container a rarefraction of -40 mm Hg was produced for 20 min; and then after a break the rarefraction was increased up to -80 mm Hg. The subjects were exposed to continously LBNP for 10 to 20 min, unless signs of immenent syncope called for earlier termination. X-ray kymograms of the heart were made in a front to rear projection while holding the breath in the average inhalation position with the use of a multislit-kymograph and emplying a "Nonofos" device. The photographs were taken prior to the exposure to LBNP and during the 10th-16th min of -40 and -80 mm Hg. The diastolic and systolic and stroke volumes of the heart were computed in accordance with the existing methods (12)

RESULTS

Analysis of the X-ray kymograms revealed that LBNP influnces considerably all dimentions of the heart, intensity of its shodown and tilt.

The most significant alterations developed in the width and length of the hear The cross section was decreased at the expence of both the right (averaging 10.9% at -40 mm Hg, and 17.3% of -80 mm Hg) and the left dimension (averaging 14.4 and 22.7% respectively). This is supported by a reduction in venous inflow into the pulmonary heart and a reduction of blood inflow into the left ventricular. Under the influence of LBNP of -40 and -80 mm Hg the length of the heart reduced by 7.1 and 12.3% respectively; the corresponding figures for the cross-section of aorta were averages of 4.2 and 5.7% respectively height increased by 2.2 and 5.7% respectively. While with a reduction in heart size there was a considerable reduction in the intensity of its shadow and also the shadow intensity of the large vessels.

Under the influence of LBNP of -40 mm Hg the angle of the heart tile elevated by an average of 4° and at - - 80 mm Hg by 5°. During LBNP an appreciable reduction in cardiac systolic, diastolic and stroke volumes developed. There the minute volume, despite an increase in the frequency of cardiac contractions, also increased. Under a LBNP of 40 mm Hg the diastolic volume reduced on the average by 13.6% and at -80 mm Hg -- by 22.5%, systolic volume-- by 12.9 and 19.4% respectively, stroke volume -- by 19.7 and 43.3%, and minute volume -- by 21.8 and 18.6% (Table 1).

An analysis of the obtained data on X-ray kymograms of the heart made it possible to determine an alteration in contractability of the myocardium: during the ex-

Table 1. Alterations of Cardiac Volume
Under Different Levels of LBNP

Volume of Heart	Level of LBNP			Difference (in %) Between		Reliability of Difference Between	
	A A Initial Data	B -40 mm Hg	C -80 mm Hg				
	M±m			A and B	A and C	A and B	A and C
Diastolic	1235±75	1065±55	954±88	-13,7	-22,7	1,8	2,4
Systolic	1098±73	955±56	882±84	-13,1	-19,6	1,5	1,9
Stroke	68,6±3,3	54,9±4,6	38,7±2,1	-19,9	-43,5	2,4	7,6
Minute	4656,8±262	3641±303	3780±211	-21,8	-18,8	2,5	2,6

posure to LBNP the amplitude of the cardiac waves reduced considerably. Under a well-expressed tachycardia the waves of the left ventricle exhibited a reduction in the diastolic "knee", as a result of which aquired a haystake-like form. A significant index of the reliability of the cardiovascular system is the contraction factor (ratio of cardiac diastolic to stroke volume). According to the existing data (11-14) the alterations in the contraction factor make it possible to determine the conditions of the tonic and contractile capacity of the myocardium. An increase in this coefficient is considered as an index of attenuation of the tonic and contractile activity of the cardiac muscle fibers, whereas a decrease is regarded as an expression of the possitive dynamics of these activities.

Computing the cardiac contraction coefficient it was possible to determine that under the influnce of LBNP of -40 mm Hg it increases on the average by 7.5% and at -80 mm Hg -- by 35.8%. Evidently an impairment of the tonic and contractile activity of the myocardium is exhibited under the influnce of LBNP.

Thus in response to LBNP there were mediated the subsequent changes: an alteration in the size of the heart and large vessels, a reduction in cardiac diastolic, systolic, stroke and minute volumes, an impairment in the contractile activity of the myocardium, and an elevation in cardiac tilt. These data were found to compare well with the experimental data obtained by other investigators (15,16). The mechanism of these alterations is evidently related to blood deposition during the exposure to LBNP, a decrease in venous return to the heart and a reduction in its blood filling, as is showed by a decrease in the size and intensity of the cardiac shadow.

After the foregoing it can be said that under the influnce of LBNP the reliability of the cardiovascular system manifests a considerable impairment.

REFERENCES

1. Lamb, L. E., Stevens, R.M., 1965. Aerospace Med. 136:1145.
2. Murray, R.H., J. Krog, 1965. Aerospace Med. 8:238.
3. Zorbas, Y. G. et al., 1972. in Man's Reliability Under Hypokinetic Stress ed. M. Delaportas, Athens.
4. Murray, R. H., 1967. Aerospace Med. 38:243.
5. Brown, E., J. S.Goei. 1966. J. Physiology. 183:606.
6. Show, D. B., F.Cinkotai, M. L.Thomson. 1966, Aerospace Med. 37:154.
7. Milledge, R. 1967. in 38th Annual Sci. Meeting of Aerospace Med. Washington.
8. Zorbas, Y. G. et al., in Man's Reliability under Hypokinetic Stress, ed. M. Delaportas, Athens, 1972.
9. Balakhovskiy, I. S., V. G. Voloshin, and O. A. Virovets. 1970. Kosmicheskaya Biol. (Cosmic Biology). 5:25.
10. Zorbas, Y. G. et al., 1972. in Man's Reliability Under Hypokinetic Stress. ed. M. Delaportas, Athens.
11. Zorbas, Y. G., Ibid.
12. Shik, Ya. L., 1940. Klin.Med. 7:80.
13. Arobey, I. L. Problems of Cardiology at Resorts. 1965. Kislovodsk
14. Lysholm, H. 1934. Acta Radiol.
15. Mansurov, A. R. 1965. J. Military Med. 10:59
16. Agadzhanyan, N. A., Mansurov, A. R. 1962. Bulletin of Exper. Biol. 4:42

VIBRATION AND DECOMPRESSION GAS BUBBLES

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INTRODUCTION

Vibrations are created in many vehicles on the ground, at sea and in the air. Transportation with helicopters may expose both pilot and passengers to vibrations. Helicopters are often used in ambulance flights, and thus in the transports of divers with decompression sickness to recompression therapy. In these cases the helicopter offers the advantage of a fast transport with lowest possible cabin altitude to avoid worsening of the symptoms of decompression sickness (6). Vibrations in the helicopters, as well as in other vehicles, may have adverse physiological and psycological effects on man (5, 7). Muscle exercise as well as passive limb movements may release trapped gas bubbles from the body tissues during decompressions in connection with diving or flying (1, 2, 3). Muscular exercise is also known to increase the risk of decompression sickness (4). The purpose of this study was to investigate if vibrations, similar to those that may appear in helicopter flights, might also release decompression gas bubbles after diving.

METHODS

Ten healthy male SCUBA-divers between 18 and 38 years old (mean 28±7 years) were used as subjects (mean length 184±7 cm, mean weight 79±10 kg). They were given a hyperbaric exposure at 250 kPa (15 m diving depth) for 100 min in a hyperbaric chamber while performing an intermittent (2 min work, 2 min rest) bicycle ergometer work of about 75 W. Such an exposure allows no-stage decompression to surface level pressure. This exposure was followed within 5 min by a hypobaric exposure at 70 kPa (3000 m flying altitude) for 2 h in a hypobaric chamber. These exposures are known to cause decompression gas bubbles in a great amount (3). Under the hypobaric condition the subjects were exposed to 15 min periods of 0.23 g_z (peak) vibrations of 15 Hz (simulating single rotor helicopter vibrations) alternated with 15 min periods of non-vibrations. The subjects were seated in an upright position in a vibration chair. Subjects 1-5 started with the non-vibration condition; subjects 6-10 with the vibration condition. Some further subjects were similarly exposed to 0.64 g_z 25 Hz vibrations (simulating twin rotor helicopter vibrations). All subjects had a hyperbaric oxygen treatment at 200 kPa for 30 min for safety reasons (see Fig. 1).

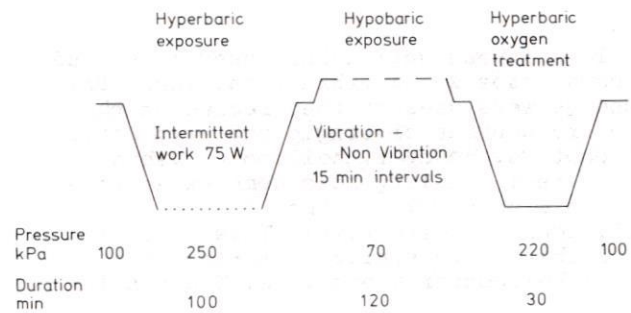

Fig. 1. Pressure exposure profile.

Venous gas bubbles were detected by the precordial Doppler ultrasound technique (2) during the hypobaric exposure. The duration of the bubble signal in seconds was registered under the vibration and non-vibration conditions, thus giving an estimate of the amount of bubbles.

RESULTS AND DISCUSSION

No significant difference in the release of decompression gas bubbles could be found between 15 Hz vibration and non-vibration conditions (Fig. 2). Neither in the few experiments using 25 Hz vibration could there be detected any differences between vibration and non-vibration conditions. The noted differences in appearance of bubbles are inter-individual and unaffected by vibration (Table I).

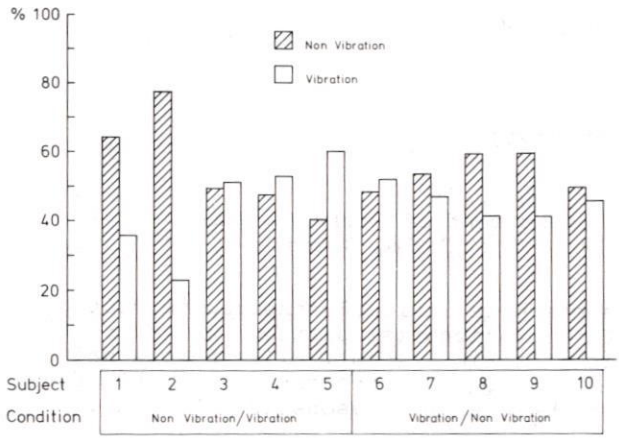

Fig. 2. Relative bubble duration.

This investigation only used 15 Hz and in some cases 25 Hz vibrations. These frequencies were used as they represent the main frequencies of single and twin rotor helicopters. However, helicopter vibrations are in reality more complex as they contain a spectrum of other frequencies, amplitudes and directions. This makes it impossible to generalize the results to actual helicopter vibrations. The conclusion of this study can only be that there is no effect on the release of decompression gas bubbles with the used types of vibration. If other single frequencies or compound spectra of frequencies and if other amplitudes and directions of vibrations may release such bubbles remains to be investigated.

Passive movements of extremities or muscle exercise may release showers of trapped decompression gas bubbles from the tissues (1, 2, 3). This effect is in a great contrast to the lack of bubble release with the used vibrations. The question of a possible increased risk of decompression sickness by vibration, like that of muscular exercise (4), also remains to be investigated.

TABLE 1. Registered bubble duration (s)

condition / subject	vibration	non-vibration
1	4	7
2	30	103
3	33	32
4	2911	2527
5	154	103
6	13	12
7	1915	2179
8	115	165
9	209	300
10	1118	1334

REFERENCES

1. Adams, J.D., R.M. Olson, and G.A. Dixon. 1979. Use of the Doppler precordial bubble detector in altitude decompressions. Preprint of 1979 Annual Scientific Meeting, Aerospace Medical Association, Washington, D.C. pp. 260-261.
2. Balldin, U.I. 1978. Intracardial gas bubbles and decompression sickness while flying at 9000 m within 12-24 hours of diving. Aviat. Space Environ. Med. 49:1314-1318.
3. Balldin, U.I. 1980. Venous gas bubbles while flying with cabin altitudes of airliners or general aviation aircraft 3 hours after diving. Aviat. Space Environ. Med. (in press).
4. Fryer, D.I. 1969. Subatmospheric decompression sickness in man. The Advisory Group for Aerospace Research and Development, NATO Technivision Services, Hough, England.
5. von Gierke, H., and N.P. Clarke. 1971. Effects of Vibration and Buffeting on Man. In Aerospace Medicine, 2nd edition (Ed. H.W. Randel). The Williams and Wilkins Company, Baltimore, Md., USA. pp. 198-223.
6. Reddick, E.J. 1978. Movement by helicopter of patients with decompression sickness. Aviat. Space Environ. Med. 49:1229-1230.
7. Rotondo, G. 1978. Workload and operational fatigue in helicopter pilots. Aviat. Space Environ. Med. 48:430--436.

RELATION BETWEEN PHYSIOLOGICAL EFFECTS OF GRAVITATIONAL FORCES AND THAT OF MAGNETIC FORCES

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INTRODUCTION

In terrestrial environment, there are many kinds of geographical and artificial electric magnetic fields. Off course, there are various intensities of magnetic fields in various places in outer space. On terrestrial bioclimatological effects of them, there is a review by Dr.Wladimirskij, which served as an impetus to conduct more precise physiological studies.

We conducted an experiment on the attitudes of the metabolic and cardiovascular functions of albino rats during and after low magnetic field (LM) exposure under simulated hypogravic conditions[5].

In this paper, we would like to report on experimental results on the attitudes of the physiological functions of mice during and after LM exposure, under simulated hypogravic conditions. The results were compared to those of geomagnetic field exposure.

EXPERIMENTAL METHODS AND RESULTS

For the experimental method, we selected a LM of about 1% intensity of geomagnetic fields (NM) by active shielding by means of Helmholtz coils. At the 30 cm^3 space of the center of the apparatus, the oscillation value of the magnetic force, measured with the magnetometer sensor fixed toward the total intensity was less than 2 mG, that is 1% of the intensity of the terrestrial magnetic field (NM), therefore the intensity of the (LM) space was considered unnecessary in this experiment.

I. WOUNDS HEALING TEST

EXPERIMENTAL METHODS

The 6-unit confining containers were used for experimental mice of D. D. Strain. Each unit measured 6 cm x 10 cm x 8 cm high. One container was set in the geomagnetic shielding apparatus, the rests were not. The container placed the mice in a somewhat hypokinetic condition. After a period of time under such condition, 2 kinds of experiments were performed—— a wound healing test and a swimming capacity test.

The special hole puncher was used for making same-size wounds on the dorsum skins of the mice. The punches were made on mice exposed to 1 week of LM and on control NM mice. And the rate of healing of each group was compared by measuring the surface area of the wounds of the same time each day. Measurement was made by tracing the size of the wound on a transparent acetate film, enlarging it 100 times with a photo enlarger, and determining the area with a planimeter.

RESULTS

For the healing ratio of the wounds, following the wound healing law of Okuda[1], the process for the wound surface area of each day after wounding, expressed as the percentage of the values of just after punching.

The process of the healing ratio of 2 groups of mice was drawn on the semi-logarithmic scale. We can see in the figure, the fluctuating period by Okuda is 7 days in NM mice and 8 days in LM mice. After those days the healing curves decreased logarismically and perfect healing was attained in 11 days for the NM mice average, and in 14 days for the LM mice average. Wound healing coefficient K of each group is 0.41 and 0.40 respectively. The comparison of the healing curves shows a slightly better effect of NM than that of LM, for the healing mechanism.

II. SWIMMING CAPACITY TEST

EXPERIMENTAL METHODS

For the swimming capacity test, G.F.Kiplinger's methods[2] was used. The pool used measured 10 cm x 180 cm x 30 cm deep. Water depth was 13 cm and water temperature was kept at 17-19°C. Twenty swimming trials were conducted without rest for mice following exposure to different dynamic and magnetic conditions, they were low dynamic (LG) and normodynamic (NG) in low magnetic condition (LM) for 1,2, 3 and 4 months. Time was measured for the swim between two beams placed 150 cm apart.

RESULTS

Fig. 1 shows the swim times for mice forced to swim 20 trials across the above-mentioned swimming course, following exposure to different dynamic and magnetic conditions, expressed as the average values of each successive 5 trials. The control (dot-dash line) is a more than 3 months normodynamic and geomagnetic(NM·NG)exposure group, that is not hypodynamic, standard group. There are also two hypodynamic groups, they are low magnetic and hypokinetic group (LM·LG), drawn by solid line, and geomagnetic and hypokinetic group (NM·LG), drawn by dotted line. The former group is exposed for 1 month to low magnetic and hypokinetic conditions following 0.6 month geomagnetic and hypokinetic exposure, and the latter group is exposed for 1.6 months to geomagnetic and hypokinetic conditions.

Through the all stages of the swimming trials, geomagnetic and normokinetic (NG·NM), that is standard group, was holding the best records.

On the records during the 1st 5 trials, the low magnetic exposure group (LM) under hypokinetic exposure (LG) group is worst and NM·LG is medium, and the each group showed a highly significant difference with each other, and the standard group.

Such ordination of each group is not inconsistent with the Russians' (Dr. Mastryukaova et al.[3]) results on the comparison of NM·NG to LM·NG as far as the relation between NM and LM conditions is

concerned.

And after the 3rd 5 trials, NM·LG group declined to the worst speed, and separated strongly, very significantly, from the NM·NG group, clearly expressing the deteriorative effects of LG.

But LM·LG group was still keeping the medium value during the 2nd to 4th 5 trials, and through all the course of swimming test, LM·LG group has a tendency to approach to NM·NG group standard value, that means LM·LG group deteriorates more than NM·NG group, and it is understood as an expression of the attenuate effects of LM to the deteriorative biological influences of LG.

In this stage of 1 month exposure to LM, no changes were found between the body weight of each group.

The low magnetic exposure was conducted for 2 months in LM·LG group. LM·LG group and NM·LG group express the 2 month low magnetic and hypokinetic exposure following 0.6 month geomagnetic exposure and 2.6 month low magnetic and hypokinetic exposure group respectively.

The standard group was best through all experimental trials, as above mentioned. And every group is going to ameliorate by the progress of trials, but during 10 trials LM·LG and NM·LG group is significantly worse than standard group. And during 5 trials NM·LG is better than LM·LG significantly. And after 10 trials, LM·LG group ameliorates and closes to NM·NG group. But during this end half of the trial run, difference between NM·LG and NM·NG is very large. The difference between NM·LG and LM·LG is very highly significant.

For the next step, the low magnetic exposure was conducted for 3 months in LM·LG group. This duration is the same length as that of Russians' LM·NG experiment. LM·LG group and NM·LG group express the 3 month hypokinetic and low magnetic exposure following 0.6 month hypokinetic and geomagnetic exposure and 2.6 month hypokinetic and low magnetic exposure group respectively.

In this experiment, all groups closed to each other during all trials but still significancy of delaied swimming time of LM·LG and NM·LG group continued all trials run, but there is no significant difference between LM·LG and NM·LG.

But the body weight of NM·LG groups increase from the end of 3rd month of LM·LG exposure period.

We conducted the same experiment with 4 month low magnetic exposure in its LM·LG group. And we recognized the swim times of all groups come to be entirely the same without the 1st 5 trials. The bad swimming ability of LM·LG during the 1st 5 trials entirely ameliorates during all period, after the 1st 5 trials. The significant deterioration of NM·LG group during all experimental run will be explained by the increasing of body weight of the group as above mentioned.

Such results clear amelioration of LG group with the prolongation of hypokinetics exposure, and may suggest the effective adaptation to the hypokinetic conditions. But the LM condition clearly prevents such detelioration during 2 month hypokinetic exposure.

From the previously reported results[5] on the fluctuation of K excretion rate of rats during 3 weeks hypodynamic exposure and 5 weeks ambulatory life after the exposure under geomagnetic field (NM)and low magnetic field (LM), we learned that increase of the urinary excretion value of K during the hypokinetic period was more remarkable in the NM group than in the LM group. And return to the pre-exposure control level was faster in the LM group than in the NM group. Such correlation is recognized again in this result.

SUMMARY

We have reported some findings on low magnetic field (LM) on mice. And the results obtained were summarized as follows:

1) Healing process of the wounds inflicted with a punch to the dorsum of mice under hypokinetic field, during geomagnetic field (NM) and LM exposure showed the significantly better healing effects of NM than LM exposure.

Such results on the multiplication speed of the tissue cells of higher progressed animals, such as mammalia, shows an interesting contrast to our last reports on some primitive bacteriological cells[4].

2) Swimming capacity of mice showed that the deterimental effects of hypokinetics were attenuated by LM.

But the deterioration of swimming capacity of mice by hypokinetic exposure seems to be adapted by the prolongation of exposure time. The deterioration ameliorated and vanished following the time course after 3 month hypokinetic exposure.

And those results combined with the reported results[5] suggest the attenuative effects of LM on the responses of rats and mice by hypokinetics.

REFERENCES

1) Yanagi, S. 1940. A review on wound healing. J. of Jap. Assoc. of physical med., balneology and climatology 6: 1-22.

2) Kiplinger, G. F. 1967. The effects of drugs on the rate of development of fatigue in mice. Texas Reports on Biology and Medicine 25: 531-540.

3) Nakhilnitskaya, N., V. M. Mastryukaova, L. A. Andvianova and A. G. Borodkina. 1977. Animal responces to the zero magnetic field. 28th IAF meeting, Plague.

4) Saiki, H., M. Saiki, M. Nakaya, M. Sudoh and M. Abe. 1978. Biological responces induced by the low magnetic field. Jap. J. of Biometeor. 15:36.

5) Saiki, H., M. Saiki, M. Nakaya, M. Sudoh, M. Abe and J. Nakajima. 1979. Environmental physiological study on low intensity magnetic fields. 50th Annual Scientific Meeting, Aerospace Medical Association, Washington, D. C., 193-194.

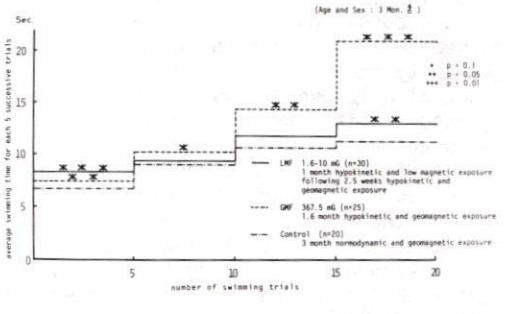

Fig. 1. Swim times for mice forced to swim 20 trials across the 1.5m swimming course, following different dynamic and magnetic conditions exposure.

ORTHOSTATIC TOLERANCE AND EXERCISE RESPONSE BEFORE AND AFTER 7 DAYS SIMULATED WEIGHTLESSNESS

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4 volunteers underwent orthostatic tests before and after a 7 days period of prolonged bedrest in antiorthostatic position (-4°). A +85° tilt test (head up) during 20 minutes and a squat-stand test were performed. Tests on an ergometric bicycle were also performed. Concerning orthostatic tolerance after the simulation, we noted a greatly increased intolerance among 2 of the volunteers, that can be translated as a presyncopal state with severe arterial hypotension, whereas the other 2 volunteers showed a paradoxical reaction with a hypertensive spike. The exercise response was also perturbed after, specially for one of the volunteers for whom the test had to be stopped.
We feel that these results confirm the value of such a position where one intends to simulate the cardio vascular changes that occurs during weightlessness.

Our experiment involves the effects on orthostatic tolerance and exercise response created by prolonged bedrest in antiorthostatic position. The objective of this work was to confirm the value of such a position where the intention is to stimulate the cardio vascular danges that occur during weightlessness.

MATERIAL AND METHODS

4 healthy male volunteers with an everage of 25 were selected after undergoing medical and psychological tests. The lenght of the period of bedrest was fixed at 7 days and studies of basal conditions were performed during the 24 hours preceeding the experiment ; the cardio vascular readaptation was studied during the four days following the end of the period of bedrest. The antiorthostatic position chosen was an inclination of -4° (head down). Before the experiment, the different tests were performed twice ; for four days after the end of the experiment, identical tests were carried out twice daily for the first two days, and then once a day on the third and fourth days.
Two tests were carried out for study the orthostatic tolerance, the first with the patient passive on the tilting table at 85° for a period of 20 minutes, the second, an active test, the squatting standing up test during which a continuous recording was made of carotid circulation velocities and cardiac frequency (1,3,4).
The effort aptitude test required the building up to 200 W on an ergomatic bicycle, by steps of 50 W at a time, lasting 5 minutes, with continuous ECG monitoring being carried out and with blood pressure being taken at the end of each step. During the recovery periods (10 minutes), blood pressure was taken every 30 secondes.

RESULTS

Before the experiment, no anomalies were found in the 4 patients either in orthostatic position or in conditions of effort. Orthostatically, after the experiment, we noted the following : -firstly, on examination on the tilting table ; two patients showed a big increase in hypertension with tachycardia, these symptoms appearing progressively and persisting throughout the 20 minutes test period. This only occured at the first examination after the bedrest period. We think that it may be accounted for in terms of a vagotonic response. The other two patients showed considerable arterial hypotension producing a pre-syncope state in one and syncope in the other (in the latter case during the first five minutes of the test). - Secondly, the first of the squatting-standing tests to be carried out after the experiment was disturbed in all four patients, producing delayed resumption of normal carotid velocity curves in excess of 15 seconds (21" ± 3) and considerable tachycardia (127 ± 11) compared to the pre-experiment 82 ± 7,6 (figure 1) The following tests produced lower than normal results. - The post experiment effort aptitude test was also disturbed. Increased tachycardia occured (+22%) with smaller differential blood pressure, and the recovery phase was also longer. One subject presented a syncope during this phase. The symptoms persisted for 48 hours.

CONCLUSIONS

The results are in agreement with those of the different authors in both bedrest studies and immersion simulations, whether total or with head out of water (2,5). The same modifications are found on return from space flight.
We feel that these results confirm the value of such a position where the intention is to simulate the cardio-vascular changes that occur during weightlessness.

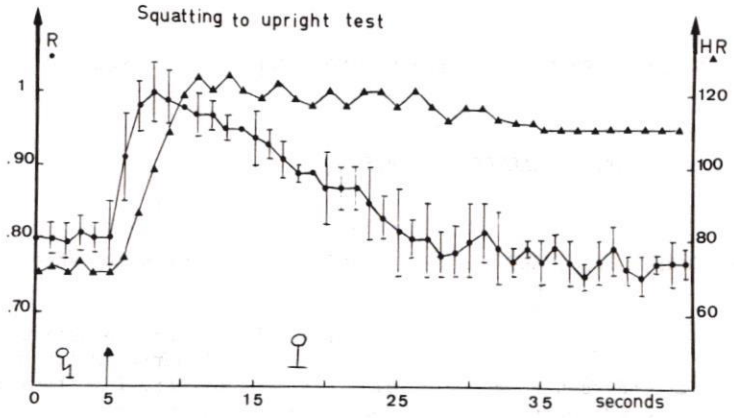

Figure 1 : Squatting to upright test after the bedrest period (mean values of the 4 subjects).

BIBLIOGRAPHY

1.BES A., GUELL A., BARRERE M., BRAAK L. and G. GERAUD, 1979 : Doppler carotid velocity measurements as an index of total C.B.F. In : Cerebral Circulation and Neurotransmitters. Edit. A. BES , G. GERAUD, Ph. JAUZAC, A. GUELL, EXCERPTA MEDICA, pp 93-99.

2. GRAYBIEL A., CLARCK B. (1961) : Symptoms resulting from prolonged immersion in water. The probleme of zero G asthenia. AEROSPACE MED., 32, 181.

3. GUELL A., BRAAK L., ARNE-BES M.C., FANJAUD G. and A. BES, 1979 : Simulation d'absence de pesanteur : modifications circulatoires au niveau de l'extrémité céphalique. In : CIRCULATION CEREBRALE, TOULOUSE, Septembre 1979. Edit. A. BES & G. GERAUD, pp 271-274.

4. GUELL A. BRAAK L., BARRERE M., JAUZAC Ph., A. BES, 1978 : Correlation between C.B.F. Modifications and variations in blood velocity as measured by Doppler Sonography. IXe International SALZBOURG Conference, Edit. J.S. MEYER, H. LEACHNER and R. REIVICH, EXCERPTA MEDICA, pp 93-98.

5. HYATT K.H., SMITH L.G., KANENESTKY and VOGEL J.M., 1970 : A study of post recumbency orthostatism and prophylactic measure for prevention of this phenomenon. NASA, Contractor's Report N° T 2856-6.

POSTURAL CONTROL RELATED TO THE DIFFERENT TILTING BODY POSITIONS

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ABSTRACT

The effects of 5 minutes exposure to different body tilting positions on the patterns of postural control were investigated. By means of stabilographic method the influence of recumbent (supine) position - head horizontal 0°, head up +60°, head down -15° and sitting position head +90° was measured. The evaluated parameters of the posture stability were: statokinesigram area (A) and the velocity index of body sways (I). 31 healthy men aged 18-24 were tested after all tilting exposures with eyes open and eyes closed. Romberg quotient (R) was computed from data obtained. Horizontal position 0° induced the mildest decrease of postural stability. The most destabilizing effect after +60° tilting was found. The significant changes were manifested only in subjects with eyes closed. It was the R for A, which reflected the reduced ortostatic tolerance most sensitively.

INTRODUCTION

During standing on feet the earth´s gravity acts on an individual in the direction of the long body axis. The vertical body position being frequent in human subject results in an appropriate afferent inflow, neural integration and efferent performance. During the prolonged bed rest - a procedure simulating gravitational stresses - a change of gravity vector occur and different motor disturbances take place (Brjanov et al., 1976). The tilt-table test can be used to demonstrate the importance of the change of the gravity vector in relation to the long axis of the body as well. This study is concerned with the effects of short-term different passive body positions upon the immediately following upright posture regulation.

METHODS

The upright posture control have been studied by means of stabilographic method (Gurfinkel et al., 1965). From the stabilograph - a force-sensitive plat - form, which reacts to horizontal body sways - sagittal y(t) and lateral x(t) stabilograms were obtained. Statokinesigram consisted of both, sagittal and lateral stabilograms, registered on XY recorder. Statokinesigram area (A) was evaluated by means of a planimeter and was used as a parameter of body equilibrium regulation. The second parameter, the velocity index of body sways (I) was calculated from sagittal, y(t) and lateral, x(t) stabilograms. Velocity index is the sum of the integral of the absolute value of the first derivative of the stabilograms y(t), x(t) during 30s.

Test subjects were 31 healthy men aged 18 - 24. They were exposed to the following four experimental body positions: 1. sitting on chair, head up +90°; 2. recumbent (supine), head horizontal 0°; 3. tilting head up +60°; 4. tilting head down -15°. Last three positions were achieved using the tilt-table technique. After each 5 minutes lasting position body sways of the test subject standing on the stabilograph during 30s with eyes open and eyes closed were registered. The role of visual afferentation in upright posture control was evaluated for both parameters, A and I by means of Romberg quotient (R) according to van Parys and Njikiktjien (1976). Romberg quotient is a relative, percentual value, the ratio between values found with eyes open and values with eyes closed.

RESULTS

In all experimental situations it was seen, that 5 minutes exposure of subject to different passive body positions exhibited no significant influences on the subsequent postural reactions, when comparing the findings with eyes open. However, tilting head up +60° evoked relatively the most pronounced changes of the postural stability. The A was increased and I as well. On the other hand, the postural control with eyes closed made the influence of preceeding body position evident. The horizontal position produced minimal changes in the subject´s postural reactions. The most marked effect was evoked after the position head up +60°. The posture stability after tilting head down -15° and sitting head

+90° compared with the horizontal position was reduced as well. The above mentioned findings with regard to the parameters A and I are shown using relative values on Fig. 1. Head horizontal showing the mildest orthostatic intolerance was taken for 100 r.u.

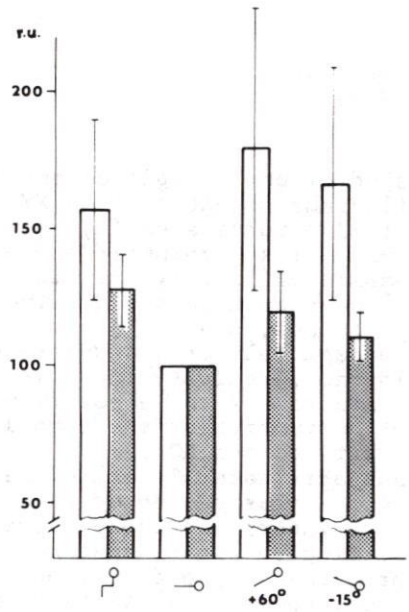

Fig.1. The relative changes in statokinesigram area ▭ and velocity index ▨ of body sways during upright posture control with eyes closed vs different body positions.

The evidence of the visual information involved in the equilibrium control was clearly demonstrated by means of R. There were significant differences in the mean values for different experimental body positions as related to the parameter A (Fig.2) and I as well.

According to data obtained we can conclude that the postural control system of a healthy men does compensate the preceeding short-term influence of the gravity vector direction if the posture regulation is accoplished with eyes open. Depriving the subject of the visual afferentation produces marked orthostatic reactions reflecting the effect of gravity stimuli. Tilting head up +60° altered most importantly the subsequent postural task (23 % decrease of R as related to head horizontal). It seems to be in accordance with references about the use of the tilting test as a measure of orthostatic tolerance (Hyatt et al.,1975).

The results of the present study underline the role of the visual afferent system in the orthostatic control, which make manifest the influence even of a short-term gravitational stres on the human body.

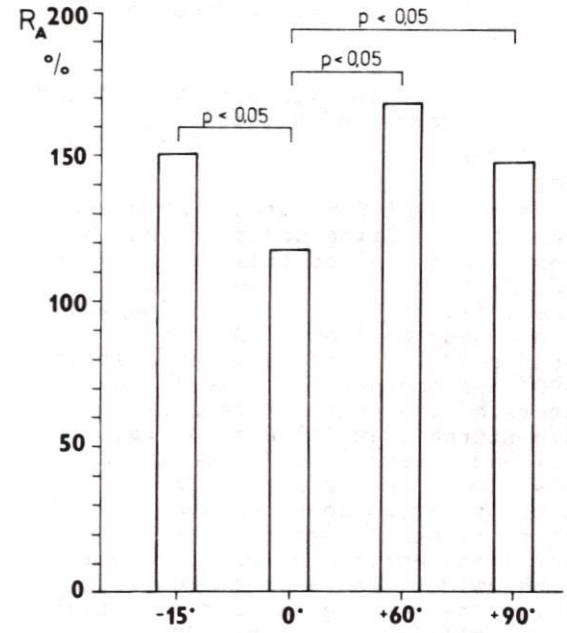

Fig.2. Romberg quotient for statokinesigram area as related to different body positions: -15° tilt head down, 0° - recumbent head horizontal, +60° tilt head up, +90° - sitting positions.

REFERENCES

1. Brjanov,I.,I. et al.,1976. Osobennosti stato-kinetičeskich reakcij. In: Kosmičeskije polety na korabljach "Sojuz". Gazenko,O.,G. et al. (Eds.) 1.ed. Nauka. Moskva. p. 195-229.
2. Gurfinkel,V.,S., Koc,J.,M., Shik,M.,L. 1965. Reguljacija pozy čeloveka. 1.ed. Nauka. Moskva. p.256.
3. Hyatt,K.H., Jacobson,L.B., Schneider, V.S., 1975. Comparison of 70° Tilt, LBNP and Passive Standing as Measures of Orthostatic Tolerance. Aviation, Space and Environmental Medicine, 46: 801 - 808.
4. van Paryjs,J.A.P., Njiokiktjien, Ch. J., 1976. Romberg sign expressed in a quotient. Agressologie, 17, B: 95 - 99.

THE REACTION OF SIMULATED AND TRUE WEIGHTLESSNESS ON DIGESTIVE TRACT OF RATS

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ABSTRACT

In rats we found after 15-30 days of hypokinesia similar modifications as after in 18,5 days space flight:decreased mucopolysaccaride secretion in different digestive organs and increased of secretion of some enzymes of intestinal wall,increased secretory activity of antral and duodenal G (gastrin secreting) cells,as may be concluded from histochemical investigations.In soil hypokinetical conditions the gastric acid and pepsin secretion and the plasmatic corticosterone were also increased.In the flight these reactions are due to a stress reaction and only in a reduced manner to the weightlessness.

INTRODUCTION

To provide for the changes produced in the organism by weightlessness, different kinds of simulating it at soil level are employed. Limiting movements are between the most employed. But in the rat as in other species restraining movements induced complex functional and morphological changes that depend also on a stress reaction. The most important modifications produced by gravitational changes occured in skeletal and myocardial muscles and in bones, especially interested in movements. The tissues of digestive tract respond to restraint with a nonspecific reaction.

The purpose of this study was to evaluate the changes produced by restraining the movements of the rats on the digestive tract and to compare it with those produced in a true space flight in order to better differentiate the reactions produced by stress and weightlessness.

METHODS

Rats movements were limited 1, 2, 7, 14, 15, 30 and 60 days by introducing them in special cages. The following histochemical parameters of the gastrointestinal tract were investigated: the mucopolysaccarides (MPS) of submaxillary glands, gastric mucosa and of the intestinal goblet cells; the G gastrin secreting cells of the antrum and duodenum; different enzymes of the small intestine, especially the leucine aminopeptidase (LAP) and acid phosphatase(AP), which showed the most modifications. The same changes were investigated also in rats recovered after a 18,5 days space flight on Soviet Cosmos 936 satellite.

At soil level the gastric acid and pepsine (Shay method) and the plasma corticosterone values (Vernikos-Danellis method) were also determined.

RESULTS

Hypokinesia by restraining movements in special cages determines a decrease of MPS secretion at all investigated levels : submaxillary glands, gastric mucosa and small intestine. These reac-

tions were maximal after 15-30 days of hypokinesia. 60 days later a near complete recovery could be noticed. LAP and AP secretions of small intestine showed also a maximal increase 15-30 days after beginning of the experiment, returning to normal value after 60 days. These changes may be interpretated as a stress reaction, in accord with others(1). In same median interval the antral and duodenal G cells showed an increased activity. In this interval the gastric acid and pepsine secretion presented also a maximal out put.

These changes were in relation with the corticosterone plasma concentration, That was maximal after 7-14 days, interval in which they were investigated. It is known that glucocorticoids decreased mucus secretion an increased that of digestive enzymes (2).

We found similar changes in rats recovered after a 18,5 days space flight as in rats after 15-30 days of hypokinesia: reduction of MPS secretion of submaxillary glands, stomach and small intestine, and an increased secretion of LAP and AP.

These changes were judged by comparing them with the rats maintained at soil level under similar environmental conditions as during the space flight and centrifugated at soil level with + 1 Gx. During the flight a group of rats was also centrifugated.

The MPS decreased and the enzymes increased activity were more important in rats centrifugated in flight. These gradations may be explained by the degree of the stress. The only benefit of the centrifugation was a slight restoration of submaxillary and intestinal sialomucins, but not that of neutral mucopolysaccarides.

After 15-30 days of hypokinesia and in rats recovered after the space flight in the submucosa, there was a marked accumulation of eosinophiles, effect characterising the circumstances which hypersecretion of corticoid hormones take place.

From complementary investigations results that the AP of B pancreatic cells presented an increased reaction, corresponding with an insuline hypersecretory activity in both circumstances, i.e. after 15-30 terrestrial hypokinetical conditions and in the space flight. The B cells increased activity is in accord with the hyperglicemia that appeared in rats in terrestrial hypokinetical conditions and with the increased glycolysis.(3).

REFERENCES

1. Portugalov V.V., 1978. Arkh.Anat. (Moskova). 75: 23
2. Gray S.Y., Benson I.A., Reinfenstein E.W., Spiro A.N., 1951. JAMA 147:1529
3. Kaplanski, A.S., Durnova G.H., Portugalov V.V. 1977. Biul.exp. Biol.Med., 4:485

PRELIMINARY ANNOUNCEMENT

IUPS COMMISSION ON GRAVITATIONAL PHYSIOLOGY
THIRD ANNUAL MEETING
SEPTEMBER 30 - OCTOBER 2, 1981
INNSBRUCK, AUSTRIA

The Third Annual Meeting of the Commission of Gravitational Physiology of the International Union of Physiological Sciences will be held in Innsbruck, Austria, September 30-October 2, 1981. The meeting will be in conjunction with a regional meeting of the German and Austrian Physiological Societies, which have kindly agreed to act as hosts.

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

The Commission Meeting will also comprise a one-day symposium by invited speakers on several topics in gravitational physiology. The topics will include graviperception in animals and plants, metabolic scale effects in mammals, physiological effects of restraint in animals, and recent results from the USSR Manned Flight Program and Kosmos Biosatellite Program.

It is planned to publish the Proceedings of the 3rd Annual Meeting in The Physiologist. As previously, the Proceedings will contain the contributed papers and symposium papers presented at the Meeting.

Your participation in the Commission Meeting is welcomed. If you are interested in the particulars, please complete the form below and send it to Dr. Orr E. Reynolds, Commission Business Officer, by January 15, 1981.

- -

Airmail to: Dr. Orr E. Reynolds, American Physiological Society,
9650 Rockville Pike, Bethesda MD, 20014, U.S.A.

- -

I wish further information about the 3rd Annual Meeting of the IUPS
[ ] Commission on Gravitational Physiology to be held in Innsbruck, Austria,
September 30-October 2, 1981.
[ ] I plan to attend.
[ ] I plan to present a paper at the meeting.
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THE NASA SPACE BIOLOGY GRANT PROGRAM

The objective of NASA's Space Biology research program has been to use gravity to solve relevant terrestrial biological problems and to enhance our capability to use and explore space. 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.

Goals

The goals of the Space Biology 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 effects of gravity on development, adaptation and evolution.

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 depend upon 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 Ca-mediated physiological mechanisms and in Ca metabolism?

4. What is the gravity sensing mechanism? How does it perceive information? How is the information transmitted to evoke a response?

5. How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology and behavior of organisms? Or how do gravitational and other environmental stimuli interact in their control and direction of living forms? Can the action of gravity be replaced by different stimuli?

Strategy

The strategy so far has been to manipulate gravity on earth and develop weightless simulation to: (a) develop and test gravitational hypotheses, (b) identify gravity sensitive biological systems and interacting environmental response mechanisms, (c) address valid gravitational biological questions on earth when possible, and (d) plan and design future space experiments. As longer flight missions, both manned and unmanned, become available more emphasis will be placed on flight experiments. Biological questions that require longer periods of micro-gravity will also be stressed.

Program Content

The program has been divided into the following three broad areas:

1. The role of gravity in development, maturation, senescence 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.

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 effects of gravity and other stimuli and stresses on the physiology 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:

Dr. Thora W. Halstead
Chief, Space Biology Program
Life Sciences Division
NASA Headquarters
Washington, D.C. 20546

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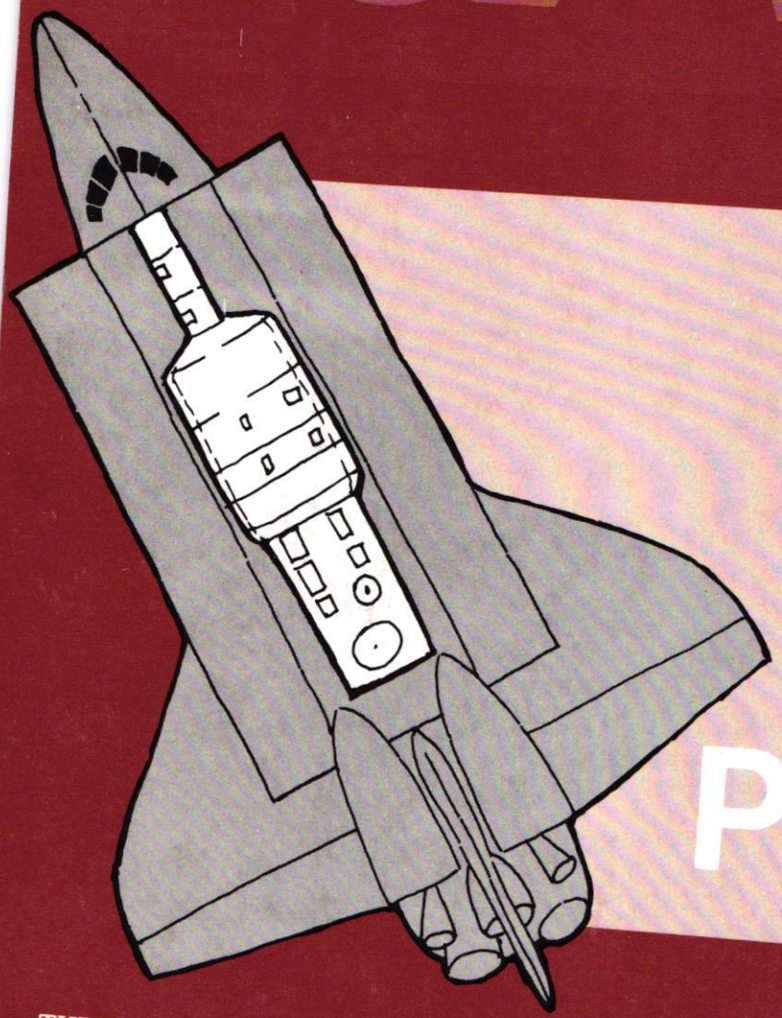

SPACE BIOLOGY GRANT PROGRAM

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