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THE EFFECT OF DYNAMIC FACTORS OF SPACE FLIGHT ON ANIMAL ORGANISMS

by

A. M. Genin, Editor

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16. Abstract

Results are presented in this book of the physiological, biochemical and morphological studies made on the Kosmos-782 biosatellite. The animals (rats) exposed on the biosatellite for 19.5 days were examined immediately after completion of the flight and also during the 25-day period of readaptation to Earth's conditions. Correlation of the data obtained is very important for understanding the mechanism of the effect of factors of space flight, primarily weightlessness, on the organism.

The book is of interest to specialists working in the field of space biology and medicine and also to a broad circle of scientists studying the effect of extreme factors.

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TABLE OF CONTENTS

Foreword 1
A.M. Genin

Conditions for Conducting the Experiment, Selection and Preparation of the Animals 3
L.V. Serova, Ye. A. Il'in, A.D. Noskin, V.I. Milyavskiy, N.A. Chel'naya, V.K. Golov, V.B. Pishchik

Condition of the Animals in Flight and in the Postflight Period

Temperature Homeostasis and Motor Activity of the Animals During Flight 10
V. Ya. Klimovitskiy, V.S. Oganov, Ye. A. Il'in, A.D. Noskin, V.V. Verigo, V.S. Magedov, L.M. Murashko, Yu. A. Rastopshin, S.A. Skuratova

General Condition of the Animals Immediately After Flight and During the Period of Readaptation to Earth Gravitation 14
L.V. Serova, N.A. Chelnaya, I.I. Britvan, Yu. P. Chivkunova

Feeding the Animals During Flight and Study of Certain Indices of Metabolism in Them 19
Yu. I. Kondrat'ev, N.A. Ilyushko, Ye. G. Besedina

Results of Postflight Studies

Nervous System

Morphological Study of the Central Nervous System 24
V.V. Portugalov

Higher Nerve Activity 25
N.N. Lifshits, Z.I. Apanasenko, M.A. Kuznetsova, Ye. S. Meyzerov

Certain Neurochemical Characteristics of Rats 28
O.G. Gazenko, N.N. Demin, A.N. Panov, D.A. Rashevskaya N.L. Rubinskaya, R.A. Tigranyan

Synaptic Excitability of Motor Neurons of the Spinal Cord of Rats 32
A.P. Gokin, N.N. Preobrazhenskiy, A.V. Shapovalov

The Composition of Solute Proteins of the Spinal Cord and Intervertebral Nodes 37
A.V. Gorbunova

Microelectrophoretic Study of LDH Isoenzymes of the Intervertebral Nodes 39
N.V. Petrova

Conclusion

Exchange of Substances

Total Gas Exchange and Energy Consumption 42
Ye. A. Kovalenko, V.I. Korol'kov, V.F. Zenin, V.L. Popkov, M.P. Bobrovnitskiy, A.M. Gordilenkova

Change in Certain Biochemical Indices of the Blood 42
R.A. Tigranyan, M.I. Belyakova, N.F. Kalita, L.B. Sochilina, N.G. Tuzova

Protein Synthesis *in vitro* in the Liver 46
L.D. Sabo, L. Kyeryeshi, Y. Kholland

Changes in Desoxyribonucleoproteides and the Quantity of Nucleic Acids in Certain Tissues 49
Ye. Mishurova, R.A. Tigranyan, K. Kropachova, M. Praslichka

Intensity of Synthesis of DNA in Certain Organs 54
F.T. Guseynov, I.A. Yegorov, Ye. S. Komolova, R.A. Tigranyan

Activity of Certain Enzymes in the Liver and Processes of Lipogenesis in Fatty Tissue 56
L. Makho, Sh. Nemesh, V. Shtrbak, R.A. Tigranyan

The Effect of Weightlessness on Lipids of the Plasma and Tissues 61
I. Alers, R.A. Tigranyan, Ye. Alersova, E. Paulikova, M. Praslichka

Conclusion 63

The Endocrine System

Reaction of the Cortex in the Myeloid Layer of the Adrenal Gland 64
R. Kvetnyanski, R.A. Tigranyan, T. Torda, D. Repchikova, Ye. Yakhnova, K. Murgash

Morphologic Studies of the Adrenal Glands 67
Ye. A. Savina, Ye. I. Alekseyev

Morphometric Studies of the Cortex of the Adrenal Glands at the Ultrastructural Level 71
S. Baran'ski, S.V. Baran'ska, Ye. A. Savina, V. Shimkovyak 71

Study in the Hypothalamus of Catecholamines and Enzymes, Their Synthesis and Decomposition 74
R. Kvetnyanski, R.A. Tigranyan, T. Torda, D. Babushikoba, Ye. Yaknova, N.F. Kalita, V. Gigash

Morphological Study of the Hypothalamus-Hypophiseal Neurosecretory System 78
Ye. A. Savina, Ye. I. Alekseyev

Thyroid and Parathyroid Glands 81
G.I. Plakhuta-Plakutina

Conclusion 84

The Muscle System

Cytochemical and Morphometric Studies of the M. Quadriceps Femoris 85
S. Baran'ski, V. Baran'ska, T. Rymashevska-Kossakovska, A. Slivinski

Morphometric and Cytochemical Studies of the Nerve-Muscle Synapses 93
S. Baran'ski, Ya. Godlevska-Yendzheychik, M. Martynyak

The Effect of Space Flight on the Skeletal Musculature and Nerve Apparatus of the Muscles (Morphologic and Cytochemical Study) 100
Ye. I. Il'ina-Kakueva, N.V. Petrova, V.V. Portugalov

The Effect of Space Flight on the Metabolism of the Skeletal Muscle Tissue 106
M.S. Gayevskaya, Ye. A. Nosova, R.A. Belitskaya, N.A. Veresotskaya, N.S. Kolganova, Ye. V. Kolchina, L.M. Kurkina

The Effect of Space Flight on Bioenergetics of the Skeletal Muscles of Rats 112
E.S. Mailyan, Ye. A. Kovalenko

Free Amino Acids of Muscle Tissue 114
A.S. Ushakov, T.F. Vlasova, V.B. Miroshnikova

Activity of Glycogensynthetase, Glycogenphosphorylase, the Content of Glycogen and Electrolytic Composition of Skeletal Muscles 117
V.N. Nesterov, Z.P. Zheludokova, L.A. Kuznetsova

Study of Functions of Skeletal Muscles in Experiments on Biosatellites 121
V.S. Oganov, A.I. Potapov

Protein Fractions and their Enzyme Activity in the Myocardium 128
M.S. Gayevskaya, N.A. Veresotskaya, N.S. Kolganova, Ye. V. Kolchina, Ye. A. Nosova

Histologic, Histochemical and Electron Microscope Study of the Myocardium 131
A.S. Kaplanskiy, K.D. Rokhlenko, P. Ye. Mul'diyarov, N.V. Petrova

Conclusion 133

Vestibule Motor Reactions, the Vestibular Apparatus

Static Endurance and Vestibular Motor Reactions 135
A.R. Kotovskaya, A.A. Shipov, G.S. Ayzikov, A.S. Markov

Structural and Functional Organization of the Vestibular Apparatus 142
Ya. A. Vinnikov, O.G. Gazenko, L.K. Tirova, A.A. Bronshteyn, V.I. Govardovskiy, R.A. Pevzner, F.G. Gribakin, M.Z. Aronova, T.A. Kharkevich, T.N. Tsirulis, G.A. Pyatkina, D.V. Lychakov, L.R. Palm'bakh, V.F. Anichkin

Conclusion 151

The Bone System

Quantitative and Qualitative Characteristics of Inorganic Substances in Certain Bones of the Skeleton 152
K. Ostrovski, A. Dzedzits-Gotslavska, V. Stakhovich

Mineral Exchange in Calcified Tissues 154
A.A. Prokhonchukov, A.G. Kolesnik, N.A. Komissarova, L.L. Novikov, N.N. Sirotkina

Changes in Bones of the Skeleton 158
V.S. Yagodovskiy, G.P. Gorokhova

Changes in the Mechanical Properties of the Bones of the Skeleton 167
G.P. Stupakov, V.V. Korolev

Conclusion 173

The Hemogenic System

The Hemopoietic Function in Weightlessness 174
V.N. Shvets

Morphologic Study of Lymphoid Organs 178
G.N. Durnova, A.S. Kaplanskiy

Conclusion 182

Immunologic Reactivity of the State of Autoflora

Reactivity of B-Lymphocytes of the Spleen in Relation to the Polyclonic Mitogen 182
I.V. Konstantinova, M.T. Martines, N.N. Sakayan, G.M. Zhuravel'

Microbiological Investigation 186
V.I. Korol'kov, V.M. Shilov, N.N. Liz'ko, L.N. Petrova

Conclusion 187

Internal Organs

The Digestive and Resorptive Function of the Gastrointestinal Tract 188
K.V. Smirnov, A.M. Ugolev, L.G. Goland, I.L. Medkova, N.K. Permyakov, R.A. Pechenkina, T.A. Sokolova

Structure and Function of the Parietal Cells of the Mucous Membrane of the Stomach 190
A.A. Pokrovskiy, M.M. Gapparov, I.A. Morozov, G.Yu. Mal'tsev

Morphologic Changes in the Stomach and Liver of Rats 195
A.S. Loginov, L.I. Aruin, V.V. Ul'yanova, V.S. Gorodinskaya, V.I. Yakovleva

Morphologic Study of the Small Intestine 199
R.A. Brodskiy, Ye.M. Parshkov, L.N. Bandurko, V.Ye. Vazilo, N.A. Pogudina, N.D. Yakovleva, A.N. Tsygankov

Histologic Examination of the Kidneys 205
A.S. Pankova

Histologic Examination of the Lungs 207
V.I. Yakovleva

Morphologic Study of the Testicles 209
G.I. Plakhuta-Plakutina

Conclusion 210

Conclusion 211
L.V. Serova

References 217

THE EFFECT OF DYNAMIC FACTORS OF SPACE FLIGHT ON ANIMAL ORGANISMS

A. M. Genin, Editor
Doctor of Biological Sciences

Foreword

The primary purpose of biological studies on rockets and /5* artifical Earth satellites was the obligation to protect the lives and health of man in such unusual conditions as weightlessness, cosmic radiation and other factors. This goal was achieved at the end of the 1950's and by 1961 the era of direct mastery of outer space by man had begun. Biological experiments have acquired a secondary value because it has been assumed that the most important practical problems can be solved by a method of multifaceted research of man in sequential flights which increase in duration.

However, difficulties have arisen in this approach because in the experiments made earlier on animals. the mechanisms of weightlessness and other flight factors affecting them were not discovered; only the general phenomenology of this effect was clarified. Therefore, progress in increasing the duration of flights for cosmonauts has been extremely slow and to a considerable degree blind. To make these studies on man was impossible due to the limitations of existing research methods. Due to this, at the beginning of the 1970's, again it became necessary to conduct a broad circle of biological experiments in space. Rats were used as the test animals. This made it possible to expose 25-40 animals in each flight and as a result to obtain more valid data.

After the flight, the animals were subjected to detailed biochemical and morphological studies; also physiological studies were used, particularly on the Kosmos-782 satellite. The results of physiological, biochemical and morphological studies of animals who had completed flight on the Kosmos series of biosatellites produced the material for this monograph.

Experimental data obtained on the Kosmos-782 biosatellite which was launched in December 1975 and remained in flight for 19.5 days was used as the basis. Research results on preceding biosatellites (Kosmos-605 and Kosmos-690) have already been published in periodicals and we will use them in this book only for comparison and discussion of experimental materials.

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*Numbers in the margin indicate the pagination in the foreign text.

Studies on the Kosmos-782 biosatellite were carried out with the broad cooperation of scientists from different scientific institutions of the Soviet Union, the Hungarian Peoples Republic, the Polish Peoples Republic, the Czechoslovakian Socialist Republic and the USA.

At the moment of launch of the Kosmos-782 biosatellite, an adequate amount of data had been accumulated which made it possible to concentrate attention of the researchers on the /6
study of the most urgent questions. In accordance with this, in this experiment those organs and systems of the organism were studied which can, to the greatest degree, show changes due to factors of space flight. In the book, materials on each such system are combined in different sections. Conclusions are written by A. M. Genin. The greatest attention was devoted to presenting factual data in order to present the reader with material for comparison and construction of hypotheses.

The basic results are presented in the conclusion of the book, questions are posed requiring study and problems for further work are noted.

A large collective of authors, specialists in different fields, representatives of science in the USSR, the Czhechovakian SSR, the Hungarian Peoples Republic, the Polish Peoples Republic and the USA all participated in the creation of this monograph. These participants are: Laureate of the State Prize of the USSR, academician O. G. Gazenko; Laureate of the State Prize of the USSR, corresponding member of AMN SSSR [Akademiya meditsinskikh nauk, SSSR, Academy of medical sciences, USSR] V. V. Portugalov, leading specialist in histochemistry of the nervous system; professor S. Baran'ski, director of the center of training for Polish cosmonauts; corresponding member of the Slovakian Academy of Sciences, L. Makho, famouns specialist in biochemistry of the endocrine system; L. D. Sabo, coworker of the Scientific Research Institute of radiobiology and radiation hygiene in Budapest, specialist in the field of the study of protein systhesis. A considerable part of the material was presented by coworkers of the Ames Research Center of NASA (USA): H. A. Leon, R. Ye. Grindeland, E. Kraft, E. M. Kholton, S. U. Esling, G. Vernikos-Dannelis, P. A. Braun and Ye. D. Philpott and others.

For convenience in presenting the factual material in the book, the following abbreviations and symbols have been adopted.

For the groups of animals: flight experiment -- F, synchronous control -- SC, vivarium control -- VC.

For indices of the degree of accuracy, variations (P): with the synchronous control -- P_{SC}, with the vivarium control -- P_{VC}.

Laureate of the State Prize,
Doctor of biological sciences
A. M. Genin

Conditions for Conducting the Experiment, Selection and Preparation of the Animals. /7

Conducting experiments with mammals on biosatellites has specific difficulties involving the need to provide for a long period of time in a completely automated experiment comfortable conditions for a large number of animals, maintenance of the gaseous atmosphere and microclimate, providing periodic supplies of water and feed, removal of waste, etc. For preparing experiments for the Kosmos series of biosatellites, different variations of systems for housing the rats in flight were developed and tested experimentally; these began with systems designed for special fixing of the animals relative to the food and drink containers, the source of light and the sanitation device and ending with systems with free housing of the animals in individual cages whose volume allowed them to change position without special effort. When testing the systems, they were evaluated not only for technical reliability of the separate units but also for their "biological reliability". The task of the experimenters came down to selecting systems for long term (30 days) housing in which the organism would have minimum deviation from the physiological standard so that when conducting the actual space flight, an analysis of the experimental material would not be complicated by the necessity to differentiate physiological factors of weightlessness from effects due to housing conditions of the animals on board. The results of several series of laboratory tests was the selection for the experiments on the Kosmos biosatellite of the BIOS system shown in Figure 1 and described in detail earlier (Serova, 1975; Il'in et al., 1976). Thirty-day housing of the animals in cages in this system did not inhibit growth; when studying the blood, lymphoid organs and adrenal glands, no signs of stress reactions were apparent; there was no change in the behavior of the animals. Moreover, during histologic and histochemical studies, atrophic changes were detected in certain muscles of the rear extremities similar to changes observed during hypokinesia but apparent to a lesser degree.

The BIOS system was used in experiments on the 605, 690 and 782 biosatellite Kosmos series and in all cases the conditions necessary for maintaining the life activity of the animals in flight were provided. Here, according to the results of the first two experiments in this system, certain technical developments and improvements were made providing improved sanitary and hygienic conditions for housing the animals. For the experiment on the Kosmos-782 biosatellite a set of equipment was created including the following elements:

five housing blocks (BIOS) for 25 rats with automatic supply of food and water, ventilation, assembly of the experiments and control of systems for the state of the animals in flight according to indices of breathing activity and body temperature; /8

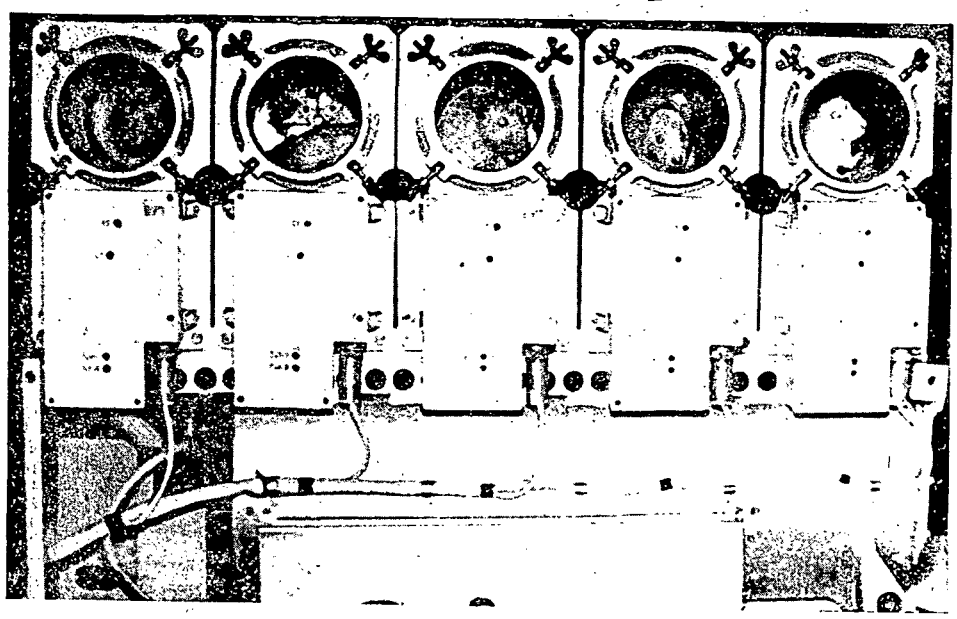


Figure 1. An overall view of the research block and the housing.

a regeneration system for purifying the atmosphere of carbon dioxide and harmful microadmixtures and for providing the animals with oxygen;

an analyzer for oxygen, moisture content and carbon monoxide in the atmosphere of the hermetically sealed containers with the animals;

a cooling and drying unit constructed in the thermal regulation system of the biosatellite for maintaining temperature and humidity conditions in the hermetically sealed container.

Mature male Wistar-SPF rats (free of pathogenic factors) obtained from the breeding nursery of the Institute of Endocrinology of the Slovakian Academy of Sciences (Bratislava) were used for the experiment. During the selection period, the animals were housed in a vivarium in cells made of polypropylene with dimensions 40 X 30 X 18 cm, with 3-4 animals per cage. The light day lasted for 12 hr. Air temperature in the vivarium varied within limits of 23 $\pm$ 1°, relative humidity was from 60 to 70%.

Selection of the animals was made in two stages. The problem of the first stage was to evaluate the general resistance and reactivity of all the batches of animals; the problem of the second stage was individual selection.

Results of observation of the general condition and behavior of the animals in the vivarium and also the results of pathological and anatomical discoveries and special laboratory studies showed that the Wistar-SPF rats have definite advantages

over the Wistar rates from the Stolbovaya breeding nursery used in the experiments conducted earlier. The animals, free of pathogenic factors, were less susceptible to different infectious diseases and also had a smaller individual spread in physiological and biochemical indices. Besides the fact that these animals usually are housed in adequately comfortable conditions, experience in using automated systems of life support was absent and therefore it was impossible to exclude the fact that in a number of situations they were weaker than the ordinary Wistar strain animals. However, it was established that the Wistar-SPF animals did not differ from the Wistar strain of rats from the Stolbovaya breeding nursery in their resistance to hypoxic hypoxia evaluated during their stay at an "altitude" of 12,000 m and according to statistical endurance with a sampling of six (Shipov, Markin, 1977). No differences were detected between the two groups of animals in their reactions to experimental hypokinesia evaluated according to general conditions and according to the degree of the stress reactions found in these conditions (change in weight of the lymphoid organs and adrenal glands, changes in the blood). All of this made it possible to conclude that it was possible to use the Wistar- SPF strain of rats in the experiment on the biosatellite. /9

The criteria of individual selection were the clinical condition of the animals evaluated using the results of otoscopy and microbiological studies; rate of growth; the picture of the peripheral blood; behavior of the animals evaluated by observation in the vivarium and in a closed labyrinth proposed by Ya. Dombrovskiy (1966). In accordance with the preflight program, the animals became accustomed to the body temperature transmitters and the injections necessary for certain subsequent studies were made. Below, the measures taken in the preflight period are listed.

Measure	Time before launch, days
Attachment of the body temperature transmitters (intraperitoneally; weight -- 3.5 g; dimensions -- 35X15X6 mm)	19
Injections	
C-14-glycine (intraperitoneally)	15.5
Declomycin (intraperitoneally)	3
Dead culture (in the plantar padding of the rear extremity)	6
Transfer from vivarium feed to a special ration,p. 19)	12

Measure	Time before launch, days
Beginning of training	11
Housing in the BIOS cages	20

During flight the animals were housed in individual cages of cylindrical shape 26 cm long and 9.5 cm in diameter. Each cage (Figure 2) was equipped with a feed trough, drinking bowl, lighting fixture and sanitation device whose design made it possible to collect and store waste from the animals separately for each two day period up until the end of the flight experiment. Five of the cages functioned as a single block.

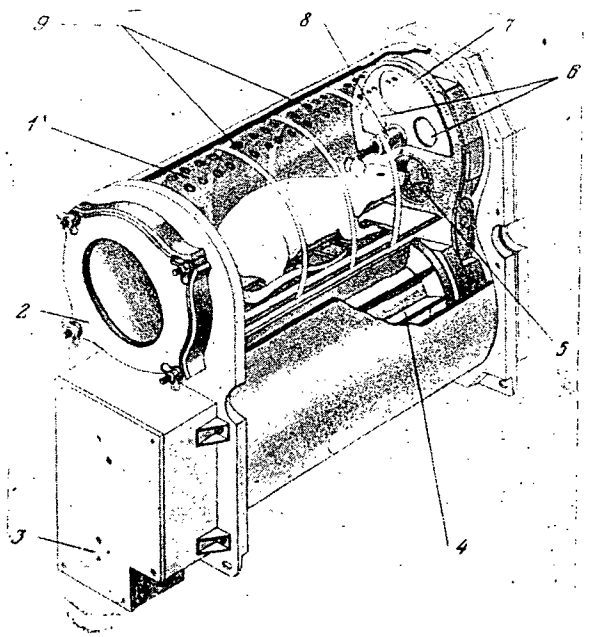


Figure 2. A cell for individual free housing of the animals /10

1 - cage with filter;
2 - landing hatch;
3 - reinforcement and conversion block;
4 - collector of body waste;
5 - feed container;
6 - illumination cartridges;
7 - device for removing the tail;
8 - drinking bowl;
9 - ventilation apertures.

Control of the animals in flight was accomplished by their spontaneous motor activity; for five of the animals it was done according to body temperature.

The light day in flight lasted for 12 hours, from 0800 to 2000. The animals received 40 g of paste type feed in the form of four separate portions during the course of 24 hours at 0200, 0800, 1400 and 2000 hours.

The air temperature in the housing area for the animals varied within a range of 20-22 degrees, on certain days in the first third of the experiment, the temperature dropped below 20°; at the end of the experiment a brief increase in temperature to 24-25° was noted (Figure 3).

Relative humidity varied within a range of 40-65% (Figure 3). The partial pressure of oxygen varied in a range of 150-210 mm mercury column; on the second day of flight, a brief increase in pO_2 occurred to 250 mm mercury column (Figure 4). Partial pressure of carbon dioxide varied in a range of 1-8 mm mercury column (Figure 4).

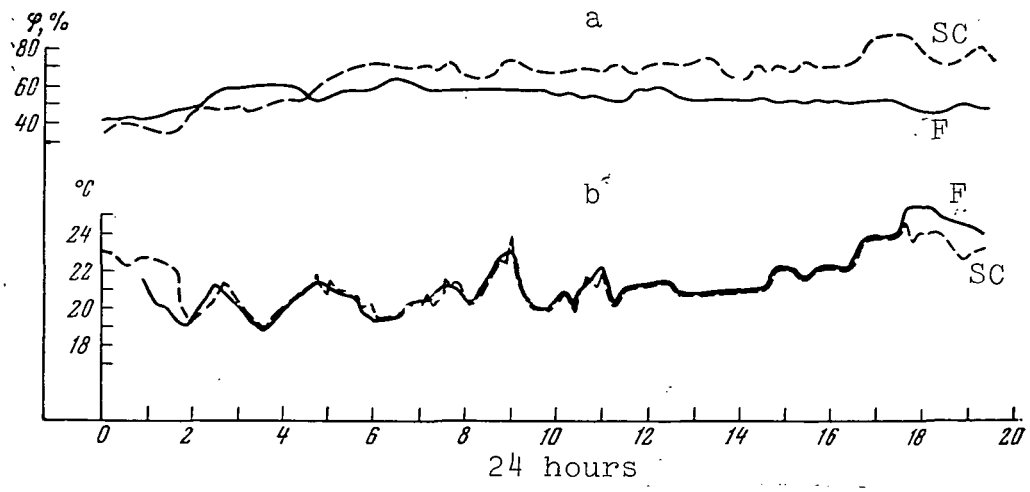


Figure 3. Change in humidity (a) and temperature (b) in the atmosphere of the hermetically sealed containers with the animals.

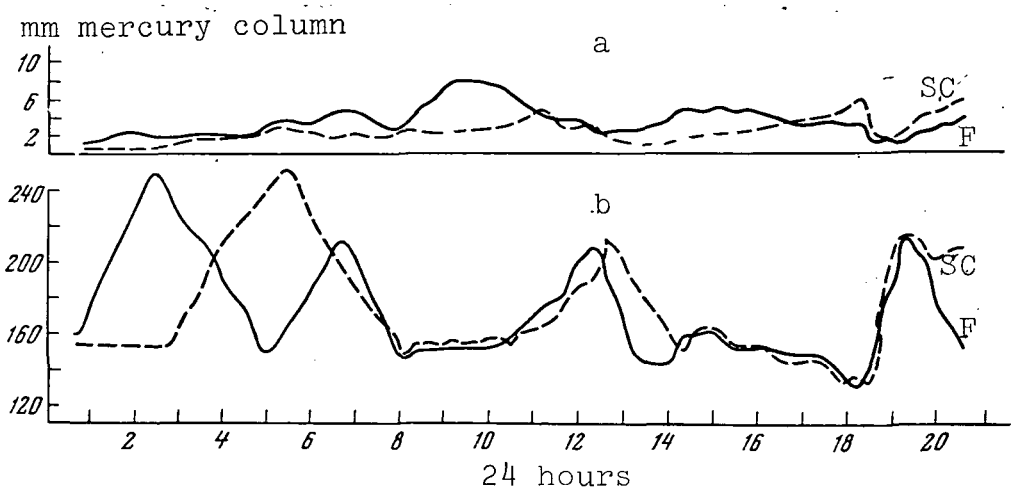


Figure 4. Change in partial pressure of carbon dioxide (a) and oxygen (b).

The results of the flight experiment were compared with the results of two control groups, the vivarium and synchronous controls. The animals in the vivarium control were housed in the conditions described above in housing where animals of the entire group had been kept during the selection period. They received the same paste type feed as the animals on the biosatellite but as a single portion in 24 hours (at 0900-1000 hr.). /10

The synchronous control experiment was conducted in a mock-up of the ascent vehicle of the biosatellite. Its purpose was to differentiate the effects of weightlessness from the

effects of accompanying factors. The following physiologically significant effects were modeled for the synchronous experiment.

In the "introductory section":

vibration at 50-70 Hz (amplitude 0.4 mm, time 10 min);

acceleration four units (time 10 min, plateau 7 min); /11

Noise 110 db (time 10 min).

During the experiment:

housing the animals in cage systems providing life activity;

supplying food and water according to the standard cyclogram;

maintaining the parameters of the microclimate similar to those in the cabin of the biosatellite in flight.

In the "landing section""

impact loads of 50 units - 10 s;

linear acceleration 6 units - 5 min, plateau - 3 min.

Twenty-five animals were used in the flight and synchronous experiments and 40 animals in the vivarium control group. The age of the rats in all three groups was 63 days before beginning the experiment. Data on weight is given in Table 1. /12

TABLE 1. AVERAGE WEIGHT (IN GRAMS) OF THE RATS BEFORE AND AFTER FLIGHT (M ± m).

Group	Preflight	Immediately after flight	Growth	25 days after flight
F	212±1,5	255±1,9	43±1,9	321±4,7
SC	213±5,2	283±4,2	70±2,3	322±6,0
VC	217±4,4	276±3,3	59±2,5	322±11,0

After completion of the flight and synchronous experiments, the animals were examined in two time periods: after 5 to 11 hours and after 25 days (Table 2).

[Note: Commas in Table 1 and all subsequent Tables are equivalent to decimal points.]

TABLE 2. TIME PERIODS AND DATES OF KILLING THE EXPERIMENTAL ANIMALS

Group	First Killing		Second Killing	
	Date	Time after completion of the experiment, hours	Date	Time after completion of experiment, days
F	15.XII 1975	5—7 и 9—11	10.I 1976	25
SC	20.XII 1975	5—7 и 9—11	15.I 1976	25
VC	21.XII 1975	5—7 и 9—11	12.I 1976	22

The several hours during which the first killing lasted can be important for the degree of changes shown in the animals killed at the beginning and end of this period. In order to keep the effect on results of this factor to a minimum, in each group of rats killed in the first time period, they were divided into two subgroups. The first group contained six animals who were killed after 5 to 7 hours; the second group contained six animals killed after 9 to 11 hours. Making a time period for the first killing more precise was taken into account by the experimenters when interpreting the data and when necessary to indicate it in the material presented.

Work with the animals in field conditions where the landing unit of the biosatellite came down was done in a special laboratory complex and was carried out in adequately comfortable conditions with an air temperature of 20-23°. The material obtained when killing the animals, depending on the purpose of the further studies, was placed in histologic fixing agent or in liquid oxygen (or dry ice) and transported to the laboratory.

The remaining animals for studying the readaptation period for Earth's gravitational field were housed in a vivarium under the conditions described above. The rats who had returned from space were housed in one of the cages with dimensions 40X30X18 cm; the animals from the synchronous and vivarium controls, 3 and 4 to a cage. All of the animals continued to receive paste-type feed; the daily ration in this period was increased to 45 g which was given once a day.

Condition of the Animals in Flight and in the Postflight Period /13

Temperature Homeostasis and Motor Activity of the Animals During Flight

Physiological control of the condition of the animals during flight on the Kosmos-782 biosatellite was done by recording motor activity and body temperature.

It was expected that the conditions of thermal balance of the organism in weightlessness would be different than in Earth conditions due to changes in the structure of muscular energy consumption -- the absence of cognitive-tonic work and decreased force load during motor activity. The possibility of involving the system of thermal regulation in the total complex of reactions of the organism to weightlessness had been considered earlier (Klimovitskiy, 1972) -- due to the results of an orbital experiment on monkeys made by American scientists (Hahn et al., 1971; Meehan, 1971).

The dynamics of total motor activity were used in this experiment as the criterion of behavioral adaptivity of the animals. The distribution of levels of motor activity and body temperature in a 24 hour cycle also made it possible to draw conclusions as to the presence of normal organization of the circadian rhythm. The material obtained can be used for solving a number of theoretical questions applying to energetics during motor activity and temperature homeostasis of the organism in weightlessness.

Measurement of body temperature in five rats during flight and in the same number in the synchronous experiment was done using transmitters attached to the abdominal cavity of the animals. The transmitter contained a power source, a microcircuit and thermistor. It was established in preliminary studies that the presence of the transmitter in the peritoneal cavity of the rats did not affect the condition of the surrounding tissue or the general condition of the animals. The total number of motions was recorded in each of the 25 animals in flight and in the synchronous control in the form of voltage level. During an analysis of motor activity (MA) the flight data were compared with the results of preflight observations and with the data of the synchronous experiment. Inasmuch as the capacity of the memory cell on the recording channel, as was discovered during experiment, was inadequate for storing the entire range of possible values of motor activity, the frequency of filling the memory cells of the recording system in each measurement session was used as the index.

Both body temperature and the MA were recorded every two hours; however, the first index is only on the even and the second on the odd days of the experiment.

The average daily values of temperature in the rats in flight progressively decreased from the start of the experiment, reaching a minimum on the sixth day (Figure 5). In all of the /14

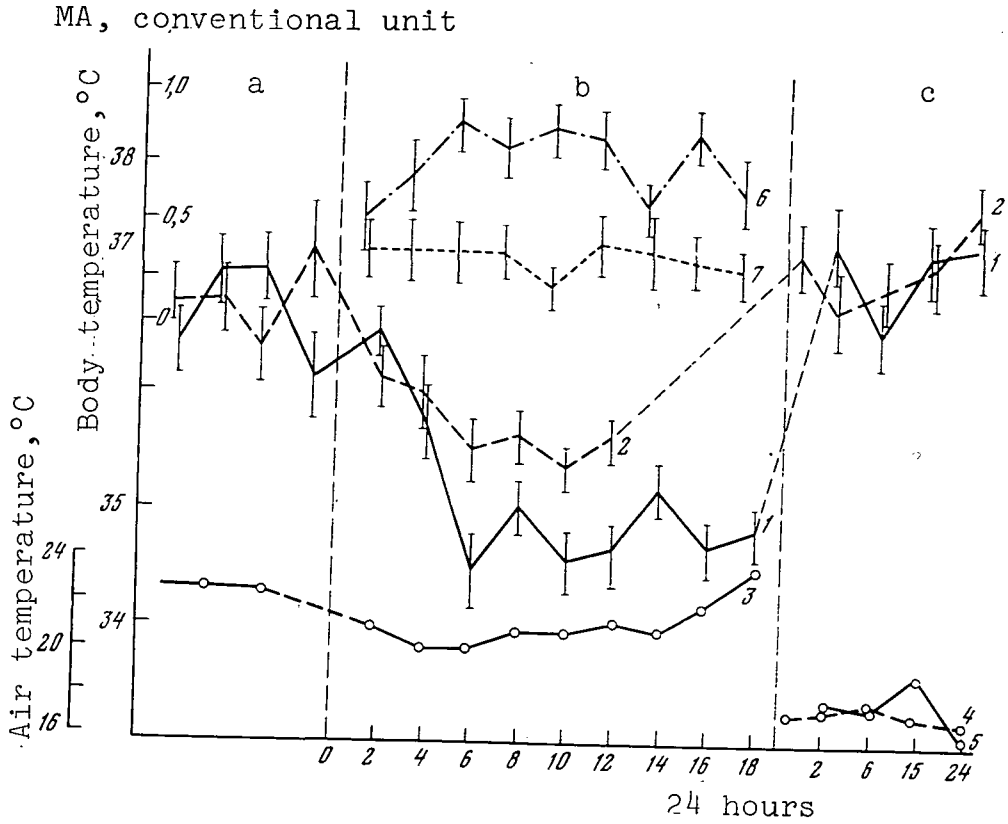


Figure 5. The dynamics of average daily body temperature and motor activity (MA)

a - background; b - flight; c - postflight; body temperature of the rats: 1 - flight group; 2 - synchronous control group; air temperature: 3 - in the cabin of the biosatellite, 4 - in the containers for the flight group of animals; 5 - for the animals in the synchronous control group; motor activity of the rats; 6 - flight group; synchronous control group.

rats on the 2nd-6th days, body temperature was decreased on the average by 1.5-1.7°; in some animals it decreased by 3-3.5° in relation to the initial level. During this same period, the lowest values of air temperature were noted in the cabin of the biosatellite. In subsequent flight days, interior temperature increased somewhat. At the same time the average body temperature of the rats increased. However, beginning on the sixth

day and up to the end of the flight, this index did not return to normal and remained lower than in animals of the synchronous control group in spite of the fact that in the latter one also observed a certain decrease in body temperature.

The average daily total MA of the animals in the flight experiment proved to have a higher level than the index for the synchronous control group. This difference was maintained for the entire length of the experiment except for the first days. In contrast to body temperature, the total MA in the initial /15
period increased monotonically to the fifth day and then was maintained at the increased level with two local minimums on the thirteenth and seventeenth days. It was established that with a shift in one day (due to technical conditions of reporting) there was complete inverse correlation averaged for each 24 hour value of total motor activity of all 25 animals in the flight group and the average daily values of body temperature of the five rats in the same group (Figure 5).

The results of spectral correlation analysis of the materials of the flight experiment make it possible to conclude that the animals had a marked variation in body temperature and motor activity in a 24 hour period. Apparently a less marked 6-hour rhythm occurred caused by periodicity in supplying the food. A more noticeable rhythm was observed in periodic changes of motor activity in the synchronous experiment where the comparatively low level of this index makes it possible to differentiate the corresponding extremes.

Distribution of the phases of change of both parameters in 24-hour periods was the same: both body temperature and motor activity were higher during the dark part of the 24 hours and lower when the lights were switched on. After completion of the flight and synchronous experiment, temperature measurement of the animals in both groups was continued with the same instruments on days 2, 6, 15 and 24. The temperature level did not differ from the initial, however, the 24-hour rhythm was not observed with continuous 24-hour observation of body temperature and motor activity.

Calibration of the body temperature transmitters removed from all the animals in the experimental and control groups makes it possible to conclude that the actual body temperature of the animals in flight was no higher and in the synchronous experiment no lower than that observed. In this way, body temperature of the animals in the area of the transmitters during flight was lower than in the rats in the synchronous experiment. The following variations in interpreting this result are possible. "The regulating point" (Hardy, 1972) of the thermal regulation system could not be changed but the power of the compensatory mechanisms maintaining body temperature around this level was inadequate.

As was expected, the amount of motion of the rats in weightlessness was higher. In this situation, heat insulation of the body decreases (Khaskin, 1975). Moreover, it is possible that in unsupported space, realization of a compact position characteristic for a rat during sleep was difficult. It is pointed out that the muscle work in motor acts can replace incontractible thermogenesis and chills with a tendency toward cooling (Bazhenov, 1971). But physical activity is the least effective in adapting to the effect of myogenic mechanisms for maintaining body temperature (Khaskin, 1975). Perhaps, due to these circumstances, an increase in motor activity seeming paradoxical at first glance, in the final analysis intensifies hypothermia which is reflected by the inverse correlation of the level of total MA and the average daily body temperature.

The hypothesis as to the stress activity of physiological mechanisms of restoring the normal body temperature level is supported in this case by a number of objective symptoms of cold adaptation (decrease in the rate of weight gain, activation of lipid metabolism, increase in requirement for oxygen), observed in other studies made simultaneously. /16

As is well known, rats generally are capable of maintaining a close to normal body temperature at extremely low environmental temperatures (Hart, 1957). However, the most important component of adaptation to conditions in which chilling is possible is the cognitive control of heat exchange. The impossibility of using this factor in combination with the absence of cognitive-static work can result in the development of hypothermia. This has to mean that the effective comfort zone (taking into account required position) for rats in weightlessness shifts toward higher temperatures.

One can also allow that the "regulating point" of thermal regulation in animals in flight shifted downward. The normal combination of MA in body temperature in the circadian cycle and retention of a dynamic range usual for the rats in regulating body temperature during a 24-hour period (1.5-2.5°) shows that thermal regulation remained physiologically a control process, however, regulation occurred at a lower level. The organism maintains temperatures corresponding, as a rule, to the power generated by them. Paying attention to characteristic changes in the muscles noted in other similar experiments, one can propose that in spite of the larger number of motions completed, the power developed by the muscular system of the rats in weightlessness was smaller on the average than in the synchronous control rats. Full atony of the muscles at rest and in the lowest force load during motion theoretically are an adequate basis for a certain downward shift of the range of working temperatures. But if the actual body temperature corresponds to the "regulatory point" there is no reason for the appearance of reactions to cold adaptation and then the decrease in the rate of growth, mobilization of the lipids and increase in the requirement for oxygen would require a different explanation.

Finally, it is possible that the shift of the "regulating point" actually occurred, but the actual body temperature, particularly in these zones, was even lower. After the period of significant hypothermia, the average daily body temperature increased somewhat and was even maintained at higher values. This does not exclude the fact that the level reached expressed the final state of balance for the studies considered. Settling this can be done only with a longer stay of animals in orbit. It is very possible that the energy cost of functions in this state was higher.

As has already been noted, a certain decrease in body temperature occurred in the animals in the synchronous control group. It is possible that the cause for this was the housing conditions, in particular, limitation in mobility, the absence of collective thermal regulation, the presence of an air current and the presence of the mass of metal. However, the changes in body temperature observed in this group were proven to be less significant than those recorded in flight.

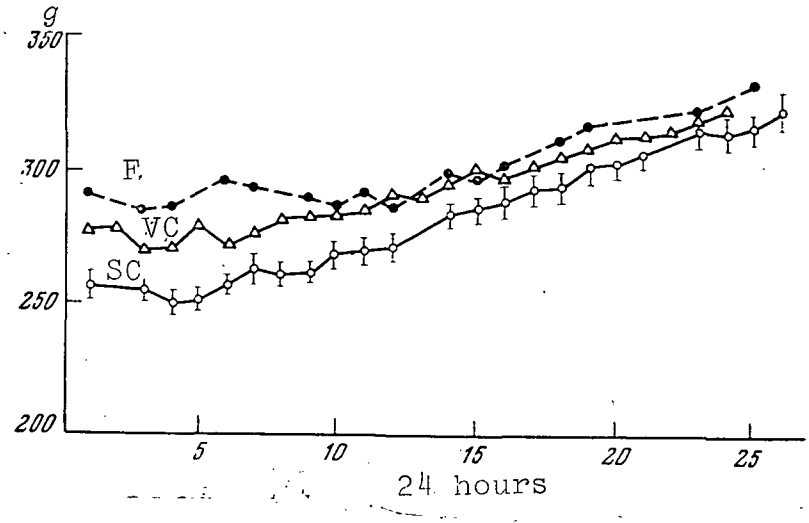
General Condition of the Animals Immediately After Flight and During the Period of Readaptation to Earth Gravitation. /17

Examination of the animals and cages directly in the landing area for the descent vehicle of the Kosmos-782 biosatellite made it possible to evaluate the conditions for housing the animals in the flight as being completely satisfactory. The cages were adequately clean, the walls and floor were hardly contaminated with fur or food residue. The general condition of the animals was completely satisfactory: the visible mucous membranes and skin where not covered by fur was a light pink color, clean, without damage. Average contamination of the fur was observed in only some of the rats. Moreover, in the first flight days attention was given to flabbiness, a decrease in general tone and motor activity of the animals; regardless of the health regime they were observed for functional hypokinesia which made readaptation to Earth's gravitational force after weightlessness easier. We observed this same picture after the experiment on the Kosmos-605 biosatellite (Il'in et al., 1976). It is interesting that similar phenomena, physiological limitation of mobility, was observed by Bengele (1969) in rats when changing from Earth's gravitational force to hypergravitation (continuous rotation on a centrifuge at 1.7 and 3 *g*).

Before the beginning of the experiment on the biosatellite, the average weight of the animals in the entire group was approximately the same. After 19.5 days, the animals who had been in flight weighed 43 *g* and the animals in the vivarium control 59 *g*; but the animals in the synchronous control weighed 70 *g* (see Table 1). Thus, the animals in the flight group lagged behind the animals in the vivarium control in weight and

to a greater degree behind the rats in the synchronous control group. Here the requirement for food by the animals in flight and the ground synchronous control was practically identical but assimilation of food in the animals in the flight group was even higher than in the control.

Figure 6 shows the weight dynamics of animals in the test and the two control groups for the 25 day period readaptation.



/18

Figure 6. Dynamics of animals in the period of readaptation to Earth's gravitational force.

Attention is given to the fact that in the first few days after /17
flight the weight of the animals in the test groups was practically unchanged. A similar picture was observed earlier in the experiment on the Kosmos-605 biosatellite (Serova, 1975). In both cases, the animals in the first few days after space flight did not have any sharp increase in weight which usually is observed after flight in man which is due to compensation for the water deficit which occurs in flight. In distinction from this, the weight of the animals in the first postflight days not only did not increase but in certain cases even dropped. Here one must note that the rats, a constantly growing animal, have a weight loss in conditions of weightlessness causing a lag in growth because of this.

The lag in growth in the animals in the vivarium control group in the first days after completion of the experiment obviously involved the large load caused by numerous and varied physiological studies conducted in this period. After the experiment on the Kosmos-605 biosatellite, the volume of studies /18
in the readaptation period was considerably smaller, no lag in growth in the animals was detected in the vivarium control animals. Instability in weight dynamics on the sixth day of

TABLE 3. CERTAIN ERYTHRON INDICES IN THE POSTFLIGHT PERIOD (M±m)

Time after flight, 24 hours	Group	No. of animals	Hemoglobin, g%	Erythrocytes, mln/mm^3	Reticulocytes 0/00	Resistance of erythrocytes (in % of whole erythrocytes) to solutions of NaCl concentrations.				
						0.48%	0.50%	0.52%	0.54%	0.60%
2nd	VC	10	11,4±0,6	6,1±0,14	28±3,3	12,8±1,6	20,6±3,0	28,7±3,7	37,2±0,54	92,9±2,4
	F	12	12,0±0,5**	6,3±0,14**	27±1,1	10,9±1,3**	18,0±1,8**	29,0±2,9**	—	86,0±2,3**
	SC	13	13,1±0,1	6,7±0,10	24±1,1	22,6±2,8*	35,5±4,1*	48,9±3,9*	59,0±3,7*	95,7±2,5
5th	VC	13	11,5±0,1	6,1±0,06	28±2,7	14,6±1,7	22,2±2,0	34,5±4,1	49,3±4,0	91,9±1,5
	F	12	12,6±1,3	5,9±0,20	25±1,7	26,9±2,2*	40,5±4,0*	55,9±4,3*	69,0±4,3*	95,4±0,6*
	SC	10	11,3±0,8	6,2±0,20	28±2,4	29,6±4,4*	42,7±5,5	50,0±5,8*	59,3±5,6	88,0±3,2
10th	VC	12	12,5±0,3	6,5±0,10	26±1,0	13,5±2,3	23,3±4,0	35,7±5,3	52,2±5,3	91,6±2,9
	F	12	12,8±0,1	6,6±0,09	25±1,0	21,1±4,4	31,0±5,6	40,5±6,5	53,3±6,8	90,5±3,5
	SC	—	—	—	—	—	—	—	—	—
20th	VC	13	13,7±0,3	7,0±0,20	24±1,9	8,9±1,2	14,7±1,7	23,8±2,9	40,4±5,3	84,9±2,9
	F	11	12,8±0,1*	7,0±0,08	26±1,1	18,9±2,0*	29,7±3,0*	43,1±4,2***	57,0±3,8***	94,1±1,4***
	SC	11	12,4±1,3	6,9±0,05	26±1,5	14,2±3,1	18,6±3,7	25,5±4,3	34,8±4,2	84,2±3,1

*Proven difference with VC. **Proven difference with SC. ***Proven difference with VC and SC.

[Commas in the tabulated material are equivalent to decimal points.]

the readaptation period was noted in the rats of the synchronous control group due to the appearance of sick animals in this group (4 out of 11). Beginning on the fifth to seventh days of the readaptation period, the weight of the animals who had returned from flight increased constantly and by the 23rd day their lag behind the control group had been equalized. On the second day of the readaptation period, no significant changes were detected in comparison with the two control groups in the concentration of hemoglobin, erythrocytes and reticulocytes in the peripheral blood or in the resistance of the erythrocytes to osmotic hemolysis in vitro (Table 3). On the 5th, 10th and 20th days of the readaptation period the animals who had returned from flight had increased resistance of the erythrocytes to osmotic hemolysis which is due, probably, to adaptation of the erythroid hemogenesis during the readaptation period, and consequently, to regeneration of the peripheral link of the erythron. Immediately after flight, suppression of erythropoiesis was found in the bone marrow and spleen (see pages 174 and 178).

Attention should be directed at the high stability of the erythrocytes in the synchronous control animals involving the most rapid acute hypoxia which, due to failure of technical systems, part of the animals of the synchronous control were subjected to before the experiment began.

During analysis of leukocytes of the peripheral blood 5 to 6 hours after the flight, a picture was observed which is typical for stress reactions of an organism: an increase in the percentage of segment nucleus neutrophils, lymphopenia and eosinopenia (Table 4). Here, the total number of leukocytes

TABLE 4. THE NUMBER OF LEUKOCYTES OF THE PERIPHERAL BLOOD DURING THE READAPTATION PERIOD. /20

Group of animals	Time period of the test	No. of animals	No. of leukocytes, $1000/mm^3$	Segment-nucleus neutrophils, %	Lymphocytes	Eosinophils of cells/mm
VC	—	13	7,7±0,3	16±1,4	76±1,4	112±6.2
F	5–7hr	15	5,8±0,9	54±3,2	41±3,2	0
	2 days	12	6,1±0,5	17±1,6	75±1,8	51±6
SC	5–7hr	13	10,6±0.7	30±3,9	63±4,1	96±10
	2 days	13	3,3±0,4	23±2,6	70±2,7	114±10

in the blood was not increased. In the synchronous control animals one observed only an insignificant increase in the percentage content of lymphycytes and eosinophils. The total concentration of leukocytes was higher than in animlas of the flight group and the vivarium control group although it did not go beyond the limit of physiological standards (Table 4). /18

When examining the animals on the second day after completion of the flight, one observed a practically complete normalization of all the indices. Eosinopenia was retained in this time period and only on the fifth day was the number of eosinophils normalized. /20

In the experiment on the Kosmox-605 biosatellite, the first analysis of the blood was made on the second day after completion of flight; here, besides neutrophilesis and lymphopenia, one observed a tendency toward an increase in the total concentration of leukocytes; this picture lasted to the fifth to seventh days after flight. The less marked stress reaction in the experiment on the Kosmos-782 biosatellite can be due both to the improved conditions for housing the animals in flight and to the peculiarities of the different populations of animals.

The results of observations on the general condition and behavior of the animals returned from flight and also experimental materials obtained when examining them make it possible to draw conclusions as to the adequately favorable course of the period of readaptation to Earth's gravitation. Only in the very first days after flight did we observe changes in animals involving the process of readaptation to Earth's gravitational course; this primarily is a lag in growth in the first six days and also the limitation of motor activity, regardless of health conditions which the animals created after flight. From the first days of the period of readaptation, gradual normalization of the state of the organism began, ending on the 20th to the 25th days. Here one should note that having reached the normal, separate functional indices did not go beyond the limit, that is, normalization was stable. Discomfort due to return to Earth in organisms adapted to weightlessness, under conditions of Earth's gravitation, are perceived only in the first days after flight and later on in the period of readaptation one did not note functional phenomena which would make it possible to show a chronic stress effect.

Speaking of the favorable conditions of the animals after flight, one should remember that all of the studies we are talking about were done in a state of rest. Moreover, the general volume of activity of the animals who had undergone space flight was less than in the control and mainly this adaptive reaction, of physiological limitation of mobility, apparently provided the organisms with the capability for maintaining homeostasis and retaining physiological standards for

most of the organs and systems in the readaptation period. /21
Observations of the clinical and physiological state and behavior of the animals after flight, and also the reaction to random injury (sluggish course of inflammation, slow healing of wounds, etc.) permits one to assume that the maintenance of homeostasis at rest requires a considerable stress on the organism of the animals who have undergone flight. This does not exclude the fact that the regulatory mechanisms mobilized by the organism during adaptation to weightlessness and readaptation to the force of Earth's gravity adequate for maintaining homeostasis at rest appeared to be inadequate for retaining its biological capabilities under stressful activity. For testing this hypothesis, it is proposed that one evaluate the state of the organism returned from weightlessness as the basis for further studies not in a state of rest but in different load situations, as well as an evaluation of the degree of stress of physiological functions maintaining homeostasis under load.

Feeding the Animals During Flight and Study of Certain Indices of Metabolism in Them.

A special ration balanced according to the physiological requirements of rats was used for feeding the animals. The ration was a paste type mass including easily assimilated food polymers: milk casein, corn starch, sugar, sunflower oil fortified with vitamins A and D, a preparation of dry brewer's yeast, a saline mixture and water. Most of the components listed were prepared so the food had maximally high adhesive properties (Kondrat'yev, 1973). The composition of the daily ration is presented below.

	For one rat		For one rat
Protein, g	3.06	Vitamins	
Fats, g	1.79	B_1, µg	64.8
Carbohydrates, g	9.61	B_2, µg	62.4
Mineral substances, mg		B_3, µg	240.0
		B_6, µg	50.5
Na	60.9	PP, µg	493.6
K	67.1	E, µg	1380.0
Cl	20.4	A, IU	60
P	86.3	D, IU	6
Ca	84.3		
Fe	3.19		
Zn	0.08		

With a daily standard of 40 grams and moisture content 60% (16 g of air dried substance) the calorie content of the ration was 68.7 kcal.

Sorbic acid was used to preserve the food ration and drinking water which were used in the life support system. The food containers were pasteurized at a temperature of 100°. The constancy of the chemical composition of the feed and also its paste like consistency, preventing loss of food during eating, made it possible with adequate precision, to carry out balance studies when investigating metabolism. /23

During background studies and during experiments on life support systems the rats received approximately 40 g, and in the readaptation period, approximately 45 g of food per 24 hours. The amount of food required by the animals when housed in the life support system was determined according to the difference in weight of the food containers before the experiments and after their completion and also during operation of the pumps supplying the food. An individual collection of solid excrement made it possible to determine the dynamics of generation of life support products and assimilation of food as a whole during the flight.

On days 1, 3-5, 11-12 and 21-22 after completion of the flight and after ground control experiments, metabolism was studied in 5-7 animals from each group. For this purpose they were placed in special cages (Il'ushko et al., 1975) which made it possible to have an individual count of the amount of food eaten, water drunk and also separate collection of solid and liquid excrement. /24

The content of nitrogen, potassium, sodium, calcium and magnesium were determined in the food, urine and solid excrements. In the food and solid excrement, also the content of moisture, fat and salts was analyzed. The content of carbohydrates was determined by a calculation method. The nitrogen was determined according to Kjeldahl using a Konvey cup, the fat by extracting in a Soxhlet unit, salts by burning the suspension in a muffle furnace, the content of potassium, sodium, calcium and magnesium by a method of flame spectrophotometry (Pokrovskiy, 1969).

The animals in flight and in the ground control experiments did not differ in the quantity of food required. Assimilation of the food by the rats in flight (97.0±0.1%) proved to be 1% higher than in the synchronous experiment (96.0±0.1%); this difference is statistically proven ($P<0.01$). This aspect of differences in assimilation of food was observed in the experiment on the Kosmos-605 biosatellite. Assimilation of food in the flight animals remained somewhat higher than in the control animals and in the period of readaptation (Table 5).

The nitrogen balance in the animals of all groups in all the study periods was positive; in the flight animals it basically exceeded the level of the control rats and amounted to 203.8-260.5 mg/24-hrs.

TABLE 5. THE QUANTITY OF FOOD REQUIRED (IN g, CALCULATED FOR THE DRY SUBSTANCE) AND ITS ASSIMILATION (IN %) IN THE READAPTATION PERIOD. /22

Group of Animals	Background		1-5 Days	
	Requirement	Assimilation	Requirement	Assimilation
F	14,52±0,58	96,0±0,60	16,74±0,13	96,4±0,38
SC	14,64±0,0	96,0±0,43	16,16±0,49	96,4±0,34
VC	14,79±0,20	96,1±0,30	16,77±0,10	94,6±0,24

TABLE 5 (continued)

Group of Animals	11-12 Days		20-21 Days	
	Requirement	Assimilation	Requirement	Assimilation
F	17,02±0,08	96,6±0,45	16,64±0,17	97,10±0,32
SC	14,82±0,70	95,6±0,55	16,40±0,20	96,4±0,48
VC	16,7±0,24	96,6±0,30	16,69±0,14	95,8±0,56

At the beginning of the readaptation period, the animals in the flight group showed a greater requirement for water and a decrease in its elimination in comparison with the control groups (Table 6). /24

The balance of sodium, potassium, calcium and magnesium in the readaptation period in the animals in all groups was positive (Table 7). Changes in the balance of sodium were determined to a considerable degree by conditions for housing the rats in the life support system. The calcium balance in the entire observation period of the flight animals was characterized by a significant (from 33 to 50%) lag in this element; normalization in calcium exchange did not occur up to the end of the observation time. At the beginning of the readaptation period, in the flight animals one noted a lag in potassium whose balance was normalized in subsequent days. In the balance of magnesium there were no significant differences among the groups.

TABLE 6. INTAKE AND ELIMINATION OF WATER IN THE READAPTATION PERIOD /22

24 hours after flight	Group of animals	No. of animals	Weight of animals	Quantity, mℓ		Generation of urine, % of total intake of water
				Water drunk	Water eliminated with urine	
3rd	F	6	242,6±3,7	13,2±2,65	18,3±2,29	45,0
	SC	7	297,8±10,3	7,6±0,76	16,1±1,81	49,0
	VC	10	303,0±4,5	3,6±0,75	21,2±0,90	67,0
4th-5th	F	6	254,0±5,3	6,4±0,88	16,2±0,96	47,0
	SC	7	306,4±9,7	5,5±1,06	18,0±2,75	56,0
	VC	10	316,4±3,9	4,0±1,28	19,1±1,96	60,0
11th-12th	F	6	281,8±6,3	5,9±0,88	15,8±0,87	52,0
	SC	7	328,4±8,6	5,2±1,07	16,0±2,20	54,0
	VC	10	335,8±3,5	5,2±1,47	18,1±2,25	56,0
21st-22nd	F	9	308,2±6,9	5,8±0,86	16,0±1,93	51,0
	SC	7	348,2±8,5	5,2±2,01	16,6±2,55	50,0
	VC	10	352,2±5,9	5,5±1,51	19,1±1,51	57,0

TABLE 7. BALANCE OF SODIUM, POTASSIUM, CALCIUM AND MAGNESIUM IN THE READAPTATION PERIOD /23

24 Hours after flight	Group of animals	No. of animals	Sodium		Potassium	
			Balance, mg	% of lag	Balance, mg	% of lag
5th	F	6	10.4±2,5	16,0	14.2±1.9	18,0
	SC	7	12,3±1,6	20,0	6,4±2,5	10,0
	VC	6	5,1±1,3	8,0	7,2±2,0	11,0
11th-12th	F	6	14,0±2,4	22,0	13,9±2,5	17,0
	SC	7	14,6±1,9	26,0	10,0±2,5	17,0
	VC	6	8,9±2,3	14,0	10,1±3,1	15,0
21st-22nd	F	6	10,7±2,2	18,0	11.1±3,5	14,0
	SC	7	11,5±2,4	18,0	8.8±2.7	14.0
	VC	6	7,4±1,3	12,0	7,3±2,0	12,0

TABLE 7. (continued)

/23

24 Hours after flight	Group of animals	No. of animals	Calcium		Magnesium	
			Balance, mg	% of lag	Balance, mg	% of lag
4th-5th	F	6	33,0±4,4	46,0	6,1±0,3	53,0
	SC	7	17,7±4,1	25,0	6,0±0,5	53,0
	VC	6	20,8±1,5	28,0	6,0±0,4	53,0
11th-12th	F	6	29,2±3,9	40,0	6,81±0,8	57,0
	SC	7	16,3±3,3	24,0	5,0±0,6	48,0
	VC	6	11,1±1,5	15,0	5,9±0,8	49,0
21st-22nd	F	6	22,2±3,8	35,0	6,4±0,4	55,0
	SC	7	15,1±3,9	21,0	6,3±0,7	55,0
	VC	6	11,5±1,8	16,0	5,9±0,7	50,0

/24

Thus, the most significant shift in the animals in the flight group were a certain increase in assimilation of food both during and after flight which is due, apparently, to an increase in metabolism and also a deficit of water, sodium, potassium and calcium. Then, normalization of the exchange of water, sodium and potassium occurred fairly rapidly whereas reestablishing exchange of calcium had not occurred up to the end of the observations (the 22nd day after completion of the flight and the synchronous experiment).

Results of Postflight Studies /25

Nervous System

Morphological Study of the Central Nervous System

Samples of tissue from different sections of the brain in the rats were studied by a histologic method 9 to 11 hours and 25 days after completion of the space flight and the synchronous experiment and also in the rats of the vivarium control group. A total of 33 animals was examined.

After the cranium was removed it was cut into three parts and fixed in a mixture of mercuric chloride and formol and neutral Formalin. Then the tissue samples from the brain were immersed in the usual way in paraffin and series sections 5 μm thick were prepared. The preparations were colored with Van Gizon toluidine blue and hematoxylin eosin. During studies of the preparation, particular attention was devoted to the condition of the cellular structures and the vascular vessels of the cortex of the optical and sensomotor regions of the large hemispheres, the hypothalamus, the interior and exterior nuclei of the optical eminence, the pads of the optical eminence, the caudate, lens shape and almond shape nuclei, the cornu Amonnis, the cortex and central nuclei of the cerebellum, nuclei of the reticular substance of the stem, the external articulated body, the red nuclei, the nuclei of the block and eye motor nerves, the soft brain sheathing.

A comparative study of sections of the rat brain in all three experimental groups did not show any kind of differences. Rats from the flight group and the synchronous control group showed no differences in the cyto- and angioarchitectonics, in the degree of filling the vascular vessels with blood, and no peculiarities were observed in the structural organization and tinctorial properties of the nerves and glial cells and the walls of the blood vessels. In the animals in all groups, in different cell formations of the brain, one encountered irregularly placed cells possessing marked hyperchromia, and also nerve cells with diffusely colored nuclei and an absence of clots of the chromatophil substance. These changes can be due to the method of killing the animals by mass blood letting and, apparently, a certain delay in starting the fixing of the samples of brain tissue. Thus, with the methods used of histologic analysis in the brain tissue of the test rats exposed for 19.5 days on the biosatellite, no kind of shifts were determined in structural organization of the cellular structures and cytoarchitectonics in the cell formations studied which could relate to the effect of flight factors. An exception is data ob- /26
tained when studying paraventricular and sympaoptical nuclei

of the hypothalamus in which symptoms of activation of the hypothalamo-hypophysis of the neurosecretory system were observed. The corresponding data are presented in a special section.

Higher Nerve Activity

To obtain a complete concept of the state of the higher sections of the central system in man during space flight and in the postflight period, experiments are necessary on animals in which the physiological effects of space flight are not complicated by the stress of work activity and social and emotional factors. Earlier it was pointed out (Gazenko et al., 1975) that long exposure of rats in a biosatellite causes certain changes in higher nerve activity. The task of this work was to expand and deepen knowledge on this question. To do this, in the studies on animals exposed on the Kosmos-782 biosatellite, criteria of varying degrees of complexity were used.

The development and existence of habits for reaching the goal in closed labyrinths with a food reward were studied. During the two weeks before flight the animals had their first practice in a labryinth proposed by Ya. Dombrovskiy (1966) consisting of four parallel alleys; there were four doors in each alley three of which were locked and through a fourth (closed but not locked) the animals could go into the next alley. In each test, the problem was repeated three times and the number of errors made by the animals with complete passage through the labyrinth was calculated; the time expended in reaching the goal; the number of failures in cases of inadequate behavior in the labyrinth.

Each of the seven animals in the flight group had two control partners with similar indices of higher nerve activity. At flight time there was a break in the work with the control animals housed in the vivarium. After landing of the biosatellite, on the 2nd and 12th days the rats were studied for retention and reestablishment of the habits developed before flight for behavior in the labyrinth. On the 7th day a test was made with the addition of an extraneous stimulant, a bell, switched on when the rats were passing through the first alley of the labyrinth. On the 11th and 13th days in this same labyrinth the rats were studied for fatigue with increased functional load on the central nervous system. The number of tests in the labyrinth was increased to 16 for this. On the 15th to 20th days, the capability of the animals to use previous experience in the new situation ("transfer of experience") was studied. This capability was evaluated according to acceleration of developing the second habit in the labyrinth in comparison with the first, developed before flight. The sequence

of the locked and unlocked doors was changed in the labyrinth for this purpose. At the next stage of observation (20th to 25th days) the rats were run in a more complex labyrinth with a mainly new system. The animals were run through the complex labyrinth two times in the test. For a comparison of the data obtained, the criterion of verification of differences in two series of regressions was used (Plokhinskiy, 1975).

The study showed that retention and restoration of a previously learned habit in the rats of the flight group was worse than in the control. A larger number of errors made when passing through the entire labyrinth was verified (Figure 7, curve 1) up to the moment of the opening of the first door in the feed section (Figure 7, curve 1a) and also in the final section of the labyrinth (after opening the door in the feed section). The

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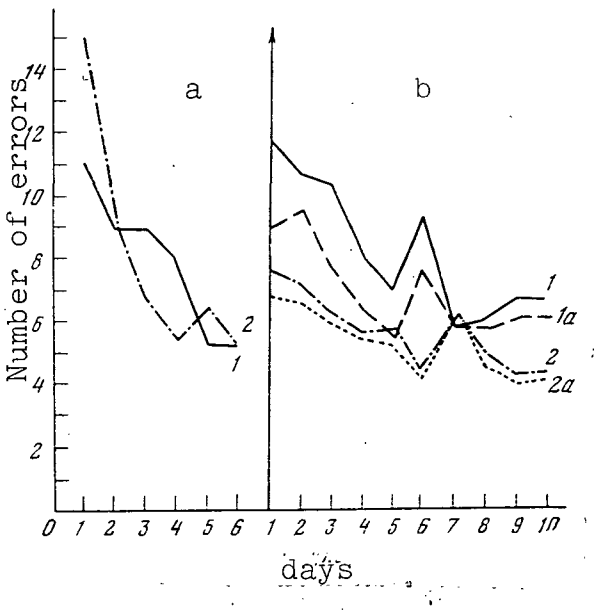

Figure 7. Average number of errors in the labyrinth.

a - before exposure on the biosatellite (before the break for rats in the control group);
b - after flight (after the break for the rats in the control group);
1 - test;
2 - control when passing the animals through the labyrinth to the feed section;
1a- test;
2a- control when passing the animals through the labyrinth until opening of the last door.

last parameter is defined as the difference between the first two, and in Figure 7 it is characterized by a proven difference in the path of curves 1 and 1a in comparison with curves 2 and 2a corresponding to the control test. The rats of the flight group had a verified longer time for passing through the labyrinth (Figure 8) and also a larger number of failures in completing this task.

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The effect of the extraneous stimulant (bell) on the rats in the flight group did not cause significant changes whereas in the control group a tendency was noted toward an increase in the number of errors and a proven increase in time for running the labyrinth (see points corresponding to the seventh day of the experiment in Figures 7 and 8).

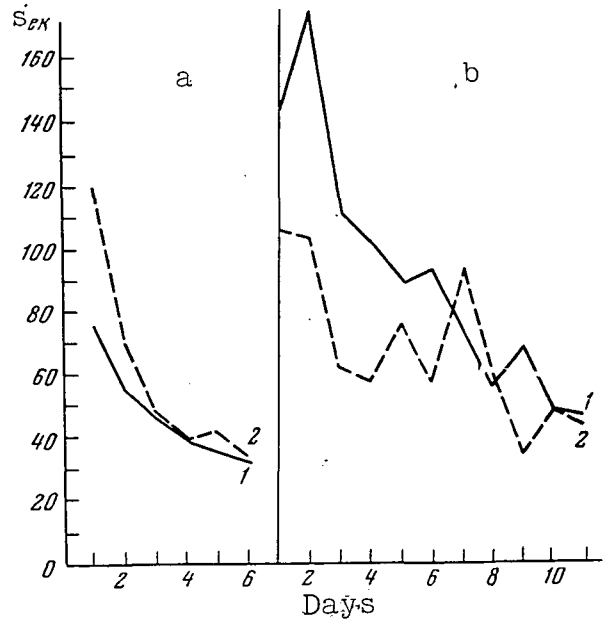

Figure 8. Average time for passing through the labyrinth

Symbols the same as in Fig. 7.

When increasing functional load on the central nervous system the number of failures, according to increase in the number passing through the labyrinth, was increased in the flight group of rats to a lesser degree than in the control.

The study of "experience transfer" showed that the number of errors in the time for passing through the restructured labyrinth in the test and control rats did vary significantly. Failures in passing through the labyrinth in this period did not occur in any of the indicated groups. The number of neurotic states in the rats exposed in the biosatellite was here proven to be smaller than in the control.

When studying the behavior of animals in a complex labyrinth it was observed that the first runs in each experiment were made by the test and control rats uniformly. In the second run, there was a statistically proven difference between the groups. The test rats reached the goal by a longer and more complex pass (Figure 9). On the next day observations were made of the test with a large number of coworkers in the laboratory at the ordinary time of day. These conditions had an effect only on the rats in the flight group.

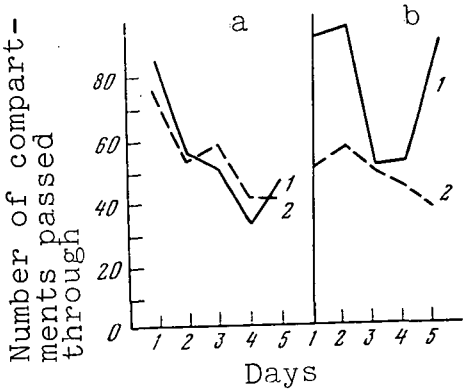

Figure 9. The average length of the pass in the complex labyrinth. Symbols are the same as in Figure 7.

Naturally one assumes that a decrease in food stimulation and also muscle weakness observed in the rats after space flight could have an effect on the characteristics of higher nerve activity being studied (Gazenko, et al., 1975). However, an analysis of the material does not show a correlation between the desire for food and behavior of the animals in the labyrinth. The best transfer cability of the test animals to functional (second runs in the labyrinth) excluded the hypothesis as to the existing significance of muscle weakness because this test requires the largest amount of muscle work.

This is the basis for proposing that weakening of the basic nerve processes is the basis for worsening in retention and restoration of previously learned habits in the flight group of rats. When we talk about weakening of the nervous system of rats who have undergone flight we are talking about an increase after flight in the number of errors in the next stage of the labyrinth which attests to the depth of the neurotic state and also the number of errors caused by neuroses (completed up to the moment of opening the door leading to the food compartment).

At the later stages of observation, disturbance in rats /28
in the flight group resulted in completing only the same work as in the preceding tasks (complex labyrinth). Easier tasks were completed by the test rats better than the control rats. This can be due to weakening of reactions to random stimulants one of which is a decrease in reaction to the bell. This compensatory restriction of attention is inadequate however, for solving complex problems.

The absence of changes in the parameters studied with the first run in the complex labyrinth indicate retention in the animals of the closing function of the cortex; the retention of capability to transfer experience attests to this. Therefore, one can consider than an increase in the number of errors in the second runs in rats exposed in the biosatellite was the result of fatigue of functional structures of the brain responding to higher nerve activity. The phenomena of fatigue and asthenia in the postflight period is well known in humans (Beregovkin et al., 1976).

The changes in higher nerve activity described are not pathological. They can be considered as the result of protective slowing down which is, as is well known, a universal mechanism protecting the nerve system from damage by unfavorable factors.

Certain Neurochemical Characteristics of Rats

The following were selected as the biochemical characteristics: concentration (according to optical density) and absolute content of ribonucleic acid (mainly ribosome) and proteins in the cytoplasm of the Purkinje's cells of the cerebellum and in their glial cell satellites, the content of sulfhydryl /29
groups in the tissue of the cerebellum, the midbrain and cortex of the large hemispheres, and also activity of cholinesterase of these same sections.

The total content of proteins and ribonucleic acids in separate cells was determined by a method of cytospectrophotometry (Gazenko et al., 1976). The content of sulfhydryl groups in the

homogenate of corresponding sections of the brain were determined amperometrically, by titrating the solution of nitric acid of silver (Sokolovskiy,1962). The activity of acetycholinesterase (ACE) and cholinesterase (CE) was found by the Ellman method (1959) using acetylcholine and butyrylthiocholine as the substrates.

Several hours after landing, changes in concentrations and also absolute content of ribonucleic acids were noted only in the bodies of the neurons but not in the glyocytes. The concentration of ribonucleic acids in the Purkinjes's cells of rats who were in flight decreased by 17% in comparison with the same index in the vivarium control and by 14% in the synchronous control. The absolute content of ribonucleic acids in the neurons was decreased both in the flight group of rats (by 20%) and in the synchronous control animals (by 10%); no proven difference was detected between the animals of these two groups according to this index (Table 8). Neither the content nor the concen-

TABLE 8. CONCENTRATION AND ABSOLUTE VALUE OF RIBONUCLEIC ACIDS AND PROTEINS IN THE PURKINJE'S CELLS AND GLIAL CELL SATELLITES (% OF DEVIATION FROM THE VIVARIUM CONTROL) /30

Component, type of cells	F		SC	
	Optical Density	Absolute Content	Optical Density	Absolute Content
	9-11 hours after flight			
Ribonucleic acid				
Purkinje's cells	−17	−20	−4	−10
Glia	−6	−6	+3	+3
Proteins				
Purkinje's cells	0	−3	+2	−4
Glia	−4	−4	+1	+1
	25 days after flight			
Ribonucleic acid				
Purkinje's cells	−4	−3	−4	−3
Glia	0	0	−6	−5
Preteins				
Purkinje's cells	−3	−2	−2	−1
Glia	+2	+2	+2	+2

tration of the total quantity of proteins both in the neurons and in the glial cells of the cerebellum was changed in the animals of the groups studied. Twenty-six days after landing no changes were noted in the indices studied in comparison with the vivarium control. /29

Figure 10 shows a comparison of data obtained when studying animals who had been on the Kosmos-782 and Kosmos-605 biosatellites. One can see that they are very close. In this way, at

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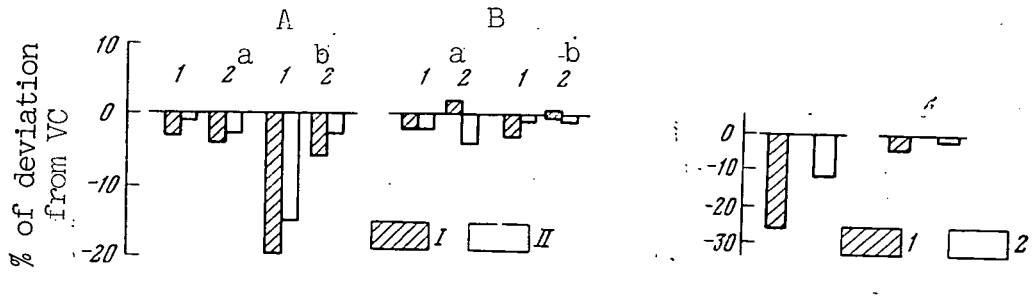

Figure 10. The content of proteins (a) and ribonucleic acid (b) in the cytoplasma of the Purkinje's cells (1) and their glial cell satellites (2).

A and B - first and second observations; I - animals exposed on the Kosmos-782 biosatellite; II - animals exposed on the Kosmos-605 biosatellite.

Figure 11. The content of sulfhydryl groups in the homogenates of the frontal section of the cortex of the large hemispheres of the brain of the rats

a and b - first and second observation time period; 1 - F; 2 - SC.

least by the content of proteins and ribonucleic acid in the cerebellum cells, transportation of the rats after landing and the delay in studying them which occurred in the Kosmos-609 test did not have a noticeable effect. /29

Immediately after flight in the rats there was a significant (26% in comparison with the vivarium control and 14% in comparison with the synchronous control) decrease in the quantity of the sulfhydryl groups in the frontal section of the cortex of the hemisphere (Figure 11). In the synchronous control, this index decreased less significantly. No changes were discovered in the remaining tests on the sections of the brain. Twenty-five days after landing, the content of the sulfhydryl group in the frontal section of the cortex of the hemisphere did not differ from the same index in the vivarium control rats. /30

Immediately after landing, the activity of acetylcholinesterase (ACE) (Figure 12) in the flight group animals was proven to be decreased in the frontal section of the cortex and in the cerebellum; the cholinesterase (CE) activity -- in the frontal and rear sections of the cortex and in the cerebellum. A proven decrease in ACE activity only in the frontal section of the cortex and CE activity in the midbrain was even somewhat increased remaining unchanged in other sections of the brain. Twenty-five days after landing, a proven decrease in ACE and CE activity in the animals of the flight group was /31

discovered in the rear section of the cortex and in the midbrain. In the frontal section of the cortex, a decrease in

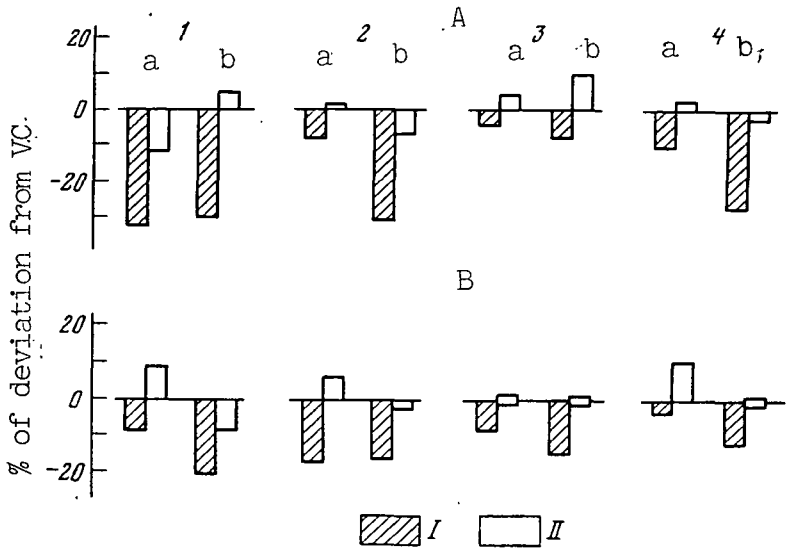

Figure 12. Activity of acetylcholinesterase (a) and nonspecific cholinesterase (b).

1 - frontal; 2 - rear section of the cortex of the large hemispheres; 3 - midbrain; 4 - cerebellum. Other symbols are the same as in Figure 10.

ACE and CE activity was not verified. On the basis of these data one can infer that the change in cholinesterase activity observed immediately after landing has leveled off by the 26th day to the content in animals in ground conditions, to a significant degree. /32

Thus, the lengthy stay of rats in conditions of space flight (weightlessness, isolation, limitations in mobility, etc.) cause a certain suppression of metabolism of the brain particularly marked in those sections whose function involves motor activity: in the cerebellum and motor (frontal) zone of the cortex of the large hemispheres. A decrease in the absolute content of ribonucleic acid in the Purkinje's cells of the cerebellum while retaining the usual content of proteins attests to the drop in intensity of protein metabolism in these neurons, that is, a decrease in their function. The decrease in ACE and CE activity indicates this. In the motor zone of the cortex, both the content of sulfhydryl groups and ACE and CE activity decrease; these both can reflect functional inactivation of the central structures.

Attention should be given to the fact that the content of sulfhydryl groups and ACE activity (enzyme involved with neurons) in the frontal section of the cortex in the synchronous animals was also proven to be lower than in animals in the vivarium

control. Probably, this was due to conditions of limited mobility. It is important that in the other sections of the brain, a similar difference between the synchronous and vivarium control groups was not detected. One can infer that shifts in metabolism of the brain in the flight group rats basically was the result of the effect of space flight conditions; however, their directivity was the same as in the animals in the synchronous control group where changes were significantly smaller.

Return of the animals to ordinary conditions of housing on Earth resulted basically in normalization of the aspects of brain metabolism which we studied.

Synaptic Excitability of Motor Neurons of the Spinal Cord of Rats

As is well known, an important factor in regulation of motor activity of an organism is the level of synaptic excitability of motor neurons; the latter is maintained due to synaptic effects delivered to the motor neurons on an afferent path from the receptors of motor activity, skin and internal organs, and also due to pulsation from the super-segment structures. One can expect that in conditions of weightlessness the character of synaptic flows undergoes significant changes. This is why it is advantageous to analyze the synaptic excitability of motor neurons of the spinal cord of rats after space flight. The studies were conducted on five rats exposed on the Kosmos-605 biosatellite, on five rats in the synchronous control and also somewhat earlier on the rats housed in the biosatellite mock-up.

Preparation of the animals for the study included tracheotomy; laminectomy in area of the fourth lumbar - first sacral vertibrae and exposure of the corresponding segments of the spinal cord; preparation of the ventral (VR) and dorsal (DR) /33
roots of the vertibrae segments and preparation of the fibular (FN) and tibial (TN) nerves of the rear extremeties, which innervate the flexor and extensor muscles of the talocrural joint. After operative preparation, the animal was placed in a special stereotaxic device which we had developed for neurophysiological studies on the rats.

In all of the tests, reflector stimuli to the ventral root of the sixth vertibra segment were conducted causing stimulation of the dorsal root and peripheral nerves and also using microelectrode equipment (Kostyuk, 1960) -- antidromic and orthodromic responses of separate motor neurons of this segment. A VR and nerve stimulus was used for antidromic excitation of motor neurons and their identification; for the orthodromic excitation -- stimulation of VR and nerves.

Determination of the rate of conducting in the afferent fibers of the nerves to the DR fibers showed that with adequate force of stimulation, the afferent wave going from the surface of the spinal cord consisted of several components showing excitation of the fibers at different rates. Calculation showed that the maximum rate of conducting in the afferent TN fibers for different animals amounted to 38.8-59.4 m/s, minimum -- 13.6-17.9 m/s, for the FN -- respectively, 43.2-50.2 m/s and 16.1-20.0 m/s. According to bibliographical data (Bezhenaru, 1971; Gokin, 1971; and others) one can assume that the afferent fibers which have a maximum rate of conducting, belong to the very rapid muscular afferent of rats. Synaptic excitation of motor neurons was studied using their removal from reflector stimuli (mass stimuli) and also intracellular responses of separate motor neurons. Recording of reflector stimuli made it possible to obtain a concept simultaneously of the entire population of motor neurons studied. When stimulating the nerves or the DR in the VR segments studied, responses were recorded consisting, as a rule, of several components. The earliest of these were monosynaptic stimuli followed by polysynaptic. When increasing the frequency of stimulation above 0.2-0.5 in 1 s, amplitude of the mono and polysynaptic responses dropped progressively. In Figure 13a, where the relationship of amplitude of monosynaptic response to frequency of stimulation is presented for the flight and control tests, it is apparent that the course of this suppression in both cases is practically identical.

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The use of a method of double stimulation made it possible to obtain a general concept of the time changes of excitability in the motor neuron population. In Figure 13b curves are presented of excitability which describe the relationship of amplitude of the response to test stimuli to the value of the interval between them and the condition stimulus. Each of the stimuli separately caused a marked monosynaptic response. It is apparent that all the curves are made of two sections: alleviation of the test responses (interval 8-14 m/s) and their suppression (interval 300-500 and more m/s). It is important to know that the character of the curve is uniform in the control and flight groups of animals.

During stimulation of VR in the motor neurons studied (32 cells in rats in the flight group and 28 in the control rats) an antidromic potential of effect occurred (PE) which had a characterestic bend in the ascending phase (Figure 14a). The amplitude of PE was 50-70 mV. Measurement of the hidden periods of antidromic PE made it possible to calculate the rate of conduct ~~to~~ the axons of the motor neurons studied which was equal to 15-33 m/s.

The average amplitude and duration of the sequential hyperpopulation (Figure 14,b,c) did not differ in animals in the flight and control group.

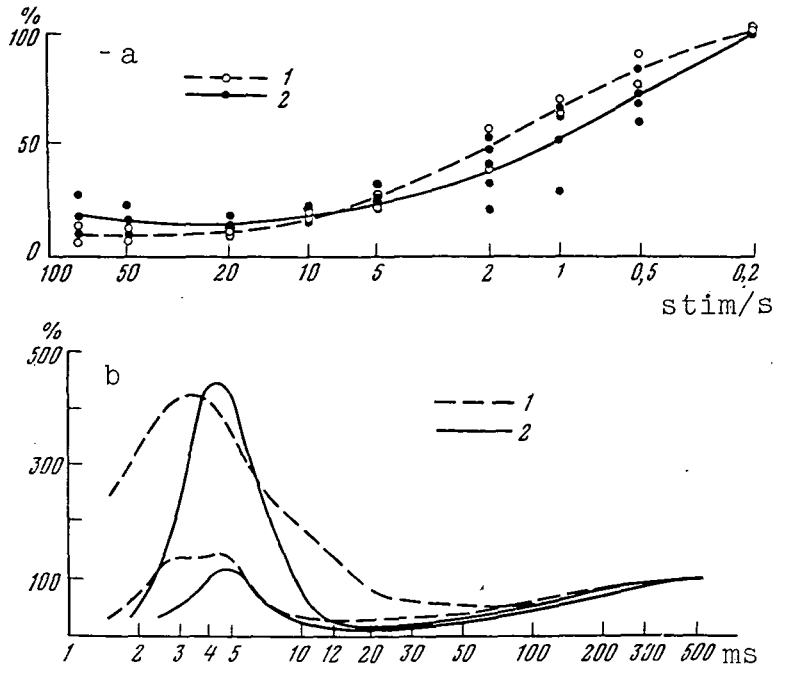

Figure 13. The relationship of amplitude of a monosynaptic stimulus to the frequency of stimulation of the tibial nerve (a) and curves of excitation obtained by graph averaging (b).
a: ordinate -- amplitude (% of observations with frequency of stimulation 0.2 stim/s); abcissa -- frequency of stimulation; 1 - control; 2 - flight;
b: ordinate -- the value of the test stimuli (in percentage of the initial test response); abcissa -- intervals between stimuli; 1 - control; 2 - flight.

Between the animals in both groups there were also no proven differences in amplitude and duration of monosynaptic excitation postsynaptic potentials (EPSP) which occurred earliest in the motor neurons during orthodromic activation of low threshold afferents. During stimulation with a series of stimuli, monosynaptic EPSP were easily totaled (Figure 14,d). An increase in frequency of stimulation of the afferent paths resulted in a progressive depression of monosynaptic EPSP in both series of tests (Figure,e,f). In Figure 14g, averaged curves of this depression obtained in flight and control series of experiments are shown. The path of the curves in both cases is similar.

Activation of high threshold afferents resulted in the motor neurons in polysynaptic potentials (PSP) both in the TN motor neurons and in the FN motor neurons one observed excitation (Figure,a) and retardation (Figure 15,b,c) PSP.

The results of this work indicate that the change, due to flight, in synaptic excitation is insignificant. Also coordination of the simple spinal reactions of the flexor type was not

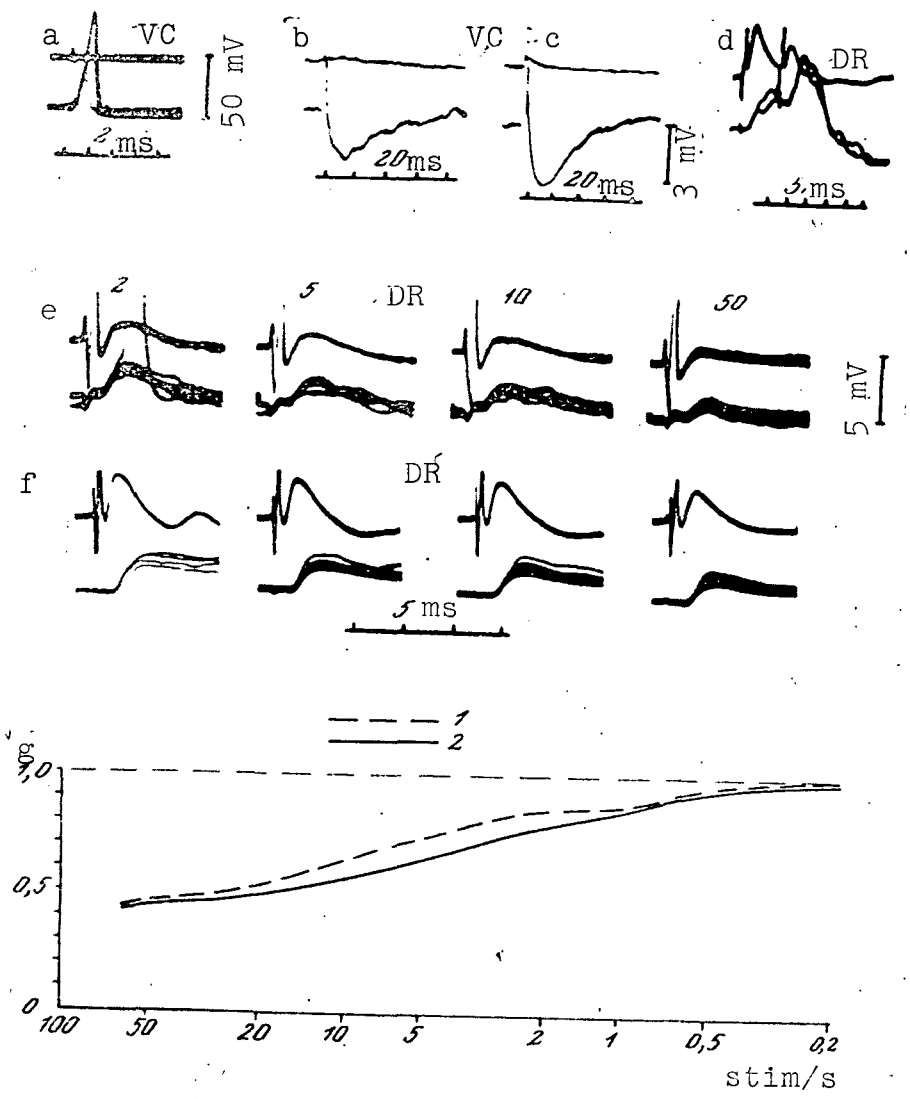

Figure 14. Successive and monosynaptic processes in separate motor neurons.

a -- antidromic potential effect (PE) of the motor neurons during stimulation of the ventral root (VR); b,c -- antidromic PE and successive hyperpopulation in animals of the control (b) and flight (c) groups; d -- summation of monosynaptic EPSP with dual stimulus of the dorsal root (DR); e,f -- monosynaptic EPSP of the motor neurons of animals in the flight (e) and control (f) groups with different frequency of stimulation of DO; the numbers under the curves -- frequency of stimulation (in stim/s) (the upper path is the potential of the dorsal surface, the lower -- is the intracellular response; g -- the relationship of the amplitude of monosynaptic EPSP to frequency of stimulation; ordinate -- amplitude; in portions from observation at a stimulation frequency of 0.1 stim/s; abcissa -- frequency of stimulation; 1 -- control; 2 -- flight.

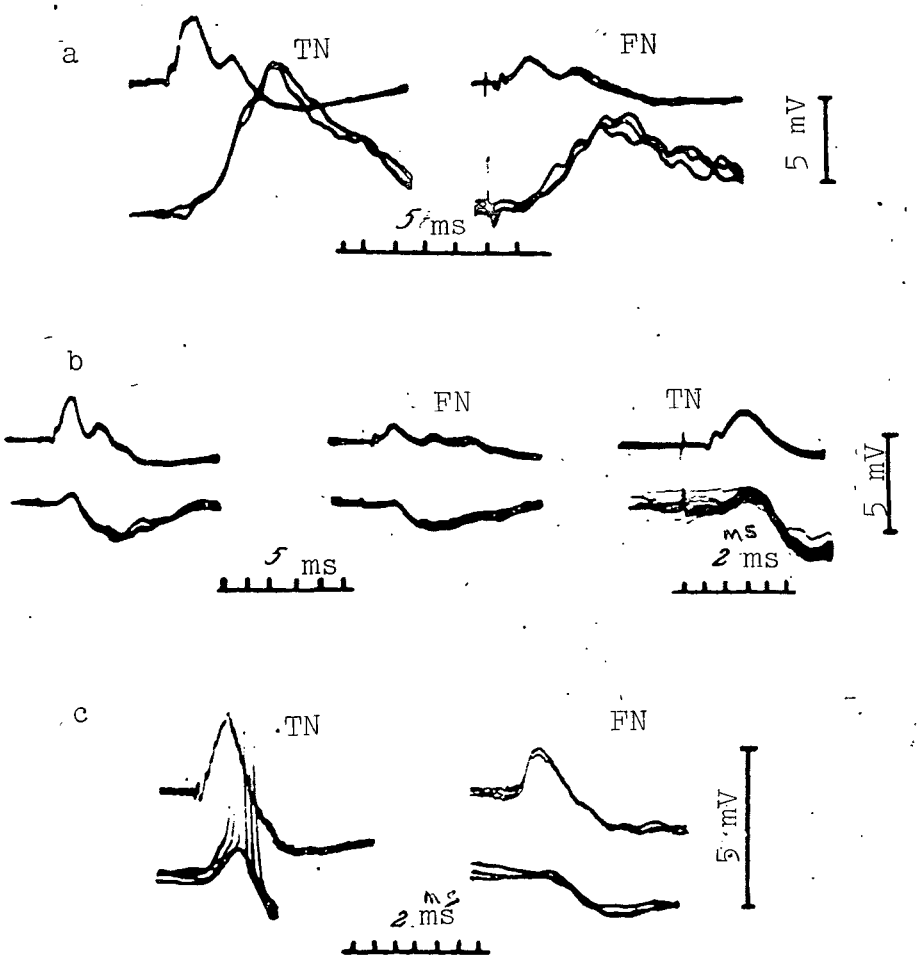

Figure 15. Synaptic processes in the motor neurons.

a -- polysynaptic EPSP in the motor neurons of the tibial nerve (TN) caused by stimulation of the TN and the fibular nerve (FN); b,c -- polysynaptic retardation of PSP in two motor neurons of the TN in rats in the flight (b) and control (c) groups.

disturbed. No significant changes were detected in the characteristics of monosynaptic EPSP of the motor neurons which are the basis for the synaptic mechanisms of the stretching reflex.

The results presented make it possible to propose that as /37
a whole the mechanisms of synaptic transmission to the spinal cord due to the effect of space flight does not undergo significant changes. The similarity of properties of the monosynaptic EPSP and stimuli of the motor neurons in both series of experiments attests to the fact that functioning of the presynaptic endings is not disturbed. The absence of differences in amplitude and duration of the sequential hyperpopulation in monosynaptic EPSP indirectly indicates the retention of the electrical properties of the postsynaptic membranes and the ion mechanisms of generation of the electrical activity.

The Composition of Solute Proteins of the Spinal Cord and Intervertebral Nodes.

It has been pointed out (Gorbunova, Portugalov, 1976) that rats in weightlessness conditions have a decrease in the content of proteins of the cytoplasm in the motor neurons of anterior horns of the spinal cord and the neurons of the intervertebral nodes which innervate the muscles of the rear extremities. In this experiment, the content of separate protein fractions in different parts of the vertebral nodes of the spinal cord is differentiated according to histologic structure and functional value: the gray matter (anterior, lateral and posterior horns) where the motor neurons of the centers of cerebrospinal reflexes of anterior extremities and intercalary neurons are grouped; the white matter comprising the nerve conducting paths; the intervertebral nodes where the sensitive neurons are concentrated.

After 9-11 hours and 25 days postflight, in the animals in all three groups the spinal cord was taken at the level of the vertebral node with the intervertebral nodes adjacent to it. The spinal cord was divided into white and gray matter under the MBS-2 microscope. Examples of the brain and nodes were placed in special polyethylene centrifuge test tubes and homogenized with ten times the volume of distilled water at a temperature of -4°. Separate protein fractions subsequently were removed with distilled water, 0.85% solution of NaCl, 0.1 n solution of NaOH. The homogenates were centrifuged with cooling at 15,000 *g* for a period of 60 minutes. The content of protein in the centrifuged specimens was determinted according to Lowry's method (Lowry and co-authors, 1951). The concentration of protein then was calculated for 1 mg of fresh tissue. The parameterless criterion of Van-der-Varden was used when statistically processing the numerical materials (1960). A comparison was made with indices of the animals of both control

groups. The results are summarized in Table 9. The data

TABLE 9. THE CONTENT OF PROTEINS (γ/mg) IN THE SPINAL CORD AND INTERVERTREBRAL NODES (4-8 ANIMALS WERE USED IN THE TEST) /38

Tissue	VS	9-11 hrs after completion of the tests		25 days after completion of the tests	
		F	SC	F	SC
	Water soluble proteins				
Gray matter	37,0	30,0*	35,5	43,0*	39,0
White matter	28,0	20,0*	26,0	27,5	30,0
Intervertebral nodes	43,0	37,0	41,0	47,0	57,0
	Alkali soluble proteins				
Gray matter	47,0	46,0	49,0	54,0	57,0
White matter	38,0	39,0	37,0	45,0	48,0
Intervertebral nodes	21,0	30,0	29,0	37,0*	47,0
	Salt soluble proteins				
Gray matter	6,0	7,0	7,0	6,0	8,0
White matter	6,0	7,0	6,0	5,0	6,0
Intervertebral nodes	8,0	9,0	9,0	7,0	10,0

*verified difference from VS

obtained in rats in the vivarium control at the two time periods for the study did not differ; because of this they were combined in a single sampling. /37

9-11 hours postflight in the white and gray matter of the spinal cord, a proven decrease in the content of water soluble proteins occurs. In the intervertebral nodes, a decrease in the fractions of water soluble proteins was not verified. The content of salt soluble and alkali soluble proteins in the structures of the spinal cord and intervertebral nodes in the given time period of study were proven to be unchanged. Twenty-five days postflight, the concentration of water soluble and alkali soluble proteins in all structures studied was higher in comparison with the preceding time period and in two cases the level was proven to have increased in the vivarium control. /38 The content of salt soluble proteins remains unchanged in this time period.

One of the factors which causes a breakdown in the motor functions in weightlessness is possibly the decrease in flow

of proprioreceptor impulses from the skeletal musculature as a result of hypokinesia and weakening of antigravitation muscles. The level of proprioreception affects the exchange of substances in the structures of the motor analyzer, adapting it to conditions where the organism exists. The dysfunctions described possibly are due to a detected decrease in concentration of water soluble proteins in the structures of the spinal cord and intervertebral nodes.

When returning to conditions of the Earth's gravity, which after adaptation to weightlessness becomes a stronger stimulus for the gravitation receptor apparatus of the muscle, the afferent pulsation can increase which results in an increase in functional activity of the structure of the motor analyzer. However, in a number of works it has been pointed out that with intense hyperfunction one can observe a decrease in protein synthesis due to competition for energy because in the process of adaptation reactions, the energy supply of the functions must be realized much more rapidly than the plastic. Data exist on the use of cellular proteins of the brain as an energy source under certain conditions (Abood, et al., 1958). All of this can be the cause of the fact that after flight ends the content of water soluble proteins in the neuron structures remains decreased.

An accumulation of water soluble proteins in the gray matter of the spinal cord on the 26th day postflight we related to an increase in synthesis of protein and considered it as a compensatory reaction of the biosynthetic apparatus of the nerve cells to the decrease in content of neuron proteins. /39

Microelectrophoretic Study of LDH Isoenzymes of the Intervertebral Nodes.

The study of the metabolism of intervertebral nodes was directed toward establishing the relationship between the dysfunction detected earlier (Portugalov, Petrova, 1976) in exchange of substances in the muscles of rats and the state of the afferent systems.

The isoenzyme composition of lactate dehydrogenase (LDH) in the intervertebral nodes was determined with a method of electrophoresis in polyacrylamide gel. For the research, the inteverte-bral nodes were taken at the level of the fifth to sixth lumbar vertebrae after 9-11 hours and 25 days postflight both in the synchronous experiment and also in the animals from vivarium control. The tissue was frozen in dry ice and stored at a temperature of -70° for several days until the test.

We used the method of microelectrophoresis developed on a modified (Dietz, Lubrano, 1967) Davis method (1964) described

earlier (Portugalov, Petrova, 1976). The Van-der-Varden parameterless criterion was used when statistically processing the material (1960). The results of the studies are presented in Table 10. In the first hours after landing a shift was dis-

TABLE 10. THE PERCENTAGE RATIO OF ISOENZYMES OF LDH OF INTERVERTEBRAL NODES (4-6 ANIMALS WERE USED IN THE TESTS) /40

Isoenzymes	VC	9-11 hrs after conclusion of tests		25 days after conclusion of tests	
		F	SC	F	SC
LDH_1	15,3	19,7*	14,0	19,7	13,4
LDH_2	35,0	35,5	31,7	34,0	35,9
LDH_3	35,2	31,4	40,7	33,0	38,7
LDH_4	12,9	9,5	11,4	11,3	11,0
LDH_5	1,6	3,9*	2,0	2,0	0,9

*proven difference with VC

covered in the homgenates of the intervertebral nodes in relation /39 to activity of isoenzyme fractions of LDH -- a proven increase in activity in the LDH_1 and LDH_5 fractions. Twenty-five days postflight, the relationship of activity of LDH isoenzymes had returned to normal. In the synchronous control rats at no time period were their changes in the isoenzyme spectrum of LDH of intervertebral nodes in comparison with the control.

A study with methods of light and electronic microscopy of the muscles of the rear extremities of the rats exposed on board the Kosmos-605 biosatellite showed the presence of atrophic and dystrophic processes whereas shifts in the isoenzyme spectrum of LDH were not detected. The latter is due, obviously, to the relatively large time period (2 days) which had passed after completion of flight before killing the animals for the indicated experiment. In our experiment, it was possible to discover the presence of metabolic shifts in the nerve structures which participate in the generation and conduct of afferent pulsation.

One of the causes, which occur in space flight, leading to the development of dysfunctions of the muscles is a decrease in the activity of the proprioreceptors (Murav'ev, 1967). The state of the muscles can affect functioning of the structures of the spinal reflex arch. On the Kosmos-605 biosatellite, the metabolic shifts were recorded in nerve elements which provide both afferent and efferent innervation (Gazenko et al., 1976; Gorbunova, Portugalov, 1976).

The data obtained indicate the presence of metabolic shifts in the structure of primary afferent neurons. However, one must note that in the intevertebral nodes there are groups /40
of neurons which differ in dimension and apparently in function and also cells of the capsular glia. A separate study of the isoenzyme composition of LDH in the neurons and neurologic cells involves great method difficulties. The relationship of the LDH fractions in the homogenates which we have studied reflects the total spectrum of different cellular and other structures of the intevertebral nodes. The contribution of neurons and cells of the capsular glia in the changes described can be clarified only be microchemical analysis made at the level of separate cells.

Conclusion

The high sensitivity of the central nervous system to a change in exterior conditions is apparent in the shifts established by physiological methods in higher nervous activity which occurred as a result of the protective retardation, a universal adaptive reaction of this system.

At the histologic level, in the largest part of the structure of the brain, there are no signs of effect of space flight factors. During a biochemical study a decrease in the metabolism of the cells in the brain was detected, particularly in those sections involving motor functions (the motor zone of the cortex, cerebellum). A decrease in the concentration of water soluble proteins is apparent in the spinal cord which obviously also reflects changes occurring in response to a decrease in the flow of proprioreceptor impulses from the skeletal musculature.

By using electrophysiological methods on the rats exposed on the Kosmos-605 biosatellite, it was established that the synaptic excitability of motor neurons of the spinal cord and also functioning of the presynaptic endings and postsynaptic membranes was retained.

Shifts in the activity of the LDH isoenzymes was detected in the sensitive neurons of the intervertebrate nodes; their value can be evaluated only by studying separate neurons.

A large part of the deviations observed were caused by hypokinesia whose effect was aggravated by weightlessness. The changes were reversible and by the 26th day of the post-flight period the indices were normalized.

Exchange of Substances /41

Total Gas Exchange and Energy Consumption

The question of change in the external gas exchange due to space flight factors remains in dispute up to the present time. It has been established by many scientists (Voronin, 1969; Berry, 1971b; Kas'yan, Makarov, 1974) that the direction of the changes can vary. The study of external gas exchange can give a definite concept of the total level of exchange processes in space flight. Studies were made on 23 animals from all three experimental groups in the preflight period and also on the 2nd, 5th, 10th and 20th days after completion of the flight. A closed chamber method was used. The gas composition of the atmosphere in the chamber was continuously determined for 15-20 min on the Corning-165 automatic gas analyzer. The following were calculated: requirement for oxygen, generation of carbon dioxide, the breathing coefficient and energy consumption of the animals.

On the 2nd, 10th and 20th days of the postflight period, a proven decrease (by 14-22%) in the demand for oxygen and generation of carbon dioxide was observed in comparison with the background data. The breathing coefficient decreased insignificantly. The decrease in the demand for oxygen involved the decrease in energy consumption of the organism which was statistically proven on the 2nd and 20th days. Similar changes took place in animals in the synchronous and vivarium control groups which make it impossible to relate the changes described to the effect of flight factors. In all probability, the increase in external gas exchange in all three series of tests was the result of an increase in the weight and dimensions of the bodies of the animals during flight. It is generally known that with an increase in weight in animals their total gas exchange which occurs per unit of weight, accordingly with a decrease in the relative body surface, gradually decreases; this agrees completely with Rubner's law. This hypothesis does not remove, however, the question of the possible effect on external gas exchange from the complex of flight factors.

Change in Certain Biochemical Indices of the Blood

The experiments on the Kosmos-605 and Kosmos- 690 biosatellites indicated that a long term stay for rats in weightlessness conditions is accompanied by definite changes in the biochemical parameters of the blood (Tigranyan, 1974; Tigranyan et al., 1975, /42
1976). Due to the peculiarities of setting up these experiments, the studies were made only 24 hours after landing. In this work one attempts to clarify whether changes in biochemical indices in the rats were observed in the earliest time period after the long space flight.

Determining the biochemical indices was done in both postflight time periods for studying rats of all groups. The following were studied in the plasma: the content of glucose (Bittner, McCleary, 1963), lactates (Barker, Summerson, 1941); corticosterone (Popene et al., 1962), sodium, potassium, calcium and magnesium (by a method of atom-absorption spectrophotometry); the activity of alkaline phosphatase (KP 3.1.3.1) (Morgenstern, 1965); aspartate aminotransferase (AST; KP 2.6.1.1) and alanine aminotransferase (ALT; KP 2.6.1.2) (Rush et al., 1970) creatinphosphokinase (CPP; KP 2.7.3.2) (Siegel, Cohen, 1966); lactate dehydrongenase (LDH; KP 1.1.27) (Wroblewsky, La Due, 1955) and its isoenzymes (Smirnov et al., 1971). The results are presented in Table 11.

A few hours after completion of the flight, a significant increase was noted in the concentration of glucose and lactate absent in rats in the two control groups. The increase in concentration of glucose in the blood occurred probably as a result of stress reactions. Twenty-four hours after completion of the 22-day flight on the Kosmos-605 biosatellite, the concentration of glucose in the rats was unchanged (Tigranyan et al., 1975). In all probability, the increase in concentration of glucose in the blood occurs due to weightlessness but is fast acting and can be detected only in the first postflight hours.

The increase in concentration of lactate attests to a possible increase in the specific weight of anaerobic processes in providing energy to the organism in the weightlessness condition.

The concentration of corticosterone immediately after completion of flight significantly exceeded the control level but in distinction from the two preceding indices it was increased in the synchronous control animal group. One can assume that the increase in concentration of corticosterone is the result of events occurring during landing of the biosatellite. In the rats who had undergone flight on the Kosmos-605 biosatellite, 24 hours after completion of the flight, a significant decrease was noted in the concentration of corticosterone (Tigranyan et al., 1975). Consequently, the first hours after completion of space flight are accompanied by considerable increases in the level of corticosterone in the plasma and in the succeeding time period the level of corticosterone in the plasma decreases; after 24 hours it is slightly less than that of the control level. One can think that in the first minutes after completion of the flight, the peak of the rise in concenteration of corticosterone in the plasma was higher than 9 to 11 hours postflight.

No changes were detected in any group as to the content of sodium, potassium, calcium and magnesium. Similar data were

TABLE 11. THE CONTENT OF GLUCOSE AND LACTATE (mg%) CORTICOSTERONE (µg%) and THE ACTIVITY OF LACTATE DEHYDROGENASE (LDH AND ALKALI PHOSPHATASE (m-unit/mℓ) IN BLOOD PLASMA

Substance		9-11 hrs postflight			25 days postflight		
		VC	F	SC	VC	F	SC
Glucose	$(M \pm m)$	179,0±4,9	256,0±8,5*	187,0±1,1	169,0±2,6	165,0±6,4	171,0±4,2
	n	5	4	5	5	5	5
Lactate	$(M \pm m)$	19,7±0,73	32,3±3,54*	19,4±1,70	18,6±1,04	17,4±0,38	18,8±0,54
	n	5	4	5	6	6	6
Corticost-erone	$(M \pm m)$	27,2	40,6±4,25*	38,5±3,13*	36,4±3,76	35,7±4,01	30,3±2,15
	n	4	4	5	5	6	6
LDH, total activity	$(M \pm m)$	1361±20	1279±30*	1165±95	1019±83	1142±73	1010±85
	n	4	4	4	6	6	6
Spectrum of isoen-zymes of LDH in %	$(M \pm m)$						
	LDH	5,2±1,9	4,6±2,6	2,5±1,2	14,1±1,3	4,8±0,7*	5,2±0,7*
	LDH	20,5±2,7	18,3±4,8	14,4±2,2	21,5±1,6	19,3±1,0*	20,8±0,3*
	LDH	19,6±3,2	14,9±2,3	15,9±1,9	20,4±2,1	16,6±1,3	20,7±0,2
	LDH	24,9±2,3	21,6±3,4	24,5±1,1	18,9±2,1	28,6±3,4*	26,1±0,3*
	LDH	29,8±4,8	46,0±7,5	42,7±3,9	22,1±2,4	31,1±2,7*	27,2±2,7
Alkali phosphatase	$(M \pm m)$	101,6±1,7	145,0±6,3	90,4±3,4	99,8±3,9	101,3±1,4	95,0±4,7
	n	5	4	5	6	4	5

*verified difference from VC

obtained in experiments on the Kosmos-605 and Kosmos-690 biosatellites. /42

The total activity of lactate dehydrogenase immediately after completion of the flight is somewhat below the control value and at the end of the readaptation period, equally for rats in the synchronous control, it does not differ from that of the intact animals. When studying the activity of isoenzyme fractions of LDH in all groups of animals, in the first observation period, no changes were noted in comparison with the intact control. Changes in the activity of the isoenzyme /44 fractions of LDH apparent 25 days after completion of the experiment were approximately the same both for animals in the flight group and animals in the synchronous control group but in all probability they involved changes in the relationship of the isoenzyme fractions of LDH in the corresponding group of the intact control.

Immediately after completion of the flight, a significant increase was noted in the activity of the alkali phosphatase; at the end of the readaptation period, equally for rats in both groups of the synchronous control, activity of the enzyme indicated did not differ from that of the intact animals. The changes obtained make it possible to assume that an increase in the activity of alkali phosphatase is due to remaining in conditions of weightlessness and can attest to the presence of certain changes in the metabolism of the bone tissue.

As to the activity of creatinphosphokinase, aspartate and alanine aminotransferase, both in the flight group and the synchronous experiment, no changes were detected in comparison with the intact animals.

Consequently, one can assume that an increase in activity of the processes of gluconeogenesis occurs in flight animals immediately after cessation of flight. A single increase in the level of corticosterone and glucose in the blood plasma detected 9-11 hours after completion of flight is the result of inhibition of the processes utilizing glucose: similar data were obtained during immobilization stress (Nemeth, 1973).

Thus, in rats 6-10 hours after completion of space flight, one observed a significant increase in the concentration of glucose, lactate and corticosterone and also in the activity of alkali phosphatase in the blood plasma.

All of this attests to the functional character of restructuring the metabolic processes during long space flights.

Protein Synthesis *in vitro* in the Liver

Before processing all of the samples of the liver they were frozen for an hour. The liver samples from six rats each from three groups were combined and homogenized in a medium of twice the volume of a special liquid (Holland, Antoni, 1970). The microsomes and ribosomes are isolated by a method of differential ultracentrifuging. The so-called insoluble fraction pH 5 was isolated from the precipitated liquid (Hoagland et al., 1958).

Determining the functional activity of isolated microsomes and ribosomes was done in an amino acid incorporation system (Holland, Antoni, 1970). Incubation was carried out in a different time period at 37°; the reaction was established using frozen trichloroacetic acid. Isolation of labeled proteins was done mainly by the Siekevitz method (1952). The residue was collected on a membrane filter made of glass fiber (Vatman GF/A). After drying the filter, the radioactivity of the protein residue is determined using a liquid-scintillating method on a Packard-Tricarbspectrometer. /47

During the study, the functional activity of ribosomes (polysomes plus monomers) and also microsomes (ribosomes plus endoplasmatic reticula) isolated from the rats' livers were compared. Inclusion of the mixture of the amino acids, labeled C14 and also separate amino acids(C14-phenylalanine, C14-valine) were measured in the system described (Holland,Antoni, 1970). In this system, inclusion of amino acid gives us information on the ribonucleic acid isolated along with the particles and the structural and functional interaction with it (the so-called endogenic i-ribonucleic acid). Moreover, inclusion of phenylalanine was studied induced from within by an artificial messenger, polyuridilic acid (poly-U) which can interact with the ribosomes which do not contain endogenic ribonucleic acid. The concentration of poly-U in the test systems used was 50 µg/mℓ; this concentration as the preceding studies showed causes maximum induction.

It was apparent that in the first time period (5-7 hours after the test) there was no verified difference between the groups in the activity of protein synthesis in the subcellular organellae isolated from the liver. Twenty-five days after the end of the experiment one noted proven differences in the functional activity in the ribosomes and microsomes of the liver; the aspects of change in the ribosomes and microsomes was not uniform. Endogenic inclusion of amino acids in the ribosomes was decreased in rats of the flight group both when using the amino acid mixture (Figure 16) and when using the C-14 valine separately (Figure 17) or the C-14-phenylalanine (Figure 18). In each of the three tests, the ribosomes from the synchronous control group of animals occupied the center position between the flight group and the vivarium control. The fact that in

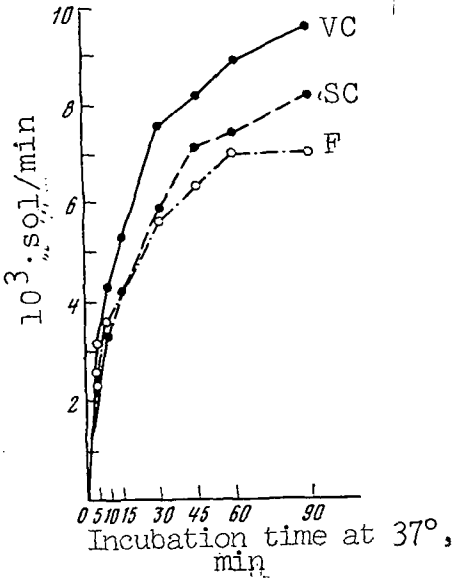

Figure 16. Inclusion of a mixture of C-14 amino acids with ribosomes.

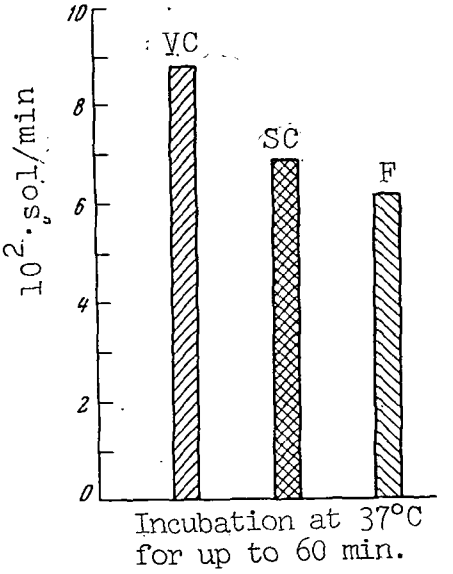

Figure 17. Inclusion of C14-valine with ribosomes

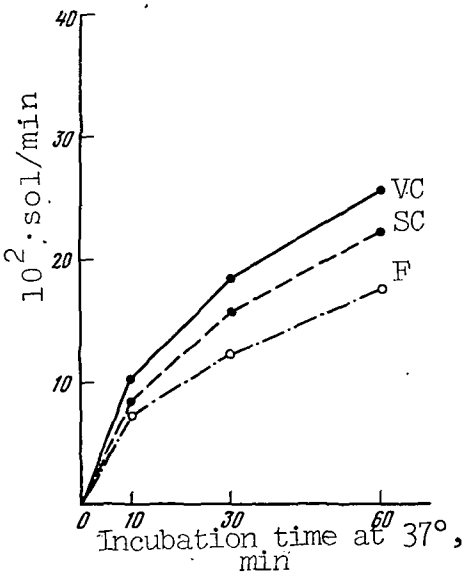

Figure 18. Inclusion of C14-phenylalanine with ribosomes

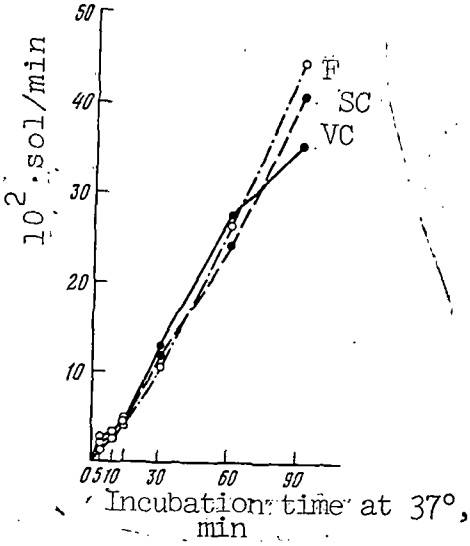

Figure 19. Inclusion of C14-phenylalanine with ribosomes in the presence of poly-U.

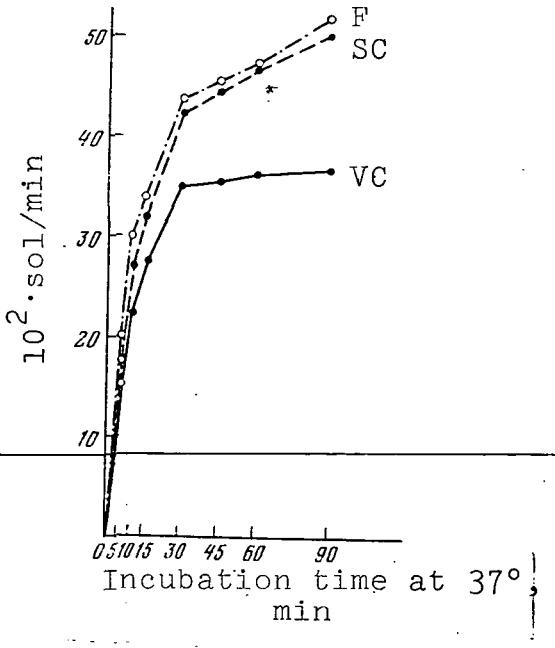

Figure 20. Inclusion of C14-valine with microsomes

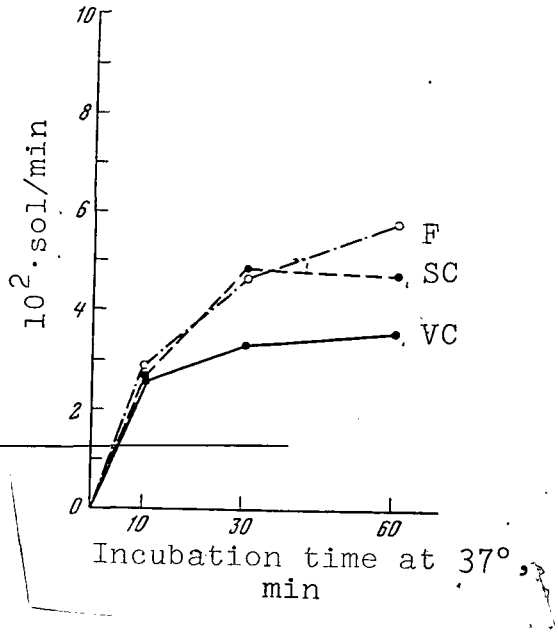

Figure 21. Inclusion of C14-valine with microsomes

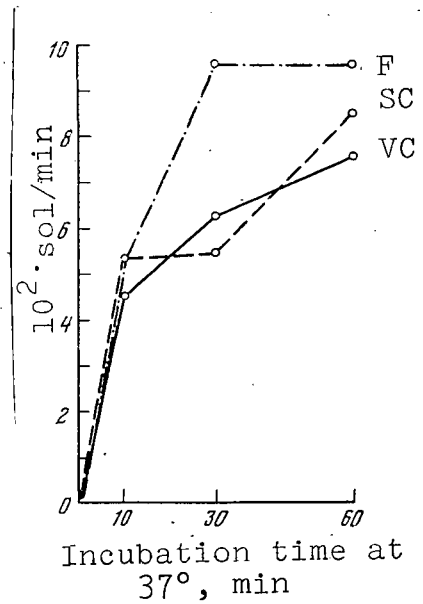

Figure 22. Inclusion of C14-phenylalanine with microsomes

the value of inclusion of phenylalanine induced by exogenic information of ribonucleic acid (Figure 19) there was no difference between the groups attests to the unchanged functional capability of the ribosome monomers. /47

Functional activity of the microsomes changed antithetically in comparison with the activity of the ribosomes. In the animals of the flight group there was a significant increase in inclusion both of the mixture (Figure 20) and the separate amino acids (Figures 21, 22). The microsomes from the livers of the rats in the synchronous group showed increased activity when using the C^{14}-amino acid mixture, to a lesser degree during inclusion of the C^{14}-valine; with the C^{14}-phenylalanine the intensity of inclusion did not exceed the level of the vivarium control.

Thus, changes in functional activity of the ribosomes and microsomes of the liver in the animals 26 days after completion of the flight and of the synchronous experiment were in opposite directions. The possible explanation of this difference involves the following. The microsome level, regulatory factors (Szabo et al., 1968; Holland, Antoni, 1970; Szabo et al., 1976) which are located in the membranes of the endoplasmic reticula can be active; these factors are capable to a certain degree of changing the rate of translation so that a decreased function of the ribosomes causes over-compensation. The accuracy of such a hypothesis can be supported only by further studies.

Changes in Desoxyribonucleoproteides and the Quantity of Nucleic Acids in Certain Tissues. /48

Under the effect of certain damaging factors in hemogenic tissues, a change occurs in desoxyribonucleoproteides (DNP) which are apparent in the increase in their solubility in a physiological solution (Cole, Ellis, 1957; Skalka et al., 1965; Yermolayev, Vodolazskaye, 1970). The soluble fraction is a polydesoxyribonucleotide, whose increased level is due to pyknosis and death of the cells. The quantity of DNP (DNA) in the tissues correspondingly decreases (Cole, Ellis, 1957; Mišurova et al., 1967).

The task of our work was to study the effect of factors of the space flight on the DNP of the spleen, the liver and leukocytes of the blood and also on the quantity of nucleonic acids in the spleen, thymus, brain, liver and leukocytar mass of the blood.

The changes in the DNP are judged according to their concentration in the tissue and the level of solute polydesoxyribonucleoproteides (Cole, Ellis, 1957). The concentration

of absolute number of nucleic acids was determined according to the method described by Misurova and co-authors (1967).

Of the organs examined, the most significant changes were detected in the spleen. In the flight group animals, the level of polydesoxyribonucleotides in the spleen increased after landing by almost 30 times (Figure 23). The increase in

/49

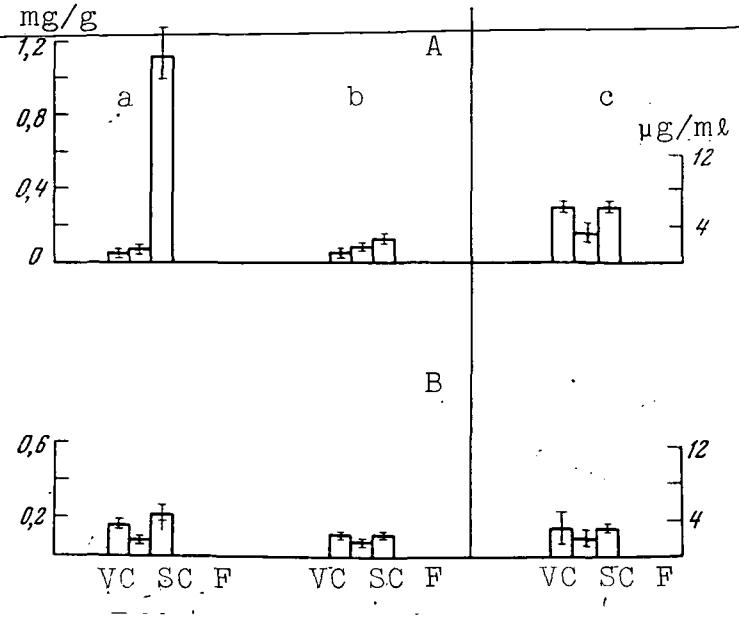

Figure 23. Concentration of polydesoxyribonucleotides in the spleen (a), liver (b) and blood leukocytes (c). A -- 5-7 hours; B -- 25 days.

level of polydesoxyribonucleotides which attests to a breakdown in DNP of part of the cells was combined with the decreased concentration of DNP by 28% in comparison with the control values (Table 12). By the 26th day, the level of polydesoxyribo- /48

TABLE 12. CONCENTRATION OF DNP IN THE SPLEEN, LIVER (mg DNA/g OF RAW TISSUE) AND LEUKOCYTES OF THE BLOOD (mg DNA/mℓ) (M ± m)

Tissue	5-7 hrs after landing			25 days after landing		
	VC	SC	F	VC	SC	F
Spleen	12,17±0,30	10,89±0,54	8,79±0,41	10,37±0,38	9,63±0,70	8,76±0,22
Liver	1,80±0,05	1,72±0,08	1,78±0,12	1,87±0,19	2,47±0,15	2,27±0,14
Blood	0,049±0,002	0,053±0,005	0,071±0,018	0,038±0,005	0,063±0,006	0,038±0,005

nucleotides was normal but the concentration of DNP had not been restored. No changes in DNP were detected in the synchronous test. Changes in DNP in the liver and leukocytes of the blood were only slightly noticeable. The content of ribonucleic acid DNA in the spleen of rats in the flight group was significantly increased in comparison with the control (Figure 24); here, the quantity of ribonucleic acid remained decreased 25 days after landing. In the animals of the synchronous group

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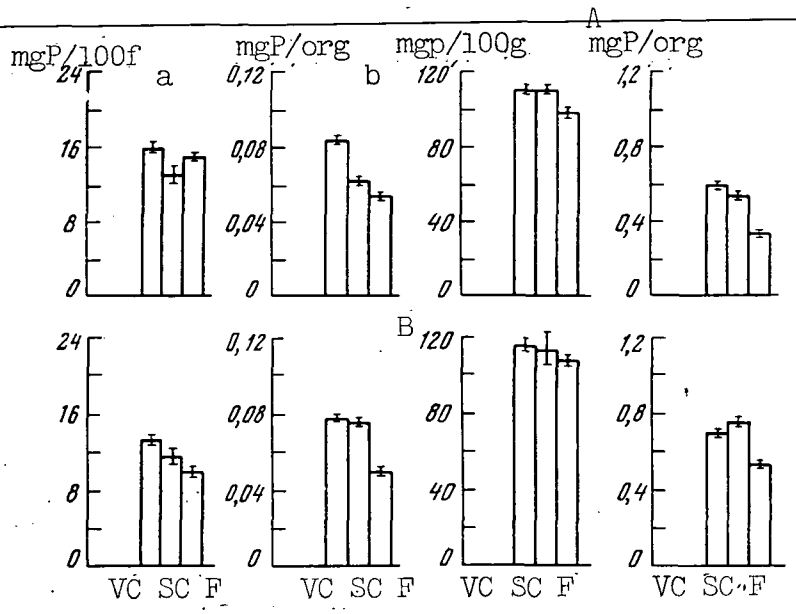

Figure 24. Concentration (in mg P/100 g) and total content (mg P/org) ribonucleic acid (a) and DNA (b) in the spleen.
A -- 5-7 hours; B -- 25 days.

the decrease in concentration and absolute content of ribonucleic acid occurred only in the first observation period; at the same time the quantity of DNA was unchanged. /48

As the data presented in Figures 25 to 27 indicate, in the other organs the indices which characterize the content of nucleic acids were unchanged or were changed to a lesser degree than in the spleen. /51

The increase in level of polydesoxyribonucleotides in the liver several hours after landing reflect the breakdown in DNP which occurred in the last phases of space flight. The level of polydesoxyribonucleotides increases from the second hour after the harmful effect and reaches a maximum after 4-8 hr. The

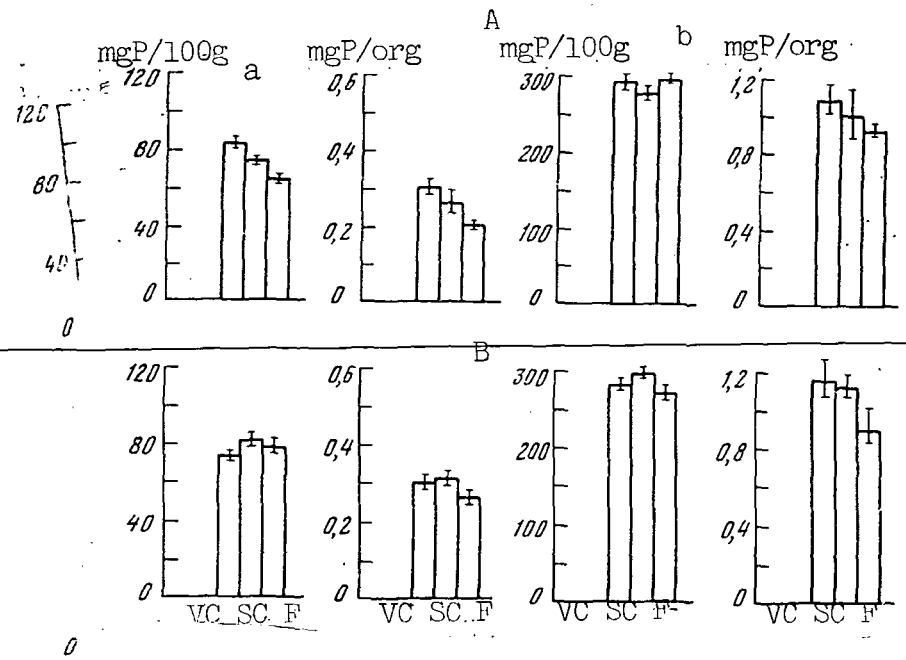

Figure 25. Concentration (in mg P/100 g) and total content (in mg P/org) of ribonucleic acid (a) and DNA (b) in the thymus. A -- 5-7 hours; B -- 25 days.

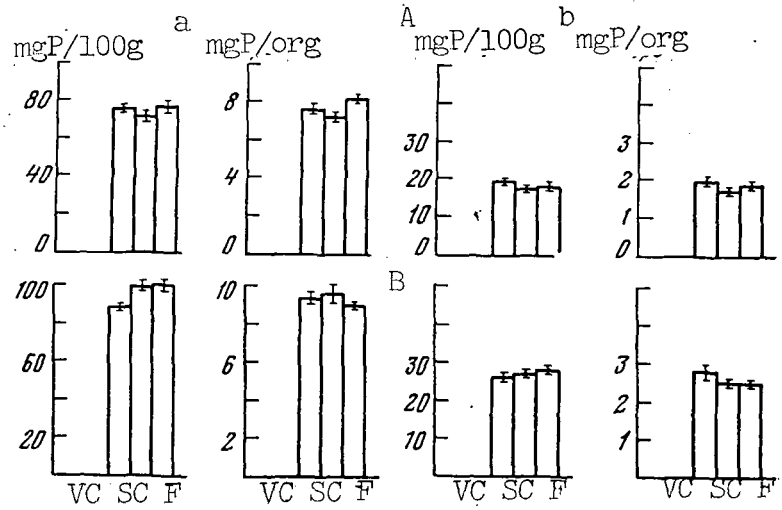

Figure 26. Concentration (in mg P/100 g) and total content (in mg P/org) of ribonucleic acid (a) and DNA (b) in the liver. A -- 5-7 hours; B -- 25 days.

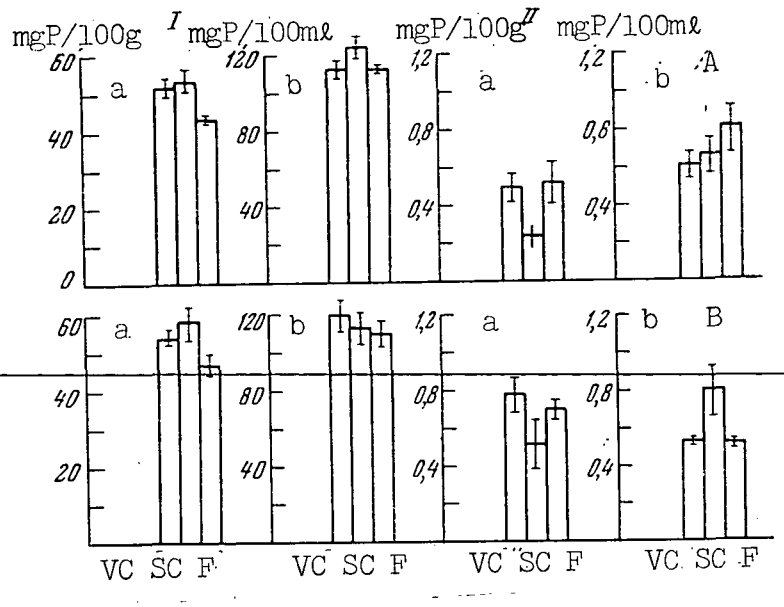

Figure 27. Concentration of ribonucleic acid (a) and DNA (b) in bone marrow (I) and leukocytes of the blood (II). A -- 5-7 hours; B -- 25 days.

polydesoxyribonucleotides formed break up and are removed by the blood; their level in the tissues decreases and reaches normal in a period of days. Such changes of this index were not observed in the rats of the synchronous experiment and due to this one can assume that the increase in the level of polydesoxyribonucleotides in the flight group of rats was the result of gravitation stress.

The simultaneous increase of concentrations of DNP and DNA in the spleen of the flight rats attests to removal of part of the lymphocytes, cells with large nucleus cytoplasmic ratios in which the DNP is the main source of polydesoxyribonucleotides (Skalka, et al., 1965). The histologic picture is confirmed by hypoplasia of the lymphatic tissues of the spleen.

The content of DNA which is a definite biochemical indicator of the cellular state of the organs also indicates significant damage to the spleen which, during 25 days, is only partially restored. Changes in the quantity of nucleic acids in the thymus and bone marrow were less marked than in the spleen. No significant breakdown in DNA or nucleic acids existed in the spleen and leukocytes.

Intensity of Synthesis of DNA in Certain Organs

/52

After space flights, one does not successfully detect significant changes in content and polymer state of the DNA of lymphoid organs and the liver (Guseynov, 1975; Makeyeva, et al., 1976). Moreover, an analysis of cytomorphologic characteristics indicate a number of reversible shifts in the organs and systems important to life (Il'in et al., 1976). It is obvious that for a clear understanding of processes which occur in the organs and tissues of the organism in the postflight period, additional data are necessary particularly about those indices which are the most sensitive. From this point of view, there is interest in studying the intensity of synthesis of DNA.

Analysis of the intensity of synthesis of DNA in the liver, spleen and thymus was made at both time periods when killing animals of all three groups.

The rats, 4 hours before slaughter, received an intraperitoneal injection of 5-methyl-N^3-thymidine (specific activity 11.8 OUF/mM[1]) from a calculation of 1 μOUF per 1 g of weight. The DNA was extracted by hot 0.5 n. chloric acid after preliminary hydrolysis of ribonucleic acid with alkali and removal of the acid soluble products from suspensions of the air dried degreased tissue powder (Monroe, Fleck, 1966).

In the experiments radioactivity was determined (using a liquid scintillation SL-30 counter) and concentration of DNA (spectrophotometrically). Five-six rats in each group were used. In each of the animals 3-4 determinations were made of specific radioactivity and the data obtained were averaged.

The results of the experiments are presented in Table 13.

TABLE 13. INCLUSION OF N^3-THYMIDINE IN DNA. SPECIFIC ACTIVITY OF THE DNA (IN PULSE/MIN·μg)

Group	Parameters	Liver		Spleen		Thymus	
		9-11 hrs	25 days	9-11 hrs	25 days	9-11 hrs	25 days
VC	$M \pm m$	13,1±0,04	28,5±3,06	58,1±12,41	109,6±14,22	18,2±1,49	22,5±2,29
SC	$M \pm m$.	20,0*±2,07	21,2±3,51	49,5±3,56	116,2±40,52	12,7*±0,76	27,5±2,61
	% of VC	152	75	85	106	69	122
F	$M \pm m$,	14,4±1,34	17,3*±1,03	22,9**±2,55	114,8±13,09	12,5*±0,72	27,9±2,10
	% of VC	110	61	39	105	67	124

*proven difference from VC
**proven difference from VC and SC

1. Oxygen utilization factor.

Six-ten hours after completion of the experiment, the specific radioactivity (SR) of the DNA of the liver of animals in the flight group did not differ from that of the animals in the vivarium control group whereas in the liver of animals in the synchronous experiment group this index was approximately 50% higher than the level in the vivarium control. After 25 days, the intensity of synthesis of DNA in all of the groups was increased and to the greatest degree in animals of the vivarium control group.

In the spleen of animals in the flight group, after 9-11 hours after landing, the SR of the DNA was significantly (2-2.5 times) lower than in animals in both control groups. A small (15%) decrease was observed in animals of the synchronous control group. After 25 days, the intensity of DNA synthesis not only returned to the level of the vivarium control but somewhat exceeded it. /53

A similar picture was observed in the thymus. However, the absolute value of the specific radioactivity of DNA was considerably lower here and proven suppression of the inclusion of N^3-thymidine in the DNA in the first hours after completion of the experiment occurred in the animals of the synchronous control group. After 26 days, the SR of the DNA in the thymus of animals in the flight group and animals in the synchronous control group was fully restored and even exceeded the level in the vivarium control by 20%.

The data obtained agree with the results of determining the content of DNA in the liver of rats 24 hours after completion of the space flight on the Kosmos-605 biosatellite and the appropriate synchronous experiment (Guseynov, 1975). One can consider that one of the causes of the increased content of DNA in the liver of animals in the synchronous control experiment is the increase in activity of its biosynthesis due to specific conditions of housing the animals among which, apparently, hypokinesia plays the leading role. The absence of similar changes in the liver in the flight animals can be explained by the effect of weightlessness which probably, prevents intensification of DNA synthesis and an increase in its content.

In distinction from the liver, in the spleen and thymus of the animals of the flight group, immediately after landing one observes a considerable suppression of the intensity of inclusion of a radioactive precursor in the DNA. Changes which are similar in direction are observed in animals of the synchronous experiment group; however, the degree of suppression of the inclusion of labeling in the DNA of the spleen in these conditions is less marked. One can propose that the complex of factors accompanying space flight and absence in the synchronous experiment (in particular, weightlessness) intensifies the suppressing effect of hypokinesia. In the thymus of

rats in the flight group and in the synchronous control group, the degree of suppression of DNA synthesis is uniformly expressed. It is obvious, due to high sensitivity of this organism to the extreme effects which take place in flight, as in the synchronous experiment, differences involved with the effect of other factors are equalized.

Comparing the data obtained with the results of cytomorphologic studies of lymphoid tissue and the system of hemogenesis of the animals who had completed a 22 day flight on the Kosmos-605 (Durnova et al., 1977), one can conclude that one of the main causes resulting in the decrease of concentration of lymphocytes of the peripheral blood and hypophasia of the spleen and thymus in the early time periods after landing is the suppression of intensity of DNA synthesis and consequently, proliferation of appropriate populations of cells.

By considering the results obtained on the 25th day after completion of the experiment, one should note that as a whole in all of the organs studied and in all groups, including the control, the level of inclusion of N^3-thymidine in the DNA is higher than several hours after flight. The most probable explanation of this is the decrease in the degree of thinning of the isotope after its introduction. This can be due to the decrease with an increase in the endogenic supply of thymidine or the lag in weight increase of internal organs relative to the total weight of the animal with age. /54

After 25 days, the directivity of changes of the index studied in animals of the flight and synchronous experiment groups relative to the vivarium control was contradictory to that which occurred in the first examination time period (5-7 hr postflight). This picture reflects, apparently, the complex problem of interorgan regulation of the intensity of the biosynthetic processes. In the later time period, after completion of the experiments in conditions of hypofunction of the adrenal glands and a decrease in concentration of glucocorticoids, the exchange processes, to compensate, intensify in the lymphoid tissue and slow down in the liver.

Activity of Certain Enzymes in the Liver and Processes of Lipogenesis in Fatty Tissue

Studies of the effect of long term space flight indicated the presence of changes of certain physiological functions, the morphologic state of the organs and processes of exchange of substances in the organisms of humans and test animals (Alers et al., 1976; Gayevskaya, Ushakov, et al., 1976; Gazenko et al., 1976; Il'in et al., 1976; Portugalov, Savina, et al., 1976; Tigranyan et al., 1976). Then, morphologic and biochemical characteristics were detected indicating a stress state

in the organism caused by flight factors. Therefore, one of the problems of our studies was to investigate in the homogenate of the liver the activity of these enzymes whose functions depend on the level of corticosterone in the plasma. It was also pointed out (Il'in, 1976) that a 22-day stay of rats in conditions of space flight led to a lag in the animals weight and a decrease in fatty tissue in the subcutaneous cellular tissue and in the fatty deposits. Therefore, we also studied the activity of the enzymes of lipogenesis in the liver and processes of lipogenesis in the fatty tissue.

The material was taken in animals of all three experimental groups. Below we enumerate the bibliographical sources in which the methods used in the work are described.

Index	Source
Activity of enzymes in liver homogenates	
tyrosine-aminotransferase (TAT)	Diamondstone 1966
tryptophan-pyrolase(TP)	Knox, Auerback, 1955
alanine-aminotransferase (ALT)	Farbtest
aspartate-aminotransferase (AST) phosphoenolpyruvate-carboxykinase (FEPCK)	Nordlie, Lardy, 1963
fruktoso-diphosphatase (FDP)	Taketa, Pogell, 1965
glucoso-6-phosphatase (G-6-P)	Harper, Bergmayer, 1962
lm-alat-NADP-oxidoreductase	Ballard, Hansen, 1967
decarboxylizing (malik-enzyme, ME)	/55
glycerolphosphate-oxidase (GP)	Hemon, 1967
ATP-citrate-liase (ATP-CL)	Srere, 1959
serine-dehydrase (SD)	Goldstein et al., 1962
Intensity of inclusion of C^{14}-glucose in lipids of subcutaneous fatty cellular structure	Macho, Saffran, 1967
in fractions of lipids after their separation by a method of thin layer chromotography	Ditto

The two indices listed last were determined only in the second investigation period (on the 26th day postflight).

Data on the activity of enzymes in exchange of amino acids in the liver of rats in the control and flight group are presented in Figure 28 a and b. A significant increase in TAT and

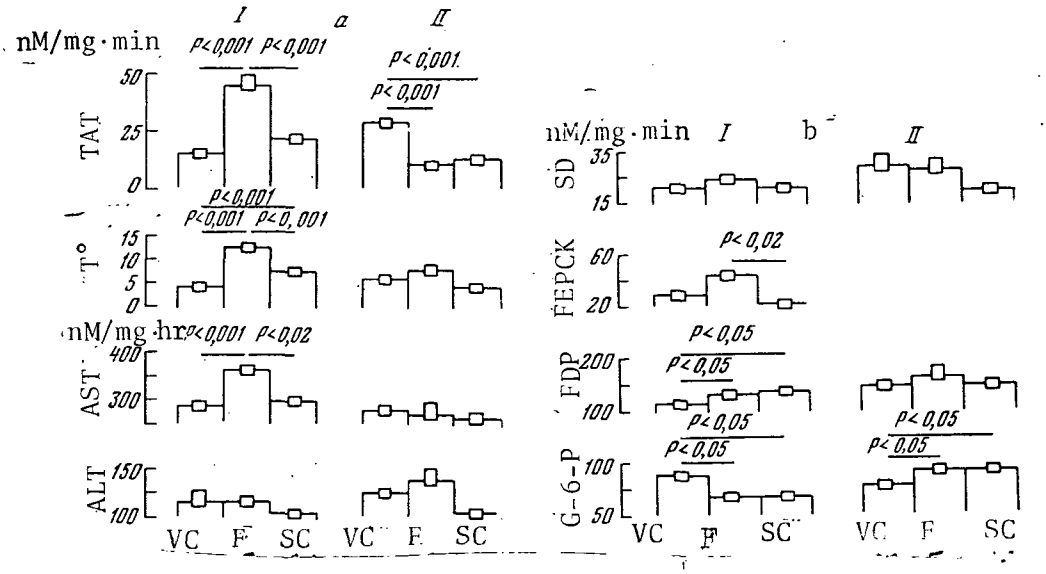

Figure 28. Activity of the enzymes in exchange of amino acids in the liver.
a - TAT -- tyrosine-aminotransferase, AST aspartate aminotransferase, TP -- tryptophan-pyrolase, ALT -- alanine-aminotransferase; b -- SD -- serine-dehydrase, FEPCK -- phosphoenolpyruvate-carboxykinase; FDP -- fruktosos-diphosphatase, G-6-P -- glucoso-6-phosphatase; I -- 5-7 hours; II -- 25 days.

TP activity was observed in rats who had undergone flight and had been killed 5-7 hours after its conclusion. The activity of these enzymes in the liver, to a large degree, depends on the level of hormones of the adrenal cortex and increases even after a single injection of corticosterone (Nemeth, 1973). A small increase in activity of both enzymes in the liver was detected in rats of the synchronous experiment group. Twenty-six days after completion of the flight, the TP activity in rats in all groups was similar whereas the TAT activity in rats of the flight group and rats of synchronous control was lower than the level of the vivarium control. It is obvious that the decrease is caused by an increase in TAT activity in rats of the vivarium control group because when comparing the rats of the vivarium control killed 5-7 hours after completion of the test, the difference between the groups is not apparent. AST activity immediately postflight in animals of the flight group is higher than in animals of both control groups. There are no differences among the groups in ALT and SD activity. /55

After flight, only a small increase in FDP and FEPCK occurred. The FDP activity was higher in the synchronous experiment. The G-6-P activity in the animals in the first hours after conclusion of the flight and the synchronous experiment was lower and after 25 days higher than in the animals of the intact control group. These results indicate that glucose, synthesized in gluconeogenesis processes, to a large degree, were used for synthesis of glycogen. At the end of the readaptation period, as a result of increased G-6-P activity, glucose was isolated in the blood. Determination of concentrations of glucose in the blood showed a significant increase of it in the rats killed 9-11 hours after completion of the experiment (Tigranyan, et al., 1976). A parallel increase in the concentration of corticosterone in the plasma (Tigranyan et al., 1976) is a symptom of stress in which inhibition of the process of utilization of glucose occurs as well as an increase in its quantity in the blood (Nemeth, 1973).

Activity of lipogenesis -- ME enzymes (Figure 29) and ATP-CL in the liver significantly decreased in animals of the flight group and the synchronous control group. After 25 days,

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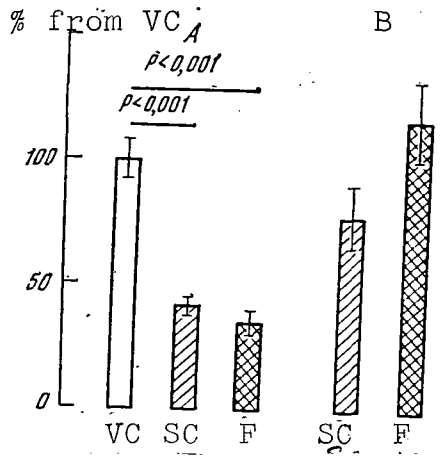

Figure 29. Activity of malik-enzyme in the liver. A -- 5-7 hrs; B -- 25 days.

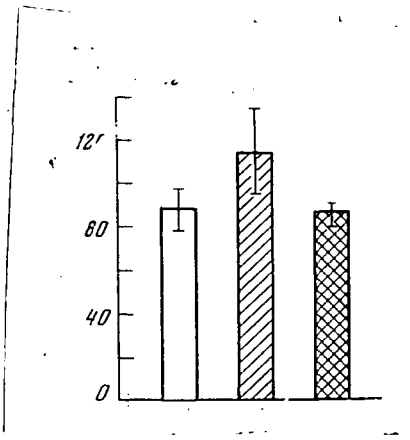

Figure 30. Inclusion of glucose in lipids of fatty tissue 25 days after completion of flight.

ME activity did not differ from that of the control, that is, the housing conditions of the rats in the biosatellite and its mock-up had an inhibiting effect on the processes of biosynthesis of fatty acids, but after the readaptation period, biosynthesis of lipids in the liver was normalized. Changes in the malik-enzyme activity do not depend on the effect of hormones in the thyroid gland, because GP activity does not

/57

change after flight and this enzyme is very sensitive to the effect of thyroxine (Hemon, 1967).

After the readaptation period, no differences were apparent in the rate of biosynthesis of lipids in the fatty tissue (Figure 30). However, distribution of the lipids in fractions (Figure 31) indicated that in the flight animals glucose to a large degree is included in the fraction of triglyceride and the "start" fraction (probably, polarlipids, phospholipids); in connection with this inclusion in the fraction of fatty acids is increased.

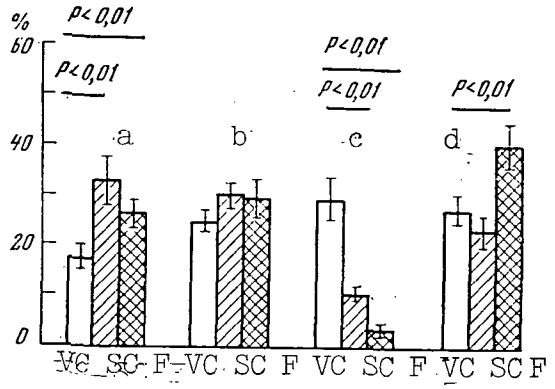

Figure 31. Inclusion of glucose in fractions of lipids of the fatty tissue. Ordinate -- % of radioactivity of a given fraction of the total radioactivity; a - "start" fraction; b - mono and diglycerides; c - fatty acids; d - triglycerides.

The results obtained in this experiment indicate that in the flight animals first of all there is an increase in activity of the enzymes (FEPCK, TAT, TP), whose quantity in the tests with acute stress increases even after a single injection of corticosterone. On the other hand, activity of these enzymes whose content increases in the liver only with repeated injection of corticosteroids or after repeated stress (Nemeth, 1973), in animals after space flight and the ground synchronous experiment did not differ from the control. Consequently, an increase in enzyme activity in the liver is due primarily to the effect of the complex of factors of acute stress in the final stage of the experiment and not the effect of long term weightlessness during space flight.

As was pointed out, in weightlessness conditions and in the synchronous experiment, activity of lipogenetic enzymes decreased in the liver and synthesis of fatty acids as well. This does not coincide with data obtained on the Kosmos-605 biosatellite where an increase was detected in rats of the flight group and the synchronous control group of the concentration of triglycerides in the liver and nonesterified fatty acids in the plasma and fatty tissue (Alers et al., 1975). One can assume that the increase in lipomobilization in fatty tissue is the cause of increase of nonesterified fatty acids and triglycerides in the liver, although the processes of lipogenesis in this organ are decreased. Activity of the enzymes of lipogenesis in the liver at the end of the /58

readaptation period basically has returned to normal. Inclusion of glucose in the general lipids in the test animals also did not differ from that in animals of the intact control group. After completion of flight, in the fatty tissue of the rats there was an increase in the inclusion of triglycerides and fractions containing phospholipids. One can assume that glucose is included first of all in the molecules of glycerin of these lipid fractions. Later on it is necessary to devote attention to these changes in the exchange of lipids in the fatty tissue.

The Effect of Weightlessness on Lipids of the Plasma and Tissues

Only data on the content of cholesterol in the blood of cosmonauts during and after completion of space flights exists relative to the effect of weightlessness on the metabolism of lipids of live organisms (Balkhovskiy, 1973). The lipids of /59
plasma and certain tissues were studied on the rats exposed on the Kosmos-782 biosatellite.

In the plasma, liver, white (epididimal) and brown (interscapular) fatty tissue, the nonesterified fatty acids (NEFA) were determined according to the Dole and Meinertz method (1960), in the plasma, liver, thymus, bone marrow -- triglycerides (TG) according to the Eggstein and Kreutz method (1966) and phospholipids (PL) according to the Bartlett method (1959), in the plasma and liver -- the total cholesterol (C) according to the Zlatkis and co-author method (1953). Observations were made in both time periods using 5-6 animals from each experimental group.

The data obtained are presented in Tables 14 and 15. The most noticeable changes are in the quantity of NEFA which in the flight group animals and the synchronous control group were increased in the plasma and in the liver (Table 14) and also in the fatty tissue (Table 15). In the plasma and fatty tissue, the rats of the flight group still had an increase in this index after 25 days; after 5-7 hr postflight in the animals of these groups there was an increase also in the content of TG in the plasma and the liver, whose quantity had normalized by the 25th day. The cause of the high content of TG in the bone marrow of the animals of all three groups is unclear; it exceeded the established norm by 3-4 times (Alers et al., 1976) for this tissue. As to the quantity of cholesterol and phospholipids as seen from the data of Tables 14 and 15, no regular changes were observed.

Thus, a few hours after completion of flight symptoms of increased lipolysis and increased lipomobilization were noted; however, the determinations were made on satiated animals. The changes in metabolism of cholesterol were not caught; however, without studying synthesis using labeled precursors, it

TABLE 14. COMPOSITION OF LIPIDS OF PLASMA AND THE LIVER (M±m) /58

Group	NEFA	TG	C	PL
	Plasma			
	Eqv. per 1000 mℓ	Mg per 100 mℓ		
	After 5-7 hr.			
VC	688±98	107,6±11,5	110.2±7,9	189,8±6,8
SC	1104±152	366,3±25,0	127,3±13,1	211,4±21,9
F	1392±233	231,8±28,3	110,9±5,6	155,3±6,1
	After 25 days			
VC	452±103	186,7±42,8	95,7±14,1	159,6±10,6
SC	1376±133	156,3±11,1	106,7±6,1	198,5±6,9
F	1182±360	139,0±10,4	113,7±10,2	187,5±15,6

Group	Liver			
	Eqv. per 1 g	mg per 1 g		
	After 5-7 hr.			
VC	3,74±0,48	15,73±0,85	4,33±0,23	33,46±0,64
SC	3,92±0,44	26,05±1,53	4,29±0,25	35,4±1,34
F	5,91±0,75	26,53±2,00	4,89±0,22	33,4±0,95
	After 25 days			
VC	3,76±0,33	12,33±0,88	4,23±0,13	34,0±2,00
SC	4,33±0,51	12,88±0,30	4,30±0,18	35,8±1,68
F	3,24±0,39	14,38±1,27	5,21±0,44	37,1±3,15

TABLE 15. COMPOSITION OF LIPIDS OF THE THYMUS, BONE MARROW AND FATTY TISSUES (M±m) /59

Tissue	Index	Group of animals		
		VC	SC	F
		After 5-7 hr.		
Thymus	TG mg/gr	43,5±8,5	82,4±10,2	63,3±9,3
	PL mg/g	13,8±0,2	13,6±0,2	13,7±0,6
Bone marrow	TG mg/g	84,7±12,3	103,3±14,0	87,3±9,9
	PL mg/g	20,0±1,4	24,3±1,1	12,9±0,9
White fatty tissue		3,29±0,57	6,89±1,15	10,00±0,75
Brown fatty tissue	NEFA eqv/g	2,45±0,49	10,54±2,27	18,75±1,62
		After 25 days		
Thymus	TG mg/g	43,0±8,4	67,7±9,6	51,9±12,0
	PL mg/g	14,8±0,3	14,5±0,7	14,4±0,5
Bone Marrow	TG mg/g	117,4±17,9	99,1±14,2	101,7±16,6
	PL mg/g	12,7±1,7	11,4±0,6	11,9±0,5
White fatty tissue		3,38±0,59	2,55±0,17	5,95±0,46
Brown fatty tissue	NEFA eqv/g	8,30±1,14	5,51±0,54	15,75±1,44

is impossible to draw final conclusions. In the cosmonauts, the cholesterol of the plasma during flight was not changed or decreased slightly (Balakhovskiy, 1973). /60

Evaluating the value of the observed shifts, one should note that they occur in a small range of values and attest to a small degree of stress effect.

Conclusion

When studying gas exchange, it was established that with recalculation per unit of body surface it is practically unchanged in the animals who have returned from flight in comparison with the control animals.

A series of shifts apparent in the blood, liver and fatty tissue indicate the presence of a stress effect from space flight factors. With the effect of stress factors one also sees a breakdown in the exchange of nucleic acids, to a larger degree apparent in the lymphoid organs. The data obtained show that the stress effect was mainly due to factors accompanying the launch of the biosatellite. Moreover, certain changes absent in the animals of the synchronous control group (increase in content of glucose and lactate in the blood, a decrease in the level of nucleic acids and desoxynucleoproteides in the spleen, with simultaneous increase in the content of polydesoxyribonucleotides) indicate the primary effect of weightlessness.

Reaction of the Cortex in the Myeloid Layer of the Adrenal Glands

The stress effect of long term space flight on the function of the adrenal glands was studied. Here, the content of corticoids, catecholamines and catecholamine-synthesizing enzymes was determined. Below bibliographical sources are listed in which the methods used are presented.

Index	Method
Content of corticosterone	Guillemin et al., 1959 (with modifications)
Content of adrenaline and noradrenaline	Euler, Lishajko, 1961 (with modifications)
Activity of tyrosine-hydroxylase (TH)	Nagatsu et al., 1964 (radioisotope)
Activity of phenyl-ethanolamine-N-methyl transferase (PNMT)	Axelrod, 1962
Activity of dihydroxyphenylalanine-β-hydroxylase (DBH)	Molinoff et al., 1971

The studies made show changes in the animals killed only 5-7 hours after completion of flight. In the flight group of animals, a proven increase was noted in the weight of the adrenal glands in comparison with the same index in the vivarium control and synchronous control rats (Figure 32). The concentration of corticosterone in the adrenal glands in rats of the flight group was proved to be higher than in animals of the synchronous control group; but in comparison with the vivarium control it was proven unchanged (Figure. 33).

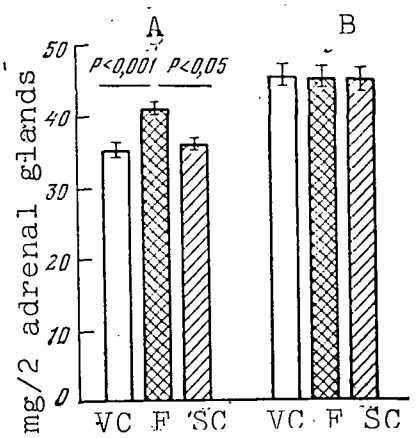

Figure 32. Weight of the adrenal glands (M±m). A - 5-7 hr; B - 25 days.

In content of adrenaline in rats of the flight group and the synchronous control group, one observed a tendency toward decrease; in the content of noradrenalin there was no difference between the groups (Figure 34).

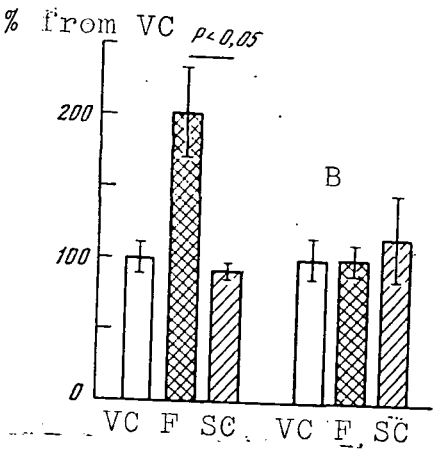

Figure 33. Concentration of corticosterone in the adrenal glands (M±m)
VC: M=8.5 mg/g of tissue; A - 5-7 hr; B - 25 days

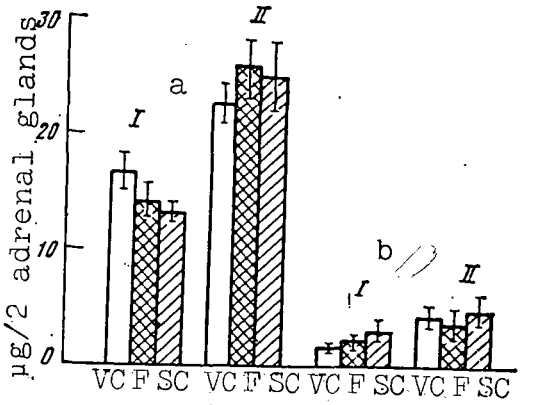

Figure 34. Content of catecholamines in the adrenal glands (M±m). a -- adrenaline; b -- noradrenalin ; I - 5-7 hr; II 25 days.

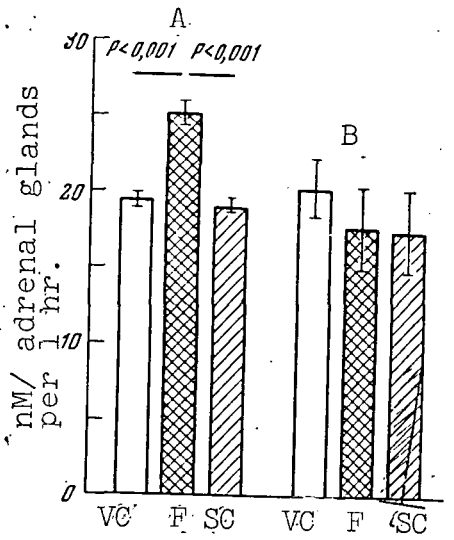

Figure 35. TH activity in the adrenal glands (M±m)
A - 5-7 hr; B - 25 days.

The TH activity which is an enzyme limiting the rate of biosynthesis of catecholamines, there was a significant ($P<0.01$) increase in the rats of the flight group in comparison with the same index in the control and synchronous groups (Figure 35). /61

The DBH activity in the adrenal glands of rats in the flight group was noticeably increased in comparison with both control groups (Figure 36), but in view of the small number of animals, this change was not verified. No marked changes in PNMT activity was detected in rats of the flight group. By the 26th day all existing shifts had been equalized.

Biochemical indices studied in our work, up until now have not been investigated in rats with long term space flight. Therefore, there is no possibility for direct comparisons of our results with the bibliographical data. /62

Activation of the adrenal cortex in a rat shows an increased secretion of corticosterone and its content in the adrenal glands and after a long term effect, increased weight of the adrenal glands. The two latter characteristics were apparent in the animals of the flight group. An increase in weight in the

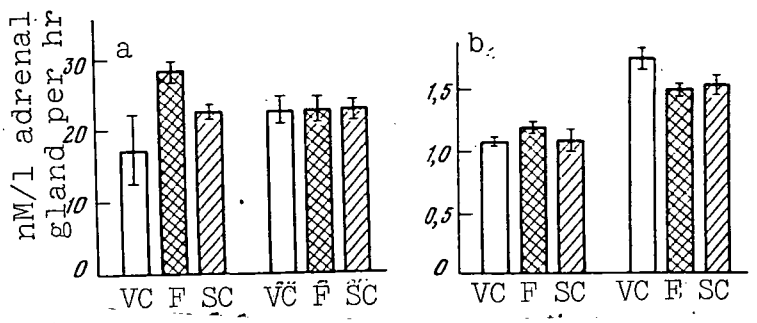

Figure 36. DBH (a) and PNMT (b) activity in the adrenal glands 25 days postflight (M±m)

adrenal glands, accompanied by an increase in the volume of nuclei in the fascile zone took place in the rats after flight on the Kosmos-605 biosatellite (Portugalov, Savina, et al., 1976).

An increase in the content of corticosterone in the adrenal glands in this experiment, during a comparison with certain models of stress, was less marked. A significant increase in the content of corticosterone in the adrenal glands was discovered, for example in rats subjected to traumatization in the Nobel'-Kollip drum (Mikulaj, Kvetnansky, 1966) and rats during immobilization (Mikulaj, Mitro, 1973). One should take into account that in this space experiment the animals were killed 5-11 hours after landing when strict activation of the adrenal cortex can be ended. Moreover, conduct of a number of manipulations with the animals before killing them must have had a stress effect. However, the increase in weight in the adrenal glands attests to the long term effect of the stressor due to which one can attribute activation of the cortex of the adrenal glands to the effect of weightlessness. There are indications (Leach et al., 1973) of an increase in the level of cortisol in the urine of three cosmonauts during a 56-day space flight onboard the Skylab-3 orbital station; here the level of cortisol in the plasma was almost unchanged and the level of ACTH was markedly decreased. Similar results, which do not contribute to a clear picture of the increase in production glucocorticoids, were found in cosmonauts from the Soyuz-9 (Dlusskaya et al., 1973) and the Gemini-7 (Lutwak et al., 1969) spacecraft.

The absence of proven changes in the content of catecholamines in the adrenal glands of rats in the flight group can be explained either by the insignificant intensity of the stressogenic impulse or by the long term effect of this impulse. A decrease of catecholamines in the adrenal glands was detected after different intense stress impulses, for example, after strict immobilization (Kvetnansky, Mikulaj, 1970).

Determination of the activity of catecholamine synthesizing enzymes makes it possible to judge the intensity of synthesis of the catecholamines. A proven increase in TH activity, in comparison with the same index in the control and synchronous groups, can indicate a long term stimulation of the myeloid substance of the adrenal glands during space flight. Increased /64
activity of catecholamine synthesizing enzymes in the adrenal glands was detected after different stress effects (Kvetnansky, 1973; Bhaga, Horenstein, 1976; Pfeifer, 1976 and others). In this experiment, the factor of weightlessness active for a period of 20 days of space flight caused small shifts: TH activity in the adrenal glands increased only by 25% and DBH and PNMT activity generally was proven unchanged.

From the data presented it is apparent that the state of weightlessness in this experiment was not an intense stress effect although during flight activation of the adrenal glands occurred. In comparison with results obtained on humans these data indicate that weightlessness causes an insignificant but proven activation of the sympatico-adrenal system in rats.

Morphologic Studies of the Adrenal Glands

The study of the adrenal glands is of interest inasmuch as they are one of the indicators of stress and reflect the manifestation of the adapatation reactions of the organism.

A histologic study was made of one of the adrenal glands of each of ten rats in the flight group and the corresponding number of animals from the synchronous and vivarium control groups. The adrenal glands were removed from the fatty cellular structure, weighed, and fixed in Bouin's fluid and immersed in paraffin. Sections 4 μm thick were colored with hematoxilin-eosin. Part of the adrenal glands were fixed in calcium-formal for showing lipids according to the Berg method. The volume of nuclei in the glomerular fascicle zones in the myeloid substance were determined; for this, caryometric measurements were made. Using a drawing apparatus (RA-6) projections of the nuclei were outlined (for 100 nuclei in each zone magnified 2,000 times). The length and short diameters of the nuclei were measured and their volume was determined according to the generally used method of caryometry (Khesin, 1967).

The absolute and relative (calculating body weight) weight of the adrenal glands of the animals killed in the first hours after completion of the flight was proven to be (by 20%) increased in comparison with the same index and synchronous and vivarium controls (Table 16). The adrenal glands of rats killed 9-11 hours after landing, in general histologic structure of the cortex and ratio of separate zones do not differ significantly from those in the vivarium control. However, the cells of the fascicle and reticular zones had

TABLE 16. WEIGHT OF THE ADRENAL GLANDS (M±m) /65

Group	No. of animals	Weight of adrenal glands		Group	No. of animals	Weight of adrenal glands	
		Absolute, mg	Relative mg/100g of body weight			Absolute, mg	Relative, mg/100g of body weight
		After 9-11 hr				after 25 days	
F	12	42,35±1,55 $P_{VC}<0,002$ $P_{SC}<0,002$	16,40±0,64 $P_{VC}<0,001$ $P_{SC}<0,001$	F	11	46,75±1,25	14,6±0,63 $P_{VC}<0,05$
SC	12	36,00±0,84	13,20±0,37	SC	11	45,78±1,69	13,46±0,33
VC	11	35,36±1,24	13,10±0,47	VC	11	43,86±1,6	12,9±0,47

a denser eosinophil cytoplasm and contained fewer vacuoles. /64
Particularly sharp differences between the adrenal glands of rats in the vivarium control and the flight group were discovered when showing lipids according to the Berg method (Figure 37). In the animals of the flight group, the

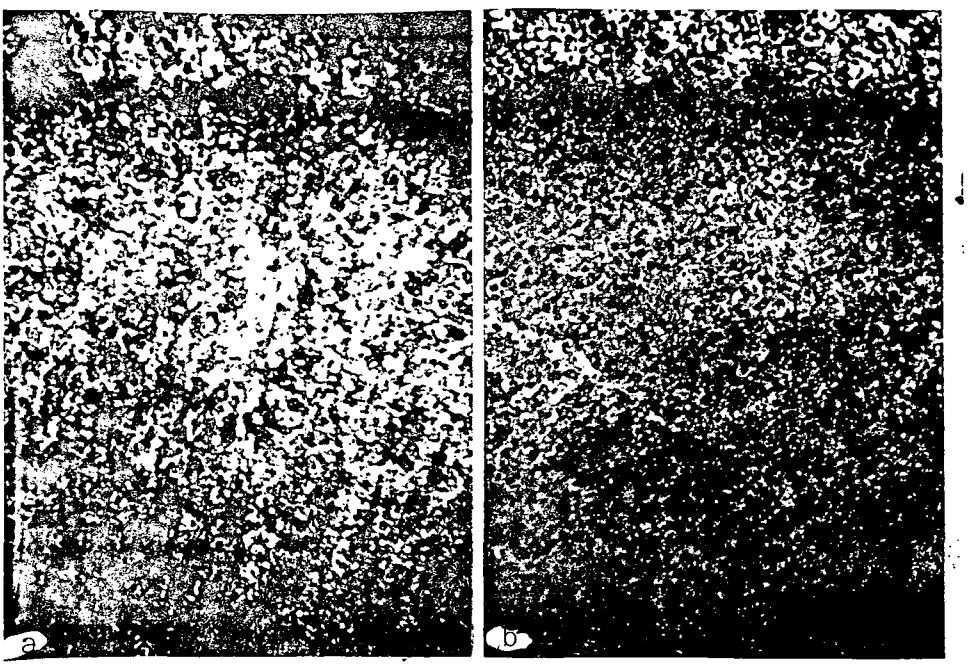

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Figure 37. Cortex of the adrenal glands (prepared according to the Berg method, magnification 20)
In rats of the vivarium control group (a) the cells of the glomerular and fascicle zones are filled with large drops of lipids. In rats of the flight group (b) the content of lipids in the cells of the fascicle zone is decreased.

glomerular zone was isolated on a background of the remaining cortex by a large number of nuclei of bright drops of lipids. Then the subcellular layer of excess lipids was traced. In all of the animals one observed a reduction of lipid drops and delipoidization of the fascicle zone (particularly the center and interior sections of the bands) and of the reticular zone. The degree and extent of delipoidization were nonuniform in different animals. In some of the rats, a small quantity of finely dispersed drops of lipids were determined only in the very exterior sections of the fascicle zone whereas in another, extension of the fasicle and reticular zones showed no lipid inclusions. In other rats, the external sections of the fascicle zone were richer in lipids and delipoidization extended mainly to the interior and partially the center sections of the bands. Delipoidization of the cells of fascicle zone was combined with a proven increase in volume of their nuclei in comparison both with the vivarium and with the synchronous controls (Table 17). In the synchronous experiment,

/64

/65

TABLE 17. THE VOLUME OF NUCLEI (IN μm^3) OF CELLS OF THE CORTEX AND MYELOID SUBSTANCE OF THE ADRENAL GLANDS (M±m) (NUMBER OF ANIMALS IN EACH GROUP -- 5)

/67

Group	Cortex		Myeloid Substance	Group	Cortex		Myeloid Substance
	Glomular zone	Fascicle zone			Glomular zone	Fascicle zone	
	After 9-11 hr				After 25 days		
F	87,0±3,99	130,5±4,10***	140,8±5,17	F	78,0±3,68*	100,0±3,67*	99,4±4,92
SC	89,3±2,73	89,4±3,60	130,6±3,65	SC	65,3±2,14**	84,3±2,97**	89,4±6,38
VC	82,2±5,40	84,5±2,65	136,5±3,24	VC	78,8±3,46	101,7±2,42	107,6±5,44

* Proven difference with the SC.
** Proven difference with the VC.
*** Proven difference with the SC and VC.

the content of lipids in the fasicle zone of the adrenal glands was also decreased in comparison with the vivarium control but delipoidization was basically focal and less extensive than in rats of the flight group; but the volume of nuclei of the cells of the fascicle zone did not differ from that in the vivarium control. The results presented of the study of lipids basically agree with the data of electron microscopy. Certain differences in the content of lipids in separate zones involve, apparently, the characteristics of the methods used. Thus, electron microscopic studies showed a number of important details not captured at the optic level (for example, an increase in the number of finely dispersed drops of lipids in the reticular layer in rats of the flight group). Moreover, the smallest dimension of sections of tissue studied during electron microscopy did not make it possible to discover focal delipoidization in fascicle and reticular zones observed on all sections of the adrenal glands in the animals of the synchronous experiment group.

/65

During histologic examination of the myeloid substances of the adrenal glands, no - significiant structural differences were detected among the groups. The volume of nuclei of the cells in rats after flight did not differ from that in the synchronous experiment or the vivarium control (see Table 17).

Twenty-five days postflight, the absolute weight of the adrenal glands did not differ from that in both control groups. The relative weight was slightly but verified, as increased in comparison with vivarium control (see Table 16). The latter is due, apparently, to the fact that in the indicated time period the animals of the flight group lagged behind the rats of the vivarium control in body weight. In the synchronous control, there were no differences from the vivarium control in absolute and relative weight of the adrenal glands. Histologic study of the adrenal glands at this slaughter period also did not show significant differences among the groups, did not differ from the data of the vivarium control for the volume of nuclei of glomerule, fascicle zones and the myeloid substance in rats of the flight group. However, in the latter one observes larger, than in the rats of the vivarium control group, individual variations in the thickness of separate layers of the cortex, content of lipids in them and also a certain wearing of the interzonal boundaries. /66

In the synchronous experiment, in most of the animals killed on the 26th day, the architectonics of the cortex did not differ from that in animals in the vivarium control group. In some of the animals, the cortex of the adrenal glands was richer in lipids. It is necessary to note that at this time period, in the synchronous control, there was a proven decrease in comparison with the vivarium control and also in comparison with the flight group, the volume of nuclei of the cells of the fascicle and glomerule zones. When studying the myeloid substance differences among the groups were not noted in histologic structure and volume of nuclei.

Thus, in rats killed 9-11 hours after landing of the satellite, one detected a proven increase in the absolute and relative weight of the adrenal glands, an increase in the volume of nuclei of the cells of the fascicle zone and delipoidization of the cortex layer. The combination of hypertrophy and delipoidization of the cortex of the adrenal glands is a morphologic manifestation of stress reaction and an increase of their functional activity. This is confirmed by studies of lymphoid organs (Durnova et al., 1977) and agrees with the biochemical data, an increase in content of corticosterone in the tissue of the adrenal glands and the blood (Kvetnyanski et al., 1976; Tigranyan et al., 1976). /67

An analysis of data obtained when studying the adrenal glands and a comparison of them with the results of studying lymphoid organs makes it possible to consider that an increase in volume of nuclei of the cells of the fascicle zone and delipoidization of the cortex layer are the result of acute stress reactions which develop during descent and after landing. An increase in the weight of the adrenal glands with time also can occur after return of the animals to Earth. However, the absence of symptoms of acute structural restructuring of the cortex and marked plethora give us the basis for assuming that an increase in weight of the adrenal glands can occur in the flight process.

Morphometric Studies of the Cortex of the Adrenal Glands at the Ultrastructural Level

Certain organellae and cytoplasmic inclusions in the cells of the cortex of the adrenal glands were studied by morphometric methods at the ultrastructural level, attempting to find the connection between the functional disturbances and the morphologic picture.

Five rats from each of the three groups were used and the study was conducted after 9-11 hr and 25 days after conclusion of the experiment. The material was processed according to the standard method (Konwinski et al., 1974), samples of tissue of the adrenal glands were fixed in a 3% glutaraldehyde and 1% osmic acid and poured into the Epon-812. The ultrathin sections were prepared on the Reichert UM-3 ultramicrotome and after processing with solutions of acetic uranyl and lead citrate they were studied on the JEM-100C electron microscope. /68 Morphometric analysis of the ultrastructure of cells was done by microstereology methods (Weibel et al., 1966) modified for our material (Konwinski, Szimkowiak, 1975). Four hundred fifty electronograms made with a magnification of 24,000 were used. The photographs were made on randomly selected cell sections. Determination of the relative volume of the mitochondria, lysosomes, contours of the endoplasmatic reticulum and drops of lipids were made. In separate layers of the cortex of the adrenal glands, the shape of the mitochondria was evaluated according to the magnitude of the ratio of their axes which is considered as a mean coefficient of the shape of the mitochondria. All of the results were statistically processed according to the St'yudent method using a Hewlett Packard computer.

An analysis of the results presented in Tables 18 and 19 indicate that in rats of the flight group the most marked shifts were observed in the cells of the fascicle zone. They are expressed in the increase of volume and number of mitochondria, the appearance of mitochrondria with bubble type crystals, an increase in the volume of the smooth endoplasmatic

TABLE 18. RELATIVE VOLUME OF THE MITOCHONDRIA (MC), DROPS OF LIPIDS (DL), LYSOSOMES (LS) AND SMOOTH ER (GℓER) IN THE CELLS OF THE CORTEX OF THE ADRENAL GLANDS (IN %)

Cortex zone	Cytoplasmatic structure	VC	F	SC
Glomerular	Mc	14,52±1,92	16,94±1,13	25,79±1,25 *
	DL	23,91±2,43	13,85±1,06 *	5,49±0,56 *
	GℓER	10,01±1,01	9,61±1,22	15,52±1,28 *
Fascicle	Mc	14,35±1,11	19,55±1,15 *	19,44±1,03 *
	LS	1,33±0,22	1,59±0,18	1,26±0,22
	DL	11,95±2,11	1,03±0,42 *	4,93±0,91 *
	GℓER	12,60±1,30	17,71±1,42 *	17,86±0,91 *
Reticular	LS	16,54±0,96	16,88±1,12	22,96±1,14 *
	DL	1,89±0,79	1,79±0,18	1,79±0,18
	GℓER	4,04±0,25	1,02±0,79 *	7,91±1,26 *
		13,60±0,93	16,32±0,80 *	17,32±0,93 *

*Proven difference with VC

TABLE 19. NUMBER OF CYTOPLASMATIC STRUCTURES OCCURRING PER 100 μm^2 OF AREA OF THE CELLS OF THE CORTEX OF THE ADRENAL GLANDS (M±m) /71

Cortex zone	Cytoplasmatic structure	VC	F	SC
Glomerular	MS	15,25±1,71	11,08±0,74 *	14,38±0,86
	DL	5,60±0,66	12,25±1,18 *	5,54±0,60
Fascicle	MS	16,10±1,48	21,09±1,20 *	18,72±1,04
	LS	5,65±0,83	4,20±0,53	2,32±0,29 *
	DL	3,70±0,75	0,43±0,10 *	3,12±0,43
Reticular	MS	14,87±1,24	20,17±1,16 *	20,65±0,89 *
	LS	3,26±0,37	3,26±0,37	2,23±0,30 *
	DL	0,74±0,24	1,76±0,31	4,41±0,46 *

*Proven difference with VC

reticulum (ER), a decrease in dimensions and number of the lipid drops. The Golgi apparatus in the cells was very clearly marked and one observed a significant quantity of glycogen grains (Figure 38). /68

The most significant differences, in comparison with the vivarium control, are an increase in the number and the degree of dispersion capability of the lipid drops.

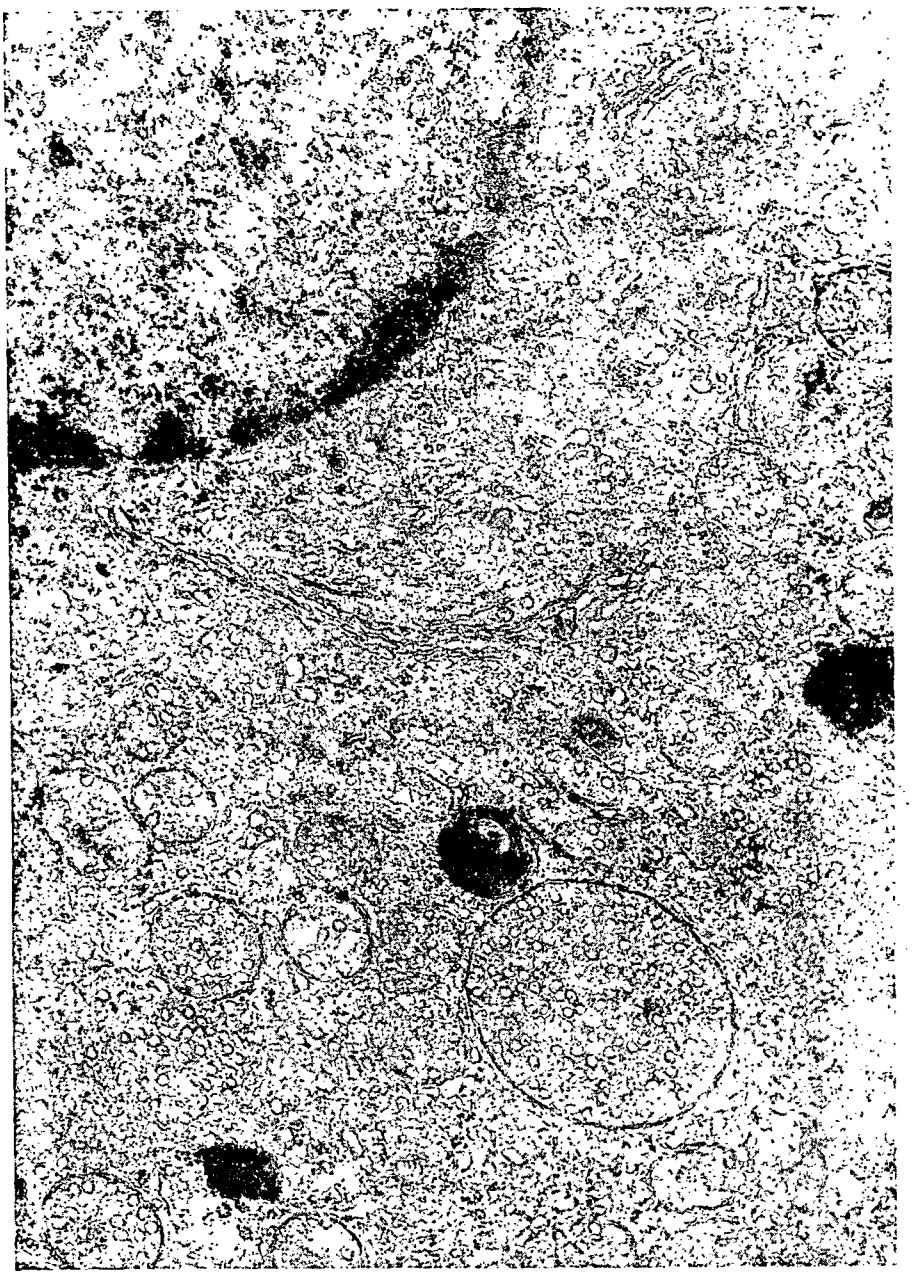

Figure 38. Fascicle layer of the cortex of the adrenal glands 9-11 hr postflight. Magnification 24,000.
In the near nucleus zone, the Golgi apparatus is well developed. Grains of glycogen are visible.

In the cells of the reticular zone one detected an increase in the volume of the endoplasmatic reticulum and number of mitochondria, the average number of the latter was not changed. An increase in the number of mitochondria with the absence of change of their volume can be explained by the increase of the number of small dimension mitochondria in rats of the flight group. The drops of lipids in the cells of this zone were significantly decreased in volume; however their number exceeded the level of the vivarium control. One sporadically encounters myelin-type structures found in contact with the ER in the reticular zone (Figure 39). /68 /71

The number of mitochondria was decreased in the glomerular layer. Their size was not significantly changed but their shape in the rats of the flight group and the rats of the synchronous control were different. The coefficient of the shape of the mitochondria in the rats of the flight group was 1.45 ± 0.25 and in the rats of the synchronous control group 1.25 ± 0.025, that is, the mitochondria in the cells of the glomerular zone in animals of the flight group were elongated. In animals of the synchronous control group, similar changes were basically observed (Table 18, 19); there were individual differences, as a rule, but only in degree.

Thus, both in the rats of the flight group and in the rats of the synchronous control group an increase occurred in the number of mitochondria and volume of the ER, and also a decrease in the relative volume of lipid drops. These characteristics attest to the increased production of steroid hormones (Sabatini et al., 1962; Gospodinow, Kietz, 1967; and others). The mitochondria and the ER are organoid which provides synthesis of a large number of necessary enzymes; cholesterol itself, which is contained in the drops of lipids is the initial plastic material. Reduction of the lipid drops indicates an increased demand of it for steroids in the synthesis process. Taking into account all that has been presented, one can conclude that in the rats who underwent flight and also in animals subjected to the effect of flight factors, except for weightlessness, there is a marked stress reaction.

Study in the Hypothalamus of Catecholamines and Enzymes, Their Synthesis and Decomposition /72

Up until now, the content of catecholamines in the hypothalamus of rats after space flight has not been studied. Moreover, the necessity for such a study is obvious if one takes into account the regulatory role of the hypothalamus in reactions of other organs of the endocrine system on the effect of extreme factors.

In the hypothalamus of rats, the content of catecholamines was determined and also the activity of enzymes of their synthesis

Figure 39. Reticular layer of the cortex of the adrenal glands 9-11 hr postflight. Myelin-type structure. Magnification 24,000.

(tyrosine-hydroxylase and dihydroxyphenylalanine-β-hydroxylase (DBH)) and decomposition (monoamino-oxidase). /72

Six rats from each experimental group were used. The bibliographical sources are listed below in which the methods used in this work are presented.

Index	Method
Concentration of catecholamines (CA)	Coyle, Henry, 1973 (with modifications)
Tyrosine-hydroxylase (TH)	Saavedra et al., 1974
Activity of dihydroxyphenylalanine-β-hydroxylase (DBH)	Molinoff et al., 1971 (in modification of Saavedra and co-authors 1974)
Activity of monoamino-oxidase (MAO)	Wurtman, Axelrod, 1966
Quantity of protein	Lowry et al., 1951

The catecholamines determined were basically from noradrenalin because, in the hypothalamus, there is an extremely small quantity of adrenaline.

The results obtained are presented in Figures 40-43.

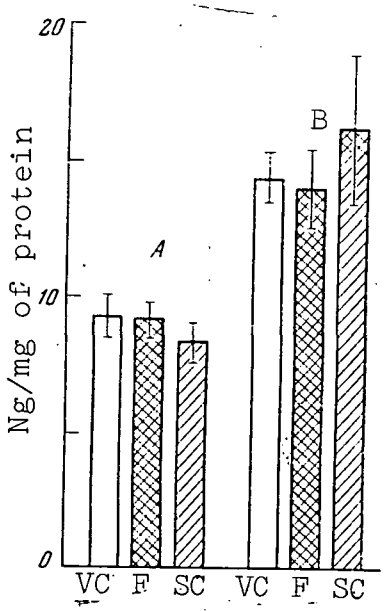

Figure 40. Concentration of catecholamines. A -- 5-7 hr; B -- 25 days.

/73

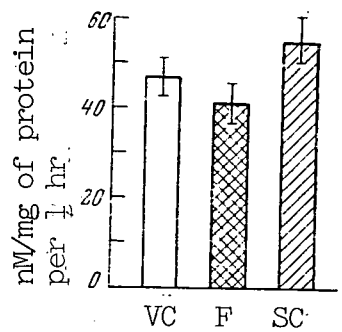

Figure 41. Activity of tyrosine-hydroxylase in the hypothalamus after 5-7 hr of flight

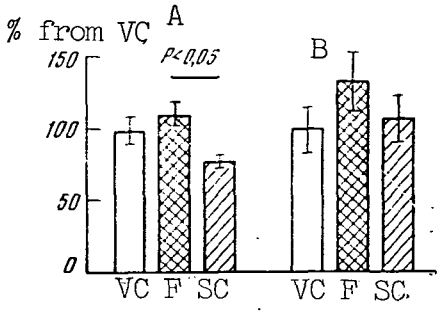

Figure 42. Activity of dihydroxyphenylalanine-β-hydroxylase
VC: M=3.69±0.56 nM/mg of protein per 1 hr; A -- 5-7 hr. B -- 25 days.

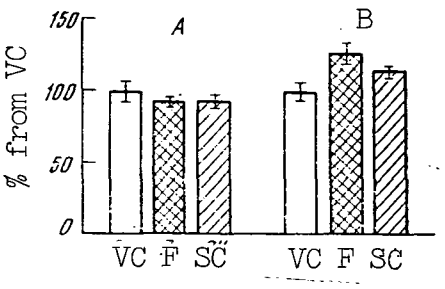

Figure 43. Activity of monoamino-oxidase
VC: M=70.66±4.56 nM/mg of protein per 1 hr; A -- 5-7 hr; B -- 25 days.

In no one of the research periods in the rats in the flight group was any difference detected in the content of CA in comparison with the control groups; however, after 25 days after the tests, this index was increased in the rats of all three groups (Figure 40). /72

The TH activity (Figure 41) in the hypothalamus of rats in the flight group immediately after flight also did not differ from that in the animals of both control groups. Twenty-five days after landing, for technical reasons, study of this index was impossible.

The DBH activity (Figure 42) was not changed in animals of the flight group in comparison with the vivarium control, but in the first time period the study proved an increase ($P<0.05$) in comparison with the synchronous control.

There were no proven changes in rats of the flight group in MAO activity (Figure 43).

It is well known that intensive acute stress causes a significant decrease in the concentration of CA in the hypothalamus (Kvetnansky et al., 1976; Matlina, 1976; and others). Such a phenomenon can occur in the initial phases of flight. It is possible that later on synthesis of catecholamines increases in compensation as a result of which, at the moment of the study, the concentration of these compounds did not differ from the control level.

Many researchers have pointed out a certain increase in the concentration of CA in the hypothalamus of rats after secondary stress, caused, for example, by immobilization. /73 With unchanged content of CA, activity of TH and DBH increased (Kvetnansky et al., 1976). An increase in activity of these enzymes in the hypothalamus of rats after secondary stress

indicates increased synthesis of CA and explains why the level of the latter was not decreased. No changes either in the content of CA in the hypothalamus or in the activity of enzymes studied in their synthesis and decomposition was found. Obviously, long term space flight, whose main condition is weightlessness, did not cause an acute stress effect on the production of CA in the hypothalamus.

To explain the increase in concentration of CA in the hypothalamus of the rats of all groups killed on the 26th day after completion of flight is still impossible. This can be due to aging of the animals and also conduct in this period of certain additional studies. Also an increase was detected at this time period in the content of catecholamines and the activity of the phenylethanolamino-β-methyl-transferase in the adrenal glands.

Morphological Study of the Hypothalamus-Hypophiseal Neurosecretory System /79

As is well known, in the cosmonauts during transition to Earth's gravitation a decrease in diuresis was noted and an increase in antidiuretic activity of the blood. In rats exposed on the Kosmos-605 biosatellite, two days after flight, morphological characteristics were observed in the increase of functional activity of the hypothalamus-hypophiseal neurosecretory system (Savina et al., 1976). An increase in the diameter of the nuclei of the neurons of the superoptical nuclei is noted in the Karmanchikov mice after flight on the Apollo-17 spacecraft (Ordy et al., 1975). Due to the fact that the hypothalamus-hypophiseal neurosecretory system (HHNS) participates not only in regulation of water exchange but also in establishing adaptation reactions of the organism as a whole, there is interest in studying it in the immediate hours after landing of the animals.

The histologic study of supraoptical nuclei of the hypothalamus and the hypophisis was made in 5-6 animals of each of the three groups in both time periods of postflight examination. The hypothalamus and the hypophisis were fixed in a mixture of mercuric chloride and Formalin (9:1) and immersed in paraffin. The sections were colored with hematoxylin-eosin according to the method of Gomor and Eykarson. The volume of the nuclei of the neurons was determined by measuring the length and short diameter on the contour projections on the nuclei obtained using the RA-6 drawing apparatus with a magnification of 2000 times. Subsequent calculations were made according to the method generally used in caryometry (Tsanev, Markov, 1960; Khesin, 1967), using a logarithmic method of dividing by class. /80

In studying the supraoptical nuclei of the hypothalamus in rats killed 9-11 hr after landing, the following characteristics were noted in comparison with the animals in the vivarium control group. In all the rats of the flight group, plethora of the capillaries was more marked. The neurosecretory cells were increased, and more unitypical. The perinuclear zone of cytoplasm in most of the cells was noticeably expanded, transparent, but along the periphery, sharp rings of chromatophil substance were determined; thanks to this, the boundaries of the cells are well outlined. Particularly clear is the increase in dimensions of the secretory neurons and their nuclei are apparent in the preparations colored according to Eykarson. In rats of the flight group, the peripheral rings with basophilic substance from Nissel were broad, determined almost along the entire perimeter of the neurons at the same time that in the control animals they were narrow and less clear. The volume of nuclei of the neurons in the animals of the flight group was proven to be increased in comparison with the vivariym control (405.6 ± 15.5 μm^3 and 323.9 ± 6.7 μm^3, respectively). Larger nucleoli were established.

The study of neurosecretion showed that in animals of the flight group the content of Gomor-positive granules (GPG) in studies of separate HHNS was decreased in comparison with the vivarium control. The neurosecretory cells are poor in granules and in distinction from the control rats, in the rats of the flight group the GPG are very fine, dustlike, palely colored and do not form precise accumulations around the nucleus. In the majority of neurons, the small quantity of GPG is determined on one side of the nucleus or single granules concentrated through the entire cytoplasm. Often one encounters cells of excess neurosecretion. In most of the studies, axon at the level of supraoptical nuclei is determined in the form of separate threadlike or striplike partially vacuolized fragments which contain a small quantity of granules optically denser than in the neurocytes.

Both the quantity of capillaries found and the degree of their plethora were incresed in the hypophisis of animals in the flight group. Besides the expansion of the capillaries, the total content of neurosecretion was decreased. Not only the number of Herring small bodies decreased but also the density of distribution of granular neurosecretory substance. In the Gerring bodies, retained between the granules there are vacuoles; due to this, expansion of the axons gradually acquired a reticular structure and blended it with the background. In most of the neurons of the supraoptical nuclei, one should turn attention to the high precision of differentiation of cytoplasm on the eosinophil perinuclear zone and the rings of the chromatophil substance. The perinuclear eosoniphil

zone in part of the cells was broader than in the control animals; here such neurons were somewhat increased in dimensions, the rings of the chromatophil substance were broader and more intensely colored; due to this, the boundaries of the cells were very sharply outlined. When inspecting the preparations colored with gallocyanin, one observed a certain increase in the dimensions of the cells; more often, than in the control one encountered cells of oval or pear shape. The ribonucleic /81
acid rings in most of the cells were determined throughout the periphery of the cytoplasm and were broad and homogeneous. In some of the cells, one noted a noticeable enlargement of the nucleolus, and an increase in the number of 2-nucleolus nuclei. Determination of the volume of the nucleus of the neurons showed a tendency toward their increase; no proven differences existed in comparison with the vivarium control. When studying neurosecretory substances in the bodies of neurons of the supraoptical nuclei and their axons at the nucleus level, there is the impression that the content of GPG, at least in some of the animals, is higher than in the control and more so in animals of the flight group. The neurosecretory granules formed precise rings or broader spindles around the nuclei or cone shaped accumulations on one side of the nucleus. In some of the animals one observed a precise tendency toward concentration of the granules and decrease in their dimensions and optical density. In most of the animals in the hypothalamus-hypophiseal tract, at the nucleus level, a large quantity of fragments, axons, polymorphous in shape and dimensions were apparent with a high content of GPG. The posterior part of the hypophisis, in composition of the blood filling vessels in content of neurosecretion as a whole were similar to those in the vivarium control rats. However, in distinction from the latter, in this group of animals one observes great variation in the number and dimensions of Herring bodies and the content of granular neurosecretion. In some of the rats, one noted uneven distribution and focal decrease in the content of neurosecretion.

Twenty-five days after flight, one could not successfully show significant differences in the state of the neurons of the supraoptical nuclei in comparison with the vivarium control. In the rear portion of the hypophisis, one observed an accumulation of a large quantity of Gerring bodies; here they predominated over the granular neurosecretions. In three of the five animals in the synchronous control group, one observed a decrease in the content of GPG in the bodies of the neurons and the terminals of the axons in the posterior part of the hypophisis.

Thus, the study made showed that 9-11 hr after landing, in the hypothalamus-hypophiseal neurosecretroy system of the rats, morphological characteristics were observed which,

according to data in literature (Voytkevich, 1967; Polenov, 1971; Zhuraleva et al., 1976, and others) attest to the increase in functional activity of HHNS and are observed under the effect of extreme factors of different types including the G-force (Andrianova, 1968; Podymov, 1970). In the synchronous experiment, these characteristics were less marked. An analysis of the data makes it possible to consider that the changes described in time can develop by the 9th to 11th hours after landing. This gives us a basis for proposing that the shifts detected show the HHNS reaction in response to a complex of stress effects during descent and landing of the satellite. A significantly high degree of change in rats in the flight group, in comparison with the synchronous experiment, are due in all probability not only to this more complex set of factors acting during descent and landing of the satellite but also to the return to Earth's level of gravitation after a long stay in weightlessness conditions. The shifts observed in HHNS in the rats in the first hours after flight have a transitional character. In rats from the Kosmos-605 satellite, the morphological characteristics of the increase in functional activity of the hypothalamus-hypophiseal system are retained for two days after flight (Savina et al., 1976). However, after 25-27 days, the state of the neurons is normalized. /82

Thyroid and Parathyroid Glands

During space flights and after their completion, in cosmonauts and in animals, an increased secretion of electrolytes was recorded, in particular calcium, a decrease in mineral saturation of bony tissue, and also the phenomenon of osteoporosis (Balakhovskiy et al., 1973; Heany, 1974; Yadogovskiy et al., 1977).

The thyroid and parathyroid glands participate in the development of metabolic dysfunctions in the bone tissue and in the exchange of calcium; therefore it is considered expedient to show the effect of the factors of space flight on the morphologic characteristics of these glands.

The thyroid and parathyroid glands were studied by histologic methods in six rats from each group 9-11 hr after completion of the experiments. Twenty-five days after landing, material was taken from five rats from each group. The thyroid gland, along with the parathyroid glands were fixed in Bouin's fluid and immersed in paraffin. The sections were colored with hematoxylin-eosin, hematoxylin-light green, and with azane according to Geydengayn's method. The parafollicular cells (C-cells) were shown by a method of impregnation with silver according to the De Grandi method. On the series sections in the central parts of the thyroid gland, the number of C-cells was counted using an ocular grid (ten measurements per nominal unit of area of the section of thyroid gland with a

magnification of 40X7). The numerical material was processed statistically and a comparison was made according to the St'yudent criteria.

Nine-eleven hours after completion of the flight, in the thyroid and parathyroid glands, a marked capillary plethora was noted, a small edema of the connective tissue stroma. The thyroid glands of the flight group of rats were characterized by a fairly unitypical morphologic picture close to that of the rats in the synchronous experiment and vivarium control groups. It was only successfully noted that the thyroid glands in the flight group of rats were distinguished by greater uniformity of structure and dimensions of the follicles, the colloid was of average density, with occasionally encountered resorption vacuoles. Proliferation of interfollicular tissue occurred more intensely than in the control; this can be judged by the increase in dimensions of the interfollicular islands (Figure 44). The C-cells were encountered primarily

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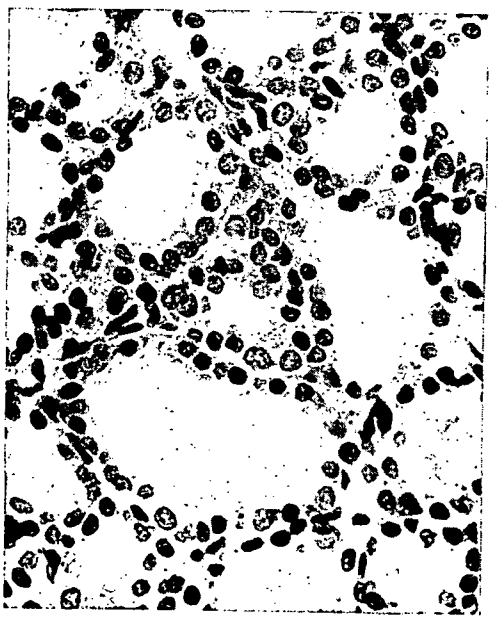

Figure 44. The thyroid gland of rats 9 hr after flight. Magnification 400. Interfollicular islands are increased. Coloration with hematoxylin-light green.

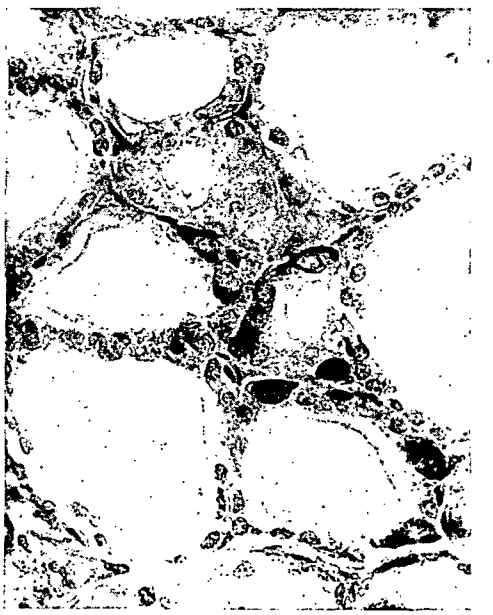

Figure 45. The thyroid gland (control). C-cells. Magnification 400. Impregnation according to the De Grandi method.

in the central sections of the gland; they were located in groups of 3-4 cells around the follicles close to the vessels. In the wall of the follicles there were apparent, as a rule, single C-cells. The results of counting the C-cells showed /82

a certain increase in their number in rats of the flight group but proven differences in the indices of the groups studied could not be obtained. Nine-eleven hours after flight, the number of C-cells per unit of area of the section of the thyroid gland amounted to 12.6 ± 1.0; in the synchronous control -- 11.3 ± 1.0 and in the vivarium control -- 10.3 ± 1.1. With impregnation of the sections of the thyroid gland according to De Grandi in the cytoplasm of the C-cells, secretory granules were apparent (Figures 45, 46). /84

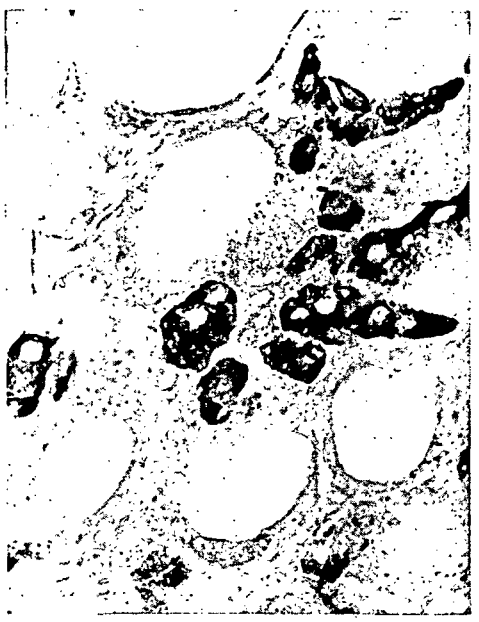

Figure 46. Thyroid gland of a rat 10 hours after flight. Magnification 400. The number and dimensions of the C-cells were increased. Impregnation according to the De Grandi method.

The density of distribution of the cytoplasmatic granules varied; however, in the flight group of rats one most often encountered C-cells at different stages of degranulation whereas in the animals from the vivarium and synchonous control groups the cytoplasm of almost all of the C-cells was more densely filled with granules.

The parathyroid glands in the flight group of rats had an ordinary structure; however, separate sections were encountered consisting of parathyreocytes with large round nuclei and clarified foamy vacuolized cytoplasm. Sections of hypertrophied main cells can be seen in animals of the synchronous experiment group but their prevalence in this case was small.

Twenty-five days after flight, the morphologic picture of the thyroid and parathyroid glands was the same as in rats from the synchronous and vivarium control groups. The number of C-cells in the thyroid gland of the flight group rats, as in the first study, were somewhat increased; however, there were no proven differences among the various groups apparent.

Thus, with histologic examination of the thyroid and parathyroid glands in rats after a 19.5-day flight, no significant morphologic changes were noted. The discovery of C-cells of the thyroid gland according to the De Grandi method showed a certain increase in the number in rats in the flight group and significant variation in the content of secretory granules in the cytoplasm. Inasmuch as it is known that degranulation of parafollicular cells is a specific response to hypercalcemia (Matsuzawa, Kurosumi, 1967; De Grandi, 1970), this increase in

the number of calcitonin cells accompanied by degranulation of some of them, can be evaluated as a manifestation of a reaction directed at increasing the discharge of hormones of thyreocalcitonin with a decrease in the content of calcium in the blood plasma and slowing of resorption by the bones.

Conclusion

Endicrinological studies directed mainly at investigating the hypophiseal-adrenal system showed that the effective factors of space flight do not cause sharp dysfunctions. Part of the indices were not changed in the animals of the flight group (content of catecholomines in the hypothalamus, hormone growth, thyreotropic hormones and other glycoprotein hormones in the hypophisis), change in others (content of corticosterone in the adrenal gland, a number of histologic and morphometric criteria of the state of the adrenal glands) was insignificant and indicated a weakly expressed stress reaction. Basic attention then was turned to flight factors active during descent and landing of the object on Earth.

A study of the structure of the thyroid and parathyroid glands which participate in exchange of calcium did not show changes which would indicate damage to these organs.

The Muscle System

Cytochemical and Morphometric Studies of the M. Quadriceps Femoris.

In the test models it was discovered that limitation of muscular activity results in atrophy and degenerative changes in muscle fibers; the expression of this is not uniform in muscles which differ in morphology and function (Mann, Salafsky, 1970; Cooper, 1972). These changes to various degrees are also apparent in three types of muscle fibers differint in metabolism and cytoenzymatic characteristics (Engel, 1970; Tomanek, Lund, 1974).

In this study, in the animals of all three groups, in both time periods for slaughter, the character and degree of structure and cytochemical changes in the m. quadriceps femoris were studied.

The histologic structure was evaluated and also the percentage ratio between muscle fibers of different types which were classified according to the intensity of histochemical reaction to succinate dehydrogenase (Stein, Pedycula, 1962). According to this nomenclature, the thick muscle fibers containing relatively small quantities of the reaction product and evenly distributed on the surface of the lateral section of the muscle are classified as fiber A. Fibers which are smaller in diameter with a large quantity of reaction product also uniform, are designated as fiber B and the finest fibers with the largest content of products from the histochemical reaction, localized mainly under the sarcolemma are designated as fiber C.

After the histochemical reaction was made, five randomly selected fields of vision were photographed containing about 200 fibers each. The relationship among the three types of fibers is expressed in percent. Morphometric analysis of the histologic pictures was done using the automated Quantiment-B analyzer. In the muscle of each animal, about 300 lateral sections of separate fibers were measured. In each section, the area of the fiber and its diameter were determined. Using a computer program developed for this purpose, the average area and average diameter of the cross section of fiber was calculated, as well as dispersion and standard error for these values and also the percentage distribution of the magnitude of diameters and areas of the cross sections of muscle fibers in separate animals and in separate groups of animals. Electron-microscope study was made for which sections of the quadriceps muscle were fixed in 3% glutaraldehyde per 0.1 M phosphate buffer solution for a period of 24 hours and then

in a 1% osmic acid. The material after dehydration in alcohols with increasing strength and acetone were poured in the Epon-812.

Ten randomly selected blocks were used from the poured material corresponding to each group of animals for preparation of ultrathin sections on the Reichert microtome. The sections were colored with acetic uranyl and nitric acid lead. The work was done on a JEM-100C microscope. Morphometric studies for quantitative evaluation of the electronograms was done according to a method of microstereology (Weibel et al., 1966). One hundred electronograms were used for each group of rats for morphometric analysis. The length and relative volume of the fibers and separate structural components of the sarcomere were measured. Attention was given to the morphologic picture of the mitochondria, the sarcoplasmatic network, the content of lipid drops. The results of morphometric measurements were processed according to the St'yudent method. /86

Data on the content of fibers of types A, B and C are given in Table 25. Im the first time period of the study, statistically proven differences were not detected among the three groups of animals studied; because of this, this determination was not made 25 days after the flight. The absence of changes in the relationships among the three basic types of muscle fibers was established by other scientists. The increase in percent of fiber type C, interpreted as the result of atrophy described only in the red slower muscle in which earlier and to a greater degree than in the white rapid muscle, atrophy processes developed of a neurogenic character (Tomanek, Lund, 1973).

TABLE 25. CHARACTERISTICS OF MUSCLE FIBERS OF TYPES A, B AND C IN THE M. QUADRICEPS FEMORIS

Type of Fiber	VC	SC	F
	% of composition		
A	45,0±3,1	46,5±2,4	45,1±2,5
B	21,9±1,2	18,4±1,2	19,9±1,0
C	33,1±2,8	35,1±1,7	35,0±2,4
	Average diameter, μm		
A	55,6±0,5	56,1±0,5	57,5±0,5
B	42,8±0,4	43,4±0,4	48,5±0,5
C	33,4±0,3	34,8±0,3	36,6±0,4
	Average area of cross section μm^2		
A	2172±38	1991±21	2309±26
B	1236±13	1299±15	1553±22
C	761±10	844±10	942±16

The morphologic characteristic of the m. quadriceps femoris in rats 9-11 days after flight was distinguished by the presence of two peculiarities absent in animals in the vivarium and synchronous control groups. In the rats of the flight group, on the cross section of muscles, 80% of the surface was occupied by wide hollow spaces located between separate muscle fibers. The latter, on the cross sections, are close to a circular shape (Figure 47a) in distinction from fibers of polygon shape in rats of the vivarium control group (Figure 47b).

Figure 47. Distribution of succinate dehydrogenate in muscle fibers of the quadriceps muscle. Magnification 600.

A -- 9-11 hr after flight; free spaces form between the muscle fibers which have a circular shape; B -- vivarium control; fibers having a polygonal shape, densely packed.

When using hematoxylin-eosin coloration it was discovered /86
that the free spaces between the muscle fibers do not involve hypertrophy of the endomysium and infiltration of exogenic cells (in particular, lymphocytes) which occurred in the m. quadriceps and the m. soleus in rats with hypokinesia (Portugolav et al., 1971) and also in the m. soleus muscles in rats exposed for 22 days on the Kosmos-605 biosatellite (Il'na-Kakueva et al., 1976). In our study, one did not observe histologic changes in any of the muscle fragments studied 25 days after landing of the animals. /88

As is apparent from the data of Table 25, during morphometric examination, statistically significant differences are not detected in the average diameters of muscle fibers of different types. An evaluation of the average dimension of the area of the cross section of muscle fibers proved a more sensitive test and showed, in the 9-11 hr time period, a difference between the flight group and the two other groups of animals. The area of the cross section of muscle fibers of types B and C was proven ($P<0.01$) to be increased.

In the diagrams (Figures 48, 49) a distribution of the diameters of muscle fibers and the areas of their cross sections are presented; there is clearly visible a large disparity in rats of the flight group in comparison with both control groups; this applies here to all three types of muscle fibers.

It is possible that the histologic changes we discovered and also the increase in area of the cross section of muscle fibers in the flight group of animals was the result of edema involving redistribution of fluids in the organism after return to condition of Earth's gravitation. These disturbances can also occur as the result of hemodynamic disorders during space flight. The absence of these changes in the synchronous control group indicate a primary connection with weightlessness and not with hypokinesia. The histologic changes described above have a reversible character; after 25 days postflight, they are absent. The stereologic studies on the ultrastructure level which did not indicate marked differences among the groups in the first time period, were not made in the 25-day period.

During morphometric study of the electronograms, no differences in length and volume of the sarcomeres was discovered nor stria of the A, I red and white muscle fibers in the rats of the flight group in comparison with the control (Table 26).

However, in the flight animals, both in the white and in the red fibers, a marked decrease in the volume of sarcoplasmatic reticula was noted (Table 27), and also an increase in the /89
quantity of triglycerides. The relative volume of the mitochondria was unchanged. As part of the decrease in average values

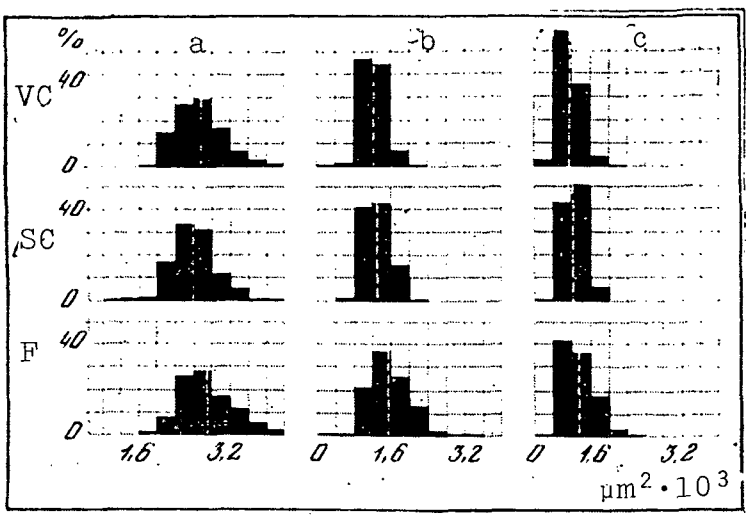

Figure 48. Distribution of muscle fibers of different types according to the magnitude of diameters a, b, c -- A-, B-, C-types of fibers

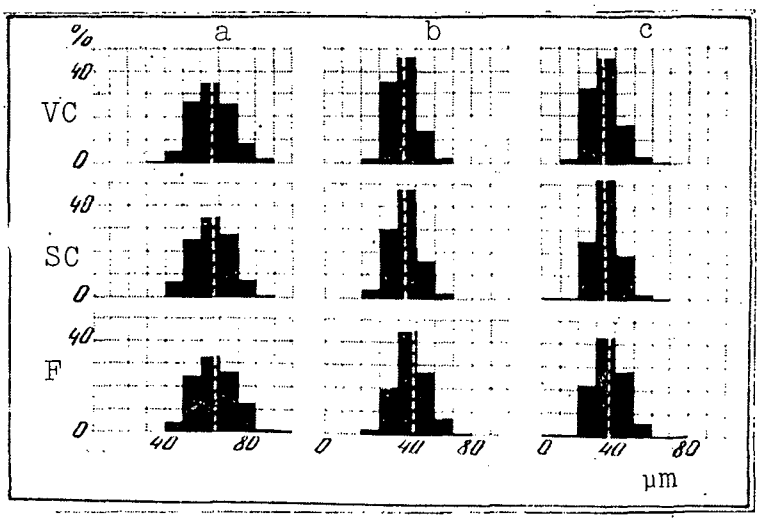

Figure 49. Distribution of muscle fibers of different types according to magnitude of the area of their cross sections. a, b, c -- A-, B-, C-types of fibers.

of the volume of the sarcoplasmatic reticulum, in the muscles of rats in the flight group one encountered sections most frequently distributed on the peripheral fiber where the volume of the sarcoplasmatic reticulum was increased (Figure 50) and also sections where the sarcoplasmatic reticulum was almost absent, but there were many free unchanged mitochondria.

TABLE 26. RESULTS OF MORPHOMETRIC STUDIES OF SARCOMERES (M±m)

/88

Parameter	White Fibers		Red Fibers	
	F	VC	F	VC
Length of sarcomere, μm	2,47±0,02	2,44±0,02	2,39±0,03	2,43±0,02
Length of stria, μm				
A	1,88±0,01	1,89±0,01	1,83±0,02	1,82±0,02
I	0,52±0,01	0,48±0,01	0,46±0,02	0,52±0,01
Z	0,07	0,07	0,10	0,09
Relative volume of stria,%				
A	76,29±0,40	75,38±0,40	76,78±0,68	76,81±0,68
I	20,92±0,40	21,00±0,40	18,94±0,70	18,01±0,70
Z	2,79±0,02	2,80±0,02	4,28±0,08	4,25±0,08

TABLE 27. RELATIVE VOLUME (IN %) OF THE CYTOPLASMIC STRUCTURES (M±m)

/92

Structure	VC	F	P_{VC}	Structure	VC	F	P_{VC}
	Red Fibers — a				White fibers		
Mitochondria	12±1,0	14,0±1,2	>0,05	Mitochondria	6,6±0,2	6,9±0,4	>0,05
Sarcoplasmic reticulum	32,7±0,9	23,7±1,6	<0,001	Sarcoplasmic reticulum	32,0±0,8	26,5±1,2	<0,001
Drops of lipids	0.6±0,1	1,5±0,3	<0,01	Drops of lipids	в 0,24±0,07	0,26±0,07	>0,05

The mitochondria in an edema state and also mitochondria containing myelinlike structures (Figure 51) are a rare phenomenon. The changes observed in the ultrastructure and the decreasing volume of the sarcoplasmatic reticulum in the muscle fibers of rats of the flight group can involve a decrease in intensity of metabolic processes. Hydration of the sarcoplasmatic reticulum was encountered primarily in the peripheral sections of the fibers. This phenomenon can be related to beginning edema which is a manifestation of hemodynamic disorders characteristic for transition from conditions of weightlessness to conditions of gravitation. These observations are in accord with the results of measurements of the area of the cross section of muscle fibers.

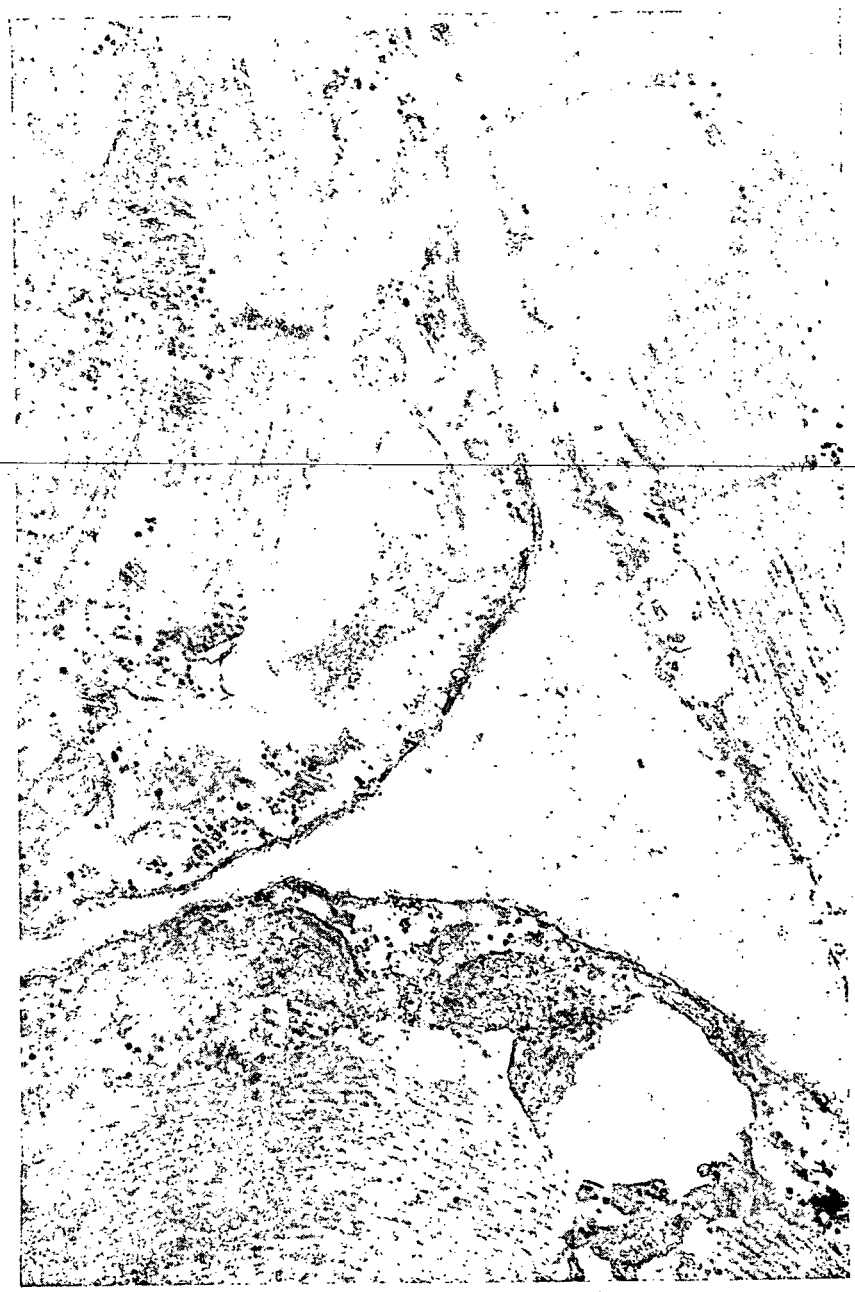

Figure 50. Increase in the volume of the sarcoplasmatic reticulum in the peripheral sections of muscle fibers 9-11 hr post flight. Magnification 24,000.

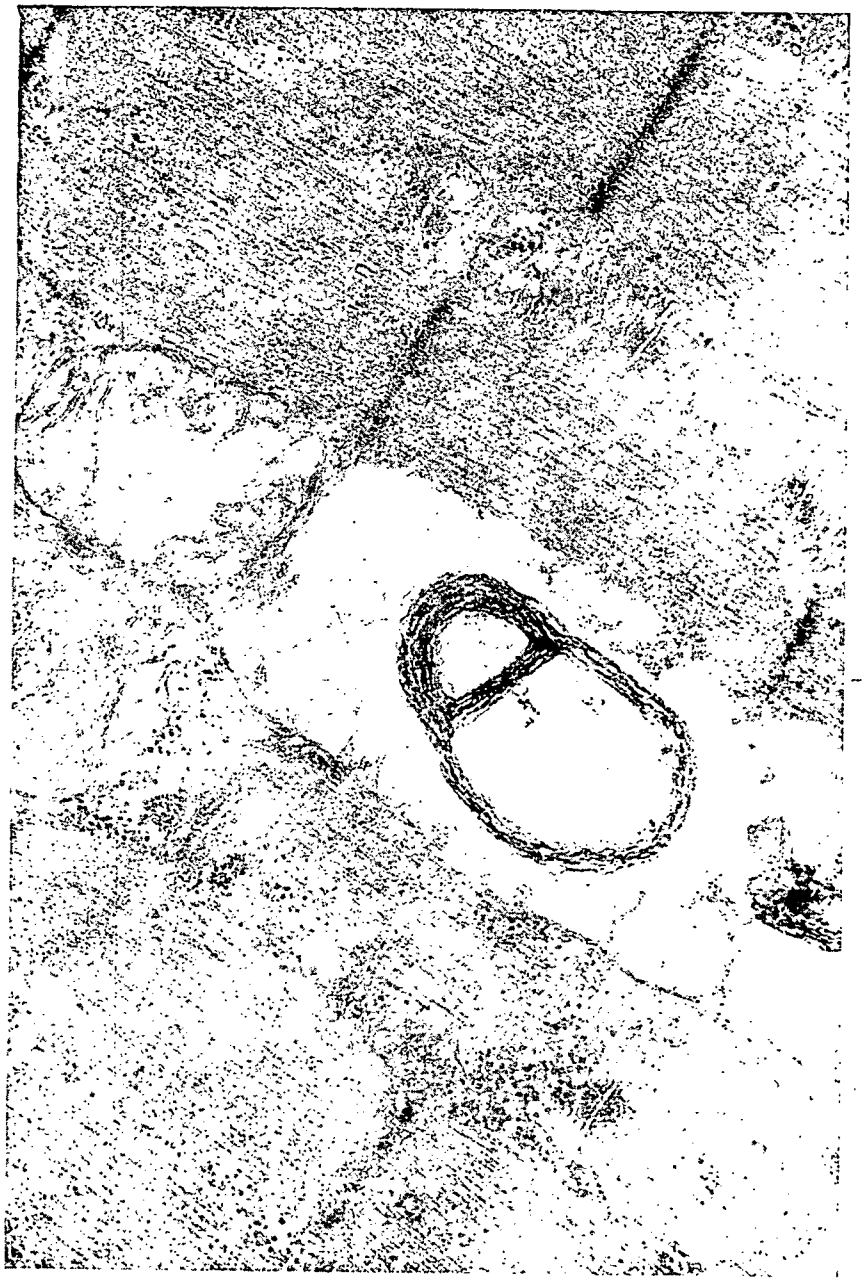

Figure 51. Edema of the mitochondria and the appearance of myelinlike structures 9-11 hr postflight. Magnification 24,000.

Thus, the histologic and the chemical studies we made did not show atrophic changes in the structure of the m. quadriceps of the rats exposed on the biosatellite for 19.5 days. A decrease in volume of the sarcoplasmatic reticulum discovered by methods of morphometric analysis at the level of the ultrastructure. Damage to other structures was absent or very rarely encountered.

Morphometric and Cytochemical Studies of the Nerve-Muscle Synapses.

There is interest in discovering whether or not a decrease in the functional activity of the skeletal muscles in conditions of weightlessness results in a change in concentrations of enzymes which participate in carrying out nerve and muscle transmission of impulses and also change in ultrasonic structure of the motor plaque.

Nerve and muscle synapses were studied in the m. quadriceps femoris in animals of all three groups 5-11 hours and 25 days after completion of the test.

On electronograms, a qualitative evaluation was made of the structure of the motor plaque and the number of synaptic sacs and mitochondria in them was studied as well as relative volumes of their structure, ratio of their surface area to volume. In the motor plaque, also the concentration of /93
acetylcholinesterase and cholinesterase were determined. The method consisted of inhibition of the enzymes labeled according to tritium with diisopropylfluorphosphate (N^3-DFP) and subsequent reactivation. On the radioautographs, the average number of grains of reduced silver was counted in the structures of the motor plaques in 100 fields of the measurement grid.

As is apparent from the data presented in Figure 52, in the motor plaques of the diaphragm and m. quadriceps femoris the concentration of all esterases, blocking DFP, remained unchanged in comparison with the control. Quantitative relationships among the enzymes studied were uniform in the rats of all groups both in motor plaque of the diaphragm and in the m. quadriceps femoris (Figures 53, 54).

In the literature we will not find data on the content of active centers of cholinesterase in motor plaque of animals found in weightlessness conditions. In conditions of similar /94
limitation of mobility and with full loss of muscle function in the animals, activity of cholinesterase decreases in the nerve and muscle synapses (Cooper, 1972; Tomanek, Lund, 1974). It is proposed that a 19.5 day stay in a state of weightlessness is too short a time period for any changes to occur in concentration of cholinesterase localized in the motor plaque.

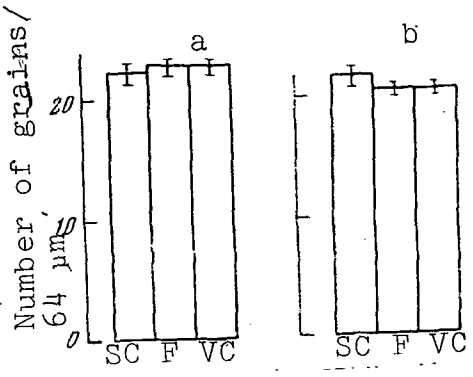

Figure 52. Concentration of active centers of esterase blocking H^3-DFP.
a -- m. quadriceps femoris; b -- diaphragm.

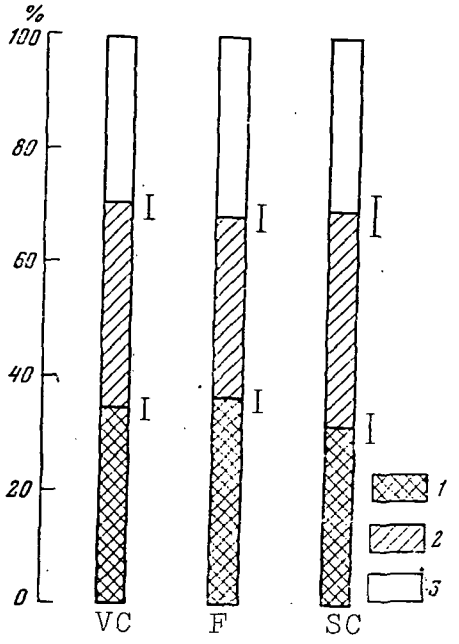

Figure 53. Percentage relationship of DFP-sensitive esterases in the motor plaque of the diaphragm.
1 + 2 -- cholinesterase;
1 -- acetylcholinesterase;
3 -- simple esterases.

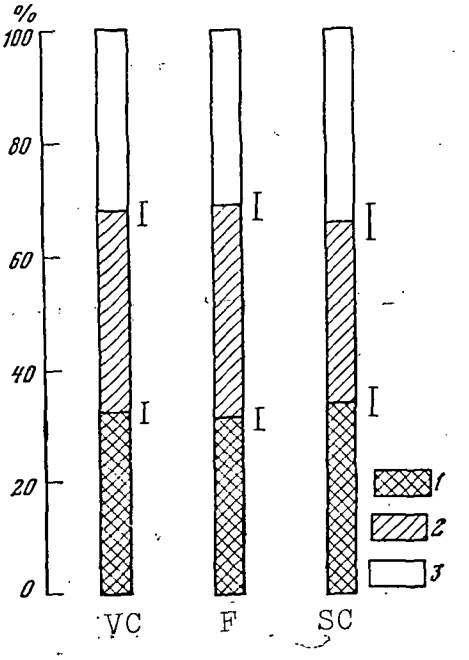

Figure 54. Percentage ratio of DFP-sensitive esterases in motor plaque of the m. quadriceps femoris.

Symbols the same as in Figure 53.

Quantitative studies of ultrastructures in motor plaque (see below) showed a decrease in the relative volume of synaptic sacs, but no statistically significant differences were observed in the relative volumes of the mitochondria. /94

	Mitochondria	Synaptic Sacs
Relative volume, %		
VC	19,5±2,3	28,8±2,3
F	16,7±2,3	17,0±1,6
Area of the surface per unit of the volume, $\mu m^2/\mu m^3$		
VC	2,2±0,3	16,5±1,8
F	2,4±0,3	17,3±1,8

Morphometric determination showed that the average number of profiles of the mitochondria and also of synaptic sacs existing on a cross section of one motor plaque was significantly decreased in the flight group of rats: 9.1 and 205.6, respectively, as opposed to 16.6 and 350.6 in the vivarium control. This attests to the fact that a decrease in volume of the fractions of synaptic sacs involved a decrease in their number. Moreover, an absence of changes in relative volumes of the mitochondria with uniform decrease in the average number of their profiles can attest to degeneration of part of them and edema of the remaining.

A qualitative analysis of the electronograms showed a small difference in the degree of change in animals of different groups. Pictures were observed of edema of the terminal sections of the axons accompanied by the appearance of identical vacuoles (Figure 55, a) and also edema of the mitochondria (Figure 55, b). The latter were characterized by melting of the cristae and their fragmentations. On certain electronograms shortening and thinning of the synaptic folds were observed which resulted in a decrease in the contact area of the terminal axon with the muscle fiber. Only in a few cases were wrinkling of the axon terminals, nonuniform distribution of the neurofibrillae in them observed. Sometimes within the nerve endings dark structures were detected, apparently, residue of the sheathing of the decomposed mitochondria (Figure 55, c). On several electronograms fragmentation of the axon terminals was observed surrounding on all sides the cytoplasm of the Shvannovskiy cells, completely separating them from the muscle fiber (Figure 55, d). In single cases, full autolysis of the terminals took place.

In the cytoplasm of the Shvannovskiy cells, residue of the sheathings of the mitochondria, multivesicular structures, myelin-like bodies and lysosomes were discovered. These changes of the ultrastructure of the neuronal component of the motor plaque,

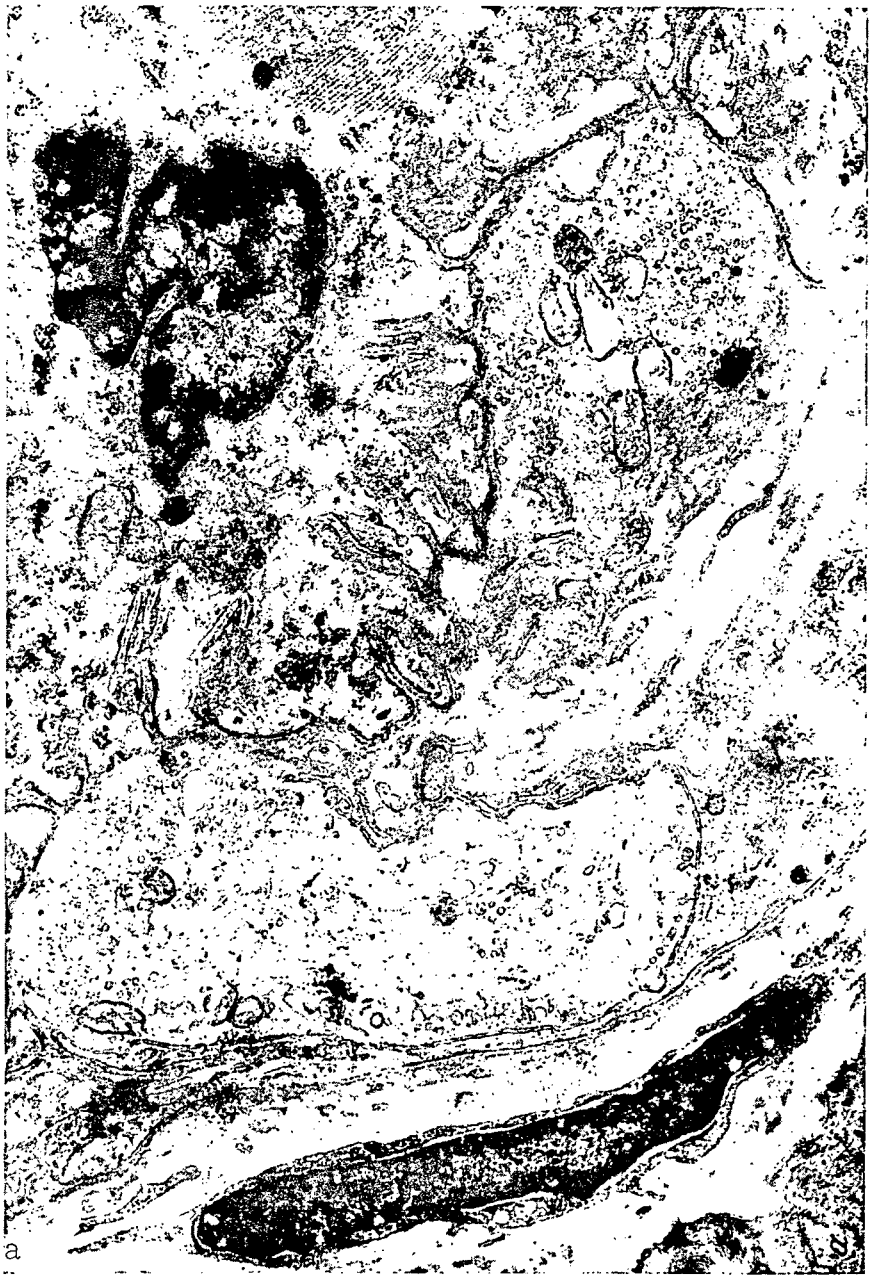

Figure 55. The m. quadriceps femoris 9-11 hr post-flight.
a -- motor plaque. One observes edema of the terminal section of the axon and the presence of vacuoles in it. Magnification 10,000.

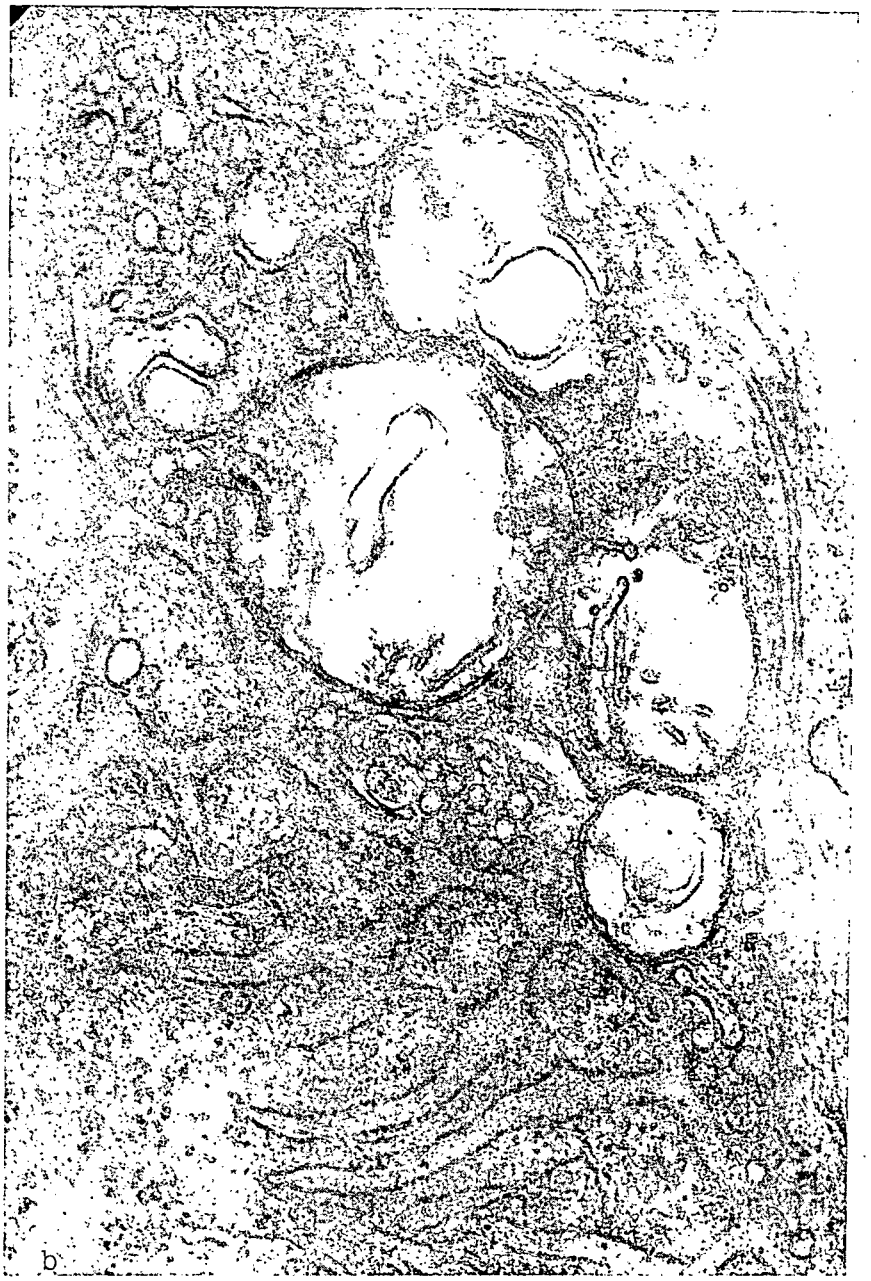

Figure 55. (continuation)
b -- Swelling of the terminal section of the axon is apparent: the number of mitochondria is decreased. Magnification 40,000.

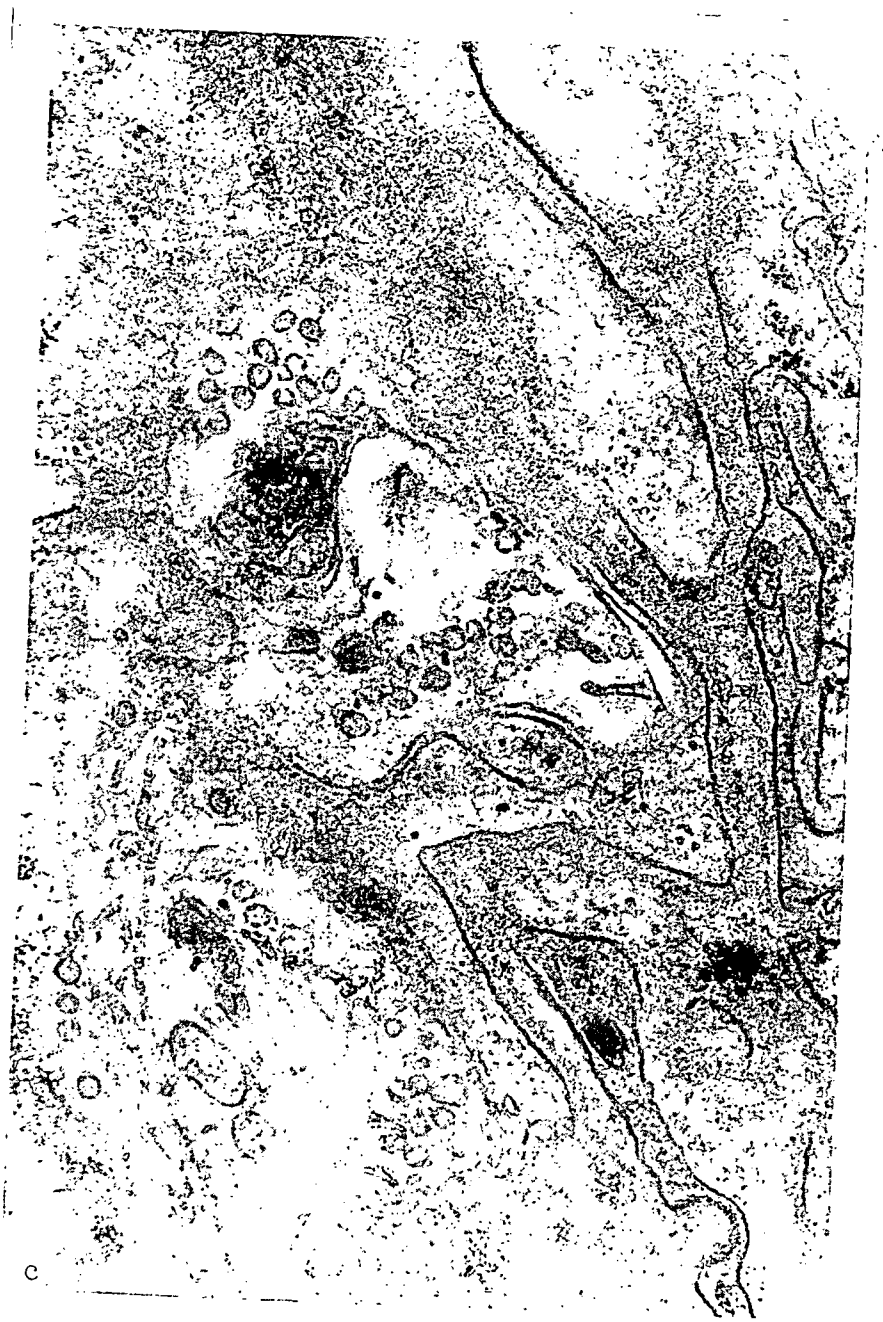

Figure 55 (continuation)

c -- fragment of degenerate change in the motor plaque. Magnification 40,000.

Figure 55 (continuation)

d -- Visible breakdown of the nerve endings separated from the muscle fiber of the Shvannovskiy cells.

in the opinion of many authors, is characteristic for the initial period of the degeneration when the process affects only the axon terminals (Nickel, Waser, 1968; Lentz, 1972; Pappas, Purpure, 1972). /95

Thus, the histochemical studies using an isotope label did not show changes in localization and the number of active centers of acetylcholinesterase and cholinesterase in the motor plaques. At the same time, morphometric studies at the ultrastructural level showed a decrease in relative volume of the synaptic sacs and a decrease in the average number of their structures and mitochondria occurring on one section of the nerve ending. Qualitative electron microscope analysis showed the presence of certain changes of destructive character in separate nerve endings.

The Effect of Space Flight on the Skeletal Musculature and Nerve Apparatus of the Muscles (Morphologic and Cytochemical Study)

Studies of the skeletal muscles of the rats who had completed 22.5 and 20.5 day flights on artificial satellites in the Kosmos series showed structural and metabolic breakdowns expressed in different degrees and shape in functionally different muscles (Illyna-Kakueva et al., 1976; Portugalov , Petrova, 1976; Ilyna-Kakueva, Portugalov, 1977). The purpose of the present study was to evaluate in rats who were on board the Kosmos-782 biosatellite for 19.5 days, the state of the two two muscles most damaged by the effect of hypokinesia and weightlessness -- the m. soleus and m. gastrocnemius, and also the state of peripheral motor, sensor and sympathetic nerve endings in these muscles. The latter is of particular interest due to the fact that in the m. soleus, in distinction from other muscles after a 22.5-day flight, symptoms were noted of breakdown in hemodynamics which is, as is well know, controlled by the vegetative nervous system (Abdulayev, 1967).

The histologic and histochemical studies were made on the m. soleus and m. gastrocnemius, and cytochemical -- on the m. soleus. The material from six rats was studied 5-7 hours and from six rats 25 days after completion of flight; in these same time periods and with the same number of rats from the synchronous control group and from 24 rats in the vivarium control group. The cryostatic sections of m. soleus and m. gastrocnemius were colored with hemotoxylin and eosin according to Mallory's method, the structures of the nerve tissue were colored according to the Bil'shovskiy-Gross method in a Kampos modification; they also showed acetylcholinesterase and nonspecific cholinesterase according to the Kelle method in the Gomori modification and catecholomines according the Ye. M. Krokhina method (1973). The isoenzyme composition

of LDH of the m. soleus which makes it possible to judge the condition of carbohydrate exchange, were studied using microelectrophoresis in a polyacrylamide gel (Portugalov, Petrova, 1976). Density measurement of flat gels was conducted on the IFO-451 instrument. The area of separate fractions on the densitygram was determined according to a planimetry method. The results obtained were processed statistically, using the parameterless criterion of Van-der-Varden (1960).

Five-seven hr after landing, the weight of the m. soleus and m. gastrocnemius had decreased in comparison with the same index in the vivarium control (Table 28). /100

TABLE 28. WEIGHT OF THE MUSCLES (IN mg) (M±m)

Group	5-7 hr. after completion of the test		25 days after completion of the test	
	m. soleus	m. gastrocnemius	m. soleus	m. gastrocnemius
F	79±3*	1416±21*	155±12	1900±49
SC	107±4**	1610±71	149±7**	1942±31
VC	129±5	1610±46	170±5	2072±86

* Proven differences with VC and SC.
** Proven differences with VC.

In the m. soleus one should turn attention to the clearly expressed thinning of muscle fibers, the increase of the number of nuclei in them which often is located centrally and in the form of a chain. Single muscle fibers have undergone dystrophic changes (Figure 56) and phagocytosis. In part of the muscle fibers, the coloration properties of the cytoplasm have changed. The quantity of connective tissue has increased in the endomesia.

In the m. soleus significant changes have occurred in the relationship of activity of LDH fractions (Table 29).

TABLE 29. THE SPECTRUM OF ISOENZYMES OF LDH IN THE M. SOLEUS (IN %) AFTER THE TEST

Isoenzyme	VC ($n=5$)	F		SC	
		5-9 hr ($n=3$)	25 days ($n=2$)	5-9 hr ($n=3$)	25 days ($n=2$)
LDH_1	28,9	23,0	26,1	31,0	33,1
LDH_2	34,3	26,2	33,6	32,5	33,6
LDH_3	24,2	30,0	25,0	21,8	21,3
LDH_4	9,7	17,2	10,7	12,2	9,6
LDH_5	2,9	3,6	4,6	2,5	2,4

An increase in activity of the "muscle" fractions (LDH_4 and LDH_4) and a decrease in activity of the "center" fractions (LDH_1 and LDH_2) led to a change in the spectrum of isoenzymes from the "center" in standard to the intermediate.

The nerve-muscle endings in the m. soleus differed in structural forms. In most of the motor plaques of the axon terminals, thickening is observed and there are excess argyrophilia (Figure 57). The number of cells of the pad in it varied considerably.

/101

Figure 56. m. soleus 5-7 hr postflight.
a -- VC; b -- F; c -- SC. More marked atrophic changes are noted in the animals of the flight group.

The neurofibrillae of the axons show different fibers. Changed nerve-muscle synapses are encountered. In the nerve conductors entering into the composition of the nerve cores, no kind of deviation from normal is detected. /100

The activity of acetylcholinesterase and nonspecific cholinesterase in the tests and control was identical. /102

In the m. gastrocnemius in animals during flight, changes developed similar to the changes in the m. soleus but they were less marked than those earlier encountered. No structural characteristics or changes in activity of cholinesterase in the nerve-muscle synapses in this muscle were established.

On the 25th day after landing, the weight of the m. soleus and m. gastrocnemius did not reach the control level, but there were no verified differences. During histologic examination, no changes were discovered except for small foci of reparation of muscle tissue in the m. soleus in sections where, in the flight period, destruction of the muscle fibers was observed (Figure 58). In this time period in the muscle, a normal relationship of the LDH fraction is reestablished.

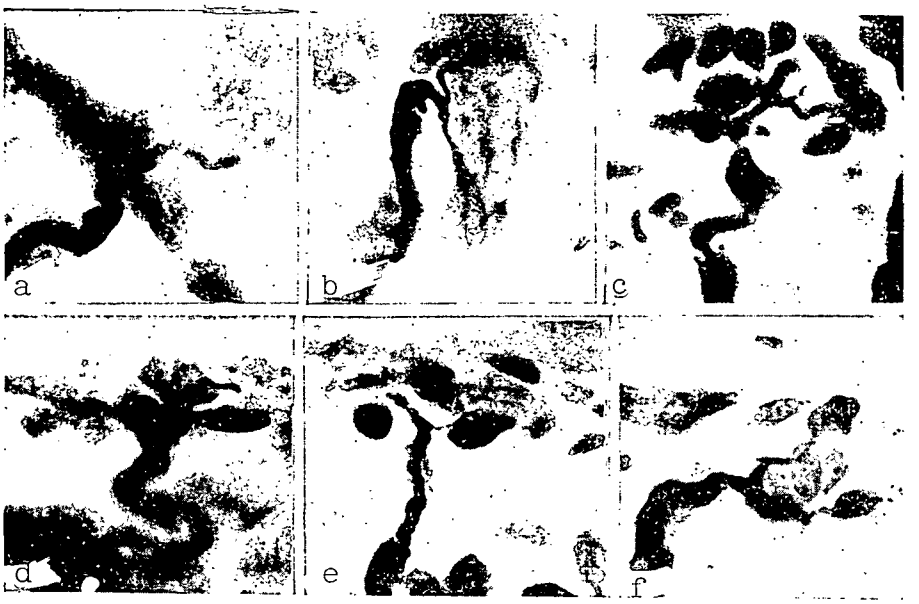

Figure 57. m. soleus of rats 5-7 hr after completion of flight. Magnification 640.
a, b -- VC; c, d, e, f -- F; sharply expressed argyrophilia, thickening of the axon terminals in the motor plaques of the flight group of animals. Preparation was obtained by impregnation with silver salts according to the Bil'shovskiy-Gross method in a Kampos modification.

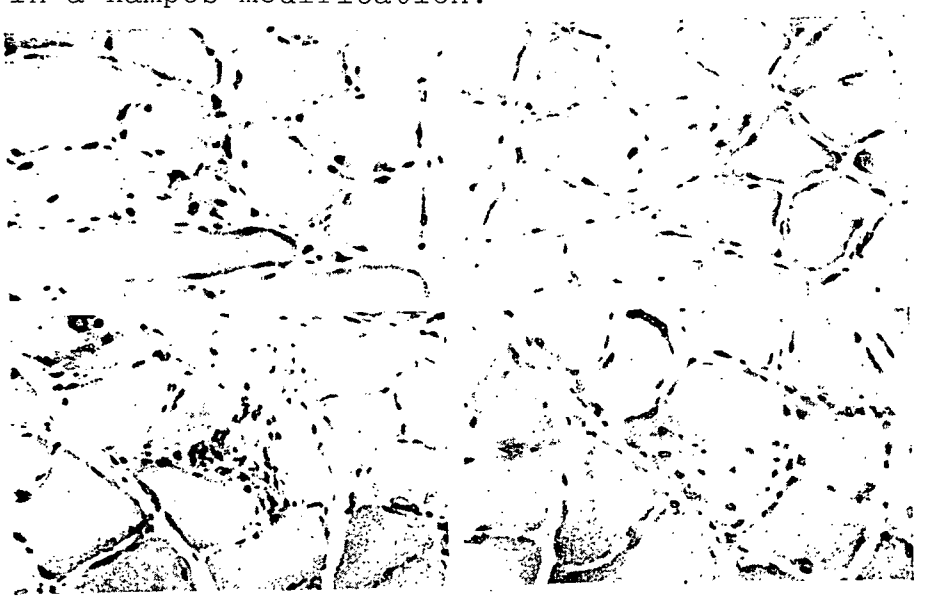

Figure 58. Sections of the m. soleus in rats 25 days post-flight. Magnification 1260.
Small foci of reparation of muscle tissue are visible in the destruction zone of the muscle fibers. Coloration with hematoxylin-eosin.

In both muscles, the structure of the motor plaque and activity of cholinesterase in it does not differ from the control.

In animals from the synchronous control group, 5-7 hr after completion of the test there was verified but to a lesser degree than in the animals of the flight group, that there was a decrease in weight of the m. soleus. The weight of the m. soleus does not differ from that in the control. The value of the diameter of muscle fibers of the m. soleus in rats of the synchronous control group occupied a center position between the corresponding parameters of animals in the flight group and in the vivarium control group. A number of muscle fibers had a /103
circular shape in which the fibers of the nucleus were located centrally, sometimes in the form of a chain. In both time periods of the study, no noticeable changes were observed in the animals of the synchronous control in the relationship of activity of LDH fractions in comparison with the vivarium control. No structural dysfunctions were discovered in the motor plaques or changes of cholinesterase activity in them.

In the m. gastrocnemius, there were no differences from normal in the structure of the tissue in the synchronous control rats.

Twenty-five days after completion of the synchronous test, the weight of the m. soleus continued to have a proven decrease in comparison with that index in the vivarium control (see Table 28). The diameter of the muscle fibers was somewhat smaller than in the vivarium control. In certain fibers, the nuclei were located centrally.

In none of the experimental groups of animals did one find structural changes in the nerve samples of the nerve-muscle spindles. When disclosing catecholomines in all time periods of the study, the muscles both in the animals of the flight group and in the synchronous control group and in the vivarium control group showed single fluorescent nerve fibers following the course of the intramuscular vessels. The nerve conductors of the rats of the flight group did not differ from the control in intensity of luminescence.

The material obtained attests to the fact that rats in the process of a 19.5-day flight and in conditions of the synchronous control for the same duration develop atrophy of certain muscles of the extremities. Both in the flight and in the synchronous experiments, atrophic changes were apparent in the slow red m. soleus. In spite of the fact that in the rats of the flight group this muscle was changed to a greater degree than in the rats of the synchronous control group, its structural recovery in the animals of the flight group set in more rapidly.

This study once more has confirmed that weightlessness aggravates the severity of the atrophic process developing in the muscles under the effect of hypokinesia.

It was pointed out earlier that the structural changes in the muscles of the animals affected by hypokinesia develop on a background of certain metabolic dysfunctions (Portugalov et al., 1971; Ilyna-Kakeuva, Portugalov, 1977; and others). The materials obtained when studying the spectrum of LDH isoenzymes support this position. The character of changes in the relationships of LDH fractions in the m. soleus of rats in the test on the Kosmos-782 biosatellite coincide with those obtained earlier in the experiment on the Kosmos-605.

The morphologic and metabolic dysfunctions in the muscle tissue are accompanied by changes in the structure of motor nerve-muscle synapses whereas the receptor instruments of the muscle remain intact. It is well known that the terminals of motor nerve fibers, having high plasticity, react fairly rapidly to the state of the central neuron and to the state of muscle fibers innervated by them (Falin, 1935), /104 whereas the nerve-muscle spindles are more stable (Cooper, 1960; Ilyna-Kakueva, 1974). Dysfunction of the structure of motor plaque and also disorders in metabolism of muscle tissue occurred only with its very marked atrophy. Motor plaque fully restores its structure when ordinary functioning of the muscle begins.

The rats which completed flight on board the Kosmos-782 satellite, in distinction from the rats exposed on the Kosmos-605 and Kosmos-690 satellites, have in the m. soleus, foci of edema and intense growth of the connective tissue in the endomysium. It was proposed earlier that this type of change in the muscle, whose occurrence involves peculiarities of blood supply, appeared on the 21-22 day of flight and almost in the same time period in the synchronous experiment conditions (Ilyna-Kakueva et al., 1977) and that at this critical time period the compensatory mechanisms of blood flow regulation break down in the muscle. The materials obtained in this experiment force one to reconsider this point of view and to put forward the hypothesis that the changes observed in the muscles occur not during flight (or correspondingly, the synchronous experiment), but on the second day after its completion. This version is based on the fact that edema and proliferation of the connective cells were observed only in cases where the animals were studied on the second day after cessation of flight or the synchronous experiment and that the phenomena mentioned are absent in rats in the experiment on the Kosmos-782 biosatellite who were killed after 5-7 hr. The occurrence of hemodynamic disorders leading to stases of the blood and edema, in this case, can be explained by the fact that the venous system of muscles which are out of shape, with transition of the

animal from a state of hypokinesia to active behavior begins to operate with the demands made on it.

Taking into account that the trophic processes and the process of hemodynamics in the muscle are partially under the control of the vegetative nervous system (Abdulayev, 1967), one could consider obtaining information which explains the dysfunctions described by studying the adrenergic innervation of the muscle vessels. However, the adrenergic nerves of the fiber in the muscle were very scarce and the content of catecholamines in them, inasfar as one could judge from the histochemical preparation, did not change in comparison with the control.

The Effect of Space Flight on the Metabolism of the Skeletal Muscle Tissue.

A study of metabolism and the morphologic structure of tissue of the skeletal muscles showed that under the effect of space flight, changes occur in them which are atrophic and dystrophic and the expression of these changes in different muscles is not uniform (Gazenko et al., 1975; Portugalov et al., 1975; Gayevskaya, Ushakov et al., 1976; Portugalov, Savina et al., 1976). Similar changes in the skeletal muscles are observed with limitation of mobility of the animals in conditions of Earth's gravitation (Portugalov et al., 1968; Amdiy et al., 1969, 1973). In animals exposed on the Kosmos-782 biosatellite, in the subskeletal fractions of the tissue of the skeletal muscles, the content of proteins, their enzyme activity and also the content of phospholipids were studied.

Changes in the content of sarcoplasmatic proteins and actomyosin, as a result of space flight, were not detected either at 9-11 hr or by the 25th day (Table 30). The quantity of proteins with fraction T also was not changed after 9-11 hr, but after 25 days there was a proven but slight increase in the control level. After 9-11 hr postflight, the adenosine triphosphatase activity of the myosin of the m. quadriceps in three rats did not differ from the control but in them there was an increase which was due to a proven increase of the corresponding mean index. After 25 days postflight, the adenosine triphosphatase in all of the rats was at the control level. In the rats of the synchronous control group, in both time periods of observation, the content of sarcoplasmatic proteins and T fraction proteins in the m. quadriceps was not changed. The quantity of actomyosin and adenosine triphosphatase activity of the myosin in the first time period of the examination were increased and after 26 days did not differ from the control. /105

The activity of aspartataminotransferase (AST) of the sarcoplasmatic proteins (Table 31) after 9-11 hr and 25 days postflight was not changed. The activity of alaninaminotransferase (ALT) during observations after 9-11 hr showed

TABLE 30. THE CONTENT OF PROTEIN IN PROTEIN FRACTIONS (IN g PER 100 g OF MOIST TISSUE) AND ADENOSINE TRIPHOSPHATASE ACTIVITY (IN μg Φ_N PER 1 mg OF PROTEIN FOR 10 MIN AT 37°) OF MYOSIN OF THE M. QUADRICEPS FEMORIS (M±m)

Group	Time after completion of the tests and the number of animals (n)	Sarcoplasmatic fraction	Actomyosin	Fraction T	Adenosine triphosphatase activity of the myosin
VC	Both time periods combined (n=10)	5,71±0,12	8,34±0,12	2,31±0,08	140,5±1,8
F	9-11 hr (n+6)	5,75±0,13	8,68±0,22	2,34±0,15	155,5±4,4*
	25 days (=5)	5,80±0,11	8,52±0,23	2,87±0,09*	142,5±2,0
SC	9-11 hr (n=6)	5,50±0,11	9,56±0,26*	2,48±0,09	158,8±2,3*
	25 days (n=6)	5,85±0,10	8,02±0,11	2,25±0,08	140,9±2,8

*Proven difference from VC

TABLE 31. /106

Group	Time period after completion of tests and no. of animals (n)	AST	ALT	LDH total activity	LDH_1	LDH_2	LDH_3	LDH_4 + LDH_5
		μm of pyruvate per 1 mg of protein after 1 hr at 37°		μM of $NADN_2$ per 1 mg of protein after 1 min at 26°				
VC	Both time periods combined (n=10)	14,69±0,03	5,11±0,13	7,52±0,18	0,61±0,04	1,08±0,04	1,91±0,06	3,92±0,12
F	9-11 hr (n=6)	14,05±0,92	6,64±0,19*	6,50±0,29*	0,68±0,03	0,86±0,05*	1,49±0,09*	3,48±0,16*
	25 days (n=5)	16,59±0,53	5,56±0,21	6,62±0,20*	0,61±0,07	1,01±0,05	1,68±0,08*	3,33±0,08*
SC	9-11 hr (n=6)	20,34±1,44*	7,43±0,23*	7,79±0,10	1,07±0,06*	1,12±0,03	1,90±0,04	3,65±0,08
		14,26±0,85	5,75±0,30	6,91±0,29	0,70±0,08	1,12±0,10	1,71±0,12	3,38±0,04*

*Proven difference from VC

an increase, and after 25 days, it returned to normal. The total LDH activity of sarcoplasmatic proteins in the first time period of observation was decreased due to a decrease in activity of four of the five of its isoenzymes and remained somewhat depressed in the second observation period. /105

In rats of the synchronous control group, in the first observation period, in the sarcoplasmatic proteins of the m. quadriceps, one observed an increase in activity of AST and ALT, the total activity of LDH was not changed whereas the relationship of its isoenzymes was changed atrophically. In the second observation period, the AST, ALT and total LDH activity did not differ from the control but the changes in the isoenzyme spectrum of LDH were significantly leveled out.

Thus, the stay of the rats in flight did not lead to significant changes in the content of protein in the protein fractions of the m. quadriceps which corresponded to the absence of proven changes in its weight (1768±102 mg in the control rats and 1676±21 mg in the rats after flight), also the normal relationship of LDH isoenzymes. In the rats of the synchronous control group, one noted an increase in the content of actomyosin and a proven decrease in the muscle weight (1472±40 mg) with a change of atrophic type in the relationship of LDH isoenzymes. Obviously, the increase in quantity of actomyosin, like the increase in activity of transaminase of sarcoplasmatic proteins can be considered as a result of the compensatory reaction of the muscle tissue to activation in the processes of catabolism. The single direction of changes in adenosinetriphosphatase activity of the myosin in m. quadriceps of the rats in the flight and synchronous experiments attests to the fact that these changes are not involved with the effect of weightlessness. /106

Data of the study of m. soleus are presented in Tables 32 and 33. It is obvious that no changes significant in magnitude in the content of protein and protein fractions either in the flight or in the synchronous experiment, in both periods of observation, were observed. Twenty-five days postflight and in both examination periods after the synchronous experiment, there was a significant decrease in adenosinetriphophatase activity of the myofibrillar proteins whose cause could be the changes in structure of the protein complex of actomyosin and other myofibrillar proteins inasmuch as determination was not made in the pure fraction of myosin.

Changes in AST activity of the sarcoplasmatic proteins of the m. soleus was not noted in any of the experimental groups of animals (Table 33). ALT activity of this fraction of protein was increased in the first examination period both after flight and after the synchronous experiment and remained increased 26 days postflight indicating a compensatory increase

TABLE 32. CONTENT OF PROTEIN (IN g PER 100 g OF MOIST TISSUE) IN PROTEIN FRACTIONS AND ADENOSINETRIPHOSPHATASE ACTIVITY (IN µg PER 1 mg OF PROTEIN AFTER 10 MIN AT 37°) OF MYOFIBRILLAR PROTEINS IN THE M. SOLEUS

Group	Time period after flight	Number of animals	Sarcoplasmatic fractions	Actomyosin	T protein fractions	Adenosinetriphosphatase activity of the myofibrillar proteins
VC	Both time periods combined	5	5,06±0,13	6,01±0,04	3,33±0,37	95,7±1,1
F	9-11 hr	3	5,46±0,17	6,29±0,34	2,54±0,03	117,6±12,3
	25 days	3	5,54±0,22	7,04±0,94	4,31±0,28	47,1±1,8*
SC	9-11 hr	3	4,50+0,18	6,12±0,10	2,97±0,11	50,3±4,0*
	25 days	3	5,60	7,35	2,65	50,2
			0,07*	0,70	0,50	1,3*

*Proven difference from VC.

TABLE 33. ENZYME ACTIVITY OF SARCOPLASMATIC PROTEINS IN THE M. SOLEUS (M±m)

Group	Time period after flight	No. of animals	AST	ALT	LDH, total activity	LDH_1	LHD_2	LDH_3	LDH_4 + LDH_5
			µM of pyruvate per 1 mg after 1 hr at 37°		µM NAD-N_2 per 1 mg of protein after 1 min at 25°				
VC	Both time periods combined	6	19,61±1.20	6,87±0,43	2,76±0,10	0,68±0,02	0,82±0,01	0,67±0,03	0,59±0,06
F	9-11 hr	3	17,30±0,77	8,55±0,18	3,13±0,12	0,64±0,02	0,68±0,01*	0,79±0,03*	1,02±0,04*
	25 days	3	21,34±1,60	8,03±0,42*	2,62±0,30	0,52±0,04	0,70±0,05	0,65±0,09	0,75±0,11
SC	9-11 hr	3	21,27±0,44	9,38±0,31*	2,72±0,33	0,74±0,10	0,73±0,09	0,63±0,07	0,61±0,09
	25 days	3	17,17±1,94	7,70±0,74	2,52±0,20	0,61±0,01*	0,67±0,03*	0,49±0,02*	0,49±0,07

*Proven difference from VC

in intensity of metabolism.

The total LDH activity of sarcoplasmatic proteins significantly was unchanged in all groups but the spectrum of its isoenzymes was changed atrophically only in rats who had undergone flight. The loss in weight of the m. soleus corresponded to this; in rats of the flight group it amounted to 39% and in the synchronous control group only 17%. Consequently, in spite of the decrease in muscle mass, in the remaining tissue of the m. soleus, the normal protein composition was retained.

In this experiment, first the tibial muscle and the long extensor of the digits was studied. In both muscles in the flight group and in the control group of animals, in both observation periods, no changes in content of protein in the protein fractions was noted. Adenosinetriphosphatase activity /108
of the myofibrillar proteins isolated from these muscles, in opposition to what was observed in the m. soleus, showed a certain increase in the first observation period; changes were absent in the relationship of LDH isoenzymes atrophically; the changes observed in the isoenzyme spectrum could attest to the fact that, on the other hand, there is an increased functional load on the muscles indicated. The ALT activity of the sarcoplasmatic proteins of the tibial muscle and the long extensor of the digits, as in the other muscles, was increased in both observation periods both in the animals of the flight group and in the synchronous control group.

Table 34 shows the results of studying the content of phospholipids in the subcellular fractions isolated from the mixed sample of tissue of the m. gastrocnemius and the m. quadriceps. As is apparent from the data, the content of

TABLE 34. THE CONTENT OF PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF THE SKELETAL MUSCLES (IN μg OF PHOSPHORUS PER 1 mg OF PROTEIN

Parameter	VC	F⁻		SC	
		9-11 hr	26 days	9-11 hr	26 days
		Microsome			
$M \pm m$	17,76±0,57	13,99±0,92	12,43±0,45	13,21±1,03	13,97±1,22
n	9	5	5	6	5
P_{VC}		<0.01	<0,001	<0,01	<0,05
		Mitochondrial			
$M \pm m$	3,16±0,76	12,60±0,63	14,42±1,23	14,85±0,45	13,42±0,93
n	8	6	5	6	5
P_{VC}		<0,01	>0,05	>0,05	<0,05

phospholipids in the microsome fraction of these muscles in the first period of flight and after the synchronous experiment, was decreased by approximately 20% in comparison with the vivarium control. Taking into account the changes in content of phospholipids in the microsome fraction were the same in muscles of rats in the ground and flight experiments, one can assume that not only the state of weightlessness was significant but also such factors as the G-force, limitation of mobility, the artificial gas medium, etc.

These data confirmed observations made in the experiment on the Kosmos-605 biosatellite in which the rats after a day postflight had a decreased content of phospholipids in the microsome fraction; to a lesser degree this was true after the ground experiment. Twenty-six days after the flight and the ground synchronous experiment, the content of phospholipids in the microsome fraction still remained somewhat decreased which attested to the significant and long term change in composition and probably properties of the membranes of the sarcoplasmatic reticulum.

In the mitochondrial fraction, the content of phospholipids in the first time period decreased in the rats of the flight group by approximately 20% in comparison with the same index in animals of the vivarium control. In the synchronous experiment rats, no verified decrease in the phospholipids in this fraction was detected. This difference between the flight group and the synchronous control group attests to the fact that weightlessness had an effect on the content of phospholipids in the mitochondria. /109

Twenty-six days postflight, the content of phospholipids in the mitochondrial fraction was normalized whereas in three of the five animals of the synchronous control group, one detected a decrease in the content of phospholipids which caused a low average level of the index. At the present time, it is impossible to explain the cause for this phenomenon.

When comparing the data presented in Table 34, one can conclude that the content of phospholipids in the mitochondria, in comparison with their content in the sarcoplasmatic reticulum was affected by weightlessness but normalizes rapidly.

Thus, in the skeletal muscles of the posterior extremities of rats, with varying degree of atrophy, caused by space flight, the content of protein in the protein fractions was unchanged in spite of the loss of muscle mass whereas changes in the enzyme activity of sarcoplasmatic proteins correlated well with the degree of atrophic damage. The effect of space flight factors also led to significant changes in the content of phospholipids of the intracellular membranes of the skeletal muscles which could cause a disturbance in the function of the mitochondria and the sarcoplasmatic reticulum.

The Effect of Space Flight on Bioenergetics of the Skeletal Muscles of Rats

It is well known that almost all types of energy consumption at rest and with activity includes expenditure of energy for overcoming Earth's gravitation. Theoretical calculations make it possible to consider that energy consumption directed at completing motion and work in weightlessness must be significantly less (Kovalenko, 1974). However, the actual studies of the effect of weightlessness on man and animals had contradictory results. The effect of short term weightlessness during flights on aircraft and spacecraft, and also when simulating weightlessness on an unsupported test stand and in conditions of immersion causes an increase in the requirement for oxygen and the level of energy expenditure (Kas'yan, Makarov, 1969; Kas'yan et al., 1971; and others). With an increase in the duration of the effect of weightlessness, a tendency arises toward a decrease in energy consumption (Genin et al., 1967; Berry, 1971b; and others). A sharp increase in gas exchange and energy expenditure was found when simulating weightlessness in conditions of hypokinesia and on test stands simulating decreased gravity (Wortz, Prescott, 1966; Katkovskiy, 1967; Mikhasov et al., 1969).

The results of studying gas exchange and energy consumption in cosmonauts and astronauts was affected particularly in the first days of flight by emotional stress, disturbance in coordination of movement, and later on by intense physical training. The absence of these factors which mask the effect of weightlessness in the flight experiments on animals makes it possible /110 to obtain more precise data.

In this study, results are presented of investigating tissue breathing and the processes of oxidizing phosphorylation in skeletal muscles, which to a considerable degree determine the level of total gas exchange and energy consumption.

The rats were studied during the 22 days of exposure on the Kosmos-605 biosatellite and also the animal groups of the synchronous and vivarium controls.

Studies were made on homogenates of the muscles of the posterior group of the femur. Tissue breathing was determined on a Varburg apparatus. The requirement of oxygen (ΔO), assimilation of inorganic phosphorous (ΔP) and the coefficient of phosphorylation (P/O) were studied. The tissue preparations were incubated for 15 min at a temperature of 26°; the substrate of oxygen was 0.02 M solution of succinic acid. The quantity of inorganic phosphorous was determined to the Lowry method (1951) and V. P. Skalachev's modification (1962).

The results are presented in Table 35 and Figure 59.

TABLE 35. THE INTENSITY OF OXYDIZING PHOSPHORYLATION IN HOMOGENATES OF THE SKELETAL MUSCLES OF RATS IN THE POSTFLIGHT PERIOD (ΔO AND ΔP -- IN μA PER 100 mg OF TISSUE AFTER 1 HR). (M±m)

Group of animals	Time period after completion of the tests, days	n	ΔO	ΔP	P/O
VC	—	18	7,30±0,29	8,07±0,59	1,11±0,22
SC	2	7	5,46±0,56*	5,71±0,68*	1,06±0,12
F	2	4	3,37±0,36**	3,34±0,28**	1,02±0,10
				$P_{VC} < 0,01$	
SC	26	6	6,11±0,56	8,63±0,91	1,43±0,11
F	26	5	7,59±0,31	10,60±0.96	1,40±0,11

*Proven difference from VC
**Proven difference from VC and SC

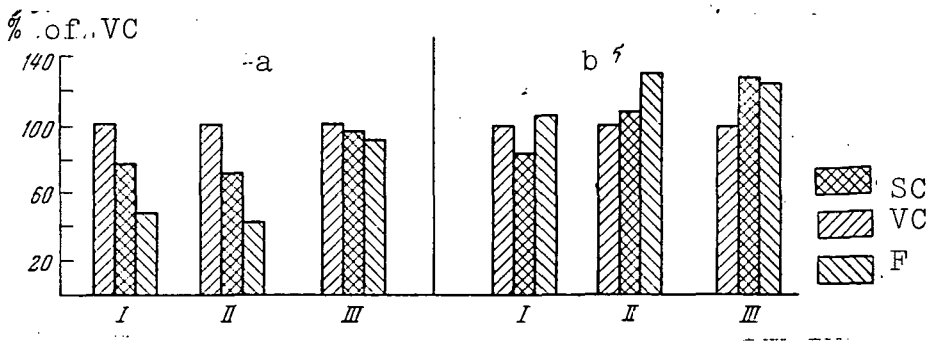

Figure 59. Oxidizing phosphorylation in homogenate of skeletal muscles.
I -- oxygen requirement; II -- assimilation of inorganic phosphorous; III -- coefficient of phosphorylation; a -- second day; b -- 26th day.

From the data presented it is obvious that the second day after completion of flight is characterized by a sharp decrease (by 40-50%) both in the breathing and in the phosphorylation activity of skeletal muscle homogenate[5] in animals of the flight group. The P/O coefficient here remains unchanged and consequently the conjugation of these processes is not disturbed in spite of their sharp suppression. On the second day postflight, both the breathing and the phosphorylation activity were decreased in rats of the synchronous control group. It is obvious that the conditions of the content also are reflected on the course of processes of biologic oxidation in the muscles, causing their suppression. On the 26th day after flight, no kind of deviations were detected in the demand for oxygen and assimilation of inorganic phosphorous in the rats. /111

The disturbances described in the tissue oxidizing processes can, to a significant degree, decrease the magnitude of the energy potential of the cell. Weakening of capability for regeneration of microergs cannot result in a significant disorder of biosynthesis, specific functions of the cells and the course of exchange reactions involving consumption of energy. These changes, according to all the evidence, are the basis for this functional insufficiency of skeletal musculature which is detected in the first postflight days in man (Nevedov et al., 1972).

This decreased level of bioenergetic processes possibly is an adequate response of the organism to weightlessness. But after returning to Earth it is inadequate and a sharp decrease in breathing and phosphorylation activity becomes a factor which stimulates the exchange processes directed at adaptation to the conditions of Earth's gravitation in their functioning level. As a result, breathing and phosphorylation capability in the skeletal muscles is reestablished on the 26th day and even increases somewhat in comparison with the factors in the control animals.

Data obtained at the tissue breathing level agree well with changes in general gas exchange and body temperature established in the same animals and also reminds one of the principles obtained on the hypokinesia models in rats (Mailyan et al., 1970).

Free Amino Acids of Muscle Tissue

Data obtained in space flight and model experiments attest to the occurrence of multiple changes in the process of protein exchange in the skeletal muscles (Revich et al., 1975; Gayevskaya, Ushakov et al., 1976; Portugalov, Savina et al., 1976; and others). One of the criteria for evaluating intensity and direction of protein exchange is the content in the

tissues studied of free amino acids. In accordance with this, a study was made of the content of free amino acids in the m. quadriceps femoris. Animals from the flight group, the synchronous group and the vivarium control killed 9-11 hr and 25 days after completion of the test were used. Determination was made by a method of ion exchange chromatography on an automatic analyzer for amino acids (Vysotskiy et al., 1973).

Regular changes in the concentration of free amino acids occurred under the effect of flight (Table 36). Nine-eleven hr /112

TABLE 36. THE CONTENT OF FREE AMINO ACIDS (IN Mm PER 1 g OF MOIST TISSUE) IN THE M. QUADRICEPS FEMORIS (M±m)

Amino acid	VC	F	% of VC	SC	% of VC
		9-11 hr			
Isoleucine	0,10±0,02	0,17±0,04	170	0,12±0,01	120
Leucine	0,14±0,02	0,29±0,07	207	0,19±0,02	131
Valine	0,11±0,02	0,22±0,04	200	0,15±0,02	136
Threonine	1,34±0,09	0,96±0,11 *	72	1,38±0,13	103
Methionine	0,12±0,02	0,18±0,05	150	0,11±0,03	92
Phenylalanine	0,07±0,01	0,16±0,03 *	229	0,12±0,01 *	171
Tyrosine	0,08±0,01	0,16±0,03	150	0,10±0,02	125
Aspartic	0,15±0,04	0,15±0,07	100	0 26±0,03 *	173
Glutamic	0,74±0,05	0,66±0,14	89	1,18±0,01 *	192
Serine	0,57±0,10	0,52±0,08	99	0,80±0,11 *	140
Proline	0,38±0,04	0,32±0,07	84	0,44±0,04	116
Glycine	1,93±0,14	1,42±0,16 *	74	2,30±0,35	119
Alanine	1,63±0,18	1,57±0,21	96	2,26±0,23	139
Total	7,36	6,74	92	9,41	128
		25 days			
Isoleucine	0,12±0,02	0,13±0,03	108	0,07±0,01 *	58
Leucine	0,15±0,01	0,18±0,04	120	0,09±0,01 *	60
Valine	0,21±0,01	0,20±0,04	95	0,15±0,02 *	125
Threonine	1,71±0 17	1,44±0,12	84	0,92±0,21 *	54
Methionine	0,11±0,02	0,10±0,02	91	0,09±0,01	82
Phenylalanine	0,11±0,01	0,10±0,02	91	0,11±0,01	100
Tyrosine	0,11±0,01	0,11±0,01	100	0,07±0,01 *	64
Aspartic	0,14±0,02	0,15±0,02	107	0,12±0,02	86
Glutamic+Proline	2,25±0,19	2,27±0,13	101	1,71±0,10 *	76
Serine	0,95±0,04	0,85±0,08	90	0,56±0,10 *	59
Glycine	2,43±0,30	2,91±0,26	120	2,08±0,78	86
Alanine	2,60±0,20	2,55±0,14	98	2,11±0,25	81
Total	10,89	10,99	101	8,08	74

*Proven difference from VC

after completion of the flight, a significant increase was detected in comparison with the control of the content of isoleucine, leucine, valine, phenylalanine and tyrosine. At the same time, there was a proven decrease in the content of threonine and glycine. The total content of free amino acids

essentially was unchanged.

Twenty-five days after completion of flight, the content of free amino acids in the m. quadriceps femoris of the rats basically does not differ from the control values and as a total amounted to 101% (see Table 36).

In the animals of the synchronous control group, in the first examination period, the content of most of the free amino acids was increased; here for four of them (phenylalanine, aspartic and glutamic acids, serine) the increase was proven. The total content of free amino acids in the muscle tissue amounted to 128% of the control. Twenty-five days after completion of the synchronous experiment, a significant decrease was detected in the content of most of the free amino acids; a decrease in the quantity of isoleucine, leucine, threonine, tyrosine, glutamic acid and serine was proven. In this case, a total content of free amino acid was decreased to 74% of the control level. /113

Thus, as a result of having undergone flight, in the m. quadriceps femoris, the total content of free amino acids was unchanged whereas in their relationships, one observed sharp shifts -- the content of some of them increased and others decreased. The noticeable changes were leveled off by the 26th day after completion of flight. In the synchronous control group one observed significant differences from the changes in the flight group of rats; here the differences not only were quantitative but also had a qualitative character. In the animals of the synchronous control group, a significant change was indicated in the content of amino acids in the muscles studied whereas a proven increase was noted basically for these amino acids whose content (excluding phenylalanine) was not increased in the flight group of rats. Significant differences were observed also 25 days after completion of the experiment when in the animals of the synchronous control group there was a marked decrease in the content of the majority of free amino acids.

These data attest to the fact that changes in content of free amino acids in the flight and ground experiments occurred due to different causes which determine the functional state of the muscle tissue. In conditions of weightlessness, the m. quadriceps femoris of the animals of the flight group was in a state of decreased functional activity which caused development of catabolic processes in the proteins of the muscle tissue. This process had, obviously, a compensatory character; the absence of significant changes in the content of proteins in them (see Table 30), changes in the total concentration of free amino acids, and also restoration of the pool of free amino acids by the 26th day postflight all attest to this.

The increase in content of most free amino acids in the muscle tissue of the animals in the synchronous control group which was detected during examination in the first time period must be considered as a result of their decreased use due to limitation of motor activity and a decrease in intensity of synthetic processes. The decrease in content of free amino acids after 25 days of the ground experiment can be interpreted as intense utilization of them in the process of synthesis of muscle proteins when reestablishing the mass of muscle tissue. In this case, changes in amino acid metabolism were similar in character to the shifts observed in the skeletal musculature of rats in conditions of hypokinesia (Revich et al., 1975; and others).

One should note that the results of determining free amino acids in the m. quadriceps femoris of animals who have undergone flight on the Kosmos-605 and Kosmos-782 biosatellites varied significantly. As a result of the first flight, changes occurred /114
of a hypokinetic type which can be explained by nonidentical conditions in the housing of rats in flight and during the time samples were taken for the experiment.

One should note the changes in content of free glycine in the muscle tissue. It is well known that glycine participates in synthesis of purine bases and in this way plays an important role in the synthesis of nucleic acid and nucleoproteides. Unidirectional changes in the content of this amino acid in the m. quadriceps femoris of rats who have undergone flight on the Kosmos-605 and Kosmos-782 biosatellites, attests, apparently, to a decrease in its use in the first days postflight (a decrease in content of glycine in tissue) and activation of synthetic processes on the 26th day after their completion (increase in content of glycine in the tissue).

Activity of Glycogensynthetase, Glycogenphosphorylase, the Content of Glycogen and Electrolytic Composition of Skeletal Muscles.

Conditions of space flight can disturb normal neurohumoral and neuroendocrine regulation of the motor apparatus and cause a number of biochemical and structural-functional changes in muscle tissue (Kovalenko et al., 1975; Oganov et al., 1976). Characteristic changes can occur in carbohydrate-phosphorous exchange at the level of synthesis and decomposition of glycogen, in mineral, water and other types of exchange of substances.

In the skeletal muscles of rats exposed on the Kosmos-782 biosatellite, the activity of enzymes was studied which catalyze synthesis and decomposition of glycogen -- glycogensynthetase and glycogenphosphorylase and also the total content

of glycogen, water and ions of Na^+, K^+, Mg^{2+} and Ca^{2+}.

The following muscles were studied: m. soleus, the diaphragm muscle, m. gastrocnemius and m. plantaris. Phosphorylase activity was determined by a modified Cori method (Cori et al., 1955); the activity of glycogensynthetase -- spectrophotometrically, using a modification of methods by L. A. Kuznetsovaya, M. N. Pertsevaya (1974 and Barber (Barber et al., 1976); the total content of glycogen in the tissues was determined according to the Kemp method (Kemp et al., 1954); the content of water -- by drying the tissue at 105° to a constant weight. Determination of the concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} was done by a method of atom-absorption spectrophotometry.

In none of the examination periods were proven changes successfully detected in the content of glycogen in the muscle tissues of the flight group of rats (Figure 60). One can note only a certain tendency toward an increase in the content of of this polysaccharide in animals killed 9-11 hr after landing.

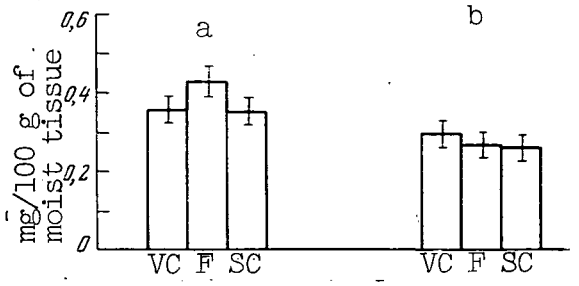

Figure 60. The content of glycogen in the m. gastrocnemius.
a -- 9-11 hr; b -- 25 days.

A statistically proven ($P<0.01$) increase was detected in the active form of phosphorylase (glycogenphosphorylase "a") in the muscles of rats in the flight group at both examination periods (Figure 61). The total activity of glycogen phosphorylase (form "t") in the flight group animals in both examination periods does not differ from that of the control groups (Figure 62). Obviously, in conditions of space flight, only redistribution of active and inactive forms of the enzyme occur. /115

The complex process of change of activity of phosphorylase with transition of it from form "b" to "a" is a chain of mutually dependent enzyme reactions. One of the links in this chain is the accumulation of cyclic 3', 5'-AMP which activates the enzyme of phosphorylase-b-kinase which, in turn, catalyzes the conversion of phosphorylase "b" to phosphorylase "a". Solution of the question as to whether or not sensitivity of this section of enzyme interaction is retained, after the effect of flight factors, to an increase in cytoadenosine monophosphate in the muscles, is important for explaining changes which occur in phosphorylase activity. Due to this, a study was made of phosphorylase activity of the muscles with the addition of cAMP in tests *in vitro*. No deviations in sensitivity of skeletal muscles to cAMP in animals of the flight group were detected.

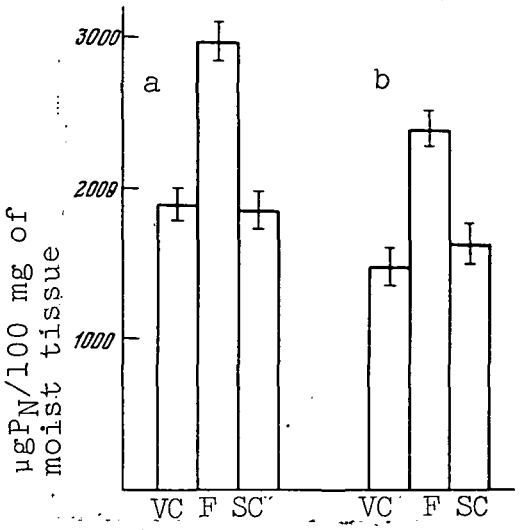

Figure 61. Activity of glycogen phosphorylase "a" in the m. gastrocnemius.
a - 9-11 hr; b - 25 days

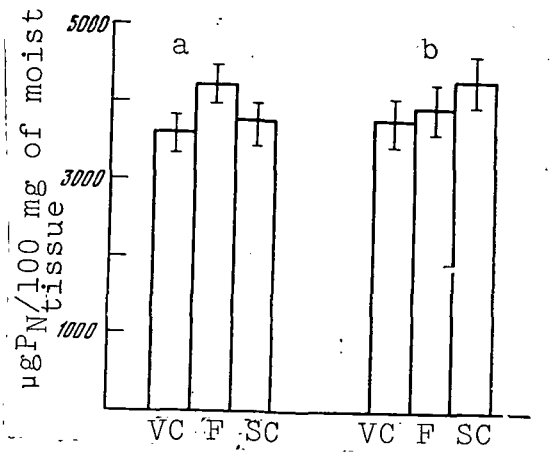

Figure 62. Activity of glycogen phosphorylase (form "t") in the m. gastrocnemius.
a - 9-11 hr; b = 25 days.

Figure 63 shows the results of determining activity of glycogen synthetase which exists in two mutually converting forms -- dependent on and independent of glucose-6-phosphate (G-6-P). Transition of the independent I-form to the dependent D-form occurs under the effect of cAMP (Soderling et al., 1970). From the data of Figure 63 it is apparent that the activity of both types of enzyme, that is, its sensitivity to cAMP is practically identical in the flight and control groups. On the drawing itself one can see that ions of calcium, inhibiting both types of enzyme, have a similar effect on glycogen synthetase of the muscles of the rats in the flight and control groups. /116

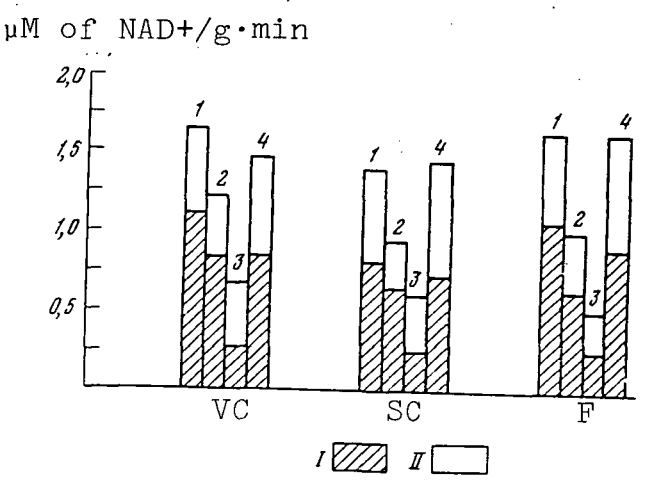

Figure 63. Activity of glycogen synthetase in the m. gastrocnemius.
I - independent of G-6-P; I-form of the enzyme; II - dependent D-form; 1 - control; 2, 3 - effective C^{2+} on the concentration of 2.5 mM (2) and 5.0 mM (3); 4 - effective cAMP in a concentration of 0.5 mM.

The question arises as to why with unchanged activity of glycogen synthetase and an increased activity of glycogen phosphorylase, the content of glycogen in the skeletal muscles of the rats who have undergone flight was not decreased. It is possible that

in flight conditions changes occur in the glycogen molecule which make catalysis of this polysaccharide of phosphorylase difficult. Also one should not exclude the fact that with the phosphorylase molecule conversions which are unknown to us occur; as a result of this, the enzyme partially loses its affinity to the substratum and an increase in the activity of the enzyme in these conditions is a reaction of the organism directed at providing normal activity of skeletal musculature. Both these hypotheses require testing and comparison with other data obtained when carrying out this experiment.

Determination of the tissue content of the Na^+ and K^+ ions in the muscles of rats in the flight group did not show sharp changes (Table 37) but a tendency was observed toward a relative decrease in the content of K^+ ions in comparison with Na^+ ions in the m. soleus for which the posture-tonic function is the basis. Similar data were obtained in the experiment on the Kosmos-690 biosatellite. These results support the fact that antigravity muscles are subjected more to the effect of weightlessness because being in a state of decreased functional activity, they are deprived of the necessary trophic effect of the nervous system. Apparently, the 19.5-day flight caused only initial reversible atrophic changes in the m. soleus. This does not exclude the fact, however, that with a significant lengthening of the time of space flight these changes can result in a loss in static and dynamic work capability and also lead to a marked degnerative process in the contractile tissue. /117

In the tissue content of Mg^{2+} and Ca^{2+} ions, in the skeletal muscles of rats who have undergone flight, there is an absence of proven changes in comparison with muscles of the control animals.

As in the experiment on the Kosmos-690 biosatellite, proven dehydration of the m. plantaris and the diaphragm muscles are observed. Redistribution of the ions of alkali metals in the muscles benefiting from Na^+ attest to the regressive changes in the intracellular system of ion homeostasis (breakdown in the system of active transmembrane transition of ions, atrophic changes resulting in a decrease in the number of fixed negative charges on the intracellular protein polyelectrolytes, etc.). Going from the data obtained one can assume that dehydration of the skeletal muscles during space flight is a reflection of adaptation reactions which protect the organism and in particular the skeletal musculature, from damage.

TABLE 37 CONTENT OF H_2O (IN mℓ/kg OF MOIST WEIGHT), Na^+, K^+, Mg^{2+} AND Ca^{2+} (IN MEQV PER 1 kg OF MOIST WEIGHT) IN THE SKELETAL MUSCLES (M±m)

Group	Time period after completion of tests	No. of animals	H_2O	Na^+	K^+	Mg^{2+}	Ca^{2+}
			m. Soleus				
F	9-11 hr	6	766±3	28,5±2,1	82,0±4,1	21,6±2,4	3,1±1,0
SC		9	758±3	24,1±1,9	90,5±3,7	19,2±2,6	2,7±0,2
F	25 dats	6	771±4	25,4±2,5	85,0±4,4	20,1±0,4	3,0±0,3
SC		6	758±5	23,5±2,5	88,6±4,1	16,6±1,1	2,8±0,2
VC	Both time periods combined	10	765±2	25,3±1,7	86,5±2,9	19,5±1,0	2,5±0,2
			Diaphragma				
F	9-11 hr	11	735±3	27,0±2,0	89,1±3,9	18,9±2,1	3,6±0,7
SC		11	751±4	27,2±1,8	90,8±3,3	20,2±1,9	3,0±0,9
F	25 days	7	751±4	26,9±2,2	91,6±4,3	21,4±1,1	3,7±1,2
SC		5	761±5	27,4±2,4	89,7±4,7	18,6±1,9	3,0±0,7
VC	Both time periods combined	20	751±3	26,4±1,3	39,4±2,3	21,3±1,2	3,3±0,3
			m. Plantaris				
F	9-11 hr	6	750±2	18,6±2,2	103,2±4,5	26,3±2,2	3,3±0,7
SC		6	760±2	18,1±2,1	106,6±4,7	26,2±2,1	3,1±0,6
F	25 days	6	766±4	17,6±2,7	104,4±5,7	29,2±3,4	4,1±1,4
SC		5	767±5	18,9±2,6	96,3±5,1	27,8±2,4	4,3±1,3
VC	Both time periods combined	14	768±3	18,1±1,7	104,1±3,4	26,2±1,1	3,5±0,2

Study of Functions of Skeletal Muscles in Experiments on Biosatellites. /118

In this section we will present results of studing the contractile and biomechanical properties of the skeletal muscles in animals who have been in weightlessness conditions for 20-23 days on board the Kosmos-605 biosatellite (first experiment) and the Kosmos-690 (second experiment). The general conditions of the experiment on the Kosmos-605 biosatellite were described earlier (Il'in et al., 1976). In the second experiment on a 10-day flight, the animals were subjected to a single gamma radiation with a dose of 800 rads.

Functioning of the muscles was studied *in vitro* on the 2nd and 26th days after completion of the flight. The animals were anesthetized with thiopenthane and decapitated. The m. soleus removed and the long extensor of the digits were placed in a saline solution at a temperature of 26° (first experiment) or

in a 199 medium with a temperature of 37° (second experiment). In both cases the solutions were oxygenated with a mixture of O_2 (95%) and CO_2 (5%).

In the process of registration of isometric contractions of the muscles in response to single and tetanic stimulation with an electrical current, the time and force characteristics of contractions, stability and fatigue were studied. The mechanical properties of the muscles (the "length-stress" relationship were studied during their passive extension to 1.3 of their initial length. The change in weight indices of the muscles was taken into account. The methods of recording data and their methods of processing were described earlier (Katinas, et al., 1974).

In each of the two experiments, the results of the studies of the animals of the flight group were compared with the data of the vivarium and synchronous control groups.

In the period after landing, the animals showed significant differences from the control in the condition of the m. soleus and the long digital extensor. These differences were apparent primarily in the changes in the weight indices. On the second day postflight in the first and second experiments the weight of the m. soleus was decreased by 28-40% in comparison with the same index in the vivarium and synchronous control groups. A decrease in weight of the "rapid" muscles in both experiments was twice as small. In the second examination period, the weight of both muscles did not differ significantly from the control value although in the m. soleus restoration was less effective.

On the second day after flight in the first experiment in both muscles an increase in the time of development of a single contraction and the time of semidecrease (the time of weakening to half the amplitude of the contraction) was noted. In the m. soleus, these changes were much less noticeable. The time characteristics of single responses of the muscle in the second experiment changed insignificantly. In the second time period, the observations of the time parameters of single responses of the muscles in both experiments did not differ from the control values.

The changes of time characteristics of contraction of muscles with tetanic stimulation were detected only in the m. soleus. Here they had the opposite direction in comparison with changes of single responses. Both the time of development of tetanic contraction (to one-half its amplitude) (Figure 64) and time of its half decrease (statistically valid only in the second experiment) were decreased. In the second experiment, it was established that there was a decrease in the frequency of coalescent tetanus in the m. soleus (Table 38). /119

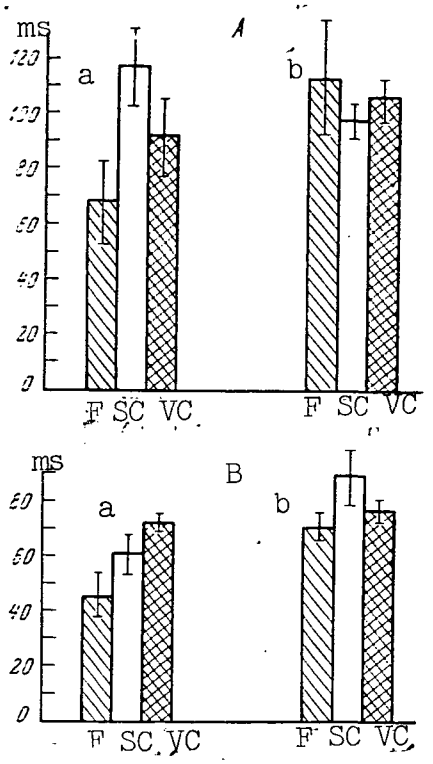

Figure 64. Time of development of tetanic isometric contraction of the m. soleus in rats on the 2nd (a) and 26th (b) days after flight on the Kosmos-605 (A) and Kosmos-609 (B) biosatellites.

In both experiments, the values of most of the time parameters of tetanic contractions of the m. soleus in the second observations did not differ from the initial level.

The results of studying the power characteristics are presented in Figure 65 in the form of dynamics of change of the A/A_o index which is calculated as the ratio of the amplitude of the tetanic (A) and single (A_o) responses of the muscles. It is apparent that on the second day after the flight of the Kosmos-605 biosatellite, in both of the muscles studied, there was a decrease in the value of the A/A_o ratio. In the m. soleus, this tendency was retained up to the second observation period. No significant differences were noted in the changes of amplitude of single responses in the muscles studied in the test and control groups of animals. Therefore, the results presented in Figure 65 give evidence apparently of a decrease under the effect of space flight in the power of tetanic contractions of the muscle which is

TABLE 38. CHANGE IN FREQUENCY (IN IMP/S) OF TETANUS OF THE SKELETAL MUSCLES AFTER FLIGHT ON THE KOSMOS-690 BIOSATELLITE

Time period after completion of tests, days	F	SC	VC	Time period after completion of tests, days	F	SC	VC
Long extensor of the digits				m. soleus			
2	150±4	142±4	146±3	2	56±3*	66±6	65±2
26	138±5	141±3	143±5	26	44±5*	59±4**	70±4

*Proven difference from VC and SC
**Proven difference from VC

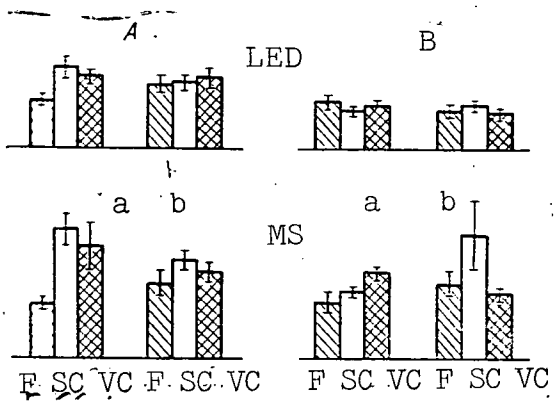

Figure 65. The value of the ratio of amplitude of the tetanic and single contractions of the long digital extensor (LED) and the m. soleus (MS) of rats on the second (a) and 26th (b) days after completion of flight on the Kosmos-605 (A) and Kosmos-690 (B).

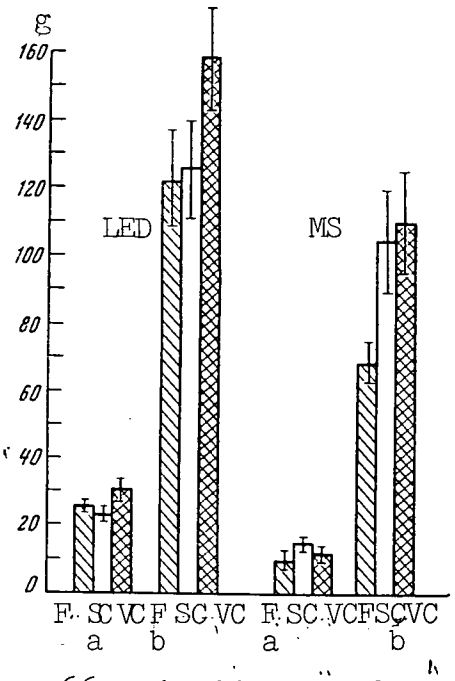

Figure 66. Amplitude of single (a) and tetanic (b) isometric contractions of the long digital extensor (LED) and the m. soleus (MS) of rats on the 26th day after completion of flight on the Kosmos-605 biosatellite.

/120

confirmed by direct data presented in Figure 66. In the first experiment after 26 days postflight, the amplitude of tetanus of the m.soleus of the flight group animals was confirmed to be smaller than in the animals of the two control groups. With this value of tetanic isometric force per 1 mg of m. soleus in animals of the flight group it amounted to 0.65 ± 0.06 g and in the animal groups of the synchronous and vivarium controls, respectively, 1.0 ± 0.09 and 0.96 ± 0.08 g. The value of A of the long extensor of the digit in the flight group and in rats of the synchronous control group also remained below that in the vivarium control group.

/119

The picture described is added to by the results presented in Table 39 of measurements of force characteristics of contraction of both muscles in the second experiment. It is apparent that the results of this experiment basically are similar to that described above. One should also note a decrease in A of the m. soleus in the synchronous control group and somewhat better restoration of strength in the second observation. The value of the tetanic isometric force of the m. soleus per unit of weight (1 mg) on the second day postflight amounted to 0.50 ± 0.1 g; in the synchronous and vivarium control groups it was equal, respectively, to 0.60 ± 0.08 and 0.82 ± 0.08 g. On the 26th day postflight, the relative strength of the muscle in the corresponding groups of animals amounted to 0.65 ± 0.06, 0.74 ± 0.05 and 0.65 ± 0.03 g.

/120

TABLE 39. CHANGE IN AMPLITUDE (IN g) OF SINGLE AND TETANIC CONTRACTIONS OF THE MUSCLES AFTER FLIGHT ON THE KOSMOS-690 BIOSATELLITE

/121

Type of contraction	Time period after completion of tests, days	F	SC	VC	Type of contraction	Time period after completion of tests, days	F	SC	VC
	Long extensor of digits					m. soleus			
A_0	2	24,5±3,5	24,8±2,8*	30,6±2,0	A_0	2	7,9±1,4	9,5±1,3*	10,5±0,6
	26	29,6±4,7	30,8±3,9	35,3±3,0		26	11,2±1,4	10,8±1,8	13 3±0,9
A	2	96±8	82±13*	109±4	A	2	37,0±6*	54,0±7*	74,0±5
	26	101±14	106±11	110±10		26	69,0±8	99,0±13	78,0±5

*Proven difference from VC

Figure 67 shows results of studying the biochemical properties of muscles with passive tension (experiment on the Kosmos-605 biosatellite). It is apparent that on the second postflight day, elasticity of the m. soleus is increased in the flight group and the long extensor digit in the flight group and the vivarium control group. Elasticity of the experimental "rapid" muscle remained elevated in the second observation period.

/120

/121

For evaluating the resistance of muscles to fatigue, we used the value of the ratio of amplitude of the responses expressed in percentage values at the end and the beginning of a 30-second series of stimulating impulses transmitted with a frequency of 3 Hz. In the first experiment, the statistically valid increase in fatigue in both muscles in the flight group animals was established on the second day postflight and its recovery to the level of the control values.

The results of the studies make it possible to state that after animals stay in space flight for 22 days (Kosmos-605), certain contractile functions and biomechanical properties of the muscles studied acquire significant changes which are expressed in retardation of single isometrically responses, loss of strength and elasticity, a decrease in functional mobility and resistance to fatigue. In the m. soleus, besides the

markedness of the changes, one also notes acceleration of the process of developing a tetanic isometric contraction. The basic characteristics of functional activity of muscles on the 26th day the animals remain on Earth does not differ statistically from the level of control values except for certain indices of strength and elasticity.

The results of similar studies on the Kosmos-690 biosatellite coincide with the main tendencies presented above. The basic differences are slower recovery of the weight index and certain deviations in the function of the m. soleus in the synchronous control group. The latter, apparently, is due to the presence in this experiment of an additional effect (ionizing radiation) as a result of which, however, they are realized as nonspecific effects inasmuch as the muscle tissue, as is well known, has fairly high radioresistance.

Disturbances were large in the m.soleus. As is well known, this muscle, opposed to the long digital extensor, consists primarily of fibers which are characterized by slow development of the contractile process, by an aerobic type of metabolism /122
and high resistance to fatigue thanks to the high content of myoglobin (Stein, Padycula, 1962; Devanandan et al., 1965; Close, 1967; Fyedorov, 1970). It is possible, consequently, to propose that the m. soleus capable of developing and maintaining long stress and functionally designed for capability in fulfilling on Earth work against the force of gravity, in conditions of weightlessness, to a large degree, are inactive in comparison with the long extensor of digits; this apparently causes the more noticeable changes in it which can be considered as functional phenomena of atrophy or inactivity. Also changes more marked in comparison with "rapid" were detected histo- /123
logically and histochemically in the m. soleus (Portugalov, Savina et al., 1976; Ilyina-Kakueva et al., 1977).

The data which indicate selective acceleration of the process of tetanic contraction in the m. soleus agree well with the results of studying the spectrum of isoenzymes of lactate dehydrogenase which also indicate restructuring of the metabolism in this muscle toward a decrease in activity of oxidizing processes existing in it and activation of the glycolytic paths of metabolism (Portugalov, Petrova, 1976), that is, in a direction bringing this muscle closer to the "rapid" muscles. The results of studying strength characteristics of the muscles which are described above give evidence of the possibility of such restructuring. It is well known that each type of motor unit corresponds to a certain level of the A/A_0 ratio and its value is larger in the "slow" muscles (Lindquist, 1973, Proske, Waite, 1974). From this point of view, the data which attest to a decrease in the A/A_0 value can also be considered as the result of transforming parts of slow fibers of m. soleus to rapid. The decrease in the pliability of its elastic components

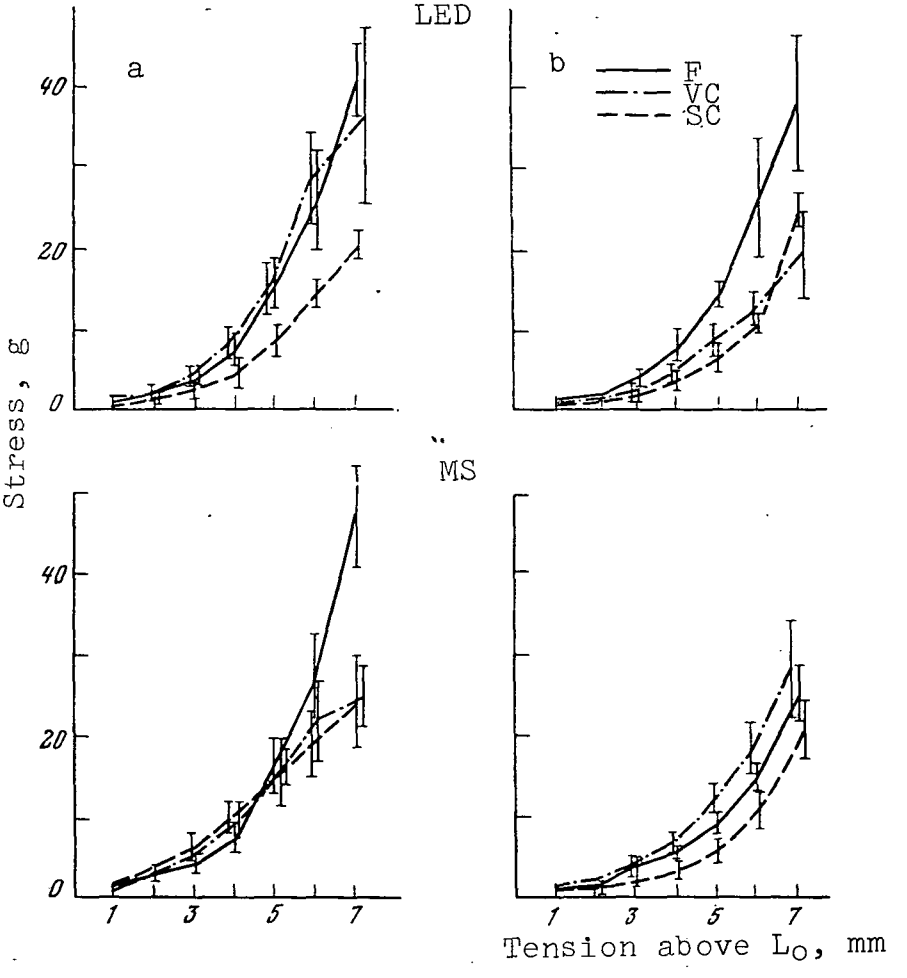

Figure 67. The relationship of "length-stress" with passive tension of the long digital extensor (LED) and the m. soleus (MS) of rats on the second (a) and 26th (b) day after completion of flight on the Kosmos-605 biosatellite.

plays a definite role in accelerating the process of tetanic contraction. This agrees with the increase in rigidity in the m. soleus established in these studies. /123

Theoretically it is possible that retardation of the single isometric contractions described above can be due to an increase in the length of activity of the state of the contractile apparatus of the muscles (Hill, 1953, Jewell, Wilkie, 1960). In favor of this hypothesis one can present information, according to the opinion of certain authors, on the decrease in frequency of the unified tetanus of the muscles

established in our studies in the first experiment. In this case, this circumstance indicates the known weakening of the power of the calcium pump and the supply of its mechanisms which mainly determine the character of development and duration of the active state of the muscle (Lesch et al., 1968; Bendol, 1970; Nicolet, 1972).

Thus, space flight this way or differently shows many structural and functional links of the contractile process to a larger degree in "slow" muscle. At the level of motor functioning of the entire organism, this can be in particular in the form of a decrease of static endurance established in the experiment on the Kosmos-782 biosatellite (see page 135). Moreover, the changes which occur in the majority are reversible and some of them have an adaptive character.

A comparison of the data presented and the results of certain model studies in hypokinesia conditions (Oganov, Potapov, 1973) and hypodynamia (Katinas et al., 1974; Oganov, Potapov, 1975) make it possible to assume that in the set of reasons which cause the development of functional disturbances in the skeletal muscles during space flight, the main role belongs to the force load of the muscle which decreases the tonic component of muscle activity.

Protein Fractions and their Enzyme Activity in the Myocardium. /124

Even the first studies made on the biosatellites show that in the myocardium of rats, 24 hours after landing, there are significant changes in enzyme activity of protein fractions (Gazenko et al., 1975; Gayevskaya, Veresotskaya et al., 1976). Due to the peculiarities of conducting experiments, the observations were made on a small group of test animals and require confirmation.

In this work we attempted to discover whether changes were detected in the protein fractions and their enzyme activity in the myocardium of rats in the earliest time periods after return to Earth from multi-day space flight and to add new observations to the preceding data.

The methods of isolating the protein fractions and determining the activity of enzymes has been described earlier (Gayevskaya, Veresotskaya et al., 1976).

In the myocardium of rats, 9-11 hr postflight, one noted a decrease in the content of sarcoplasmatic protein (Table 40), and after 26 days it was normalized. In the rats of the synchronous control group, the content of sarcoplasmatic proteins of the myocardium at the same time period for examination was unchanged. The quantity of actomyosin in the first time period

TABLE 40. THE CONTENT OF PROTEIN IN PROTEIN FRACTIONS (IN g PER 100 g OF MOIST TISSUE) AND ADENOSINETRIPHOSPHATASE ACTIVITY (IN μg P_N PER 1 mg OF PROTEIN AFTER 10 MIN AT 37°) OF THE MYOSIN OF THE MYOCARDIUM (M±m)

Group	Time after completion of tests and no. of animals (n)	Sarcoplasmatic fraction	Actomyosin	T fraction	Adenosinetriphosphatase activity of the myosin
VC	Both time periods combined (n=6)	6,10±0,16	4,32±0,12	2,41±0,09	109,5±1,1
	9-11 hr (n=3)	5,27±0,20*	3,26±0,46*	2,26±0,25	73,4±2,7*
F	25 days (n=3)	6,43±0,12	4,36±0,30	2,99±0,11*	117,8±1,9
	9-11 hr (n=3)	6,31±0,33	5,27±0,14*	2,46±0,16	126,3±2,5
SC	25 days (n=3)	5,80±0,00	4,26±0,10	2,85±0,19	110,4±2,7

*Proven difference from VC

of examination after flight also was decreased and in the second time period it returned to that of the control level. In the synchronous control group of animals, the content of actomyosin in the myocardium was proven to be increased in the first examination period but on the 26th day it had decreased to its initial value. The content of proteins in the T fractions in most of the animals was unchanged and even after 25 days postflight it had increased insignificantly.

A decrease in the content of protein of the sarcoplasmatic fraction and the actomyosin fractions in rats examined 9-11 hr postflight apparently can be explained by the effect of weightlessness because such changes are not observed in rats of the synchronous control group. Adenosinetriphosphatase activity /125
of the myosin was significantly increased in rats 9-11 hr postflight, whereas in all the other animals it remained at the control level. The aspartateaminotransferase (AST) activity of the sarcoplasmatic proteins in the myocardium postflight was unchanged (Table 41). Twenty-four hours postflight on the Kosmos-605 biosatellite, it was significantly increased. It is possible that in the latter case a strengthening of metabolism is apparent as a reaction to transition to conditions of the Earth's gravitation whereas at 9-11 hr this was inadequate.

In the myocardium of rats in the synchronous control group, the AST activity was increased in the first observation period. This, like an increase in the content of actomyosin could indicate an increased functional load.

TABLE 41. ENZYME ACTIVITY OF SARCOPLASMATIC PROTEINS IN THE MYOCARDIUM (M±m)

Group	Time period after completion of tests and number of animals (n)	AST	ALT	LDH total activity	LDH_1	LDH_2	LDH_3	LDH_4 + LDH_5
		μM of pyruvate per 1 mg of protein after 1 hr at 37°			μM $NADN_2$ per 1 mg of protein after 1 min at 26°			
VC	Both time periods combined (n=6)	19,6±1,3	3,01±0,20	4,14±0,15	0,98±0,03	1,43±0,04	1,09±0,06	0,59±0,05
F	9-11 hr (n=3)	18,1±0,8	4,44±0,10*	4,23±0,04	1,05±0,02	1,38±0,02	1,11±0,01	0,69±0,02
	25 days (n=3)	21,6±0,2	3,69±0,17	3,73±0,03*	0,89±0,03	1,24±0,02*	0,99±0,01	0,61±0,01
SC	9-11 hr (n=3)	22,3±0,6*	6,31±0,12*	4,33±0,05*	1,05±0,02	1,48±0,03	1,11±0,02	0,68±0,02
	25 days (n=3)	21,5±0,7	5,98±0,11	4,18±0,09	1,14±0,03	1,47±0,05	1,03±0,04	0,55±0,02

*Proven difference from VC

The activity of alaninaminotransferase (ALT) of sarcoplasmatic proteins of the myocardium was increased in comparison with the vivarium control in the rats of the flight group and the synchronous control group in both observation periods; this can be evaluated as a sign of certain strengthening of the intensity of energy exchange directed at maintaining synthetic processes in the tissue of the myocardium with changing functional activity.

When studying the total activity of LDH and the activity of its isoenzymes in the sarcoplasmatic fraction of the myocardium in rats 9-11 hr after completion of flight, no differences from the control were detected. Twenty-five days postflight, the LDH activity and its isoenzyme LDH_2 was insignificantly decreased. In the ground synchronous experiment in both observation periods, changes in the indices were not detected in the myocardium of rats.

Thus, a study made on rats who had undergone a 19.5-day flight on the Kosmos-783 biosatellite confirmed the fact of decrease in adenosinetriphosphatase activity of myosin of the myocardium due to weightlessness which was established earlier. Decreased activity of adenosinetriphosphatase of the myosin and a decreased content in the myocardium of contractile and /126

sarcoplasmatic proteins 9-11 hr after completion of flight could be the result of lack of load on the muscle apparatus of the heart in weightlessness and probably was an adaptive reaction. The data of electronmicroscopy which shows flattening of the disks in the muscle fibers also indicates lack of load on the myocardium during flight (Portugalov, Mul'diyarov et al., 1976).

Return to Earth's gravitational force can show a lack of correspondence between the necessary force contraction of the myocardium and the possibility of providing it with decreased adenosinetriphosphatase activity of the myosin. Considering the time of half decay of the myosin of the myocardium as equal to 5-8 days, reestablishment of normal adenosinetriphosphatase activity can be expected in approximately the same time period.

Histologic, Histochemical and Electron Microscope Study of the Myocardium.

The histologic and histochemical studies of the hearts of rats killed two days after completion of the 22.5-day flight onboard the Kosmos-605 biosatellite did not show significant structural and exchange dysfunctions. However, shifts in the exchange of the cardiac muscle could normalize two days after completion of flight. Because of this, and also for further more detailed information on the state of the structure of the myocardium, the following study was made. The hearts of four rats killed 9-11 hr and 3 rats killed after 25 days postflight was used as the material. Also, the hearts of seven rats from the synchronous control group and the hearts of seven intact rats from the vivarium control group were used. Immediately after killing and removing the heart with a razor blade, it was cut into two sections in a plane perpendicular to the long axis. Part of the heart, including the auricle, the valve apparatus part of the ventricle, were fixed with neutral Formalin, immersed in parrafin and used for histologic studies (coloration with hematoxylin-eosin, azane according to Geydengayn's method, iron hematoxylin according Geydengayn, toluidine blue). Part of the heart consisting only of the ventricles were frozen in solid carbon dioxide and sections of the myocardium prepared in a cryostat were used for discovering the activity of dehydrogenase of succinate, malate, isocitrate, B-oxybutyrate, α-glycerophosphate, $NADN_2$, and also phosphorylase A and B and the content of lipids. An electrophoretic method (Dietz, Lubrano, 1976) was used for studying the isoenzyme composition of LDH of the left ventricle, using, for density measurement flat gels of the recording IFO-451 microphotometer. Determination of the area of separate LDH fractions was done by a method of planimetry. Samples of the left

and right ventricles and the left auricle were fixed for 3.5 days in a 2.5% solution of glutaric aldehyde prepared on a 0.33 M solution of saccharose, degreased in alcohols and immersed in araldite were used for conducting the electron microscope study. Ultrathin sections of the heart sections named were studied in the JEM-7 electronic microscope.

During histologic and electron microscope study of the hearts of rats, killed 9-11 hr and 25 days after completion of flight, neither in the myocardium, the valve apparatus nor in the vessels were any significant changes detected in comparison with animals of the control group. Both in the test and in the control rats, most of the muscle cells retained the structure normal for them with a clearly marked longitudinal and lateral striation and only in two of the four rats in the flight group killed in the first time period were isoline myosin protofibrillae detected oriented tangentially and even perpendicular to the axis of the main mass of protofibrillae. Such protofibrillae were localized usually under the sarcolemma or between the myofibrillae. Moreover, in the time period indicated, in the rats of the flight group one noted a certain thickening of the intercalary disks in the muscle cells of the right ventricle which was expressed in a decrease in the height of fingerlike growths. The structure of the intercalary disks was retained. /127

Both in the tests and in the control rats, in the myocardium separate muscle cells or groups of cells were counted with a homogeneous poorly colored eosin cytoplasm which, after coloration with azane and iron hematoxylin acquired, respectively, a raspberry-red and black color. On the electronograms of these fibers one noted the uniform contraction of the myofibrilla sarcomers and formation of contracted bands. The indicated changes in structural and dye properties of the muscle cells involve excessive contraction of the muscle cells at the moment of death of the animals and are well known in literature by the term "contracting degeneration" (Naddachina, Smol'nikov, 1964; Rappoport, Tinyakov, 1969; Kochetov, 1972; Nikitova, Zagrebin, 1972). Signs of decomposition of the protofibrillae, formation of nucleus detritis and also the increase in the number of lysosomes and other bodies (lipofuscin granules) were not noted in the muscle cells.

In the myocardium of rats in the flight and both control groups, also sections were encountered with expanded full blood capillaries and veins, less often small hemorrhages where one observed unchanged erythrocytes; this indicated the occurrence of a recent hemorrhage. In the muscle cells belonging to the hemorrhaging section, changes were observed of hypoxic character in the form of edema of the subsarcolemma sarcoplasm , swelling of the mitochondria with transparency of their matrix, a decrease in the number of

glycogen granules. Inasmuch as these morphologic signs of disorder in microcirculation were noted both in the test and in the control rats, one can consider that they occurred at the moment of death. In certain cases, in the myocardium diffuse proliferation of the connective tissue elements of the stroma occurred and at the base of the valves one observed neoplasms of the cartilage.

During histochemical investigation of the myocardium in rats of the flight group killed 9-11 hr after landing of the biosatellite, an increase in phosphorylase activity was discovered whereas activity of the other enzymes studied did not differ from that of the control level. Electrophoretic study of the isoenzyme composition of LDH of the myocardium was not apparent in rats of the flight group to have proven differences in comparison with animals of both control groups. Free lipids in the myocardium of rats in the test and control groups were not apparent. Twenty-five days after completion /128
of the experiments, the phosphorylase activity in the myocardium of rats in the flight group had normalized. The increased activity of this enzyme noted in the first time period involved, probably, an increased content of adrenaline in the myocardium because the presence of a direct relationship between the content of adrenaline and activity of phosphorylase is well known. An increase in the content of adrenaline involves, in turn, the development of acute stress, whose morphologic symptoms were detected when studying the lymphoid organs (Durnova et al., 1977) and the adrenal glands (Savina, 1977). The occurrence of acute stress is explained by the effect on rats of the complex of extreme factors accompanying landing of the biosatellite. Thus, the data obtained attest to the fact that a 19.5-day space flight does not result in the development of significant structural changes in the heart and does not cause rough disturbances in the myocardium exchange. It is necessary, however, to note that the results of this study cannot be fully extrapolated for humans inasmuch as in a difference between humans and rats, during their stay in conditions of weightlessness one does not observe a marked redistribution of blood in the organism.

Conclusion

Muscles which differ in structure and functional characteristics were studied.

In the skeletal muscles, changes were detected indicating activity of catabolic processes (a change of atrophic type in the activity of the LDH isoenzymes, a decrease in breathing and phosphorylizing functions, a decrease in exchange of the sarcoplasmatic network, a decrease in weight, atrophic changes in the histologic structure). The muscles which carry

out the antigravitation function in terrestrial conditions underwent the most changes.

In the animals exposed on the Kosmos-605 and Kosmos-690 biosatellites, mainly in the m. soleus and to a lesser degree in the long digital extensor, a decrease was observed in functional full value when studying *in vitro*.

In the cardiac muscle, histologic, histochemical and electron microscope studies did not show dysfunctions. However, when studying the metabolism of the myocardium, a decrease in adenosinetriphosphatase activity of the myosin was detected and also the content of myosin and sarcoplasmatic proteins.

The changes detected in the muscle system are absent in animals killed 25 days postflight.

Static Endurance and Vestibular Motor Reactions

After long flights of man on spacecraft and in orbital stations, a decrease in the volume of skeletal muscles a drop in their tone, vestibular characteristics, unsteadiness in walking, dysfunction of posture stability (Gurovskiy, Yegorov, 1976) were detected, attesting to the series of definite changes in the systems of motor and vestibular analyzers.

In the animals who spent 22 days in weightlessness conditions on the Kosmos-605 satellite, morphofunctional changes were noted in the skeletal muscles of the extremities, mainly in the antigravity musculature and also in the long tubular bones, that is, in those tissues which [were not functionally] under load due to the disappearance of body weight (Portugalov, 1976). When setting up physiological experiments on the Kosmos-782 biosatellite, basic attention was directed at finding changes in those systems of the organism whose functioning in terrestrial conditions to one or another degree is determined by the force of gravity. Due to this, it was justified to make an evaluation of endurance to static force, the reflex of turning over during free fall and the landing reflex and also the equilibrium function.

Endurance in rats to static force (static endurance) was evaluated as the maximum time of maintaining animals on a rod suspended vertically below a platform attached to a mount 120-180 cm high (Shipov, Markin, 1977). The turning over and landing reflex during free fall was evaluated using a film record of the fall of the rat from the rod and subsequent frame analysis of the film. The state of function of equilibrium was studied on a device which is a swing with equal projections, mounted at a height of 80 cm over the level of a table. A weight is suspended on one of the ends of the swing putting the swing at an angle of 30-40° from the horizontal. The animal is placed on the swing; after a certain time it is placed in such a way that the angle of slope of the swing from the horizontal was minimum. The state of the equilibrium function was evaluated according to the time for the animal to level the swinging platform.

Evaluation was made with particular care of the general state of the animals, their neurological status, the character of walking and other natural motions as well as their reaction to the environment.

Studies were made on eight animals before the flight and after 6 hours, 2, 6, 10, 15, 24 days after its completion.

The data obtained were compared with the results of examining 8 animals in the synchronous control group and 8 in the vivarium control. /130

After landing, the condition of the animals was completely satisfactory, but motor activity was sharply decreased. All of the motions were distinguished by caution, slowness; it was as if the animals were in a state of functional hypokinesia.

In the first three days after completion of flight, a considerable change was noted in coordination of random motions. The animals limped and rested on their rear paws putting most of their weight on them for support. When examining objects which were located high up the animals never stood on the toes but rested on the heel and often lost their equilibrium.

Restoration of disturbed equilibrium was accomplished in the animals using the front extremities which were used for grasping food and "washing".

When lying on their stomach, one observed an unnatural angle of the rear feet to the trunk. Turning of the trunk was accomplished mainly by shifting support on the front paws.

The changes noted gradually leveled out and by the 10th to 15th days of observation, had disappeared in most of the animals completely. Similar changes in the animal group in the synchronous control were weakly expressed.

An analysis of the posture and walking of the animals attested to the fact that after flight the function of the rear extremities is noticeably lost whereas the function of the front extremities remained fairly stable. This conclusion is supported by observations of the behavior of rats on a pole. Usually the animals maintain themselves on the pole primarily with their rear paws leaving the front feet relatively free. After space flight, the rats stayed on the rod using all four extremities.

A morphologic evaluation of the effect of flight factors on muscle structure of rats also showed the muscles of the rear extremities are the most vulnerable (Portugalov, 1976).

Probably the reason for this differentiation effect in the animals with four legged locomotion involves the fact that extensors of the rear extremities which have the largest muscle mass and fulfill the basic function during walking, jumping, taking vertical positions, are also the most sensitive to detraining during artificial limitation of mobility or conditions of weightlessness. The front paws which grasp food and retain constant insignificant coordination motions, make the least contribution to total energy consumption of the organism and undergo the effect of detraining conditions

the least. In space flight, the role of the front extremities in grasping food, in the "toilet" are retained but specific weight in the moving functions, due to their large coordination-adaptive capabilities, undoubtedly increase. In conditions of weightlessness constant control is necessary of random motions due to the inertia moments which arise. In such cases, only the front paws whose function is more cortical, can provide viably necessary motor acts. This gives them the advantage of keeping in shape during flight conditions.

Preliminary studies of static endurance showed that the /131
maximum time for the rat to stay on the pole depends on the body weight.

Figure 68 shows a normogram, or the required values, of static endurance for male rats of the Wistar line, the SPF-Wistar and mongrel animals. It is obvious that with an increase

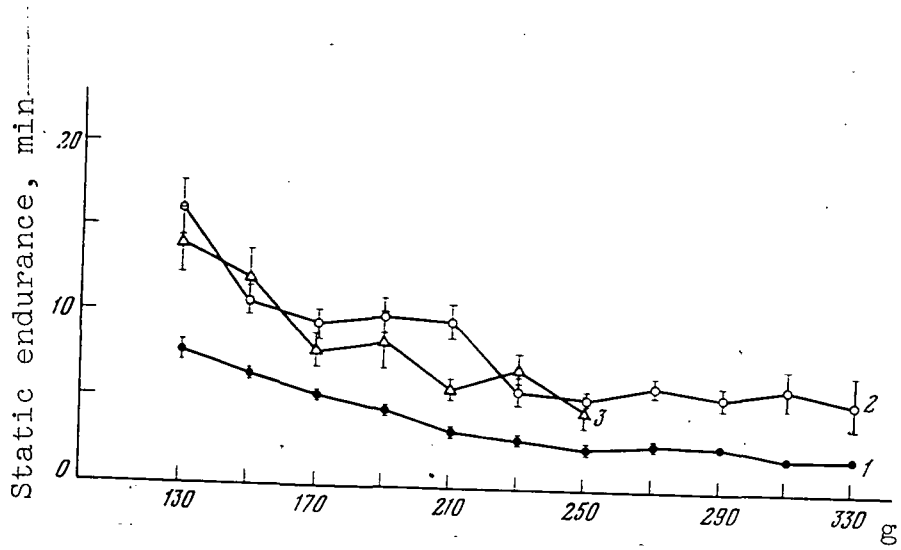

Figure 68. Static endurance of rats of different species depending on body weight.
1 -- SPF-Wistar; 2 -- Wistar; 3 -- mongrel.

in weight, static endurance began to drop sharply; then the rate of fall would slow down and with the weight of the animals more than 250 g remains practically unchanged. A comparison of the /132
normograms indicates that the required values of static endurance of rats of the SPF-Wistar species is proven to be lower than these indices in rats of the other types in all the range of weights considered and differ least in variability.

Figure 69 shows a graph of changes of static endurance of rats of the flight group and of the synchronous control group and also the required values of endurance corresponding

to weight of the animals on the days of the observations. Inas-

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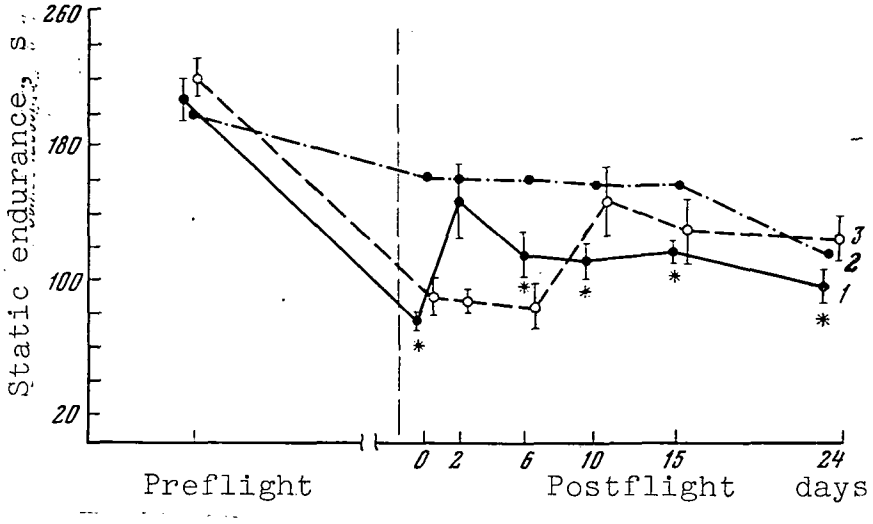

Figure 69. Static endurance of rats.
1 -- F; 2 -- required value; 3 -- SC. The asterisks indicate the points with proven difference from the required value.

much as the weight of the rats exceeded 250 g, the required values of endurance of both groups of animals did not differ and are presented in Figure 69 in the form of a single curve. /132

During preflight observation, no proven differences were found in endurance of the rats of the flight group or the synchronous control group from the required value. Just six hours after completion of flight, endurance of the animals was almost three times below the initial value and also the required value. A similar decrease was noted in animals in the synchronous control group. However, endurance to static force in the animals of the flight group remained decreased in comparison with the required value even on the 24th day after completion of flight whereas in the animals of the synchronous experiment, restoration of static endurance had begun by the 10th day. This difference in the time periods for recovery should be explained by the effect of weightlessness. Compensation for the effects of weightlessness was a process which is more complex and of longer duration in comparison with compensation for the effect on the organism of the housing conditions on the biological satellite. A decrease in resistance of the animals to static force occurs, in all probability, due to the total astheniza-tion and detraining of the organism in weightlessness conditions. A similar phenomenon is described after hypokinesia and orbital flights in studies with man (Cherepakhin, 1970;

Cherepakhin, Pervushin, 1970), and also after hypokinesia in animals (Kovalenko et al., 1975).

The peculiarity of dynamics of restoring static endurance in the rats both in the flight group and in the synchronous control group which involves the presence of an adequately sharp increase in endurance (see Figure 69), at the present time is difficult to explain. It is possible that this increase in static endurance is the result of increasing biological activity of the muscles observed by certain scientists after space flight in man and animals (Taranov, Panferova, 1965; Gazenko, 1968; Kakurin et al., 1970). It is possible to propose that with the launch mechanism, a similar phenomenon is a sharp change in the character of proprioceptive pulsations during return of the animals to natural life conditions. The sharp increase in static endurance in rats after a 22-day hypokinesia observed by us earlier is confirmation of this (Figure 70). It occurred on the 4th to 5th days of the readap-

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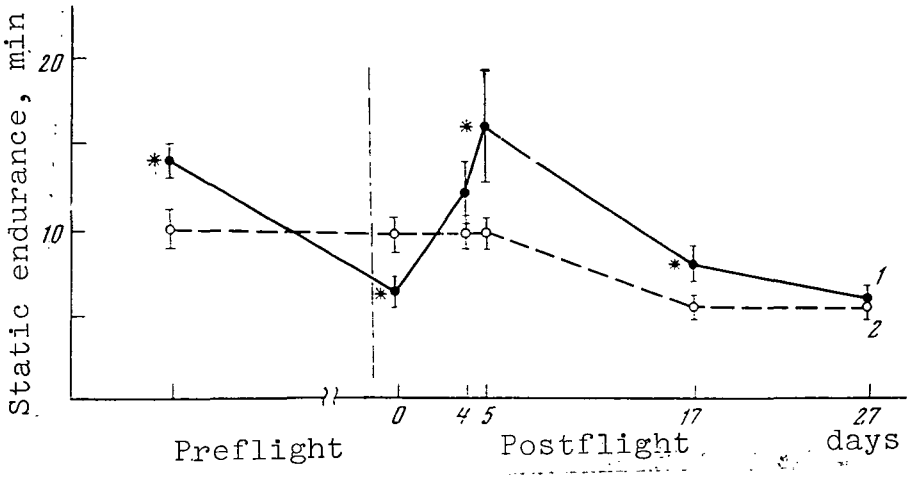

Figure 70. Static endurance of rats of the Wistar species in a test with 22-day hypokinesia; 1 -- hypokinesia; 2 -- required value. The asterisks -- same as in Figure 69.

tation period, that is, later than in animals of the flight group in this experiment, but considerably earlier than in animals in the synchronous control group. It is probable that the more severe the conditions for limiting mobility, the closer the dynamics of recovery of static endurance are to those observed after a long stay in weightlessness. /132

The existence of a reflex of turning over usually involves the labryrinth function (Magnus, 1962). An evaluation of the

reflexes of turning over and landing were made in animals of the flight and synchronous experiments. The animals of the synchronous control group, like those in the earlier experiments with 22-day hypokinesia, when falling from the rod turned over in the air and landed softly on their paws (Figure 71a). As a rule, rats who are on a rod gradually slip and are

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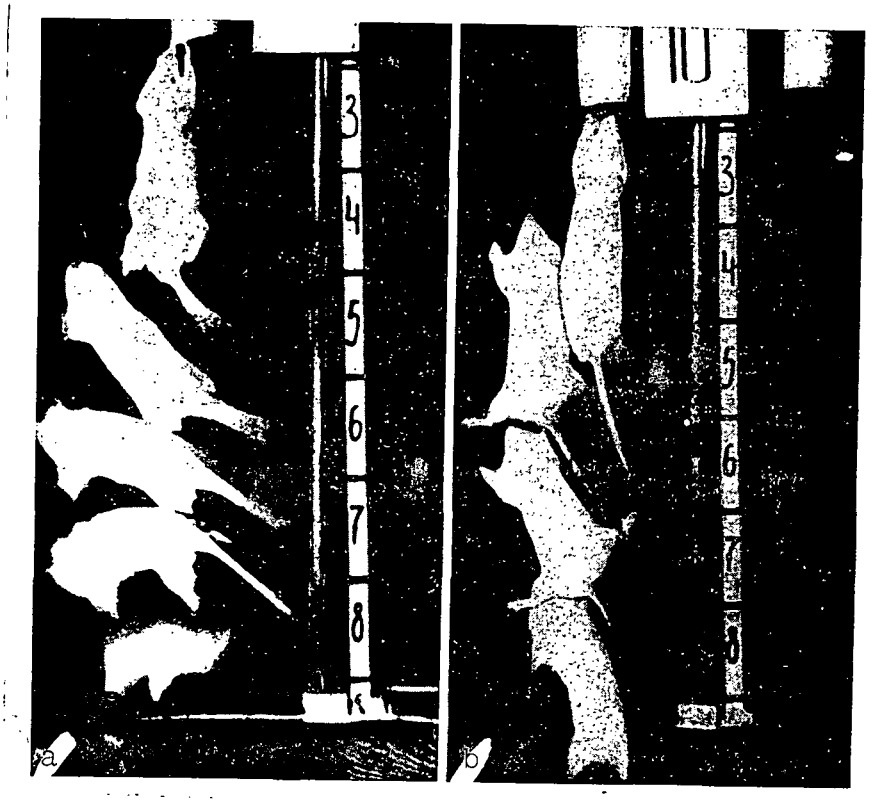

Figure 71. Films of rats falling from a rod.
a -- third day after the synchronous experiment;
b -- third day after flight.

in a position with their backs downward, holding on with their forepaws. Suspended in this position for several seconds and having made one or two unsuccessful attempts to regain their position on the rod, the rats hold on with their front feet and then drop downward. After separation from the support, the phase of preparation for turning begins with inclination of the head. Then turning is accomplished by ventroflexion of the trunk (the animals is hunched together). Preparations for landing begin after the trunk was rotated around the lateral axis. All phases were accompanied by rotation of the tail which stopped at the moment the paws touched the support.

/134

After space flight, when falling from a rod, even some of the animals unsucessfully attempted to take the correct position in the air. Rotation of the body remained incomplete and the animal, as a rule, landed on the tail (Figure 71,b). By the 10th to 15th days after landing, in most of the animals the principal changes had disappeared completely.

In the postflight period, one observed worsening of the function of equilibrium. An increase in the time for finding stable equilibrium was noted on the second day, and by the sixth day it had increased by two times and in its average value was proven to differ both from the background data and from the data of the synchronous experiment (Figure 72).

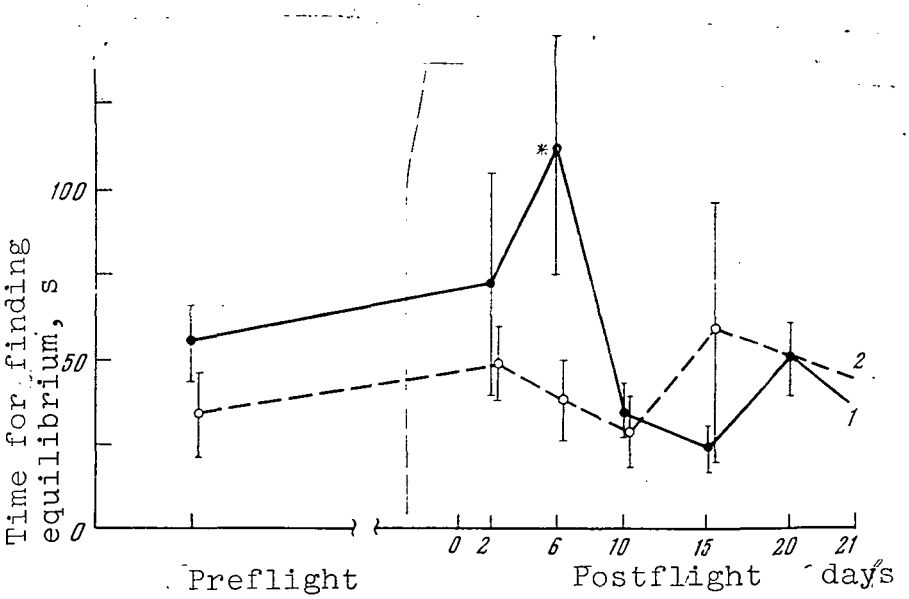

Figure 72. Function of equilibrium of animals. 1 -- flight group; 2 -- synchronous control group. The asterisk indicates the point with proven difference from SC.

The animals in the synchronous control group showed an insignificant increase in the time for finding stable equilibrium even on the second day after completion of the experiment. It is well known that the equilibrium function is an integral characteristic of interaction of a number of sensor systems of the organism, primarily motor, visual and vestibular analyzers. In experiments from the 22-day hypokinesia function, equilibrium, evaluated by the time for finding a horizontal position, acquired changes in time similar to those observed after flight, although less marked. This gives us the basis for assuming that the greatest contribution to dysfunction of equilibrium noted after flight involves changes in the motor analyzer. /135

The results obtained, in the aggregate, attest to the occurrence in animals, affected by long term weightlessness, of the time functional changes in the motor and vestibular analyzers systems.

Structural and Functional Organization of the Vestibular Apparatus.

As studies made of the vestibular apparatus in recent years have shown, in the peculiarities of the utriculus of higher and lower vertebrates, acceleration causes a certain cycle of cytologic shifts in the receptor cells which take a long time to reestablish (Vinnikov et al., 1971, 1972). If these changes are applied during space flight to shifts caused by weightlessness, then they can make an analysis of the effect of the latter on the vestibular apparatus very difficult. Due to this, studies were made on the embryos of fish and amphibians in which the vestibular apparatus was still absent when going into space (Vinnikov et al., 1972, 1976; Vinnikov, 1974). The results obtained indicated that during a stay in conditions of space flight for 40 hours, 6-16 days, the embryos of fish and amphibians continued to develop; a normal auditory sac developed and then labryrinth (otocones in the amphibians and atoliths in the fish). Cytologic differentiation of the receptor cells was typical to a fair degree. Undesirable shifts caused by acceleration were absent. All the same on the 6th and 16th days of staying in weightlessness, certain intumescence of the tissue more marked in comparison with the control, a dysfunction of the correct crystal shape of the otocone in the amphibians and disorganization of the cyclicity of daily rings of the otolith in the fish were observed. Similar results were obtained on the Fundulus heteroclitus embryos, kindly sent to us by our American colleagues. /136

When analyzing the vestibular apparatus in rats we attempted to differentiate between shifts caused by acceleration and those changes caused by the effect of weightlessness. /137

Particular attention was also devoted to the structure of the otocone and otolith membranes.

In all, 19 rats were studied, 9 of them control and 12 experimental. The vestibular apparatus was removed according to the method by Ya. A. Vinnikov and A. K. Titovaya (1961); it was placed in a solution of glutaraldehyde and the appropriate sample was inspected on sections of light and electron microscopes. Then, the time periods for taking the material were considered as well as the duration for keeping it in the fixing agent. One should point out that in all of the experimental rats, when uncovering the bone labryrinth, hemorrhages

were observed in the area of the cochlea in the perilymph where it makes a transition to the vestibular perilymph. /139

In the light guide of the microscope, good retention and correct markings of the vestibular apparatus were apparent, the otolith membrane and otoliths were observed in animals of the flight group. The thinned otolith membrane with the otoliths lay close to the surface of the receptor epithelium of the utriculus (Figure 73), which was the result of the effect of acceleration. Both the light optical and electron

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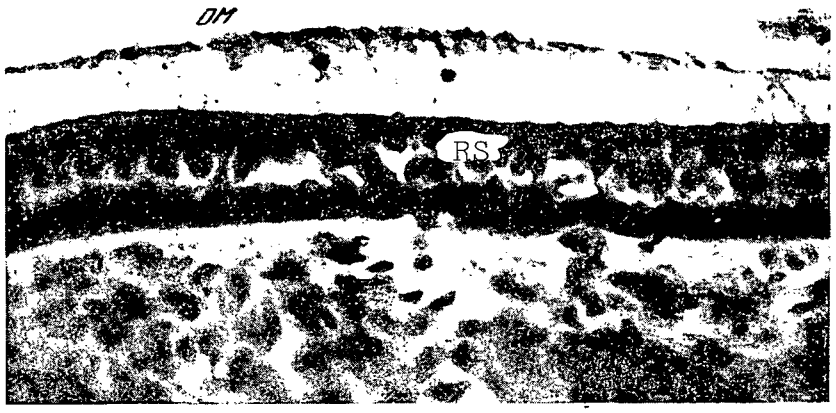


Figure 73. The utriculus of rats in the flight group. Magnification 70. The otolith membrane (OM) lies next to the receptor surface (RS) of the utriculus. Vertical section. Microphotography.

microscope observations showed increased intumescence of the tissue (Figure 74,a) and vacuolization of the cells (Figure 74, b; 75) in comparison with the control. Marked stasis took place in the capillaries of the connective tissue. Dish-shaped and bud-shaped nerve endings around the type I and II receptor cells, and also the synaptic structures did not undergo any coarse disturbances, however many of them were intumescent (Figure 75). The nerve fiber and the support cells were well retained. The mitochondria, as a rule, were dense although sometimes their swelling and vacuolization occurred, in particular in the efferent and afferent endings. The receptor cells acquired more marked changes. One observes deviation from their apical surface of characteristic protoplasmatic growths which, in the form of "protuberances" set in in the endolymph under the otolith membrane. The "protuberances" were surrounded by an extension of the plasmatic membrane (see Figure 74, b; 75); in their cytoplasm, separate swollen mitochondria were detected and also basal kinocillin globules, although, as a rule the bundle of stereocillin and kinocillin opposite it were well retained. The cytoplasm of receptor /139

Figure 74. Receptor epithelium of the utriculus. Magnification 4000. /136

a -- overall view (rats of the control group); b -- specific section (rat of the flight group); RC -- receptor cell; DNE -- dish-shaped nerve ending; SpC -- support cell; ST -- stereocillin; P -- protoplasmatic "protuberences"; NE -- nerve endings. Electronogram.

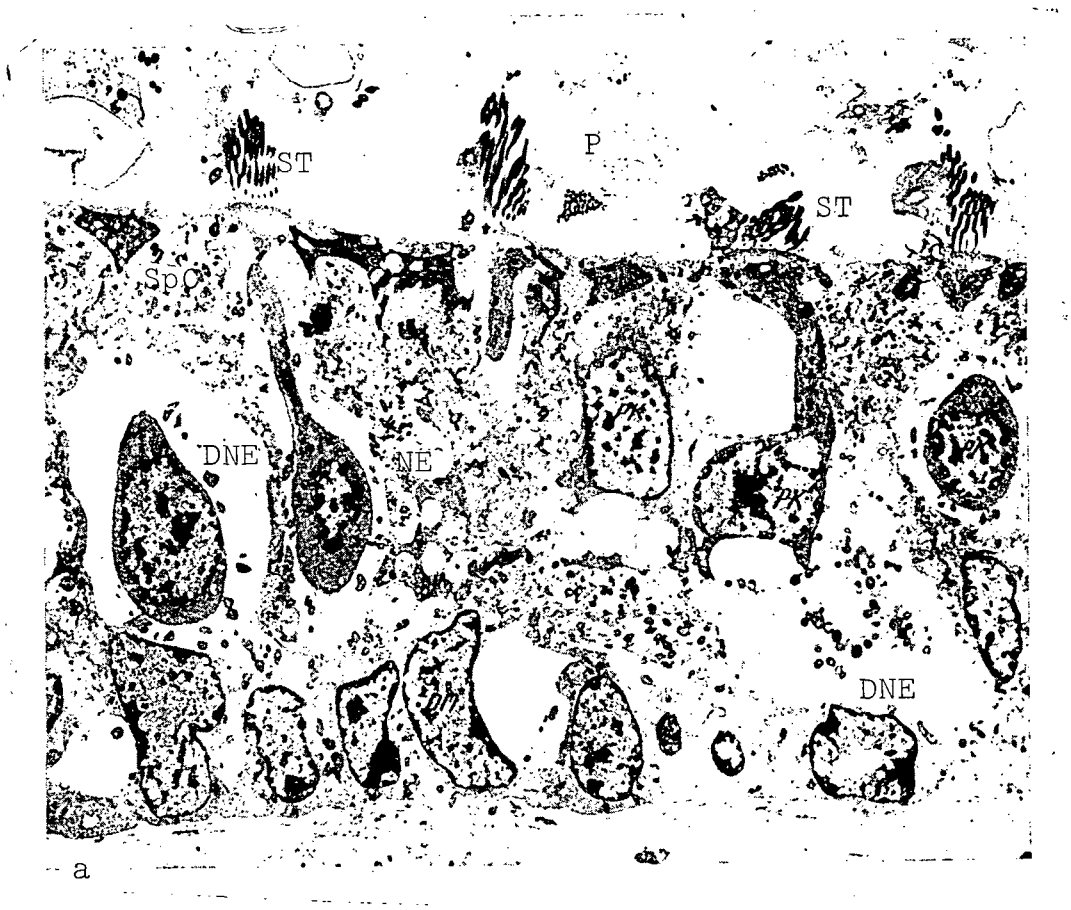
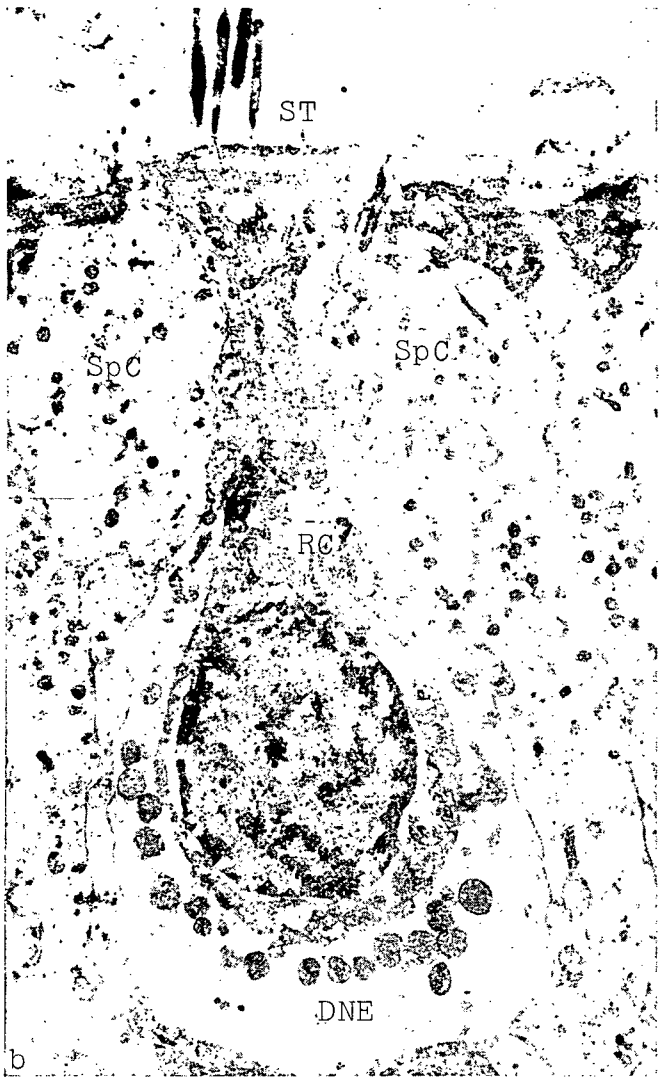


Figure 74.
(cont.)



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cells of types I and II were distinguished by increased electron density. The mitochondrial apparatus and the remaining cytoplasmatic organellae of the receptor cells were not changed in comparison with the control; the nucleoli in the nuclei of the receptor cells were discovered in the extreme position which usually is the result of the acceleration effect (Vinnikov et al., 1971). /139

Along with such slightly changed receptor cells, one encountered cells (mainly of type I) with pronounced size of degeneration and even necrobiosis (see Figure 75). As a rule, these cells are located within the dish-shaped nerve endings which were sharply vacuolized.

The otolith membrane which in the control (Figure 76,a) has a granular structure, in the animals who have undergone flight acquires a more diffuse, gel type character (Figure 76,b).

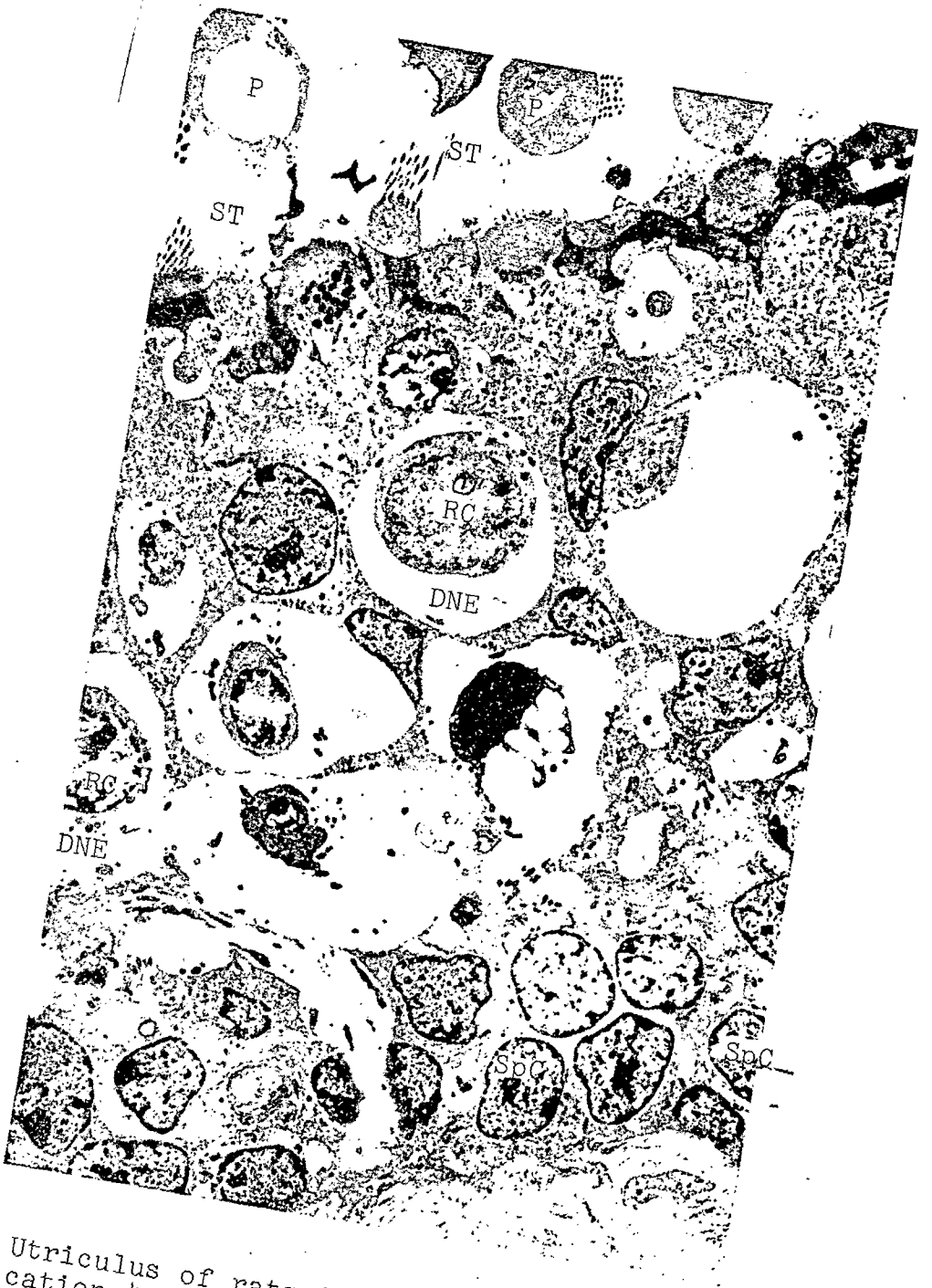


Figure 75. Utriculus of rats in the flight group. Magnification 4000.
Symbols the same as in Figure 74. One observes edema of DNE, death of RC of the first type. Electronogram.

Figure 76. The otolith membrane (OM) and the otocone (OT). Electronograms. /140
a -- rat of the control group; magnification 9000; b -- rat of the flight group, magnification 3700.

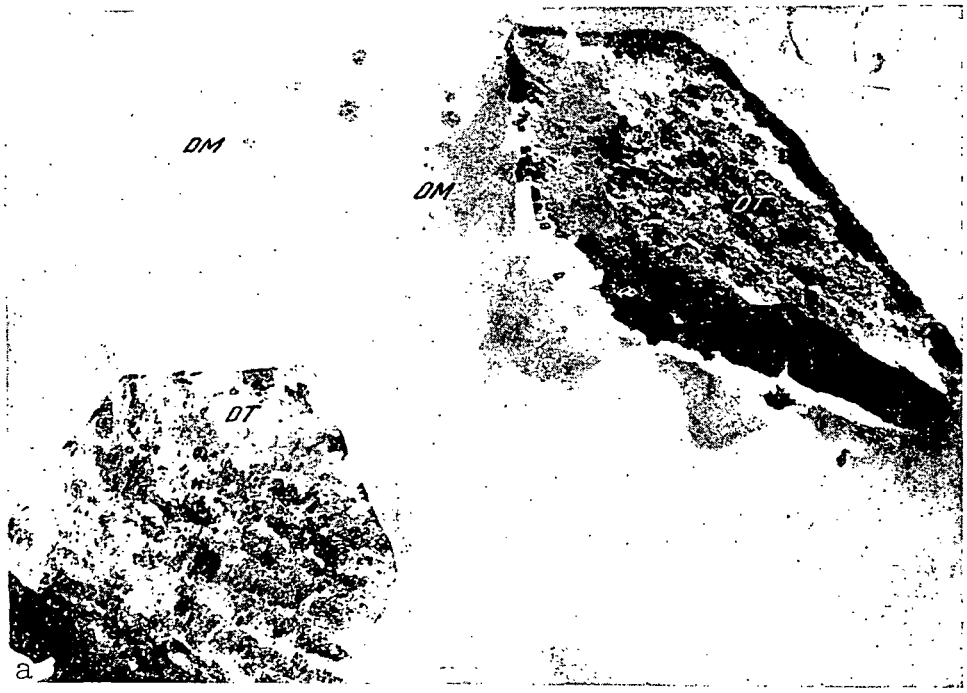
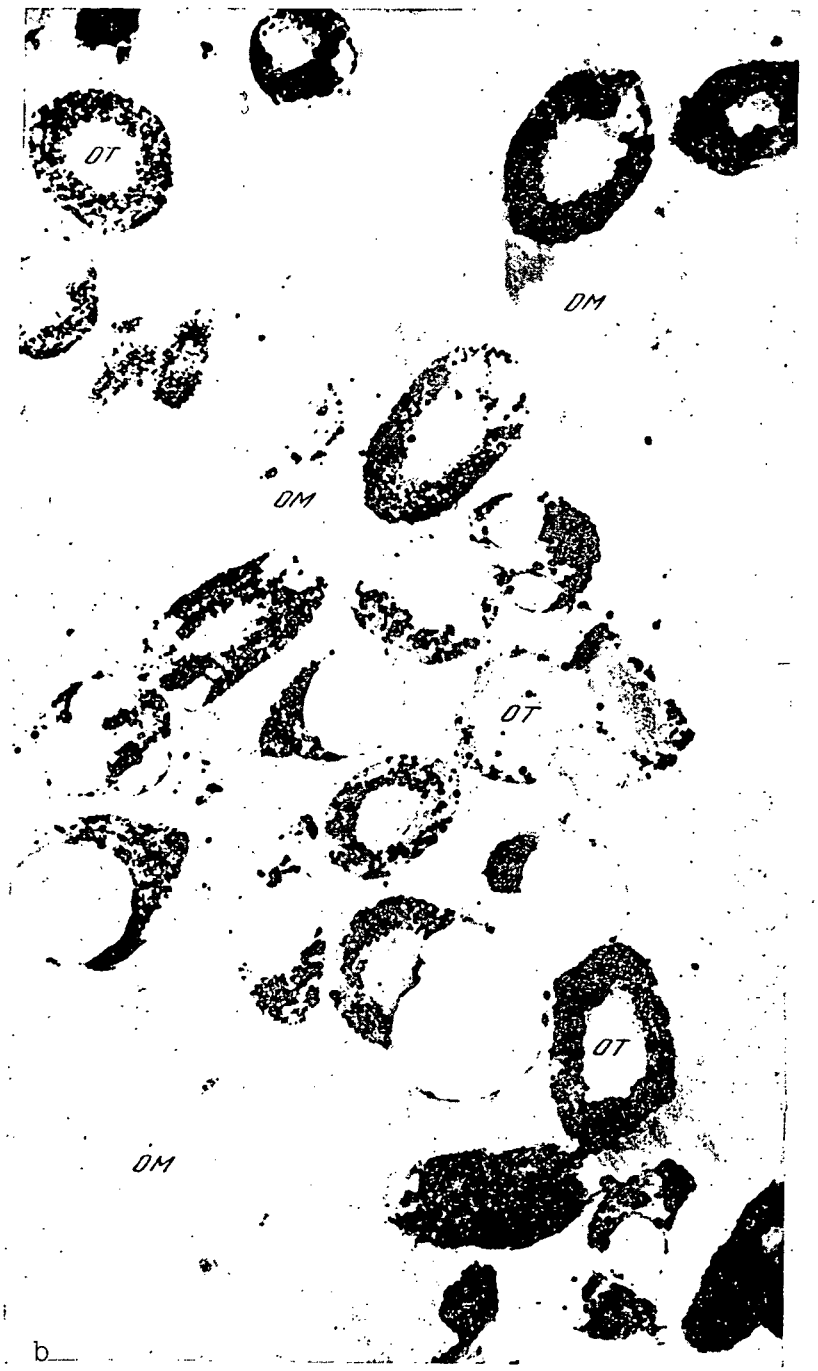


Figure 76 (continued)

/141



The otocone, which in the control group has the correct shape of polygon crystals with uniform alternation of light and dark granular substances (Figure 76,a), in the flight group of animals was sharply changed. As a rule, they had an oval-round shape (Figure 76,b), light and dark substances were distributed nonuniformly. The center of the otocone was light and the periphery -- a dark substance. The latter consisted of markedly different granules located loosely or compactly. Certain otocones were vacuolized, the vacuoli were located in a field of light substances and, running together, formed bulges which gave some of the otocones grape-like outlines. /139

Studies of the sacculus in the flight animals showed in their structure organization of shifts similar to those described for the utriculus. The cristæ were comparatively more stable, nevertheless, there were breaks observed in them from the receptor epithelium of the cupula base, for example, in the gravitation semicircular canal (Figure 77). These receptor cells and their innervation is practically unchanged in comparison with the control (Figure 78), although due to the break of the cupula, the stereocillin is curved at its base. /140

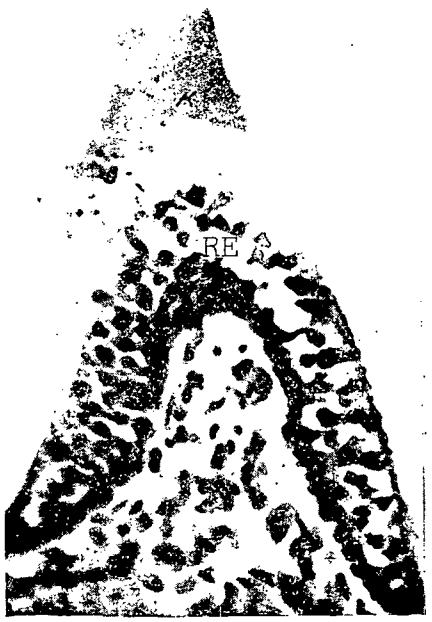


Figure 77. A break in the cupula in the ridge of the horizontal canal in rats of the flight group. Microphotography. Magnification 70. K -- cupula; RE -- receptor epithelium.

Further thorough studies are necessary, in particular, to clarify the question of reversibility of the changes described.

The data presented indicate that a 19.5-day stay of animals in conditions of weightlessness and a two time effective acceleration is undifferentiated for the structural and functional organization of the vestibular apparatus. Reinforcement of the function of the vestibular apparatus during acceleration is changed by its disengagement or a change in weightlessness conditions. Apparently, it is just this that results in the fact that, besides a large intumescence of the tissue involv- breakdown and formation of outflow of the endolymph and perilymph, one observes death of separate receptor cells mainly of type I. Also particular attention is attracted to the change in the otocone: the loss of the

Figure 78. Apex of the receptor cell with stereocillin ridge of the horizontal semicircular canal after break of the cupula. Rat of the flight group. Electronogram. Magnification 9000.

correct crystal shape and change in light and dark substances in them which separate them one from the other. We propose that the dark substance is organic, and the light -- an inorganic ingredient of the otocone. Consequently, redistribution of the substances attests to breakdown in calcium metabolism within the limits of the otolith and the otolith membrane. This hypothesis also comes from observations which we made earlier (Vinnikov et al., 1976) on the otocones and otoliths of amphibians and fish and also from bibliographical data on the dynamics of calcium exchange in the otocones of the otolith membrane of mammals, in particular rats (Lim, 1973, Ross, Reacar, 1975).

Conclusions /143

Physiological studies showed that after space flight, the animals had a marked disorder in motor coordination. Noticeable breakdowns were apparent in resistance to static force and also in reflexes of turning over and landing during free fall. In the animals of the flight group, these disturbances were not fully leveled out even at the end of the postflight observation period. To a large degree, the functions of the rear extremities were disturbed in ground conditions; these play the basic role when moving the body and have the largest muscle mass. In the flight group animals, significant changes also occurred in the equilibrium function, which, in the synchronous control group, was disturbed only slightly.

Histologic and electron microscope study of the vestibular apparatus showed intumescence of the tissue involving disturbance in the formation and discharge of endolymphs and perilymphs, and also degeneration of separate receptor cells. The characteristic changes apparently involve disturbance of calcium exchange; they were observed in the structure of the otolith memberane and particularly in the structure of the otocones.

Quantitative and Qualitative Characteristics of Inorganic Substances in Certain Bones of the Skeleton.

Under weightlessness conditions, an also under conditions of limited mobility, a significant decrease in the content of calcium in the bones is observed.

Of the two fractions which make up the mineral part of the bone, the crystal hydroxyapatite and amorphous phosphorane of calcium, the amorphous fraction is considered more labile; due to this, the loss of calcium in weightlessness conditions can, in turn, affect this fraction particularly.

Methods which have been developed recently (Ostrowski, 1972, 1974) make it possible to measure the ratio of crystal fractions to amorphous in the bone tissue. This method is based on the spectrometry of electronic paramagnetic resonance (EPR) of constant paramagnetic centers induced in the crystal lattice of the hydroxyapatite by ionizing radiation. This radiation does not cause changes in the amorphous fraction. Thanks to this method, one can quantatively determine the so-called coefficient of crystallinity, which is the ratio of the quantity of paramagnetic centers induced by ionizing radiation to the volumetric quantity of mineral residue of the bone.

The loss of calcium from the amorphous fraction in weightlessness conditions must affect the value of the coefficient of crystallinity which theoretically must reach higher values.

Using these suppositions, the shoulder bone and the bone of the cranial crown were studied in rats of all groups in both examination periods. After lyophilization of the samples and degreasing in a mixture of alcohol and ether (1:1), the bones were mechanically ground and subjected to the effect of the gamma rays of Co^{60} in a dose of 10 Mrad (power of the dose 0.11 Mrad/hr). The concentration of spins was measured by the spectrometer which was connected to a computer. The samples were burned at a temperature of 90° and the coefficient of crystallinity was calculated using the method described earlier (Ostrowski, 1972, 1974). The results obtained are presented in Tables 42 and 43.

The low values of standard errors attest to the high quality and uniformity of the experimental material. There is no difference in the concentration of sols and in the concentration of spins in animals belonging to different experimental groups. Also there were no differences in the data obtained on the tubular bones which bear the load in Earth's gravitation conditions and on the bones of the cranial crown which do not support such a load. Theoretically one could expect a large effect in the first case.

TABLE 42. THE CONCENTRATION OF SPINS, THE CONTENT OF SOLS AND THE COEFFICIENT OF CRYSTALLINITY OF BONES OF THE CRANIAL CROWN (M±m)

/145

Group	Time period after completion of tests	Number of Animals	Number of spins (rel. unit) (S)	Content of sols, % of weight (A)	Coefficient of crystallinity (S/A)
F	5–7hr	6	2380±60	65,8±0,4	36,2
	25 days	4	2300±50	66,0±0,5	36,2
SC	5–7hr	6	2320±50	64,9±0,6	35,7
	25 days	5	2290±30	66,2±0,8	34,6
VC	both time periods combined	11	2315±70	66,75±0,7	34,7

FIGURE 43. CONCENTRATION OF SPINS, THE CONTENT OF SOLS AND THE COEFFICIENT OF CRYSTALLINITY OF THE SHOULDER BONE (M±m)

Group	Time period after completion of tests	Number of Animals	Number of spins, (rel. unit) (S)	Content of sols, % of weight (A)	Coefficient of crystallinity (S/A)
			Epiphysis		
F	5–7 hr	6	1590±70	59,95±0,52	26,52
	25 days	4	1560±55	61,33±0,87	25,44
SC	5–7 hr	6	1500±60	61,30±1,47	24,47
	25 days	5	1640±40	62,95±0,71	26,06
VC	Both time periods combined	16	1630±35	62,02±0,53	26,28
			Diaphysis		
F	5–7 hr	6	2720±40	65,50±0,30	41,5
	25 days	4	2670±30	65,56±0,59	40,7
SC	5–7 hr	6	2540±30	66,17±0,25	38,4
	25 days	5	2800±80	65,59±0,90	42,7
VC	Both time periods combined	16	2680±40	66,31±0,22	40,4

The absence of changes in the quantity and quality of minerals in the bone corresponds to data obtained by other researchers. In rats, a decrease in the quantity of the inorganic substance of the bone by a total of 3% was observed only after six weeks of hypokinesia (Jankowich, Lange, 1971). Man, in conditions of strict bedrest, loses about 0.5% of the total content of calcium during one month (Donaldson, et al., 1970); according to other data (Krasnykh, 1969) -- about 12% of the bone mineral after 73 days.

Apparently, the length of this experiment was inadequate in order to discover changes in the condition of the inorganic substance of bony tissue.

The method used in this work can be expediently used in the future for flight experiments of greater duration.

/146

Mineral Exchange in Calcified Tissues

A disturbance in calcium exchange in the organism, a loss in calcium salts from calcified tissues, occurs under the effect of factors of space flight (Hatter, McMillan, 1968; Biryukov, Krasnykh, 1970; Mack, 1971; Vogel, Whittle, 1974).

In ground tests with experimental hypokinesia in animals using radio isotopes, roentgenophotometric and other methods, disturbance of calcium exchange and a decrease in the degree of mineralization in the bones and teeth was discovered (Lobachik, Yegorov, 1968; Prokhonchukov et al., 1970; Volozhin et al., 1973; Pavlova et al., 1975).

The hypothesis was made on the basis of these studies that increased generation of calcium from the calcified tissues under conditions of space flight depend to a large degree on weightlessness although hypokinesia plays some part (Prokhonchukov et al., 1977). An experiment on the Kosmos-782 biosatellite facilitated solving this problem; weightlessness was the basic determining factor of this space flight.

The humerus, femur, ulnar, and radius bones, the ulna, the mandibular bone and the teeth (molars and incisors) were used as the material for the study. The content of sols, the content of phosphate, of stable calcium and the intensity of inclusion of radioactive calcium ($Ca^{45}Cl_2$) in the sols of calcified tissues were determined. The isotope was introduced four hours before death, intraperitoneally, calculating 50,000 pulses per minute for 1 g of body weight. The content of phosphate, calcium and the inclusion of Ca^{45} were studied by the method described in the preceding work (Prokhonchukov et al., 1977).

The studies made showed that the more marked changes were in the humerus and femur bones. Because of this, as an illustration (Tables 44-46) data are presented, obtained when studying only these bones inasmuch as the study of other bones gave mainly similar results.

TABLE 44. THE PERCENT OF SOL CONTENT IN THE FEMORAL AND HUMERAL BONES (M±m)

Group	Femoral Bones		Humeral Bones	
	Epiphysis	Diaphysis	Epiphysis	Diaphysis
	9-11 hr			
F	47,4±1,4*	66,8±1,2	54,8±1,9*	63,7±1,7
SC	59,4±1,2	69,8±0,6	60,4±1,4	70,7±0,8
VC	60,0±1,3	70,8±0,5	62,8±0,7	70,5±0,8
	25 days			
F	58,4±1,5	69,2±1,6	63,6±1,4	68,9±1,3
SC	64,7±1,4	71,6±0,8	63,1±2,7	70,9±1,5
VC	60,1±2,3	78,0±1,1	66,5±2,4	70,5±1,1

*Proven difference from VC

TABLE 45. CONTENT OF CALCIUM (IN % OF DRY WEIGHT) IN THE SOL OF THE FEMORAL AND HUMERAL BONES (M±m) /147

Group	Femoral Bones		Humeral Bones	
	Epiphysis	Diaphysis	Epiphysis	Diaphysis
	9-11 hr			
F	38,1±0,5	37,3±0,8	38,4±0,4	37,7±0,3
SC	38,1±0,6	36,5±0,8	38,2±0,5	37,7±0,5
VC	38,0±0,7	37,7±0,6	38,2±0,6	38,1±0,4
	25 days			
F	37,5±0,6	37,7±0,9	37,8±0,3	37,7±0,3
SC	37,9±0,4	36,9±1,0	37,6±0,3	38,2±0,5
VC	37,6±0,6	37,3±0,8	37,8±0,4	37,6±0,4

TABLE 46. INCLUSION OF Ca45 IN THE SOL OF THE FEMORAL AND HUMERAL BONES (M±m)

Group	Femoral Bones		Humeral Bones	
	Epiphysis	Diaphysis	Epiphysis	Diaphysis
		9-11 hr		
F	6055±265	2741±105	6318±228	3818±174
SC	4616±254	2922±98	5595±159	3348±306
VC	3598±159	3174±131	4802±395	3704±228
		25 days		
F	2416±90	1961±301	2865±222	1809±76
SC	3317±110	1500±57	3840±209	1999±93
VC	2705±45	1331±49	2828±165	1838±166

The coefficient of mineralization (% of sol content) of the teeth and bones immediately after completion of space flight is slightly decreased. The proven decrease in comparison with results obtained in the vivarium control was detected in the epiphises of the femoral (b 15%) and the humeral (by 8%) bones (see Table 44). In the diaphyses of the bones, the coefficient of mineralization decreased to a lesser degree. In most of the tissues studied, this index remained decreased even 25 days after completion of the experiment.

No significant changes were detected in the content of calcium in comparison with the vivarium control. However, as is apparent from the data of Table 45, in the first examination period, there is a tendency toward a certain increase in the content of calcium in the epiphyses in comparison with the diaphyses, that is, one observes a redistribution of the calcium sols in the limits of certain sections of bone which are distinguished by a different rate of renewal of mineral components. /148

The phenomenon of redistribution was clearly apparent from the data of Table 46 where the intensity of inclusion of Ca45 in the sol of the femoral and humeral bones is indicated. In the epiphysis of the bones of the flight group of animals and the synchronous control group the Ca45 was included to a much greater degree than in the diaphysis whereas in the vivarium control one did not observe this sharp difference.

In the diaphyses of the femoral and humeral bones, after flight, one observed a decrease and in the readapatation period for terrestrial conditions -- a marked increase in the inclusion of Ca45. The picture has an opposite character in epiphyseal sections of these same bones. A comparison of data

with the results of the studies of the coefficient of sol content and the content of calcium makes it possible to assume that under the effect of weightlessness disturbance in calcium exchange and also normalization occur primarily in the sections of the bone which are characterized by a more labile exchange (epiphysis).

When studying the exchange of phosphorous one does not find significant deviation from the vivarium control. Marked redistribution of the content of phosphorous in the femoral and humeral bones is noted -- a decrease in the diaphysis and an increase in the epiphysis.

An analysis of the results of the experiment showed that due to the effect of space flight, not only quantitative but also qualitative changes occur in mineral exchange and the degree of mineralization both in different sections of the calcified tissues of the skeleton and in the limits of the same bone (for example, the epiphysis and diaphysis of tubular bones). Thus, after space flight, one observes a redistribution of mineral salts in the skeleton.

For a comparison of groups in results expressed in points, non-parameter criteria were used; before the use of an evaluation with plus or minus points, they were converted into numbers expressing the relative portion of pluses. An analysis of the condition of the bone was done by projection-weight morphometry of cross sections of the center of its diaphysis. In these same sections, using an ocular micrometer, the single largest and smallest diameters of osteocytic lacunae were determined and the areas of the latter were calculated according to the product of these diameters. All of the data obtained were processed statistically, using, as a rule the Wilcoxon-Mann-Wheaton (U criteria).

In the metaphysis and ephysis of the femoral bones of rats in the flight group and in the synchronous control group, 9-11 hr after the test, atrophic changes were detected (osteoporosis) of the spongiosis (Table 53, Figure 85, a b; Figure 86, a, b). They were particularly clear in the bones of the /169
flight group of animals which retained only one-third of the spongioses of the metaphysis and approximately one-half of the spongioses of the epiphysis. Our data correlate with the results of testing femoral bones of rats of the flight group for bending (see p. 167). These bones supported a much smaller load than the control and breaks were limited once again to the area of the distal metaphysis, that is, the zone where the most acute osteoporosis was detected by a morphometric method. In rats of the synchronous control group, spongiosis of the metaphysis and epiphysis, correspondingly, amounted on the average to 2/3 and 3/4 of the spongioses of rats in the vivarium control group. After the readaptation period, statistically valid differences occurred only when comparing the flight and the vivarium control groups.

Reduction of spongioses in the metaphysis in rats of the flight group was accompanied by a considerable (approximately at 710 in comparison with the vivarium control) decrease in mass of primary spongioses in direct proximity to the cartilage of the growth plate (see Table 53, Figure 86, a, b). Inasmuch as formation of primary spongioses is one of the manifestations of growth in length of the bone, we proposed, as when studying bones in the rats of the Kosmos-605 biosatellite that during flight, retardation of growth of the long tubular bones occurs in their length. This position was supported when measuring the length of bones done 25 days postflight; the bones of the animals of the flight group proved to be somewhat, statistically significant, shorter than in the rats of the two other groups (Table 54). In the metaphyses of rats of the synchronous control group, one discovered only a comparatively small decrease in the mass of primary spongiosis close to the cartilage of the growth plate (approximately by one-sixth in comparison with the control). After the readaptation period, the difference among the three groups in the experiment in the volume of primary spongiosis was practically absent.

Changes in Bones of the Skeleton /165

It has been pointed out (Jagodovski et al., 1976), that a 22-day space flight causes the development of osteoporosis of the porous sections of long tubular bones. The data of light and electron microscopy gave use the basis for suspecting the occurrence of periosteocytic osteolysis in flight conditions. This study used preceding works for making the data more precise and in order to obtain quantitative information on the status of certain parameters of the bones in rats. This was accomplished using morphometric methods. /166

The right femoral bones of rats were studied in all three groups 9-11 hr and 25 days after completion of the experiment. The material was fixed in an 0.5% alcohol-Formalin solution of cytopyridine chloride, was decalcified in a 10% solution of Trilon B and immersed in paraffin. The sections of distal halves of the femoral bone cut along its longitudinal axis through the center of the epiphysis, metaphysis and diaphysis were studied as well as the cross sections through the center of the diaphysis. The media were colored with hematoxylin-eosin, picrofuchsin (according to van Gizon) and Alcyone blue (with precoloration of the nucleus with red carmine. In the longitudinal sections of bone, with a method of paired sequentional comparisons (Yagavskiy, 1970) the degree of osteoporosis of the spongiosis of the metaphysis, epiphysis and also the cortical plates in the zone of the metaphysis were studied. The essence of the method is alternating comparison of any one of the features or characteristics of the morphologic picture studied in each of the m polished micropreparations with analogous peculiarities of characteristics in the other m-1 preparations. Thanks to a single comparison, just two preparations presented the possibility of a precise evaluation of differences. When evaluating the preparations in one of them in which osteoporosis was much weaker, a "plus" mark was given and a drug with a much weaker osteoporosis was marked with a "minus" sign. With uniform characteristic of both preparations, the "plus" was used. The number of all the comparisons m of the preparations is equal to the number of total combinations from m elements in twos and can be expressed according to the formula

$$C_m = \frac{m!}{2!(m-2)!}, \text{ or } C_m = \frac{m(m-1)}{2}.$$

Each of the preparations, according to its sign, received m -1 points within whose limits it is theoretically possible to have any combination of pluses and minuses (a total of m combinations). The evaluation consisting of only pluses was given to a preparation on which osteoporosis was much weaker or absent and also one with all minuses -- to a preparation with maximally marked osteoporosis. The other preparations, in their evaluation, occupied intermediate positions. The method made it possible to distribute the preparations according to rank, reflecting the degree of expression of any sign of the phenomenon studied.

TABLE 53. A MORPHOMETRIC EVALUATION BY A METHOD OF PAIRED COMPARISONS OF THE DEGREE OF OSTEOPOROSIS OF THE DISTAL AND FEMORAL BONE AFTER COMPLETION OF THE TESTS

No. of rats	Spongiosis of the metaphysis as a whole			Peripheral spongiosis of the metaphysis in direct proximity to the growth plate			Spongiosis of the epiphysis			Cortical plate in the metaphysis region		
	VC	F	SC	VC	F	SC	VC	F	SC	VC	F	SC
					9-11 hr							
7	1	0,43	0,64	1	0,14	0,71	1	0,58	—	0,79	0,79	1
8	1	0,21	0,50	1	0,29	0,71	1	0,33	0,58	0,93	0,57	0,64
9	1	0,21	—	1	0,29	—	1	0,67	—	0,93	0,64	—
10	0,93	0,35	0,93	0,93	0,36	1	1	0,58	1	0,93	0,14	0,86
11	1	0 29	0,57	1	0,36	0,93	—	0,50	0,67	1	0,57	0,29
12	—	0,21	—	—	0,36	—	—	0,58	—	—	0	—
		$P_{VC}<0,005$ $P_{SC}<0,01$	$P_{VC}<0,05$		$P_{VC}<0,005$ $P_{SC}<0,01$	$P_{VC}=0,05$		$P_{VC}=0,005$ $P_{SC}>0,05$	$P_{VC}=0,5$		$P_{VC}=0,005$ $P_{SC}>0,05$	$P_{VC}>0,05$
					25 days							
19	1	0,50	0,64				1	0,86	0,86	1	0,79	1
20	0,64	0,50	0,93				0,86	0,64	0,93	1	0,57	0,21
21	0,86	0,29	0,43	No difference among the groups			0,93	0,71	0,93	1	0,79	0
22	0,79	0,86	0,93				0,86	1	0,93	0,79	0,79	0,57
23	0,93	0,57	0,43				0,93	0,29	0,71	0,79	—	0,64
		$P_{VC}<0,05$ $P_{SC}>0,05$	$P_{VC}>0,05$					$P_{VC}=0,05$ $P_{SC}>0,05$	$P_{VC}>0,05$		$P_{VC}>0,05$	

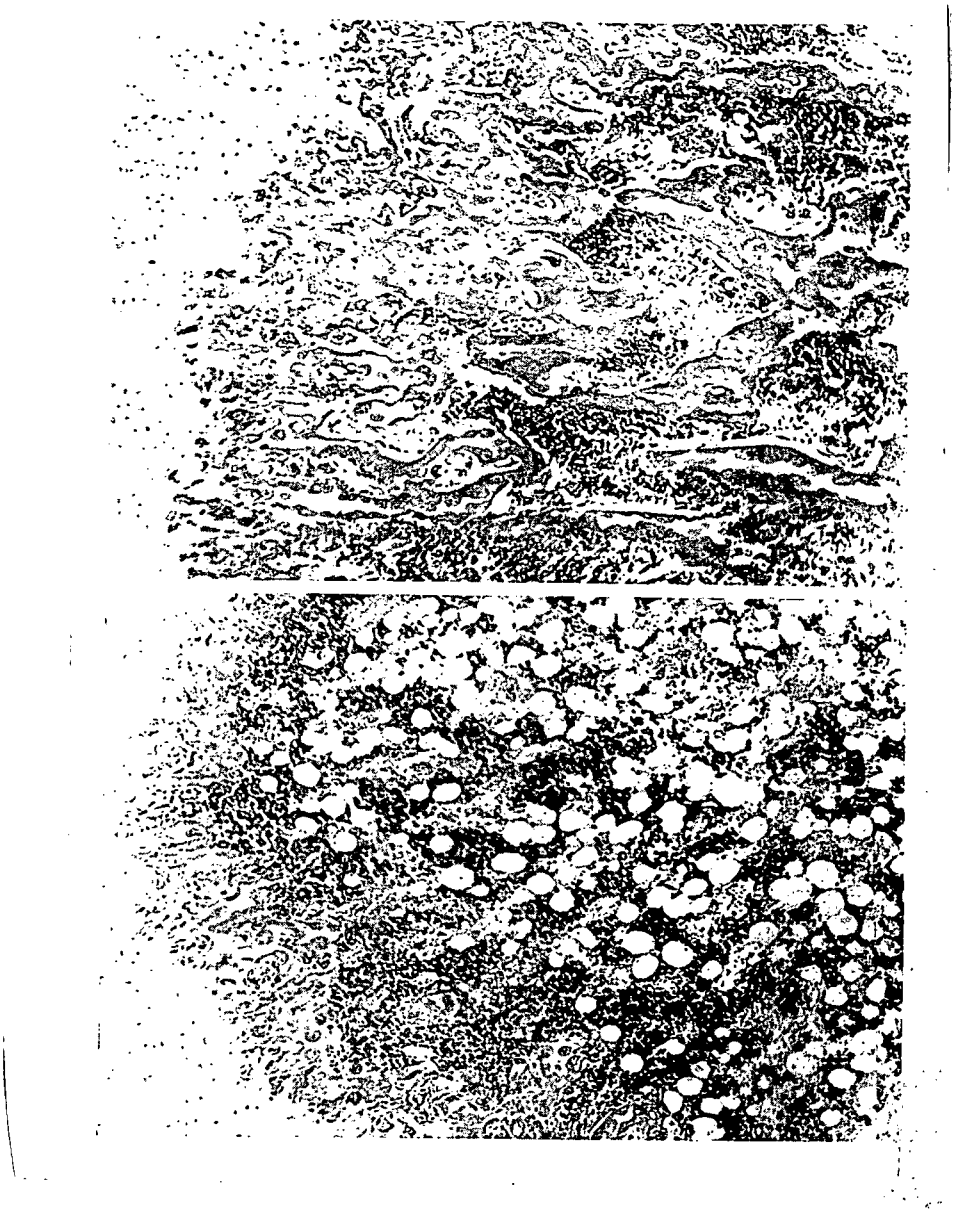


Figure 85. Distal metaphysis of the femoral bone. Coloration with picrofuchsin. Magnification 75.
a -- VC. Well developed spongiosis including peripheral; b -- 9-11 hr postflight. Sharp thinning of the spongiosis. Decrease in mass of primary spongiosis close to the cartilage of the growth plate.

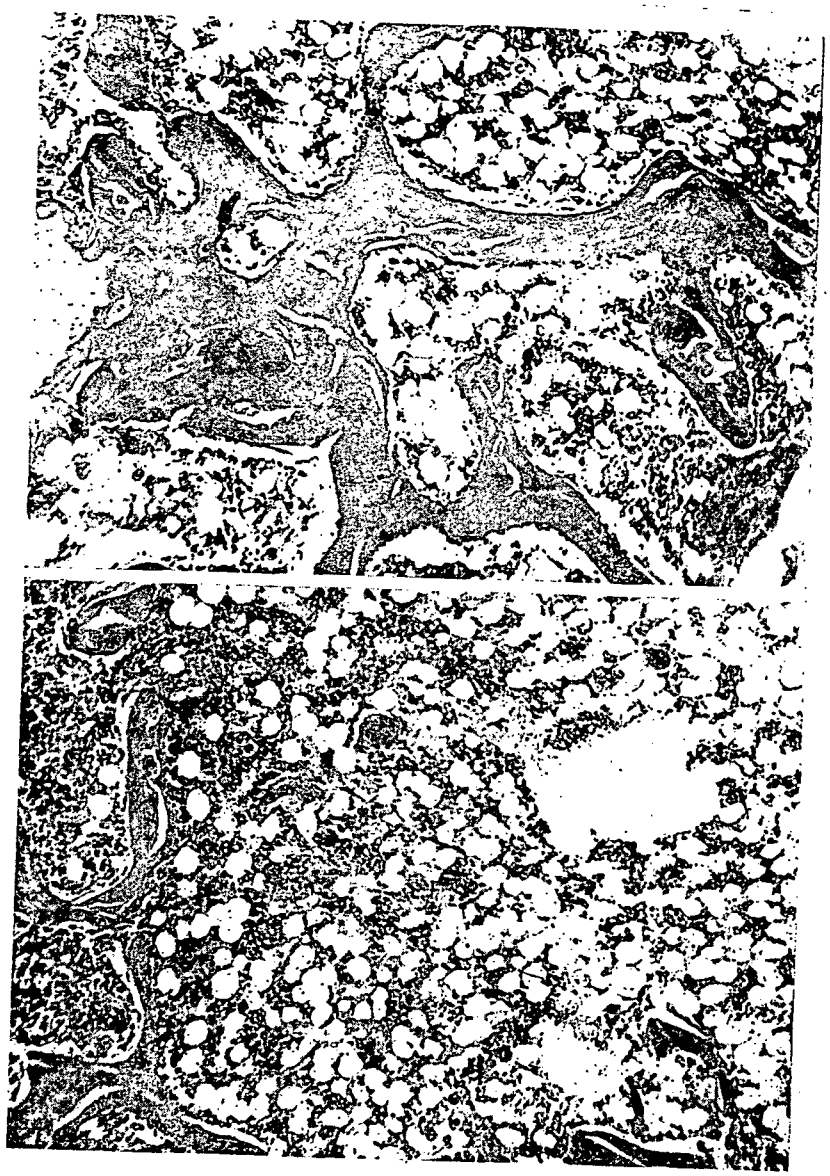


Figure 86. Distal epiphysis of the femoral bone. Coloration with picrofuschsin. Magnification 75.
a -- VC. Well developed spongiosis; b -- 9-11 hr postflight. Significant reduction in spongiosis.

TABLE 54. LENGTH OF THE FEMORAL BONES (IN mm) 25 DAYS AFTER THE EXPERIMENT

/169

No. of rats	F	SC	VC	No. of rats	F	SC	VC
19	35,9	36 3	36,3	22	35,5	36,5	36,6
20	35,3	37 0	35,5	23	35,8	34,8	37,0
21	34,5	37,5	36,7		$P_{VC}<0,05$	$P_{VC}>0,05$	
					$P_{SC}=0,05$		

Only in the rats of the flight group does one find a statistically valid osteoporosis of the cortical bone plate in the region of the metaphysis in comparison with that of the vivarium control (see Table 53). This thinning of the plates occurred due to expansion of its vascular channels and the foci of subperiosteal resorption of the bony substance (Figure 87, a, b).

There was no sign of marked osteoporosis of the cortical plate of the bone diaphysis in animals of the flight group or the synchronous control group. These facts agree with the fact that the sol content and mineral absorption of fragments of the cortical plate of femoral bones in rats from the indicated groups is practically unchanged (see p. 154). The small expansion of the vascular channels of the cortical plate of the diaphysis of the bone in its longitudinal sections was detected by us only near the metaphysis in two of the six rats in the flight group and in one in the synchronous control group.

/172

A morphometric analysis of cross sections of the center of the diaphyses of bones showed (Table 55) that according to the average data the areas of the cross section of the bone and the bone marrow canal in the synchronous and flight experperiments was somewhat smaller than in the vivarium control but these differences were not statistically significant possibly due to the small number of animals studied and the variability of individual indices. The growth of the bone in width is accompanied by new formations of bony structure on the periosteal surface of the cortical plate and resorption of them on the endosteal side. Retardation in growth must lead to a decrease in the area of the cross section of the entire bone and its bone marrow canal. Therefore, if further studies support the validity of the changes described in rats of the flight group, then one can confirm that during flight certain retardation of growth of the bones in width occurs.

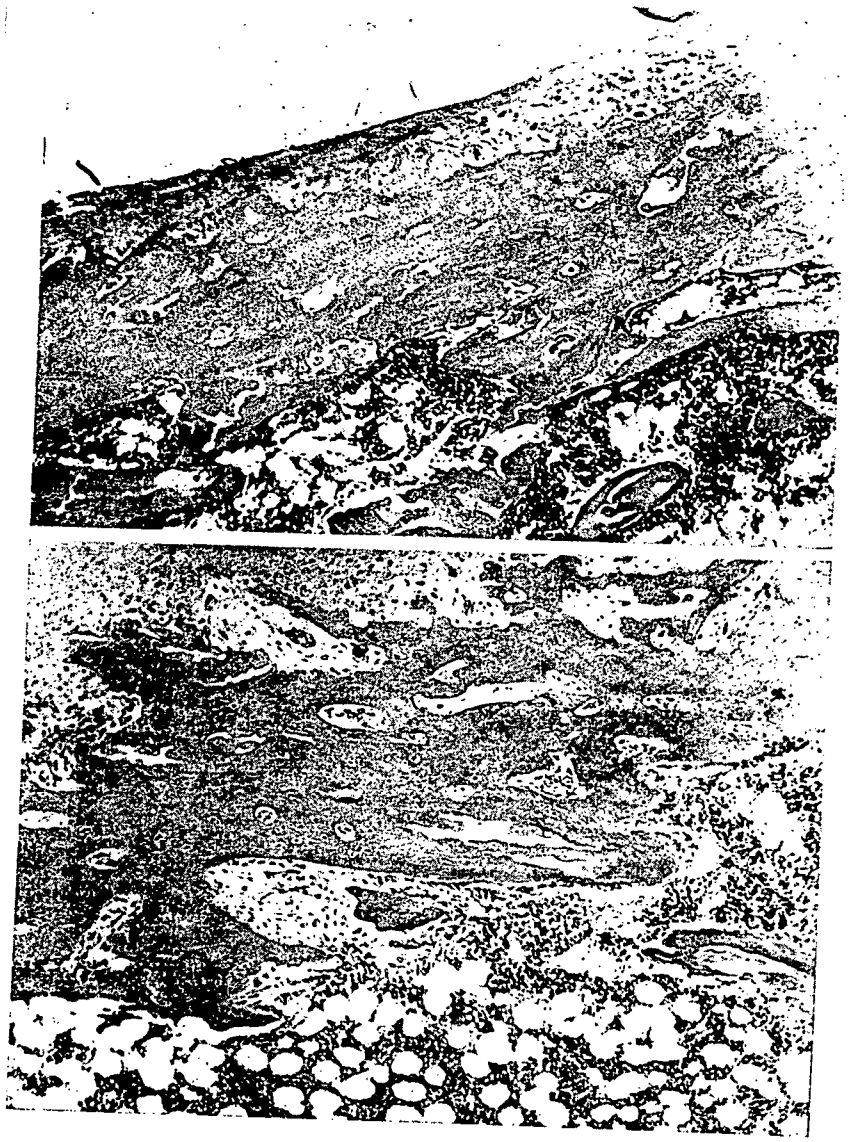


Figure 87. The cortical plate in the region of the distal metaphysis of the femoral bone. Coloration with picrofuchsin. Magnification 75.
a -- VC. Vascular channels of the plate, ordinary width; b -- 9-11 hr postflight. Marked expansion of the vascular canals of the plate.

TABEL 55. DATA OF PROJECTION-WEIGHT MORPHOMETRY (IN CONVENTIONAL UNITS) OF THE CROSS SECTIONS OF THE CENTERS OF THE DIAPHYSES OF THE FEMORAL BONES (M±m)

/172

Group	Area			Portion of the area of the cortical plate from the area of the cross section of the bone
	Cross section of the bone	Bone Marrow channel	Cortical plate	
		9-11 hr		
F	947±40	437±21	510±38	0,54±0,23
SC	881±19	433±16	448±10	0,51±0,11
VC	932±44	478±31	504±20	0,51±0,14
		25 days		
F	1050±125	508±67	542±59	0,51±0,12
SC	1089±63	509±38	579±30	0,53±0,14
VC	1070±37	515±21	555±17	0,52±0,05

Apparently, one can also assume that in rats during flight the retardation of the periosteal bone formation is relatively predominant over slowing the endosteal resorption. This hypothesis is based on the fact that in the rats in the flight group the areas of cross section of the bone and bone marrow channel, approximately, to the same degree are decreased in comparison with the vivarium control: the area of the cortical plate in rats after flight and in the control animals is practically identical; the portion of the area of the cortical plate of the area of the entire cross section of bones in rats of the flight group has a somewhat higher value than in the control. One should note that our hypotheses as to the development during flight of slowing down of periosteal bone formation is confirmed by the studies of Holton and Behling (see p. 157) which were made using declomycin.

/173

Twenty-five days after flight, all of the parameters of the cross sections of the diaphyses of the bone in animals of all three groups did not differ significantly. Measurement of the areas occupied by osteocyteal lacunae in the cross sections of the bone show that after 9-11 hr postflight, the osteocyteal lacunae are, statistically valid ($P<0.05$), broader (51.9±3.12 conventional units) than in the control rats (42.04 ±1.92 conventional units). These data attest to the development during space flight of periosteal osteolysis, that is, the opinion is supported as given earlier (Jagodovski et al., 1976).

From the data obtained in this experiment on animals of the flight group, one can extrapolate to man the results causing changes in the terminal sections of the bones studied, that is, in the metaphyses and epiphyses. These changes, including sharp thinning of the spongiosis and to a lesser degree of the cortical plate in the field of the metaphyses, are not specific for the effect of weightlessness because qualitatively unitypical phenomena were detected in rats in the synchronous control group.

A significant expression of changes in the bones of the flight group of rats, in comparison with animals in the synchronous control group gives us the basis for considering that the main factor responding to their difference is not hypokinesia but weightlessness, which deprives the bones of their support function. This confirmation corresponds to data according to which the loss of calcium in bones in the astronauts can be detected earlier than it is determined in persons who have been in hypokinesia conditions (Vogle, 1973).

It was found in the experiments that the bone, as an adequately strong system retains the rigid limitation of motor activity of animals after 100 days (Mirolyubov et al., 1975) at the same time that elimination of the support function of the extremities leads to a rapid decrease in the strength of the bones (Rybakova, 1966).

The mechanism of development of osteoperosis of the terminal sections of the bones which occurs during space flight, in our opinion, involves primarily activation of resorption of the bone structures and not retardation of their new formation. This hypothesis agrees with the facts which give evidence of the presence of a negative balance of calcium in the cosmonauts (Lutwak et al., 1969).

Besides this, one should note that in distinction from persons during flight in space, these constantly growing animals, as rats are, have a retardation of growth of the bone in length and width caused, obviously, by slowing down the enchondrial (in the metaphyses) and periosteal (in the diaphyses) bone formations.

The periosteal osteolysis which develops or increases during space flight obviously also is a response to the loss of calcium in the cosmonauts inasmuch as in the total mass of the skeleton the quantity of osteocytes in tremendous.

The factors causing the changes described above in the bones, do not involve the hyperfunctioning of parathyroid glands, because we did not find such symptoms in our experiment (see p. 81).

The changes discovered are of potential danger in the occurrence of breaks in the area of the end sections of bones, particularly acute under increased load on a skeleton immediately after landing of the cosmonauts. /174

In the 25-day period of readaptation, the main changes which developed during flight, in distinction from those observed in the synchronous control have leveled off completely and obviously they are completely reversible.

Changes in the Mechanical Properties of the Bones of the Skeleton.

During long term space flight or bedrest, in a healthy person a negative calcium balance develops (Lutwak et al., 1969; Parin et al., 1970) as a result of the occurrence of osteoporosis (Krasnykh 1974) which probably involves a functional lack of load on the skeleton. A decrease in bone mass can result in a decrease in strength of the bones. Moreover, in the experiment with limitation of dynamic or kinetic functions of the skeleton, nonuniform and sometimes contradictory results are obtained on the change of mechanical properties of the porous and compact bones. Some authors have indicated a decrease in the corresponding indices (Rybakova, 1966), others have indicated their increase (Jankovich, Lange, 1971; Mirolyubov et al., 1975), and a third group have indicated an absence of any kind of changes (Semb, 1976).

In rats who have undergone a 22.5-day flight on board the Kosmos 605 biosatellite, according to our data, the volumetric content of the mineral components in the compact bone of the diaphysis of the femur was unchanged but decreased by 8% in the porous tissue of the distal epiphysis. The development of osteoporosis in the metaphyses was discovered by a histologic method. Resistance of the tibial and femoral bones to mechanical load during bending was decreased by 20-30% while most of the breaks of the bones in the rats of the flight group occurred in the area of the metaphyses. Work of elastic deformation was changed appropriate to the index of destructive load but the value of the modulus of the elasticity remained at the control level. During compression tests a decrease in strength of the compact bones cut from the center of diaphysis in the form of columns did not occur. Consequently, the decrease in resistance of the femoral and tibial bones to the effect of force during bending apparently involves the development of osteoporosis in the metaphyses.

The purpose of our study was to further study the effect of the space flight factors on mechanical properties of bones in the skeletons of rats. After killing the rats and skeletonizing the femoral bones from them, the distal epiphysis was

separated by a blunt method and through punctures in the distal and proximal metaphyses, a neutral buffer solution was poured into the bone marrow channel. Then the femoral and intact tibial bones were placed in an 0.5% solution of neutral Formalin. A quantity of the material studed is presented in Table 56.

TABLE 56. A NUMBER OF ANIMALS FROM WHOM BONY MATERIAL WAS OBTAINED

Group	Femoral Bones: From separate distal epiphyses	Femoral Bones: Distal epiphyses	Femoral Bones: Heads	Tibial bones	Group	Femoral Bones: From separate distal epiphyses	Femoral Bones: Distal epiphyses	Femoral Bones: Heads	Tibial bones
	Background					25 days			
—	6	—	—	—					
	9-11 hr				VC	8	11	10	5
VC	7	—	1	5	F	8	12	6	5
F	6	—	3	5	SC	8	10	10	5
SC	7	—	—	5					

The femoral bones were X-rayed in the sagittal and frontal projections on Mikrat-200 film, applied in the cassette from a single layer of black light proof paper. According to the X-rays of the bones, osteometry was done at the center of their length under the MBS-2 microscope with an ocular-micrometer. The total thickness of the cortical layer on two sides was measured, the width of the diaphysis and the width of the bone marrow channel were calculated. Precision of measurement was ± 0.025 mm. Each parameter was determined three times and a mean value was taken. /175

Testing the bones for strength was done no later than five days after killing the rats on the standard M-40 machine using reverse. Error in indices of the power measuring device did not exceed 1% of the measured load. Taking into account the data of studies we made earlier of mechanical properties of the bones in rats after a 22.5-day flight, in the present experiments, the values of elastic deformation of the modulus of elasticity were not determined.

The study of strength of bones was done by testing for static bending (Figure 88,a) and for compression on the head of the femur by load using a cylindrical shaped penetrator which has a cross section area of 2 mm^2 (Figure 88,b). The rate of moving the active head of the machine in the bending tests was 35 mm/min, in the compression test -- 1 mm/min.

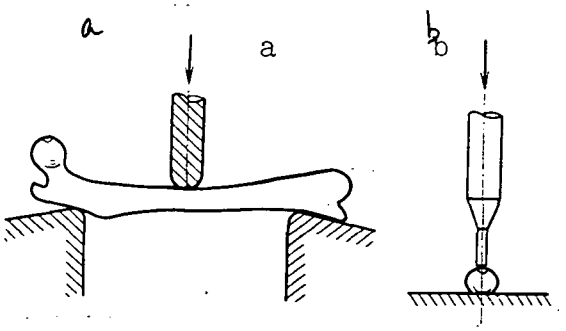


Figure 88. Testing bones for strength (diagram).
a -- for static bending;
b -- for compression

The content of the mineral component in relation to the weight of the bone (sol content) and units of its volume (mineral saturation) is determined in a dehydrated and degreased fragment of the cortical layer of the femur shaped in the form of a ring and in the distal epiphysis. The volume of the fragment was determined according to Archimedes law by weighing it in the air and in distilled water on torsion scales with a precision of +0.05 mg. A small quantity of washing substance is added to decrease the surface tension of the water. Calcination of the bone is carried out in a muffle furnace at a temperature of 700° per 7 hr. The sols are weighed and the index of sol content and mineral saturation is calculated.

Changes in the anteroposterior and lateral dimensions of the femoral bone in the rats of different test groups was approximately the same. Changes in the lateral dimensions are presented in Table 57.

Nine-eleven hours postflight, the thickness of the cortical layer was the same as during the background examination of the control rats at an increase by 10%. Inasmuch as the /176
width of the bone marrow channel in rats of the flight group did not differ from that of the rats in the vivarium control group, the relative thinning of the cortical layer is probably due to retardation of apositional growth of the bone, that is, to slowing down of bone formation.

The thickness of the cortical layer of bones in the synchronous control group of rats was close to that of the animals in the vivarium control group. A decrease in the width of the diaphysis and the bone marrow channel is due, obviously to significant individual variations in the group (σ = 0.278).

TABLE 57. CROSS-SECTIONAL DIMENSIONS OF THE FEMORAL BONES IN A MEDIAL-LATERAL DIRECTION.

Group	Thickness of the cortical layer		Width of the diaphysis		Width of the bone marrow channel	
	M±m	% of VC	M±m	% of VC	M±m	% of VC
			Background			
—	1,01±0,015	—	3,63±0,0065	—	2,62±0,063	—
			9-11 hr after the test			
VC	1,11±0,024	—	3,66±0,057	—	2,54±0,050	—
F	0,99±0,007*	89,1	3,55±0,055	97,2	2,56±0,061	100,7
SC	1,09±0,017	97,8	3,55±0,051	96,9	2,40±0,105	94,5
			25 days after the test			
VC	1,21±0,019	—	3,91±0,067	—	2,63±0,057	—
F	1,16±0,011	96 5	3,62±0,078*	92,8	2,42±0,065*	92,0
SC	1,19±0,013	98,5	3,81±0,061	97,6	2,62±0,067	99,6

*Proven difference for VC

Twenty-five days postflight, the thickness of the cortical layer was increased but did not reach the control level. Judging from the width of the diaphysis and the bone marrow channel, thickening of the cortical layer occurred both in the periosteum and in the bone marrow cavity. In the rats on the Kosmos-605 biosatellite, after restoration of all the parameters studied, no differences were noted from the control indices. The causes for this development in the dynamics of recovery of the bone in the postflight period are unclear. The dimensions of the bone in the rats of the synchronous control group 25 days after completion of the experiment did not differ from the parameters of the vivarium control. /177

In spite of thinning of the cortical layer, the content of the mineral components in the compact bone tissue of the diaphysis in the flight group rats was unchanged (Table 58). This fact confirms the hypothesis as to thinning of the compact substance not due to increase in processes of resorption but due to the slowing of the processes of bone formation. The fact that the index of sol content is identical in the test group and in the vivarium control group is evidence of retention of the degree of mineralization of the organic matrix and the value of mineral saturation -- the absence of significant changes in dimensions of the cavities of the vessel channels of the cortical plate in relation to the total mass of the bony substance.

TABLE 58. THE SOL CONTENT AND MINERAL SATURATION OF THE FEMORAL BONE (M±m)

Group	Cortical layer of the diaphysis		Distal epiphysis	Group	Cortical layer of the diaphysis		Distal epiphysis
	Sol content, %	Mineral saturation, g/cm^2	Mineral saturation, g/cm^2		Sol content, %	Mineral saturation, g/cm^2	Mineral saturation, g/cm^2
	Background				25 days		
—	0,639±0,004	1,218±0,014	—				
	9-11 hr						
VC	0,656±0,001	1,288±0,007	—	VC	0,660±0,004	1,322±0,012	0,505±0,011
F	0,646±0,009	1,310±0,013	—	F	0,655±0,006	1,310±0,025	0,462±0,000
SC	0,656±0,006	1,317±0,017	—	SC	0,667±0,005	1,341±0,030	0,494±0,009

The content of the mineral component in the cortical layer was unchanged in rats of the synchronous control group.

The content of the mineral component in the distal epiphysis in the flight group rats in the first time period was studied due to the absence of appropriate material. Twenty-five days postflight, the mineral saturation of the distal epiphyses remained at 8.5% less in comparison with the vivarium control (see Table 58). These data agree with the results obtained in the test on the Kosmos-609 biosatellite and indicate the development of osteoporosis in sections of the bone which show porous tissue.

During the bending tests, the femoral and tibial bones of rats in the flight group withstood a load of approximately 30% less than these bones in the control animals (Table 59). Fracture of the bones in the rats occurred in the area of the distal (femoral bone) and the proximal (tibial bone) of the metaphyses. A decrease in maximum strength of the head of the femoral bones which have, like the metaphyses, porous structure was close to the degree of change in the long bones.

In the rats of the synchronous control group no characteristic significant decrease in the resistance of the femoral and tibial bones to the effect of mechanical load was noted. /178

TABLE 59. MECHANICAL PROPERTIES OF BONES

Group	Fracture load, kgf				Maximum strength of head of femoral bone	
	For the femoral bone		For the tibial bone			
	$M \pm m$	% of VC	$M \pm m$	% of VC	$M \pm m$	% of VC
			Background			
—	6,12±0,25	—	—	—	1,28±0,11	—
			0-11 hr			
VC	7,00±0,38	—	7,32±0,38	—	3,10±	—
F	5,09±0,48*	72,7	5,07±0,26*	69,5	1,89±0,15	61,0
SC	7,64±0,73	109,1	6,60±0,36	90,2	—	—
			25 days			
VC	12,60±0,39	—	10,66±0,49	—	4,29±0,30	—
F	10,10±0,51*	80,2	7,48±0,69*	70,2	3,38±0,32	78,8
SC	11,06±0,45*	87,9	11,39±0,94	106,6	3,92±0,24	91,5

*Proven difference from VC

The strength characteristics of the bones in the rats of the flight group after 25 days postflight remained 20-30% lower than the index of the vivarium control. In the rats of the synchronous control group at this same time one noted a certain decrease in the indices of strength of the femoral bone and its head. These data require additional experimental verification inasmuch as the strength characteristics of the bones in animals of the synchronous control group in the experiment on the Kosmos-605 biosatellite somewhat exceed the indices of the control.

The results of the studies made make it possible to conclude that due to factors of space flight lasting 19-22 days, the rats, in bones carrying the weight load developed osteoporosis which primarily affected those with porous substances involving probably the high metabolic activity. Osteoporosis in the cortical layer of the long bones develops with a certain delay. Osteoporosis of a relatively low degree causes a significant decrease in strength characteristics of the bone. The postflight period lasting 25 days is inadequate for eliminating characteristics of osteoporosis and restoration of bone strength. This is the basis for considering that mainly development of osteoporosis and the decrease in strength of the bones due to factors of space flight is due not so much to limitation of muscular activity as to the absence of a weight load on the skeleton.

Conclusion /179

The quantity and quality of inorganic substance in the bones carrying the weight load in terrestrial conditions (humeral bone) and not supporting this load (bones of the cranial crown) do not undergo significant changes. One notes only redistribution of mineral sols between different sections within the limits of a single bone and also among different parts of the skeleton.

A clearer deviation was found when studying processes of growth and restructuring of bones. The growth of bones both in length and width was slowed down in the animals of the flight group whereas resorption of bony tissue which accompanies restructuring of bone occurred more intensely than in the control animals.

The main factor causing this disturbance is weightlessness.

Osteoporosis developed as a result of increased resorption and retardation of the new formation of bone, although it was insignificant; it caused a significant decrease in the strength of the femoral bone. Twenty-five days proved to be inadequate for full recovery of the changes described.

The Hemopoietic Function in Weightlessness.

Part of the program on the Kosmos series of biosatellites is clarifying the genesis of the hematologic changes which are continuously noted in the crews of spacecraft , beginning with the Gemini-5. The first results which give evidence of a proven decrease (approximately by 10%) of the mass of erythrocytes was explained basically as an oxygen effect. Subsequently, on the basis of tests with hypokinesia (Shvets, Portugalov, 1976) the concept arose that a decrease in the mass of erythrocytes can be due to other mechanisms: shortening of the length of the life cycle of the erythrocytes, depression of bone marrow hemopoiesis (Johnson et al., 1975; and others). At this time, facts have been accumulated which indicate that the leading mechanism in the change in the erythrocyte mass in weightlessness conditions is the drop in bone marrow erythropoiesis. The results of histologic analysis of the bone marrow of the sternum and ribs of Karmanchikov mice exposed on the Apollo-17 spacecraft (Ellis, et al., 1975), the femoral bones and spleen of rats in the Kosmos-605 biosatellite (Durnova et al., 1977; Portugalov, Savina et al., 1976) apply to this. Ellis connects the decrease in erythropoiesis to the effect of hyperoxia which occurs on spacecraft of the Apollo type. An outstanding characteristic of the Kosmos type biosatellite (except for the Kosmos-605 on which temporary unplanned hyperoxia occurred) (Il'in et al., 1976) is the normalized oxygen medium which makes it possible to evaluate the effect of "pure" weightlessness on bone marrow hemogenesis.

In this section data will be presented of the study of bone marrow in the femoral bone. The material was fixed in Bouin's fluid, decalcified in a 5-7% solution of nitric acid and immersed in paraffin. The sections, 5-6 μm thick, were colored with hematoxylin-eosin. The state of erythropoiesis was evaluated on histologic sections in ten fields of vision (ob. 90, oc. 7, 2.5) with a total number of cells $2 \cdot 10^3$. In certain tenths of the field of vision, 100 megakaryocytes were counted; the quantity of these which were pathologically changed were determined.

A decrease in erythropoiesis was observed in the rats 5-11 hr after landing of the biosatellite. The erythroid growth in the bone marrow was found to be small numerically and with rarely encountered groups of cells. The quantity of morphologically identified erythroid elements on the sections was 15±3% of the total cells containing nuclei with 26-32±2% in the control. In the postflight period, erythropoiesis was established and at 25 days, the quantity of erythroid elements was close to the control level (23±2%). In rats of the synchronous control group, at both observation periods,

no changes were established in erythropoiesis in comparison with rats of the vivarium control group. Thus a decrease in bone marrow erythropoiesis in the flight group rats is reversible. /181

Two types of megakaryocytes were encountered in the bone marrow of the flight group rats. The first group are a normal type of megakaryocytes with rich, structured nuclei and granulated cytoplasm; the second group are megakaryocytes with dense nuclei and sharp eosinophilic structureless cytoplasm. The quantity of changed megakaryocytes, 5-11 hr after landing, increased in the rats to 27±3% of the total number of these cells whereas in the animals of the synchronous control group their number did not exceed 6±2%; in the rats of the vivarium control it was 2±1%. Twenty-five days after completion of flight, the quantity of changed megakaryocytes did not exceed the level of the vivarium control. These observations attest to the fact that destructive changes in megakaryocytes in the flight group are completely reversible. Similar data were obtained when studying the population of megakaryocytes in rats with hypokinesia. The latter excludes the connection of degenerative changes in the megakaryocytes and the effect of weightlessness. The mechanism of this phenomenon is still unknown.

Although temporary hyperoxia occurred on the Kosmos-605 which was absent on the Kosmos-782 biosatellite, in both experiments one observed in the bone marrow uniform changes in erythropoiesis. Like the American scientists (Berry, 1974; Ellis et al., 1975), we considered the effect on erythropoiesis of the gas medium in the spacecraft as important. The reasons given at first for the change in mass of erythrocytes in astronauts on the Gemini and Apollo satellites was the hemolytic effect of O_2, which was later confirmed by data obtained when studying the erythrocyte mass in ten members of the crew of the Skylab orbital station. The oxygen effect was not confirmed in studies of the metabolism of erythrocytes. No symptoms of overoxidation of the lipids were observed in the membranes of the erythrocytes (Mengel, 1974). The hypothesis that a decrease in mass of erythrocytes involves the lack of effectiveness of erythropoiesis or decrease in the lifetime of the erythrocytes, also was not confirmed inasmuch as changes were not found in kinetics of inclusion of Fe59 and C14-glycine and the half-removal of Cr51 (Johnson, 1974). The change in content of similar shapes of erythrocytes (transformation of discocyte to echinocyte) apparent during flight on the Skylab orbital station also cannot be considered the basis for explaining the decrease in erythrocyte mass (Kimzey et al., 1974).

Thus a decrease in erythrocyte mass which is continuously observed both in space flight and in conditions of hypokinesia

indicates the presence of a mechanism common to these two effects which does not involve absorption of oxygen from the atmosphere. The most probably cause of decrease in the mass of erythrocytes is a decrease in the hemopoietic function of the bone marrow. The latter presupposes the existence of a mechanism with an inverse connection between the number of erythrocytes in the blood and activity of the bone marrow. The presence of this connection is well known in practical medicine (Uzhanskiy, 1968). In experiments with bloodletting or with experimental polycythemia, one observes respectively either activation or suppression of bone marrow erythropoiesis. Then, in the population of erythrocytes, primary changes occur /182
mediated thru the erythropoietic system according to the canonical system: erythrocytes -- potential of O_2 in the tissues (kidneys) -- erythropoietin -- bone marrow (erythropoietin-sensitive cell) -- erythrocytes. According to this same system, probably, regulation of the hemopoiesis occurs with increased or decreased muscle work. A decrease in muscle load which occurs in weightlessness can be the decisive condition for decreasing erythrocyte mass. This is supported in tests with hypokinesia. Moreover, in accordance with the effect of the inverse connection of the mechanism, the decrease in mass of erythrocytes must be accompanied by activation of the hemopoietic function of the bone marrow which compensates for the lack of erythrocytes. Obviously with a deficit of muscle load, the mechanism which provides oxygen to the tissue with a given mass of erythrocytes is involved due to an increase in the O_2 potential in the tissues. Thus, a relative hyperoxia is created in the tissues which results in blocking synthesis of the erythropoietin or its components. Then, the value of the initial mass of erythrocytes loses its importance and the erythron transfers to a lower economic level of functioning adequate for providing O_2 to the tissues. From the position of a mechanism of inverse connection, the latter must be accompanied by a decrease in bone marrow erythropoiesis which is established for conditions of weightlessness and during hypokinesia.

At the present time, we cannot propose however that the data indicate a correlation between the change in mass of erythrocytes and activity of the bone marrow in the same biological object. Thus, information on change of the erythrocyte mass was obtained in humans but the data on decrease in activity of the bone marrow is for animals. To a known degree this makes it difficult to evaluate the facts obtained. Moreover, the question is still not solved as to whether erythropoietins are a constant regulator of erythropoiesis or are a stimulating agent used by the organism in critical situations.

The mechanism described above makes it possible to explain the decrease in mass of erythrocytes in weightlessness and during hypokinesia. It also gives us a basis for assuming

that in the erythrocyte populations morphological shifts do not occur which could explain the decrease in mass of erythrocytes. The decrease in bone marrow hemopoiesis which we have established in weightlessness, leading obviously to a decrease in erythrocyte mass forces the researchers to pay attention to the need for studying bone marrow, primarily proliferation and differentiation of hemopoietic trunk cells. The latter are very sensitive to the effect of extreme factors and their proliferation and differentiation is controlled by a series of systems in the organism. One of these systems is the immunity T-system. The role of the T-lymphocytes in regulating erythropoiesis is well documented and shown experimentally (Goodman, Shinpock, 1972; Svets, 1976). Thus, the bone marrow erythropoiesis can depend on the condition of T-system immunity (T-dependent erythropoiesis). Inasmuch as the population of T-lymphocytes changes more sharply under stress, this must affect the erythropoietic function of the bone marrow. This effect is observed in experiments when injecting animals with hydrocortisone and during hypokinesia (Portugalov, Shvets, 1976). The connection between the trunk cells and T-lymphocytes during stress is the redistribution of T-lymphocytes in the organism and, in particular, their migration to the bone marrow; as a result of this, the center of immunopoiesis shifts (Cohen, 1972; Moorhead, Cleman, 1972; Fanci, 1975). An evaluation of the T-system of immunity was made in astronauts from Apollo-7 to Apollo-13 spacecraft and the astronauts from the Skylab space station using PGA. No changes were noted here in the blastotransformation reaction, in synthesis of ribonucleic acid and DNA by the lymphocytes of the peripheral blood (Fischer et al., 1972; Ritzman, Levin, 1973). However, one should keep in mind that the test with the PGA does not give as precise an understanding of the potentials for realization of immunologic properties as the entire population of T-lymphocytes or their separate subpopulations.

/183

There is a great deal of data giving evidence of the fact that stress always accompanies space flight (Leach, Rambaut, 1974; and others). This does not exclude the fact that bone marrow erythropoeisis in space flight can affect stress reactions during which migration of the T-lymphocytes to the bone marrow occurs. But with interaction of T-lymphocytes and trunk cells, acceleration of the erythropoietic properties of the latter occurs whereas in weightlessness suppression of bone marrow erythropoiesis develops. Consequently, changes in homopoiesis in rats in the first two days after completion of 19.5-22.5-day flights, obviously, is caused not by stress reaction but is due to other mechanisms, primarily hypotrophy of the support muscle apparatus. Thus, it is found that the state of erythropoiesis, especially during long term space flights, will be determined more rapidly by the volume of functions fulfilled by the support motor apparatus than by the effect of stress factors in space flight. Because of this,

the complex of prophylactic and therapeutic measures recommended for cosmonauts must be directed at compensating for the hematologic shifts and not toward eliminating inadequacies in functioning of the support-muscle apparatus.

Morphologic Study of Lymphoid Organs. /186

It was pointed out earlier that a 22.5-day space flight leads to involution of the lymphoid organs in rats as a result of stress developing which is caused by the extreme factors of space flight (Durnova et al., 1977). Due to the fact that the rats were not killed until late (second day) in this experiment it is not possible to establish whether or not stress occurred at the moment of landing of the biosatellite or at which stages of space flight preceding the landing. The changes in lymphoid organs as a result of acute stress caused by landing of the biosatellite can disappear after two days. Due to this, the purpose of our study was to investigate the lymphoid organs of rats killed a few hours after landing, at a time period when the phenomenon of acute stress can still be detected.

The spleen, thymus and inguinal lymphatic nodes were used as the research material in 12 rats of the flight group, 12 rats of the synchronous control and 11 rats in the vivarium control group. The samples of organs were taken for study 9- /187
11 hr and 25 days after completion of the experiment.

The spleen, thymus and lymph nodes were weighed, fixed in Karnui liquid and immersed in paraffin. The sections of the lymphoid organs were colored with hematoxylin-eosin, methyl green-pyronine and according to the Perls method. For cytologic study, smears were prepared from the spleen cells which were colored according to the Pappenheim method.

One sees from the data presented in Table 61 that after 9-11 hr postlanding, one observes a decrease both in the absolute and in the relative (calculating per body weight) weight of the spleen and thymus in the rats and a fairly marked tendency toward a decrease in weight of the inguinal lymph nodes. After 25 days had passed, the weight of the spleen and lymph nodes was normalized whereas the weight of the thymus still remained decreased. The weight of the lymphoid organs in rats of the synchronous control group 9-11 hr and 25 days after completion of the test did not differ from the corresponding data of the vivarium control.

Histologic study of the spleen showed that 9-11 hr after landing, hypoplasia of the white and red pulp was detected in the rats. The dimensions of the lymphoid follicles and their

TABLE 61. ABSOLUTE (I) AND RELATIVE (II) WEIGHT (IN mg) OF THE LYMPHOID ORGANS

Group	Spleen		Thymus		Lymph Nodes	
	I	II	I	II	I	II
			9-11 hr			
F	351±9**	1,37±0,03	309±9**	1,20±0,04	31±3	0,12±0,01
SC	450±13*	1,78±0,15	391±26	1,43±0,10	38±4	0,13±0,01
VC	510±38	1,90±0,17	362±17	1,34±0,06	32±7	0,16±0,02
			25 days			
F	509±15**	1,59±0,05	339±14*	1,06±0,05*	42±3	0,13±0,01***
SC	609±26	1,59±0,06	389±25	1,14±0,07	48±5	0,16±0,01
VC	595±19	1,75±0,06	419±17	1,23±0,04	49±6	0,14±0,02

* Proven difference from VC
** Proven difference from VC and SC
*** Proven difference from SC

light centers were decreased and the parafollicular zone was contracted. In the red pulp of the spleen, there were no foci of erythroid hemorrhaging and the quantity of lymphocytes was decreased. Moreover, the red pulp was diffusely infiltrated with neutrophils. The content of hemoxyderin and also the number of plasmocytic elements in the red pulp of the spleen in flight group rats did not differ from the normal.

The data of cytologic analysis of the spleen correlated well with the results of a histologic study and showed that 9-11 hr after completion of flight one observes a proven decrease in the percentage content of lymphocytes in the rats (vivarium control group -- 90.0±1.8; flight group -- 84.0±1.9) and a proven increase in the percentage content of segment-nucleus neutrophils (vivarium control group -- 7.5±1.7; flight group -- 13.9±1.4). /188

Microscopic study of the thymus showed that 9-11 hr after landing, in the rats the cortical substance of the gland is found to have a massive breakdown of lymphocytes and an accumulation of nucleus detritis (Figure 89, a,b); a typical picture of the thymus with distribution on the cortical and brain substance was retained here.

In the lymph nodes 9-11 hr after landing one noted thinning of the cortical substance, a decrease in the dimensions of lymphoid follicles, combining of the pericortical zone of the lymphocytes, a decrease in the number and dimensions of light centers of the follicles and also a decrease in the number of plasmocytes in the spinal cords. In three of the six rats examined, decomposed lymphocytes were detected in the

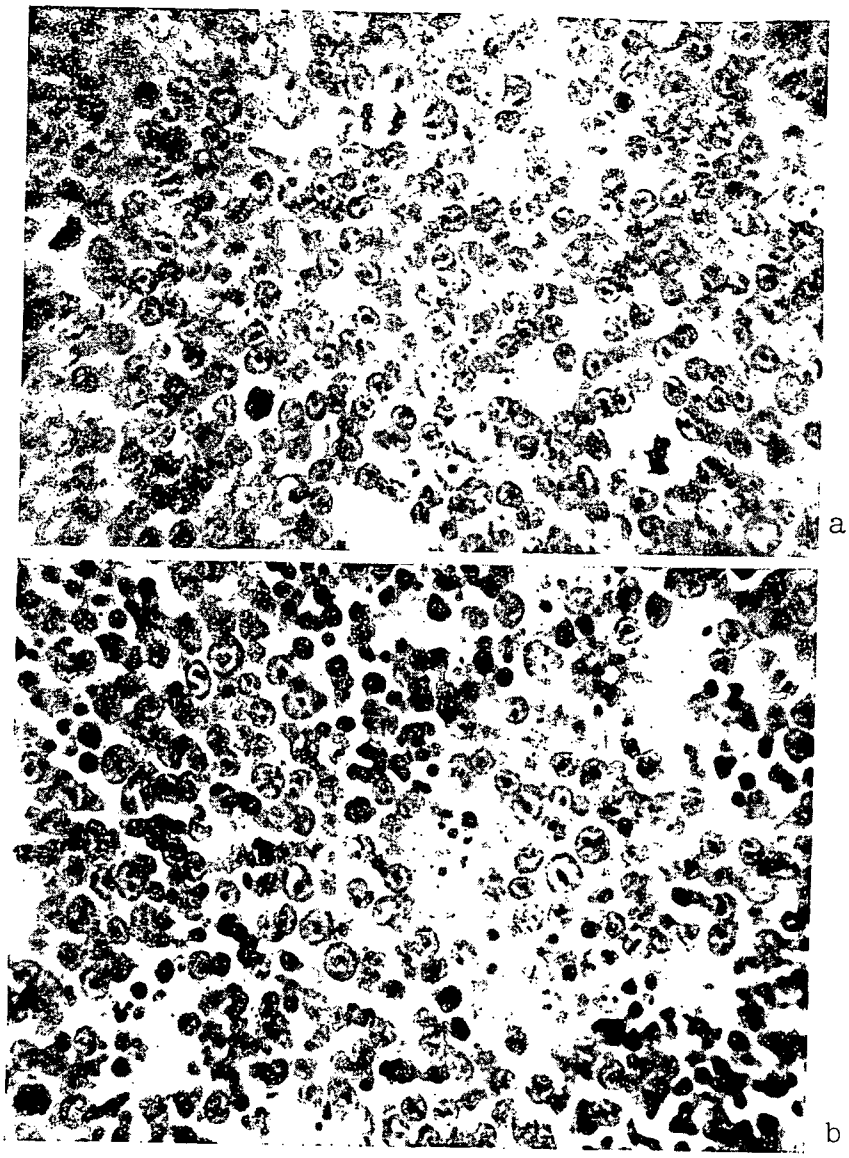


Figure 89. Thymus. Coloration with hematoxylin-eosin. Magnification 240.
a -- VC. In the cortical substance among the lymphocytes separate dividing cells are visible; b -- 9-11 hr postflight. One observes massive decomposition of lymphocytes and an accumulation of cellular detritis in the cortical substance.

thickness of the cortical substance of the lymph nodes. /188

Twenty-five days later, the structure of the lymphoid organs in the flight group rats as a whole was normalized and the intensity of erythroid blood formation in the spleen even exceeded that in the intact rats.

In distinction from the animals of the flight group, in rats in the synchronous control group examined at the same time, the histologic picture of the lymphoid organs differed very little from normal and only in the thymus in two rats killed 9-11 hr after completion of the experiment did one encounter some nuclear detritus; in the lymph nodes, the cortical zone was somewhat contracted and the dimensions of the lymphoid follicles were decreased. Twenty-five days later, the picture of the lymphoid organs in rats in the synchronous control group did not differ from that of the vivarium control group.

An analysis of the data presented gives evidence that a 19.5-day space flight results in involution of the lymphoid organs in rats; the weight of the spleen and thymus decreases to a greater degree than the weight of the inguinal lymph nodes. Hyperplasia of the spleen is due to a decrease in the quantity of lymphocytes and erythrocytes elements in it and a decrease in weight of the lymph nodes and thymus, a decrease in the number of lymphocytes. Involution of the lymphoid organs has a transitional character and after 25 days a partial or complete regeneration of weight, structure and cellular composition of the lymphoid organs occurs. The changes detected in the lymphoid organs of the flight group rats have been divided into two categories according to its characteristics. Decomposition of lymphocytes in the thymus and the lymph nodes and neutrophil infiltration of the spleen occurs in the first group. In the second group, atrophy of the lymphoid follicles and their light centers, a decrease in the cortical substance of the thymus and lymph nodes occur. The changes occurring in the first category are morphologic evidence of acute stress and usually develop during several hours after the effect of the factor causing the stress (Selye, 1936; Daughterty, White, 1944; Baker et al., 1951; La Pushin, Harven, 1971). In this experiment, the source of stress apparently can be considered as a complex of extreme effects accompanying landing of the biosatellite and possibly, transition from weightlessness to Earth's gravitation. In distinction from changes in the first category, hypoplasia of lymphoid tissue basically causing a decrease in weight of the lymphoid organs probably has a later origin and is evidence of the effect of certain stress factors in the earlier stages of space flight.

Conclusion /192

A study of the state of the erythron, a vulnerable link of hemopoiesis in weightlessness conditions, showed an increase in spontaneous hemolysis and also a decrease in the erythroblastic growth of the bone marrow. Unfortunately, due to the corresponding conditions of the experiment, an increase in hemolysis possibly was established only in relation to erythrocytes which reached their mature growth during flight.

The mechanism of the damaging effect of weightlessness on the state of the erythron remains unclear. It is possible that a decrease in the level of erythropoieses is not a direct result of the effect of weightlessness but is due to a sharp decrease in load on the muscle system and a decrease involving the requirement of the organism for oxygen.

A study of the thymus, lymph nodes and spleen showed a marked stress effect. Here, besides symptoms of acute stress (decomposition of lymphocytes, neutrophil infiltration of the spleen) phenomena are observed (atrophy of the lymph follicles, contraction of the cortical substance of the thymus and lymph nodes) which are evidence of long term stress effect which can be ascribed to weightlessness.

Immunologic Reactivity of the State of Autoflora /193

Reactivity of B-Lymphocytes of the Spleen in Relation to the Polyclonic Mitogen. /196

B-lymphocytes, or lymphocytes of bone marrow origin, are cells capable of identifying antigens using specific immunoglobulin receptors on their surface and synthesizing humoral antibodies of different classes. In the composition of the population of B-lymphocytes, there are also cells without /197 immunoglobulin receptors on the surface (null-cells), K-cells and predecessors of the B-lymphocytes.

In distinction from the T-lymphocytes, whose reactivity during the space flight was frequently the object of analysis (Fuks, Konstantinova, 1973), the capacity of the B-cells for activation under the effect of specific antigens and nonspecific polyvalent mitogens has not been studied. Meanwhile, the reactivity of the B-cells is an integral quantitative factor characterizing both the state of metabolism of separate B-lymphocytes and the reactivity of the set of B-lymphocyte clones.

In recent years it was pointed out that lipopolysaccharide (LPS) E. coli is the polyvalent mitogen for B-lymphocytes (Gary

et al., 1972; Anderson et al., 1973; Parker, Metcalf, 1974; and others). This mitogen was used in our work.

The equipment of the experiment was used ahead of time on spleen cells from intact white Wistar rats.

When setting up the basic experiment, four groups of animals were studied. The first three groups comprised rats who had been on flight in the Kosmos-782 biosatellite and rats of the synchronous and vivarium control groups.

The flight group rats were killed a few hours after landing; the spleens removed were transported to the laboratory in a culture medium of RPMI-1640 at 0°. The time from killing the animals to beginning the cultivation of isolated cells was 36 hours. Due to this, the rat spleens in the synchronous and vivarium control groups, after the animals were killed, also were left in a culture medium at 4° for 36 hours. The fourth group comprised rats contained in the vivarium but killed immediately before preparing the culture (an additional control group). A total of spleens from 22 rats were studied for reactivity of lymphocytes.

The suspensions of spleen cells were prepared in an RPMI-1640 medium containing glutamine, antibiotics and also a 10% fetal bull serum. The cellular suspension containing $1 \cdot 10^6$ cells per 1 mℓ of culture medium was placed in penicillin flasks. The spleen cells of each rat were cultivated without mitogen (two parallel samples) and with the addition of mitogen -- lipopolysaccharide E. Coli in a concentration of 50 μg/mℓ (four parallel samples). The mitogen was prepared in the following way. LPS from the Difco firm was dissolved in a balanced Henks saline solution. Then the LPS solution was dialyzed against a phosphate buffer (pH 7.1; 0.1 M) cold for 24 hours. The solutions were sterilized through a bacteria filter with a millipore membrane (0.45 μm), after which the solution was diluted to the necessary concentration of the culture medium.

To judge the reactivity of the lymphocytes, that is, their capability for blastotransformation under the effect of a mitogen, the N^3-thymidine label was used. Forty-four hours after setting up the culture, N^3-thymidine was added to each flask in a quantity of 2 μOUF/mℓ (specific activity 5OUF/mℓ). Four hours after labeling, the cells were precipitated cold for five minutes (100 rpm) and twice the isotope was washed out with a cold Henks solution. Then, in the cellular precipitate, 1 mℓ of cold phosphate buffer was added, it was mixed and 1 mℓ of 10% cold trichloroacetic acid was added. The precipitate was transferred to milipore filters and two parts of cold 5% trichloroacetic acid and two parts of cold 80-degree ethyl alcohol were added. The filters were dried and radioactivity was measured on a tricarb counter (Packard firm, /198

model 3320) in a scintillating fluid containing 2, 5-diphenyloxazole (5%) and 1, 4-bis-2(5-phenyloxazole)-benzene (0.01%). After preparing this suspension and also after completing the tests, an evaluation was made of the viability of the lymphocytes by a method supravital coloration with eosin.

Statistical processing of the data was done according to the St'yudent method. Table 64 shows data which characterize the reactivity of B-lymphocytes of the spleen in cultures made 36 hours after killing of the rats in the three basic groups and immediately after killing rats in the additional control group.

TABLE 64. RADIOACTIVITY OF THE CULTURE OF B-LYMPHOCYTES OF THE SPLEEN (PULSE/MIN FOR 10^6 CELLS) (M±m).

Number of rats	Without mitogen ($M_1 \pm m_1$)	With mitogen ($M_2 \pm m_2$)	Number of rats	Without mitogen ($M_1 \pm m_1$)	With mitogen ($M_2 \pm m_2$)
	Flight			Vivarium Control	
1	27,6±0,35	84,3±6,40	13	18,5±2,50	48,8±7,28
2	40,0±5,50	67,5±3,50	14	12,5±1,50	40,5±5,80
3	59,5±9,50	111,0±28,70	15	20,0±1,00	41,0±5,10
4	48,4±3,60	83,3±16,80	16	17,0±2,00	75,0±23,0
5	54,6±0,00	74,7±1,43	17	19,5±3,50	37,0±4,50
6	40,6±5,10	75,8±16,20	18	16,0±1,00	34,0±2,58
			19	13,5±0,50	47,0±7,78
$M \pm m$	38,3±5,50	82,80±6,18			
P_{VC}	<0,05	<0,01	$M \pm m$	16,7±1,01	46,2±1,60
	Synchronous Control			Additional Support	
7	16,4±0,35	68,8±12,54			
8	47,5±9,50	88,0±11,50	20	36,0±2,37	381,0±12,70
9	18,0±5,00	49,5±8,50	21	34,0±3,70	697,0±24,36
10	51,8±1,15	69,6±7,90	22	31,5±1,59	284,0±10,20
11	36,0±0,00	64,0±2,60			
12	45,0±0,00	59,0±8,60	$M \pm m$	34,0±1,40	454,0±124,20
$M \pm m$	35.8±6,10	66,5±7.05			
P_{VC}	<0,05	<0,05	P_{VC}	<0,05	<0,05

The average values obtained in the flight group did not differ from the data of the synchronous control group; here, there were no significant differences either in the culture with mitogen or in the culture without mitogen. Moreover, reactivity of the each of the groups indicated showed a proven increase in the level of radioactivity in the vivarium control group.

Radioactivity of B-lymphocytes from the spleens not subjected to preservation and transport (the additional control /199

group) was several times higher than in the two first groups and exceeded by almost a magnitude the level of radioactivity of the vivarium control group.

When determining the viability of cells before beginning cultivation in the suspensions from the spleens of the animals of the three basic groups, a 20-30% live cell count was determined in which the cellular suspensions of the rats in the additional group was 97-98%. A comparison of data obtained in the different groups indicates that DNA synthesis in B-cells of animals in the additional control group was activated by LPS E. coli by a magnitude or more. In the first three groups, synthesis of DNA was activated by LPS by less than three times. Thus, long term transportation of spleens from rats who have returned from flight and also the holding time for keeping the culture in the two other groups sharply decreased the reactivity of B-lymphocytes in relation to LPS E. coli. A decrease in reactivity of cells in relation to LPS correlated with a drop in their viability established according to a test with eosin. It is obvious that a drop in viability of the cells during the postflight transportation considerably complicates an evaluation of the effect of factors of space flight on the reactivity of B-lymphocytes.

A comparison of the first three groups of the animals, one to the other, makes it possible to propose that in the flight group and synchronous control group on Earth, an increase in reactivity of B-cells occurs in relation to LPS. However, apparently, this is due to their background (without LPS) activation because the cells of the animals in the flight group and the synchronous control group not stimulated by LPS included N^3-thymidine more intensely than the cells from animals in the vivarium control group.

One can propose that the level of content in rats in space flight and in the synchronous experiment on Earth shows a stimulating effect on a background synthesis of DNA in the spleen lymphocytes and as a result of this, has an effect on reactivity of lymphocytes in relation to LPS E. coli.

In parallel experiments (see page 182) on the same animals an increase was established in the flight group in the reactivity of B-cells of the spleens of rats in relation to a specific antigen and a polyvalent mitogen. These changes did not exist in the synchronous control. It is possible that in the flight experiment and in the synchronous control, we are talking about nonspecific stress reaction and activation of the B-cells could be a secondary result of activation of the T-cells. It is necessary to analyze the existence and scale of the phenomenon detected. However, such studies require considerable shortening of the time between killing the animals and making the culture.

Microbiological Investigation

At the present time, a fair amount of material has been accumulated which is evidence of changes in the composition of autoflora of the organism both in conditions of man in a hermetically sealed chamber (Shilov et al., 1972), and among cosmonauts after completion of flight (Zaloguyev et al., 1970; Taylor, 1974; and others). In this experiment, the microflora of the mouth and intestine and also the bactericidal properties of the skin were studied in 36 rats from all three groups. The microflora of the oral cavity were studied according to a method by N. N. Klemparska and G. A. Shal'nova (1966). When studying fecal microflora quantitatively, the total number of anaerobic bacteria, bacterioids, the total quanity of anaerobic bacteria, lactobacilli, intestinal bacteria, streptococci, staphylococci, spore bearing anaerobic bacteria, yeasts, and protei were all taken into consideration. A method of applying hourglasses with a 4 -- 6-hour culture of Serratia marcescens (Liz'ko et al., 1975) was used for determining the total quantity of anaerobic microorganisms, bacterioids and lactobacilli for creating anaerobiosis. The bactericidal properties of the skin were studied by a method of imprints. The sample imprints were taken from the skin of the tail, the percentage content of colonies who had died 6 and 12 minutes after landing on the skin in a culture of intestinal bacteria was determined. When studying microflora of the oral cavity, on the second postflight day, 4 out of the 12 animals in the flight group and the synchronous control group showed disbacteriosis. In the vivarium control group of rats, in this season of the year, disbacteriosis of the mucous membranes of the mouth were observed 2.5 times less frequently than in animals of the flight group. On the 22nd day after completion of the experiment, the indices of the animals of different groups were close to each other due to increased frequency of disbacteriosis in rats of the flight group and in the synchronous control group. /200

The bactericidal properties of the skin in rats of the flight group did not differ significantly from that of the animals in both control groups during both study periods.

Thus it is apparent from the data presented in Table 65, that the flight rats showed a decrease in total quantity of aerobic microbes, intestinal bacilli, streptococci and staphylococci. Similar changes were observed in animals of the synchronous control group in whom, moreover, there was a marked decrease in the number of protei and lactobacilli. Thus, a simplification of the composition of fecal microflora took place both in the animals of the flight group and in the rats of the synchronous control group. Similar deviations from normal in intestinal cenosis was noted by both Soviet and foreign scientists under ground isolation conditions (Luckey, 1968; Shilov et al., 1972) and during actual space flights (Thomas et al., 1968; Taylor, 1971). /201

TABLE 65. THE COMPOSITION OF FECAL MICROFLORA (NUMBER OF MICROBES PER 1 g OF FECES EXPRESSED IN LOGARITHMIC NUMBERS) /200

Microorganisms	VC	F	SC
Total quantity of anaerobes	9,6±0,1	9,5±0,1	9,4±0,3
Bacterioids	9,2±0,4	9,3±0,1	9,1±0,4
Total quantity of aerobes	9,1±0,9	7,0±0,7*	6,8±0,8*
Lactobacilli	8,0±0,7	8,2±0,4	6,8±0,9*
Intenstinal bacteria	7,8±0,4	6,9±0,8*	6,7±0,8*
Streptococci	8,4±0,6	6,5±0,3*	6,7±0,6*
Enterococci	6,9±0,4	6,9±0 1	6,1±0,6
Staphylococci	6,2±0,4	4,2±1,1*	4,6±1,2*
Protei	5,8±0,6	4,6±1,3	4,0±1,3*
Spore bearing anaerobic bacilli	Not detected		
Yeasts			

*Proven difference from VC

Apparently, a stay of the rats in weightlessness was not reflected in the condition of the autoflora of the organism. /201

Conclusion

Immunologic studies indicate an increase in reactivity of thymus lymphocytes (T-lymphocytes) in relation to the specific and also polyclonic mitogen. This result which is apparent only in the animals exposed on the biosatellite must be proven in subsequent experiments and cannot be used as the basis for concluding that there is an increase in the cellular immunity in conditions of weightlessness.

A study of lymphocytes of bone marrow origin (B-lymphocytes) also showed increase in their reactivity in relation to the polyclonic mitogen; this was observed both in animals of the flight group and in animals of the synchronous control group. It is possible that this reflects the nonspecific stress reaction having a secondary character in depending on primary activation of the T-lymphocytes.

Microbiologic study showed only changes in the state of the fecal microflora, a decrease in the total quantity of aerobic microbes and their separate types. This simplification of the composition of microflora took place in animals in the synchronous control group.

The Digestive and Resorptive Function of the Gastrointestinal Tract

A study of the enzyme spectrum of the gastrointestinal tract in members of the Soyuz series of spacecraft (Smirnov et al., 1970; Smirnov, Ugolev, Golland, Murashko, 1976) demonstrated the relationship of the depth of the change and duration of the regeneration period to the length of the flight. Changes in generating digestive enzymes were unitypical when modeling weightlessness in ground experiments but have a less marked character in this case (Smirnov, Ugolev, Golland, Goncharova et al., 1976).

The processes of synthesis of digestive enzymes were judged according to their activation which was determined in the homogenates of organs of the gastrointestinal tract. Translocation of the enzymes from the cells to the surface of the intestine (to the microhairs of enterocytes) was studied *in vitro* according to their activity on the mucous membrane surface. Below the indices used in this work and bibliographical sources from which the appropriate methods were taken all are listed.

Index	Source
Activity of pepsin	Korot'ko, 1965
Activity of trypsin and trypsinogen	Erlanger et al., 1961, modification by V. A. Shaternikov (1966)
Activation of proelastase	Geokas, 1967
Activation of pancreatic amylase	Smith, Roe, 1949
Activity of pancreatic lipase	Shaternikov, Sovchuk, 1966
Lipolytic activity of the mucous membranes of the small intestine, activity of invertase and glycyl-l-leucindipeptidase	Ugolev, Iezuitova, 1969
Absorption of glucose	Wilson, Wiseman, 1953

A comparison of the flight group with the vivarium control indicated that differences were observed only in the first few hours postflight whereas on the 26th day the majority of the

indices had leveled off. Table 66 shows data obtained in the first observation period. Changes in the generation of diges-

TABLE 66. ACTIVITY OF DIGESTIVE ENZYMES 9-11 HR AFTER COMPLETION OF THE EXPERIMENTS (M±m) /203

Enzyme	n	F	n	VC	n	SC
			Stomach			
Pepsin, mg%	6	2,18±0,60	5	0,94±0,26	6	2,10±0,72
			Pancreatic gland			
Amylase, units	8	82200±13270	9	263400±16210	8	39000±59870
Tripsin,	10	484,7±93,7	11	215,9±42,4	7	126,1±26,1
Tripsinogen,	6	1027,1±32,8	5	179,6±39,5	11	525,5±74,1
Proelastase, unit	4	74,4±0,8	11	30,4±8,0	4	38,4±12,0
Lipase,	11	26400±2596	11	3700±470	12	8900±1281
			Mucous membrane of the thin intestine proximal section			
Homogenate						
Lipase, μg/min·cm²	—	—	7	0,020±0,097	6	1,54±0,09
Invertase, mg%	6	102,5±8,0	2	37,25±1,35	3	80,6±10,3
Glycyl-l-leucindi-peptidase, mg%	6	113,6±7,7	2	40,0	3	60,8±3,31
Surface						
Invertase, mg%	6	165,5±11,5	2	105,7±5,7	3	163,3±27,3
Glycyl-l-leucindi-peptidase, mg%	6	138,0±11,7	2	58,2±2,2	3	105,3±5,8
			Distal section			
Homogenate						
Lipase, μg/min·cm²	—	—	7	1,42±0,07	6	1,92±0,13
Invertase, mg%	6	60,58±17,18	2	25,7±11,4	3	56,0±9,9
Glycyl-l-leucindi-peptidase, mg%	—	—	2	40,0±0	3	64,5±1,5
Surface						
Invertase, mg%	6	122,5±13,3	2	49,3±10,7	3	71,30±6,96
Glycyl-l-leucindi-peptidase,	—	—	2	50,0±2,0	3	88,6±1,3

tive enzymes were reflected in the activation of all the proteolytic enzyme systems of the pancreatic tract which can involve the predominance of catabolic reactions in the organism after flight. The results of morphologic study of the stomach agree with this (see p. 195) which indicate the presence after flight of a hypersecretory syndrome and also our studies when limiting the motor activity of men and animals which established an increase in oxygen and pepsin generation (Smirnov, Ugolev, Golland, Goncharova et al., 1976). /202 /203

No significant changes in synthesis of the intestinal carbohydrase-invertase (activity in the homogenate) were detected after flight but the activity of this enzyme on the surface of the mucous membrane was somewhat increased. This

corresponds to a sharp suppression of α-amylase, hydrolyzed 1, 4-glucocide bonds apparent after flight; this, in turn, obviously caused a compensatory increase in the generation of intestinal carbohydrase to provide hydrolysis of disaccharides.

A sharp increase in the activity of pancreatic lipase, specific for esters of glycerine and higher fatty acids are evidence of an increase in the processes of lipogenesis in the organism. Actually, in this experiment, a fatty infiltration of a number of organisms was established as well as an increase in the content of higher fatty acids in the blood plasma (see pp. 195 , 199). /204

A decrease in resorption of glucose in the small intestine involves changes of both the hydrolytic and the transport processes. We observed similar changes in the cosmonauts after long term flights (Smirnov, Ugolev, Golland, Murashko, 1976). The changes in the animals were more marked which apparently should be explained by the effect of more significant hypodynamics.

It is important to note the particularly small expression of changes in secretory functions after the synchronous ground experiment in comparison with the postflight results. This indicates the important role of weightlessness in the development of changes in the digestive system after space flight.

Fairly rapid (by the 26th day) normalization of most of the indices studied attests to the fact that the changes which occur have a functional character. The digestive system is not a limiting link in the effect of space flight factors on the organism, the changes of hypolytic and resorption processes noted are secondary and mainly involve changes in the nerve and muscle system.

Structure and Function of Parietal Cells of the Mucous Membrane of the Stomach.

It is well known that the exceptionally energy consuming process of secretion of hydrochloric acid as opposed to the tremendous concentrated gradient occurs with a close structural-functional interaction of the mitochondria and certain enzyme systems of the secretory membranes and oxyntic cells (Sachs et al., 1972; Pokrovskiy et al., 1975; and others). Here the basic role of the mitochondria leads to energy supply of secretion and the enzyme systems are responsible for translocation of hydrogen ions from the cells to the lumen of the stomach.

Consequently, a study of activity of enzyme links in the breathing chain of the mitochondria and adenosine triphosphatase activity in the secretory mucous membranes of the

stomach make it possible to judge the activity of the secretory process under different influences.

An electron microscope study was made of the stomachs of 44 rats from all three experimental groups. Pieces of the fundus section of the stomach were fixed in a 40% solution of paraformaldehyde on Henks buffer, postfixed in a 1% solution of osmium tetroxide and after degreasing in acetone the resulting concentration was put into a mixture of epoxide resins (Epon-Araldit). The sections prepared on the IKB ultramicrotome were contrasted with uranyl-acetate and lead citrate and studied under a JEM-7A/120 electron microscope. The electrographs were subjected to morphometric analysis according to a method described by A. S. Yagubov and V. A. Kats (1974) which we modified (Morozov, 1976) for studying the parietal cells. /205
Morphometry was conducted with a magnification of $4 \cdot 10^4$ times using test grids with a 10 mm step.

Homogenates were also prepared from the mucous membrane of the stomach in which the following were measured by spectrophotometric methods (Pokrovskiy, 1969): the activity of succinate dehydrogenase (SDH) and cytochromoxidase (CO), and the activity of NADN-nitrochrome c-reductase (NADN-CcR) by the Hogeboom and Schneider method (1950), activity of Na^+, K^+-adenosine triphosphatase and HCO_3^-, Mg^{2+}-adenosine triphosphatase (Emmelot et al., 1964) and also the protein content was determined (Lowry et al., 1951).

Under electron microscope study of the material, no noticeable differences were detected in the ultrastructure of the parietal cells between rats in the vivarium and synchronous control groups or between the animals in the flight group and the synchronous control group on the 26th day of the readaptation period. Differences were found only when comparing materials of the flight group and the vivarium control group (Figure 92). The lining cells of the mucous membrane of the stomach of rats exposed on the biosatellite had mitochondrias with a denser matrix and more frequent cristae. The tubule vessicles in animals of the flight group occupied a large area of the cytoplasm. The intracellular secretory canals had a much broader lumen and more developed elongated microvilli.

The results of morphometric analysis are shown in Table 67. The greatest deviations were found in the animals who had been in space flight. With an absence of noticeable swelling, the mitochondria significantly (by 16.4%) increased the area /207
occupied by the cells which is obviously due to an increase in their number. The area of the surface of the cristae of the mitochondria increased even more significantly (by 47.5%) which is due not only to the increase in number and area of the mitochondria but to a large degree to the increase in density of packing the cristae in each mitochondria. The difference in morphometric indices among the zones of the glands

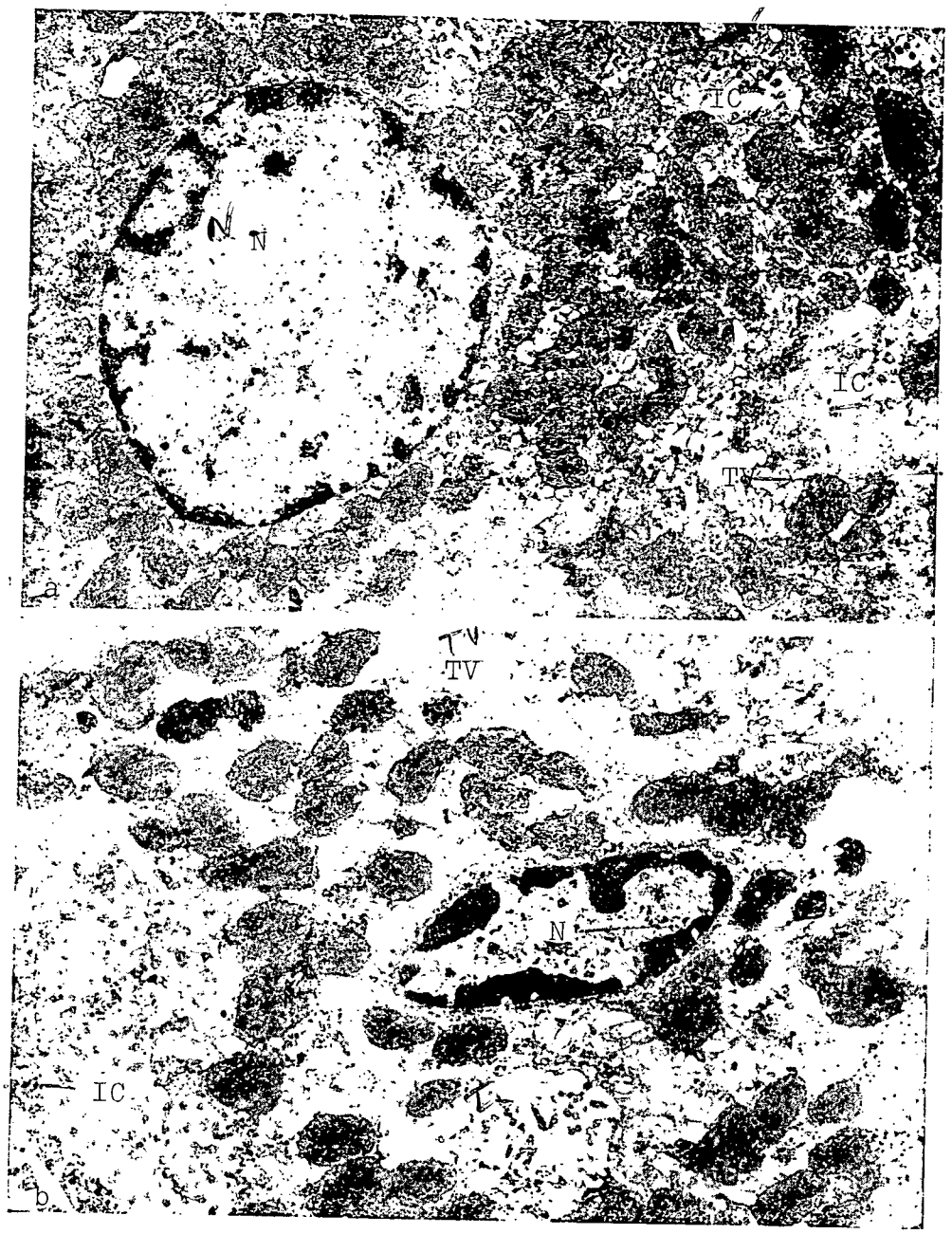


Figure 92. Parietal cells from the central section of the fundus of the pancreatic gland. Electronogram. Magnification 10,000. a -- Vivarium Control; b -- flight group; N -- nucleus; M -- mitochondria; TV -- tubule vessicles; IC -- intracellular canals.

TABLE 67. THE RESULTS OF MORPHOMETRIC ANALYSIS OF THE DATA OF AN ELECTRON MICROSCOPE STUDY OF THE PARIETAL CELLS OF THE MUCOUS MEMBRANES OF THE STOMACH (M±m)

/205

Group	Position of the cells in the gland	Area of the mitochondria %	Area of surface of the crista of the mitochondria, conv.unit	Coefficient of fragmentation of the crista of the mitochondria	Index of swelling of the mitochondria
		9-11 hr			
VC	Top	30,85±0,31	2,482±0,097	0,634±0,047	0,912±0,049
	Center	39,44±0,27	3,365±0,063	0,657±0,033	0,989±0,061
	Bottom	28,06±0,32	1,627±0,005	1,135±0,046	0,986±0,053
SC	Top	37,05±0,41	2,749±0,040	0,786±0,053	0,955±0,047
	Center	41,93±0,32	3,224±0,027	0,721±0,042	1,051±0,06
	Bottom	28,6±0,38	2,156±0,024	0,777±0,041	1,015±0,054
F	Top	39,66±0,31	3,898±0,056	0,667±0,040	0,948±0,049
	Center	39,57±0,27	3,887±0,061	0,636±0,027	0,996±0,041
	Bottom	35,23±0,37	3,236±0,047	0,637±0,024	0,848±0,039
		25 days			
F	Top	32,73±0,29	3,021±0,033	0,751±0,057	0,937±0,051
	Center	39,81±0,31	3,783±0,050	0,793±0,047	0,980±0,054
	Bottom	29,37±0,29	2,740±0,070	0,937±0,043	0,981±0,047

/207

which are distinguished by secretory activity leveled off thanks to this activation of secretion. Twenty-five days postflight, all of the morphometric indices are normal but the area of the surface of the cristae remains increased by 28%.

The data of parallel biochemical studies in homogenates of these sections of the mucous membrane of the stomach are presented in Table 68. In the group of rats who have undergone space flight, the activity of succinate dehydrogenase, cytochromoxidase, NADN-cytochrome c-reductase and HCO_3-, Mn^{2+}-adenosenetriphosphatase significantly (by 34-57%) increased in comparison with the ground control. The activity of Na^+-, K^+-adenosenetriphosphatase decreased in the flight group by 62% in comparison with the vivarium control. Twenty-five days after landing, the activity of all HCO_3-, Mg^{2+}-adenosenetriphosphatase remained increased whereas in the synchronous control group it did not differ in this time period from the level of the same index in rats of the vivarium control group. A tendency toward a decrease was observed in the

TABLE 68. ACTIVITY OF ENZYMES OF THE MUCOUS MEMBRANE OF THE STOMACH OF RATS AFTER COMPLETION OF THE TEST.

Group	Activity of enzymes, μm per 1g of protein for 1 min.				
	SO	SDH	NADN-CcR	HCO_3^-, Mg^{2+}-adenosine triphosphatase	Na^+, K^+-adenosine triphosphatase
			9-11 hr		
VC	67,4±2,3	8,55±0,95	295±21,2	2,35	1,7
SC	60.8±7,7	10,4±5,2	287±57	2,07	1,9
F	90,2±1,7*	12,8±0,95*	462±24,5*	3,2	0,59
			25 days		
VC	62,7±4,9*	22,3±0,95	285±21,5	3 1	—
SC	57,9±3,1*	12,3±2,6*	225±10,8*	3,36	—
F	68,4±3,4*	18,5±1,0*	191±9,2*	4,6	—

*Proven difference from VC

activity of NADN-cytochrome c-reductase in the second observation period.

On the basis of data on the increase of activity of respiratory enzymes localized in the secretory membranes, one can conclude that there is activation of the secretion of hydrochloric acid due to the effect of space flight. The results of electron microscope research which show an increase in the area of the internal membranes of the mitochondria and secretory membranes attest to this. At the same time, the Na^+, K^+-adenosenetriphosphatase activity was considerably decreased. This enzyme is localized in the endoplasmatic reticulum of cells and, apparently, does not have a relationship to transport of hydrogen or chlorine ions. /208

Twenty-five days after the animals stay on Earth, the activity of succinate dehydrogenase and HCO_3^-, Mg^{2+}-adenosine triphosphatase remained increased which is evidence of relative stability of the change.

According to the data of parallel morphometric and biochemical analysis, one can make a hypothesis as to the mechanism of increase of respiratory activity of the mitochondria. It occurs apparently due to an increase in the surface of the internal membranes and not due to an increase in density of packing the respiratory chains of the mitochondria.

Thus, rats staying in space flight results in a significant increase in the area of the internal membranes in the mitochondria in conjunction with sharp stimulation of activity of the enzymes of the mitochondria and the secretory membranes of the oxygen forming cells of the mucous membrane of the stomach; this attests to an increase in the level of secretion of hydrochloric acid.

Morphologic Changes in the Stomach and Liver of Rats

The study of the state of the stomach and liver in long term space flight is important not only for understanding the effect of different factors of flight on the organism but also for subsequent processing of effective regimes for nutrition of cosmonauts.

Data exist which indicate a decrease after flight in the content of mucopolysaccharides in the epithelia and mucocytes of the stomach, and also a certain increase in the quantity of lipids in the hepatocytes (Portugalov, Savina, et al., 1976). Similar changes which occur due to space flight are described in a number of references (Petrukhin, 1962; Yuganov et al., 1974; and others).

The task of this research was to study the structure and histophysiology of the liver and mucous membranes of the stomach. Rats from all three experimental groups were used. The material was fixed in a 10% Formalin buffered according to Lilli, in paraformaldehyde with postfixing in osmium tetroxide. Fragments of the stomach and liver were frozen in liquid nitrogen. They were immersed in paraffin-celloidin and Epon-812. Sections of the nonfixed material frozen in liquid nitrogen were prepared in a cryostat, ultrathin and semithin sections -- on the IKV-111 ultratome. Hematoxylin-eosin, the ShIK-reaction, Alcyone blue, toluidine blue, fatty red O, Sudan black B were all used for coloration according to the problem of the research. The activity of succinate dehydrogenase, NAD-diaphorase of acid and alkali phosphatase were discovered. Rats injected with N^3-thymidine immediately after the flight (11 hours before killing) were used for the autoradiographic study of DNA synthesis. A count of the number of labeled nuclei was done on sections coated with deparaffinized emulsion P exposed for 24 days at 4° and colored with hematoxylin-eosin.

No ulcers or erosions were detected in the stomach. In the flight group rats, a certain flattening of the surface epithelium was detected and also a significant decrease in content of neutral mucopolysaccharides in the mucous cells

of the neck of the fundus glands (Figure 93,a). In one of the rats of the flight group, in the upper third of the fundal gland, a crista was found lining the flattened prismatic epithelium (Figure 93,b). In rats in the flight group and /209

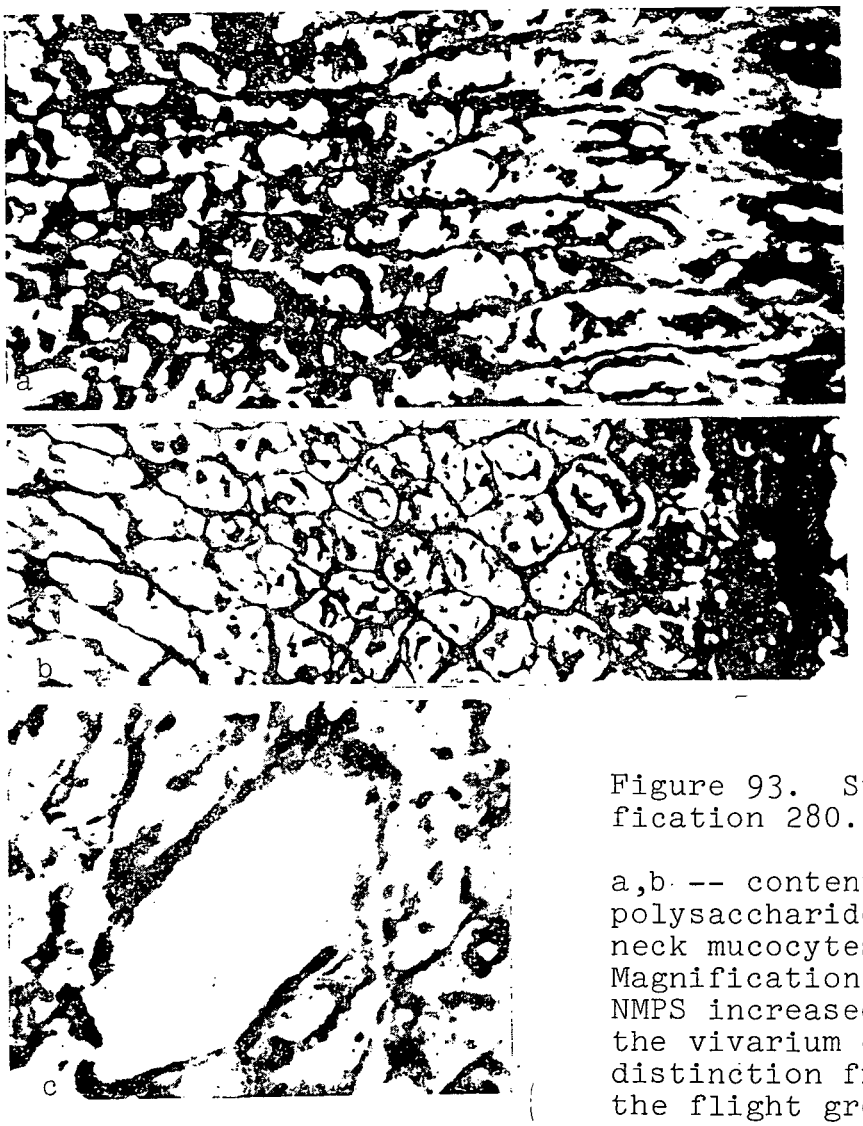


Figure 93. Stomach. Magnification 280.

a,b -- content of nuetral polysaccharides (NMPS) in the neck mucocytes, ShIK-reaction. Magnification 200. Content of NMPS increased in animals of the vivarium control (a) in distinction from animals of the flight group (b) in whom much of the mucoid is visible on the surface of the mucous membranes; c -- retention crista in the fundus section. Coloration with hematoxylin-eosin.

the synchronous control group, the parietal cells located in the region of the glandular neck had vacuolized cytoplasm and pale chromatonuclei. The activity of succinate dehydrogenase and NAD-diaphorase in the parietal cells shows a slight decrease in the flight and synchronous experiments in comparison with the vivarium control.

During histoautoradiographic study, a decrease was detected in the rate of DNA synthesis in the synchronous control group (label index 1.6±0.5) and in the flight group (label index 2.1±0.4) in comparison with the vivarium control group (label index 5.7±2.3). /210

All of the changes described relate to a picture of stress reaction and are similar to changes which occur under the effect of various stress agents and also when injecting glucocorticoid hormones (Lambert, Martin, 1969; Lev et al., 1970; and others).

A decrease in the content of mucopolysaccharides in the mucocytes of the fundus section is due not to a breakdown in synthesis but to increased excretion of mucus which indicates its accumulation in the lumen of the fossa and on the surface of the stomach.

One can assume that the crista found in one rat is retained and retention of secretion occurs as a result.

Similar, although less marked, changes in animals in the synchronous control group are evidence that the stress reaction is caused not only by the factors of space flight but also by isolation, a decrease in motor activity and unusual feeding conditions. The suppression of DNA synthesis is a clearly expressed stress reaction. This effect is well known due to different stress agents and during the injection of glucocorticoids (Lahtiharjue et al., 1964; and others).

There are no differences in the intensity of DNA synthesis between the animals of the flight group and the synchronous control group; this is evidence of the absence of the effect factors which are specific for space flight on this index. No changes were found in the mucous membrane of the pyloric section. Twenty-six days postflight and after the synchronous test, the picture of distribution of mucopolysaccharides did not differ from that in the vivarium control. These data attest to the reversibility of changes in synthesis in secretion of neutral mucopolysaccharides.

Many small and large lipid drops located basically centrolabilly were detected in the liver of rats in the flight group (Figure 94,a). There were fewer close to the portal tracts (Figure 94,b). They were encountered in a large quantity in the fatty cristae (Figure 94,c).

During electron microscope study, large lipid inclusions were found in the sinusoids (Figure 94,d); lipids filled significant sections of the cytoplasm of the hepatocytes (Figure 94,d). The organellae of the hepatocytes have the usual

form and glycogen is retained. Approximately the same picture was observed in the synchronous experiment.

/211

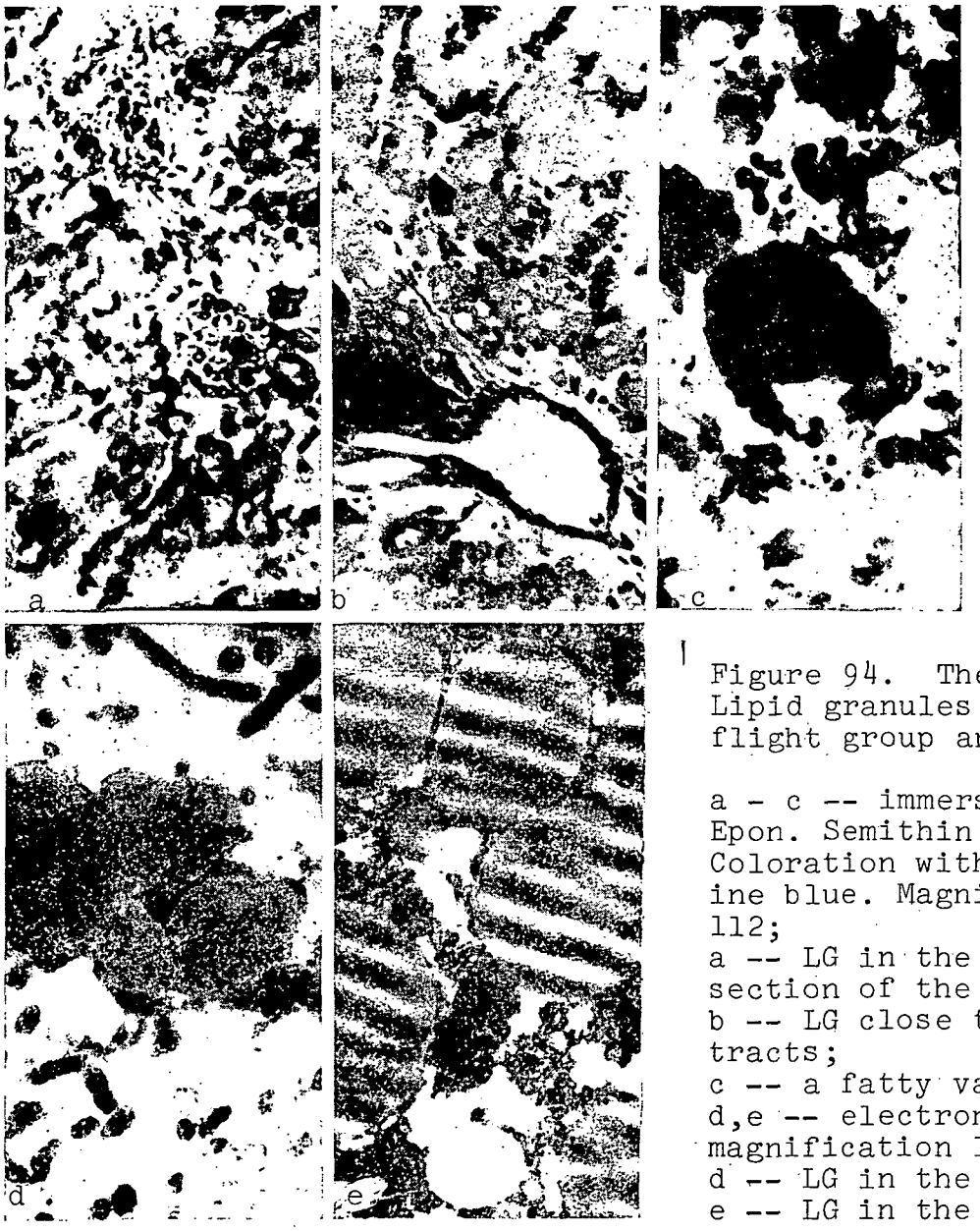


Figure 94. The liver. Lipid granules (LG) in flight group animals.

a - c -- immersed in Epon. Semithin section. Coloration with toluidine blue. Magnification 112;
a -- LG in the central section of the lobules;
b -- LG close to the portal tracts;
c -- a fatty vacuole;
d,e -- electronograms, magnification 15,000.
d -- LG in the sinusoids;
e -- LG in the hepatocytes.

/210

Retention of the ultrastructure of hepatocytes and the presence of lipid granules in the Disse spaces, their capture by star-shaped reticuloendothelial cytes can truly be considered as proof of infiltration fatty degeneration of the liver. The cause for this can be the stress reaction during which the

energy resources of the organism are mobilized. Their source could be not only glucose but also free fatty acids which form during hydrolysis of a neutral fat. Then, the content of non-esterified fatty acids increases in the blood; this can cause fatty infiltration of the liver. Apparently this must be considered during long term space flights and lipotropic substances which provide synthesis of phospholipids must be introduced into the diet.

Twenty-five days postflight, one also observes this picture in the control animals which is evidence of the reversibility of fatty infiltration. /211

Thus, the data obtained a few hours postflight and after completion of the synchronous experiment indicate the presence of separate symptoms of stress reaction apparent in the increase in excretion of mucus by the mucocytes of the mucous membrane of the stomach, a decrease in DNA synthesis by stomach cells, and in fatty infiltration of the liver. All of the shifts indicated were reversible and were not detected in the study 25 days later. /212

Morphologic Study of the Small Intestine

Bibliographical data on the effect of space flight factors on the structure of the small intestine are scarce, have been made basically on a light optical level and due to this do not give adequate information.

In white rats and mice the changes existing after space flight corresponded to a breakdown in hemodynamics and an increase in tissue hypoxia (Petrukhin, 1962; Portugalov, 1976; and others).

An analysis of bibliographical data gives us a basis for considering that in the small intestine of the rats, there will be no visible destructive changes at the light optical level. Due to this, in our work, besides histologic studies histochemical, electron microscope and hemographic studies were conducted of segments of the duodenum, the proximal, center and distal sections of the small intestines of rats from the different groups.

During histologic study of the small intestine, as one was led to expect, no destructive changes were detected in the mucous membrane, the submucous, the muscle and serous membranes. The relief and reticular ostia were unchanged; the fiber structures and cells responsible for their operation, the argyrophils and basal membrane precisely delimited the epithelial plast from the membrane itself (Figure 95). The ShIK-positive material is apparent in the strigillate edge of the enterocytes, in the cup-shaped cells and the basal membrane. /214

/212

Figure 95. Small intestine of rats in the flight group 9-11 hr postflight. Magnification 70. Relief of the mucus unchanged. Hematoxylin-eosin coloration.

Figure 96. Center section of the small intestine activity of leucine-aminopeptidase increased in the flight group rats (b) in comparison with the same index in the vivarium control group rats (a).

/214

In all sections of the intestine of the animals in the groups studied, the activity of oxygen-regenerative enzymes -- NAD-diaphorase, SDH, NADP-diaphorase -- essentially were unchanged. Also the proximal-distal gradient of activity of these enzymes was not broken down. At the same time, the activity of glucoso-6-phosphate-dehydrogenase, NAD dependent α-glycerophosphate-dehydrogenase increased in rats of the flight group in comparison with the control.

During histochemical detection of hydrolytic enzymes, no marked changes were detected in the activity of alkali, acid phosphatase and nonspecific esterase; the activity of leucine-aminopeptidase was sharply increased in the center section of the small intestine of the flight group rats. This increase was retained, although to a lesser degree, after the readaptation period (Figure 96a,b). When determining zinc in the epithelium it was discovered that immediately postflight its quantity decreased in the Panatovsky cells in all sections of the intestine but increased in the strigillate edge and in the sections adjacent to its cytoplasm. Similar changes were observed in the synchronous control group.

/215

During electron microscope study of the wall of the duodenum and jejunum of rats killed immediately after flight and in the synchronous test, changes were observed in some of its structures basically similar in both groups. For enterocytes accumulation of a large quantity of mitochondria, ribosomes and their complexes -- polysomes was characteristic as well as compaction of their hyaloplasm. The basal part of most enterocytes of the villi is filled with free ribosomes and is subject to fragmentation of the clasmatosis type (Figure 97). In certain cells one also notes fragmentation and complete disappearance of microvilli (Figure 98).

/213

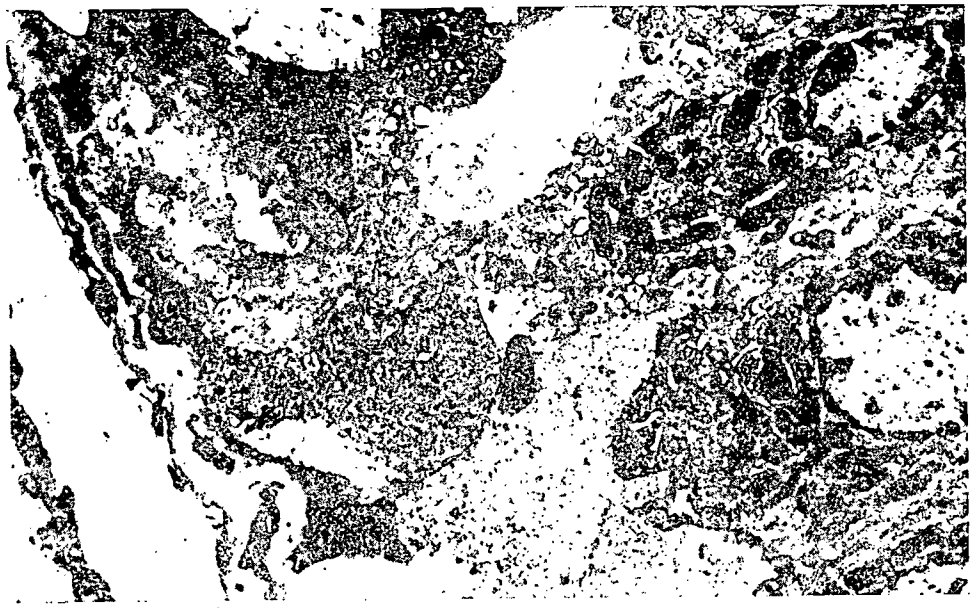


Figure 97. The center third of the villi of the duodenum of rats in the synchronous control group. Magnification 6480.
Fragmentation of the cytoplasm and lysis of the content of fragments in the enterocytes are observed.

For these animals, also the presence of lipids in the enterocytes of the fiber, the intercellular spaces, in the basal membrane of the layer itself between the nearest cells and in the lumen of the lymphatic vessels are also characteristic. They are considerably larger in the enterocytes of the large intestine in the flight group animals. /215

In the vascular and lymphatic vessels of the small intestine in rats killed immediately after the experiment, thinning and compaction of the cytoplasm of endothelial cells was apparent as well as an increase in compaction of fibrillar structure. /216

/213

Figure 98. The center third of the fiber of the duodenum of rats in the synchronous control group. Magnification 16,920.
Fragmentation of the microvilli of the enterocytes is observed.

in the precapillary connective tissue. The fenestrated capillaries making up the basal membrane of the mucous membrane itself form, moreover, a large number of microfoliations (see Figure 97). The appearance of fenestra on the side of the capillaries which form at the center of the fiber is also characteristic. The endothelium of the central lymphatic vessel, besides compaction of the cytoplasm, loses the precise contours of the membranes in certain sections and forms a broad "window." In the nerve apparatus of the wall of the small intestine, changes result in increased swelling of most of the nerve fibers in the muscle, submucous and mucous membranes (Figure 99). Then, one notes a breakdown in the limiting membranes of the axon as a result of which broad lacunae form. In such axons, one observes disappearance of the neurofibrillae and the majority of specific granules. The cytoplasm of the Shvannovskiy cells undergoes a significant change: it becomes edematous, combines with organoids, and certain of its sections break down. The nerve-muscle contacts expand and noticeably lose the vesicular structure. Twenty-six days after flight and the synchronous experiment, changes in the wall of the duodenum and jejunum are retained; some of them acquire a different character. For the enterocytes of all groups, a decrease in the quantity of ribosomes, mitochondria and also a decrease in hyaloplasm density are characteristic. Fragmentation of the cytoplasm of the basal part of the enterocytes of villi and microvilli of certain cells is retained. The quantity of lipids in the duodenum of the animals in

/216

/217

/214

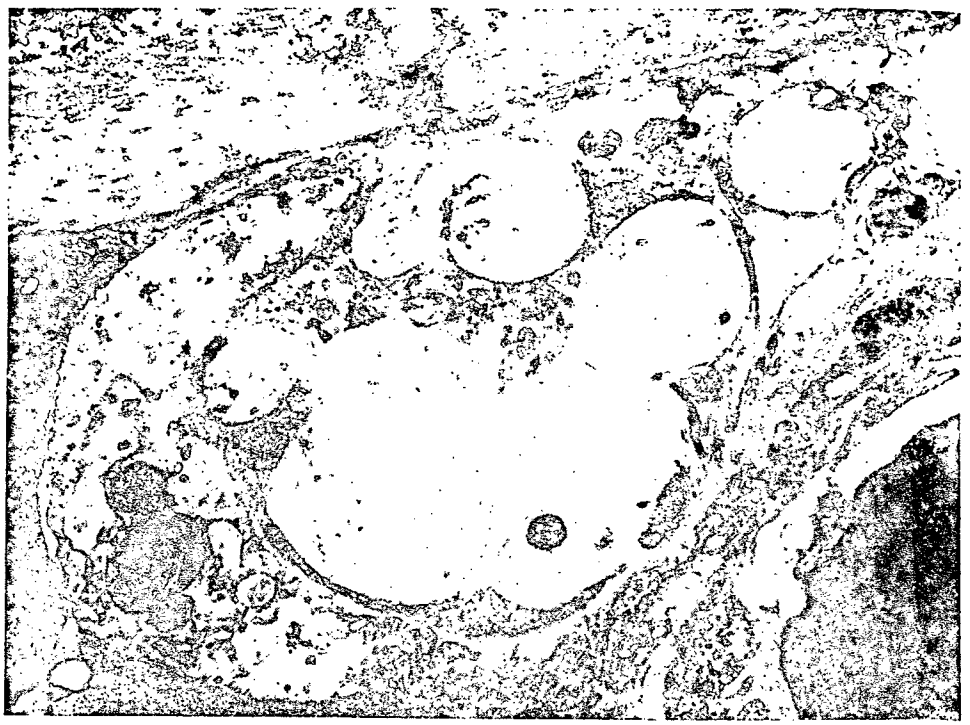


Figure 99. The duodenum of a rat of the flight group. Intermuscular nerve trunk. Magnification 18,960.

/217

both groups and in the jejunum of the animals in the synchronous control group remain at the level of the preceding examination although in the jejunum of the flight group of rats it increases sharply (Figure 100). In the endothelium of the vessels, a decrease occurs in compaction of cytoplasm. The fibrillar structure of the perivascular connective tissue becomes thinner and its viscosity decreases. The facts mentioned are characteristic for rats of the flight group and the synchronous control group. However, in the latter the ultrastructure of the vessels reminds one more of that of the intact animals. In the same examination period the Shvannovskiy cells and most of the axons have an ultrastructure close to normal; however, in the nerve flexus, the large nerve trunks and separate axons are observed to have myelin shapes (Figure 101). The ultrastructure of most of the nerve-muscle contacts in the rats of these groups is close to normal; however, certain active zones remain hollow and contain myelin shapes.

Analyzing the data obtained one can conclude that in the changes observed in the small intestine of rats of the flight group and the synchronous control group, there are no main differences although in the former they are more intense and disappear in the postflight period more slowly than in the latter. Thus, on the one hand, the morphologic changes found

/215

Figure 100. Jejunum of a rat in the flight group 25 days postflight. Magnification 18,240. One observes fragmentation of the microvilli and an accumulation of lipoids in enterocytes.

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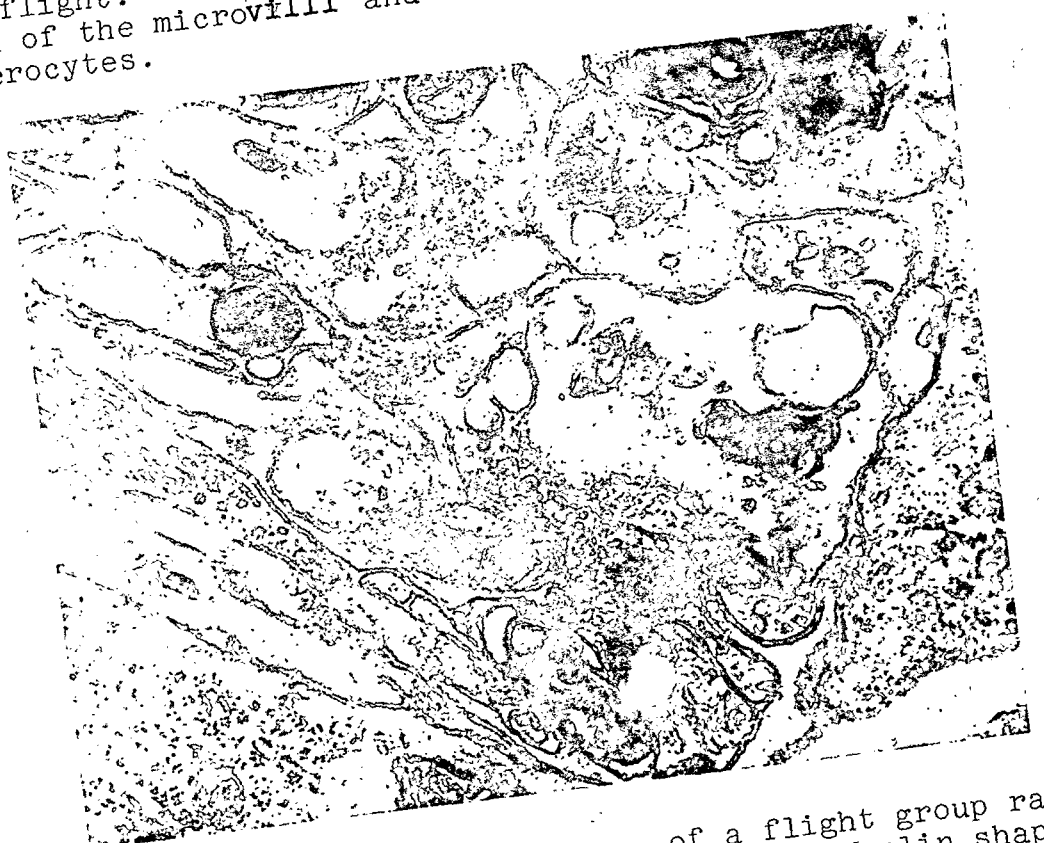


Figure 101. The duodenum of a flight group rat 25 days post flight. Magnification 238,000. Myelin shapes are observed in the intermuscular nerve trunks

are specific for the effect of weightlessness and on the other hand weightlessness intensifies the effect of other factors of space flight. This does not exclude the fact that destructive changes in the vascular-stromal, nerve-muscle component of the wall of the small intestine can be one of the causes for gastrointestinal pathology occurring much later after space flight. /217

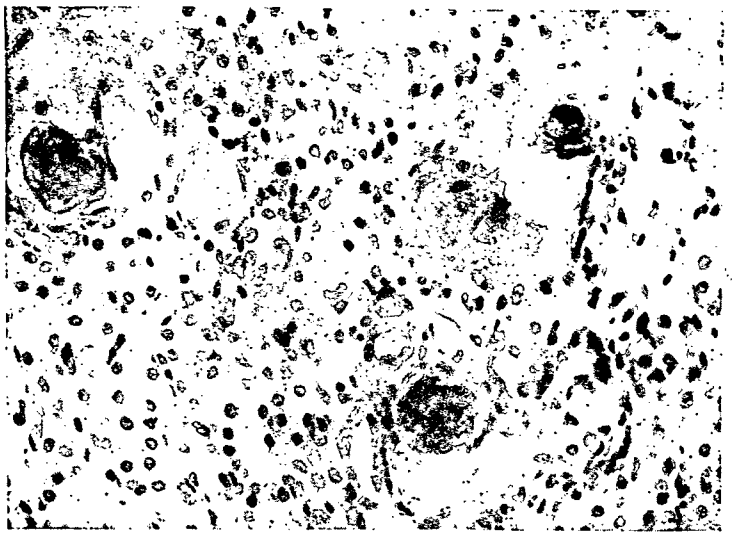
Histologic Examination of Kidneys /219

At the present time, a good deal of material has been accumulated which gives evidence of shifts in the water-saline equilibrium and changes in functioning of the kidneys in cosmonauts during flight and in the first days after landing. In animals exposed on the Kosmos-605 and Kosmos-690 satellites, a proven increase in absolute and relative weight of the kidneys was noted as well as an increase in the activity of the **juxtaglomerular** apparatus (Savina et al., 1976). At the same time, during histologic study in the parenchyma, no pathologic changes were detected in the interstitial tissue of the kidneys which could be the cause for an increase in weight of the organ. This data was advantageous.

The kidneys of 23 rats exposed on the biosatellite and killed 5-11 hr (12 animals) and 25 days (11 animals) after completion of the space flight were studied. The data obtained were compared with the results of studying animals in the synchronous control and vivarium control groups. The weight of the kidneys was determined and the characteristics of their histologic structure. They were fixed in Karnui fluid, Tsenker-formal, in a 10% solution of neutral Formalin, and immersed in paraffin. The material was colored by hematoxylin-eosin, toluidine blue according to Van Gizon. The calcium salts were detected according to the Koss method, and the granules in the **juxtaglomerular** apparatus, according to Bovi.

During macroscopic study of kidneys of rats killed at the landing area, it was noted that in 4 out of the 12 rats (numbers 3-5, and to a lesser degree number 9) the kidneys were flaccid with nonuniform dimensions: one decreased and another increased sharply. In both kidneys, on the cross section one noted expansion of the pelvic cavity. In the increased kidneys this was accompanied by thinning of the cortical layer. In the bladder of these animals, whitish loose, sometimes coagulated pale gray clots were found. In the synchronous control group, similar changes were detected in one case (rat no. 9) and in the vivarium control group in rat no. 8.

During microscopic study in 11 of the 12 rats in the flight group one noted an expansion of the canals primarily on the boundary of the cortical and cranial substances. In the lumen of these **canals,** laminar or lumpy masses were detected with an admixture of calcium salts (Figure 102).



/220

Figure 102. The kidney of a rat 9-11 hr postflight. Magnification 200.
Protein-calcium casts are visible in the canal lumen. Coloration is with hematoxylin-eosin.

The degree of these changes varied, the greatest deviation /219
was observed in a rat whose kidneys had the phenomenon of hydronephrosis detected under macroscopic investigation. In such kidneys, crista expansion of the canals was observed not only in the boundary zone but also for a considerable extent. In some of the cases, the content of the canals was infiltrated with leucocytes (particularly in rat no. 5 in the flight group) and leucostasis of the adjacent peritubular capillaries was observed. The protein-calcium casts were in other sections of the cortex and cranial layer (proximal channels, connecting tubes). In such kidneys, where under macroscopic investigation one did not discover changes, the cystic expansion of the canals had had a focal character. In the synchronous experiment, in rats nos. 8, 9, 10, and 11, there were similar microscopic changes and in rats nos. 7 and 12, the cystic expansion of the canals was not observed but one encountered separate scattered protein-calcium casts of the microlyte type; these same microlytes were found in 6 of the 10 rats in the control group. In one of the control /220
rats (no. 8) one detected a picture of more advanced atrophy of the channels.

When studying the glomeruli in some of the rats of the flight group, changes were found, typical of internal hydronephrosis (glomerulohydrosis) -- a strain on the Bowman's capsule by the filtrate. In these cases, more often one encountered groups of proximal canals with expanded lumen. No other pathological changes were detected in the glomeruli. In spite of the presence of protein-calcium casts in the canals, expansion of the connective tissue around them was not noted.

Twenty-five days postflight, the protein-calcium casts in the canals were noted in the kidneys of rats of all three groups studied. Then, both in frequency of the changes observed and in their extent, there were no differences among the groups.

The changes described in the kidneys made it difficult to evaluate the state of the juxtaglomerular apparatus. It is possible only to note an almost complete absence of granules in the hydronephritic kidneys and marked degranulation of the Juxt-cells in the other animals.

A proven increase in weight of the kidneys was detected in rats of the flight group (Table 69).

TABLE 69. WEIGHT OF THE KIDNEYS (M±m) /221

Group	Weight of kidneys, g		Group	Weight of kidneys, g	
	Absolute	per 100 g of body wt		Absolute	Per 100 g of body weight
	5-11 hr			25 days	
F	2,33±0,05 ($P_{СК}<0,001$)	0,9±0,02 ($P_{ВК}<0,001$)	F	2,28±0,03	0,71±0,03
SC	1,86±0,05	0,68±0,01	SC	2,30±0,68	0,66±0,02
VC	1,90±0,05	0,70±0,01	VC	2,26±0,77	0,68±0,02

Thus, the changes which could cause a breakdwon in the drainage function of the canalis system were discovered in a significant number of rats from all three experiment groups. /220
One can hypothesize that this pathology is a species characteristic of the Wistar-SPF line. In the bibliography there are indications of the development of hydronephrosis in such animals in a 90 day test period and in 2% one noted even tumors of the kidneys with pelvic involvement (Beach, 1967; /221
and others). The established increase in the weight of the kidneys in animals in the flight group corresponds to observations made in the experiments with the Kosmos-605 and Kosmos-690 biosatellites; however the cause of this phenomenon remains unclear.

Histologic Examination of the Lungs

A study of the state of the pulmonary parenchyma in the preceding flight experiment (Portugalov, Savina, et al., 1976) was difficult due to the fact that a large part of the animals

in the flight group were discovered to have spontaneous chronic pneumonia. From this point of view, a study of the pulmonary parenchyma is necessary of a microbe-free SPF-Wistar line.

The material for histologic examination was samples of lung tissue from 23 rats killed 5-11 hr (12 rats) and 25 days (11 rats) after completion of space flight. This quantity of samples of the lungs was taken from animals in the two control groups.

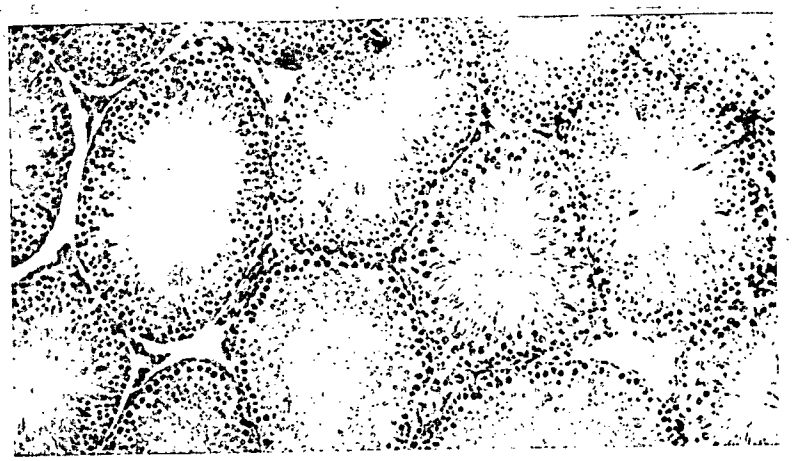
The material was fixed in a 10% neutral Formalin and immersed in paraffin. The preparations were colored with hematoxylin-eosin and Van Gizon picrofuchsin. No peculiarities in blood accumulation and distribution of blood in the vessels of the pulmonary parenchyma were apparent in comparison with animals from the control group according to histologic methods in the pulmonary tissue of rats killed in the first time period. The artery and vein walls of all of the animals examined were without peculiarities, their lumen had average expansion, and contained erythrocytes as a rule. No symptoms were discovered of acute or chronic stagnant plethora of the blood. In the lungs of 5 rats in the flight group killed after 9-11 hr, one noted a significant increase in the quantity of segment-nucleus neutrophils in the interalveolar barriers for a large extent on the pulmonary parenchyma. Leukocytes were found then not only in the capillaries but also outside of them; due to this the alveolar membrane was somewhat thickened. In the lungs of rats killed in the later time period (5-7 hr) also an accumulation of leukocytes occurred in the capillaries of interalveolar membranes but the latter was thin and the process of migration of the leukocytes from the capillaries was less marked. /222
In animals of the control group, the interalveolar membranes, as a rule, were thin; segment-nucleus leukocytes were encountered in a small or insignificantly increased quantity. In the lungs of the animals of the synchronous control group, the interalveolar membranes were insignificantly thickened; in some areas one encountered cells of a lymphoid-histocytic type and in other areas neutrophil leukocytes predominated, located primarily in the capillaries. Twenty-five days later in most of the rats of the flight group (9 out of 11) in the capillaries and membranes, one encountered a small number of neutrophils and also eosophyllic glucocytes but the membranes, as a rule, were thin. In 2 rats (nos. 20, 23) one noted a focal increase in the number of leukocytes. The lungs of the animals of both control groups did not differ from the lungs of most rats in the flight group. Thus, a characteristic change was the large number of neutrophil leukocytes in the interalveolar membranes observed mainly in animals of the flight group killed 9-11 hr after landing. Apparently, this phenomenon, to a lesser degree noticeable in the control groups, expressed neutrophilia in the peripheral blood (see Table 4 on page 16). As is well known, the latter is characteristic of an acute stress reaction.

Morphologic Study of the Testicles

In the experiment on the Kosmos-605 biosatellite, no disturbances in the reproductive glands were noted (Plakhuta-Plakutina, 1976). This work discusses a further study of the effect of space flight factors on the state of spermatogenesis.

The testicles of rats killed 9-11 hr and 25 days after landing were studied. Six animals of each group in the first and five animals in the second for the time periods indicated were used. In the rats of all the groups, the weight of the testicles was determined and their histologic structure was studied. The samples of tissue were fixed in Bouin's fluid and Ca-formal . The paraffined sections with thickness 5-7 μm were colored with hematoxylin-eosin according to Van Gizon, the lipids were discovered by Sudan black B. For judging the condition of spermatogenesis, besides the histologic structure, the cellular composition of the spermatogenic epithelium was studied.

Nine-eleven hours after completion of flight and the synchronous experiment, the weight of the testicles of rats was somewhat decreased in comparison with the same index in rats of the vivarium control group but these differences were not proven. During histologic examination, the rats of the flight group and the synchronous control group noted a venous plethora, swelling of the endothelium of the capillaries, a small edema of the connective tissue. In the testicle canalis, containing layers of cells, 4-6 rows, all of the generation of cells of the spermatogenic epithelium was traced. In the lumen of the slightly constricted canalis, one encountered spermatozoids fairly often (Figure 103). The canalis of the testicle appendage was filled with a mass of mature spermatozoids. In



/223

Figure 103. Testicles of a rat from the flight group. Normal structure of the canalis.

structural peculiarities and content of different forms of reproductive cells in the testicle canalis, the spermatogenic tissue of the rats in the flight group essentially did not differ from that of the rats in the synchronous and vivarium control groups. The Sertoli and Leydig cells retained a normal structure and one could only note an increase in quantity of finely dispersed lipid drops in their cytoplasm. On the 26th day, there were no differences among the groups in the condition of the spermatogenic epithelia. Thus, the stay of the animals in conditions of weightlessness for 19-25 days did not result in a significant change in the spermatogenic tissue or a breakdown in the process of spermatogenesis. The small venous plethora and edema of stroma noted in rats of the flight group and synchronous control group 9-11 hr after completion of the test must apparently involve the effect of the G force to which animals of both of the groups indicated were subjected.

Conclusion

Certain functional and structural disturbances were noted in the organs of the gastrointestinal tract. An increase in secretion of hydrochloric acid and pepsin was noted in the stomach as well as the formation of mucus by the mucocytes. The activity of pancreatic lipase and activity of all the proteolytic enzymes of the gastrointestinal tract were increased significantly. Destructive changes were detected in the vascular-stroma and nerve-muscle elements of the intestinal walls. Changes in the gastrointestinal tract were observed in animals of the synchronous control group, where, however, they were less marked. In a special study it was established that there were no ulcers of the stomach which are a manifestation of severe stress.

On the 26th day of the postflight period, most of the indices in which various changes had been observed were normalized.

In the lungs, kidneys and testicles, there were no significant changes found indicating the effect of space flight factors.

Conclusion /231

The results of the physiological experiment on the Kosmos-782 biosatellite supported conclusions drawn earlier as to the possibility of adaptation of mammals to long (19.5 days) weightlessness and the absence in these conditions of pathological changes in the basic vitally important organs and tissues.

Moreover, attention is given to a number of fairly serious structural and functional changes which occur during weightlessness and after return to Earth's gravitation. These are primarily nonspecific changes which make it possible to evaluate weightlessness and the stress effect. Some of these are a lag in growth in animals in flight in the first days after return to Earth; lymphopenia, eosinopenia and neutrophilia in the blood in the first days after flight; hypertrophy of the adrenal glands, delipoidization of their cortical layer, an increase in the concentration of corticosterone in the adrenal glands as well as activity of tyrosine-hydroxylase, a key enzyme in the synthesis of catecholamines. An increase in the concentration of corticosterone in the blood plasma and an increase in phosphorylase A and B in the myocardium as well as hypoplasia in the lymphoid organs relate to a manifestation of stress reactions: a decrease in weight of the thymus, spleen and inguinal lymph nodes, atrophy of the lymphoid follicles and their light centers, a contraction of the cortical substance of the thymus and lymph nodes.

The accompanying factors which occur during landing of the biosatellite (linear and impact G-load and others) and also rapid transition from weightlessness to Earth's gravitation all make a definite contribution to stress reactions. An increase in functional activity of the neurosecretory cells of the hypothalamus and the hypophysis, decomposition of lymphocytes and neutrophil infiltration in the lymphoid organs are some of the manifestations of acute stress reaction.

One should note here the fact which is of practical importance that 19.5-day weightlessness caused an average stress effect. The absence of serious changes in metabolism of catecholamines, the absence of changes in the hormonal status of the hypophysis, the absence of ulcers and a preulcer state in the mucous membranes of the stomach and also average expression of all these reactions which have been discussed above are evidence of this.

Changes in the support-motor apparatus, the myocardium and the erythrocyte system are some of the most significant specific manifestations of the effect of weightlessness.

During analysis of the set of changes detected in the skeletal muscles, a principle was established including the

fact that the expression of changes in different skeletal muscles correspond to the degree of participation of one or another muscle in antigravitation functions on Earth. Strictly speaking, this relationship is determined to a greater or lesser degree by a predominance of slow (red) fibers in the muscle. The capability of the muscles to develop and withstand long term stress and resist fatigue depends on this; these are properties which provide, besides strength, adaptation of the entire support-motor apparatus in functioning under conditions of Earth's gravitation. This applies particularly to the m. soleus and the most marked changes occur in it after a 19.5-day space flight. With histologic and histochemical methods on the level of light microscopy and also with electron microscopes, marked changes are noted in all the structural components of the muscles. The dimensions and localization of the territories with a breakdown in structure encompass the field of the sarcomer up to the entire muscle fiber. Morphologic symptoms of atrophy are significantly less marked in the m. gastrocnemius which is the basic synergist of the m. soleus but differs from the latter in the much smaller quantity of slow fibers and, consequently, the functional designation. At the same time, thorough morphometric and electron-microscope studies did not show any kind of significant characteristics of atrophy in the m.quadriceps femoris which, like the m. gastrocnemius is mixed; their activity apparently to a lesser degree than the activity of the m. soleus involves maintenance of posture. /232

The results of biochemical studies which correspond well with data of morphologic observations showed that in the m. soleus the greatest change occurs in activity of the enzyme. It is established, in particular, that there is a change in the spectrum of LDH isoenzymes in the form of an increase in activity of the "muscle" fractions (LDH_4 and LDH_5) with a simultaneous decrease in the activity of the "core" fraction (LDH_1 and LDH_2). This can be evidence of activation of the glycolytic processes in the m. soleus in which normally fiber with an oxidation type of metabolism predominates.

The results of morphologic and biochemical studies of the muscles in an experiment on the Kosmos-782 biosatellite basically coincide with similar data obtained earlier on the Kosmos-605 biosatellite. Unfortunately, in this experiment the functional characteristics of the muscles are not studied. Therefore, it is expedient to remember that in the experiment on the Kosmos-605 biosatellite, besides symptoms of functional atrophy (loss of strength, increased fatigue), noted primarily however in the muscle-antagonists-m. soleus (slow) and the long digital extensor ("rapid"), a decreased time for development of the tetanic contraction of the m. soleus was established down to a value comensurate with this index in the

"rapid" muscles. This set of muscles could appear at the level of the entire organism in the form of disturbances of those motor functions which involve the development of long term stress with posture activity. Actually, in the experiment on board the Kosmos-782 biosatellite, a decrease in static endurance was established (when making tests on "staying on a rod") in the animals after the flight. Similar changes in animals in the synchronous control group were reversed much more rapidly. /233

Data on the increase in adenosinetriphosphatase activity of the myofibrillar proteins in the m. quadriceps femoris and a similar tendency in the m. soleus with the absence of such changes in other muscles studied, primarily the rapid muscles, are indirect evidence, using changes in the contracting properties of the muscles in animals in the experiment on the Kosmos-782 biosatellite.

Taking into account the relationship described of the changes found in muscles to the degree of their participation in posture, and also taking into account the reversibility of these changes one can conclude as we have shown that the structural and functional restructuring which is observed in the muscle system after animals stay in space flight for a period of 20-22 days, has an adaptive character and that some of these, in particular acceleration of the process of contracting the "slow" muscles, apparently are biologically advantageous. Moreover, one can propose that according to a certain index, fundamental for vital activity and functioning of the muscle cells, these restructurings are compensated for in the indicated time period. Such results of biochemical studies as the absence of significant changes in the quantity of muscle proteins in the five types of muscles studied, the insignificant decrease after flight and full restoration in the readaptation period of the content of free amino acids and also only the insignificant increase in activity of transaminases (ALT and AST) in all the muscles studied are evidence of this. One must note that the degree of expression of atrophic changes in the muscles in the experiment on board the Kosmos-782 biosatellite was less than in animals who had been on the Kosmos-605 biosatellite. Atrophy of the muscles in this case was not accompanied by proliferation of the connective elements in the endomesium, foci of dystrophic regeneration of the muscle fibers did accompany atrophy in this case. In distinction from research data in the experiment on the Kosmos-605 biosatellite, an increase in the content of free amino acids was established which also is an index of the depth of atrophy. The differences indicated can involve a certain difference in the length of flights on the Kosmos-782 and Kosmos-605 biosatellites (19.5 and 22 days) and the peculiarities of different populations of animals. This does not exclude the fact that one of the causes for these differences was a change in the level of motor activity and body temperature noted during the flight among the animals ~~in the~~

in the experiment on the Kosmos-782 biosatellite.

Data obtained when studying the bone system agree well with the results of studying the muscles. The absence of long-term stress in the muscles in weightlessness conditions and long term load on the skeleton resulted in retardation of growth of the bones in length (reduction of spongiosis in the area of the epiphysis of the cartilage plate), osteoporosis of the porous sections of the bone, suppression of periosteal bone formation. Also slowing down of mineralization of the bony tissue was detected which is a symptom of change in the metabolism (for example, an increase in activity of alkali phosphatase in the blood plasma) and a decrease in the strength of the bones.

One can note that some of the changes described in the support-motor apparatus are similar in aspect to the effects of certain model situations, for example, hypokinesia. In particular, in this they were noted not only in flight but also in the synchronous control experiment although their expression in this case was considerably less. Moreover, some of the functional phenomena described above are very reminiscent of the effects of modeling a decreased force load on the muscle (hypodynamia). This makes it possible to assume that the mechanism of the specific effect of weightlessness like a situation of relative "nondemand" on the support-motor apparatus involves not so much the deficit of motion as the strength of the "relief" of the support-motor apparatus due to the absence of necessity for developing and maintaining long term muscle stress. /234

Keeping in mind the practical applications of some of the results obtained in the experiments on animals on board the Kosmos series of biosatellites, one can assume that in man in space flight similar physiologically expedient (adaptive) changes occur in the structure and function of the skeletal muscles, particularly in cases where for one or another reason the cosmonauts cannot take special prophylactic measures or cannot carry them out completely. One can expect that such changes, depending on the degree of their development, can be the cause for a decrease during space flight of physical capability and possibly show secondary dysfunctions in the blood circulation system. One can also propose that they are one of the causes of disorganization of motion and other deviations of motor functions which are observed in cosmonauts after landing. The results of studies on animals as a whole confirm the scientific basis and practical expediency of measures taken at the present time for manned space flights directed at prophylaxis of undesirable effects of weightlessness. Moreover, they indicate an expediency of adding certain new measures to the system in the form of differentiated exercises for certain muscles or muscle groups.

Besides changes in the support-motor apparatus, including specific effects of weightlessness, one can relate a decrease in the content of contracting and sarcoplasmatic proteins and a significant decrease in adenosenetriphosphatase activity of the myosin of the myocardium which occurs as a result of lack of load on the muscle apparatus of the heart in weightlessness and also changes in the erythrocyte system: suppression of erythroid hemogenesis and a decrease in the lifetime of erythrocytes due to spontaneous hemolysis.

One should note that all of the changes observed in animals in the first hours after landing were reversible and when studying groups of animals killed 25 days after completion of flight, almost all of the indices do not differ from the control values.

The broad experimental material obtained in the experiment with rats exposed on the Kosmos-782 biosatellite shows a detailed concept of changes occurring due to space flight factors in all of the organs and tissues of the mammals. Because of this, it /235 can be used not only as the basis for theoretical generalizations and construction of a hypothesis as to the mechanisms of weightlessness effects, but also during the practical search for measures to prevent undesirable effects.

Establishing the fact that weightlessness is a stress effect makes it possible to use all of the material concerning the mechanism of stress reactions in ground conditions for analyzing it. Because 20-day weightlessness showed stress reactions which were average and which have an adaptive character, the question of their prophylaxis has hardly any practical value.

Moreover, a portion of the nonspecific (stress) components in the reaction of the organism to the effect of other factors of flight definitely does not include the possibility that it determines a large part of the functional and structural restructuring which occurs in these conditions. Therefore, for an analysis of the mechanism of the weightlessness effect, there is undoubted interest in flight and ground (model) experiments on animals when removing or modifying the stress component of the reaction.

Moreover, it is necessary to note that in all of the studies made in this experiment, the animals are in a state of rest. Moreover, the total volume of activity of the animals who have undergone flight was less than in the control. This is the basis for thinking that this adaptive reaction, a physiological limitation of mobility, is one of the factors giving the organism the capability to maintain homeostasis and to retain physiological standards during the readaptation period for most of the organs and systems.

The resistance of an organism which has undergone 20 days of flight is decreased. Although this decrease was insignificant, it is very probable that when creating load situations during the readaptation period, we encounter a significant decrease in reserve capabilities of the organisms who have undergone space flight; incapability of maintaining homeostasis during loads is one of these. With increased time periods for flight, the threat of such changes will be more acute and important. Therefore, one of the problems of further study is investigation of reactions of the organism subjected to space flight not in a state of rest but in different load situations primarily an evaluation of the degree of stress of physiological functions when maintaining homeostasis under load.

Changes in the muscles and bone apparatus noted in animals after flight are mainly similar to those which were observed in model experiments on Earth (hypokinesia, hypodynamia), although to a much lesser degree. This makes it possible to expect that they can be eliminated by using special sets of physical exercises under conditions of weightlessness. Prophylaxis of such specific changes as a change in the myocardium (decrease in adenosine triphosphatase activity of the myosin) and in the erythrocyte system (suppression of the erythroid hemogenesis, increased decomposition of erythrocytes) obviously is particularly complex.

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