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Ontogenesis of Mammals in Microgravity

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Preface

At the turn of this century, when spaceflight was viewed as a fantasy, K. E. Tsiolkovsky outlined a grandiose plan for a stepwise conquest of outer space. He prophesied the launch of artificial Earth satellites, the creation of a normal environment within spacecraft, and the establishment of space stations and "ethereal settlements." K. E. Tsiolkovsky regarded all this as a necessary prerequisite for man to live and work outside the Earth, to spread across the entire solar system, and to develop to an unprecedented extent the science, industry, and culture in a "Solar Fraternity."

Today, almost a century later, we admire the striking accuracy of his scientific prophesies. Space technology, biology, and medicine have scored great achievements and are now tackling problems related to space stations, the precursors to long-duration space colonies. Researchers are facing new questions, including whether Earth's organisms can grow and develop for extended periods of time in an altered field of gravity, and what the limits might be for the normal existence of various plant and animal species in microgravity.

This book presents the results of an embryology experiment flown on the biosatellite Cosmos-1514, which for the first time demonstrated that the fetus can develop in the maternal body exposed to microgravity.

The novelty and scientific value of the problem attracted a large number of research groups to the study. The participants in the study were the Institute of Biomedical Problems, USSR Ministry of Health; Sechenov Institute of Evolutionary Physiology and Biochemistry, USSR Academy of Sciences; Koltsov Institute of Developmental Biology, USSR Academy of Sciences; Bakh Institute of Biochemistry, USSR Academy of Sciences; Brain Research Institute, Center of Mental Health, USSR Academy of Medical Sciences, and others. In addition to Soviet scientists, researchers from Bulgaria, Hungary, the German Democratic Republic (GDR), Poland, Rumania, Czechoslovakia, France, and the USA took part in the study.

The concerted efforts of this large international group led to the development of unique methods for assessing the health status of the mother and her litter. This study was a very detailed and comprehensive pioneering investigation in mammalian developmental physiology. The animals that returned to Earth after spaceflight were examined at every level—from the organismic to the subcellular—using various physiological, biochemical, and histological methods. This study furnished data about normal mammalian development and development in response to the effects of microgravity and other spaceflight factors. These data should be of interest to many scientists dealing with developmental problems.

We would like to extend our thanks to all of our colleagues, both Soviet and foreign, who took an active part in this study. It is hoped that the international research team that was formed will remain functional in the future. This is very important because, among other things, the experiments described in this book form only the first stage of a challenging task—to study the complete cycle of mammalian development in space and to produce several generations of animals on spacecraft.

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Abstract

This report is an English translation of a Russian report, *Ontogenez Mlekopitayuschikh v Nevesomosti* (Moscow: Nauka Press, 1988), prepared by a group of authors from the USSR, Bulgaria, Hungary, the GDR, Poland, Czechoslovakia, France, and the USA. It presents results of the first microgravity experiment on mammalian embryology performed during the flight of the biosatellite Cosmos-1514 and in ground-based simulation studies. An overview is provided of the data available about the role of gravity in animal growth and development, and future studies into this problem are discussed. A new introduction has been provided for the English version.

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A report within a series, initiated in 1976, on Scientific Results of Spaceflight Research

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Introduction

This report, translated from the Russian, describes the results of a remarkable and significant set of spaceflight experiments connected with the Cosmos-1514 satellite mission in 1983. Before introducing this particular mission, it is appropriate to describe the Cosmos Biosatellite program more generally.

The Cosmos Biosatellite program is the most important continuous source of basic data for nonhuman spaceflight research in the life sciences. Beginning with the orbital flight of Cosmos-605 in 1973, and including the flight of Cosmos '92 scheduled for late 1992, the Cosmos program will have conducted ten dedicated life sciences missions. Use of the Cosmos satellites, the Vostok launch vehicles, and recovery operations were generously provided by the USSR, and we are grateful to that government for this valuable contribution to world space science. Through the Institute for Biomedical Problems in Moscow, multinational experiments were assembled, typically with investigators from both eastern and western Europe as well as the United States.

Cosmos missions are dedicated to life sciences research and have involved a wide range of biological specimens, including both plants and animals. Many animal species have been flown: fish eggs, fruit flies, rhesus monkeys, and jellyfish. All nine of the Cosmos flights flown to date have carried rats (*Rattus norvegicus*). The varied specimens sent from the Earth represent the spirit of comparative methods in biology. These spaceflights not only provided unique biological material but also brought together scientists and engineers from different cultures, and challenged them to negotiate the sharing of limited resources for common goals.

Cosmos-1514 can be considered a pinnacle of flight experiments in space biology and biomedicine. The offspring of the ten pregnant rats aboard Cosmos-1514 were the first mammalian specimens to undergo gestation in the absence of Earth-normal gravity. Cosmos-1514 was a pioneering study in developmental biology in space. This study touched on several key issues in the space life sciences.

First, for more than a century, developmental biologists have considered and debated the possible roles of gravity in ontogenetic processes. At one extreme are those who predict that development cannot proceed normally in the absence of Earth-normal gravitational forces. Others predict no difference in the face of microgravity. Still others anticipate modest or system-specific effects. The debates will continue until empirical answers bring us to new levels of understanding.

As plans for Space Station Freedom continue in the United States, the life sciences are increasingly gaining prominence. Indeed, much of the scientific value of a space station laboratory is in the ability to maintain animals for multiple generations and to study their developmental processes. Cosmos-1514 was the first probe in this direction.

Many scientists see special value in studying development in space, because many central questions about adaptation during life and throughout evolution involve ontogenetic processes. Ontogenesis also provides an especially clear or magnified view of basic mechanisms. The large-scale

changes involved in the growth and differentiation of bones, muscles, and organs can provide information on the cellular mechanisms in the maintenance and functioning of these systems throughout life, in health and disease. Ultimately, the key to understanding the role of gravity in life processes is in the systematic study of the life cycle, including conception and aging. During periods of rapid growth and development, active processes serving maintenance may display variations that in adult systems are too subtle to be clearly recognized. Thus, even investigators whose interest in development *per se* is minimal often turn to developmental studies for a clearer view of processes that continue in other stages of the life cycle.

It must be acknowledged that the Cosmos-1514 experiments, though pioneering, were an extremely modest first excursion into a vast scientific frontier. The investigations focused on only a small portion of prenatal development, in one species. The 5-day flight of Cosmos-1514 exposed fetuses to microgravity conditions for less than one-fourth of the gestation period. Early critical events such as implantation, placentation, and axis formation, as well as later events such as pulmonary development and numerous aspects of neural development were spared from the challenges of microgravity. Furthermore, in rats, there is an extended period of postnatal maturation that will require experimental scrutiny in space. Clearly, there is a need for additional direct, systematic study in order for us to appreciate the roles of gravity in Earth-normal development and to learn about Earth-evolved life in the context of the space environment.

The success of the Cosmos-1514 activity, particularly the attainment of unsurpassed levels of cooperation, is due largely to the generosity and cooperation of our colleagues, especially Academician Oleg Gazenko (then director of the Institute for Biomedical Problems), Dr. Anatoly Grigoriev (his successor), Dr. Eugene Ilyin (director of Cosmos programs), and Dr. Lyubov Serova (chief scientist for the ontogenesis project, which included approximately 50 experiments from nine nations). Their commitment, vision, and unrelenting energy is reflected in the important legacy of Cosmos flights. The late Edward W. Gomersall from NASA Ames Research Center was the project manager for the final phase of the Cosmos-1514 mission.

The role of the United States in the Cosmos-1514 rodent experiments was also noteworthy. The Cosmos studies involve intercontinental negotiations, detailed explanations of protocols, and careful attention to many different contingency plans—all set forth in carefully prepared documents, in two languages, which are signed ceremoniously. The depth and success of these negotiations and the level of cooperation that was achieved during the Cosmos-1514 negotiations are a tribute to the dedication of the scientists and project managers from both countries. With this study, conducted in the pre-Glasnost Soviet Union, American investigators for the first time worked directly with the flight animals, in a jointly established laboratory. Traditionally, United States participation in Cosmos was confined to the sharing of data, examination of flight specimens in laboratories in the United States, and contribution of equipment for joint studies. Cosmos-1514 experiments included American and Soviet investigators working side by side in a jointly established laboratory at the Institute for Biomedical Problems, studying living flight specimens.

Cosmos-1514 returned to Earth after a five-day flight at the end of 1983. Since then, there have been three additional Cosmos biosatellite flights and several United States Space Shuttle missions which included nonhuman life science experiments. When we look back at these missions, it is apparent that most of the fundamental questions in developmental biology that were opened with the Cosmos-1514 mission have not been addressed. Only additional studies in space can provide answers.

NASA is planning a Space Shuttle flight as early as 1993 that will carry pregnant female rats, making it possible to repeat and extend some of the studies introduced with Cosmos-1514. This will be an extension of NASA's Physiological and Anatomical Rodent Experiments (PARE), but it will be the agency's first excursion into mammalian developmental studies. NASA has invited the participation of Russian investigators in the PARE mission; it is hoped that some of the same investigators who led the Cosmos-1514 studies of development in microgravity will participate. This would be beneficial with regard to scientific continuity and as a gesture of goodwill and cooperation—to acknowledge past successes and affirm a future of international cooperation and sharing of dreams, ideas, and knowledge. In addition, NASA will fly an experiment on the Space Shuttle in September 1992, to investigate the effects of microgravity on the fertilization and development of amphibians. This experiment, like the Cosmos-1514 rodent experiments, will open new areas of research in developmental biology in the unique environment of space.

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Chapter 1

History of Studying the Physiological Effects of Microgravity and Hypergravity from the Ontonogenetic and Evolutionary Aspects

Human beings evolved several million years ago, while some animals appeared on the Earth much earlier. During this time period, living creatures have been constantly exposed to Earth's gravity field. The ties between gravity and life are so close that before real spaceflights were launched, it was hard to predict how biological processes would develop in the absence of gravity, whether they would occur at all, or if life could exist in the microgravity environment.

It was previously postulated that gravity affects the shape and size of living organisms, and that gravitational changes, both to an increased or a decreased level, might cause serious organismic alterations (Tsiolkovsky, 1882, 1885; Thompson, 1917; Brovar, 1960; Korzhuev, 1965, 1971).

K. E. Tsiolkovsky (1882, 1885) described the specific environment which man might find in outer space and predicted the various changes that might be experienced, e.g., loss of spatial orientation, decline of sensorimotor function, changes induced by blood shifts. K. E. Tsiolkovsky believed that man would be able to adjust to microgravity, but nevertheless, he suggested spacecraft rotation to generate artificial gravity.

At the end of the XIX century, K. E. Tsiolkovsky and A. Salathe (1877) initiated animal experiments in hypergravity. In the mid-1940s, biological experiments were conducted in and beyond the higher layers of the atmosphere, first using balloons and geophysical probes, and later artificial Earth satellites (Genin and Pestov, 1974; Gerd and Gurovsky, 1965; Petrukhin et al., 1976). The goal of the early studies was to evaluate the effect of microgravity and cosmic radiation on biological objects at various levels of organization, and to discover whether highly organized animals could survive the effects of spacecraft launch, orbital flight, reentry, and landing. The objective was to experimentally verify the plausible hypothesis that microgravity could induce catastrophic malfunction of vital physiological systems; another objective was to discover whether man would be able to undertake spaceflight.

Since then, dozens of various biological objects were flown into space: viruses and molds, plants and seeds, and different animals, including unicellular animals, multicellular invertebrates, cold-blooded vertebrates, bird eggs, and mammals (Parfyonov, 1967, 1982a,b; Gazenko et al., 1974; Vaulina, 1976; Gazenko, 1982; Ilyin, 1984; Jenkins, 1968). The very selection of biological objects suggests that serious changes were expected to arise as a result of exposure to microgravity. Together with animals that have a sufficiently high sensitivity to environmental effects, highly resistant objects were used (Sirotinin, 1981); also flown were small-sized objects, whose high tolerance to the effects of increased or decreased gravity was theoretically demonstrated (Polard, 1965; Smith, 1975).

Thompson (1917) advanced a theory that animals can be subdivided into three categories in terms of their susceptibility to gravitational effects:

1. microorganisms that are mainly controlled by viscosity, Brownian movement, etc.;

2. small animals (e.g., insects) that are primarily governed by surface tension rather than by gravitational forces; and

3. large animals that are mostly dependent on gravity effects.

The results of subsequent experiments in microgravity and hypergravity have verified this hypothesis.

Flown microorganisms showed no changes in their growth, development, or survival rate as compared to ground-based controls. Exposure to microgravity produced no deviations in spontaneous mutagenesis of microorganisms (Parfyonov, Lukin, 1967; Tairbekov and Parfyonov, 1981).

A large number of experiments have been performed using the fruit-fly *Drosophila melanogaster*, a classic object of genetic research. The experimental results are described in detail in several review papers (Parfyonov, 1967; Gazenko et al., 1974; Gazenko and Parfyonov, 1982).

An original *Drosophila* experiment was flown by Soviet and American scientists on Kosmos-782, to measure its developmental rate in orbital flight, and survival rate and lifespan after flight, as well as to perform anatomical and biochemical examinations (Miquel et al., 1979; Parfyonov et al., 1979). The flight experiment continued for 19.5 days with some fruit-flies being exposed to 0 g and others to 1 g, as generated by a centrifuge.

Postflight examinations did not reveal any significant differences in the developmental rate between 0 g and 1 g insects or between flown flies and synchronous controls. The lifespan of weightless fruit-flies tended to increase; however, the difference between the flight and control insects was not statistically significant. These groups did not show significant differences in terms of their aging rate. Postflight observations demonstrated a two-fold

increase in the latent period of copulation of the flown animals as compared with the controls, as well as a decline in their motor activity measured in terms of negative geotaxis. These changes cannot, however, be associated with the effects of microgravity because they occurred both in the weightless and centrifuged insects. Electron microscopic examinations did not reveal any differences in muscles, including wing muscles, of flown and ground-based insects.

Kosmos-1129 carried a *Drosophila* study in which gravitational preference was determined. The flies were allowed to choose an area where the gravity field varied from 0 g to 1 g in flight and from 1 g to 1.71 g on the ground. The response was measured in terms of the population density in different areas (Parfyonov and Rostopshina, 1980). Both the flight and ground-based experiments allowed the conclusion that gravitational variations from 0 g to 1.71 g exerted no effect on *Drosophila* development and behavior.

K. Wunder et al. (Wunder, 1955; Wunder et al., 1959, 1963) staged a *Drosophila* experiment in which the insects were exposed to high g. At 500 g, the growth rate increased by 25% as compared to the controls; at 1,000 g, the growth rate began to go down; however, a slight growth of insects occurred at 5,000 g as well. At 3,000 g, the survival rate of the population was 100% within 48 hours.

Kosmos-605 flew an embryological beetle (*Tribolium castaneum*) experiment. Specimens at different developmental stages—embryos, pupae, and larvae—were launched for a 22.5-day flight. Postflight observations allowed the conclusion that larval hatching, pupa formation, and metamorphosis developed normally in microgravity. The survival rate at all developmental stages in microgravity was the same as on the ground (Tairbekov and Parfyonov, 1981; Gazenko and Parfyonov, 1982).

The purpose of the *Tribolium castaneum* experiment flown on Salyut-6 was to study the activity, morphogenesis, and mutability of beetles exposed to microgravity during several generations. The experiment demonstrated that the complete cycle of development—from fertilization to the emergence of a new generation—occurred normally in microgravity (Parfyonov, 1981; Gazenko and Parfyonov, 1982).

The goal of hypergravity, microgravity, and clinostating experiments on fish and amphibians was to detect specific features of their embryonic development in those environments. They are reviewed in the papers by A. Smith (1975), L. R. Palmbakh (1976), Ya. G. Dorfman, V. G. Cherdantsev (1977), and E. M. Cherdantseva (1983).

These experiments have a long history that began with the publication of E. Pfluger (1883), who reported that amphibian eggs can spontaneously be oriented in such a way, that the axis connecting the anomalous and vegetative poles remains parallel to the gravitational vector, and that the first cleavage is located parallel to this vector, regardless of the position in which the egg is fixed. On the basis of these observations, E. Pfluger came to the conclusion that the gravity field is the factor that determines embryogenesis. Conversely, B. Roux (1884) demonstrated that amphibian eggs in a clinostat developed in a normal manner and inferred that gravity exerts no effect on embryogenesis. Since then the role of gravity has been an issue of great controversy. What part does it play at different stages of embryonic development of fishes and amphibians? What contribution does it make to the formation of bilateral symmetry and other processes? It should however be noted that the data obtained recently in centrifugation, clinostating, and orbital flight experiments give greater support to the concept of B. Roux rather than E. Pfluger. It cannot be ruled out however that this discrepancy may be caused by methodological constraints, especially at very early stages of animal development.

The first flight experiment with amphibian eggs (*Rana pipiens*) was performed on Biosatellite-2 in 1967 (Young et al., 1971). The experimental design was such as to expose eggs to real microgravity prior to the first cleavage. However, the launch date slipped and the very early stages of development occurred on the ground. Visual and histological examinations revealed no differences between flown and control larvae. The developmental rate remained identical at 0 g and 1 g.

The aquarium fish *Brachydanio rerio* maintained a normal rate of development in microgravity as well (Vinnikov et al., 1983). Histological and electron microscopic examinations did not detect any marked differences in the otolith apparatus of the flown and control species. Similar observations were made on *Rana temporaria* and *Xenopus laevis* eggs (Vinnikov et al., 1974, 1983). Summarizing the results of fish and amphibian experiments in space, the authors concluded that larval development from fertilized eggs was normal, showing no marked morphological changes, and that the vestibular apparatus evolved similarly in space and on the Earth (Vinnikov et al., 1983).

According to E. M. Cherdantseva (1983) who staged clinostating and centrifugation experiments with *Brachydanio rerio* eggs, the gravitational field had no impact on the early morphogenesis of this fish species. The only exception was weak—thermolabile—eggs. In normal spawnings, the course of development at early and larval stages remained unaffected by clinostating or

altering the egg orientation in the gravity field. The resistant spawnings showed no abnormalities when exposed to 50 g whereas susceptible layings exposed to 50 g for 4–9 hours displayed a substantial or total death of embryos. Resistant embryos remained viable when exposed to 500 g for 24–36 hours at early stages of development, beginning with the first cleavages. In spite of certain changes in the blastoderm and periblast during cleavage and a significant delay of the cleavage and gastrulation stages, the embryos proved to be almost normally developed by the time of the axial complex formation. The resistance of embryos to high g values increased continuously with their growth.

There is other evidence showing that eggs of Teleost fishes are resistant to centrifugation and that abnormalities at early stages of their development emerge only in response to the effects of 300–500 g (Shaver, 1951).

An interesting series of experiments using *Fundulus heteroclitus* eggs was carried out by Soviet and American scientists as a joint study (Scheld et al., 1979; Belousov et al., 1979).

The first experiment in the series was flown on Skylab-3. The fry that hatched from 50 eggs flown in microgravity did not exhibit any signs of spatial disorientation.

The next experiment on the same fish species was carried out during the 9-day Apollo-Soyuz joint flight. Embryos at different developmental stages were launched. The fry that developed in microgravity did not show any vestibular changes. Histological and electron microscopic examinations did not detect any differences in the central nervous system, vestibular apparatus, eye, or cardiovascular system of the fishes that were in orbit or remained on the ground.

The next experiment in this series was flown by American scientists on the Soviet biosatellite Kosmos-782. The age of the embryos launched varied from 32 to 128 hours. Some eggs were exposed to 0 g while others were subjected to 1 g generated by an onboard centrifuge. A large number of control studies were also planned. Unfortunately, due to technical problems unrelated to spaceflight effects, a substantial amount of both flight and control biosamples died; however, the larvae that survived the flight were in a better condition than the controls. This led to the conclusion that the development of *Fundulus heteroclitus* that followed the gastrula stage was not affected by microgravity. Histological examinations did not show any differences in the structure and development of otoliths and sensory compartments of the vestibular system of the flight and control animals; the same held true of their motor activity (Scheld et al., 1979).

The Soviet experiment on Kosmos-782 was designed similarly (Belousov et al., 1979), except that the *Fundulus heteroclitus* eggs were exposed to clinostating before being placed onboard the biosatellite. Beginning with the 52nd hour of development, clinostating and then microgravity did not induce any developmental abnormalities. However, exposure to clinostating beginning at the stage of mid-blastula caused a large amount of developmental abnormalities, due to the inhibiting and discoordinating effects on the epiboly, gastrulation, and differentiation of the axial complex. The inhibitory effect on the differentiation of the trunk compartment was more pronounced than on the head compartment, which resulted in abnormally short embryos. At later developmental stages, these abnormalities were, to a large extent, compensated, at both 0 g and 1 g. After hatching however, 23% of larvae that developed in microgravity, following preliminary exposure to clinostating beginning at the blastula stage and later, showed signs of body asymmetry, including body curvature, underdeveloped pectoral fins at the side facing the curvature, asymmetry of opercula, and sharply distorted position of the internal organs.

Thus, microgravity simulation by means of clinostating at the blastula stage led to "nonspecific inhibition and mismatch of various types of cell movements" (Belousov et al., 1979). According to the authors, this can be caused by inhibition of segregation and transport processes in the ooplasm. Beginning with the gastrula, no developmental abnormalities were observed in real or simulated microgravity. A similar pattern of changes was reported during clinostating of amphibian eggs (Dorfman and Cherdantsev, 1977).

V. V. Popov et al. (1975) clinostated frog (*Rana temporaria*) eggs for 2.5 hours beginning with the fertilization stage and detected a large number of developmental abnormalities, e.g., irregular cleavage, lowered rate of epiboly, etc. At later stages of development (neural tube, tail bud), various changes in the axial complex, a partially closed or completely open neural tube, and others variations were observed. Similar changes were seen in clinostating experiments with eggs of other frog species—*Xenopus laevis* and *Rana pipiens* (Young et al., 1971). O. P. Melekhova et al. (1976a) exposed developing *Rana temporaria* eggs to clinostating and also found various abnormalities, some of which disappeared later.

An embryology experiment with the viviparous guppy fish *Poccilia reticulata* (Parfyonov et. al., 1985) was flown on Kosmos-1514. At the launch day, the embryos were at the stage of a completed axial complex and onset of brain differentiation. The flight duration was 5 days. The amount of biosamples was limited (3 fishes each in

the flight and in the control study); however, the results were unambiguous and led to the conclusion that microgravity produced no effect on the guppy development, beginning with the stage of organogenesis. One flown female fish was allowed to survive until natural delivery; it produced 25 normally developed fry versus 20 fry produced by the control. Histological observations did not reveal any differences between the flown and ground-based newborns. Some of the young fish that developed in microgravity were allowed to reach sexual maturity; they, in turn, produced a next generation of fry which also did not differ from the controls.

Thus, many fish and amphibian experiments have convincingly demonstrated that simulated (clinostating) and real microgravity did not exert any effect on their development, beginning with the gastrula stage. Clinostating at earlier stages resulted in the emergence of a large number of various developmental abnormalities, some of which later disappeared. However, some of the abnormalities never disappeared which deserves further study. Special attention should be paid to the data obtained by E. M. Cherdantseva (1983) who observed attenuated or thermolabile egg spawnings; these spawnings can be viewed as a natural analog of provocative tests and controlled injuries that are used in mammalian experiments to detect pronounced responses to environmental stress-effects.

With respect to reptiles, space experiments have been performed on turtles. Steppe turtles (*Testudo horsfialdii*) were flown for different periods of time, viz. from 6 to 60 days (Petrukhin et al., 1976). Histological and electron microscopic examinations performed postflight revealed serious changes in the nervous system, muscles, skeleton, and visceral organs that increased with flight time. These included a reduction of nuclei and mitochondria of myofibers; focal dissolution of the sarcoplasm and appearance of myelin-like figures and lysosomes in the foci; glycogen build-up in hepatic cells; hemosiderosis of the liver, spleen, and kidneys; early stages of fat infiltration of the myocardium; and bone demineralization.

K. Wunder et al. (1963) performed another experiment in which turtles (*Pseudemys scripta*) were exposed to 30 g for 36 hours. The authors did not detect any adverse effects of centrifugation. However, they reported a significant (two-fold) increase of turtle growth during chronic exposure to 5 g, an interesting observation which so far remains inexplicable.

Birds, as was the case with fish and amphibians, were mostly used in embryological experiments in the interest of gravitational biology.

Kosmos-1129 flew an experiment with Japanese quail eggs. Unfortunately, due to technical reasons unrelated to spaceflight effects, a large portion of the eggs perished. The remaining biosamples were histologically examined, and the results suggested that the normal development of eggs in microgravity was possible (Gazenko, Parfyonov, 1982; Shepelev et al., 1980).

Bird eggs are more sensitive to the effects of hypergravity than those of poikilothermic vertebrates. A. Smith (1981) investigated the development of embryos of chick eggs laid at 2 g and centrifuged at 2 g during the first 24 hours. He found that during subsequent incubation of the eggs at 1 g, the death rate increased. The differences between the experimental and control samples were most significant during the first 8 days and during incubation days 18 through 21.

A. Smith carried out another experiment in which eggs were laid at 1 g and then centrifuged at 2 g or 4 g. In this case, exposure to 2 g did not increase the death rate of embryos but exposure to 4 g led to developmental abnormalities (distortion of the axial skeleton, delayed growth, etc.) and death of some embryos. The effect of hypergravity of 4 g was most pronounced on the earliest (from fertilization through day 4) and latest (from day 18 to the hatching day) developmental stages.

O. P. Melekhova et al. (1976b) exposed bird eggs to clinostating and reported many developmental defects in embryos, with the death of a number of them. The authors also observed a changed orientation of the cephalo-caudal axis of the embryo relative to the long axis of the egg. They attributed these changes to the fact that during clinostating, embryonic cells were not in contact with the yolk.

Adult birds also showed a sufficiently high sensitivity to hypergravity. R. Burton and A. Smith (1965) exposed chicks of varying age to acceleration of 2–2.4 g for a prolonged time and observed chronic acceleration sickness, which was characterized by body mass loss, blood changes, adrenal enlargement, and sometimes death. The authors noticed a certain range of resistance to acceleration and subdivided the chick population into five groups, varying from highly susceptible chicks, who died during the first 12 hours at 2 g, to highly resistant chicks, who tolerated the exposure without any changes in their health state.

A. Smith and R. Burton obtained over 20 generations of chicks during chronic exposure to 2 g (1980). It is important to note here that even the first generation included birds that were highly susceptible to acceleration. The authors used selection, by mating the most resistant specimens, which survived and delivered

offspring during chronic centrifugation, thus forming a population better adapted to environmental changes.

A large number of investigations into the effects of microgravity and hypergravity were performed on mammals. Different mammalian species, e.g., guinea-pigs, rats, mice, rabbits, dogs, and monkeys, were launched into space (Gazenko et al., 1962, 1964, 1974, 1980; Parin et al., 1968; Ilyin, 1984; Jenkins, 1968).

In early space flights, animal holding units and ground-based controls were certainly inadequate, and may explain the serious changes in the health state of animals observed after flight. In all likelihood, they were associated with the high temperatures and dynamic factors (vibration, etc.) that were characteristic of the launch and recovery stages (Arsenyeva et al., 1962).

The first dog flights demonstrated that highly organized mammals can survive launch and orbital flight (Gazenko et al., 1962). On the basis of these observations it was concluded that man-in-space flight was possible. Subsequent manned space flights, the duration of which increased from 108 minutes to 247 days, confirmed that conclusion.

A great amount of factual data concerning the effects of microgravity on mammals has been derived from Wistar rat experiments flown on Kosmos-605, 690, 782, 936, 1129, 1514, and 1667.

Animal holding units, life support systems, and physiological data recording systems were specially designed and developed to support these flights (Adamovich et al., 1979). In order to discriminate the effects of microgravity from other spaceflight factors, control studies were designed to meet several conditions; these included the use of intact (untreated) controls and synchronous controls in a biosatellite mock-up, where physiologically significant parameters typical of launch and landing (noise, vibration, linear acceleration, and impact acceleration) were simulated (Serova et al., 1979). The flight animals, as well as controls, were fed with a specially prepared paste-like diet (Kondratyev et al., 1979). All this provided uniform and statistically significant data that allowed unambiguous and reliable scientific conclusions, which were repeated from flight to flight.

Rat experiments flown on Kosmos biosatellites showed that these mammals can survive spaceflights of 18 to 22 days in duration, approximately 1/50 of their lifespan, without any irreversible pathologies in the viscera (Ilyin, Serova et al., 1976; Gazenko et al., 1978, 1980; Portugalov et al., 1976). Orbital flight did not lead to mutations in somatic or sex cells (Serova et al., 1982; Benova, 1985), shorten rat longevity (Serova et al., 1978), or cause marked changes in response to the additional stress-effects, which the animals were exposed to after flight (Serova, 1980). Microgravity did not exert any noticeable effects on radiation sickness in rats that were exposed to gamma-irradiation at doses of 2.2 and 8 Gy. The coefficient of the modifying effect of microgravity on the radiation sensitivity of most physiological systems was close to 1.0; it was 1.2 only for some blood parameters (Grigoriev et al., 1977; Kalandarova et al., 1978; Portugalov et al., 1978; Grigoriev et al., 1976; Kalandarova et al., 1976).

At the same time, some organs and systems of rats exhibited reversible changes which were subdivided into two categories: specific, related to the effects of microgravity *per se*; and nonspecific, related to the development of a stress-reaction (Gazenko et al., 1978, 1980; Serova, 1977, 1979).

Specific, microgravity-induced effects included muscle, bone, erythrocyte, and myocardium changes. Muscular changes included muscle atrophy, restructuring of myofibers, and molecular composition of contractile proteins, which was adequate to the spaceflight environment. The level of changes was closely correlated with the degree of muscle involvement in the antigravitational function on the Earth. The most significant changes were seen in the soleus muscle (Gaevskaya et al., 1976, 1979; Ilyina-Kakueva et al., 1977, 1979, 1981; Oganov et al., 1981; Oganov, 1984).

As with muscles, the greatest skeletal changes were seen in bones that perform the weight-bearing function in the Earth's gravitational field, particularly femoral bones. Bone studies revealed osteoporosis of spongy compartments, moderate thinning and loosening of cortical plates, significant loosening of the metaphyseal spongiosa, decline of periosteal formation, delayed osteoid maturation and bone mineralization, delayed bone growth in length, and decrease of bone strength (Yagodovsky et al., 1977; Stupakov et al., 1979; Holton, Baylink, 1979; Kazarian, 1980).

It should be noted that changes in the musculoskeletal system of rats produced by actual microgravity were similar to those observed in simulation studies. This similarity can be ascribed to the unloading of the musculoskeletal apparatus, which is characteristic of both actual microgravity and ground-based hypokinesia. One of the major causes responsible for musculoskeletal changes seen in microgravity is an altered pattern of activity of some muscles, and a lack of static load on large groups of muscles and the skeletal elements responsible for posture maintenance and the formation of tonic movements on the Earth. It is very probable that the latter is accompanied by changes in the pattern and quality of proprioceptive impulses, which in a normal environment

are necessarily involved in motor control, and muscle structure and function control (Portugalov, 1978a, b; Oganov, 1984).

Biochemical investigations of the heart performed by M. S. Gaevskaya et al. (1976, 1978, 1979) revealed a decline, by 44% on the average, of myosin ATPase. According to the authors, this could result from the underloading of the muscular apparatus in microgravity (an adaptive response to an "easier" work). Having in view the fact that the half-life of cardiac myosin is 6–8 days, the authors postulated that myosin with a lower ATPase activity was synthesized in an underloaded heart. As a consequence upon return to Earth's gravity, there can be a discrepancy between the required force of cardiac contraction and its support.

During 18–22 day flights, the above changes in the heart were reversible and disappeared entirely by the 26th postflight day. However, during Kosmos-690 flight, in which some of the animals were exposed to irradiation, the changes did not return to normal, although irradiation alone (in the ground-based synchronous control study) induced an increase rather than a decrease of myosin ATPase activity (Gaevskaya et al., 1978). This suggests that in a microgravity/irradiation experiment, and possibly in an extended flight, cardiac metabolic recovery may take longer, and may impact the cardiac function, and consequently, the health status and work capacity in general. Functional unloading may be responsible not only for musculoskeletal and cardiac changes, but also for erythrocyte shifts.

Blood examinations of Kosmos rats flown for 18–22 days showed a decrease of erythropoiesis in bone marrow (Kalandarova et al., 1981; Shvets et al., 1877, 1984; Kozinets et al., 1983; Kalandarova et al., 1976; Shvetz and Portugalov, 1976), a reduction of the number of hemopoietic stem cells (Vacek et al., 1982, 1983), and an increase of spontaneous hemolysis *in vivo* (Leon et al., 1978, 1980). Hemolysis and other parameters of red blood cells of Kosmos rats were investigated *in vivo* (Landaw et al., 1970). The original method used required measurement of ^{14}CO in the exhaled air and preflight labeling of some red blood cells with 2-^{14}CO-glycine. In the Kosmos-936 study, two groups of animals were examined: one was exposed to actual microgravity; the other, to artificial gravity of 1 g generated by an onboard centrifuge. The data obtained suggest that artificial gravity prevented changes in the life cycle of red blood cells which occurred in the flight animals, indicating that the blood changes could be ascribed to microgravity. Spontaneous hemolysis of red blood cells in the weightless rats increased to 1.32% versus 0.32–0.35% in the ground controls ($p < 0.05$) and 0.21% in the flight centrifuged animals. The mean red blood cell lifetime of the weightless rats decreased to 39.9 days versus 53 days in the controls ($p < 0.02$). The lifetime of red blood cells was shortened due to the fact that many died incidentally, while a smaller portion of the population "survived to reach a natural death," although the potential lifetime in the flown and control animals was identical (Leon et al., 1980).

Biochemical investigations of the viscera of rats demonstrated reversible changes in almost all types of metabolism, including that of nucleic acids. These changes included a decrease of RNA in Purkinje cells of the cerebellum, in large neurons of spinal cord intervertebral ganglia, and in the liver and spleen (Gazenko et al., 1976a, 1979; Gorbunova et al., 1977; Makeeva et al., 1976; Komolova et al., 1977, 1978, 1982). The general trend of these changes supports F. Z. Meerson's hypothesis (1963, 1967), that hyperfunction stimulates the synthesis of nucleic acids and proteins, while hypofunction inhibits it.

Muscle examinations showed changes in the enzymic activity of sarcoplasmic proteins which were closely correlated with the degree of atrophic lesions detected histologically. The level of atrophy of different muscles depended on their involvement in the antigravitational function (Gazenko et al., 1978, 1980; Ilyina-Kakueva et al., 1977, 1979; Oganov et al., 1979, 1981).

Spaceflight induced marked changes in lipid metabolism, including activation of lipolytic and lipogenetic processes (Ahlers et al., 1979, 1980, 1982a, b).

Changes in carbohydrate metabolism were evidenced by changes in skeletal muscle LDH isoenzymes (Petrova, 1977, 1978) and in the blood content of glucose, lactate, and pyruvate (Tigranyan et al., 1979).

Changes in fluid-electrolyte metabolism included water and electrolyte losses. Postflight examinations demonstrated an inadequate reaction to a potassium loading test; in spite of potassium deficiency in the body, the ability for its retention was poor (Natochin et al., 1979; Ilyin et al., 1980).

It is important to note that during the flights, the durations of which were about 1/50 of the life expectancy of the rats, both catabolic and anabolic processes were activated. Stimulated anabolism was evidenced by a high food utilization (Kondratyev et al., 1979) and a high oxygen consumption (Golov et al., 1977) during flight. It appears that in flights of this duration, the animal body is capable of compensating catabolic reactions which emerge as a response to musculoskeletal underloading. However, in spite of a phylogenetically predetermined conservative level of anabolic reaction stimulation, it can be asserted

that this response may disappear without adequate reinforcement. As a result, uncompensated progressive activation of destructive metabolic reactions may develop (Gaevskaya et al., 1976).

Nonspecific changes identified postflight included signs of a moderate stress-reaction in the hypothalamic-pituitary-adrenal system, lymph organs, and blood. These changes were: an elevated activity of neurosecretory cells of the hypothalamus; an increased activity of ACTH in the anterior pituitary; a decreased content of the neurosecretory substance in the posterior pituitary; enlarged adrenals; an increased volume of cell nuclei in the fascicular area; lipid decrease in the cortical layer; and an increased concentration of corticosterone (Savina and Alexeyev, 1979; Kvetnansky et al., 1979). Signs of a stress-reaction in lymph organs seen after flight included weight loss, a reduction of the total number of thymus and spleen cells, and an increase in the number of pycnotic nuclei and the concentration of polydeoxyribonucleotides (Durnova et al., 1977, 1978, 1979; Serova, 1975, 1977; Misurova et al., 1979); changes observed in the blood involved an increased concentration of corticosterone and glucose in plasma, a change in the lipid spectrum, lymphopenia, and neutrophilosis (Ahlers et al., 1979; Serova et al., 1979; Tigranyan et al., 1979).

Postflight examinations did not reveal significant changes in catecholamine metabolism. The flight and control animals did not show any differences in terms of epinephrine and norepinephrine concentrations in the hypothalamus and adrenals, as well as enzyme activity, synthesis, and degradation. There was only one case (Kosmos-782) when flown animals showed a significant increase in tyrosine-hydroxylase in the adrenals. At the same time, during the first days postflight, the animals exposed to an additional immobilization stress (5 times of 2.5 hours each) displayed a greater decrease of epinephrine and a higher increase of tyrosine-hydroxylase in the adrenals, when compared to ground-based controls (Kvetnansky et al., 1981).

Analysis of the data obtained and general concepts of adaptation suggest that the stress-reaction develops during the first days of flight, an acute period of adaptation to microgravity, and is then followed by a partial stabilization of the health condition. Another acute stress-reaction occurs at the time of landing, during transition from microgravity to 1 g. Examinations of the animals that were exposed to postflight immobilization gave evidence that health stabilization by the end of flight did not return to normal, but rather resulted in a new steady-state. The response to this additional chronic stress varied from that of ground-based controls in terms of several metabolic parameters, specifically catecholamines in adrenals.

Kosmos-936 was the first flight in which the physiological effects of artificial gravity were assessed, utilizing an effective countermeasure against the adverse effects of microgravity (Gazenko et al., 1980). Artificial gravity of 1 g was generated by an onboard centrifuge. The results demonstrated that artificial gravity can help control such changes as reduced activity of cardiac myosin ATPase, decreased bone strength, deteriorated contractility of skeletal muscles, and others.

Most Kosmos studies were performed using adult, sexually mature rats. The first attempts to study physiological mechanisms of mammalian adaptation to microgravity in terms of ontogenesis were made in Kosmos-605 and -1129 flights, after which small groups of flown rats were allowed to survive until their natural death. The Kosmos-1514 flight carried a dedicated embryological experiment, the results of which will be discussed in detail in this book.

A large body of embryological data has been accumulated in hypergravity experiments. Yu. G. Simakov et al. (1973) exposed nonadapted female mice to 2 g 10–12 hours after their fertilization, and observed abortions, the death of embryos, and lysis of blastomeres. Ishay (1977) exposed rats to centrifugation beginning with pregnancy day 2 or 10 and reported abortions or a reduction of the number of pups in the litter beginning with exposure to 1.43 g.

V. I. Baranov and A. A. Gurjian (1963) exposed rats to 1.5 g or 3 g beginning with their first days of life for 4–6 hours a day. They detected abnormalities in the estrous cycle, which included an extended resting phase and a shortened estrus phase. However, both experimental and control groups included rats whose pregnancies and offspring were normal. Unfortunately, the authors did not record the number of pregnancies, reproductive function, or the health state of newborns. Additionally, exposure to centrifugation was intermittent, making it difficult to compare this data with other observations.

J. Oyama and W. Platt (1967) evaluated the reproductive function of rats exposed to 2.5, 3.5 or 4.7 g. Prior to copulation, the animals were rotated for over 2 months and, consequently, adapted to the environment. Despite adaptation, none became pregnant at 4.7 g. At 3.5 g, 69% of inseminated females became pregnant, but the litter only numbered six pups, which died soon after birth. At 2.5 g, 86% of inseminated females became pregnant; the average litter numbered nine pups. Some survived, but only when the female and her offspring were removed from the centrifuge for 1618 hours per day during the first three weeks after delivery. The offspring of mice that

were born at 2.5 g in a similarly designed experiment survived even when they were not removed from the centrifuge (Oyama et al., 1967).

L. Janer and J. Duke (1984) allowed female mice to adapt to 1.82.6 g for several weeks, mated them with intact males at 1 g, and then returned pregnant animals back to the centrifuge. The number of pregnant females in the centrifuged group was 11% and in the control group, 29%. However, there was no difference in the embryo death rate or litter number between the two groups; nevertheless, the pups that grew in hypergravity were smaller in size. These observations are similar to what was detected in experiments in which rats were exposed to stress-effects (i.e., repeated immobilizations), during the time period that preceded their pregnancy (Serova and Denisova, 1983).

C. Wunder et al. (1963, 1968) exposed mice of different ages to accelerations of 1.5 to 14 g, which delayed the growth rate of the animals. At 7 g, the mice survived a year, while at 2 g their longevity remained normal, and the mice were able to conceive and nurse their offspring. C. Wunder et al. (1968) made reference to the study by Matthews who obtained three rat generations while rotating them at 3 G. However, he did not publish his data; therefore, it remains obscure how the experiment was designed and when the rats were mated: during rotation or not.

J. Oyama et al. (1965) exposed rats to 2.5, 3.5, and 4.7 g, for 4.5 and 12 months. As was in Wunder's experiments, centrifugation delayed the growth rate of animals, the delay increasing with the value of acceleration. After 4.5-month exposure to 2.5 or 3.5 g, no significant changes were seen in the viscera and bones of rats; after exposure to 4.7 g, the weight of the thymus decreased while that of other organs remained unchanged. After a 12-month exposure at 3.5 and 4.7 g, no marked changes in the weight of the liver, kidneys, spleen, thymus, pituitary gland, adrenals, lungs, heart, or femurs were seen, although the body weight of experimental and control animals differed. After exposure to 4.7 g, body weight averaged 231 g, while after exposure to 3.5 g, body weight was 272 g versus 323 g in the controls. The weight difference was primarily related to differences in the adipose tissue of chronically centrifuged animals (Keil, 1969; Oyama et al., 1965; Pitts et al.. 1972).

Briney and Wunder (1962) reported an increase of the weight of the heart, diaphragm and gastrocnemius muscle of hamsters exposed to centrifugation at 45 g for four weeks. At the same time, no changes in the muscle weight of mice exposed to 4 g for 8 weeks were found (Bird et al., 1963).

Interest in the viability and longevity of animals under hypergravity conditions was probably the reason why many scientists performed chronic experiments during which animals were rotated for months, and even years, with the most important observations being made at the later stages of exposure, when the initial alterations may have already returned to normal.

Disappearance of primary changes and adaptation to hypergravity may explain the lack of significant changes in the weight and structure of internal organs of animals during a prolonged (up to 12 months) exposure to 24 g. Conversely, the acute stage, which can be termed the stage of hypergravity stress, as evidenced by a substantial weight loss during the first days of rotation, remains inadequately studied.

The experimental observations available suggest that mammals can survive an essentially indefinite exposure to 24 g without serious changes in their viability. At the same time, it should be noted that at 23 g, although on the average the longevity remained normal, some of the animals died. It was demonstrated that in principle mammals can grow and develop when the maternal body is exposed to hypergravity; nevertheless, reproduction parameters were not normal (Oyama et al., 1967; Janer et al., 1984). This points to adverse changes in the hormonal and metabolic status of the maternal body . Regretfully, no dedicated embryological experiments were conducted within the framework of the above studies.

At present, a huge amount of data has been obtained from manned space flights. The early Soviet manned missions, the durations of which were from 108 minutes to 4 days, and American Mercury flights gave evidence that man can survive in space and control the spacecraft (Volynkin et al., 1967; Yuganov et al., 1962; Dietlein, 1970).

Observations made during the Soviet Voskhod and Salyut flights, and the American Gemini and Apollo flights, the durations of which were in the range of 1 to 18 days, showed than man can adapt to relatively extended orbital flights. The results of these experiments are discussed in detail in many review articles (Kasyan et al., 1964, 1967; Yazdovsky et al., 1964; Kakurin et al., 1966; Parin and Gazenko, 1967; Gazenko, 1968; Vasilyev et al., 1969, 1974; Vorobyov et al., 1970, 1984a, b; Kovalenko, 1974; Pestov, Gerathewohl, 1975; Kotovskaya, 1976; Gazenko and Shulzhenko, 1978; Gazenko et al., 1980; Berry, 1968, 1970, 1991; Leach, 1974; Rambaut et al., 1979).

Man's adaptation to microgravity was accompanied by marked physiological changes in some organs and systems, the most important being spatial disorientation, cardiovascular deconditioning, body weight loss, red

blood mass decrease, reduction of calcium and other electrolytes, muscle atrophy, and decline of muscle strength and tone. Cardiovascular deconditioning led to a deterioration of acceleration tolerance upon return to the Earth and a decrease of orthostatic tolerance and physical work capacity. In most cases however, these changes returned to normal a short time after flight. After Apollo missions that continued for 147260 hours, 20 out of 27 astronauts displayed a decline of work capacity; however, this parameter returned to the preflight level within 2436 hours postflight (Berry, 1970; Rummel, 1975). The recovery period was the longest (about 30 days) after the 18-day flight of Soyuz-9 (Vorobyov, Egorov et al., 1970).

At the early stage of manned missions, flight time was allowed to increase in a systematic but cautious manner, although the results were encouraging, probably because scientists still expected undesirable and dramatic changes induced by microgravity exposure. It is interesting to quote here Charles A. Berry (1981):

> Prior to the 4-day spaceflight I had received a lot of telephone calls from all over the country the night before launch. I was told that the crew would die when they egressed from the space-craft, that they would not be able to maintain their upright posture or that they would be drowned when they tried to get on an inflated raft.

Nevertheless, studies performed during extended manned missions—a month, three months, six months and longer—helped to accumulate a huge amount of data and revealed no changes that would have not occurred in short-term flights. Moreover, the physiological changes that were seen in short-term flights did not increase in intensity with flight time, provided that the crew was in a comfortable environment, adhered to a rational work-rest cycle, and exercised regularly (Vorobyov et al., 1984). The positive results were primarily due to achievements in the development of countermeasures against adverse effects of microgravity. As O. G. Gazenko (1984) stated, "the strategy of flight medical support is essentially how to control the health status of cosmonauts, to be more precise, how to control the process of adaptation with the purpose of providing a moderate level of adaptation to microgravity and maintaining adjustment to the Earth's field of gravity." Thanks to these efforts, after the 175- and 185-day flights exercise tolerance and cardio-vascular responses to exercise tests were very close to the preflight level (Vorobyov, Gazenko et al., 1984).

These observations give evidence that some cosmonauts have a high degree of tolerance to the spaceflight environment, and that man can adapt quite well to spaceflight effects. After long-term flights, as with short-term flights, symptoms of orthostatic and exercise intolerance were detected; however they were transient and of comparable degree (Vorobyov et al., 1984).

In short-term flights, the level of physiological changes was correlated with orbital flight duration; in longer-term flights the level of variations was more dependent on the efficacy of the countermeasures used in space (Gurovsky et al., 1974; Gazenko et al., 1976; Vorobyov et al., 1984; Kozlovskaya et al., 1981).

In Skylab astronauts, cardiovascular parameters of the crewmembers who made a 28-day flight returned to the prelaunch level only by the 21st day postflight. Con-versely, the parameters of the crewmembers who made 56- and 84-day flights returned to the prelaunch level on the 4-5th day after recovery, attributable to the fact that they exercised regularly and intensely during flight (Dietlein, 1974).

On the average, the decrease of the red blood cell mass was 17% in Gemini astronauts , 10% in Apollo astronauts, and 8% in Skylab astronauts. The loss of red blood cell mass in crewmembers of the 28 day Skylab-2 flight amounted to 9.4%, and in crewmembers of the 84 day Skylab-4 flight, to only 5.9% (Dietlein, 1974; Kimzey et al., 1974; Johnson, 1974).

The experimental data available today appears to permit a very optimistic approach to the possibility of prolonged manned spaceflights; however, it is symbolic that when speaking about colonies in space, we are discussing the necessity of generating artificial gravity (O'Neil, 1974; Winkler, 1978), exactly as K. E. Tsiolkovsky did almost a hundred years ago. These reservations may be associated with the fact that our space achievements have been accomplished with the participation of crewmembers who passed a rigid system of selection, who proved to be most resistant and well trained, and who used various counter-measures against adverse effects of microgravity. These factors essentially prevented complete adaptation to microgravity and made it impossible to investigate microgravity-induced reactions at their peak.

At this stage (as with the earlier stages that preceded the first man-in-space flight), animal studies acquire a greater importance: they can help gain insight into the structure of the viscera and help to better assess the effects of microgravity by using a large amount of uniform bio-samples, undistorted by preventive measures and social-emotional factors. In this respect, of key interest are the Kosmos-1514 and Kosmos-1667 primate experiments, in which implanted sensors and electrodes were used to study mechanisms of physiological adaptation to

microgravity (Kozlovskaya et al., 1985; Korolkov et al., 1985; Ilyin, Gazenko, 1984).

In recent years biological objects of different levels of organization (from micro-organisms to higher animals and man) were flown in space. However, the data obtained have never been comparatively studied. A similar comment can be made about hypergravity investigations: responses of animals of different classes are mainly compared in terms of their size (Wunder et al., 1968; Smith, 1975) rather than in terms of their nervous and endocrine systems.

The only exception is the work by N. N. Sirotinin (1961). While evaluating the tolerance of various animals to the effect of radial acceleration, he found that the parameter deteriorated as the species' evolutionary development level increased.

Invertebrates (coelenterates, worms, mollusks, arthropods) survived exposure to 28,000 g for several minutes; very few died when subjected to 2,000 g for an hour. Poikilothermic vertebrates (fish, newts, frogs, snakes, lizards, turtles) showed a lower resistance to radial acceleration than invertebrates: they survived exposure to 2,000 g for several minutes and to 50 g for 60 minutes or more. Homoithermic animals exhibitted a dramatically lower resistance to radial acceleration: birds (sparrows, pigeons) died when exposed to 50 g for several minutes. Mammals of various species showed different types of resistance to acceleration: some responded like birds, while others displayed a much lower accelerational tolerance.

This decline in acceleration tolerance correlated with increased evolutionary organization was also observed in a second study. N. N. Sirotinin (1963) reported a similar pattern in resistance to hypoxia: unicellular organisms showed the highest level of tolerance; multicellular invertebrates, a lower level; and cold-blooded vertebrates, birds, and mammals, lower still. Within a taxonomic class, different levels of resistance can also be discerned, correlated with the complexity of the organization of the animal body as a whole and its nervous system in particular. For example, the altitude limit for humans is 7,000 m, and for rats, 12,000 m. These variations are, however, not very dramatic compared to those observed within the evolutionary framework.

At the same time, when animals ascend the evolutionary ladder, their resistance decreases while their susceptibility to environmental effects increases: passive resistance of invertebrates is transformed into active resistance, reaching a maximum in mammals (Sirotinin, 1981). Higher invertebrates (crustaceans, cockroaches) react to oxygen insufficiency: their breathing rate increases. Poikilothermic vertebrates (e.g., snakes) develop such a response at moderate altitudes (about 3,000 m). Mammals display more profound and more diversified responses, viz. a higher respiratory rate, increased oxygen utilization, stimulated erythropoiesis, etc.

Consistent changes in resistance and reactivity can also be distinguished in animals at various ontogenetic stages. It has been found that mammalian newborns (mice, rats, rabbits, cats, dogs) are more resistant to radial acceleration than adults (Sirotinin, 1961). However newborn guinea-pigs, which are better developed at birth than the above animals, have an almost identical resistance as adults. A similar pattern of responses can be observed in the reactions of newborns to hypoxia, histamine, and other effects (Sirotinin, 1981).

The mechanisms underlying these responses can be better understood through the study of resistance and reactivity of hibernating animals. During hibernation, animals exhibit a greater resistance to radial acceleration, oxygen deficiency, strychnine, cyanides, histamine, and other exposures (Sirotinin, 1981).

The reactivity of an animal body, its capacity to respond to environmental effects in a specific way, is related to "the degree of its development as a whole and the level of sophistication of its central nervous system, endocrine glands, reticular and endothelial system; it is also dependent, but to a smaller extent, on the status of other systems and organs; it is certainly inseparable from its metabolism" (Sirotinin, 1981, p. 132). In the course of evolution and ontogenetic development, the factors responsible for the reactivity of an individual organism, as well the reactivity itself, can vary.

A decline in passive resistance during evolutionary and ontogenetic development is essentially the price the organism pays for its sophistication as a single growing system. When the system becomes more complicated, the scope of its reactions and contacts with the surroundings increases. However this "free life," as Claude Bernard remarked, demands that vitally important constants be maintained within narrow limits and that more sophisticated feedback systems be initiated (Barcroft, 1937). When the sensitivity of the system decreases (i.e., when some of its components are shut-off as in hibernating animals, or when it is incompletely activated as in immature mammalian newborns), system resistance increases while reactivity deteriorates. The physiological potentials of the organism, specifically the scope of its contacts with the environment, decline: the system seems to step down to a lower rung of the evolutionary ladder.

Similar variations in the reactivity of animals of various classes must occur in microgravity as well. Unfortunately, the above phenomena have never been investigated from a

comparative perspective. Nevertheless, the above data are in conformity with Sirotinin's concept concerning evolutionary variations of resistance and reactivity. The lack of responses to microgravity seen in insects (Miquel et al., 1979; Parfyonov et al., 1979, 1980); the manifestation of such responses in fish and amphibians only at the very early stages of their development (Palmbakh, 1976; Dorfman et al., 1977; Belousov et al., 1979) or in weaker (i.e., having higher susceptibility) spawnings (Cherdantseva, 1983); and a wide range of reactions to an altered gravity in birds (Burton et al., 1965; Smith et al., 1980), and particularly, the responses in mammals (Gazenko et al., 1978, 1980) all suggest a similar pattern of resistance. The only deviation from this pattern are turtles, who exhibited postflight changes which were comparable and sometimes superior to those seen in mammals (Petrukhin et al., 1976).

Chapter 2

Primary Goals and Conditions of the Mammalian Embryology Experiment on Kosmos-1514

2.1 The Objectives of the Experiment: A Comprehensive Program of Postflight Examinations of Animals

The basic objective of the mammalian embryology experiment on Kosmos-1514 was an attempt to discover whether homeostasis in the mother-fetus system can be maintained and whether the fetus can develop normally in the maternal body exposed to microgravity.

The embryology experiments on insects, fish, and amphibians performed in real and simulated (clinostating) microgravity, as described in Chapter 1, were not very useful for predicting reactions of mammals because mammals are more susceptible to environmental effects (Sirotinin, 1981) and because reciprocal influences in the mother-fetus system are more complicated.

The experiment was performed on white laboratory rats which, according to G. Buckle (1986), are "the primary experimental model of mammals." The choice was made because a small number of these experimental animals, kept in a limited volume of the spacecraft, could provide a statistically significant amount of data; also, the flight results could be compared with the data obtained from other rat studies in different environments.

The preparation and conduct of the mammalian embryology experiment was difficult. The very possibility of the development of a mammalian fetus in microgravity was not undisputed. The normal function of the mother-fetus system strongly depends on the stimulation of uterine mechanoreceptors by a growing fetus; fetal growth and development are influenced by receptors in the vessels and skin of the abdomen, organs in the abdominal cavity, and abdominal wall musculature (Savchenkov and Lobyntsev, 1980). It was logical to expect that in microgravity, the information supplied via these channels would be different from that on the Earth. It was hypothesized (Arshavsky, 1979) that fetal development in microgravity was impossible. Taking this into consideration, we prepared several experimental designs. One of them was a minimum program, which allowed assessment of the reproductive ability of female rats when mated at different time intervals upon return to the Earth: this program would be implemented if no fetuses or newborns were available after flight.

Nevertheless, we expected that the flight experiment would be a success and postulated that the effect of microgravity, on the developing fetus suspended in amniotic fluid, would be mediated by the maternal body, depending on its hormonal and metabolic status.

Previous examinations of adult male rats flown on Kosmos biosatellites revealed changes which might exert an adverse effect on fetal development, if they occurred in the maternal body. These were primarily osteoporosis, involution of lymph organs, decline of erythropoiesis, and deterioration of red blood cells (Gazenko et al., 1980). The development of a fetus requires significant and rapid activation of anabolic processes. To what extent would this be possible in microgravity, when catabolic events in the adult animal body are stimulated? What would be the pathway of organogenesis, particularly of bone development, under conditions of calcium deficiency in the maternal body? These and many other questions formed the foundation of the design of the embryology study flown on Kosmos-1514.

The experimental protocol took into consideration the critical, most vulnerable stages of prenatal mammalian development. It was decided to concentrate on the third term of pregnancy, i.e., the period at which adverse environmental effects cause the lowest death rate of fetuses (Svetlov, 1956, 1960; Dyban, 1967; Arshavsky, 1960, 1964). At the same time, this is a crucial period in the formation of vital organs and physiological functions. This is why we might expect serious changes, which would help determine whether the fetus can normally develop in the maternal body exposed to microgravity.

The Kosmos-1514 flight was preceded by many simulation studies, intended to help develop adequate methods and procedures, as well as to accumulate baseline data. The latter was achieved through experiments on more than a thousand females and their offspring, exposed to both normal and extreme conditions. These observations allowed us to make unambiguous inferences from a limited amount of flight data.

The basic objective of postflight examinations was to give a complex evaluation of the mother-fetus system and to follow-up, not only immediate, but also longer term flight effects on the fetuses and newborns. Special attention was paid to the organs and systems which showed changes in previous flights. It is known that the fetus can often develop disorders in the organs that showed abnormalities in the pregnant mother. According to L. I. Gromova and E. A. Savina (1964), a lesion of a mother's organ may prematurely accelerate the development of a lesion in the respective organ of the fetus, possibly leading to organ failure.

The postflight examination included dissection of five female rats immediately following recovery, weight measurement of fetuses and placentas, assessment of the fetuses in relation to their age (Dyban et al., 1975), fixation of selected biosamples for further study of developmental abnormalities (Dyban et al., 1970), investigation of the skeleton (Dawson, 1926; Akimova, 1968), study of fluid and electrolytes in the fetuses and placentas, etc. Postflight examinations also required a detailed investigation of adult rats exposed to microgravity at the third term of pregnancy, with emphasis on the musculoskeletal system, endocrine system, and hemopoietic organs. All these studies were performed using methods similar to those used in previous Kosmos flights (Gazenko et al., 1980).

Another group of five rats was allowed to survive until natural delivery, with the purpose of examining the pups, studying the behavior of mothers during nursing, and investigating (during a three-month follow-up) behavioral responses of the litter, beginning with simple responses and ending with intricate behavioral patterns in mazes of different design. An investigation of the structure and metabolism of internal organs of rats dissected on postnatal days 15, 30, and 100 was also planned.

This sophisticated program of rat research required the participation of a large number of scientists with expertise in many disciplines. Preparation for the study included the establishment of a cooperative network in the USSR and other countries. The embryology study was made possible by scientists from different laboratories of the USSR, as well as Bulgaria, Hungary, GDR, Poland, Rumania, Czechoslovakia, France and the USA. These scientists contributed to the development of methods and procedures for evaluating the state of the mother-fetus system, different from previous approaches in terms of the number of organs and systems to be addressed. These methods and procedures were applied not only to the microgravity investigation, but also to that of other spacefligt factors in ground-based studies.

2.2 The Effect of Factors Acting at Launch and Recovery on the Prenatal Development of Rats

Prior to the embryology experiment in space, a number of experiments were carried out on the Earth to investigate the effect of vibration, linear acceleration, and impact acceleration, acting at launch and recovery on various stages of the Wistar rat prenatal development. The prenatal development of mammals includes the so-called critical periods characterized by enhanced sensitivity of embryos and fetuses to adverse environmental effects. According to P. G. Svetlov (1956, 1960), for rats these critical periods are pregnancy days 4 and 10 to 13. Many different factors have been recently tested with respect to their embryotropic effects, and it has been shown that some are hazardous throughout the entire pregnancy, whereas others produce an adverse effect only at certain stages, being stage-specific (Dyban, 1967; Arshavsky, 1960, 1964). Unfortunately, data concerning the effect of physical factors on mammalian development are scarce, while data concerning the impact of vibration, linear acceleration, or impact acceleration (which occur at launch and recovery) on the mother-fetus system are nonexistent. These data are necessary however, for conduct of a successful flight experiment, so as to take into consideration potentially critical periods, to minimize losses at launch and recovery.

Ground-based experiments were carried out on 110 pregnant rats of the Wistar strain. They were exposed to the effects similar to those seen in other Kosmos flights: vibrations of 50 to 70 Hz for 10 min; linear acceleration of 4 g for 10 min; and impact acceleration of 40 to 50 g (normal landing) and 90 g (contingency landing) for 40 msec. The animals were exposed to the effects typical of spaceflight at different pregnancy stages, beginning with day 3. The rats were kept unrestrained in flight-type cages, and their reproductive organs and fetuses were examined upon female dissection at the end of the pregnancy period, day 21.

When we exposed experimental animals to the combined action of vibration and linear acceleration (to simulate launch) at pregnancy days 3, 4, and 8, we did not record an increase in the prenatal death rate. The number of live fetuses was identical in experimental and control groups. Similar results were obtained during exposure to 40–50 g (to simulate landing) at pregnancy days 7, 9, 11, 13, 19, and 21.

However, in response to the combined effect of vibration and linear acceleration applied at pregnancy day 8, and impact acceleration at pregnancy days 7, 9, 11, and 13, most rats exhibited placenta abnormalities which were not seen in the controls (over 1000 animals were examined). These abnormalities were: fusion of one or sometimes two placenta pairs in every female, which led to a lower weight of the fetuses, probably, due to their poor supply with nutrients (fig. 1).

Placenta fusion in rats occurs extremely rarely. In the literature, only one reference to such an abnormality was found, a single spontaneous change in a large group of rats which were not exposed to any experimental effects (Kashmiri, 1977). Conversely, vibration, linear acceleration, and impact acceleration provoked this abnormality in most experimental animals. This may be attributed to visceral displacement, altered uterine contractility, or other problems requiring special study.

Thus, factors common to spaceflight (vibration, linear acceleration and impact acceleration) did not affect the mother-fetus system, particularly during the first half of pregnancy.

In our experiments, impact acceleration produced the most tangible effect. On pregnancy days 7–11, after exposure to impact acceleration of 40–50 g (which may occur at a normal landing), studies revealed not only placenta fusion, but also ectocardia (fig. 2) and umbilical hernia, abnormalities never observed in the control fetuses. However, exposure to impact acceleration of the same value at the end of pregnancy (day 19 or 21) did not reveal any serious fetal developmental abnormalities.

After exposure to impact acceleration of 90 g (which may occur at a contingency landing) on pregnancy day 19 or 21, we recorded a significant decrease in the number of live newborns and found a greater amount of pups having various abnormalities, primarily hemorrhages of different localization (table 1).

These observations led us to the conclusion that female rats should be flown, at least in the first embryology experiment, during pregnancy days 13 ± 1 through 20 ± 1. This experimental design was tested in a ground-based bioengineering study.

Ten female rats of the Wistar strain were exposed to vibration of 50–70 Hz for 10 min and linear acceleration of 4 g for 10 min on pregnancy day 13 (to simulate launch), and to linear acceleration of 6 g for 10 min and impact acceleration of 40 g for 40 msec (to simulate reentry and landing), on pregnancy day 20.

Table 2 gives parameters characterizing the reproductive function of rats dissected on pregnancy day 20, five hours after exposure to impact acceleration. In terms of total embryo death rate, number of live fetuses, and weight of fetuses and placentas, there were no significant differences between the treated and control animals.

Fetal examinations according to the method of Wilson, modified by A. P. Dyban (1970), demonstrated abnormalities in 27.3% of the test rats (mostly hemorrhages of various severity and localization) and 2.9% of the controls ($p < 0.01$). A larger number of placentas with infarcts and hemorrhages was also seen in the exposed rats ($p < 0.05$).

In spite of the higher percentage of abnormalities, primarily hemorrhages, in the exposed animals, the fetuses did not lag behind controls. As mentioned above, their weight was very similar. (table 2) The total body water content and skeleton formation, as well as the length of ossified regions in various skeletal compartments, were identical. This suggests that hemorrhages occurred immediately before dissection as a result of impact acceleration. It is probable that at later developmental stages these injuries would be compensated. Finally, the duration of pregnancy, number of pups in the litter, and weight at birth was similar in both the exposed and control animals.

The clinical state of pups, and their growth and development during the first postnatal month, were also similar in the treated and untreated animals. Results did not show any differences in the rate of body weight gain or the time of hair growth. The only difference, although insignificant, was that eye opening occurred earlier in treated animals than in the controls.

These results strengthened the recommendation that female rats at the last term of pregnancy (between days 13 ± 1 to 20 ± 1) should be chosen for the Kosmos embryology experiment. When exposed to launch and recovery factors during this period, female rats delivered offspring which did not differ from the controls with respect to the total number of pups, the physiological maturity at the terminal fetal stage and at birth, nor the rate of growth and development during the first postnatal month of life.

2.3 The Effect of Hypergravity on the Development of the Mammalian Fetus

In addition to the above experiments, which simulated the effects of launch and recovery on the mother-fetus system, we also carried out a centrifugation study at 2 g.

It was previously shown that a modification of the gravity field within ±1 g as related to the Earth's gravity would induce not only specific, but also nonspecific changes, viz. growth delay, involution of lymph organs, and lymphopenia, which are typical symptoms of a stress-reaction (Serova, 1977). These observations suggested that a study of the effect of 2 g on the mother-fetus system would be a suitable model for predicting changes that can be expected in microgravity.

Ten female rats of the Wistar strain (aged 5 months) were rotated in a centrifuge during pregnancy days 14 through 21. The radius of the centrifuge measured 141 cm, and its rate of rotation was 33.3 rpm. Rotation was terminated once a day (from 9.00 to 9.30 a.m.) to feed the animals and to clean the facility. The reproductive function of the centrifuged animals was compared with that of synchronous controls, which were exposed to the effects concomitant with rotation (noise, air flow) but not rotation *per se*, and with that of vivarium controls. The animals were dissected 5–10 hours after they were removed from the centrifuge on pregnancy day 21.

In the course of their exposure to 2 g from pregnancy day 14 through 21, the animals lost on the average 3 g. Similar changes were seen in the synchronous rats, while vivarium animals gained about 60 g. As compared to the vivarium controls, both centrifuged and synchronous rats showed an identical thymus involution. They exhibited no significant differences in the absolute or relative (as calculated per 100 g body weight) weight of the kidneys, liver, heart, adrenals, or spleen.

The centrifuged, synchronous, and vivarium rats also showed no differences in the content of water, sodium, potassium, calcium, copper, or iron in the heart, liver, or tibia. However the kidneys, particularly the papillary area, of both the centrifuged and synchronous rats displayed a reduction of water, iron, and copper, and an increase of sodium and potassium (Denisova et al., 1983). These changes could be ascribed to the effects of nonspecific stress-factors, which also affected animals during centrifugation.

Pregnancy induced anemia, and the hemoglobin level in blood and the count of erythroblast cells in bone marrow in the centrifuged rats was significantly lower than in the synchronous and vivarium controls. Bone marrow lymphocytes in the centrifuged rats were also markedly lower than in both control groups. The lymphocyte/ neutrophil ratio in blood declined during pregnancy, with fewer lymphocytes and more neutrophils, but remained identical in the centrifuged, synchronous and vivarium rats (fig. 3).

Study of nucleic acid metabolism in the liver detected no differences in terms of RNA and DNA per weight unit,or RNA-polymerase activity between the centrifuged and synchronous rats (Makeeva et al., 1983).

There were substantial differences between the centrifuged and synchronous rats with respect to connective tissue metabolism. Bone and skin changes were of different types. The centrifuged animals showed an elevated concentration of total collagen, specifically of insoluble collagen in the femur of centrifuged rats. The content of soluble collagen varied insignificantly, although the relationship between different types changed markedly: the content of type I collagen decreased, whereas that of type III collagen, which is characteristic of an actively growing connective tissue, tended to increase; the content of glycoprotein-bound collagen also grew. Bone glycoproteins increased due to an elevation of the percentage content of fibronectin (adhesive protein binding cells and other elements) and to a decrease of acid α-glycoproteins. In the skin, these changes were found only in glycoproteins: the total content remained unchanged, while fibronectin concentrations increased and α-glycoprotein decreased (table 3).

In summary, the pregnant female rats centrifuged at 2 g exhibited two kinds of changes: type I changes included growth delay, thymus involution, and the like, which were seen both in the centrifuged and synchronous rats and associated with a stress-reaction; type II changes included exacerbation of pregnancy-related anemia, decreased content of lymphocytes in bone marrow, and metabolic changes of collagens and glycoproteins in bone, all detected only in the centrifuged animals.

Centrifuged and control rats did not show any differences in the total death rate of embryos or in the percentage of abnormal fetuses and placentas (table 4). A slight decline of these parameters in comparison with the vivarium controls was probably related to the stress-reaction of the maternal body, which developed both in the centrifuged and synchronous animals. However, the number of live fetuses in the centrifuged and control animals was essentially the same, and their morphological characteristics were appropriate to their calendar age.

The weights of fetuses and placentas of the centrifuged rats were significantly lower than the weights of the synchronous controls (3.71 g versus 4.24 g, $p < 0.01$). The decline in fetus weight was mainly caused by a decrease in dry weight, while water content, as calculated per kilogram of dry weight, remained unchanged (fig. 4).

In our studies, special attention was given to electrolyte changes in the developing fetuses (see fig. 4). In spite of body weight losses, the total content of potassium, calcium, and magnesium, as well as copper and iron, did not decrease. Therefore the concentration of potassium, calcium, and iron, when calculated per wet weight, and especially by dry weight unit, proved higher in the centrifuged than in the synchronous or vivarium rats. Although the total content of calcium in the fetuses of centrifuged rats remained unchanged, they displayed a delayed skeletal development, which may have been caused by a delayed calcium incorporation into bone. This delayed skeletal growth in the centrifuged rats manifested as a significant reduction of the ossified compartments of the fore- and hindlimb bones, tail shortening, and fontanel enlargement, as compared with the synchronous and vivarium controls (tables 4 and 5).

A comparative analysis of liver metabolism of the fetuses in the centrifuged and synchronous rats showed no significant differences with respect to RNA, DNA, or RNA-polymerase (Makeeva et al., 1983).

In summary, exposure of female rats to 2 g during the last term of pregnancy did not prevent their delivery of a normal number of live fetuses, although the maternal body developed a stress-reaction which involved a significant activation of catabolic processes and delayed

growth. The differences between the centrifuged and control fetuses were insignificant and essentially limited to delayed growth and skeletal development.

Since our previous studies had demonstrated that hypergravity and microgravity can cause similar nonspecific changes such as delayed growth, thymus involution, and the like (Serova, 1977), the above centrifugation observations allowed us to predict that mammalian fetuses exposed to microgravity, during the last term of pregnancy, would develop in a normal manner. The results of the embryology experiment flown on Kosmos-1514 verified this prediction.

2.4 Experimental Conditions on the Kosmos-1514 Biosatellite.

The Kosmos-1514 experiment used female Wistar line rats from the "Stolbovaya" nursery aged approximately 4 months with body weights of 280–310 g. The animals were prepared for flight in accordance with a special program, which included clinical and physiological examination (evaluation of overall state, weight gain pattern, hematological and microbiological examination), assessment of estrous cycles, habituation training, and formation of a test group to evaluate compatibility 10–12 days before flight.

The female rats of the flight group were impregnated on the ground before launch. Females with regular estrous cycles were placed in cages with males, whose fertility had already been confirmed, in a ratio of 1 male to 3 females. The first day of pregnancy was counted as the day when spermatozoids were found in a vaginal smear. Launch occurred at the beginning of the 13th day of pregnancy and reentry at the beginning of the 18th day of pregnancy.

During the 5 day flight, 10 pregnant female rats were housed in a Bios-vivarium unit (fig. 5) measuring 160 x 220 x 660 mm. The animals received a special paste-like food (Kondratyev et al., 1979) in the quantity of 55 g (94.5 Cal) per rat per day and water *ad libitum*. The cage was illuminated for 16 hours daily during flight. Air temperatures fluctuated within the range of 20–24°C; pO_2 varied in a range of 150–210 mm Hg, with pCO_2 up to 1.5 mm Hg.

Three groups of control animals were used: a vivarium control group (n = 25); a baseline control (n = 30), dissected on the day of launch for evaluating initial fertility and the general state of the animals; and a synchronous control group (n = 10), housed in a mock-up of the biosatellite and exposed to the effects similar to launch (vibration, linear acceleration) and recovery (short-term impact acceleration, generated when the parachute container is jettisoned).

In accordance with the experimental design, on the day of reentry, 5 female rats of the flight group were dissected, providing approximately 60 fetuses for study. The remaining 5 females were allowed to continue their pregnancy to term, and approximately 50 living neonates were obtained from them. These neonates were observed postnatally for several months, until reaching sexual maturity.

Chapter 3

State of Female Rats Exposed to Microgravity During Pregnancy

3.1 General State of the Animals: Body and Organ Weight, Blood Profile

Before the flight, the state of the rats was fully satisfactory, growth was good, and blood profiles were normal. The control animals dissected on the launch day did not show any visceral changes or pregnancy abnormalities. When the rats were removed from the Bios-vivarium unit following biosatellite reentry, it was noted that their coats and tails were soiled with food. Nevertheless, the state of the animals was satisfactory: they were active and mobile. When 5 of the animals were dissected, no pathological changes were noted in the organs of the thoracic or abdominal cavities, nor were any instances of damage to the bones of their legs or organs, or signs of internal bleeding, seen.

During the flight, pregnancy days 13 through 18, the flight animals gained 5 g in weight, while the synchronous animals gained 65 g, a normal gain for this period of pregnancy. Consumption of food by flight and synchronous groups was virtually identical, averaging 54 and 57 g per rat per day, respectively.

Concentration of hemoglobin in the blood of the female rats of the flight group was 9.8%, significantly below that of the vivarium and synchronous control groups. The reticulocyte count in the flight animals was scarcely more than one quarter of that in the vivarium control, and half that of the synchronous control (table 6).

When the animals were examined several hours after reentry, the flight rats displayed a significant increase in the leukocyte count in peripheral blood (table 6), mainly attributable to an increase in the count of band neutrophils; the lymphocyte/neutrophil ratio decreased to 0.5, with the corresponding values being 2.1 in the synchronous group and 3.8 in the vivarium control. Such changes, representing an acute reaction to spacecraft reentry and return to Earth's gravity, were also observed in previous biosatellite experiments, in male rats exposed to microgravity for 18–22 days (Serova et al., 1979).

Animals in the flight group dissected on the day of reentry, day 18 of pregnancy, displayed a decrease in thymus weight to 238 mg, while the analogous weights were 338 and 273 mg for the vivarium and synchronous control groups, respectively ($p < 0.05$). A similar pattern was observed when the total number of lymphocytes in the thymus was counted: in the flight animals there were 738 mln., while there were 1149 mln. in the vivarium, and 1044 mln. in the synchronous control group.

The weight of the adrenal glands in the flight rats was elevated to 92.8 mg, the adrenal weight being 82.3 g in the vivarium, and 79.8 mg in the synchronous control group; however, the difference between the groups was not statistically significant ($p > 0.05$).

The flight animals displayed a significant decrease in the weight of the liver and myocardium (table 7); the absolute weight of the kidneys in females of all groups was the same, but since the flight rats showed virtually no weight increase, the relative weight of the kidneys was greater in the flight than in the control animals ($p < 0.02$) at the end of the flight.

3.2 Concentration of Hormones in Blood Plasma

As known, microgravity affects neuroendocrine regulation in adult animals (Gazenko et al., 1978, 1980a). In this context, a spaceflight lasting approximately 3 weeks acts as a stressor of moderate intensity on male rats. Pregnancy alters certain endocrine functions and environmental sensitivity; the most likely of these functions to respond to stress during pregnancy are somatotropic, lactotropic, and adrenocorticotropic.

Secretion of somatotropic hormone (STH) in rats is regulated by the interaction of the stimulating hypothalamic hormone, somatoliberin, and inhibiting hormone, somatostatin. The secretion of STH is pulsed; over the course of a day there are 8 peaks of secretion elevation, which alternate with low levels of STH in blood (Tannenbaum et al., 1976). Secretion of prolactin is regulated by the stimulating factor, prolactoliberin, and the inhibiting factor, dopamine. The level of prolactin in unstressed animals is low, due to the inhibiting effects of dopamine (Weimer and Gannong, 1978). Secretion of corticosterone in adult rats also undergoes diurnal oscillations: in the morning the level of corticosterone is low, while in the second half of the day it is significantly elevated (Krieger, 1979).

Secretion of the above named hormones is sensitive to any stressor, with corticosterone (Mikulaj, 1973) and prolactin increasing the most rapidly. The level of growth hormone in rats decreases in response to stress as a result of intensified somatostatin secretion (Terry et al., 1976); similarly secretion of insulin also decreases (Vigas et al., 1973). It was of unquestioned interest to determine the level of these hormones in rats exposed to microgravity on Kosmos-1514 on days 13–18 of pregnancy.

Blood for analysis was taken when the animals were decapitated several hours after flight. Prolactin and

somatotropic hormone were determined by RIA, using a specific antibody or activated charcoal to separate the free and bound forms of the hormone. Hormone preparations and antiserum were provided by NIH MDDK (Bethesda, U.S.A.). Corticosterone was measured using the protein binding method. Insulin was measured by RIA, using a RIA OPIDI (Poland) kit.

The female rats of the flight group did not manifest any significant changes in plasma growth hormone (fig. 6); however, individual variability of this parameter was significantly less in the flight group than in controls ($p < 0.001$), which may have resulted from the disappearance of rhythmic fluctuations in STH secretion in the flight animals, as a consequence of discoordination between somatoliberin and somatostatin.

When plasma corticosterone was measured, all three groups—flight and synchronous and vivarium controls—showed no significant differences (fig. 6). The level of prolactin in flight animals was significantly higher than in both control groups (fig. 6). Elevated levels of prolactin in the flight group may be summation of the effects of moderate stress and circulating estrogens; on day 18 of pregnancy the level of estrogen in rats is very high. Estrogens increase sensitivity to lactotropic impulses (Leong et al., 1983), which is probably the reason why spaceflight factors, which did not affect the levels of growth hormones or corticosterone, had a marked effect on prolactin.

Concentration of insulin in blood plasma of the flight rats was not significantly different from that in the vivarium controls. In the synchronous control group the level of insulin was significantly greater than in both the vivarium and flight groups. The concentration of glucose in the plasma of the flight animals was significantly lower than in both controls (fig. 7).

3.3 Sympatho-Adrenal System

The status of the sympatho-adrenal system of female rats flown on Kosmos-1514 during days 13–18 of pregnancy was evaluated on the basis of concentrations of catecholamines in plasma and adrenals and the activity of their metabolic enzymes in the adrenals. Epinephrine, norepinephrine, and dopamine were determined using Peuler and Johnson's method (Peuler and Johnson, 1977); activity of tyrosine-hydroxylases was measured using Nagatsu's method (Nagatsu et al., 1964); and activity of phenylethanolamine-N-methyltransferase was measured using Axelrod's method (Axelrod, 1962). The results obtained are presented in Figure 8.

In the adrenal glands, a significant decrease occurred in the concentration of epinephrine in the flight animals compared to the synchronous and vivarium controls, while concentrations of norepinephrine and dopamine were unaltered. The activity of tyrosine-hydroxylase—the key enzyme of catecholamine synthesis—was elevated in the flight group, while the activity of phenylethanolamine-N-methyltransferase was the same as in the controls. There were no significant changes in concentrations of epinephrine and norepinephrine in the blood of flight animals.

It should be noted that changes in metabolism of catecholamines detected in pregnant rats after the 5-day space flight were significantly greater than the changes observed in males after flights on Kosmos-936 and Kosmos-1129 (Kvetnansky et al., 1981), both lasting about 3 weeks, and also after a 7-day flight of male rats on Kosmos-1667 (Kvetnansky et al., 1986). This suggests a greater stress reaction in the female rats exposed to microgravity during pregnancy.

3.4 Thyroid Gland

The state of the thyroid gland of animals exposed to microgravity during pregnancy was evaluated on the basis of concentrations of the major hormones—thyroxine and triiodothyronine.

The thyroid gland was homogenized in buffer solution (0.04 M Tris-hydroxy methyl aminomethane; 0.11 M NaCl; 0.001 M 2-mercapto-l -methyl-imidazole, pH 8.4, 0.25 ml per gland). Complete hydrolysis was achieved by using the enzyme pronase (2 mg in 50 µl buffer solution and 0.4 ml homogenate). One drop of toluene was added to the mixture, which was stirred constantly during incubation for 16 hours at 37 °C. After completion of hydrolysis, the samples were diluted with buffer solution, and concentrations of thyroxine and triiodothyronine were determined by RIA. Concentrations of hormones were computed per mg protein, determined according to the method of Lowry (Lowry et al., 1951).

The results (table 8) show an absence of changes in the thyroid glands of animals exposed to microgravity: concentrations of thyroxine and triiodothyronine were identical in the flight animals and vivarium controls. In the synchronous control group, both parameters were significantly decreased.

3.5 Hemopoietic Stem Cells

Hemopoietic stem cells are a sensitive target to the effects of a variety of chemical and physical factors. Changes in the size of the stem cell pool in bone marrow have been described in rats after exposure to hypergravity and microgravity (Vacek et al., 1982, 1984).

This work investigated the effects of microgravity on the quantity and distribution of hemopoietic cells (CFUs) in female rats exposed to microgravity during days 13 to 18 of pregnancy. The methods developed by Till and McCulloch (1961), as modified by Vacek et al. (1975, 1976) were used for the rat experiment. Femur bone marrow and spleens from flight animals were homogenized in M-199 medium and injected intravenously into recipient rats (aged 21–28 days), irradiated at a dose of 9.0 Gy 2 hours before transplantion. Suspended cells were counted in a Goryaev chamber; depending on the cell count in individual groups, 15–20 recipients were used, each of which was injected with 2×10^7 spleen cells or 2×10^6 bone marrow cells.

On day 11 after transplantion of the hemopoietic tissue, the spleen was removed from the recipients and fixed in the Carnoy solution; macroscopically discernable cell colonies were counted on its parietal surface.

The total number of karyocytes in the bone marrow of flight animals was lower than in vivarium controls, but did not differ from the synchronous controls At the same time, the concentration of CFUs was significantly lower in flight animals than in both control groups; as a result, the total number of CFUs in femoral bone marrow was decreased in flight rats (fig. 9).

The total number of splenocytes in the flight spleens was also significantly lower than that of the vivarium controls but did not differ from the synchronous controls. The concentration of CFUs (per 10^6 splenocytes) was virtually identical in the flight and control groups, while the total number of CFUs was lower in the spleens of flight and synchronous control animals than in the vivarium group (fig. 9).

Thus, exposure of pregnant female rats to microgravity for 5 days was accompanied by a significant decrease in the concentration and total count of CFUs in bone marrow. The data obtained are consistent with the results obtained previously in examinations of male rats after space flights of greater duration (Vacek et al., 1982) and demonstrate that even exposure to microgravity for as little as 5 days can alter the quantity of CFUs. The reason for such changes may be migration of CFUs from bone marrow, their death, or a decrease in the number of the hemopoietic cells that generate them.

The total number of CFUs in the spleens of animals exposed to space during pregnancy was also decreased, and a similar decrease was noted in the synchronous control group.

3.6 Concentration of Fluids and Electrolytes in Tissues

Study of fluid-electrolyte balance in animals exposed to microgravity during pregnancy included measurements of the concentrations of water, sodium, potassium, calcium, and magnesium in the liver, kidney, skin, and bone (tibia).

The biosamples were weighed on VLAO-100 scales, placed on quartz glass, and dried at 105° C until a constant weight was reached, and the content of water and dry matter was determined. The samples were then placed in quartz test tubes; concentrated nitric acid was added; and the tubes were subjected to a dry-air bath at 80° C, until the organic substances dissolved completely. After the acid was evaporated, the samples were diluted with distilled water. Sodium and potassium were measured by a Zeiss-III flame photometer in an air-propane flame; calcium and magnesium were measured in an air-acetylene flame using a Hitachi (model 508) atomic adsorption spectrophotometer.

The concentration of water in the examined tissues of the flight animals was the same as in the controls. No significant differences were noted in concentrations of potassium, sodium, or magnesium in the liver, kidney, skin, and bone. However, the level of calcium in the liver and kidneys of the flight animals was decreased by almost a factor of two, the change in the level of calcium in the skin of the flight animals was less pronounced than in the liver and kidneys, while no significant changes were found in tibial bone (fig. 10).

It is interesting to note that these results differ from the data obtained in the male rats after a 7-day space flight on Kosmos-1667. Unlike females, the males showed no significant changes in the calcium concentration in any of the organs studied.

3.7 Concentration of Electrolytes in the Coats and Tails of the Animals

The levels of calcium, magnesium, strontium, iron, phosphorus, zinc, copper, manganese, potassium, and sodium were measured in the coats of the animals exposed to space flight, using an atomic-emission spectrometer with an inductively bound flame (Plasma-100) (Lyuderits et al., 1983). Analysis was performed on samples of the coat (0.2–0.3 g) taken from the back and the tip of the tail (ca. 1.5 g). The results obtained are presented in table 9.

No significant changes were noted in the tail samples of the flight animals with respect to levels of mineral elements in comparison with the synchronous control group. The coats of the flight animals showed a

significant elevation in the content of potassium, iron, phosphorus, copper, and manganese compared to both controls.

3.8 Lipid Metabolism

It has previously been established that exposure of animals to microgravity induces marked changes in lipid composition (Ahlers et al., 1980, 1981; Ahlers, 1984). When male rats were examined several hours after spaceflight lasting approximately 3 weeks, elevated triglycerides (TG) and cholesterol (C) were detected in blood serum, and elevated nonesterified fatty acids (NEFA) were found in serum and white (epididymal) and brown (interscapular) adipose tissue. The liver showed a biochemical pattern of moderate fat infiltration (elevated TG and C). In the thymus, the TG concentration increased, while that of phospholipids (PL) remained unchanged or decreased; if only the content in a single organ was considered, then TG was unaltered and PL decreased significantly. The greatest changes were detcted in bone marrow (femur bones) of rats and consisted of a significant accumulation of TG and a moderate decrease of PL. By day 6 postflight, the degree of hyperlipidemia NEFA hypermobilization declined and changes in the lipid content of the liver and thymus normalized partially. By day 25 postflight, lipid metabolism parameters returned to normal, with the exception of TG accumulation in bone marrow (Ahlers et al., 1980, 1981a; Ahlers, 1984). Exposure of rats to artificial gravity of 1 g has prevented TG accumulation in bone marrow (Ahlers et al., 1981b).

Pregnancy can be considered a kind of functional stress on the maternal body; since lipids are one of the major sources of energy rapidly mobilized in response to various environmental effects, evaluation of tissue lipids in rats exposed to microgravity during days 13–18 of pregnancy was of great interest.

On the day of reentry (pregnancy day 18), the concentration of triglycerides (Eggstein, Keutz, 1966) in the liver and thymus, the concentration of NEFA (Dole, Meinertz, 1960) in white and brown adipose tissue, the concentration of cholesterol (Zlatkis et al., 1953) in the liver, and the concentration of phospholipids (Bartlett, 1959) in the liver and thymus were measured in the flight rats.

The flight group displayed an increase in the concentration of NEFA in white and brown adipose tissue and an accumulation of triglycerides in the liver; concentrations of cholesterol and phospholipids were not altered in the liver. In the thymus of the flight animals, there was a tendency for triglycerides to decrease and phospholipids to increase (fig. 11). The changes described were similar to the pattern observed previously in the male rats after longer duration spaceflights (Ahlers, 1984). The major changes—accumulation of NEFA in adipose tissue and triglycerides in the liver—are indicative of the biochemical patterns seen in fatty livers.

3.9 Concentration of Nucleic Acids and Polydeoxyribonucleotides in Tissues

Certain experimental treatments (e.g., irradiation, injection with cytostatic agents or glucocorticoids) induce decomposition of deoxyribonucleoproteins (DNP) in lymphoid and hemopoietic tissues (Skalka et al., 1965; Matyasova et al., 1966: Yermolaeva et al., 1970). Decomposition of DNP is accompanied by an elevation in the level of polydeoxyribonucleotides, which are a mixture of oligonucleotides, and a decreased level of DNA. The level of polydeoxyribonucleotides is elevated for 2 to 18 hours, with the maximum rise occurring 6 hours after treatment. An increased level of polydeoxyribonucleotides is a good indicator of acute damage to the sensitive cells, occurring a short time before the animals were studied. Following repeated exposures, this reaction may be identified only after regeneration of sensitive cells (mainly lymphocytes and erythroblasts), i.e., approximately 7 days after the first treatment (Skalka et al., 1965).

Experiments on male rats exposed to microgravity for approximately 3 weeks and examined several hours after reentry, revealed a decrease in the concentration of nucleic acids in the spleen and thymus, and an increase in the level of polydeoxyribonucleotides, attesting to DNP damage during recovery, the last phase of the flight (Misurova et al., 1979, 1982a). The Kosmos-1514 experiments contained a similar study of pregnant rats.

This study involved measurements of the concentration of nucleic acids in the liver, spleen, thymus, and blood leukocytes, using a modification of the method developed by Tsanev and Markov (1960), and also an evaluation of DNP decomposition on the basis of polydeoxyribonucleotide levels. The most pronounced changes were found in the spleen. In the flight group, concentrations of nucleic acids were within normal limits, but due to the decreased weight of the organ, the total levels of RNA and DNA were decreased when compared to the vivarium control group, virtually by a factor of two. In the synchronous control group, decreases in the concentration of nucleic acids were more moderate (figs. 12 and 13). The level of polydeoxyribonucleotides in the spleen of the flight animals was the same as in the vivarium controls and higher than in the synchronous controls, where levels were very low (fig. 14).

Concentrations of DNA and RNA in the thymus of the flight rats did not differ significantly from the control values (figs. 12 and 13). At the same time, the total amount of nucleic acids in the organ was lower in the flight than in the vivarium group. For DNA, similar changes were also observed in the synchronous group. The level of polydeoxyribonucleotides in the thymus was lowered to an equal extent in the flight and synchronous control groups (fig. 14).

In blood sediments of the pregnant flight rats, the concentration of DNA was within control limits, while the concentration of RNA decreased in comparison to the vivarium controls by approximately one half. Decreases in RNA concentrations were less substantial in the synchronous control group (figs. 12 and 13). It can be postulated that the decreased concentration of RNA in blood sediments is mainly a consequence of the lower admixture of reticulocytes; their count in the flight group was half as much as in the synchronous control and one third that of the vivarium control (see table 6).

The livers of flight rats showed no changes in the concentration and total amounts of DNA compared with both control groups (fig. 13). The concentration of RNA was somewhat elevated, while the total amount in the organ was diminished due to a decrease in liver weight (fig. 12). The concentration of polydeoxyribonucleotides was unaltered (fig. 14).

The changes described above correspond to the data obtained from studies of male rats after longer duration spaceflights (Misurova et al., 1979, 1982a,b). In the spleens of these males, the level of RNA was decreased by 25–45%, while in the pregnant females from Kosmos-1514 it was diminished by 50%; the level of spleen DNA was lowered by 30–50% in the males and 59% in the Kosmos-1514 females. In the thymus, the RNA level was decreased 0–40% in the males and 40% in the pregnant females, while DNA was depressed 0–33% in the males and 36% in the females. When these figures are compared, it is evident that the decrease of nucleic acids in the pregnant animals after a 5-day flight is at the lower limit of the spectrum obtained for the males after flights of approximately 20 days. The differences may be considered as either an indication of the higher sensitivity of pregnant females to spaceflight conditions, or as one of the indirect demonstrations of damage inflicted upon sensitive cells during launch and transition to microgravity. The second hypothesis was confirmed by the absence of signs of DNA degradation in the spleen and thymus of animals after a short-term flight on Kosmos-1514. The low level of polydeoxyribonucleotides and the lack of capacity for a second response to repeated stimulation (during reentry) are typical of the stage preceding regeneration of sensitive cells (Skalka et al., 1965). After a longer exposure to microgravity, sensitive cells damaged at launch evidently regenerated; DNA degradation induced by transition from microgravity to hypergravity manifested as an increase in the level of polydeoxyribonucleotides, several hours after completion of a 20-day flight (Misurova et al., 1979, 1982a, b).

3.10 Biosynthesis of Nucleic Acids

In 10 experiments with male rats on Kosmos biosatellites, both acute and chronic reactions to space flight factors were identified in the genetic system of cells of the liver and lymphoid organs (Makeyeva et al., 1976; Komolova et al., 1977, 1982; Guseinov et al., 1979). The goal of the present investigation was to study these reactions in female rats, exposed to space on Kosmos-1514 on days 13–18 of pregnancy.

The rate of RNA synthesis was determined in nuclei of hepatocytes, and that of DNA and RNA synthesis in spleen lymphocytes. Concentrations of nucleic acids and protein were measured in the liver. To study liver RNA synthesis, nuclei were isolated in a dense sucrose solution. A portion of the nuclei was used to determine transcription, and the remainder, to obtain a solubilized RNA-polymerase (Roeder, Rutter, 1970). The enzyme activity and RNA synthesis in the hepatocyte nuclei were measured with respect to the incorporation of a radioactive precursor, ^{3}H-UMP, into acid-insoluble products of RNA-polymerase reactions.

Rates of RNA and DNA synthesis in spleen lymphocytes were determined on the basis of an *in situ* incorporation of radioactive precursors (Komolova, 1980). Nucleic acids were measured spectrophotometrically by the method of Blobel and Potter (1968), and protein was measured according to Lowry (Lowry et al., 1951).

Figure 15 presents data on endogenous RNA synthesis in hepatocyte nuclei on the day of reentry (pregnancy day 18). These data suggest that after flight, transcription activity increased when compared with the vivarium control by almost a factor of two. In the synchronous condition, RNA synthesis also increased, but to a lesser degree (30%) than in rats of the flight group.

RNA synthesis in the cell depends to a significant extent on RNA polymerases, the enzymes that synthesize the various types of RNA on the DNA matrix. Investigation of RNA-polymerases solubilized from hepatocyte nuclei showed that in the flight and synchronous rats, the changes were similar to those seen in RNA synthesis, i.e., after space flight, enzyme activity exceeded control levels by a factor of two, while in the synchronous controls it was elevated by 37%. A positive correlation between the

rate of RNA synthesis and the activity of RNA-polymerases may indicate the dominant role of enzymatic components in the transcription changes observed.

Results of quantitative measurement of nucleic acids in the liver (table 10) are also consistent with these data. The flight animals showed an elevated level of RNA. It should be noted that in the preceding experiments performed on male rats, the opposite response was observed; concentrations of RNA were decreased. Evidently, the slight increase in RNA in this experiment was associated with the development of compensatory reactions in pregnant rats, in response to the extreme effects of spaceflight.

Table 11 presents data on the rate of incorporation of radioactive precursors ^{3}H-thymidine into DNA and ^{14}C-uridine into RNA of spleen lymphocytes. After spaceflight, syntheses of DNA and RNA were altered in pregnant rats. However, it is clear that these changes were of a different nature; DNA replication was inhibited as compared to the vivarium control by almost a half, while RNA synthesis accelerated. There were no significant changes in these parameters in animals of the synchronous control group. These data suggest that changes in the metabolism of nucleic acids in spleen lymphocytes of the flight animals were due to the effects of microgravity and not to concomitant spaceflight factors. Decreased DNA synthesis in spleen cells is most likely due to chronic stress developing in flight, due to decreased loading of the musculoskeletal system. Indeed, as was demonstrated earlier, inhibition of DNA replication in spleen cells also manifested itself in male rats both after space flights (Guseinov et al., 1979) and in simulation long-term hypokinesia experiments (Makeyeva et al., 1976). However in the male rats, RNA as well as DNA synthesis was inhibited.

Activation of RNA synthesis was a response peculiar to spleen lymphocytes of pregnant flight rats. In this case there is evidently no functional link between the changes in the rate of transcription and that of renewal of the DNA matrix. At the same time, one might postulate that acceleration of RNA synthesis in the segments of the genome was responsible for the synthesis of proteins involved in the formation of adaptive reactions. It has not been ruled out that some role is played here by the increased formation of stable ribosomal RNA. This hypothesis is partially corroborated by the increase in the RNA/DNA ratio of lymphocytes, from 0.52 ± 0.01 in the vivarium control to 1.42 ± 0.11 in the flight group.

Evidently, the RNA metabolic changes in spleen lymphocytes identified in the pregnant flight animals served to activate the trophic function of the immune system. This may facilitate the maintenance of anabolic reserves and the defensive potential of the maternal body, which is extremely important for normal pregnancy.

3.11 Activity of Liver Enzymes

In previous biosatellite experiments, activites of liver enzymes, along with other parameters, were measured to identify stress-reactions in animals (Nemeth et al., 1982, 1983). An indicator of acute stress is the elevated activity of tyrosine aminotransferase (TAT) and tryptophan pyrrolase (TP), while an indicator of chronic stress is the elevated activity of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST). In the current experiment, similar investigations were performed on pregnant rats flown on Kosmos-1514 during days 13–18 of pregnancy.

The methods used were described earlier (Nemeth et al., 1982, 1983). The results obtained are presented in table 12. Enzyme activity is given per g protein in the homogenate.

No significant differences were found between the animals exposed to microgravity and control animals in activities of TAT, TP, ALT, and AST. These results are not consistent with the data obtained earlier from examination of male rats, in which the activity of these liver enzymes was elevated after spaceflight (Nemeth et al., 1982, 1983). It should be noted that other signs of acute and chronic stress were as high (neutrophilosis, lymphopenia, etc.) or higher (delayed weight gain, changes in catecholamine metabolism) in the pregnant rats as in the previously examined males.

3.12 State of the Myocardium

Rats exposed to space on Kosmos-1514 on days 13–18 of pregnancy were dissected on the day of reentry. The heart muscle was frozen in liquid nitrogen and stored on dry ice until further analysis. After it was thawed, the weights of the myocardial ventricles and the septum were measured, along with their fluid contents (by drying to a constant weight at 80° C), concentrations of glycogen and protein, and activities of myosin ATPase .

In flight animals there was a significant decrease in total myocardial weight, attributable to a 19% decrease in the weight of the septum, a 15% decrease in the weight of the right ventricle ($p < 0.05$), and an insignificant 9.5% decrease in the weight of the left ventricle.

The concentration of fluid in the myocardium (per unit dry weight) and concentrations of protein and glycogen were identical in the flight and vivarium control groups. There were no significant intergroup differences found in the activity of myosin ATPase. Unfortunately, the

analogous parameters were not measured in the synchronous animals, and thus the flight group was compared only to the vivarium controls.

3.13 Collagen Metabolism in the Skin and Bone

Microgravity induces marked changes in the musculoskeletal system. Animals previously flown on Kosmos biosatellites displayed changes in bone structure and metabolism, along with decreased strength of limb bones and vertebrae (Yagodovsky et al., 1977; Holton et al., 1979; Stupakov et al., 1979; Kazarian, 1980). It thus appeared to be of interest to study the effects of microgravity on collagen metabolism, which to a significant degree determines the biomechanical (support) functions of connective tissue. Bone (femur, flushed of bone marrow) and skin, consisting of 50 and 40% collagen, respectively, were selected for study.

Skin (without the hair) was taken for analysis from the back of the animal. Skin samples were frozen in liquid nitrogen, mechanically homogenized, and solubilized in 0.5 M acetic acid in the presence of pepsin (Behringer) for 24 hours (three times in 24 hours, 100 mg pepsin per g at 4° C), and then processed according to Ehrlich (1979), by the method of zonal precipitation chromatography using a FRAG-300 (France, Switzerland) programmed fraction collector. Hydroxyproline was measured in individual chromatographic peaks (Stegemann, 1958), and total protein in the glycoprotein peak (Lowry et al., 1951). The total amount of insoluble collagen, obtained using ultracentrifugation with the solubilized samples, was estimated with respect to hydroxyproline and used to compute the proportion of soluble collagen.

Soluble collagen in bone samples was measured the same way. Insoluble collagen was processed with cyanogen bromide (Epstein, 1974; Hanson, Bentley, 1983), splitting collagen protein into peptides specific to type I and III collagens. The peptides released using cyanogen bromide were separated by high performance liquid chromatography, using an automatic Pharmacia system. Their molecular weight was measured using permeation pressure chromatography. The ratios of peptides with different molecular weights were used to determine the ratios of various types of collagen.

Analysis of the skin of flight animals, sacrificed several hours after the 5-day space flight, revealed significant changes in pepsin-soluble collagen metabolism as compared to control rats. No differences were found between the vivarium and synchronous controls, and for this reason their results were pooled (table 13).

Flight animals displayed a 22% elevation of soluble collagen in the skin as compared to the controls ($p < 0.01$), the ratio of hydroxyproline (i.e., collagen) to glycoproteins in the flight group was decreased by more than a factor of 20, mainly due to an increase in glycoproteins. When various types of collagen were measured in the skin of the flight animals, it was observed that the proportion of type I collagen decreased slightly, while the proportion of type III collagen increased a great deal (table 13). These results suggest metabolic activation of connective tissue; the changes found in soluble collagen in the skin of the flight rats are characteristic of the skin of young, growing animals (See Section 12.13).

Analysis of soluble collagen from bone samples of flight animals revealed no significant differences in the ratios of different types of collagen. In the flight group, percentage of type I collagen was 21%, compared to 14% in the control groups ($p > 0.05$); type II collagen amounted to 2% and 1% in the flight and control groups respectively, while type III collagen was 9 and 11% ($p > 0.05$).

Analysis of pepsin-insoluble collagen in bone revealed substantial differences between the flight and control rats. Bone samples of control animals contained only type I collagen, which is consistent with data cited in the literature (Serov and Shekhter, 1981). Bone samples obtained from the flight rats showed a decrease in type I collagen, and the appearance of type III collagen in a proportion of 35%. The decrease in type I collagen may be associated with an increase in collagenolytic enzymes, such as collagenase, catepsin, and elastase. This hypothesis is supported by the increased number of catabolic peptides in the soluble collagen of flight bone samples, which reached a level of 47%, compared to a control group value of 28% ($p < 0.05$). The presence of type III collagen in the bone samples of the flight group is an interesting observation; in normal conditions, this substance is produced by smooth muscles, fibroblasts, and reticular cells, but not by osteoblasts (Montes et al., 1982). In the past, it has not been found in bone, with the exception of inherited pathologies in connective tissue—*osteogenesis imperfecta* (Kivirikko et al., 1981).

3.14 Bone Structure and Mechanical Properties

The study of bone in rats exposed to space on Kosmos-1514 during days 13–18 of pregnancy included evaluation of bone density, the number and activity of major cellular elements, as well as mineralization parameters, the composition of the organic matrix and mineral components, and biomechanical properties. The femur, tibia, and humerus bones and the thoracic and lumbar vertebrae were studied.

The methods for processing samples and performing measurements were described in detail previously

(Vico et al., 1985; Nogues et al., 1985). Measurements made in the femur included: the distance between two periosts in the middle third of the diaphysis; the total thickness of the cortical layer (inner and outer); and the mean diameter of the marrow cavity. In the proximal epiphysis of the tibia, the trabecular bone volume was determined, calculated as the portion of the spongiosa occupied by trabeculae in the 0.8 mm wide area located below the growth cartilage (zone of neoformation), and in the area another 0.8 mm lower (remodeling zone). In the bone modeling zone, the diameter of vertical cylinders (precursors of trabeculae), and in the zone of remodeling, the mean diameter of trabeculae, were measured.

In the thoracic and lumber vertebrae, the mean thickness of the cortical layer of the ventral and dorsal surfaces of vertebral bodies, and the trabecular bone volume were measured; these values were used to compute the absolute bone volume, trabecular bone volume, and their ratios.

Activity of bone cells was evaluated using methods described by Vico et al. (1985) and Nogues et al. (1985). To evaluate osteoblast activity, the mean thickness of the growth plate in trabecular bone of the thoracic and lumbar vertebrae, proximal epiphysis of the tibia, and diaphysis of the humerus were measured. Measurements were made in zones of neoformation and remodeling. Activity of osteoclasts was estimated with respect to the amount in 1 mm^3 of the spongiosa and in trabeculae, as well as in a 1 mm^2 of the trabecular surface. In the cortical layer of the tibia endosteal plate, the surface of active resorption was measured in the modeling and remodeling zones.

The composition of the organic matrix was examined in bone samples from the forearm and fibula. The procedures for sample preparation, amino acid hydrolysis, and separation were described by Perier et al. (1984). This method is based on determination of the proportional composition of collagen amino acids: proline and hydroxyproline, and also alanine and glycine, in bone samples. After separation and measurement of these amino acids, the ratios of hydroxyproline to proline and glycine to alanine were computed.

Study of the mechanical properties, mineralization, and chemical composition of the mineral components of spongy bone tissue was performed on the proximal epiphysis of the humerus. The mechanical properties in response to compression tests were studied using the Instron device and methods descrived previously (Stupakov et al., 1979). Ultimate strain, elastic strain, relative strain, modulus of elasticity, and specific work of elastic strain were computed. Mineral content, concentration of organic substance, coefficient of mineralization (ratio of the mineral to organic component), porosity, and levels of calcium, phosphorus, magnesium, sodium, potassium, zinc, and silicon were measured (Bakulin et al., 1985). The amount of each element in one gram of ash and in unit volume of bone was calculated, and then the concentration of the element in the mineral component was multiplied by the coefficient of mineralization, to determine how much of the element was contained per unit weight of the organic matrix.

Study of osteoclast activity in the flight animals revealed a tendency for an increase in the cell number per unit volume of the spongiosa, in the zones of modeling and remodeling in the tibia, and also in vertebral trabeculae. For the thoracic vertebrae, the difference was statistically significant ($p < 0.05$). A similar tendency was observed in the number of osteoclasts per unit volume of trabeculae. Analysis of variance revealed a statistically significant increase in the surface occupied by osteoclasts in the metaphyseal plate of the humerus in the flight animals when compared to that of the synchronous control group. There was a tendency for the surface of the osteocytic lacunae to increase in the endocortical zone of the humeral diaphysis in the flight and synchronous groups, compared to the vivarium controls.

No intergroup differences were found in the osteoid thickness, either in the zone of neoformation, or in the zone of remodeling in the tibia. The amount of osteoid in the thoracic and lumbar vertebrae was too small to be measured. The results of measurement of the osteoid width in the humeral diaphysis suggest a tendency for the periosteum to decrease in the flight animals.

The data on the tibia length, diameter between tibial periosts, and mean sagittal size of the thoracic and lumbar vertebrae point to the lack of significant changes in the flight animals, when compared to the synchronous and vivarium controls.

The ratio of trabeculae of the vertebrae to the overall bone volume, and the arrangement of trabeculae, were the same in all three groups. It was shown that at the level of the tibial metaphysis, primary spongiosa tubules in the modeling zone, and bone bands of the space below it (the remodeling zone), were comparable and had the same diameters. No changes were found in the trabecular volume either in the tibia or in the vertebrae of animals in the three groups.

During the flight there were no significant changes in the porosity of spongy bone in the proximal epiphysis of the humerus. A substantial ($p < 0.05$) decrease in the concentration of organic substance in the spongiosa, of the proximal epiphysis of the humerus, was found in the flight group as compared with the synchronous control, evidently due to activation of resorption. The mineralization coefficient of the proximal epiphysis of the

humerus was higher in the flight than in the control animals. The combined changes in the mineralization coefficient, and the concentration of organic substance, increased mineral content in the synchronous control animals. No significant differences were found in this parameter between rats of the flight and vivarium control groups.

Analysis of the organic matrix of the forearm and fibula did not reveal any significant differences between the flight and control groups, in the concentrations of hydroxyproline, proline, alanine, or glycine, which suggests the absence of changes in the overall content of collagen and its composition (Perier et al., 1984).

The 5-day space flight led to a decrease in the calcium concentration in the mineral component of the proximal epiphysis of the humerus (table 14). The concentration of calcium per unit weight of organic substance was also lower in the flight than in the control groups, suggesting decreased total concentration of calcium in bone, despite the increase in the mineralization coefficient. The flight animals displayed an increase in the concentration of phosphorus as calculated per mineral, as well as per organic substance of the bone (table 14). As a result, the ratio of Ca/P was significantly lower in flight rats than in both control groups, which might indicate a relative decrease in the concentration of crystalline hydroxyapatite in the mineral component of bone. The concentration of potassium was identical in the bone samples of animals of all three groups, while the concentration of sodium in the flight rats was significantly higher than in the vivarium control, but did not differ from the synchronous controls. The concentration of silicon in the flight rats was decreased when compared to the vivarium control, while the concentration of zinc was elevated. However, these differences were not statistically significant as compared to the synchronous control (table 14).

Study of the mechanical properties of the proximal epiphysis of humerus bones revealed a significant decrease in the compression strength and maximum relative deformation in the flight rats (table 15). The modulus of elasticity showed a tendency to increase when compared to both control groups, but these differences were not statistically significant. The results obtained confirm that it is possible for bone strength to decrease while its density remains unchanged, under conditions of actual or simulated microgravity, as shown previously (Stupakov et al., 1979, 1981, 1982). Evidently this decrease is associated with a decrease in the concentration of calcium in the mineral component and the Ca/P ratio.

These results, taken together, support the conclusion that exposure of pregnant rats to microgravity for 5 days is accompanied by an increase in the number of osteoclasts, and evidently, activation of the processes of bone resorption, which did not reach the stage of the main symptom of osteoporosis—bone loss.

3.15 Physiological Properties and Metabolism of Skeletal Muscles

Research on Kosmos biosatellites established the general laws governing the effects of space flight factors on skeletal muscles in white rats. It was shown that their adaptation to microgravity is systemic in nature, with the direction and severity of symptoms depending on the extent to which muscles are involved in the anti-gravitational function, their functional profile, and biomechanical properties (Oganov, 10980, 1984). It was also demonstrated that adaptive changes in the physiological properties of the muscles are accompanied by appropriate changes in their structure, catabolic and anabolic metabolism, and changes in some types of myofibers effected by reprogramming the synthesis of contractile and regulatory proteins (Portugalov, 1978; Tacacz et al., 1978; Oganov et al., 1982). The changes described are biologically expedient, reversible, and do not exceed the bounds of physiological adaptation.

The program to study the muscle system of pregnant rats flown on Kosmos-1514 was a logical continuation of this research and included the following major areas:

1. evaluation of changes in contractile properties of muscle fibers;

2. study of Ca^{2+} transport in membranes of the sarcoplasmic reticulum and its functional state;

3. evaluation of the system providing energy to contraction;

4. evaluation of the variability of molecular composition of contractile proteins, as the basis of functional adaptation of muscles.

On the day of reentry (pregnancy day 18), skeletal muscles were removed from the rats (sacrificed by decapitation): from the forelimbs, the brachial muscle (Brach) and medial head of the triceps (Tric); from the hindlimbs, the lateral and medial heads of the gastrocnemius (L and M gastroc) and soleus (Sol) muscles; the extensor digitorum longus (EDL); the plantaris muscle (Plant); and also the gravity independent diaphragm muscle (Diaphr).

The contractile properties of skeletal muscles were studied in preparations of glycerinated muscle fibers (bundles of 3–5 fibers) in the ATP + Ca^{2+} solution, using Szent–Gyorgyi's method as modified by the authors (Oganov et al., 1981). The functional activity of the

sarcoplasmic reticulum was studied by evaluating the mobility of Ca^{2+} in the membranes of the L gastroc, Plant, and Diaphr. A model based on caffeine contracture of single, chemically scanned muscle fibers, which had first been immersed in a solution with a high concentration of Ca^{2+}, was used (Mounier et al., 1983).

Protein metabolism was studied in the Brach, EDL, Sol, and M and L gastroc. The concentrations of total protein and differentiated myofibrillar and soluble proteins, and the structure of myofibrillar proteins, were investigated using SDS-polyacrylic gel electrophoresis (Weber et al., 1969).

Muscle bioenergetics was studied by measuring the rate of mitochondrial respiration in various metabolic states, by means of oxidative phosphorylation polarography (Mailyan et al., 1983). .

Flight rats displayed a significant decrease in the weight of the majority of skeletal muscles (table 16), although their relative weight was altered insignificantly. All muscles of the flight animals, with the exception of the Plant muscle, showed a significant decrease in contraction strength, as compared to the vivarium controls (fig. 16).

The maximal rate of muscle fiber contraction development was significantly decreased in the flight animals when compared with the vivarium control, in preparations of all the muscles studied, with the exception of the EDL (fig. 17).

The work capacity of myofiber preparations, which was estimated on the basis of impulse of force, was also significantly decreased in preparations of the Sol, Tric, and Plant of the flight group, and in the Brach, and M and L gastroc of the flight and synchronous groups, compared to the vivarium controls.

Thus, there was a general trend in muscle fibers toward reduction of strength, rate of contraction, and work capacity, without appreciable differentiation attributable to the functional profile of the muscle, as was observed after longer term spaceflights (Oganov, 1980).

Amplitude of contraction of single muscle fibers was found to be decreased in the flight animals compared to the synchronous controls in the L gastroc and Plant. Comparison of the "stress–pCa" function revealed a decrease in the affinity of myofibrillar proteins for calcium in L gastroc of the flight animals, and an increase in this parameter in rats of the synchronous group. In the Plant, the sensitivity of proteins to calcium was identical in all three groups.

Mobility of Ca^{2+} in the sarcoplasmic reticulum membrane was estimated on the basis of amplitude of contraction, after treating the fibers with caffeine. This paradigm is based on the capacity of caffeine to sharply increase the permeability of the sarcoplasmic reticulum to Ca^{2+} (Mounier et al., 1983). The preparations were first "loaded" with calcium, i.e., kept for a certain period of time in a solution where the Ca^{2+} concentration (pCa = 6.8) did not induce direct activation of the contractile proteins. The duration of preliminary loading with calcium required to obtain maximal amplitude, in a standard (contracting) solution with constant concentration of caffeine, served as an indicator of the calcium pump capacity, which in the norm is determined by the Ca-ATPase activity of the sarcoplasmic reticulum.

In preliminary control experiments, it was shown that (within certain limits) the longer the exposure of preparations to the calcium loaded solution, the greater the amplitude of the contraction in response to the same dose of caffeine. Thus with the standard dose of caffeine (5 mM), duration of exposure and time needed to achieve maximum contraction amplitude are functions of the calcium pump capacity, and thus of activity of Ca-ATPase of the sarcoplasmic reticulum.

The results of this experiment testify to an increased intensity and rate of calcium pumping of the sarcoplasmic reticulum, in muscle fibers of the LGM, in rats of the synchronous control group as compared to the vivarium controls. The LGM of flight rats showed a decrease in the rate of calcium uptake (compared to the synchronous control), with the intensity of absorption unchanged. Alteration of the calcium pumping capacity of Plant in the flight group was analogous, but along with a decrease in rate, there was also a decrease in intensity of calcium absorption.

The fact that the amplitude of the caffeine-induced contraction is dose-dependent, given the standard duration of preliminary exposure to the hypercalcic solution, reflects the capacity of the sarcoplasmic reticulum membrane to release calcium, since this also occurs in natural initiation of contraction by a neuronal impulse. Results of this study show that this capacity to release calcium decreases somewhat in the fibers of the L gastroc and Plant muscles of the flight animals (compared to the vivarium control), and on the other hand, increases markedly in the rats in the synchronous control group.

Thus it may be concluded that in response to microgravity, the mobility of calcium ions decreases in the membranes of the sarcoplasmic reticulum of the gastrocnemius and plantaris muscles, thus reducing their functional activity.

Tissue respiration was studied in the anterior group of muscles of the femur. Rates of mitochondrial respiration were measured in various metabolic states: substrate

respiration, ADP-dependent respiration, and controlled respiration. Information was obtained about the phosphorylation function of the muscles by measuring phosphorylation time (Δt), and by computing parameters of energy function of the respiratory chain—respiratory control according to Lardi (RC_L) and Chancy (RC_C)—and rate of phosphorylation. The results, presented in table 17, indicate the absence of significant differences between the flight and vivarium control groups. Animals exposed to microgravity for 5 days did not display any changes in mitochondrial respiration of the skeletal muscles, neither in rate of oxygen consumption nor extent of energy accumulation in myocytes. Animals in the synchronous control group showed changes in a number of parameters as compared to the vivarium animals: a decrease in the ADP:O coefficient and rate of phosphorylation; and an increase in phosphorylation time. In the majority of cases, differences between the synchronous and flight groups were not significant. The exception was the ADP:0 coefficient, which was significantly lower in the synchronous than flight group.

In the muscle tissues of the femur, no significant differences were noted between flight and control groups, in activities of malate dehydrogenase, isocitrate dehydrogenase, or lactate dehydrogenase. Activity of the last showed a tendency to increase, which can be considered an acute reaction associated with return to Earth's gravity.

The results obtained support the idea that short-term effects of microgravity neither induce depression of tissue respiration nor decrease its energy efficiency, despite significant decreases in quantity of mitochondrial protein.

Investigation of subunits of myofibrillar proteins (Takacs et al., 1985) revealed no changes in the fractions of heavy chains of myosin or concentration of actin, as was also the case with flights of longer duration.

However, a decrease in concentration of light chains (LC) of myosin, particularly LC-3B, which at least in the Sol is not normally high, was observed in the Sol and Tric of the flight animals. Low levels of LC-3B were also observed in the Sol of the synchronous group. Elevation of levels of slow isoforms of myosin light chains (particularly LC-1M) was also observed in the flight Sol, but not the Tric. Considering that ATPase activity of myosin molecules is directly associated with the concentration of LC-3B (Weeds, Taylor, 1975), it can be concluded that the data obtained do not contradict the general direction of changes (decreases), in the rate of muscle fiber contraction after a 5-day space flight. No significant changes were noted in the ratios between slow isoforms of myosin light chain in fast myofibers.

No appreciable changes were detected in the concentrations of individual fractions of the troponin-tropomyosin complex (TN-TM). It was notable that in the flight group, changes in the concentrations of the troponin-inhibitor complex (TN-I) of myosin ATPase tended to increase in fast-twitch muscles and decrease in postural muscles. An analogous tendency was observed in TN-I in the same muscles after an 18-day space flight (Takacs et al., 1983; Oganov et al., 1982).

Thus, the results of this research suggest that, in pregnant rats, short-term (5 day) spaceflight is accompanied by appreciable changes in the contractile properties of muscle fibers, expressed as a decrease in the strength of contractile proteins, more noticeable in antigravitational musculature, and as a tendency for delay in contraction development. The results confirm the idea, that the leading factor in changes in postural muscles in response to microgravity, is an unloading of the muscles and a deficit in the tonic component of movement, as was shown for longer (18–20 day) exposures of rats to microgravity (Oganov, 1980; Oganov et al., 1980; Takacs et al., 1983).

In addition, it was established that decreases in the contraction strength of some muscles (Sol, EDL) were incommensurate with their minimal loss of weight (table 16; fig. 14). To explain this, it is postulated that the decrease in the capacity of myofibers to develop force is due not only to their atrophy, but also to the observed decrease in the affinity of contractile proteins for Ca^{2+}. Additional, indirect evidence for this theory comes from the insignificant loss in myofibrillar proteins after the 5-day spaceflight (Takacs et al., 1985).

Comparison of the results of studies performed after two short-term space flights (Kosmos-1514 and Kosmos-1667) suggests that, after the 5-day spaceflight, a decrease in the strength of muscle contraction in pregnant rats applied to all muscles, while after a 7-day flight, changes noted in muscles of male rats were not as marked and were in different directions in the postural and fast-twitch locomotor muscles. It may be hypothesized that more severe changes in the strength and speed properties of muscle fibers in pregnant rats after the 5-day spaceflight reflect factors specific to their state. Changes in the speed properties of the muscles studied and in the functional activity of the sarcoplasmic reticulum were in the same direction (decrease) for both flights.

After longer-term spaceflights, a statistically significant acceleration has been observed in the contraction process in the Sol of rats (Oganov et al., 1979, 1982), which is consistent with data on the restructuring of myosin populations of in this muscle (Takacs et al., 1989, 1983). In muscle fibers, the tendency to change in the opposite

direction after a 5-day flight was most likely due to a decrease in Ca^{2+} mobility in membranes of the sarcoplasmic reticulum, as discussed above.

Changes noted in the function of contractile proteins of fast muscles in the flight animals after short-term space flight are, for many parameters, similar to changes in the analogous muscles of the synchronous control animals. It can be hypothesized that during this period, factors associated with the conditions under which the animals are maintained continue to play a significant role. These changes in the fast-twitch muscles are in a different direction from those occurring after longer space flights (increase in strength and speed of contraction), which the authors consider a consequence of behavioral adaptation to microgravity (Oganov et al., 1981, 1982).

Thus, the structural-functional and metabolic reactions of skeletal muscles described above can attest that during short-term spaceflight, changes in the skeletal muscles participating in the postural function are limited mainly to the level of contraction regulation. In other words, the most sensitive components of the contraction mechanism are what suffer, although certain reactions observed (partial atrophy) may be adaptive in nature. Nevertheless, during this spaceflight period, there has not yet been a substantial restructuring of myosin populations and corresponding muscle structural profiles, such as that observed after longer-duration space flights (up to 22 days, Oganov et al., 1982), that would reflect the physiological adaptation of the muscles.

3.16 State of the Ovaries

Conclusions were drawn concerning the functional activity of the ovaries in rats exposed to microgravity on Kosmos-1514 on days 13–18 of pregnancy, on the basis of the state of the corpus luteum, which forms after ovulation in pregnant animals and consists of a mass of cells secreting progesterone and estrogen, both essential for supporting the uterine endometrium and allowing pregnancy to continue. Unlike humans, in rats the corpus luteum function until the termination of the pregnancy, participating in regulation of fetal development at all stages.

The ovaries of the rats of the flight and control groups were fixed in 10% formalin. Morphometric analysis, utilizing stereological (Weibel, 1969) and diffractometric (Lenczowski et al., 1980) methods, were performed on 8–10 μm cross-sections embedded in paraffin, stained with hematoxylin and eosin. A total of 40 corpora lutea were analyzed for each group.

Stereological analysis of the preparations was performed using an optical microscope with magnification of 320x. The volumes of the lutein and paralutein cells, and their nuclei, were estimated, and ratios among individual structures of the corpus luteum, lutein and paralutein cells, connective tissues, and blood vessels were determined.

To evaluate the distribution of structures and their components, a diffractometric analysis of preparations on photographic negatives (x160; input unit 22 mm) was performed. A diffractometer with a helium-neon laser (wavelength 0.63 μm) was used. The light rays passed through the negative, underwent diffraction, and converged on the focal plane of the diffractometer, creating a diffractogram. Intensity of illumination was measured by an electron detector (type DPS 1-2 Recognitions Systems, Inc., California). Signals from the detector were amplified, differentiated, transformed, and fed to a computer (CDC 6600 computer—Control Data Corporation, Minneapolis, MN).

The diffractograms had a symmetrical structure; their halves were equivalent in spectral distribution, which permitted analysis in diametrically opposed geometric coordinates: one half of the plate consisted of the central ring and 31 radially arranged half-rings, and the second of 32 angular spaces—wedges. Radial analysis of a portion of the diffractogram, divided into half-rings, provided information about the sizes of the structures and the distances among them, while angular analysis of the wedge spaces gave information concerning ordering in the distribution of structures (Lenczowski et al., 1980).

Preparations from all flight, synchronous and vivarium control groups contained lutein and paralutein cells with typical structures (fig. 18). They were divided by connective tissue containing, aside from cellular elements and fibers, sections of blood vessels. The qualitative morphological study of preparations revealed no differences between the corpora lutea of rats of the flight and control groups. The stereological analysis also failed to reveal any significant differences among the groups in the relative volume of the major components of the corpus luteum: lutein and paralutein cells, connective tissues, and vessels were similar (table 18). When the absolute volumes of lutein cells were measured, no differences were found between the flight and vivarium control animals (table 19). However, the volume of the nuclei in the flight group showed a significant tendency to be decreased compared with the synchronous control (table 19). The volume of paralutein cells in the corpus luteum of animals in the flight group was the same as that of the vivarium group, but the volume of their nuclei was significantly depressed. However, this parameter was even lower in the synchronous control group (table 19). The relative volume of nuclei of paralutein cells was significantly reduced in

the flight group, compared to that of the two control groups (table 20).

The results of angular analysis of the diffractogram indicate normal distribution of structures in the elements of the corpus luteum of animals of all three groups. The absence of differences between the flight and control animals in localization of diffractogram maxima indicates similarity in the ordering of structures. Discriminant angular analysis failed to reveal significant differences between groups.

Figure 19 presents the results of radial analysis of the diffractogram. Analysis of the decomposition of light intensity among the half rings attests to the heterogeneity of the sizes of structures of the corpus luteum in animals of all three groups. Distribution of maxima in individual half-rings was the same for flight and control groups; however, the greatest maximum for animals of the flight group was displaced to the left, compared with the vivarium control group, and there was some displacement in the synchronous group as well. This may indicate that the individual components of the corpus luteum were of different sizes for the flight and control groups. Discriminant analysis was performed to identify significant differences. When decomposition of the light was compared in the 32 half rings using discriminant analysis techniques, the greatest differences were found in half rings 1, 2, 22, 29, and 30. Decomposition of the light in half rings 1 and 2, according to the Fourier transformation, corresponds to parameters indicative of the optical density of the entire sample, which depends to a significant extent, on the processes of fixation and staining, and on subsequent photographic processing of the negative; these differences were not considered. Statistically significant differences ($p < 0.05$) were found in half rings 19, 22, 24, 29, and 30, corresponding to the elements of the corpus luteum, with dimensions in the ranges of 0.7–0.8 μm.

Comparing the results of diffractometric and stereological analyses, one might postulate that differences identified using diffractometry are associated with changes in the state of paralutein cells, which had nuclei that were significantly smaller in animals of the flight group, than those of the vivarium and synchronous control groups (table 19). The corpus luteum is a temporary organ; at the termination of pregnancy, when the functioning of the placenta reaches a maximum, the functional activity of corpus luteum decreases. The differences observed here may be a sign of differences in cellular degeneration of paralutein cells in the animals of the different groups, caused by differences in the hormonal status and metabolism of rats in the flight group as described above.

Chapter 4

Parameters of the Reproductive Function of the Animals: Fetal and Placental Characteristics

Although the flight females were significantly behind the controls in weight and showed a number of other adverse changes, the major parameters of their reproductive function were virtually unaltered. When the rats were dissected on day 18 of pregnancy, the flight group did not differ significantly from the controls in preimplantation or total embryo deaths (table 21). The number of living fetuses averaged 13 in the flight and synchronous groups, and 12 in the vivarium control group. No dead fetuses were found in any of the groups; however, the flight and synchronous females produced more fetuses and placentas with hemorrhages caused evidently by the factors associated with reentry. No other developmental anomalies were noted.

The placentas of the flight group were lower in size and weight. Histological study showed that these changes were attributable to the labyrinth portion of the placenta—the site of the most intensive metabolic interchange between the mother and fetus; the thickness of the spongy layer was not altered. Hydration of the placenta was identical for all groups. Concentrations of potassium in the placenta of flight animals were depressed, and concentrations of sodium were elevated, suggesting a diminished proportion of cellular elements. Concentrations of calcium and magnesium were the same in the placentas of animals in all groups. No differences were found between the flight and control groups with respect to concentrations of DNA, RNA, protein, or glycogen in the placenta (table 22).

The mean weight of the fetuses was 0.84 for the flight group, 0.92 for the vivarium group, and 0.94 for the synchronous group ($p < 0.05$); water content in fetal tissues was significantly higher in the flight group than in controls (table 23). This can be considered as a sign of some developmental retardation, since water content decreases progressively as the fetus grows. In fetal tissue, no differences were found among the groups in the concentrations of sodium, potassium, calcium, or magnesium. When their skeletons were measured, the flight fetuses showed a developmental retardation, manifested as a decrease in the size of ossification sites in virtually all developing bones by 13–20%, compared to corresponding parameters for the control groups (fig. 20), i.e., although concentration of calcium was normal in fetal tissues, its incorporation in developing bone was delayed.

Studies of metabolism of nucleic acids and proteins in fetal tissues (on the whole) revealed no reliable differences among the groups in concentrations of DNA, RNA, or protein (table 23). Despite severe symptoms of a stress response in the mother (including changes in catecholamine metabolism), the activity of tyrosine-hydroxylase—the key enzyme for synthesizing catecholamines in the adrenal glands of fetuses—was unaltered (table 23). It is noteworthy that a signicant decrease in the concentrations of hemopoietic stem cells occurred in the livers of flight fetuses, since at this stage of development the liver is the major hemopoietic organ (table 23).

The results of this part of the experiment demonstrate that in principle, it is possible for mammalian fetuses to develop when the mothers are exposed to microgravity in the final third of pregnancy. The flight fetuses displayed no visible abnormalities, with the exception of bleeding, which was also observed in the synchronous controls and was evidently associated with the effects of reentry. The flight fetuses were retarded in their development with respect to the controls in body weight, the extent of ossification, and tissue water content; however these differences were small. It is intersting that on day 18 of pregnancy, the total weight of the fetuses developing in each flight female was equivalent to that of the vivarium controls, equalling 11.40 and 11.47, respectively. Thus, despite the significant loss of weight in the mother, due evidently to the activation of catabolic processes, these animals were able to activate anabolic processes associated with the growth and development of their fetuses to the same extent as control mothers.

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Chapter 5

State of the Neonates

The experimental program stipulated that a portion of the rats would be allowed to give birth at full term after return to the Earth. It is interesting to note, that they began to gain weight rapidly as soon as they returned to Earth, while during the entire flight they had gained a total of 5 g (compared to 65 g in the control). During their first day on the Earth they gained 35 g! The birth process was more difficult and prolonged in the rats exposed to microgravity (pregnancy days 13 to 18) than it was for control animals. Flight rats showed greater individual differences in the birth process than observed in the control groups.

Two mothers of the flight group, the first to give birth, bore normal litters with 13 neonates in each. Two other rats (no. 16 and no. 33) had prolonged (long time interval from the beginning of bleeding) and difficult births, and each litter contained one stillborn pup. In one flight rat (no. 35), the birth process lasted 2 days, due to general asthenization, muscle weakness, and the presence of one very large fetus (greater than 7 g). Since the mother was unable to deliver (it was first in the birth canal) for a long period, the other fetuses—full- term and normally developed—died from anoxia, due to the long delay in the detachment of the placenta and their final emergence from the mother. It should be noted that we have never encountered a similar case, when a female could not give birth to a full-term fetus normal in virtually all parameters, neither in the control groups nor in other vivarium experiments, where no fewer than 1000 animals gave birth. In studying the fetuses by means of Wilson's method, the largest neonate rat, first in the birth canal, was found to have hydrocephalus as well as subcutaneous edema in the area of the head, that evidently developed during the birth process. In the litters of the four flight rats that bore living neonates, there was a mean of 12 pups per litter (three rats had 13 pups each; one had nine); mean litter size in the synchronous group was also 12 (from 11 to 14), while the vivarium control group averaged 10 (from 8 to 15).

The body weight of the flight neonates averaged 5.92 g and was lower than that of the vivarium controls (6.48 g), but higher than that of the synchronous controls (5.6 g). The total weight of the neonates from a single mother was an average of 71.0 g in the flight group, virtually the same (70.8 g) in the synchronous control, and somewhat greater than that in the vivarium control (65.1 g), due to the greater litter size in the flight animals.

A comparison of these weights with the total weight of the fetuses carried by each female after the completion of the flight (pregnancy day 18) shows that during the 5 days of Earth readaptation (through pregnancy day 23), the fetuses of the flight mothers increased their weight by 59.6 g per litter, with the comparable figure for the vivarium controls being 53.6. g. Thus, despite the fact that the individual weights of the flight neonates were less than those of the vivarium controls, the rate of weight gain in the flight group during the readaptation period was slightly higher than in the vivarium controls.

The external appearance of the flight neonates was appropriate to their calendar age; when the pups were examined and their viscera studied using Wilson's method, no abnormalities were found. The flight and synchronous neonates did not display hemorrhages observed earlier in the fetuses. This suggests that the hemorrhages were slight, and the tissue changes they caused, reversible. The ratio of males and females in the litters of the three groups were identical. After birth the litters were equalized so that each contained 8 neonates, in order to create comparable conditions for all the litters during the nursing period. Biochemical and morphological studies were performed on 16 living neonates of the flight group, 11 from the synchronous control group, and 21 from the vivarium control group.

When the skeletons of the neonates were measured, it was found that the flight animals displayed an increase in ossification sites by 10–17% compared to the controls (fig. 21), while previously the flight fetuses showed a delay in skeletal development. Thus following Earth readaptation, the developing fetuses of the flight group were not only comparable with the controls, they surpassed them in this parameter.

Liver weight of the flight neonates was significantly lower than in the controls, while the water concentration per kg dry weight was identical in all three groups. There were no differences among the groups in the concentrations of sodium, potassium, or calcium in the liver (table 24). No differences were found among the groups in liver concentration of DNA, RNA, or protein (table 25).

The weight of the myocardium of the flight neonates was 29.7 mg, being higher than both control groups. No intergroup differences were found in its water content, nor in the concentrations of sodium, potassium, or calcium. The kidney weight of the flight neonates averaged 29.2 mg and was higher than that for the synchronous control, but did not differ from the vivarium group. The water content in the kidney and concentrations of potassium and calcium were identical in all three groups. The concentration of sodium in the kidney of the flight neonates was below that of the two control groups (table 24).

Table 26 presents the results of hematological examinations of the neonates in the flight and control groups. Two to three hours after birth, the flight animals showed a reliable decrease in the concentration of hemoglobin and reticulocytes, compared to animals of the synchronous and vivarium controls. There were no intergroup differences in the counts of leukocytes or formed elements of peripheral blood. Concentrations of colony forming units (CFU) in the spleen of flight neonates were almost twice as high as in the vivarium and synchronous control groups ($p < 0.001$), while concentrations of CFUs in the livers of neonates were identical for the flight and vivarium groups. In the synchronous group, the number of CFUs was elevated at that time (2–3 hours after birth).

Results of this part of the experiment show that fetuses, that spent a portion of their prenatal ontogenesis in microgravity, were able to complete their development on the Earth in the acute period of maternal readaptation to normal gravity. No structural-anatomical abnormalities occurred; the animals developed at a normal rate and even compensated for certain changes, e.g., retardation in skeletal development, decrease in hemopoietic stem cells, and hemorrhages occurring on landing.

Chapter 6

The Behavior of Female Rats While Nursing Their Offspring

After completion of the Kosmos-1514 spaceflight and subsequent return to Earth, the female rats began to gain weight rapidly, compensating for the lag occurring during flight; when they were weighed after giving birth (day 6 of the readaptation period), their weight averaged 20 g less than the weight of the vivarium controls. This intergroup difference persisted until the end of the observation period (day 20 of the nursing period), but diminished somewhat between days 13 and 18 of readaptation.

During the first 10 days postflight, the animals continued to be fed the space diet *ad lib.* Daily food consumption by the flight and control rats is presented in figure 22. No significant differences in food consumption were noted among the groups at any point. Since the flight and control animals had different weights, it seemed relevant to compute the amount of food consumed by a rat per unit body weight, and also per unit total biomass (mother + offspring). The results of this computation revealed no significant differences among the groups on any particular day of observation or *in toto* (from day 1 to day 8 after births). From day 10 of the lactation period, the animals were put on a mixed diet in which carrots, greens, cereals, and curds supplemented the space diet, complicating exact measurements of the amount of food eaten on subsequent days.

Consumption of water, measured daily, was low due to the high water content of the food and virtually identical in the flight and control groups. Total consumption of water during the first 10 days of the lactation period was 148 ml in the flight group, 153 ml in the vivarium, and 135 ml in the synchronous controls.

Starting on the first day after the birth of the new generation, the animals were housed in special cages designed for study of maternal behavior. The cage, depicted in figure 23, consisted of a nest (A) and a feeding chamber (B). The floor of the cage contained a scale, which recorded the time the mother spent in each of the chambers. The floor of the nest contained a temperature sensor, and the roof of the cage above the nest held a microphone, for recording the intensity of ultrasound emitted by the pups when the mother left. These parameters are relatively good indicators of a mother's care of her offspring, illustrating circadian variations and closely correlating with each other. If the relationship between the mother and offspring is normal, when the mother leaves the nursery the ultrasound emitted by the pups increases in intensity; the louder the sounds, the sooner the mother returns (Grota et al., 1969, 1973; Leon et al., 1978; Plant, 1976; Rosenblatt et al., 1963).

The experimental setup included a computer which recorded and processed information from the cage around the clock, every 15 minutes, and video cameras which photographed the animals. Video data were subsequently processed by the computer.

Figure 24 depicts variations in the time the flight and control rats spent daily in the nursery, over the 18 days of observation during the lactation period. Both the flight and control females showed normal patterns of change in this parameter: a gradual decrease of the time the mother spent in the nursery, from 90% on day 1–2 after giving birth, to 60% on day 15–16. However, for virtually every day of observation, the time the flight mothers spent in the nursery was 5–10% greater than that spent by the vivarium controls. This difference is likely to be related to the smaller sizes of the mothers and offspring in the flight group.

Figure 25 presents a detailed hour-by-hour record of maternal behavior parameters for one mother from the flight and one from the vivarium group on postnatal day 6. The parameters recorded were the time the mother spent in the nursery and the intensity of ultrasound emitted by the pups. Both curves clearly demonstrate the normal behavior for the mothers: diurnal rhythms in the time spent in the nursery; and a reverse correlation between the time spent by the mother in the nursery and intensity of the sound emitted by the offspring.

Thus, rats exposed to microgravity from day 13 to day 18 of pregnancy were capable not only of giving birth to offspring, but of caring for them adequately during all stages of the lactation period. Despite the stress reaction that developed during flight and reentry, the major characteristics of maternal behavior in the flight group were within physiological limits.

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Chapter 7

Growth and Development of Newborn Rats During Their First Month of Life

During the postnatal period, eight pups were left with each mother in the flight, synchronous, and vivarium groups for physiological research. However, the death rate in the first week of life was higher in the flight group (19%) than in the synchronous (2.5%) and vivarium (0%) controls ($p_V < 0.001$; $p_S < 0.05$). There were substantial individual differences in the mothers of the flight group: the instances of postnatal death were noted not in all litters, but only in two of the four—the litters of rats nos. 16 and 33, that had the difficult and prolonged births, and one stillborn neonate in each litter.

In the litter of one of these mothers (no. 16), three neonates died during their first day of life; during the period between days 4 and 7 after birth, three more pups from the flight group died—one belonging to this same litter and two offspring of rat no. 33. The dead pups were nursed, but either did not gain weight or gained weight significantly more slowly than the other animals. At autopsy no structural abnormalities were observed. Thus the cause of death was, in all likelihood, metabolic changes leading to a decreased general resistance in the neonates. It is well-known that one of the causes for perinatal death is hypoxia, occurring during pregnancy or in birth complications (Garmasheva, 1964; Makeyeva, 1964; Skornyakova, 1964). After analyzing the duration and course of the births in individual animals, one might conclude that the difficult, prolonged births by rats nos. 16 and 33 led to a more severe hypoxia than occurs during normal birth, which was the cause for the increased perinatal death rates of the animals.

As a result of these deaths, the numbers of animals used for physiological studies during the postnatal period were 26 pups in the flight group, 40 pups in the vivarium group, and 39 pups in the synchronous controls.

In observing the pups during their first weeks of life, the major foci were: the rate of growth and development; motor activity, particularly the ability to coordinate movements during various provocative tests; and development of the sensory systems, the tactile, vestibular, hearing, vision, and olfactory systems.

On the day after birth, the neonates of each litter were tagged; they were weighed individually for 20 days between 9:00 and 10:00 a.m. on scales accurate up to 0.01 g, which were kept in the incubator at a temperature of 30–32° C (fig. 26). The weights are presented in figure 27. At all observation times, the mean body weight of the flight pups was 1–5 g below that of the vivarium controls, but higher than that of the synchronous group. This further suggests that the weight gain delay noted in the flight group is due to flight factors other than microgravity.

At the same time, a detailed analysis of the individual data obtained showed that the flight pups demonstrated a significantly greater variability in weight than the controls. At some observation intervals, the variability in the body weight of the flight group was double that of the control groups.

Three tests were used to evaluate the vestibular function in the pups: the ability to turn over from the supine position (on days 2, 3, 4, 7, and 8); the capacity to turn against gravity on a tilted (–20°) plane (on days 1, 5, 6, 9, and 15); and the response to a test on a rotating platform (on days 2, 3, 5, 7, and 9). The tests were performed in an incubator with air temperature of 30–32 °C.

Turning over from the supine position is a standard test to study the vestibular function and motor activity of neonates (Altman et al., 1975). The pups were placed on their backs on a level surface and held down through light pressure of the experimenter's finger; the finger was then withdrawn; and the time and pattern of their turning to a prone position were recorded. During the test, the behavior of the rats was observed using a video camera located directly above them. The platform on which the pups were tested was surrounded by mirrors placed at an angle (fig. 28), which allowed photography and subsequent analysis of movements from various perspectives. During the test on day 2, only 6% of the pups in the vivarium control (2 out of 32) were able to perform the movement in less than 30 seconds, while the corresponding figure for the flight group was 24%, and for the synchronous control group it was 22%. During subsequent testing, the differences between the groups diminished, and on days 4 and 7, there was no difference in the time taken by the pups of the flight and control groups to turn over from their backs. The pattern of use of different muscle groups in performing this movement was identical in the flight and control groups, being normal and typical for their age.

The next test used was to evaluate the capacity of the neonates to reorient themselves against the force of gravity, manifesting the so-called "negative geotaxis" reaction (Altman et al., 1975). On the one hand, this test is indicative of vestibular function, since it requires an appropriate perception of the head down position; on the other, the test involves both significant strength and motor coordination. The pups were placed on a head-down platform (–20°) (fig. 29). Their behavior was recorded with an above video camera; as in the preceding test, the platform was surrounded by mirrors allowing the camera

to watch the pups from all sides at once. The number of pups manifesting "negative geotaxis" in 30 seconds was recorded; the task was considered accomplished when the pups manifested active movements and turned their bodies no less than 45° from the starting head-down position. During tests on the first day of life (the day after birth), 31% of the flight pups and 63% of the vivarium pups passed. Unfortunately, at this time only a portion of the vivarium pups (16 animals in all) were tested, making conclusions difficult. On day 5, the differences between the flight and control pups on this test diminished. Performance of a full turn on a head-down platform was a relatively difficult task for the pups and required significant muscle tension. On day 6, the number of pups capable of performing a full turn was 48% in the flight group; on day 9 this figure reached 88% and was significantly greater than the control level ($p < 0.01$). On day 15 no differences were found between the groups.

The next test was performed on a rotating platform; the pups were placed in the center of a round arena 9 cm in diameter and rotated for 30 seconds at a rate of 33 rpm, and observed during rotation and after its termination, using a video camera located above the rotating platform. In response to this test, the pups typically demonstrated an unconditioned compensatory reflex: the head turned in the direction opposite to the direction of rotation (fig. 30). Tests were performed from day 1 to day 7, when the pup's eyes were still closed; thus their reaction to rotation was not corrected by vision and was determined only by perception of angular acceleration during rotation. The flight group included a greater number of pups manifesting this unconditioned reflex on days 1, 2, and 5; the intergroup differences were significant on day 5 ($p < 0.05$). At later stages, this reflex could hardly be followed because it was masked by voluntary movements of the pups.

Summarizing the results of these three tests, one can say that the vestibular system of the pups exposed to space during a portion of their prenatal period functioned normally after birth and, in individual tests (rotating platform), was even more sensitive than in control animals. The motor activity of the pups and their capacity for motor coordination in performing the above-mentioned tests were no worse in the flight group and sometimes (performance of a full turn on the head-down platform) was even better than in the control animals.

On days 12, 15, and 18 , we measured the physical work capacity of the pups as indicated by the time they were able to hold onto a cross-bar. The results are presented in figure 31. At all points the flight animals were able to support themselves for a somewhat shorter time than the synchronous and vivarium controls ($p < 0.05$); however, the intergroup differences were very small.

Tactile sensitivity was measured in the pups during the first days of life on the basis of the motor response to being touched in various body sites with a Frey irritation hair. At all observation points, no differences were found between the animals that developed in microgravity and controls.

The olfactory function was evaluated between days 3 and 15 in terms of responses to strictly graded smells. The stimulus used was amylacetate. In previous experiments, it was established that the development of sensitivity of pups to this smell, which has a broad spectrum, is close to that of sensitivity to natural, biological olfactory stimuli (Alberts et al., 1980). When the test was administered, the animals were placed in a special chamber, the air of which had previously been filtered and then warmed to 33 °C. Reaction to the stimulus was evaluated on the basis of changes in the respiration frequency and depth registered, using miniature sensors of elastic resin filled with mercury. The sensors were made in the shape of thin loops placed on the chests of the animals; sensors of different sizes were used for animals of different ages. Animals were allowed to adapt to the experimental chamber, and then baseline impedance plethysmograms were recorded for 30 seconds. The olfactory stimulus was presented for 10 seconds; subsequently the odor source was removed, and respiration was recorded for an additional 20 seconds.

No significant intergroup differences were observed either in the baseline (before presentation of the stimulus) respiration rate, which increased identically with age in all three groups, or in the magnitude of reactions to the olfactory stimulus.

A special chamber, depicted in figure 32, was used to evaluate hearing parameters. Testing was performed on day 14, immediately after the pups opened their eyes, and then on days 19 and 20 of their lives. Tones with frequencies of 4 and 40 kHz were used since, in normal development of hearing, sound perception develops from low to high frequencies. The reactions to the sound were estimated on the basis of motor activity. On day 14, 100% of the flight pups reacted to low frequency sounds, and none reacted to high ones. At this same point of development, 67% of the control rats reacted to the low tone, and 17% to the high one. On days 19 and 20, 72% of the rats in the control group and 38% in the flight group ($p < 0.05$) responded to the high tone during this time period; intergroup differences in response to the low tone were not significant. Thus, the pups that spent a portion of the prenatal period in microgravity displayed some

retardation in the development of hearing, compared to the controls.

The eyes of flight pups opened earlier than those of the controls: on day 14 the eyes of 64% of the flight animals and only 37% of the controls ($p < 0.001$) were open. To evaluate visual function on the day after their eyes opened, the pups were housed in a special unit (fig. 33), consisting of a transparent cylinder which was located in the center of a large drum, the interior wall of which was covered with alternating black and white vertical stripes projected on the animals' retina at an angle of 1.5°. Preliminary experiments showed that the angular resolution of the eyes of newborns, measured as an optokinetic reaction to slow rotation of the drum under normal conditions, is sufficient for object perception at this angle.

The test included a 20-second period of adaptation to the device, a 20-second period ofdrum rotation, during which pups' reactions were recorded with a video camera (located above the animals), and a 20-second period of drum rotation in the opposite direction. The results of the visual test showed good reactivity in the flight animals: 93% of the animals were able to perceive a moving image at a visual angle of 1.5°, while in the vivarium controls, the corresponding number was 87%. Intergroup differences were not statistically significant.

When summarizing the results of observations during early postnatal ontogenesis, it should be emphasized that there was a difference between the flight and control animals: the perinatal death rate of flight rats was increased. Since the flight rats had spent a portion of their prenatal development in microgravity, it was feared that the differences between the groups would progressively increase with their growth, as more developmental and functional demands were made on the organs. This hypothesis was not confirmed. The instances of death were virtually limited to the first week of life.

Although the Kosmos-1514 flight lasted only 5 days, the animals were exposed to microgravity for approximately 20% of the pregnancy, during a period which includes a number of critical points in physiological development. Thus it was natural to expect rather severe changes, especially in the vestibular system. However, this hypothesis was also not confirmed. The flight neonates grew and developed normally; in a number of parameters they were only slightly behind the controls; and in other parameters they even somewhat surpassed controls. The differences between the flight and control groups, when they were observed, were slight and subsequently rapidly diminished, without having serious effects on the general pattern of development.

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Chapter 8

Postnatal Differentiation of Skeletal Muscles

The structural and functional organization of skeletal muscles, of existing higher mammals, may be viewed as a result of convergence of the effects of physical and chemical evolutionary factors, and the gravitational field of the Earth (Gurfinkel et al., 1965; Zenkevich, 1944; Nasledov, 1981; Schnol, 1979).

As early as the prenatal period, under the conditions of relative microgravity, existing *in utero*, the activity of skeletal muscles in mammals—constant orthostatic (flexor) and episodic dynamic (extensor) function—is one of the major stimuli for embryonic growth (Arshavsky, 1979). An even greater role in the development of motor coordination during postnatal ontogenesis is played by the tonic antigravitational activity of skeletal muscles and locomotor activity (Smith, 1975; Arshavsky, 1979).

Rats exposed to microgravity during pregnancy displayed changes in the weight, functional properties, and metabolism of skeletal muscles. However, the study of postnatal development of skeletal muscles in animals developing after prenatal exposure to microgravity was of even greater interest. Flight, vivarium, and synchronous animals were sacrificed on days 15, 30, and 100 of their lives, and muscles of the hindlimbs (soleus [Sol], lateral and medial heads of gastrocnemius [L and M gastroc], plantaris [Plant], and extensor digitorum longus [EDL]) and forelimbs (medial head of triceps [Tric] and brachialis [Brach]) were isolated. Absolute and relative (% of body weight) muscle weights were measured. Szent-Gyorgy's method (Szent-Gyorgy, 1949), in our modification (Oganov et al., 1981), was used to investigate contractile properties of glycerinated muscle fibers. In each muscle, 20–35 bundles of fibers 200–500 μm in diameter were isolated. The maximum amplitude of isometric contractions, contractile force (per mm^2 of cross section), and impulse force of the preparations were measured, and the work capacity (area under the curve of a contraction mechanogram) and maximum rate of contraction development in the linear portion of the mechanogram (in the interval 0.2–0.6 of maximum amplitude) were also determined.

The weight of all the muscles examined increased significantly as the animals matured (table 27). For the majority of muscles, the increase in weight was parallel to increases in size and body weight; as a result the relative weight of the majority of muscles remained virtually the same for all the ages studied. No significant differences between the animals developing in microgravity and controls were observed on days 15, 30, and 100. The exception was an increase in the weight of the EDL and both heads of the gastrocnemius muscle in flight rats on day 15, an increase in weight of Sol and EDL on day 30, and an increase in Tric on day 100 in this same group.

The maximum force developed by muscle fibers in isometric contractions was twice as great in 30-day old animals as in 15-day old ones for Tric, Sol, L and M gastroc, and Plant; 1.5 times as great for EDL; and virtually identical for Brach (table 28). The strength characteristics of muscle contractions of 100-day old animals did not differ from those of 30-day olds. At no time were statistically significant differences between the flight and the control groups found in isometric contractions of skeletal muscle fibers.

The maximum rate of muscle fiber contraction was virtually identical in the 15- and 30-day rats, and more than doubled by 100 days (table 29). In flight animals, there was a reliable increase in maximum rate of contraction of EDL fibers on day 15, and in Sol and Brach fibers on day 30, and also an increase in this parameters in Sol on day 100. No other significant intergroup differences were noted.

The work capacity of muscle fibers in 30-day old rats was half of that of 15-day olds, but there were no significant intergroup differences in this parameter, with the exception of a decrease in Sol on days 15 and 100 in flight animals as compared to the controls (table 30). It is noteworthy that the work capacity of the fast twitch muscles (Brach and EDL) showed a tendency to decline with age in all groups. In contrast, the work capacity of Sol increased with age, to almost double that of its initial value.

Analysis of these data supports the assertion that time course variations in the physiological characteristics of muscles in animals of the different groups, from day 15 to 100 of life, reflect the normal process of development of contractile functions and the corresponding metabolic specialization of myofibers studied, and are compatible with other data in the literature.

Neonate rats do not display great differences in the speed of contraction of future fast and slow twitch muscles (Buller et al., 1960; Curless, 1977). At later developmental stages, the contractile rate of fast twitch muscles (EDL) progressively increases for the first 2.5–3 months, after which it plateaus in adult rats, at a level which is approximately three times higher than that of slow Sol. The speed properties of slow muscles are marked by a more or less significant acceleration, followed by a deceleration, or a monotonic acceleration (slower than for EDL), which also ceases during sexual maturation of the animals (Close, 1967; Zhukov, 1974).

Since the data cited were obtained on whole muscle preparations, special investigations were carried out to study time course variations of strength and speed muscle fiber properties, during the first 40 days of life. Figures 34 and 35 show a clear difference in the development of the contractile properties of fibers of fast and slow twitch muscles. The general tendency and ratio of speed properties of EDL and Sol fibers do not differ from those described for preparations of whole muscles during this period (Close, 1964, 1967; Zhukov, 1974).

The results described above show no signs of gross disorders in the development of the contractile characteristics of muscle fibers, in offspring of the flight and control groups. Moreover, the dynamics of changes in strength and speed of EDL and Sol fibers, the ratio between them by day 100 of life, and the patterns of change in their work capacity, all attest to a longer period of development for the specific profile of the slow Sol, in a gravitational field in early postnatal ontogenesis (Smith, 1982). Decreased work capacities of EDL by day 100 of life, and the contrasting increases in Sol, are in agreement with the restructuring of the ratios of fast glycolytic, fast oxidative, and slow oxidative fibers in these muscles, during the period from weeks 3 to 26 of development (Alnageeb, Goldspink, 1981) .

At the present time, the idea that cell development, from determination to full maturity, can be viewed as a sequence of qualitative and quantitative changes in the synthesis of protein macromolecules is generally accepted (Ulumbekov, 1981). In particular, it has been established that in the course of embryogenesis the population of myosins is replaced at least twice—in the fusion of myoblasts to form myotubes, and then in shift to postnatal ontogenesis (Ivanov et al., 1977; Kudelberg, 1976). In the myogenic cells of embryos, the first reprogramming of myosin synthesis occurs during the beginning of terminal differentiation; when in association with innervation of future muscle fibers, there is a sharp increase in the coordinated synthesis of contractile proteins. The second reprogramming is associated with the replacement of polyaxonal (polyneuronal) innervation of muscles by monoaxonal innervation in postembryogenesis (Gauthier, 1978); this process is mainly caused by external factors (Ulumbekov, 1981; Whalen et al., 1984).

The results obtained do not provide a basis for postulating significant changes in pre- and postnatal differentiation of the skeletal muscles examined in terms of the above parameters.

Comparison of the data, obtained from flight, synchronous, vivarium, and baseline control groups, allow the conclusion that animals that were prenatally exposed to microgravity did not display any gross deviations from the normal development of contractile muscle fiber properties during the postnatal period, up to the age of 3 months. The slight differences between the flight and control groups discussed above were not systematic in nature and were observed only at certain time periods. It is interesting to note that virtually all instances of differences between the flight and control animals involved increases, rather than decreases, in the weight and strength of the muscles of flight animals.

Chapter 9

Morphological and Histochemical Analysis of the Brain

The brains of the 18-day-old fetuses, and the 15-, 30-, and 100-day-old rats that developed in microgravity, as well as those from synchronous and vivarium control groups, were examined morphologically and histochemically. The biosamples were fixed using Lilly's method in a mixture of 100° ethanol, 100% acetic acid, and 40% formaldehyde (85:5:10), and embedded in paraffin. Frontal 10 μm thick sections were stained with hallocyanine-chrome alums.

Sections 15 μm thick of the rhomboencephalon of 18-day-old fetuses, frozen in liquid nitrogen, were prepared in a cryostat, and histochemical methods were used to develop the activity of alkaline phosphatase (ALP), acid phosphatase (AP), acetylcholine esterase (ACE), monoamine oxidase (MAO), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and NAD-H_2-and NADP-H_2-diaphorases. The rate of histochemical reactions for developing the activity of LDH and SDH were measured using the two-wave method with a MTsFV-1 "LOMO" cytophotometer.

Morphometric analysis of cytoarchitectonics in the neocortex of 18-day-old fetuses was performed using a TAS-"Leits" teleanalyzer, for all areas of the cortical plate (layer) in the rostrocaudal direction, with every fifth frontal section stained with hallocyanin. The widths of the wall of the hemisphere and its layers were measured in five locations.

To perform electron microscopy, the hypothalamus and pituitary were fixed in 2.4% solution of glutaric aldehyde in phosphate buffer (0.08 M for the brain of fetuses and neonates, 0.1 M for animals of the older age groups, pH 7.2), in 2% solution of osmium tetroxide, and then embedded in araldite. Ultrathin sections were examined in a "Tesla-BS-500" electron microscope.

Macroscopic study in the brain of 18-day old fetuses, and the 15-, 30- and 100-day-old pups, that had developed prenatally in space, revealed no pathological changes from control groups.

Optical microscopy of frontal sections of the brains of 18-day old fetuses of the flight and control groups revealed a well delineated cortical plate (layer) in the cerebral hemispheres, representing a layer of neuroblasts beginning to stratify. The so-called "migration paths" of cells along the fibers, growing from the thalamus tubercle into the cerebral cortex, were distinct. Periventricularly, in the zone of the medulloblast matrix covering the hemisphere and striatum from the inside, there were numerous mitoses. The level of mitotic activity of neuroblasts and glial elements in this area of the brain of the 18-day-old fetuses of the flight group did not differ from the corresponding parameters of control animals. The striatum was formed of very densely packed and relatively undifferentiated cells. The cerebellum was represented by the germinal cerebellar layer, with diffusely distributed and poorly differentiated cells. Measurements of the body diameters of the earliest differentiating nerve cells of the brain—neurons of the trigeminal nucleus—revealed no statistically significant differences between the flight and control animals.

Quantification of capillaries in the striatum of fetuses of the flight group revealed a 40 and 59% increase in their number in a unit area, compared to the vivarium and ground-based synchronous control groups respectively.

Histochemical study of enzymes in the rhomboencephalon of 18-day-old fetuses of the control groups revealed ACE activity in the reticular formation; in nerve fibers in the raphe zone, at the bottom of the third ventricle, in nuclei of pairs VII, X and XII of the cranial nerves;and in the descending trigeminal nucleus. MAO activity in the structures of the rhomboencephalon was still very low at this stage of development . Somewhat greater MAO activity was observed in the raphe area. Acid phosphatase activity was distributed diffusely in the structures of the rhomboencephalon and was high only in the area ventral to the bottom of the third ventricle. Alkaline phosphatase activity was mainly concentrated in the endothelium of the brain capillaries; it was insignificant and diffuse in the nerve tissue. Activity of NAD-H_2-diaphorase was rather high in all structures of the rhomboencephalon, and still higher in the raphe zone, at the bottom of the third ventricle in the facial nucleus, and the descending trigeminal nucleus. Distribution of NADP-H_2-diaphorase activity in the structures of the rhomboencephalon were similar to the localization of NAD-H_2-diaphorase, but its activity was lower. The activity of LDH in the rhomboencephalon structures was very high and diffusely distributed, while activity of SDH was still very low.

The level and distribution of NAD-H_2- and NADP-H_2-diaphorases, MAO, ACE, SDH, AP, and LDH, in the rhomboencephalon of 18-day-old fetuses of the flight group, did not differ from the corresponding parameters in the control groups. Alkaline phosphatase in the rhomboencephalon of flight fetuses was distributed similarly to that in the controls; however, visual evaluation revealed the level of alkaline phosphatase activity in the endothelium of the capillaries to be somewhat lower than in the vivarium and synchronous control groups, while cytophotometric data showed alkaline phosphatase activity to be higher in nerve tissue.

Qualitative analysis of the cytoarchitectronics of the neocortex of the 18-day-old fetal cerebral hemispheres revealed no differences in its differentiation in the flight and control samples. Quantitative analysis of the cytoarchitectronics of the same structures, presented in table 31, testify to the tendency for the width of the matrix to be greater and the width of the cortical layer (plate) to be less, in fetuses of the flight and ground-based synchronous groups as compared to the vivarium animals.

Electron microscopy of the hypothalamic structures showed that by the 18th day of prenatal development, the ultrastructure of the neurosecretory neurons and the neuropile of the supraoptic nucleus were relatively fully formed in fetuses of the vivarium and synchronous control groups (fig. 36). The neuronal nuclei, characterized by high density of nuclear chromatin, had a narrow perinuclear space. The cytoplasm contained many polyribosomes and mitochondria with well-delineated cristae, and well-developed canaliculi of the rough endoplasmic reticulum. Elements of the Golgi complex had the appearance of two or three flat cisternae, surrounded by many small vesicles, among which neurosecretory granules were located. In the synchronous control group, the neurosecretory cells had a higher density of distribution of nuclear chromatin and canaliculi of the rough endoplasmic reticulum, compared to the vivarium control. A large number of neurosecretory granules were located around Golgi complex elements. The neuropile of the supraoptic nuclei was formed by bundles of fine unmyelinated fibers and dendrites with well-defined microtubules, mitochondria, dendritic and axonal growth cones, and dendritic and axonal terminals, which were full of synaptic vesicles.

Compared to both control groups, the neurosecretory granules of the flight fetuses (fig. 36) showed increased densities and areas of distribution of nuclear heterochromatin, widened perinuclear spaces, and decreased numbers of polysomes and canaliculi of the rough endoplasmic reticulum in the cytoplasm. The Golgi complex was represented only by isolated large vesicles. In the neuropile, there were virtually no fine dendritic and axonal terminals with synaptic vesicles. The growth cones showed deformation and adhesion of large vesicles, and damage and thickening of the mitochondria. In the unmyelinated fibers, widened microtubules could be observed.

In the median eminence of 18-day-old fetuses in the control group, the neuropile was composed of fine unmyelinated axons with microtubules retained and a large number of growth cones which contained large vesicles, two or three neurosecretory granules and mitochondria with a dense matrix and delineated christae.

In the neuropile of the median eminence of flight fetuses, the unmyelinated fibers and growth cones of axons and dendrites had undergone changes similar to those occurring in the neuropile of the supraoptic nuclei, described above.

Neonate flight rats developed all the major elements of the ultrastructure of neurosecretory cells, characteristic of the analogous neurons of neonate rats of the vivarium and synchronous control groups (fig. 37). However compared to the neurosecretory neurons of rats in the vivarium condition, the neurosecretory neurons of the flight rats showed an elevation of chromatin density in the nucleus and an increased number of Golgi apparatus elements, microvesicles, and neurosecretory granules in the cytoplasm. In the neurons of the supraoptic nuclei of neonate rats in the synchronous control group, there was a very pronounced widening of the canaliculi of the rough endoplasmic reticulum and the cisterns of the Golgi apparatus, lightening of the mitochondrial matrix, and a significant decrease in the number of neurosecretory granules, compared to the animals in the vivarium and flight groups.

In the axonal terminals of the median eminence and posterior pituitary of flight neonates (fig. 37), there was an increase in the quantity of neurosecretory granules, the majority of which were granular, and also a sharp increase in the number of light microvesicles 40–60 nm in diameter, clustered in the central area of the terminals. In the synchronous pups, the axonal terminals of the median eminence were poor in neurosecretory granules and light microvesicles 40–60 nm in diameter.

Pituitary cells of the flight and synchronous pups displayed hypertrophy and vesiculation in the elements of the Golgi apparatus compared with the vivarium control, and an increase in the numbers of canaliculi in the rough endoplasmic reticulum and free ribosomes, a lightening of the mitochondrial matrix, and a pronounced condensation of chromatin near the nuclear membrane (fig. 37).

By day 15, the ultrastructure of neurosecretory cells in the supraoptical nuclei were well formed in the vivarium pups. Chromatin was homogeneously distributed in the nuclei. The perinuclear space was narrow. In the perikaryon, the rough endoplasmic reticulum took the form of narrow, branching canaliculi, with ribosomes on the outer surface of the membrane. A large number of free ribosomes in the form of polysomes were also localized in the cytoplasm. The Golgi apparatus took the form of three or four flat cisternae, surrounded by electron transparent microvesicles and large , 120–170 nm in diameter, neurosecretory granules with electron dense centers. The mitochondria had well-defined cristae. The axonal terminals of the neurosecretory cells, in the median

eminence and posterior lobe of the pituitary, contained synaptic vesicles 40–60 nm in diameter and 1–2 vesicles up to 180 nm in diameter, aside from neurosecretory granules with a well-defined electron-dense center.

The ultrastructure of neurosecretory neurons and their axonal terminals in the median eminence and posterior lobe of the pituitary, in 15-day-old pups of the flight and synchronous groups, did not differ from the corresponding parameters of the vivarium controls.

By day 30, there were no changes in the ultrastructure of neurosecretory cells of the supraoptic nuclei, or their axons and terminals in the median eminence and posterior lobe of the pituitary, compared to day 15. No differences were found between the flight and control animals.

Thus, macroscopic and visual light microscopic studies of the brains of 18-day-old fetuses, and then of 15-, 30-, and 100-day-old pups, that spent a portion of their prenatal period in space, did not reveal any changes in the development of brain structure up to day 100 of postnatal development.

The level of activity and distribution of key enzymes of energy metabolism (LDH, SDH, NADP-H_2, and NAD-H_2-diaphorases) and mediator metabolism (MAO and ACE) in the brain of 18-day-old fetuses of the flight group remained unaltered, suggesting normal establishment of energy and mediator metabolism in the fetal brain during the exposure of their pregnant mothers to space.

At the same time the development of rats under space-flight conditions was evidently accompanied by a decrease of metabolic transport from blood vessels to nerve tissue, which was evidenced by a decline in alkaline phosphatase activity in the capillary endothelium, and the increased activity of this enzyme in nerve tissue of the rhomboencephalon of the 18-day-old flight fetuses. An increase in the number of capillaries in the fetal striatum, evidently compensatory in nature, was another sign of some deficiency in their brain oxygenation. Insufficient oxygenation of the brain may be caused by the reduced size and weight of the placenta, as observed in the pregnant flight rats (Serova et al., 1984).

The fetuses which developed in space showed a tendency toward delayed migration of cellular elements of the neocortex from the matrix to the cortical layer (plate), which is evidenced by an increase in the matrix width, and a decrease in the cortical layer (plate) width in the wall of the cerebral hemispheres. However, a similar tendency in the brains of the synchronous fetuses implied that the reason for this was not exclusively microgravity.

Morphological study of the brain structures, receiving neural impulses from the otolith apparatus (i.e., the cerebellar nodulus and the lateral vestibular nucleus) showed that in the 18-day-old fetuses, the nodulus of the vermis was still not formed; the formation of the sulcus separating the nodulus from the remainder of the vermis occurs on day 19 of prenatal development (Dadoune, 1969), while cell differentiation of the lateral vestibular nucleus completes by day 13 of prenatal ontogenesis (Altman and Bayer, 1980). Although differentiation of type I and II hair cells in the utricular macula of rat fetuses is completed on days 14 and 15 of prenatal ontogenesis, respectively (Sans and Chat, 1982), the formation of synaptic gaps between nerve fibers and these cells, and thus the capacity to transmit impulses from the otolith apparatus to the brain, occurs only on day 18 of embryonic development (Sans and Chat, 1982), i.e., after the animals were no longer exposed to microgravity, under conditions of Earth gravity. This would explain the absence of morphological changes in the lateral vestibular nucleus, which receives impulses from the otolith apparatus in the 18-day old flight fetuses.

In the fetuses developing in microgravity, the ultra-structure of neurosecretory cells and neuropile in the supraoptic nuclei of the hypothalamus, and also the ultrastructure of the axons and terminals of these cells in the median eminence, suggest some delay in cell differentiation during the neuroblast developmental stage, and the appearance of changes in the microtubules of the unmyelinated nerve fibers and in the growth cones of axons and dendrites. Could the retardation in differentiation of nerve cells be a direct result of cell exposure to microgravity? At the present time, there is no proof of the direct effect of microgravity on the genetically determined capacity of brain cells to realize growth, migration, and differentiation—the basic processes of structural morphogenesis. Nevertheless, the evidence for the effects of microgravity on the intracellular structures of nerve cells, obtained recently in a clinostating experiment with cultured neurons from the inter-vertebral ganglia (Gruener, 1985), does not permit a final answer to this question.

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Chapter 10

Development of Behavioral Reactions and Work Capacity of the Central Nervous System

It is well known that exposure of a pregnant animal to adverse environmental factors may have negative effects on the development and function of the central nervous system of the offspring (Kotin, Ignatyeva, 1982; Gallo, 1981; Giurintano, 1974; Madsen et al., 1981; Monder, 1981; Villescas et al., 1981). For example, in rabbits and rats prenatal exposure to ionizing radiation (1.5–2.0 Gy) is accompanied by significant disorders in conditioned reflex development. Exposed animals display a delayed consolidation of conditioned reflexes, occasional complete failure to develop differentiation, and a significant increase in the number of reactions in the absence of stimulation (Piontkovsky, 1961, 1964; Kruglikov, 1961; Kolomeitseva, 1961; Rizhinashvili et al., 1978). Intrauterine hypoxia deteriorates the ability of rats to develop conditioned reflexes in a T-maze (Provodina et al., 1981). Administration of hydrocortisone to rats on days 16 and 18 of pregnancy induces altered behavior in an "open field" in their offspring (Dygalo, 1982).

These and many other experimental data suggest that prenatal exposure to the adverse factors of spaceflight may have remote effects on the nervous system and behavioral reactions of the offspring. Since the effects of microgravity on the mother-fetus system were studied for the first time on Kosmos-1514, it not surprising that this type of information is unavailable in the literature. There is scarcely more information concerning the effects of prenatal exposure to the dynamic factors accompanying spaceflight.

In considering this, observations of animals developing in microgravity included an extensive program for studying the work capacity of the central nervous system and the behavioral reactions of the animals, at various stages of their postnatal development, up to the period of sexual maturity. The results of investigations of the simplest reflexes and of the development of the sensory systems during the first month of life are presented in Chapter 7.

On days 30, 51–53, and 88 of life, assessments were made of the pups' emotionality and orienting/exploratory reflexes in a round "open field" 90 cm in diameter, divided into three zones (from the center to the periphery) and 32 areas. Parameters measured included: horizontal and vertical motor activity; frequency of entering the center area of the field and relative length of the path taken there; special orienting reactions (sniffing, standing on the hind legs); grooming movements; and amount of urine and excrement. The results obtained are presented in table 32.

When studied on day 30, the flight pups showed a significantly diminished (compared to the vivarium and synchronous controls) frequency of visiting the central zones of the field, and a decrease in relative length of the path to these zones, as well as in the number of orienting reflexes. The total motor activity (horizontal and vertical) was less in the flight group than in the vivarium control group, but a similar decrease was noted in the synchronous control group. The greatest difference between the flight and control groups was found at days 51–53. At this stage the animals that developed under conditions of microgravity showed substantial and reliable decreases in all parameters of exploratory activity in the "open field." When the animals reached the age of 3 months, the differences between groups decreased and were no longer significant for any parameter.

At 51–53 days of age the male flight rats displayed elevated emotional excitation, which manifested as a significant increase in the quantity of excrement produced in the "open field." A tendency for this parameter to be elevated was also noted in the synchronous control, but to a much smaller extent than in the flight group. The difference between the two groups was statistically significant. No differences in this parameter were found in the female rats (fig. 38). Another parameter, usually considered as a sign of emotional excitation (viz., frequency and duration of grooming), displayed no intergroup differences in the "open field" test on days 51–53. When testing was conducted on 3-month old rats, no intergroup differences were observed in terms of emotional excitation.

Analysis of the course of adaptation to "open field" conditions observed during three consecutive days (51–53 of life) showed no intergroup differences. When the rats were 3 months old, a test was conducted to measure their reactions in the open field situation to an extreme stimulus (bell); no reliable intergroup differences were observed.

When the rats were 2 months old (58–72 days of life), their behavior in a Dombrovskaya maze was studied. This maze consisted of six parallel branches and a start and goal box. Each branch had several locked doors and one unlocked door. After special preparation (familiarization with the maze environment), the animals were given the task of finding the single closed but not locked door in each branch, and proceeding to the goal box to get a food reward. During task performance, the following parameters were recorded: the number of balks (refusal to traverse the maze); duration of latent period (time it took to leave the start compartment); number of errors (attempts to go through a locked door or pushing against

the dividing partition); time to traverse the maze; number of times the animal stood up on its hindlegs (a response associated with loss of orientation); and the frequency and duration of grooming. Also, errors associated with loss of orientation, such as pushing against locked doors or partitions and movements opposite to the direction of the goal box, were recorded. Behavior was studied in the Dombrovskaya maze over 11 consecutive trials, each trial containing three tasks. After trial 8, the goal path was changed; unlocked doors were locked; and one previously locked door in each branch was unlocked.

During the second task of the fifth trial, after 5 seconds, an external inhibitory stimulus—a bell—was presented. The results obtained are summarized in table 33. The flight and control group contained the same number of animals unable to acquire the algorithm for running the maze. The only difference was that the failure of the majority of rats in the flight group was manifested sooner than those in the control group—the former group failed during the first stage of preparation when all the doors were open.

Among the animals learning the maze, the number of refusals was higher in the flight than in the vivarium group, but the former did not differ from the synchronous controls. It is interesting however, that balks occurred in both control groups in the early maze branches, while the flight animals balked toward the end of the maze; in 50% of the cases (compared to 18–20% in the control), balks occurred in the last branch before the goal box. The difference between flight and control groups in this parameter was statistically significant ($p < 0.001$).

The duration of the latency period and the number of errors occurring while running the maze were the same in all three groups (table 33); the time required to reach the goal was significantly longer for the flight animals than for both control groups.

It is noteworthy that the flight animals displayed an elevated number of grooming responses (washing, scratching, and others) in the maze as compared to the vivarium and synchronous controls (fig. 39), evidently due to intensive irradiation of excitation against the background of attenuated internal inhibition. The flight animals also showed a high number of inappropriate movements, i.e., behavior not relevant to obtaining the primary goal (obtaining food): standing up; leaning on the wall; running back and forth in the branches; running through the branches without attempting to open the doors; pushing the wall in the direction opposite to the goal box or toward blind alleys or cover of the maze; nosing the open door multiple time; etc. The number of such movements did not decrease from trial to trial, as occurred in the control group, but remained at a constant level or even increased, which was evidently associated with inertia of irradiated excitation.

The diminished flexibility of neural processes in flight animals also attests to their greater reaction to the external inhibitory stimulus. The process of switching from the reaction evoked by this stimulus was more difficult and took more time in flight than in control animals. For the former, the number of errors, time required to run the maze, number of inappropriate movements, and amount of grooming all increased substantially. The greatest differences occurred as a result of external inhibition, i.e., in the trial following presentation of the inhibitor. While in each successive trial the control animals demonstrated the expected decrease (or insignificant increase) in the number of inappropriate movements and errors, these parameters increased significantly in the flight group. Utilization of past experience in the new situation was not disrupted in the flight animals; they transferred the skill of running the maze as readily as the control animals. All the parameters recorded were no greater in the flight than in the control animals. However, the flight animals did not demonstrate a decrease from trial to trail ("disinhibition") in the number of inappropriate movements, as occurred in the control groups.

The weakness of the inhibition process, the tendency to irradiation of excitation, and the decreased flexibility of fundamental neural process fostered more frequent overexcitations and neurotic states in the flight rats.

All the changes observed were not specific (to space-related conditions) and are known to be associated with the effects of a large variety of factors: irradiation, hypoxia, drugs. Examination of the synchronous animals showed that, in this experiment, the dynamic factors associated with flight (with the exception of microgravity) also had some influence on the establishment of behavioral reactions in the offspring of exposed animals. The effects of these factors and microgravity were evidently determined by the laws underlying the combined action of several factors (Apanasenko, 1966; Livshits and Meyserov, 1966).

All the results obtained in this portion of the research support the conclusion that the effects of spaceflight factors, on developing fetuses exposed on days 13–18 of the prenatal period, do not induce gross disorders in central nervous activity. The animals that developed under conditions of microgravity were virtually normal in their ability to orient themselves to the new environment, to master the behavioral algorithms required, and to use what they had learned in a new situation. The flight group did not differ from the control group in the number of animals mastering the appropriate behavioral algorithm for the mazes, the number of balks, errors in performing

the tasks, or the latency period duration. The animals in all groups were approximately equal in their ability to transfer learned skills.

However, the behavioral reactions of the flight animals were not completely identical to those of controls. The flight rats showed shifts in subtle, easily disrupted mechanisms of internal inhibition, lowered equilibrium, and decreased flexibility of fundamental neural processes. The phenomena observed may be considered a result of attenuation of the inhibitory effects of the cortex on subcortical structures, evidently due to delayed maturation of functional cortical structures, induced by prenatal exposure to spaceflight factors. The changes noted may be the reason for the decreased work capacity of the central nervous system and diminished adaptability to environmental conditions, especially in difficult situations or limited time durations.

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Chapter 11

Reactions to Stress Tests at Various Stages of Postnatal Ontogenesis

Published data suggest that exposure to stress during prenatal and early postnatal stages affects reactivity to stress tests at later stages of development, including sexual maturity (Levine, 1962, 1967; Haltmeyer et al., 1967; Ader, 1968; Ader et al., 1969; Denenberg et al., 1971; Erskine et al., 1975; Naumenko et al., 1979). For this reason, the research of the rats exposed to microgravity during prenatal development, included stress tests presented at 30 and 90 days of age. Stress was created by placing the animals in immobilization cages designed to accord with individual body size. Duration of exposure was 2.5 hours. Stress reactions were estimated on the basis of blood analysis taken twice, immediately before and immediately after immobilization.

At both time points, control and flight animals tolerated the stress test well; their general state upon removal from the cage was satisfactory. The animals of the control group at 30-days-old already displayed the reaction typical of adult rats: a decrease in the count of lymphocytes in peripheral blood and increase in that of neutrophils. The ratio of lymphocytes to neutrophils dropped from 3.8 before the test to 1.4 after. Abnormal changes—increase in the count of lymphocytes and decrease in that of band neutrophils—occurred after the stress test in fewer than 10% of the cases, and the effects were slight when they occurred (within the limits of 1 thous./mm^3).

During the same developmental period, flight rats developed neutrophilia comparable in severity to that of the control animals. However instead of lymphopenia, as observed in the control, 83% of the flight animals showed significant lymphocytosis—the count of lymphocytes increased by a mean of 3.2 thous./mm^3. The ratio of lymphocytes to neutrophils decreased in the majority of flight rats; however in 27% of the cases, there were abnormal reactions in which the lymphocytes/neutrophil ratio either did not change after stress or increased (table 34).

When tested at 90 days, the majority of animals in both the control and flight groups displayed a normal blood system reaction to stress. At the end of the stress test, the concentration of lymphocytes in peripheral blood decreased equally in control and flight animals, and the proportions of anomalous reactions were identical (table 34). Neutrophilia was observed both in flight and control animals; however, it was significantly more severe in flight rats. Also at the end of the stress test, the lymphocyte/neutrophil ratio decreased in all animals of both groups, and no significant intergroup differences in severity of the reaction were noted.

Thus at both periods of observation, the flight animals, like the controls, satisfactorily endured the stress induced by temporary movement restraint.

When the animals were tested at the age of 90 days, an adequate reaction to the stress test—lymphopenia, neutrophilia, decreased lymphocyte/neutrophil ratio—was observed in an equal number of flight and control animals. The only difference between the groups at this age was the greater extent of neutrophilia in the flight animals. It should be noted that the initial (pre-test) count of neutrophils was identical in the flight and control animals (3.0 and 3.2 thous./mm^3, respectively). With respect to the count of band neutrophils in the blood, the response to the stress tests in the flight animals (7 thous./mm^3) was substantially greater than in the controls (5 thous./mm^3).

When the animals were tested at 30 days, the flight group contained a higher percentage of animals manifesting an abnormal stress reaction: an increased count of lymphocytes (83% of the cases, $p < 0.002$) and an increased lymphocyte/neutrophil ratio (27% of the cases, $p < 0.05$). The reaction of lymphocytes to stress occurs in two stages: the release of lymphocytes into the blood from the thymus and spleen, and their migration into bone marrow and tissues, where they serve to trigger hemopoiesis and activate the mitotic activity and as a source of anabolic materials that form during their degradation (Gorizontov, 1973, 1974; Gorizontov et al., 1983). It appears that in the 30-day-old flight pups, in contrast to the control animals, only the first stage of this reaction occurred, which may be considered an indicator of some retardation in development, since later—at 3-months old—both stages of the reaction occurred in both the flight and control groups.

It should be noted that the initial (pre-test) examination of 30-day-old flight rats revealed a lower count of lymphocytes (6.9 thous./mm^3, versus10.8 in the control, $p < 0.01$) and neutrophils (1.7 thous./mm^3, versus 3.5 in the control, $p < 0.01$), in the flight group. This may have caused the difference in the count of formed blood cells in the stress response.

The data obtained clearly show differences in the reactivity of the blood system to stress tests between flight and control animals. Unfortunately, because the number of animals that developed in microgravity was so limited, we chose to preserve them for other studies, and did not dissect them for evaluation of the lymphoid organs, bone marrow, and hormonal status, following the stress test.

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Chapter 12

Structure and Metabolism of the Organs of Animals at Various Stages of Postnatal Ontogenesis

12.1 General State of the Animals: Body and Organ Weight, Blood Profile

At every observation point, the flight and control pups were in good condition; they were active and ate well. Only two rats (one from the flight group and one from the vivarium control group) showed signs of listlessness, loss of appetite, and a delay in body weight gain. When these animals were dissected on day 30 of their lives, they both were found to have pneumonia.

In accordance with the experimental design, a portion of the flight pups and of those in both control groups were dissected at ages 15, 30, and 100 days, to study the structure and metabolism of their visceral organs and musculoskeletal system. On day 15, only female pups were dissected, and on days 30 and 100, only males. The animals were sacrificed by decapitation. Aside from the two cases mentioned above, no pathological changes were noted in the viscera after dissection.

No differences were found between the flight and control groups in the absolute or relative (in proportion to body weight) weight of visceral organs, at any of the observation points.

The weight of the thymus was 100–120 mg in the 15-day old pups and was essentially identical in all three groups; by day 30, the weight of the thymus had increased almost three times, remaining identical in the flight and control animals. Further increases in body weight were not accompanied by any substantial growth in thymus weight, with the exception of isolated cases, most likely related to individual characteristics of the animals. The relative weights of the thymus in the 15- and 30-day old pups were virtually identical—about 0.35% of body weight. By day 100 this percentage had dropped to 0.1%. No significant differences in thymus weight were noted between the flight and control groups, at any examination point.

The absolute weight of the adrenal glands increased from 8–10 mg on day 15, to 22–25 mg on day 30, and subsequently continued to increase, reaching approximately 50 mg in the 100-day old animals. The relative weight of the adrenal glands decreased with age. In all cases this parameter was nearly identical for the flight and control animals.

The absolute weight of the liver in 15-day-old pups was slightly less than 1 g; on day 30 it increased to 5 g; and on day 100, to 12–13 g, remaining identical in the flight and control animals. The relative weight of the liver did not fluctuate significantly with age, comprising approximately 3% on day 15, 5% on day 30, and 3.5% on day 100, and was also identical in all three groups.

The absolute weight of the kidney progressively increased from 150 mg in the 15 day-old pups, to 1.2–1.3 g in the 100-day-old animals. The relative weight of the kidney comprised 0.45–0.50% on days 15 and 30, and decreased slightly (0.35%) by day 100. No differences were found between groups at any point.

Hematological studies of the flight animals were also performed at various stages of the postnatal period. Such studies are of great interest, particularly those of the erythrocyte system, which is highly sensitive to the effects of microgravity; moreover, postflight examinations of the pregnant flight rats revealed signs of anemia and decreased concentration of colony forming units (CFUs) in bone marrow (table 6). In the neonates that partially developed in microgravity, concentrations of hemoglobin and reticulocytes in blood also tended to decrease; differences from the controls were significant (table 26). It was feared that during the development of the flight pups, signs of anemia might progress. However, when the rats were examined on day 15 of life, no differences from the controls were found in hemoglobin. Examinations on days 30 and 100 revealed that hemoglobin in the flight animals was actually higher than in the control groups. The difference between the groups was slight but statistically significant (table 35). The reticulocyte counts in the flight and control animals were virtually identical at these points.

At all observation points, no differences between the flight and control groups were found in the leukocyte count or in the ratio of lymphocytes to band neutrophils in peripheral blood.

12.2 Concentration of Hormones in Blood Plasma

On days 15, 30, and 100, the concentrations of hormones were measured in the plasma of the rats exposed to microgravity. The methods are described Section 3.2. The results obtained are presented in figure 40.

On day 15, the level of corticosterone in the blood of the flight rats was significantly higher than in the vivarium controls; however, similar changes were also noted in the synchronous control group. On days 30 and 100, no significant intergroup differences were noted in this hormone.

The concentration of insulin in blood was measured on days 30 and 100; no significant differences were found

between the flight and vivarium control groups. On day 100, though, the synchronous rats displayed a significant decrease in the concentration of insulin, compared to that of the vivarium control ($p < 0.05$) and flight groups ($p < 0.02$).

On days 15 and 30, the concentration of prolactin in the blood of animals in all three groups was identical and very low—at the lower limit of the method sensitivity—but commensurate with other data in the literature (Gluckman et al., 1981). On day 100, the level of prolactin in the flight and control animals was identical and corresponded to the normal levels for that age group.

The level of somatotropic hormone (STH) on day 15 was identical in the flight and control pups. On day 30, there were no significant differences between the flight and vivarium control groups, although STH was elevated in the blood of the synchronous controls. On day 100, the level of STH in the blood of the flight animals was significantly lower than in the vivarium and synchronous control groups. Since during all stages of postnatal ontogenesis of the flight animals, body and organ weights did not differ significantly from the corresponding control parameters (Section 2.1), one may hypothesize that the decreased level of STH was unstable and could be associated, not with a diminished secretory capacity of the adenohypophysis, but with an uncontrolled stress-effect occurring before sacrifice of these animals. Also, the possibility of changes in the diurnal fluctuations of STH in the flight animals cannot be ruled out.

12.3 The Sympatho-Adrenal System

The state of the sympatho-adrenal system of the flight and control animals, at various stages of postnatal development, was investigated by studying metabolism of catecholamines in the adrenals and catecholamine concentrations in plasma, using methods described in Section 3.3.

At birth, the peripheral organs of rats have inactive or absent sympathetic innervation; the secretion of catecholamines by the medullary layer of the adrenals is the main pathway of adrenergic effects (Slotkin et al., 1980; Slotkin, 1985). Since *n. splanchnicus,* which to a significant extent regulates the activity of the medullary layer of the adrenals, does not start to function before the end of the first week of postnatal development (Slotkin, 1973a), the secretion of norepinephrine is triggered by means other than the neurogenic mechanism (Chantry et al., 1982). Published data attest to the fact that catecholamines of the adrenal medullary layer play a significant role in the capacity of newborns to endure stress, and for example, to survive acute hypoxia. (Seidler and Slotkin, 1985).

In the present experiment, animals that spent a portion of their prenatal period in microgravity were examined on days 15, 30, and 100 of life.

The concentration of epinephrine in the adrenals increased as the animals matured (fig. 41), with changes in the flight, vivarium, and synchronous groups being virtually identical. The concentration of norepinephrine also increased with age; between 30 and 100 days, this increase was greater in the flight group than in the vivarium or synchronous controls ($p < 0.05$). Throughout the study, the concentration of dopamine in the adrenals was virtually identical in the flight and vivarium control groups, while it was significantly lower in the synchronous controls on days 30 and 100. (fig. 41).

The activity of tyrosine hydroxylase—the key enzyme in the synthesis of catecholamines in the adrenals—and that of phenylethanol-amine-N-methyltransferase, also increased with age, remaining, however, identical in all three groups at all observation points (fig. 42).

It should be noted that in the vivarium animals the above age-related changes in the concentrations of adrenal catecholamines and the activity of the enzymes involved in their synthesis corresponded to the results we obtained earlier (Kvetnansky et al., 1978) and other data in the literature (Philpott et al., 1969; Patrik, Kirchner, 1972; Slotkin, 1973b; Srivastava, Kapoor, 1979).

The totality of the results obtained suggest that exposure of the mother-fetus system to microgravity for 5 days during the last third of pregnancy, which induced pronounced changes in catecholamine metabolism of the maternal body, did not appear to affect the formation of the corresponding system in their offspring.

12.4 Thyroid Gland

The state of the thyroid gland in animals of the flight and control groups at various stages of postnatal ontogenesis was determined on the basis of the concentrations of thyroxine and triiodothyronine, using methods described in Section 3.4.

On day 15, the offspring of the flight females displayed a significant ($p < 0.025$) decrease in the concentration of triodothyronine in the thyroid gland as compared to the vivarium and synchronous control groups; at days 30 and 100, the differences between the flight and vivarium control group were not significant. In the synchronous control group, the concentration of triiodothyronine increased on day 30. The concentration of thyroxine in the

thyroid gland was comparable in all three groups at all points of measurement (table 36).

12.5 Hemopoietic Stem Cells

The concentration of hemopoietic stem cells was measured in the spleen and bone marrow of animals exposed to microgravity *in utero,* using methods described in Section 3.5.

In 18-day-old fetuses, the quantity of stem cells in the liver, which is the major hemopoietic organ at this age, averaged 8.2 per 10^6 cells in the flight group, 10.5 in the vivarium, and 13.4 in the synchronous control group ($p < 0.05$). At the moment of birth there were no longer any differences between the flight and vivarium control group. The concentration of CFUs in the spleen was 9.5 per 10^6 in the flight group and 10.3 in the vivarium controls.

In the spleens of the flight newborns, the concentration of CFUs was elevated and averaged 19.2 per 10^6, while the corresponding values were 10.0 in the vivarium and 8.5 in the synchronous controls ($p < 0.001$). During the first 15 days of life, the number of spleen cells in the control animals increased sharply (fig. 43), while remaining virtually unchanged in the flight animals. As a result, by day 15 the total number of spleen CFUs in the flight rats was significantly lower than in both control groups ($p < 0.02$), although the relative concentrations of CFUs were equal in the flight and synchronous control groups (table 37).

At the next stage of development—the end of their first month of life—the differences between the flight and control animals diminished, and at day 30 no significant differences among the groups were found in the total number of splenocytes, or the concentration and total number of CFUs in the spleen (fig. 43, table 37).

From day 30 to day 100, there were only an insignificant increase in the total number of splenocytes, while the concentration of CFUs decreased several times ($p < 0.01$), as did the total number of spleen CFUs. At day 100, the differences between groups in the number of splenocytes, and the concentration and total number of spleen CFUs were not significant (fig. 43).

Study of the bone marrow of pups was begun when they were 15 days old. At that point, the quantity of CFUs in 10^6 karyocytes in the flight group was significantly lower than in both control groups (table 37), but due to a greater quantity of karyocytes in the flight group, the total number of CFUs in femur bone marrow was virtually the same in all three groups (fig. 44).

Between day 15 and day 100, the number of karyocytes in the flight and control groups steadily increased, remaining virtually the same. At days 30 and 100 no reliable intergroup differences were found in the concentration and total number of CFUs in bone marrow (table 37, fig. 44).

Thus, exposure to microgravity during pregnancy was accompanied not only by changes in the number of hemopoietic stem cells in the flight mothers but also by a certain instability in this system of their offspring during the prenatal and early postnatal periods. In 18-day-old fetuses of the flight group, the concentration of CFUs in the liver was diminished. Although at birth this measurement no longer differed from the controls, the concentration of CFUs in the spleen of the flight newborns was elevated. The differences between groups were maintained until the rats were 15 days old and were completely compensated for by the end of their first month of life. At this time and later—at the end of their third month of life—no reliable differences were found in any of the parameters studied.

12.6 Concentration of Fluid and Electrolytes in Tissues

At ages 15 and 30 days, the concentrations of water, potassium, sodium, calcium, and magnesium were measured in various tissues of the flight and control animals: bone (tibia), skin, liver, and kidney. Methods are described in Section 3.6.

When the rats were examined on day 15, no significant differences were found between the flight and control groups in terms of the kidney weight, water content, or concentration of electrolytes. A similar pattern was observed in the liver, skin, and bone (table 38). Before day 15, the pups consumed only their mothers' milk; thus the fluid-electrolyte composition of their tissues was influenced by the mineral composition of the milk, which in turn reflected the mineral levels of their mothers' bodies. The fact that 15-day-old pups, that had spent part of their intrauterine development in microgravity, displayed no significant changes in mineral homeostasis, attests not only to its normal development and regulation in the offspring, but also to a definite degree of normalization of the changes noted in the females immediately postflight.

By day 30, the pups were completely weaned and could live independently from their mothers. It is during this period that latent defects in the fine control of homeostasis could appear. However, comparison of the fluid-electrolyte balances of the flight and control pups revealed no significant differences (table 38).

12.7 Concentration of Electrolytes in the Coats and Tails of the Animals

On days 15, 30, and 100, the concentrations of potassium, sodium, calcium, magnesium, strontium, iron, phosphorus, zinc, copper, and manganese were measured in the coat and tail, using methods described in Section 3.7

The coats of 15-day-old rats, that developed under spaceflight conditions, displayed a significant elevation in the level of iron, compared with the coats of both control groups. The levels of other mineral elements were the same as in the vivarium control. The tails of the 15-day-old flight pups showed a significant decrease in the concentrations of potassium, sodium, and iron, compared with the tails of the vivarium and synchronous controls.

At days 30 and 100, the flight animals did not display any changes in the concentrations of mineral elements, which could be related to the effects of microgravity during their prenatal development. In a few cases, the parameters of the flight animals significantly differed from those of the vivarium controls, but similar changes were also observed in the synchronous controls.

12.8 Lipid Metabolism

The lipd content of the flight and control pups was evaluated, using methods described in Section 3.8. The livers investigated at postnatal days 15, 30, and 100 showed no differences between the flight and vivarium control groups in the absolute amounts and relative concentrations (with respect to organ weight) of triglycerides (fig. 45), phospholipids, or cholesterol.

When the thymus was studied at days 30 and 100, no differences were found between the flight and vivarium controls in the concentration of triglycerides or phospholipids; on day 30, triglyceride concentrations in the thymus of the synchronous controls were significantly elevated (fig. 45).

On postnatal days 15, 30, and 100, the concentration of nonesterified fatty acids in the white fat of the flight animals was identical to that of the vivarium controls; in the synchronous control group the concentration of nonesterified fatty acids in the white fat was elevated on days 15 and 30.(fig. 45).

On postnatal day 15, the level of nonesterified fatty acid was elevated in the brown fat of the flight group as compared to the vivarium controls ($p < 0.05$); however this parameter was even higher in the synchronous control animals. The difference between the synchronous and flight groups was not significant. On day 30, the concentration of nonesterified fatty acids in the brown fat was identical in all three groups (fig. 45).

The results obtained show that, in spite of pronounced changes in the lipid metabolism of the rats exposed to microgravity while pregnant, the lipid metabolism of their offspring was virtually unaltered when compared to the controls.

12.9 Concentration of Nucleic Acids in Tissues

The concentration of nucleic acids in the liver, thymus, and spleen was measured in flight and control animals at different stages of postnatal ontogenesis, employing the same methods used for their mothers (Section 3.9).

The total level of RNA in the control livers increased with age: in 30-day old rats it was three times greater than in 15-day-olds; and it further increased by a factor of 2 by day 100 (table 39). In the 15-day-old pups of the flight group, the level of liver RNA tended to decrease when compared to the vivarium control group; however a similar decrease was also noted in the synchronous control group. Age-related changes in the concentration of liver DNA in the control animals were similar: an approximately threefold increase between days 15 and 30, and a greater than twofold increase by day 100 (table 39). No reliable differences were observed between the flight and control groups in the total concentration of DNA in the liver .

The concentration of nucleic aids in the thymus was measured on days 30 and 100. No substantial age-related changes were noted in the absolute or relative concentration of RNA in the organ, nor were any differences noted between the flight and control groups (table 40). By day 100 the DNA concentration in the thymus increased by approximately one third in the control animals, while the total level doubled compared with day 30. At no observation point were significant differences found between the flight and vivarium control groups (table 40).

When the relative concentration and absolute amount of DNA and RNA were measured in the spleens of 100-day-old animals exposed to microgravity *in utero*, no reliable differences were detected in comparison to either control group (table 41).

12.10 Biosynthesis of Nucleic Acids

Aside from measuring the concentration of nucleic acids in the organs, metabolism was determined from the rate of RNA synthesis in nuclei of liver cells (on day 18 of prenatal development, and days 1, 15, 30, and 100 of postnatal development) and the rate of incorporation of radioactive precursors of DNA and RNA into spleen lymphocytes, using the methods described in Section 3.10.

Figure 46 presents the results characterizing endogenous RNA synthesis in hepatocyte nuclei of the flight and control animals. No differences between the flight and control groups were found in neonates or 15-day-old pups; however on days 30 and 100, the rats exposed to space prenatally displayed a 30 to 40% elevation of RNA-synthesizing activity as compared to the vivarium and synchronous controls ($p < 0.05$). Similar results were obtained when the activity of RNA-polymerase solubilized from hepatocyte nuclei was measured. In 30-day-old flight rats there was a 62% increase in enzyme activity, compared with the synchronous and vivarium controls ($p < 0.05$), while there was a 65% elevation at 100 days ($p < 0.05$)

In the system used to determine enzyme activity, RNA-polymerases I and II functioned simultaneously to synthesize the respective precursors of rRNA and mRNA. The use of alpha-amenitine, a specific inhibitor of RNA-polymerase II, made it possible to identify the contribution made by each form of the enzyme to the total activity. It was demonstrated that when alpha-amenitine was added to the incubation medium (5 μg/ml), activity of RNA-polymerases in the liver cells of the flight animals remained higher than in the vivarium and synchronous controls ($p < 0.05$), supporting the conclusion that the elevated enzyme activity in the flight rats was due both to RNA-polymerase I and RNA-polymerase II.

Table 42 presents data on the rate of incorporation of radioactive precursors in nucleic acids of spleen lymphocytes, in 30- and 100-day old rats exposed to microgravity during their fetal period. In these age groups, the rate of DNA synthesis in the flight animals did not differ from that of the controls. The label incorporatied into the RNA of the 100-day-old flight animals was significantly decreased, compared with the vivarium controls; however, the synchronous group also displayed a decrease, so the difference between the flight and synchronous groups remained insignificant.

The data obtained demonstrate that animals, who spent a portion of their prenatal development in space, did not display any serious changes in the anabolic metabolism of nucleic acids during postnatal ontogenesis. At the same time, the fact that the transcriptional activity in liver cells was elevated in the 30- and 100-day-old flight animals is noteworthy and deserves special analysis and further study.

12.11 Activity of Liver Enzymes

The activity of tyrosine-aminotransferase (TAT) and aspartate aminotransferase (AST) in the liver of rats exposed prenatally to microgravity was measured at ages 15, 30 and 100 days. Activities of tryptophan-pyrrolase (TP) and alanine-aminotransferase (ALT) were studied on days 30 and 100. The methods used are described in Section 3.11.

Age-related changes in enzyme activity in the control animals corresponded to data in the literature (Knox, 1972; Hersfeld et al., 1976).

On day 15, activity of TAT was significantly elevated in the flight rats compared to that in the vivarium controls; however similar and even more marked changes occurred in the synchronous control group (table 43). AST activity in the liver of the flight rats was significantly below that of the vivarium controls; however decreased activity of this enzyme was also noted in the synchronous control group.

On day 30, there were no reliable differences between the flight and the control animals in liver activity of TP, ALT, or AST. TAT activity was lower in the flight animals than in the vivarium controls, but did not differ from the synchronous controls (table 43).

On day 100, the activity of ALT and AST in the liver was similar in the flight and control groups. TP activity was significantly higher in the flight animals than in the vivarium controls, but did not differ from the synchronous controls. TAT activity was elevated compared to both control groups, but only significantly when compared to the vivarium group.

Thus, exposure to microgravity during prenancy induced no serious changes in the activity of TP and transaminases in the liver of the flown mothers, nor in their offspring at various stages of postnatal ontogenesis.

12.12 State of the Myocardium

The absolute weight of the myocardium was approximately 110 mg in 15-day-old pups, 370-390 mg in 30-day-olds, and 1.0–1.1 g in 100-day-olds. The relative weight of the myocardium did not change significantly with age and was approximately 0.35% body weight at days 15 and 30, and slightly less, 0.3%, on day 100. The parameters were identical for animals of the flight and control groups. No reliable differences were found between groups when the right and left ventricle and septum were weighed separately.

The water content of the hearts of the flight animals was the same as that of the control groups on days 30 and 100. On day 30, the protein concentration in the hearts of the flight rats tended to increase compared to the vivarium controls, but this difference was no longer apparent by day 100.

12.13 Collagen Metabolism in the Skin and Bone

The goal of this research was to assess potential changes in the postnatal development of organic components in the connective tissue of the skin and bone of animals that spent a portion of their prenatal period under spaceflight conditions. Attention was focused on the differences in the so-called genetic types of collagen, identifiable in its soluble fractions released by pepsin. Biosamples (femur, skin of the back) from animals in the flight, synchronous and vivarium control groups were analyzed immediately after birth and subsequently on days 15, 30, and 100 of life. The methods are described in Section 3.13.

Biosamples from individual animals were combined to obtain amounts sufficient for analysis. This was necessary due to the low weight of bones in neonate rats and the low solubility of collagen in adult rats. For each group 2–7 biosamples were combined to generate 1–2 values, decreasing the reliability of the results and making statistical processing impossible. This shortcoming was compensated for by repeated analysis of each sample, and also by the fact that, in the control animals, postnatal changes of the parameters studied in individual samples were similar, and that parameters obtained for the vivarium and synchronous control groups were virtually identical.

The results of bone examinations are presented in figure 47(a) which shows the changes in the percentage composition of pepsin-soluble collagen in bone. The control animals displayed high proportions immediately after birth and a smooth decrease during the postnatal period. In flight animals, the concentration of soluble collagen remained high until sexual maturity. On days 15, 30, and 100, this parameter was significantly higher in the flight than in the control groups.

The ratio of hydroxyproline (collagen) to glycoproteins of the soluble fraction (fig. 47(b)) was low in the bones of control neonates but increased sharply by day15 and subsequently continued to slowly increase. The flight animals displayed the opposite pattern: high ratios of collagen to glycoproteins at birth, and their gradual decrease with age. The rate of decrease of this parameter in the flight group was virtually identical to its increase in the control groups.

The percentage of type IA collagen in the soluble fraction was at all times higher in flight animals than in the control groups; however time course variations in this parameter were identical in all groups (fig. 47(c)). The percentage of type IB collagen increased in the control animals after day 15, while in the flight animals it remained virtually unchanged. As a result, the level of this parameter on days 30 and 100 was significantly lower for the flight than the control groups (fig. 47(d)).

The level of type II collagen in the soluble fraction of the control animals was high at birth and gradually decreased during the postnatal period, reaching minimal values at 3 months (fig. 47(e)); in the flight animals the level of type II collagen at birth fluctuated, sharply increasing at day 15, decreasing at day 30, and again increasing at day 100.

The percentage of type III collagen in the soluble fraction of bones of control newborns was twice as high as in the flight group. On day 15, it increased slightly, while by day 30 it had decreased sharply (fig. 47(f)). In the flight animals, the level of type III collagen was low at birth, continued to decrease by day 15, and then sharply increased by day 30. The level of type III collagen was five times higher in the flight group than in the control at day 30. By the time the animals were 100 days old, this parameter decreased sharply in the flight group, but remained higher than that in the control animals.

Results of analysis of skin samples are presented in figure 48. Flight animals, in comparison to controls, displayed a slower decrease in the level of soluble collagen with age (fig. 48(a)) and a slower increase in the ratio of hydroxyproline to glycoproteins (fig. 48(b)).

Type IA collagen was virtually absent in the soluble fraction in the skin of the flight newborns; subsequently its level increased slowly, reaching 5% when the flight pups were 100 days old (fig. 48(c)). In the control groups, the level of type IA collagen was high at birth (approximately 20%) and subsequently decreased, reaching a level identical to the one for the flight group at day 100. Differences between groups in type IB collagen were small at all observation points (fig. 48(d)).

Type III collagen in the soluble fraction (fig. 48(e)) of the skin in neonate control groups was approximately 5% and decreased with age, reaching 1% on day 100. In flight neonates and 15-day old pups, the percentage of type III collagen in the skin was high (approximately 20%); but by the end of the first month, it dropped to the level observed at this time in control rats; and by day 100 it somewhat increased again.

Thus at all stages of postnatal ontogenesis, the animals that spent a portion of their prenatal development under conditions of microgravity displayed recognizable changes in the collagen metabolism of the skin and bone, compared with animals of the vivarium and synchronous control groups.

Collagen, the major structural macromolecule of connective tissue, is a heterogeneous system composed of

several independent proteins, differing in sodium chloride solubility, concentration of carbohydrates, and other physical-chemical properties. Type I is the major collagen of bones, ligaments, mature skin, and tendons, which determines the mechanical strength of the tissue. A new technique used in this work was the separate isolation of type IA and type IB collagen, based on the higher concentration of glycoproteins in fraction IA. Type II collagen is contained in cartilage. Type II was first discovered in the fetal skin and the uterus, and then in some parenchymatous organs and blood vessels; its level is elevated in young, growing tissues (Miller, 1973; Epstein, 1974; Montes et al., 1982).

In the present experiment, bone samples of control animals revealed age-related changes in collagen metabolism, which can be considered a result of ultra-structural maturity and stabilization: a gradual decrease in the concentration of soluble collagen; an increase in the hydroxyproline/ glycoprotein ratio; an increase in the percentage of type IA and IB collagen; and a decrease in the levels of collagen II and III. Bone samples of the flight animals displayed deviations from normal age-related changes in collagen metabolism, attesting to a delay in bone development and some degree of immaturity in adult animals. These include a higher solubility of collagen, a decrease in the ratio of hydroxyproline to glycoproteins, a delayed increase of type IB collagen, and the retention of higher concentrations of type II and III collagen in the soluble fraction.

Age-related changes in collagen metabolism in the skin of control animals were, with respect to some parameters, similar to changes in bone, and with respect to other parameters, were different. In the flight animals, there was a smaller decrease in the proportion of soluble collagen and a smaller increase in the ratio of hydroxyproline to glycoproteins with age; an increase in the level of type III collagen in the soluble fraction; and other changes attesting to disorders in the normal development of connective tissue and a lower maturity at every age point studied.

The reasons for the changes described are as yet unclear. The occurrence of the same type of changes in the metabolism of connective tissue in the skin and bone of flight animals suggest that these changes are systemic in nature.

Biosynthesis of organic components of connective tissues and their catabolism is regulated at the intra- and extra-cellular level, with a critical role being played by hormonal control (Borel et al, 1984). For example, it was shown that glucocorticoids inhibit the formation of type III collagen in cultured skin fibroblasts (Verbruggen et al, 1982). The skin of diabetic mice contained an elevated quantity of type III collagen (Kern et al., 1978). It is possible that in the current study, the reason for the differences found between the flight and control animals was a change in the hormonal status, due to the mothers' exposure to microgravity.

12.14 Structure of Cartilage

Scanning electron microscopy was used to study the cartilage structure in the area of the proximal epiphysis of the tibia on days 15, 30, and 100 of postnatal ontogenesis. The biosamples were fixed in formaline and treated using methods described by A. Boyde (1972). The use of scanning electron microscopy made it possible to provide a quantitative description of loci of primary cartilage mineralization, the so-called calcified globules or calcospherites (Ornoy et al, 1979).

Normally, growing cartilage has three differentiated zones consisting of: the cartilaginous zone, including of several layers (reserve, proliferating, hypertrophic and calcified); the bone zone, starting in the base of the cartilaginous calcified columns; and the fibrous peripheral zone (Grighton, 1978). Due to the typically high metabolic activity of cartilage, bone formation, particularly primary mineralization of the columns of the cartilaginous matrix of the hypertrophic layer, is proportional to the growth of the body (Salmon 1974; Ali, 1976; Wuthier, 1982).

In the study of biosamples under low magnification, which showed various layers of cartilaginous zones and their orientation, no significant differences were found between the flight and control animals, at various stages of postnatal development.

In the proliferative layer of the cartilaginous zone, the matrix was not mineralized and had the classic form of a collagen fiber network. Chondrocyte lacunae had smooth fibrous bottoms without visible calcified globules. In some zones there were small chondrocytes.

In the hypertrophic layer of the cartilaginous zone, the bottoms of the chondrocyte lacunae were covered with calcospherites. Their size depended on the position of the lacunae: the upper lacunae contained small calcospherites; the lower lacunae, large ones. In some zones, the multi-layer distribution of small calcospherites was distinct. The transition from a nonmineralized to mineralized matrix was more visible in 15-day old pups than in those of any other age group. No reliable differences were found between the flight and control animals at any observation time.

When the zone of bone-cartilage apophyses was investigated, no structural differences were found between the flight and control animals of any age group. The calcified cartilaginous matrix was covered with large collagen

fibers of the osseous type; no calcospherites were observed. The perichondrium vertically separating the chondrocytes were reabsorbed. Deeper within the bone, bone-cartilage apophyses were replaced by bone trabeculae, the surface of which displayed osteocytic lacunae.

The results obtained support the conclusion that the process of postnatal cartilage mineralization and bone differentiation was normal in the animals that spent a portion of their prenatal development in microgravity.

12.15 Cytogenetic Study of Sex Cells

When the male flight rats reached the age of 100 days, a cytological investigation of their sex cells was conducted. Preparations were made from their testes using a modification of Dyban's method (Dyban,1970; Yagova, 1979). A total of 12 rats were examined: 4 each from the flight, vivarium and synchronous control groups. For each animal a total of 200 metaphase spermatocytes were studied. Reciprocal translocations in the form of multivalent rings and chains were counted. At this stage of rat spermatogenesis, 21 pairs of chromosomes (bivalents) are formed. Due to the presence of metacentric and submetacentric chromosomes in the karyotype, they often have a complex configuration, which makes analysis of rat spermatocytes more difficult when compared to, for example, mice. This has been noted by other authors (Leonard, 1984). For this reason all multivalents found in the preparations were analyzed by three additional experts. The results were treated using Fisher's test.

In the flight animals, a total of 815 cells from 4 animals were analyzed. Seven cells (0.9%) were found to have translocations; no translocations were detected in the 803 cells from the vivarium control group ($p < 0.01$). An increased number of translocations was also observed in the synchronous control group (0.5%). The differences between the flight and synchronous control groups were not statistically significant ($p > 0.05$), suggesting that the changes were caused by factors other than microgravity (vibration, acceleration, etc.), simulated in the synchronous control condition.

During exposure to space (days 13–18 of prenatal development) the sex cells studied were in the gonocyte stage, characterized by its high sensitivity to adverse effects (Mandl, 1964). The presence of reciprocal translocations in the spermatocytes of the flight rats suggests that spaceflight factors may induce mutations in gonocytes. Comparison of the results for the flight and the synchronous control animals permits the conclusion that the changes noted were caused not by microgravity, but by concomitant spaceflight factors. At the same time, the results obtained do not allow us to rule out the possibility of mutagenic microgravity effects on sex cells of mammalian fetuses. It should be noted that between exposure to microgravity (days 13–18 of prenatal ontogenesis) and the detection of the anomalies (100 days of life), the cells studied passed through a large number of cell divisions and differentiations, during which the anomalous cells could have been eliminated. Moreover, although the difference between the flight and synchronous control groups was not statistically significant, the number of cells with translocations was half the size in the latter case (0.9 and 0.5%, respectively), and while translocations were found in all flight animals studied, they were seen in only two of the four synchronous controls.

Chapter 13

Reproductive Functions of Animals that Spent a Portion of Their Prenatal Development in Microgravity

When the animals flown on Kosmos-1514 during days 13–18 of their prenatal development reached 3 months of age, their reproductive functions were evaluated, by mating them with each other and other unflown animals, avoiding intrafamily contacts. Vivarium and synchronous controls were mated under the same conditions. An additional group of rats (2.5 month old females from the "Stolbovaya" nursery) was also used for mating. These animals will be referred to as intact to differentiate them from the animals of the vivarium control group, which like the animals of the other two groups, were born and raised in the vivarium of the Institute of Biomedical Problems. The following mating scheme was used:

1. Males and females from the flight group were mated with each other.

2. Males of the flight group were mated with intact females.

3. Males and females of the vivarium group were mated with each other.

4. Males from the vivarium group were mated with intact females.

5. Males and females from the synchronous control group were mated with each other.

6. Males from the synchronous control group were mated with intact females.

In mating, the females were placed with the males, which had previously been put in individual cages, in a ratio of 1 male to 4-5 females. Mating occurred during two consecutive weeks.

The results obtained are presented in table 44. No reliable intergroup differences were found in the number of females failing to be impregnated through lack of insemination or fertilization. A slight increase in this parameter, when flight and synchrnous males were mated with intact females, was evidently caused by the intact females, since the parameter remained normal when the same males were mated with flight and synchronous females. A relatively high percentage of noninseminated females in all groups may be attributed to the mating schedule, in which the animals were placed together for 4 days twice, with a 3 day interval. Since the duration of the estrous cycle in rats is 4–5 days, some of the animals may not have been in estrus during the mating period; this fact, and not reproductive inadequacy, may be responsible for the lack of insemination observed.

When the rats that did become pregnant were studied (fig. 44), no reliable intergroup differences were found in the number of live or stillborn neonates, ratio of males to females in the litters, body weight at birth, or concentration of water and dry substance in fetal tissues. When the neonates were examined by Wilson's method, developmental abnormalities were noted in only one case (hydronephrosis and hepatic petechia), in the group born from copulation of flight males and females.

The concentration of hemoglobin and leukocytes in newborns was identical in all groups. No intergroup differences were found in the weight of viscera (liver, kidneys, heart, thymus, spleen) of the pups.

When the skeleton of the pups was studied according to Dawson, no reliable intergroup differences were found in the size of ossification sites in various segments of the spinal column, hind- or forelimbs, or in the skull.

During the postnatal period, no differences were found among pups of any of the six groups, in terms of body weight gain during the first month of life, or in time for the ears to unfold or eyes to open.

It is noteworthy that in the group obtained from copulation of flight males and females, the rate of postnatal death increased to 12%, while in other groups there were either no deaths (four of the six groups), or few death cases (one group).

It should be remembered that the animals that spent a portion of their prenatal development in space, that served as parents in this mating, also showed a high postnatal death rate (19%), compared to the vivarium and synchronous control groups. There were clear individual differences among the animals in this group. Of the four rats in the flight group which gave birth, postnatal deaths were observed in only two (nos. 16 and 33) and were absent in the offspring of the two other mothers (nos. 71 and 79). Results of separate analyses of reproductive parameters for the offspring of these two groups of mothers are presented in table 45, with control data provided for comparative purposes.

Analysis of these data shows that the postnatal mortality rate was increased only in the offspring of rats nos. 16 and 33, some of whose newborns died during the days early after delivery. The postnatal death rate was 22.2% in this subgroup, in which other reproductive parameters were also deteriorated: the number of live newborns was significantly decreased and that of still-borns increased. The only case of developmental abnormality identified by means of Wilson's method was also detected in this

subgroup. It should be noted that the reproductive capacity was investigated only in the females coming from the flight litters of rats nos. 16 and 33. The males from these litters were used to investigate visceral metabolism at the different stages of postnatal development.

This observation requires special study and experimental verification. It can hardly be viewed as incidental because the high postnatal mortality rate was seen only in one of three groups of animals, i.e., the flight group, and in one of six daughter generations, viz., in the pups obtained from mating the males and females that developed in microgravity. The postnatal cases of pup death occurred mainly in females descended from families that had similar abnormalities.

Discussing the remote effects of exposing the mother-fetus system to adverse factors, many authors claim, that these factors may impact the hormonal system and gonads of a developing fetus, and through them its reproductive function (Makeeva, 1964; Pozhidaev, 1962). They believe that a typical consequence of this exposure is an elevated perinatal or intrauterine and early postnatal death rate, and that the pathogenetic cause is hypoxia during pregnancy or prolonged delivery (Garmasheva, 1964; Makeeva, 1964; Skornyakova, 1964).

Analyzing the time and course of the delivery process in the flight rats (see Chapter 5), it can be postulated that the difficult, prolonged labors that females nos. 16 and 33 experienced, involved a hypoxia that was more pronounced than in normal delivery,which might have caused an elevated postnatal mortality rate in the first and second generations.

Chapter 14

Adaptive Capabilities of the Mother-Fetus System in Microgravity

The results of the embryological experiment on the Kosmos-1514 demonstrated clearly that mammals (Wistar rats) exposed to spaceflight were capable of activating adaptive processes and mobilizing the reserves essential for fetal development. The flight was relatively short in duration, lasting 5 days, but it occupied almost 1/4 of the entire rat gestation period. The animals spent days 13 to 18 of gestation in space, during which the fetus grows rapidly, and the nervous and endocrine systems, skeleton, muscles, and visceral organs form. The rats exposed to microgravity developed successfully in all these parameters. During spaceflight the rat fetuses, whose development began on the Earth preflight, continued to grow and develop, lagging only slightly behind the controls; they completed their development after return to the Earth—during the period of readaptation of the maternal body to normal gravity—and reached sexual maturity, and in turn produced offspring. However, all this required a significant mobilization of the physiological reserves of the maternal body during spaceflight.

Exposure to microgravity during day 13 to day 18 of pregnancy induced a broad range of changes in the maternal body, some of which appeared relatively serious. An integral manifestation of these changes was delayed body weight gain of the female rats by 60 g, i.e., by virtually one quarter of their weight.

A similar delay in weight gain was observed by Lederman and Rosso (1980) in pregnant rats fed 50% of their normal diet. In our experiment, the flight animals consumed approximately the same average amount of food as the controls; the delay in weight gain was evidently associated with activation of catabolic processes in microgravity.

It is interesting to note that in the Kosmos-1667 male flight rats exposed to microgravity for 7 days did not show a decrease in body weight gain when compared with vivarium controls (table 46).

The effect of spaceflight factors on a number of other parameters was also more severe in pregnant females than in males. For example, the females showed substantial leukocytosis, while in the males the leukocyte count was unchanged postflight (table 46); at the same time, the lymphocyte/neutrophil ratio was equally decreased in both cases. The females displayed a 24% decrease of hemoglobin ($p < 0.001$) compared to the controls, while in the flight males hemoglobin tended to be elevated.

The extent of thymus involution (–30%) was greater in the pregnant flight rats than in the flight males (–15%).

Given such significant changes in the maternal body, one might expect serious changes in the major parameters of reproductive functions and viability of offspring (Gromov and Savina, 1964; Antonova, 1968; Garmasheva and Konstantinova, 1978, 1985; Barlow et al., 1978).

This first embryological experiment with mammals in space was exploratory in nature. Based on reported data concerning critical developmental periods (Svetlov, 1956, 1960) and results of preflight simulation experiments , we selected a gestation period during which exposure to adverse factors caused a minimal fetal death rate, to obtain the maximal possible number for postflight studies. In addition, we attempted to minimize the adverse side effects associated with launch and reentry (Denisova et al., 1982).

In the opinion of Arshavsky (1957, 1982), in the last third of pregnancy the mother-fetus system is most resistant to harmful factors; during this period even severe bleeding and food deprivation of the mother has little effect on fetal development. However, Barlow (Barlow et al, 1978) subjected rats to immobilization at various stages of pregnancy—from day 9 to day 20—and found that changes attributable to treatment during the latter stages of pregnancy were not less but, with respect to a number of parameters, greater when compared to immobilization during early stages of pregnancy. Basically, these findings relate the rate and nature of the development of behavioral reactions during postnatal ontogenesis. Naumenko and Dygalo (1979) injected female rats with hydrocortisone on days 16–20 of pregnancy and observed changes in the responses to emotional stress of the offspring at age 3 months.

According to Dyban (1967), exposure of rats to adverse factors on pregnancy days 13–15 can be accompanied by serious developmental abnormalities, including fetal deformity and sometimes death, while exposure to alternative agents after pregnancy day 16 may lead to disorders in the physiological systems which will manifest at various stages of postnatal life. Arshavsky, who asserts a "high protection" of the fetus at the end of pregnancy (1957), points in other works (1962, 1964) to the possibility of developmental delay of the fetus, when exposed to harmful factors during the embryo-fetal period, calling this phenomenon "physiological immaturity of the fetus."

Based on these data, one might expect that the period of pregnancy (days 13–18) we selected for exposure to microgravity, while not critical with respect to the fetal survival rate, might turn out to be critical for individual organs rapidly growing in space. One might also expect

changes in the rate of development of components of complex functional systems, which might have affected the establishment of motor reactions, development of sensory systems, and behavioral patterns (Anokhin, 1948, 1973; Garmasheva and Konstantinova, 1978, 1985; Barlow, 1978; Savchenkov and Lobyntsev, 1979, 1980; Gotz et al., 1984; Kirby et al., 1984).

The flight experiment was designed to detect, whenever possible, potential changes in the development of the flight animals, as they were observed during various stages of their postnatal life, including sexual maturity.

Summarizing the experimental data obtained during postflight examinations, it may be said that the majority of our fears were not realized. In principle it is possible for mammal fetuses to develop normally when the mother is exposed to microgravity and other spaceflight factors during pregnancy. However, when observing animals during various stages of development, we detected a number of differences between the flight animals that partially developed in microgravity and the control pups. It seems desirable to summarize and discuss these differences, since in the main text of this book, they to some extent, are "drowned" amidst the general mass of parameters, which basically were identical in the flight and control groups.

In examining the fetuses on the day of reentry—day 18 of pregnancy—the flight group showed signs of developmental delay as compared to the controls, expressed as diminished body weight, greater concentration of water in the tissues, and retarded ossification of various segments of the skeleton. The differences between the flight and control animals were small for all parameters (within 10%) but statistically significant. These may have resulted from a reduction of the flight placenta and changes in its structure.

It is interesting to consider the (initially unexpected) fact, that similar changes were observed in fetuses developing in mothers exposed to hypergravity of 2 g during pregnancy. In this situation, the centrifuged fetuses were also behind the controls in body weight and size of ossification sites; the extent of these changes was virtually identical to that occurring in fetuses that partially developed in microgravity.

Retardation of growth and development is a general reaction of the fetus to exposure of the mother to various adverse effects (Arshavsky, 1964, 1979; Garmasheva and Konstantinova, 1978, 1985). For this reason, these types of changes in the flight fetuses and pups were not unexpected. More unexpected was the fact that these changes were relatively mild and rapidly reversible. The pups born 5 days after their mother's return to the Earth did not have lower body weights than the synchronous controls, and were even ahead of both control groups with respect to the size of ossification sites.

Another difference between the flight and control groups was the longer and more difficult labor of the flight rats. Of the five flight animals allowed to give birth at natural term, only two gave birth without complications and at times corresponding to that of both control groups. In one flight rat, labor lasted two days, which, aside from exhaustion and muscle weakness of the mother, was due to the presence of an oversized fetus. Since the mother was unable to deliver this fetus, which was first in the birth canal, for a long time, the remaining fetuses—full-term and normally developed—died of anoxia due to the long interval between the placenta detachment and their delivery. It should be noted that similar cases, where the female was unable to deliver normal, full-term litters, were not observed in the control groups for this flight experiment, nor in other experiments we have conducted involving the birth of a total of no less than 1000 animals.

Two other animals of the flight group also had prolonged labor, although not as extended and difficult as the case described above. Each of their litters contained one dead neonate.

Similar complications of birth processes were reported by Garmasheva (1941), when animals were anesthetized 2 days before labor began and continuing throughout, and by Antonova (1968), in animals adrenalectomized before or during pregnancy. Although the analogy between our observations and the data of these authors is not obvious, it does focus attention on the fact that the difficult labor observed in the flight animals could be associated not only with their general weakness and with changes in the muscles of the uterus and abdominal wall, but also with disorders in the regulation of the birth process.

It should be emphasized again that the major differences between the flight and control groups, observed during this stage of the experiment, were associated with difficult labor, and not with fetal development in microgravity or during readaptation.

During the initial days of postnatal life, an additional significant difference between the flight and control group was observed. This involved an increase in the death rate of pups developing in microgravity. Cases of neonate death were not observed in all flight litters, but only in the litters of females that had prolonged, difficult labor, which was evidently the cause for the weakness and death of some neonates (Makeyeva 1964; Skornyakova, 1964; Garmasheva and Konstantinova, 1978).

The increase in the death rate of pups during the early postnatal period could be a result of the disturbed maternal behavior of the flight mothers, which is very typical of stress experiments during pregnancy (Barlow, 1975, 1978; Alberts, 1985). However, observations of the pups during nursing, with the aid of 24-hour videotapes, and quantification of maternal behavioral patterns (such as time in the nest and appropriate reactions to the cries of the pups) showed the absence of substantial differences between the groups. This fact appears especially interesting within the framework of the previously described changes occurring in the mother's body during flight and subsequent labor. Evidently spaceflight factors, including microgravity, did not disrupt the gestational dominant associated with pregnancy (Arshavsky, 1957, 1982); as a result the flight mothers were not only able to realize the fetal development program by providing the homeostasis essential for prenatal life, but were also able to maintain normal maternal behavior during the lactation period.

When the pups were examined during the first month of postnatal life, an extensive research program was conducted to assess the general rate of growth and development, physical endurance, the development of motor skills and coordination, and the developmental pattern of the sensory systems (Alberts, 1978, 1984, 1985; Alberts and Hay, 1980). In the majority of parameters, the flight animals did not differ from the controls. Indeed, it is surprising that despite microgravity-induced changes in the maternal body, the maturation and triggering of various physiological systems occurred on schedule; the formation of the "incredibly complex central nervous system in its association with various coordinations" (Ginetsinsky, 1945, p. 83) was normal.

Against the background of the indubitable "success" of the flight pups, certain differences observed during this stage of research should be noted. When the vestibular function was tested on a rotating platform on day 2, the number of pups demonstrating the unconditioned reflex of turning the head toward the side opposite to the direction of rotation was 20% higher in the flight than in the control groups. On day 14 , 65% of flight pups opened their eyes, compared to 37% of the control pups. At the same time the flight pups showed a certain delay in their hearing development as compared to control animals. In all cases, differences between the flight and control groups were small and rapidly disappeared during subsequent development, without serious effects on the overall schedule of growth and development. These alterations cannot be considered as signs of developmental delay, since while the flight animals were behind the controls in some parameters, they were ahead in others. Rather this indicates a certain degree of "instability" in the overall state during the first days of life of the flight animals. Thus, while the flight pups displayed no substantial differences compared to the controls in their postnatal growth rate, they were nevertheless slightly behind the vivarium group, but ahead of the synchronous group. However, the variability in the weight of the flight pups was greater than in the controls. The "instability" of the blood system in the flight animals, manifest in fluctuations of the hemoglobin level and number of hemopoietic stem cells at certain measurement points during the first month of life, is worthy of note.

When motor activity and motor coordination were studied in the pups during the first weeks of life, no significant differences were found between the flight and control groups, with the exception of a slight decrease in static endurance, as indicated by the amount of time the animals could hold onto a cross bar on days 15 and 18. Study of metabolism and contractile capacity of skeletal muscles, when a portion of the animals was dissected on days 15, 30, and 100 (as conducted by Oganov et al.) also did not reveal significant differences among the flight and control animals.

One of the most sensitive indicators of the maternal body's capacity to maintain homeostasis in the developing fetus is the state of the brain, especially its higher compartments, assessed at various stages of postnatal ontogenesis, by testing the animals with tasks varying in complexity (Joffe, 1969; Dorner, 1974; Barlow, 1975, 1978). Our program included a large number of such tests. A significant difference was found between the flight and control rats in their behavior in the "open field": the flight rats traversed in total a shorter distance; had fewer orienting reflexes; and made fewer trips to the center of the field. These differences were observed on days 30 and 51–53 and disappeared by the time the animals were 3 months old.

The offspring of flight and control rats displayed a virtually identical capacity to learn mazes of varying complexity. However, tests in Dombrovskaya's maze revealed changes in the flight group attesting to worsened behavioral responses: a significant increase in grooming time and in the number of secondary (goal-irrelevant) movements. Analysis of the results suggested that these changes were associated with attenuation of the inhibition process and an increase of irradiation of the excitation process. The changes observed in the flight group were not gross: the animals oriented themselves normally to their new surroundings; were able to master complex tasks; could adapt their experience to a changing situation;

and were able to tolerate significant levels of functional stress.

When the flight animals were dissected on days 15, 30, and 100, no differences were found when compared with controls, in the weight of visceral organs, fluid metabolism, electrolytes, lipids, nucleic acids, or biologically active compounds. In other words, exposure to microgravity during the fetal period, the stage of viscera formation and the mechanisms controlling them, did not affect the rate of growth of the organs or their level of metabolism, throughout the various stages of postnatal life, including sexual maturity.

The single serious difference between the flight and control group revealed in this part of the research was the alteration of collagen metabolism in the skin and bone of the animals that partially developed in microgravity (Pospisilova et al., 1985), attesting to a certain delay in development of connective tissue. The causes for these changes are still unclear. The presence of similar changes in both skin and bone suggests these alterations are systemic in nature. Regulation of biosynthesis and catabolism of the organic component of connective tissue is complex, and an important role is played by hormones (Borel et al., 1984). It may be hypothesized that the cause of collagen metabolism changes, observed during the postflight period in the flight animals , is a shift in the hormonal status of the female rats during flight. Although postflight examinations of the rats did not reveal significant variations in plasma hormone concentrations, the changes noted at this time in the target organs (thymus involution, neutrophilosis, and lymphopenia), and also the significant delay in body weight gain, suggest that during the period preceding examination—i.e., during flight—the hormonal status of the maternal bodies was significantly altered, compared to the controls.

It should also be noted that when the rats were examined at the various stages of postnatal ontogenesis, no differences were observed between the flight and control groups in body and organ weight, hormonal status, weight of lymphoid organs, or white blood formed elements, while intergroup differences in collagen metabolism in the skin and bone were retained at every examination point.

The totality of the results obtained supports a relatively high assessment of the adaptive capacities of the mother-fetus system, when exposed to microgravity during the last third of pregnancy. Spaceflight conditions were not indifferent with respect to the effects on the maternal body and required significant mobilization of compensatory mechanisms. This mobilization was rather effective: the mothers succeeded in supporting normal homeostasis in the fetus and in providing them with materials for anabolic metabolism . The delay of fetal development during flight was very slight and was rapidly compensated during subsequent development. Changes in the maternal bodies that occurred during flight were functional in nature and reversible: beginning with the first day after reentry, the female rats of the flight group started to gain weight rapidly; they maintained normal maternal behavior during the lactation period and supplied their offspring with sufficient milk. The last fact is especially striking considering that during the 5-day flight the mothers lost almost one quarter of their own weight.

The rats that spent a portion of their prenatal development in microgravity were capable of going through the entire sequence of postnatal development, up through the attainment of sexual maturity and reproduction. The differences between the flight and control animals, detected at various stages of this research, were small and disappeared rapidly, and should not be considered defects or changes in developmental rate.

The most serious changes found in the animals of the flight group were the complications of the birth process. It was the difficult, prolonged labors of the mothers, which were the cause of important differences among the flight and control newborns: the death of some neonates during labor and an increased death rate in the early postnatal period of the animals that developed in microgravity, and even in their offspring (in the first case, due to the effects of anoxia on fetal viability; in the second, evidently due to its effects on developing gonads).

Analysis of the results shows significant individual fluctuations in these parameters in the flight animals. The differences were especially clear at labor. When the mothers were dissected on day 18 of pregnancy, no essential differences were noted in the flight animals with respect to the reproductive parameters and the fetal state. At the same time, the animals allowed to give birth at natural term differed from each other, and the range of differences was great: from normal, uncomplicated labor to the delivery of a dead litter.

Significant individual differences in reactions to microgravity were also observed previously in male rats flown on Kosmos biosatellites (Serova, 1980). A similar picture was described in animals exposed to hypergravity (Burton and Smith, 1965; Smith et al., 1980; Smith 1982). The reason for the serious individual differences observed in animals exposed to microgravity and hypergravity is, evidently, that "exposure to stress differentiates the population with respect to the reactions of its members to the stress factor" (Belyaev et al., 1977, p. 57). The flight data obtained demonstrate the fact that normal mammalian fetal development can, in principle, take place

when the mother is exposed to microgravity during pregnancy, although rather serious changes (including death of offspring) are also possible in individuals. This fact should not be ignored, since it is precisely these individual differences in resistance and reactivity, which are universal animal characteristics, that, in the presence of various environmental factors, form the basis for natural selection and evolutionary development (Schmalhausen, 1942; Davydovsky, 1969; Sirotinin, 1981.)

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Chapter 15

A Comparative Analysis of the Effects of Microgravity and Hypergravity on the Prenatal Development of Mammals

A great deal of empirical material has been accumulated concerning the effects of hypergravity on living organisms. Experiments have been performed on the long-term (up to and exceeding a year) exposure of animals, of various evolutionary levels, to centrifugation at 2–7 g (Wunder et al., 1968; Pitts et al., 1972; Oyama et al., 1967; Smith et al., 1980). It has been demonstrated that even at 7 g, mice can survive for a year; at 2 g mice and rats have a normal life span and are capable of conceiving and bearing young.

Mammalian experiments in microgravity are still of considerably shorter duration than those in hypergravity. Laboratory rats were exposed to microgravity on Kosmos biosatellites for up to 22 days, approximately 1/50th of their normal lifespans. In these studies, no irreversible changes were found in the viscera of the animals (Gazenko et al., 1980); however it is difficult to say *a priori* how adaptation to microgravity would proceed, if the duration of exposure were lengthened.

When preparing future animal experiments for space-flights of longer duration, we have used various techniques for simulating the effects of microgravity on the ground, e.g., hypokinesia, tail-suspension, and others. At the same time, when analyzing the phenomena occurring during the transition from 1 g to 0 g, we have not utilized in full the abundant experimental data derived from studies in which the force of gravity increases from 1 g to 2 g and more. There are very few publications devoted to comparative analysis of the effects of microgravity and hypogravity (Gazenko et al., 1967; Serova, 1977).

In theory it can be postulated that there are two ways in which an altered gravitational force can affect a living thing:

1. Specific (mechanical) effects associated with changes in the positions of organs and tissues, relative to each other and cell and tissue components, as well as with changes in the energy required to overcome the force of gravity.

2. Nonspecific effects, such as a stress response.

The extent to which the specific effects of altered gravity manifest themselves will depend on the size of the organism and will be greater the larger the animals; the magnitude of the nonspecific effects will be determined by the level of organization of the nervous and endocrine systems of the animal, i.e., ultimately by its reactivity, and will be greater in animals higher on the evolutionary ladder (Sirotinin, 1981).

Starting from these theoretical considerations, we performed experiments on adult rats and mice, which attempted to compare the physiological effects of microgravity and hypergravity of 2 g (Serova, 1977). It was demonstrated that change in the level of gravity, within the limits ±1 g compared to that of the Earth, is accompanied by the development of a stress reaction manifest in delay in the growth of animals, involution of lymphoid organs, neutrophilosis and lymphopenia in the blood, and other changes. Nonspecific changes of the stress reaction type develop, along with specific changes in the musculoskeletal system, heart, and red blood (Gazenko et al., 1980). However the proportion of the nonspecific component is substantial, and it has not been ruled out that this component could determine the majority of functional and structural rearrangements occurring in the organism under these conditions, including the changes considered specific.

A similar pattern can be observed in the response of pregnant female rats to microgravity and hypergravity. Let us compare the results of the embryological experiment performed in space on Kosmos-1514 and a ground-based simulation experiment on the centrifuge that preceded the flight (Section 2.3). The flight experiment lasted 5 days (from days 13 to 18 of pregnancy), and the hypergravity experiment lasted 7 days (from day 14 to 21 of pregnancy). In both instances, exposure of the mother-fetus system occurred during the last third of pregnancy—at the stage of active fetal growth and organ development. At both 0 g and 2 g, we observed serious changes in mothers, an integral expression of which was delay in body weight gain by 60–65 g, i.e., by virtually one quarter of their own weight.

Nevertheless, the reproductive parameters of the animals exposed to microgravity and hypergravity were not altered; the number of implantation and resorption sites, and the number of living fetuses were identical in the experimental and control animals of both experiments. Fetal weights were decreased by 10–12% ($p < 0.05$), while water content of fetuses developing in microgravity was elevated by 6% ($p < 0.05$), and remained unchanged in fetuses developing in hypergravity.

No differences were observed in the total concentration of calcium in fetuses developing in microgravity and hypergravity compared to the controls; nevertheless, in both cases there was inhibition of skeletal development, manifested as a significant decrease in the dimensions of ossification sites in limb bones and the axial skeleton (fig. 20, table 5).

Of course, the reactions of the mother-fetus system to microgravity and hypergravity were not absolutely identical. For example, rats exposed to space during days 13 to 18 of pregnancy displayed a more than 50% decrease ($p < 0.05$) in the calcium concentration in the liver and kidneys, while rats exposed to 2 g did not undergo such changes. Other differences were also identified between groups exposed to microgravity and hypergravity. However, from our point of view, what is most important is that when gravity alters by 1 g above or below Earth's gravity, i.e. in opposite directions, the overall pattern of changes in the mother-fetus system is the same. In both cases there were serious changes in the maternal bodies, similar to those in stress reactions; however, the embryonic death rate was unaltered; and the developing fetuses were only slightly behind the controls in body weight and dimensions of ossification sites. The data obtained are similar to results of experiments on mature rats (Serova, 1977), discussed above, and again demonstrate the important contribution made by the nonspecific (stress) component in mammalian reactions to the gravitational factor, when the force of gravity alters within limits of ±1 g compared to that of the Earth. In our view, this supports a recommendation for experiments using centrifugation of 2 g as one of the models within a future research program, to study the total cycle of prenatal development of mammals in space.

When starting to implement this program, we conducted a series of experiments with rats centrifuged at 2 g, in which we tested the possibility of impregnation and early prenatal development.

The possibility of obtaining offspring from rats, mice, and hamsters under conditions of hypergravity has been described earlier in a number of works (Baranov et al., 1963; Briney et al., 1962; Bird et al.,1963; Oyama et al., 1967; 1985; Janer et al., 1984). However, these investigations used animals which had been exposed to hypergravity for a long period of time before the moment of impregnation and were adapted to those conditions; analysis of the effects of hypergravity on various stages of prenatal development in mammals has not been previously conducted.

In the first experiment, intact males and females were centrifuged. During daily cessation of rotation for cleaning and feeding the animals, we examined vaginal smears to detect the onset of pregnancy. During the 30 day experiment, all the female rats exposed to 2 g were impregnated, and their pregnancies progressed. The major difference between the flight and control groups was an increase in the elapsed time (from 5.5 to 15 days), from when the male and female animals were placed together to the time of mating. Evidently, mating occurred only after the completion of hypergravity adaptation and the stabilization of the health status of the animals under these conditions. However after impregnation, development proceeded normally in the flight group, and when female rats were dissected on day 18 of pregnancy, no significant differences were observed between the flight and controls in the number of corpi luteum, implantation and resorption sites, or live fetuses, i.e., the parameters of preimplantation and postimplantation mortality in females impregnated at 2 g were the same as those in the controls.

The weight of the fetus and placenta, their tissue water content, and the amount of amniotic fluid were identical in the flight and control groups. Visual examination of the fetuses and study of them using Wilson's method did not reveal elevated numbers of developmental abnormalities in the flight group. Morphometry of the fetal skeleton also did not reveal any intergroup differences in ossification sites in various segments of the skeleton.

In the second experiment, female rats were exposed to 2 g beginning on day 7 of pregnancy. Here the major difference between the groups was abortion of pregnancy in approximately one third of the centrifuged animals, during the first days after placement in the centrifuge. Pregnancies developed normally in the remaining rats, and dissection on day 21 of pregnancy revealed no differences between the flight and control groups in the number of implantation and resorption sites or live fetuses. In other words, when pregnant animals were exposed to 2 g during the stage of embryonic implantation *in utero*, implantation did not take place in some rats, and there was a complete abortion of the pregnancy; in the remaining rats all embryos were implanted; and the postimplantation mortality rate was identical to those in the control group.

Fetal and placental weights, their content of water, and the amount of amniotic fluid, as in the previous experiment, were identical. Developmental abnormalities, identified visually or through use of Wilson's method, were not elevated. At the same time, morphometry of fetal skeletons, exposed *in utero* to hypergravity on days 7–21 of prenatal development, showed a significant decrease in the size of ossification sites in various segments of the skeleton (fig. 49). In other words, changes occurred which were similar to those observed after exposure of the mother-fetus system to microgravity and hypergravity during the last third of pregnancy, but were not found in female rats previously adapted to 2 g.

A portion of the animals centrifuged from day 7 of pregnancy was maintained under those conditions to full term. Females of the centrifuged group bore on the average 10 normal newborns, while 12 were born in the synchronous control group ($p < 0.05$). The body weight of newborns in the experimental and control conditions were

virtually identical (5.4 and 5.7 g, respectively); the size of the ossification sites was significantly smaller in the centrifuged than in the control animals (fig. 50).

Thus, the mechanisms underlying the effects of hypergravity of 2 g at various stages of prenatal mammalian development are diverse. When animals were bred under conditions of 2 g, the major difference from controls was the increase in the time elapsed after the males and females were placed together to mating. When rats were placed in the centrifuge starting on day 7 of pregnancy, during the implantation period, the major change compared to the control involved spontaneous abortion in some of the animals. When rats were placed in the centrifuge starting on day 14 of pregnancy, no cases of abortion were observed, but experimental fetuses were somewhat behind controls in body weight and size of ossification sites in the skeleton. In the latter case, the pattern observed at 2 g was virtually similar to changes occurring in the mother-fetus system in response to microgravity exposure during this period on Kosmos-1514.

The commonality of responses of the mother-fetus system to microgravity and hypergravity during late developmental stages allow us to hypothesize, with a high degree of probability, that there is a similar commonality during early stages as well, and to predict, based on the results of 2 g centrifugation experiments, the possibility of impregnation and realization of a complete cycle of prenatal development in mammals under spaceflight conditions. For such an experiment, it would be best to use intact animals, bred under space flight conditions after completion of the acute period of adaptation to microgravity.

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Chapter 16

The Mother-Fetus System as a Model for Studying the Mechanisms of Physiological Effects of Microgravity

Animal experiments on Kosmos biosatellites were initiated when it became clear that man could live and work with adequate efficiency in the microgravity environment. By then a large body of data had been accumulated which illustrated spaceflight effects on the human body and behavior.

Nevertheless, many questions remained unanswered, the majority were related to the price that must be paid for adaptation: could microgravity cause latent pathological changes in the viscera; could it produce remote adverse effects; or could it shorten longevity? I. V. Davydovsky remarked:

> Man has overcome pain and fear, he has demonstrated that he can survive in microgravity. However, he doesn't know yet, and this is to be elucidated in the future, what price he will have to pay in order to achieve new and advanced forms of adaptation. (1969, p. 303).

Answers to these key questions can be found only with the aid of regular and comprehensive animal studies, particularly mammals, flown onboard spacecraft for varying periods of time.

Spaceflight animal studies could help investigate in great detail the structure and metabolism of many organs and tissues; these studies would utilize various and sometimes complex provocative tests, follow-up flight effects after recovery, and accumulate good statistical data.

Initially, Kosmos satellites dedicated to spaceflight experiments flew male Wistar rats, in order to evaluate their health during recovery and at rest, without any additional stresses applied. At landing, the level of their stress-reaction was moderate, and changes in the musculoskeletal system, heart, and red blood, all which developed during space flight, proved reversible—they disappeared by recovery day 25 (Gazenko et al., 1978, 1980).

One of the principal adaptive reactions of animals after their return to the Earth was diminished motor activity, a peculiar functional hypokinesia, which alleviated readaptation to the Earth gravity after exposure to microgravity. This led to the assumption that flown animals, if exposed to loading tests, no matter how gentle, would show an inadequate tolerance and maladaptation of the physiological systems responsible for homeostasis (Serova, 1975, 1979; Serova, 1980).

However, further experiments, with sufficiently demanding provocative tests applied postflight, proved this assumption false. Immediately postflight, rats could sufficiently tolerate immobilization stress-tests; moreover, their responses to repeated exposures (body weight loss, thymus and spleen weight loss, decreases in the number of thymocytes and splenocytes, reduction in the corticosterone concentration in blood and adrenals, and other changes) were very similar in the flight and control groups (Serova, 1983; Kvetnansky et al., 1981).

Our basic principle was to increase the level of provocative tests in a stepwise manner, so we initiated a search for a more general test which would, on the one hand, mobilize reserves and stimulate all regulating systems (similarity to a stress-test), and on the other, produce a specific effect on the systems and organs that develop significant, although reversible, changes in microgravity (for example, bone and red blood cells). While looking for such a provocative test, we concluded that the mother-fetus system could be effectively used as an experimental model to study the mechanisms of the physiological effects of microgravity, especially in assessing compensatory and adaptive capabilities. Our decision was supported by the knowledge that pregnancy requires a marked stimulation of anabolic processes, the mobilization of calcium to be utilized in the fetal skeleton, activation of red blood, and other changes opposite to microgravity-induced alterations. We postulated that the effects of microgravity on the fetus (immersed in the amniotic fluid) would be mediated by the maternal body and influenced by its metabolic and hormonal status.

These theoretical principles formed the foundation of our preparation and conduct of the first spaceflight embryology experiment on mammals. The results of the experiment are summarized in the preceding chapters.

The experimental data support the conclusion that animals exposed to microgravity maintain their capability to stimulate compensatory and adaptive reactions, and mobilize the reserves required for normal fetal development. Despite serious changes in the maternal body, evidenced by substantial body weight loss (almost one fourth during five flight days), the reproductive function remained essentially unchanged. The fetuses of the flown rats showed a slight developmental delay, which manifested as a lower body weight gain, slower ossification of certain skeletal areas, and a higher water content. However, the differences between the flight and control groups were small, remaining within 10%, and at later stages disappeared entirely. The pups that were born five days after reentry did not differ from the synchronous controls in terms of their weight, and were superior in terms of the size of ossified areas. It is interesting to note

that the total weight of the fetuses dissected from each flight female on the recovery day was identical to that of the vivarium controls: 11.40 g versus 11.47 g. In other words, despite a significant body weight loss in adult rats, caused by stimulated catabolism, the flown rats developed enhanced anabolic processes associated with fetal development, almost identical in both flown and control rats. During nursing, the flown mothers showed normal behavioral patterns and supplied their pups with the necessary amount of milk. In the postnatal period (till the stage of sexual maturity), the rate of body and organ weight gain was very similar in the flown and control animals.

Since both pregnancy and microgravity taken separately cause serious shifts in fluid-electrolyte metabolism, especially calcium balance, we expected that the combined effect of these two factors would lead to significant, and probably destructive, changes in calcium metabolism. In fact, we observed a substantial (over 50%) decrease of calcium in the liver and kidneys, a less marked decrease in the skin, and essentially no changes in the bones of the flown female rats ($p > 0.05$). Despite this, the maternal body maintained homeostasis for the developing embryo: its content of sodium, potassium, calcium, and magnesium, as calculated per unit of dry weight, was identical in the flight and control groups, both at pregnancy day 18 (at the recovery day) and at later stages (i.e., at different stages of postnatal development). The fact that fetal homeostasis was efficiently maintained during flight is also evidenced by the lack of noticeable differences between the central nervous and sensory systems of the flown and control animals.

The animals that spent part of their prenatal development in microgravity were dissected on day 15, 30, or 100 and displayed no changes in the viscera weight, metabolism of water, electrolytes, lipids, nucleic acids, or biologically active compounds when compared to the controls. It can therefore be concluded that exposure to microgravity of fetuses, at the stage when their internal organs and respective controlling mechanisms were formed, had no effect on the rate of growth and the level of metabolism, at various stages of their postnatal development, including the stage of sexual maturity.

The results of the embryology experiment on Kosmos-1514 and ground-based controls demonstrated that the mammalian fetus can grow and develop in a normal manner when the maternal body is exposed to microgravity. Nevertheless, the study also gave evidence that individual species can develop serious changes. Most animals tolerated microgravity exposure without irreversible pathologies or complications during the recovery period; however, some animals displayed grave abnormalities: delivery of a dead litter by one of the flown females and delivery of weak pups which died during the first days of life by another.

The individual differences seen in animal responses to environmental effects, such as hypergravity or microgravity, is far from being novel. This observation was reported and discussed by many authors (Kasyan et al., 1967; Davydovsky, 1979; Williams, 1956; Burton et al., 1965; Smith et al., 1980).

It can be hypothesized that individual variations evolve as a result of genetic changes, continuously building up in the body. They may, for a long time, remain neutral or almost neutral (Kimura, 1983), if for example the animals are kept in a vivarium without any specific provocative exposures. In other words, these changes have no impact on the life of an individual or the entire population. However, in a stressful situation, these differences may be responsible for different resistance, and in an extreme situation, they may cause death in some animals.

Separation of animals into distinguishable, quantitatively different groups, that vary with respect to their resistance to diverse environmental effects, is of great biological importance in determining their survival in a natural habitat, as well as in the onset, course, and outcome of diseases (Severtsov, 1934; Schmalhauzen, 1942; Davydovsky, 1969).

While discussing individual resistance and reactivity of mammals within the framework of extended space missions, it is important to bear in mind publications by Burton et al. (1965) and Smith et al. (1980) who obtained 20 bird generations exposed to 2 g. Even in the first generation of animals, the researchers were able to distinguish a certain amount of poorly resistant chicks. The selection was performed, as it usually takes place in nature, at the population level by means of mating subjects that were better adapted to the new environment. It is probable that a similar process could occur in space, if it is attempted to produce several animal generations there. In the future it will be important to concentrate on the development of methods which will help predict (prior to launch) individual responses of different animals to microgravity.

Chapter 17

Perspectives of Studying Mammalian Growth and Development in Spaceflight

The achievements scored by space biology and medicine in recent decades can hardly be overestimated. Many researchers can easily recall heated discussions that took place not too long ago, concerning man's ability to survive microgravity exposure. However, dozens of biological objects at different levels of organization have made orbital flights, and man has demonstrated the ability not only to live but also to work efficiently in flights of more than 200 days in duration. A huge amount of data has been accumulated about the effects of microgravity on various vital processes in the human body.

However, we are still far from being able to understand completely the mechanisms underlying the compensatory and adaptive reactions responsible for human adaptation to microgravity, and are still further from being able to elucidate the role of gravity in the formation of plant and animal kingdoms on our planet.

The Earth witnessed at least two historical periods during which its inhabitants were able to feel a change in the gravitational force: when organisms left water and found themselves on the ground, and when some returned back to the aquatic habitat. The striking diversity of the plant and animal kingdoms gives evidence that in both situations living creatures proved capable of adjusting to altered gravity, maintaining the ability to grow and reproduce offspring. It should not, however, be forgotten that at present we can observe only the results of adaptation and may only speculate about the losses demanded by various stages. Obviously, no matter how extended spaceflights may become, they will always be much shorter than the evolutionary processes that occurred on the Earth.

During Kosmos flights, Wistar male rats were in space for approximately 1/50 of their lifespan without showing substantial deterioration of their resistance or irreversible pathologies in their internal organs. The embryology experiment flown on Kosmos-1514, the results of which are summarized in this book, demonstrated for the first time that a mammalian fetus can develop in the maternal body exposed to microgravity. Animals that were in orbital flight for about one fourth of their prenatal development period proved capable to complete their postnatal ontogenesis (to reach sexual maturity and delivery of offspring), showing no significant variations from the normal rate of growth and development, metabolism, motor activity, or behavioral patterns. Nevertheless, we are still unable to discern how long different species of mammals may remain in orbital flight and still maintain their reproductive function and the normal viability of the offspring, which is known to be the basic prerequisite for colonizing a new habitat.

In principle, the possibility of these events is suggested by the previously discussed observations by Smith and Burton (1980), who obtained over 20 chick generations during chronic centrifugation at 2 g. Beginning with the first generation, some animals deteriorated, while the specimens most resistant to the chronic centrifugation were selectively mated.

Such experiments may be conducted in the future in the actual spaceflight environment. In order to stage such studies, we will have to solve not only engineering problems, but also at least, three scientific problems. We will have to: (1) study the effects of microgravity and hypergravity on various stages of pre- and postnatal development of mammals; (2) investigate the complete cycle of mammalian development in orbital flight—from fertilization to birth to sexual maturation; and (3) conduct follow-up studies on the long-term effects of spaceflight factors.

The Kosmos-1514 embryology experiment was a first step, intended to clarify the role of gravity in mammalian growth and development. The next step would be to study the effects of microgravity on earlier, more vulnerable developmental stages; then to study the complete cycle of prenatal development of mammals in orbital flight; and finally, to produce several generations of mammals onboard the spacecraft. Initially, various parts of these experiments can be flown on Kosmos biosatellites or conducted in ground-based simulation, primarily hypergravity, studies. In any event, when developing such an experimental program, it will be useful to apply methods and procedures employed in the Kosmos-1514 embryology experiment and in the postflight evaluation of the mother-fetus system.

Recently, U.S. investigators (Wolgemuth et al., 1985) conducted an original investigation to study the effects of clinostating on the mammalian fertilization and early development during embryo cell cultivation *in vitro*. They did not detect any differences between the test and control animals with respect to the fertilization frequency, pattern of fertilization, or early stages of cleavage. These observations cannot, however, rule out the possibility of changes that may occur at early developmental stages of mammals exposed to microgravity *in vivo*, primarily due to hormonal shifts in the maternal body.

When designing a life support system for animal studies in space, R. Mains and J. Alberts (1985) postulated that animal copulation and fertilization would be hampered. In any case, their experimental design included a centrifuge

and other devices. Taking into consideration simulation studies and the Ontogenesis experiment flown on Kosmos-1129, we however believe that mammals have the ability for copulation and fertilization in microgravity, but need time to become adapted to the unusual environment.

A nonspecific (i.e., stressor) component plays an important role in animal responses to microgravity and hypergravity (Burton and Smith, 1965; Serova, 1977, Serova et al., 1984). Recognition of this fact determines the experimental strategy adopted by researchers in gravitational embryology and developmental physiology. For instance, Duke et al. (1985) recommended abolishing the "nonspecific component" of hypergravity effects by using isolated embryos and their cultured organs. J. Oyama (1985) obtained similar results by exposing animals to preliminary (prior to copulation) hypergravity adaptation, beginning with the suckling period and ending with sexual maturation. In his study, exposure to hypergravity (up to 2.03 g) had no noticeable effect on rat prenatal development; marked differences were seen in the early postnatal period, provided that the animals remained on a centrifuge.

Our experience (Serova et al., 1985) indicates that the development of mammalian fetuses in hypergravity can be successful if the parents were pre-adapted to it. However, when the animals were given the chance to "choose" the duration of adaptation they need prior to copulation at 2 g, they chose a shorter time than J. Oyama claimed: approximately two weeks. In this situation, there were no abnormalities in their prenatal development (see Chapter 15).

Our observations from ground-based simulation studies suggest that in future embryology experiments, it will be reasonable to allow females to meet males on flight day 2 or 3. The animals will begin copulating only after they feel adapted; in this case, the duration of the adaptation period can be reliably measured by the time interval between their introduction and subsequent copulation.

In addition to experiments in which potential adverse effects of microgravity and hypergravity can, in part, be avoided through preliminary adaptation, it will be important to carry out studies in which unadapted rats at different pregnancy stages will be used to investigate the effects of an altered gravitational field at its peak.

The embryology Kosmos-1514 experiment demonstrated that fetal homeostasis remained within the limits that assured normal development of physiological functions, although the maternal body was exposed to microgravity. However, the maternal body developed significant changes such as growth delay, involution of lymph organs, changes in catecholamine metabolism and the like, which suggests that in microgravity homeostasis of the mother-fetus system is maintained by increased work of the regulatory systems. Having demonstrated the possibility of development of a mammalian fetus in orbital flight, we have to concentrate on the mechanisms responsible for homeostasis in the mother-fetus system exposed to micro-and hypergravity, at various stages of pre- and postnatal ontogenesis.

Of no less importance is the study of the reproductive function of mammalian males in microgravity of varying duration and readaptation to the Earth's gravity.

The first experimental study along these lines was conducted by N. L. Fedorova (1967), who detected a higher number of atypical spermatozoids in the dogs Ugolyok and Veterok after their 22-day flight. G. I. Plakhuta-Plakutina (1979) performed regular histological examinations of rat testes after 18–22-day flights and found no significant differences between the flown and control animals. Conversely, R. Kusheva and I. Baev (1978) examined Kosmos-936 rats and reported spermatogenetic changes, including the appearance of spermatids with destructive changes. Cytogenetic examinations revealed no mutagenic effects of microgravity on the testes of adult rats (Vyglenov et al., 1981) or fetuses that developed in orbital flight (Benova, 1985). Mating male rats exposed to microgravity during 1/50 of their lifespan with intact females at different time intervals after flight did not lead to an elevated number of dominant lethal mutations in mature spermatozoids and their stem cells (Serova et al., 1982). Examinations of male rats flown for 7 days on Kosmos-1667 showed no changes in the weight or cytological parameters of their testes, sexual behavioral patterns, reproductive function, and viability of the offspring (Serova et al., 1985). However, Philpott et al. (1985) found decreases in the weight of testes and in the amount of spermatogonia of rats flown for 7 days on Skylab-3.

Since the data concerning the effect of microgravity on the reproductive function of mammalian rats are scarce and controversial, pertinent studies need to be continued in both real space flights of varying duration and in ground-based simulation experiments.

Another important area of research would be to study the mammalian responses to microgravity at different stages of postnatal development and to evaluate longevity and aging rate both during orbital flight and after its completion.

When discussing the basic goals and methods of evolutionary physiology, L. A. Orbeli (1956) stated that the evolution of physiological functions can be adequately

understood provided that the researchers use four approaches, which are basically different but help reach the same objective. These are: methods of comparative physiology; use of clinical data; application of specific experimental methods; and finally, ontogenetic study. The embryology Kosmos-1514 experiments is essentially the first attempt to gain a better insight into mammalian responses to spaceflight from the ontogenetic point of view. The experimental results suggest that further investigations into the effects of microgravity and hypergravity on different stages of mammalian ontogenesis, beginning with fertilization and first cleavages, and ending with the rate of postnatal development and aging, will help provide important data not only about the role of gravity in mammalian growth and development but also about general mechanisms underlying the physiological effects of microgravity.

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Table 1. Effect of Impact Acceleration on Female Rat Reproductive Function

Rat group	n, Inital	n, died at impact	n, Hemor-hages	n, Normal birth	Pregnancy time, days	Number of live pups in litter	Weight of pups at birth, g	Abnormali-ties, according to Wilson, %
Control	9	-	-	9	22.0±0.2	10.1±1.2	5.6±0.2	11.1
Pregnancy day 19, 40 G	11	0	0	11	22.2±0.1	9.1±1.0	5.8±0.2	11.1
Pregnancy day 19, 90 G	7	1	3	5	22.6±0.2	4.8±0.8	5.9±0.12	66.7
						p<0.01		p<0.05
Pregnancy day 21, 40 G	6	0	2	6	22.3±0.1	8.0±0.7	6.0±0.2	35.7
Pregnancy day 21, 90 G	6	0	3	5	22.5±0.2	6.0±1.1	5.4±0.4	75.0
						p<0.05		p<0.05

Table 2. Effect of Factors Dating at Launch and Landing on Rat Reproductive Function

Rat group	Total Death Rate	Live fetuses, n	Weights, grams		Abnormalities according to Wilson, %	
			Fetuses	Placenta	Fetus	Placenta
Control	23.43±5.60	9.9±0.8	2.4±0.05	0.530±0.020	2.9	5.06
Test	13.94±6.05	12.0±0.8	2.59±0.07	0.535±0.20	27.3	12.5
					p<0.01	

Note: The Rats were dissected 5 hours after simulated landing.

Table 3. Connective Tissue Metabolism of Rats Exposed to 2 g During Pregnancy Days 14-21								
Rat Group	n	Total collagen, mg	Pepsin-soluble collagen, mg	Type I collagen, %	Type III collagen, %	Glyco-proteins, mg	Fibro-nectin,%	Acid alpha-glyco-protein,%
		Femur						
2 G	10	43.0±4.2	648.1±20.4	37.0±3.4	30.0±3.4	1.31±0.05	74.0±0.8	26.0±0.8
		p<0.01		p<0.05		p<0.05	p<0.01	p<0.01
Control	5	26.1±3.5	596.0±13.2	62.0±1.8	22.0±2.5	0.70±0.04	67.0±0.9	33.0±0.6
		Skin						
2 G	10	7.9±1.9	596.0±11.8	44.0±1.2	27.0±1.9	0.28*	81.0±1.0	19.0±1.1
							p<0.01	p<0.01
Control	5	8.8±2.2	558.0±9.4	46.0±0.5	23.0±2.3	0.24	26.0±10.9	74.0±10.9

Note: Due to technical problems this is a pooled sample

Table 4. Reproductive Function of Rats Exposed to 2 g During Pregnancy Days Through 21								
Rat Group	n	Yellow bodies n	Implantation sites n	Resorption n	Total death rate of embyos, %	Live fetuses n	Abnormalities, fetus	% of the placenta
2G	10	14.8±0.7	10.8±1.6	1.2±0.4	24.2±4.2 Pv<0.05	10.1±0.6	8	7
Synchronous	5	13.5±0.7	11.3±0.8	1.2±0.4	35.0±10.8	9.6±1.6	8	6.3
Vivarium	15	14.4±0.8	13.2±0.7	0.6±0.2	12.6±2.6	11.4±0.6	0	1.6

Table 5. Skeletal Development in Rat Fetuses Exposed to 2 g During Pregnancy Days 14 - 21							
Rat Group	n	Length of ossified areas, mm					Fontanel width, mm
		Femur	Tibia	Iliac Bone	Ulna	Scapula	
2G	17	2.4±0.05 Pv,s<0.01	3.0±0.05 Pv<0.002	2.0±0.03 Pv<0.01 Ps<0.02	3.5±0.04 Pv<0.001 Ps<0.02	3.0±0.04 pv<0.02	2.9±0.05 pv,s<0.002
Synchronous	10	2.6±0.05	3.2±0.1	2.1±0.02	3.8±0.1	3.1±0.05 pv<0.02	2.5±0.1
Vivarium	10	2.6±0.04	3.4±0.1	2.2±0.05	4.0±0.1	3.4±0.1	2.5±0.1

Table 6. Peripheral Blood Profile of Pregnant Rats Several Hours Postflight					
Rat Group	n	Hemoglobin, g%	Reticulocytes,o/oo	Leukocytes thous / mm 3	Lymphocytes / neutrophils
F	9	9.8±0.3 Pv,s<0.001	47.7±6.6 Pv,s<0.001	30.8±1.66 Pv,s<0.001	0.5±0.05 Pv<0.002 Ps<0.001
SC	10	13.4±0.4	116.1±9.0 Pv<0.002	9.7±0.62 Pv<0.01	2.1±0.3
VC	10	12.9±0.4	211.9±21.3	13.8±1.3	3.8±1.08

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Table 7. Body and Organ Weight of Female Rats Dissected on Pregnancy Day 18							
Rat Group	n	Body weight	Organ weights, g				
			Thymus	Adrenals	Myocardium	Liver	Kidneys
F	5	293±6.5 Ps,v<0.002	0.238±0.028 Pv<0.05	0.093±0.0035	0.676±0.012 Pv<0.05	10.3±0.65 Pv<0.01 Ps<0.001	1.86±0.07
SC	5	355±5.9	0.273±0.008 Pv<0.05	0.0798±0.0053	-	14.6±0.53	1.94±0.06
VC	7	349±11	0.338±0.026	0.0823±0.0033	0.778±0.027	13.3±0.5	1.93±0.07

Table 8. Thyroxine and Triiodothyronine Concentrations in Hydrolysates of the Thyroid Gland, μg/mg protein			
Rat Group	n	Triiodothyronine	Thyroxine
F	5	0.50±0.12	1.50±0.42
SC	5	0.11±0.02	0.21±0.009
VC	6	0.35±0.07	1.40±0.33

Table 9. Concentrations of Mineral Elements in the Tails and Coats of Pregnant Rats					
Rat Group	Ca	Mg	Sr	Fe	P
	Tail, µg/g wet weight				
F	34.1±1.07	597±16.02 Pv<0.05	16.7±1.12	36.1±3.07	15.4±0.55 Pv<0.05
SC	31.5±1.27	551±19.7	15.8±0.85	45.7±4.09	15.6±0.63 Pv<0.05
VC	32.6±0.6	553±9.9	16.3±0.44	45.5±2.65	13.6±0.31
	Coat, µg/g dry weight				
F	521±83.88	161±23.04	0.76±0.13	96.3±11.63 Pv,s<0.001	3090±594.2 Pv,s<0.01
SC	375±53.7	119±14.37	0.51±0.07	25.3±1.25	435±23.35
VC	398±14.24	118±6.86	0.51±0.04	30.2±3.65	365±21.22
Group	Zn	Cu	Mn	K	Na
	Tail, µg/g wet weight				
F S	53.2±1.43	1.42±0.05 Pv<0.01	1.46±0.16 Pv<0.01 Pc<0.05	3.04±0.34 Pv<0.01	2.60±0.21 Pv<0.045
SC	50.1±0.63	1.38±0.06 Pv<0.05	2.71±0.38	3.30±0.36 Pv<0.001	2.97±0.25 Pv<0.01
VC	50.2±1.12	1.16±0.04	2.63±0.2	1.20±0.06	1.70±0.12
	Coat, µg/g dry weight				
F	202±3.61	12.3±0.33 Pv<0.01	3.06±0.52 Pv,s<0.05	3170±652.13 Pv,s<0.01	2380±478.97
SC	206±1.84	11.1±0.5	1.41±0.22	776±86.76	2420±346.3
VC	211±8.5	11.0±0.2	1.55±0.23	531±38	2000±71.6

Table 10. Concentrations of Nucleic Acids and Protein in the Liver, mg/g wet wt				
Rat Group	DNA	RNA	RNA/DNA	Protein
F	3.3±0.17	15.3±0.60	4.6	154±7
SC	2.8±0.20	13.4±0.50	4.8	155±9
VC	3.4±0.18	13.8±0.45	4.1	150±7

Table 11. Rate of Incorpation of Radioactive Precursors into DNA and RNA of Spleen		
	Lymphocytes	
Rat Group	3 H-thymidine, cpm / μg DNA	14 C-uridine, cpm / μg RNA
F	81.9±2.8	15.6±0.7
SC	119.3±10.2	10.0±1.6
VC	141.0±2.0	10.4±0.9

Table 12. Activity of Certain Enzymes in the Livers of Pregnant Rats				
Rat Group	TAT, mmole/g/min	TP, mmole/g/hr	ALT, mmole/g/min	AST, mmole/g/min
F	21.7±10.1	17.2±6.5	54.6±26.9	273±47
SC	36.9±7.2	9.9±2.6	48.6±25.7	279±11
VC	21.5±8.6	15.4±7.5	30.0±12.9	253±23

Table 13. Collagen Metabolism in the Skin of Pregnant Rats				
Rat Group	Soluble collagen, %	Hydroxyproline, mg glycoproteins, mg	Type I, collagen, %	Type III collagen, %
F	83.7±2.7	25.7±1.6	93.88±0.89	6.12±0.89
	P<0.01	P<0.01	P<0.05	P<0.05
SC+VC	61.7±2.2	544.2±47.8	98.60±0.26	1.40±0.26

Table 14. Levels of Elements in Spongy Bone (Proximal Epiphysis of the Humerus)				
Rat Group	**Ca, mg**	**P, mg**	**Mg, mg**	**Na, mg**
	Per g ash			
F	387.4±4.55	127.7±2.42	8.37±0.325	10.14±0.63
	Pv,s<0.05	Ps<0.05	Pv<0.05	Pv<0.05
SC	416.4±3.13	112.4±1.74	18.77±5.98	9.63±0.63
VC	415.2±3.7	115.2±8.85	35.25±3.63	7.22±0.68
	Per g organic substance			
F	542.7±15.0	182.7±2.3	12.37±0.40	13.93±1.53
	Ps<0.05	Pv,s<0.05	Pv<0.05	Pv<0.05
SC	574.6±2.94	155.8±4.98	19.47±6.65	13.43±1.10
				pv<0.05
VC	566.5±12.08	154.7±12.13	45.94±4.94	10.45±0.377
Rat Group	**K, mg**	**Si, μg**	**Zn, μg**	**Ca/P**
	Per g ash			
F	1.503±0.229	46.56±2.02	771.5±45.21	3.04±0.091
		Pv<0.05		Pv,s <0.05
SC	1.592±0.200	45.25±2.02	637.2±32.89	3.70±0.091
		Pv<0.05		
VC	0.813±0.44	92.39±10.52	565.3±51.47	3.73±0.098
	Per g organic substance			
F	2.140±0.392	68.08±1.39	1135±115.5	
		Pv<0.05	Pv<0.05	
SC	2.186±0.337	60.61±2.98	845±52.4	
		Pv<0.05		
VC	1.054±0.082	130.8±10.91	766±60.8	

Table 15. Bone Mechanical Properties (Proximal Epiphysis of the Humerus)					
Rat Group	Elastic Strain, N•10 -1 /μμ 2	Ultimate Strain, N 10 -1 / μμ 2	Relative Strain, N 10 -1 / μμ 2	Modulus of elasticity , N 10 -1 / μμ 2	Specific work of elastic strain, N 10 -1 / μμ / μμ 3
F	5.70±0.66	5.83±0.24	0.0913±0.0052	74.50±7.58	0.153±0.025
		Pv,s<0.05	Pv,s<0.05		
SC	6.69±0.18	6.90±0.24	0.115±0.0028	68.38±3.22	0.195±0.023
VC	6.41±0.167	6.62±0.18	0.103±0.0026	70.35±1.92	0.188±0.0090

Table 16. Absolute Muscle Weight (mg) in Pregnant Rats							
Rat Group	Brach	Tric	EDL	Sol	Plant	M. gastroc	L. gastroc
F	162.0±8.34	126.6±4.9	118.0±3.34	108.8±4.04	229.6±13.61	494.6±30.21	556.2±39.28
	Pv<0.05	Pv<0.001	Ps<0.05	Ps<0.05	Ps<0.05	Pv<0..05	Pv<0.01
	Ps<0.05	Ps<0.01					Ps<0.01
SC	203.4±12.18	170.6±11.02	142.6±4.76	131.6±8.93	278.4±16.85	555.0±30.3	731.0±23.91
VC	192.2±6.47	161.6±4.92	129.8±8.98	123.2±9.02	254.6±14.26	596.0±28.53	776.4±37.87

Table 17. Characteristics of Oxidative Phosphorylation in Mitochondria of Skeletal Muscles (rate of respiration, nmole O2/ min/ mg protein; substrate of oxidation, 5 mM succinate)

Rat group	n, Inital	n, died at impact	n, Hemor-hages	n, Normal birth	Pregnancy time, days	Number of live pups in litter	Weight of pups at birth, g	Abnormali-ties, according to Wilson, %
Control	9	-	-	9	22.0±0.2	10.1±1.2	5.6±0.2	11.1
Pregnancy day 19, 40 G	11	0	0	11	22.2±0.1	9.1±1.0	5.8±0.2	11.1
Pregnancy day 19, 90 G	7	1	3	5	22.6±0.2	4.8±0.8 p<0.01	5.9±0.12	66.7 p<0.05
Pregnancy day 21, 40 G	6	0	2	6	22.3±0.1	8.0±0.7	6.0±0.2	35.7
Pregnancy day 21, 90 G	6	0	3	5	22.5±0.2	6.0±1.1 p<0.05	5.4±0.4	75.0 p<0.05

Table 18. Relative Volume of Components of the Corpus Luteum, %				
Rat Group	Lutein cells	Paralutein cells	Connective tissue	Blood vessels
F	41.78±2.20	35.06±2.14	16.87±0.56	2.39±0.29
SC	47.56±2.04	30.04±2.16	16.36±0.60	2.38±0.28
VC	46.96±2.04	28.47±1.94	17.21±0.65	2.60±0.33

Table 19. Volume (mm 3) of Lutein and Paralutein Cells and Their Nuclei				
Rat Group	Lutein cells		Paralutein cells	
	Cells	Nuclei	Cells	Nuclei
F	2950±171	300.34±17.45	1036.8±43.2	142.04±4.67
		Ps<0.05		Pv,s<0.05
SC	3454±186	369.94±19.9	642.3±30.2	99.43±4.67
			Pv,s>0.05	Pv<0.05
VC	3218±248	325.74±25.1	966.7±48.4	176.33±8.82

Table 20. Relative Volume (%) of Corpus Luteum Cell Nuclei		
Rat Group	Lutein cells	Paralutein cells
F	10.18±0.44	13.7±0.63
		Pv,s<0.05
SC	10.71±0.65	15.48±0.64
VC	10.12±0.5	18.24±0.93

Table 21. Parameters of the Reproductive Function of Pregnant Rats				
Rat Group	Females, n	Corpus luteum, n	Implantation sites	Resorption, n
F	5	15.8±1.0	14.8±0.7	1.2±0.4
SC	5	16.0±0.8	14.4±0.7	1.0±0.4
VC	7	14.9±0.4	13.0±0.8	0.6±0.3
Group	Live fetuses, n	Dead fetuses, n	Preimplantation mortality, %	Total embryonic mortality, %
F	13.6±0.8	0	5.8±2.6	13.4±4.1
SC	13.4±1.0	0	9.9±2.8	16.4±4.4
VC	12.4±0.9	0	12.5±4.7	16.4±4.8

Table 22. Characteristics of Placentas on Pregnancy Day 18			
Parameter	**Rat Group**		
	F	**SC**	**VC**
Weight, g	0.31±0.02 Ps<0.05	0.37±0.01	0.38±0.025
Thickness of labyrinthine compartment, mm	2.12±0.06 Pv,s<0.001	2.45±0.05	2.65±0.05
Thickness of spongy compartment, mm	0.87±0.06	0.80±0.03	0.90±0.04
Water, kg/kg dry weight	5.97±0.19	5.95±0.15	5.96±0.14
Na, mequiv/kg dry weight	504.1±12.8 Pv<0.05 Ps<0.002	431.9±16.4	466.0±11.0
K, mequiv/kg dry weight	394.2±8.5 Pv,s<0.001	436.0±6.5	441.7±6.6
Ca, mequiv/kg dry weight	57.8±11.3	41.4±4.9	52.9±7.6
Mg, mequiv/kg dry weight	71.8±3.5	69.5±2.1	76.4±1.8
DNA, mg/g wet weight	2.51±0.14	2.88±0.12 Pv<0.05	2.29±0.15
RNA, mg/g wet weight	7.50±0.16	7.15±0.21	7.31±0.19
Protein,μg/mg wet weight	76.4±5.1	59.0±31 Pv,s<0.05	83.0±3.3
Glycogen, mg/g	9.3±2.8	9.8±1.9	11.3±2.2

Table 23. Characteristics of Fetuses on Pregnancy Day 18					
Parameter	n		Rat Group		
	Mothers	Fetuses	F	SC	VC
Weight, g	5-7*	67-87	0.84±0.03 Ps<0.05	0.94±0.02	0.92±0.03
Water, kg / kg dry wt.	5-7	9-13	9.55±0.15 Pv<0.002 Ps<0.02	9.03±0.12	9.04±0.07
Na, mequiv / kg dry wt.	5-7	9-12	875.0±13.1	816.7±17.2	863.0±29.8
K, mequiv / kg dry wt	5-7	9-12	637.7±11.5	585.1±12.2	604.8±23.0
Ca, mequiv / kg dry wt.	5-7	9-12	209.4±22.2	220±17	231.5±24.7
Mg, mequiv / kg dry wt.	5-7	9-12	137.9±4.3	123.9±4.0	150.3±18.7
DNA, mg/g wet wt.	5-7	10	4.05±0.18	4.04±0.15	3.95±0.03
RNA, mg / g wet wt.	5-7	10	7.72±0.22	7.75±0.21	7.79±0.27
Protein, µg / mg wet wt.	5-7	10	60.4±3.7	64.5±2.9	63.5±3.5
Tyrosine-hydroxylase in adrenals, nmole/ hr / 2 adrenals	5-7	5-6	0.043±0.009	-	0.042±0.008
CFUs per 10^6 liver cells	5-7	5-6	8.2±0.2 Pv<0..05 Ps<0.002	13.4±1.1	10.5±0.8

* 7 rats in the vivarium group

Table 24. Weight, Water, and Electrolyte Content in Organs of Neonate Rats						
Organ	Rat Group	Weight, mg	Water kg / kg dry weight	Na mequiv / kg dry weight	K mequiv / kg dry weight	Ca mequiv / kg dry weight
Kidney	F	29.2±1.6	6.87±0.18	559.6±12.2	578.2±31.6	30.2±4.0
		Ps<0.01		Ps<0.01		
				Pv<0.02		
	SC	21.2±0.9	7.4±0.77	635±17.7	679.7±34	31.2±7.7
	VC	26.2±0.9	6.88±0.16	615.7±15.8	594.4±10.1	38.7±4.0
Liver	F	255±13	3.17±0.12	204.2±24.1	356.5±9.8	12.9±2.8
		Pv<0.001				
		Ps<0.01				
	SC	322±12	2.97±0.075	223.9±17.7	350.5±27	8.5±1.02
	VC	329±10	3.1±0.039	188±7.7	342.6±8.6	7.2±0.6
Heart	F	29.7±1.0	5.04±0.11	355.2±10.5	488.9±12.5	25.1±3.4
		Pv,s<0.001				
	SC	23.8±0.7	4.86±0.066	370.4±15.4	460.6±51.9	-
	VC	24.5±0.7	4.84±0.1	358.7±9.8	494.4±9.1	35.4±4.8

Table 25. Concentration of Nucleic Acids and Protein in Neonate Rat Liver			
Rat Group	DNA mg/g wet weight	RNA , mg/g wet weight	Protein, μg/mg wet weight
F	4.3±0.21	16.3±0.8	107±5
SC	3.8±0.18	13.5±0.4	104±6
VC	3.8±0.28	14.1±0.5	118±8

Table 26. Characteristics of Peripheral Blood and Hemopoietic Organs in Neonate Rats

Rat Group	Peripheral Blood							CFUs / 10^6 cells	
	Hemoglobin	Reticulocytes,	Erythroblasts,	Lymphocytes,	Neutrophils thous/mm 3				
	g %	o/oo	%	thous / mm 3	Bands	Rods	Juvenile	Spleen	Liver
F	10.56±0.22 Pv<0.01 Ps<0.05	874± 10.5 Ps<0.001 Pv<0.002	72±2.9	1.9±0.56	3.1± 0.58	1.26± 0.24	0.44± 0.08	19.2±0.7 Ps,v<0.001	9.5±0.5 Ps<0.001
SC	11.7±0.29	926±7.6	79±2.8	2.1±1.2	4.4±0.8	1.3±0.28	0.7±0.13	8.5±0.8	26.0±2.0 Pv<0.001
VC	11.4±0.27	931±4.9	79±2.4	1.2±0.17	2.7±0.34	1.2±0.112	0.5±0.043	10.0±0.8	10.3±1.0

Table 27. Weight of Skeletal Muscles of Rats in Postnatal Ontogenesis

Age, days	Rat Group	Muscles, mg						
		Brach	EDL	Tric	Sol	M. gastroc	L. gastroc	Plant
15	F	18.5±0.9	10.7±0.5	23.0±1.2	8.75±0.3	28.7±2.8	33.6±2.9	10.8±1.5
			Pv,s<0.05			Pv,s<0.05		
	SC	18.0±0.9	7.3±0.8	23.4±0.9	8.0±0.3	23.36±1.11	31.1±1.0	11.6±0.7
			Pv<0.05		Pv<0.05			
	VC	18.8±0.8	9.0±0.2	22.8±0.7	9.2±0.3	20.2±1.97	28.3±2.2	12.3±0.8
30	F	66.2±2.7	47.1±2.5	68.5±6.2	43.2±4.2	17.1±10.8	215.0±19.2	71.2±5.5
			Pv<0.05					
	SC	71.5±2.3	42.0±1.3	67.2±3.9	37.4±1.	177.0±12.8	216.5±9.8	70.7±3.5
	VC	64.6±4.8	40.9±2.4	61.5±2.9	37.0±2.1	153.5±14.4	189.6±13.3	62.2±4.5
100	F	229.0±7.3	188.2±3.1	202.5±8.7	151.5±3.9	767.5±23.7	1005±37.91	351.2±16.7
			Ps<0.05	Pv<0.01	Ps<0.05		Ps<0.05	Ps<0.05
				Ps<0.05				
	SC	212.3±10.1	158.5±7.6	159.9±11.1	135.1±5.9	712.0±29.5	912±22.8	292.0±9.6
					Pv<0.05			Ps=0.05
	VC	214.8±71	176.2±5.5	174.2±3.5	153.6±6.2	740.0±24.3	964±18.5	326.6±15.2

Table 28. Maximal Force (Pm,m uu$^{2}10^{1}$) of Muscle Fibers of Rats Born after 5-Day Flight

Rat Group	Muscles						
	Brach	EDL	Triceps	Soleus	M. Gastroc	L. Gastroc	Plant
	15 Days						
F	241±0.14	1.63±0.12	1.23±0.08	1.21±0.10	1.17±0.12	1.10±0.10	0.82±0.15
	Ps<0.05						Pv<0.05
SC	1.99±0.88	1.34±0.14	1.20±0.39	1.11±0.04	1.30±0.33	1.28±0.12	1.04±0.21
	Pv<0.05						
VC	2.54±0.15	1.6±0.32	1.20±0.03	1.42±0.17	1.49±0.17	1.21±0.68	1.23±0.06
	30 Days						
F	3.10±0.10	3.05±0.30	3.23±0.24	2.92±0.31	3.41±0.25	3.22±0.20	2.22±0.26
			Ps<0.01				
SC	3.19±0.25	3.13±0.44	2.61±0.05	2.74±0.13	8±0.26	3.01±0.015	2.97±0.16
				Pv<0.05			
VC	2.85±0.22	2.89±0.34	2.98±0.34	3.22±0.15	3.71±0.45	3.12±0.61	2.42±01.65
	100 Days						
F	3.05±0.30	2.69±0.12	3.38±0.47	3.14±0.08			
SC	3.01±0.39	2.51±0.12	2.75±0.31	3.65±0.20			
VC	2.74±0.38	2.76±0.27	2.91±0.20	3.59±0.28			

Table 29. Maximal Speed (Vm, mg sec -1) of Contraction of Muscle Fibers of Rats Born after 5-Day Flight								
Rat group	n, Inital	n, died at impact	n, Hemor-hages	n, Normal birth	Pregnancy time, days	Number of live pups in litter	Weight of pups at birth, g	Abnormali-ties, according to Wilson, %
Control	9	-	-	9	22.0±0.2	10.1±1.2	5.6±0.2	11.1
Pregnancy day 19, 40 G	11	0	0	11	22.2±0.1	9.1±1.0	5.8±0.2	11.1
Pregnancy day 19, 90 G	7	1	3	5	22.6±0.2	4.8±0.8 p<0.01	5.9±0.12	66.7 p<0.05
Pregnancy day 21, 40 G	6	0	2	6	22.3±0.1	8.0±0.7	6.0±0.2	35.7
Pregnancy day 21, 90 G	6	0	3	5	22.5±0.2	6.0±1.1 p<0.05	5.4±0.4	75.0 p<0.05

Table 30. Force Impulse (S, $\mu \cdot c \cdot 10^{-4}$) of Muscle Fibers of Rats Born after 5-Day Flight

Rat Group	Muscles						
	Brach	EDL	Tric	Sol	M. gastroc	L.gastroc	Plant
	15 Days						
F	8.68±0.84	5.38±0.44	4.93±0.46	3.79±0.32 Pv<0.05	6.45±0.57 Pv<0.05	6.92±0.29 Ps<0.05	6.92±0.50
SC	9.64±0.25	4.56±0.25 Pv<0.05	5.05±0.47	4.46±0.40	8.04±0.98	8.24±0.46 Pv=0.05	7.81±1.30
VC	9.03±0.44	5.32±0.22	4.83±0.37	5.12±0.40	7.72±0.26	7.21±0.25	7.40±0.76
	30 Days						
F	3.95±0.63	1.98±0.20	3.07±0.11	2.91±0.37	3.69±0.38 Ps = 0.05	3.72±0.53	2.89±0.55
SC	4.42±0.67	2.34±0.36	3.32±0.30	2.83±0.25	2.83±0.21	3.69±0.15	3.32±0.29
VC	3.75±0.30	2.08±0.33	3.53±0.35	3.21±0.22	3.26±0.16	3.35±0.23	3.69±0.15
	100 Days						
F	4.46±0.45	4.41±0.37	6.87±0.61 Ps<0.001 Pv<0.01	6.24±0.50			
SC	4.69±0.61	4.58±0.26	4.00±0.25	6.33±0.71			
VC	3.81±0.45	3.42±0.50	4.40±0.39	7.85±0.49			

Table 31. Width (μm) of Layers of Walls of the Cerebral Hemispheres of 18-Day Old Fetuses					
Rat Group	Boundary zone	Cortical layer	Substantia intermedia	Matrix	Wall of the hemishpere
F	22.7±2.1	100.2±10.4	117.4±15.6	114.4±10.9	354.7±35.3
SC	27.5±1.2	118.1±7.6	131.8±15.4	142.4±10.4	419.8±25.5
VC	23.0±3.2	115.3±21.6	125.2±21.1	88.9±9.2	352.4±48.1

Table 32. Postnatnal Ontogenesis: Exploratory Activity of Rats in "Open Field"							
Rat Group	Total "open field" distance, arb.u.	Visits to central zone, arb. u.	Path length in central zone,%	Upright wall stops	Orientation reactions	Excretion, arb.u.	Grooming time
	30 days						
F	83.6 Pv<0.05	6.9 Pv<0.01 Ps<0.001	4.2 Pv<0.05 Ps<0.01	8.3 Pv<0.05	13.8 Pv<0.01 Ps<0.05	5.5 Ps<0.05	9.8 Pv<0.05
SC	95.0	10.3	7.5	10.6	16.2	2.2	11.2
VC	103.3	8.0	5.3	13.7	19.4	1.5	5.9
	52 days						
F	73.3 Ps<0.01 Pv<0.01	6.6 Ps<0.001 Pv<0.001	4.2 Ps<0.001 Pv<0.001	8.2 Ps<0.001 Pv<0.01	10.4 Ps<0.001 Pv<0.01	1.9 Pv<0.05	33.0 Ps<0.05
SC	94.9	12.0	11.7	15.6	21.2	2.7	37.6
VC	97.8	12.1	9.0	12.5	16.9	2.0	22.0
	68 days						
F	99.8 Pv<0.05	12.6 Ps<0.05	8.4 Pv<0.05	15.1	6.8 Pv<0.05	0.06	14.2 Ps<0.05
SC	106.9	10.5	9.1	12.8	19.5	0	19.3
VC	108.5	13.5	12.7	14.9	9.7	1.8	6.0

Table 33. Behavioral Parameters of Animals in Dombrovskaya Maze					
Rat Group	Rats incapable of learning behavioral algorithm in the maze, %	Number of refusals, %	Latent period, sec	Number of errors	Solution time, sec
F	31.2	9 Pv<0.01	2.4 Ps<0.05	16.8	85.1 Pv<0.001 Ps<0.05
SC	41.8	13.6	3.5	16.3	70.8
VC	33.3	3.9	2.2	15.2	61.4

Table 34. Reaction to Immobilization Stress in Rats that Spent a Portion of Their Prenatal Period in Microgravity

		Blood Changes					
Rat Group	n	Lymphocyte		neutrophils		Lymphocytes / neutrophils	
		thous/mm 3	Abnormal reactions %	thous / mm 3	Abnormal reactions %	Abs.u.	Abnormal reactions, %
		30 Days					
F	12	+3.2±0.3 Pv<0.001	83 Pv<0.001	+3.2±0.5	17	-2.6±0.4	27 Pv<0.05
VC	12	-3.0±0.4	10	+3.4±0.4	10	-2.4±0.4	0
		90 Days					
F	12	-6.1±0.6	17	+4.9±1.1 Pv<0.05	17	-3.0±0.6	0
VC	12	-5.4±0.1	17	+2.4±0.5	17	-2.5±0.3	0

Table 35. Postnatal Ontogenesis. Profile of Peripheral Blood							
				Neurophils, thous / mm 3			
Age, days	Rat Group	Hemo-globin, g %	Lympho-cytes, Tblc/μμ3	Bands	Rods	Juvenile	Reticulo-cytes, o/oo
15	F	8.2±0.49	2.6±0.22	0.7±0.22	0.2±0.009	0.1±0.01	
	SC	7.8±2.8	1.9±0.3	1.5±0.6	0.2±0.07	0.1±0.07	
	VC	8.4±0.19	2.8±0.5	1.4±0.8	0.2±0.08	0.2±0.08	
30	F	12.2±0.27	6.8±1.46	1.5±0.30	0	0	293±7.7
		Pv<0.01					
		Ps<0.01					
	SC	11.3±0.3	5.4±0.73	1.4±0.19	0.1	0	326±8.1
	VC	10.9±0.09	5.6±0.73	1.2±0.45	0	0	305±7.5
100	F	12.8±0.31	8.8±0.23	2.5±0.8	0	0	56±1.7
		Pv,s<0.01					
	SC	10.9±0.27	9.9±1.3	3.7±0.6	0	0	55±2.3
	VC	11.0±0.36	10.1±1.9	3.14±0.8	0	0	56±2.3

Table 36. Postnatal Ontogenesis. Concentrations of Triiodothyronine and Thyroxine in the Thyroid Gland			
Age, days	Rat Group	Triiodothyronine, μg/mg protein	Thyroxine, μg/mg protein
15	F	0.02±0.005	0.25±0.05
		Pv,s<0.025	
	SC	0.04±0.004	0.14±0.04
	VC	0.04±0.004	0.20±0.02
30	F	0.02±0.006	0.08±0.02
		Ps<0.05	
	SC	0.06±0.013	0.26±0.11
		Pv=0.05	
	VC	0.03±0.04	0.12±0.04
100	F	0.18±0.04	0.27±0.12
	SC	0.20±0.004	0.23±0.08
	VC	0.08±0.04	0.15±0.08

Table 37. Postnatal Ontogenesis. Concentration of CFUs in Bone Marrow and Spleen (per 10^{-6} Karyocytes			
		CFU's In	
Age, days	Group	Bone Marrow	Spleen
15	F	4.6±0.6	5.4±0.9
		Pv<0.05	Pv<0.01
		Ps=0.002	
	SC	9.1±0.8	6.0±0.4
			Pv<0.001
	VC	8.9±1.4	10.0±0.6
30	F	14.9±0.8	9.6±0.7
	SC	13.1±1.2	7.8±0.4
	VC	16.3±1.04	8.0±0.6
100	F	15.5±1.2	3.6±1.1
	SC	13.5±1.8	1.2±0.3
	VC	12.3±0.9	2.1±0.8

Table 38 (pt. 1). Postnatal Ontogenesis. Tissue Concentraions of Water and Electrolytes						
Age, days	Rat Group	Water,kg / kg dry weight	Na	C	Ca	Mg
			mequiv / kg dry weight			
		Skin				
15	F	2.52±0.26	228.1±24.9	197.5±16.8	18.6±1.8	43.2±3.0
	SC	2.51±0.18	233.1±14.2	227.8±10.7	16.2±1.8	57.6±6.1
	VC	2.04±0.20	164.7±5.6	183.0±14.8	12.7±2.0	44.4±3.3
30	F	2.34±0.06	209.7±11.2	178.2±15.0	17.5±1.6	43.1±4.5
	SC	2.31±0.14	208.9±10.5	189.6±19.2	13.1±0.9	40.2±2.1
	VC	2.15±0.17	197.5±19.6	177.9±9.7	11.1±1.1	38.9±1.6
		Bone				
15	F	1.72±0.17	1731±124	185.6±23.7	18107±257	655.9±69.5
	SC	1.48±0.16	1837±116	167.4±20.9	15334±936	938.2±43.3
	VC	1.57±0.07	1913±141	160.3±10.7	15207±784	746.6±43.3
30	F	0.52±0.04	1444±240	73.4±5.4	10235±952	542±28
	SC	0.70±0.05	1427±46	80.7±10.9	12075±699	493±32
	VC	0.68±0.03	1331±28	84.3±7.7	10575±473	466±21
		Liver				
15	F	4.40±0.28	191.8±13.6	451.8±23.4	15.3±3.6	104.9±4.5
	SC	3.44±0.23	142.4±9.2	418.7±19.6	9.8±0.4	101.4±5.2
	VC	4.08±0.20	207.2±11.4	473.7±46.3	15.9±3.3	116.3±7.6
30	F	3.16±0.11	137.5±9.2	384.4±10.9	6.7±1.3	95.3±5.1
	SC	2.88±0.12	120.8±5.6	363.9±20.0	8.3±1.4	79.5±2.6
	VC	3.09±0.05	130.1±4.3	373.7±15.5	4.75±0.18	93.7±3.5

Table 38 (pt. 2). Postnatal Ontogenesis. Tissue Concentraions of Water and Electrolytes						
Age, days	Rat Group	Water, kg / kg dry weight	Na	K	Ca	Mg
			mequiv / kg dry weight			
		Kidney				
New born	F	6.87±0.18	559.6±12.2 Ps<0.01 Pv<0.02	578.2±31.6	30.2±4.0	137.8±3.7 Ps<0.001 Pv<0.05
	SC	7.40±0.27	635±17.7	679.7±24	81.2±7.7	170.8±3.7
	VC	6.89±0.16	615.7±15.8	594.4±10.1	38.7±4.0	150.3±4.9
15	F	4.99±0.05	427.6±14.5	488.3±9.8	19.6±4.2	115.1±1.9 Pv<0.05
	SC	4.69±0.08	386.7±7.6	491.2±8.6	19.3±2.9	111.2±1.0 Pv<0.05
	VC	5.24±0.32	458.4±29.2	536.6±34.8	28.8 (n=2)	130.3±6.2
30	F	3.53±0.06	336.0±12.3	393.5±8.2	22.3±2.5	98.8±4.2
	SC	3.44±0.05	307.7±7.3	382.8±14.5	20.6±1.8	96.5±2.8
	VC	3.62±0.09	324.4±16.2	357.0±4.5	21.1±2.0	94.0±1.7

Table 39. Postnatal Ontogenesis. Nucleic Acids in the Liver				
		Age, days		
Parameter	Rat Group	15	30	100
RNA, mg / g	F	9.4±1.2	10.2±0.5	6.0±0.4
			Ps<0.05	
	SC	10.6±0.9	7.5±0.7	5.9±0.2
			Pv<0.05	
	VC	14.8±2.9	10.9±1.0	7.7±1.6
RNA, mg / organ	F	8.7±0.7	51.8±9.9	75.3±7.1
	SC	10.2±0.8	36.1±4.4	74.4±6.5
			Pv<0.05	
	VC	14.0±2.1	53.8±5.0	101.1±23.0
DNA, mg/g	F	5.2±0.3	2.7±0.1	2.6±0.1
	SC	4.3±0.4	2.6±0.1	2.7±0.1
	VC	4.9±0.4	3.0±0.2	3.0±0.1
DNA, mg/organ	F	4.9±0.4	13.3±1.9	32.9±1.2
	SC	4.3±0.3	12.7±0.8	33.4±1.6
	VC	4.8±0.4	14.3±0.6	38.9±2.6

Table 40. Postnatal Ontogenesis. Nucleic Acids in the Thymus			
Parameter	**Rat Group**	**Age, days**	
		30	**100**
RNA, mg / g	**F**	22.1±2.7	18.6±1.4
	SC	18.6±0.7	18.4±1.7
	VC	20.0±3.3	22.7±3.5
RNA, mg / organ	**F**	9.1±0.3	6.8±0.8
	SC	8.5±0.7	6.9±0.7
	VC	7.0±0.9	10.0±1.3
DNA, mg / g	**F**	18.6±2.5	33.2±2.9
	SC	19.3±0.6	29.8±2.3
	VC	21.1±1.1	30.5±4.6
DNA, mg / organ	**F**	7.8±1.1	12.3±1.2
	SC	8.9±0.5	11.2±0.9
			Pv<0.05
	VC	7.3±0.2	16.5±2.1

Table 41. Nucleic Acids in the Spleens of 100-Day Old Rats				
	RNA		DNA	
Rat Group	mg / g	mg / organ	mg / g	mg / organ
F	8.0±0.5	13.1±1.1	11.8±0.8	19.3±1.4
SC	11.4±4.1	15.9±6.0	16.9±5.2	23.7±4.7
VC	10.7±3.0	16.6±2.1	14.7±3.8	22.9±2.4

Table 42. Postnatal Ontogenesis. Rate of Incorporation of Radioactive Precursors in DNA and RNA of Spleen Lymphocytes			
Age, days	Rat Group	3 H-thymidine, cpm/μg DNA	14C-uridine cpm/μg RNA
30	VC	133.6±4.1	11.0±1.1
	SC	149.9±13.9	8.8±0.5
	F	135.9±11.3	11.1±0.7
100	VC	137.5±2.4	14.8±0.3
	SC	139.1±5.2	12.1±1.0
	F	137.8±4.9	10.7±0.6

Table 43. Postnatal Ontogenesis. Activity of Liver Enzymes				
Parameter	**Rat Group**	**Age, days**		
		15	**30**	**100**
TAT, mmole/g/min	**F**	18.5±4.0	7.8±0.8	14.8±3.8
			Pv<0.01	
	SC	26.0±2.1	8.5±1.8	8.8±3.6
		Pv<0.001	Pv<0.05	
	VC	9.7±2.9	23.8±4.5	9.5±1.5
TP,mmole/g/hr	**F**	-	16.7±4.5	12.8±3.6
	SC	-	12.3±5.2	10.9±3.6
	VC	-	11.3±2.4	5.9±1.5
ALT, mmole/g/min	**F**	-	29.9±11.9	57.3±6.6
	SC	-	29.8±12.6	67.1±10.8
	VC	-	35.8±10.5	50.6±12.8
AST, mmole/g/min	**F**	234±49	382±120	182±29
		Pv<0.05		
	SC	289±22	329±73	176±27
	VC	399±55	278±29	196±30

Table 44. Reproductive Parameters of Animals that Spent a Portion of Their Prenatal Period in Microgravity

Rat Group	Mated Mother	Mated Father	Pregnant, n	Neonates live	Neonates dead	Males in litter, %	Postnatal deaths, %	Non inseminated, %
1	F	F	8	10.0±1.5	1.4±1.0	46	12 P2,5<0.01 P3,4,5<0.001	27
2	Int	F	5	9.2±1.0	0.4±0.2	49	0	44
3	VC	VC	9	10.1±1.1	1.2±0.8	52	2.4	25
4	Int	VC	8	10.1±0.4	0.6±0.1	55	0	25
5	SC	SC	8	9.0±1.0	0.9±0.7	46	0	33
6	Int	SC	7	8.9±0.7	0	42	0	61

Table 45. Comparison of Parameters of Reproductive Function of Pups of Individual Mothers								
Mother	Father	Pregnant, n	Neonates live	Neonates dead	Body wt, g	Anomalies, %	Postnatal deaths, %	Non-inseminated, %
VC	VC	9	10.1±1.1	1.2±0.8	6.0±0.12	0	2.4	25
F (from 71, 79)	F	4	13.3±0.85	0	6.1±0.16	0	4.2	0
F (from 16, 33)	F	4	6.5±1.45	2.8±2.1	5.9±0.29	7.7	22.2	43
			P<0.01			Pv<0.05	Pv<0.05	

Table 46. Body Weight and Blood Profile of Male Rats After 7-Day Flight on Kosmos 1667						
Rat Group	Body wt, g	Hemo-globin, g%	Leukocytes, thous / mm 3	Lymphocytes, thous / mm 3	Neutrophils, thous / mm 3	Lymphocytes / neutrophils
F	332±3	15.4±0.2	8.1±0.5	2.6±0.2	5.3±0.4	0.52±0.05
n=7		Pv<0.01		Pv<0.01	Pv<0.002	Pv,s<0.01
				Ps<0.002	Ps<0.001	
SC	349±5	14.5±0.3	10.2±0.8	7.0±1.0	3.0±0.3	2.7±0.7
n=7						
VC	334±6	14.13±0.3	8.6±1.0	7.5±0.7	2.14±0.6	3.16±0.7
n=7						

Figure 1. Fusion of placentas of two adjacent fetuses.

Figure 2. Fetus ectocardia after exposure to impact acceleration on day 7 of prenatal development.

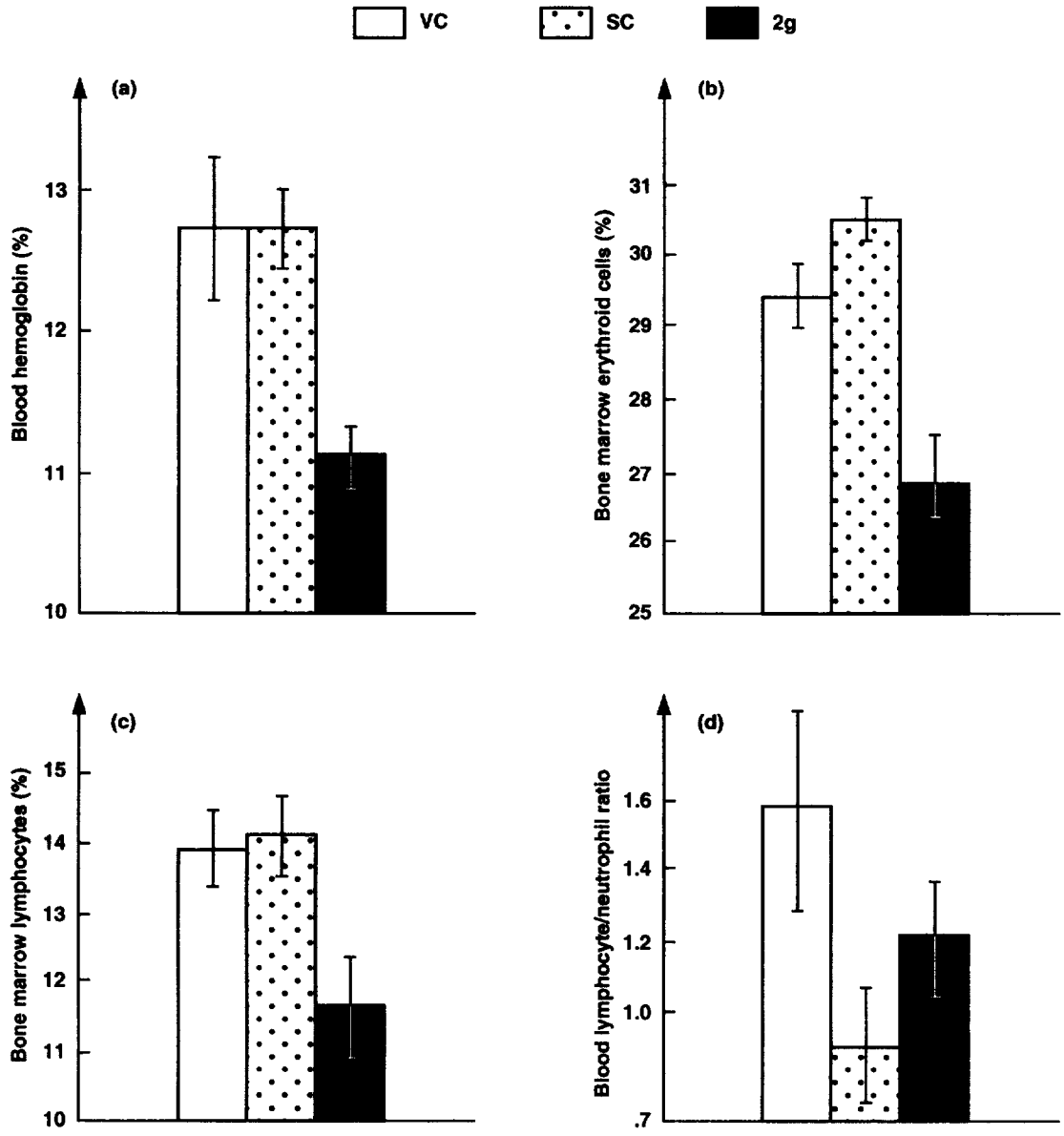


Figure 3. Hematology parameters of female rats exposed to 2 g during pregnancy: hemoglobin in peripheral blood (A), erythroid cells in bone marrow (B) and lymphocytes in bone marrow (C); lymphocyte/neutrophil ratio in blood (D). Vivarium controls = V; Synchronous controls = S; Hypergravity = 2 g.

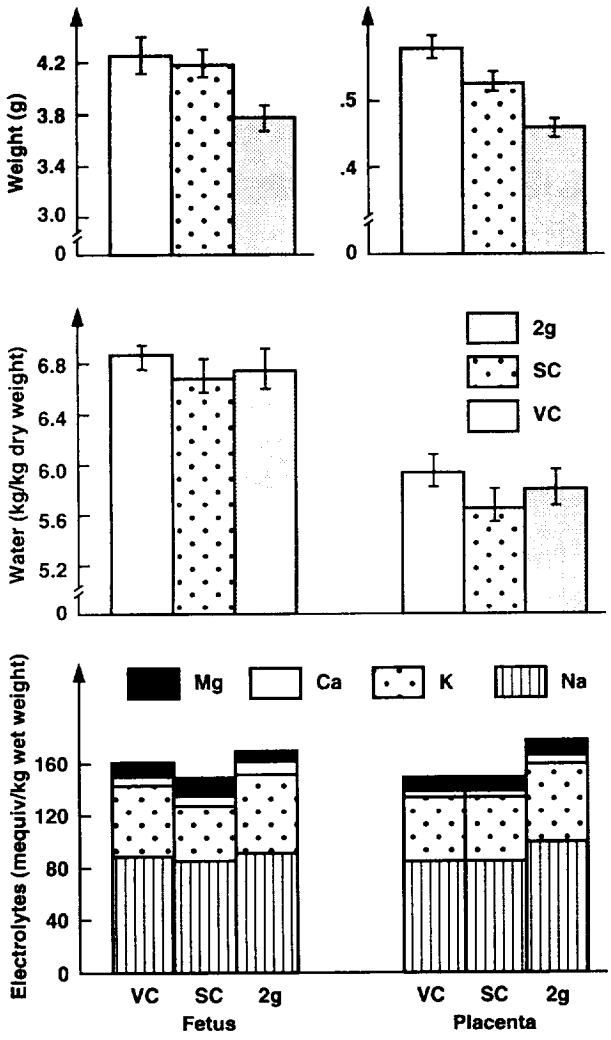


Figure 4. Effect of 2 g on the weight of fetuses and placentas and their content of water and electrolytes. Weight, g; Water, kg/kg dry matter; Electrolytes, mequiv/kg wet weight.

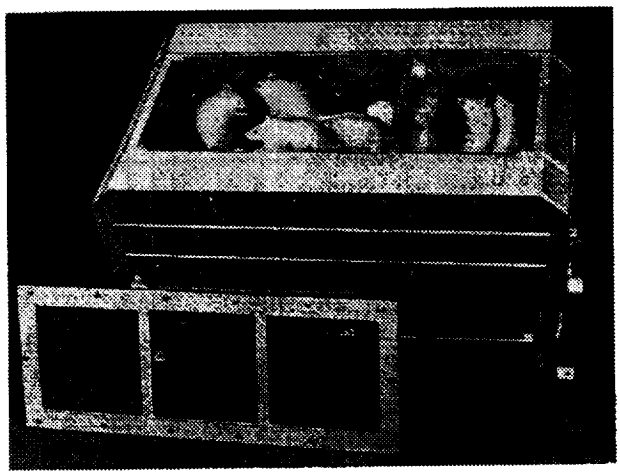


Figure 5. Bios-vivarium unit.

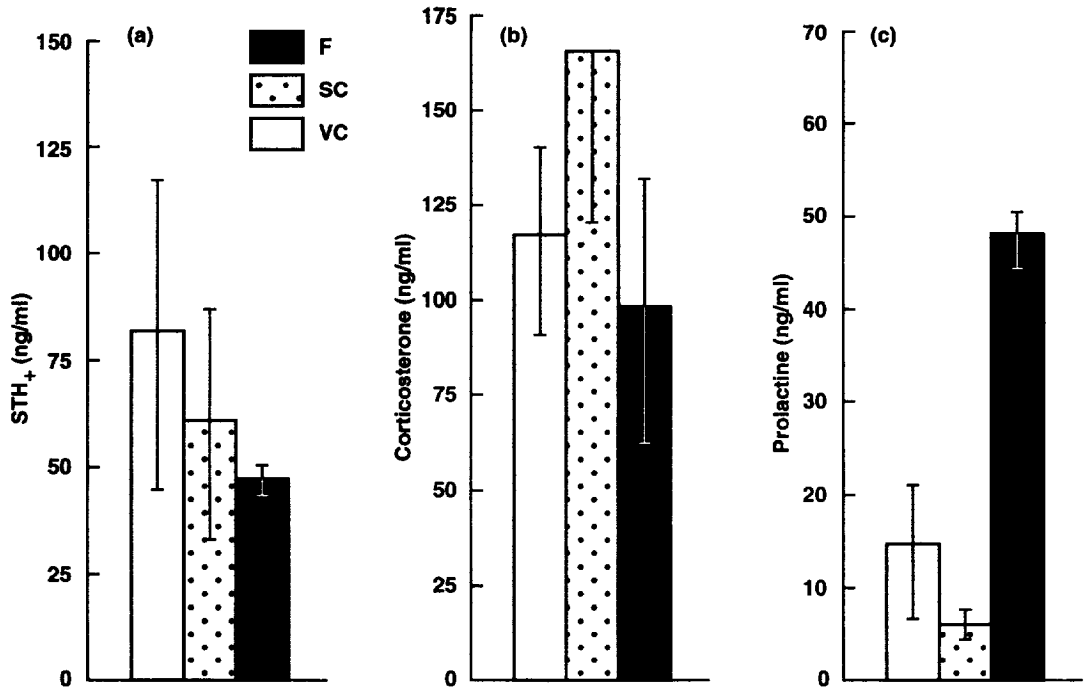


Figure 6. Concentration of somatotropic hormone (A), corticosterone (B) and prolactin (C) in plasma of female rats.

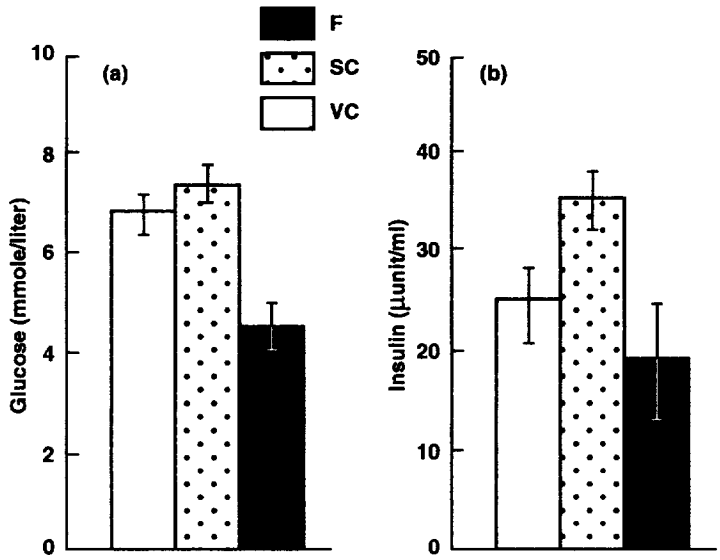
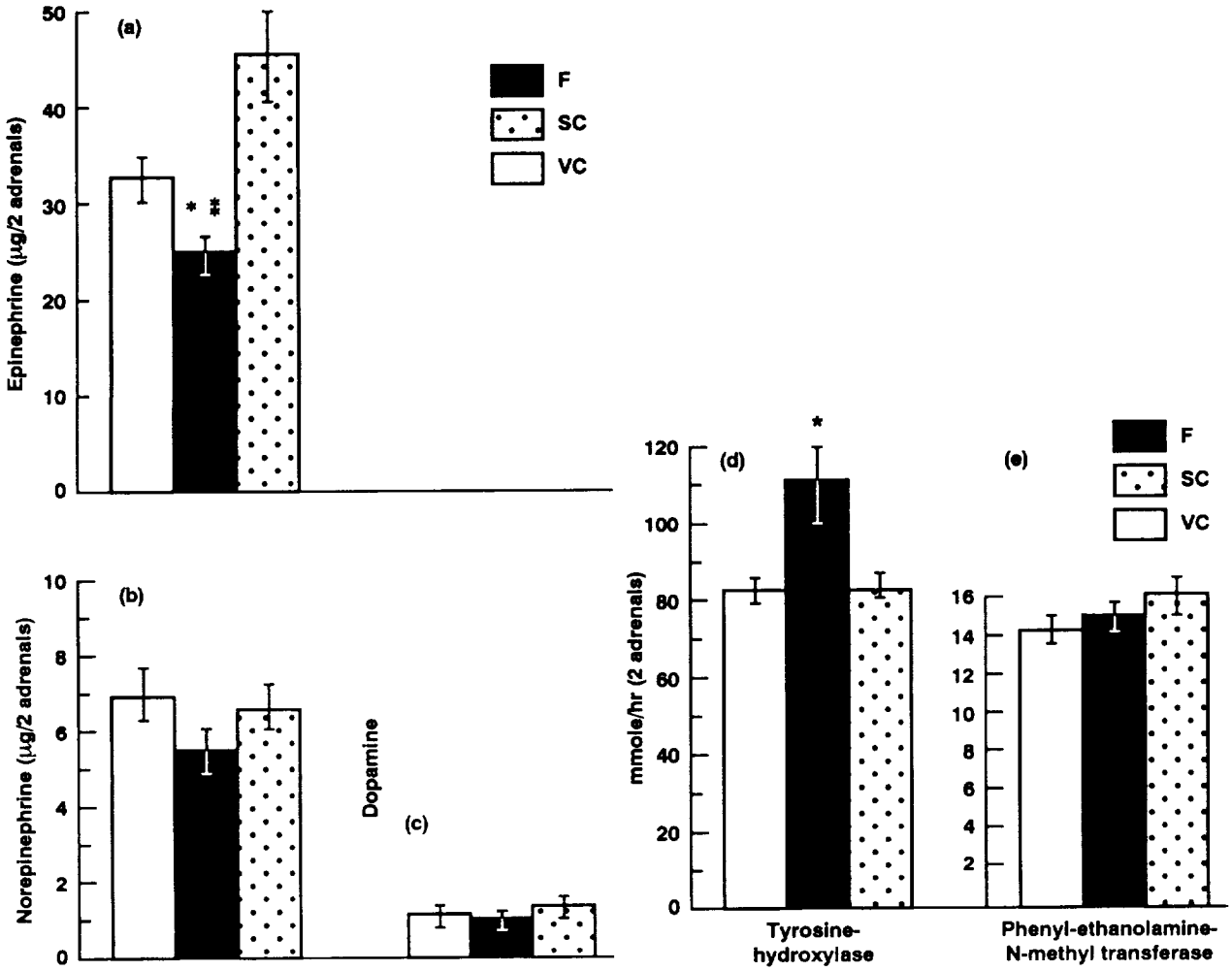


Figure 7. Concentration of glucose (A) and insulin (B) in plasma of female rats.



Asterisks indicate a significant difference between flight and experimental controls.

Figure 8. Concentration of epinephrine (A), norepinephrine (B), dopamine (C), activity of tyrosine-hydroxylase (D) and phenylethanolamine-N-methyltransferase (E) in the adrenals of female rats.

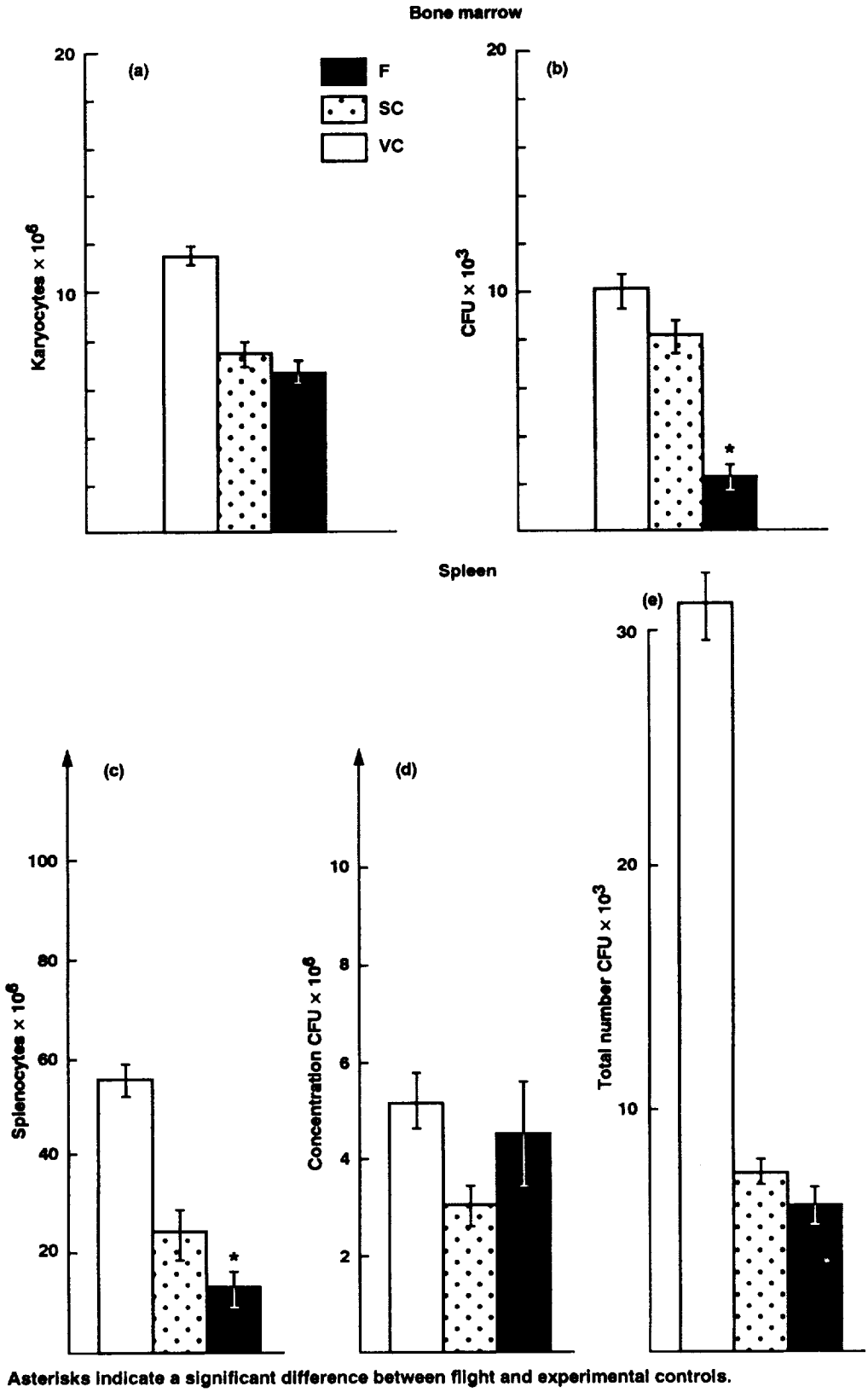


Figure 9. Characteristics of bone marrow and spleens of female rats: total number of karyocytes (A) and colony-forming units (CFU) (B) in bone marrow; total number of splenocytes (C); concentration (D) and total number of CFUs (E) in the spleen.

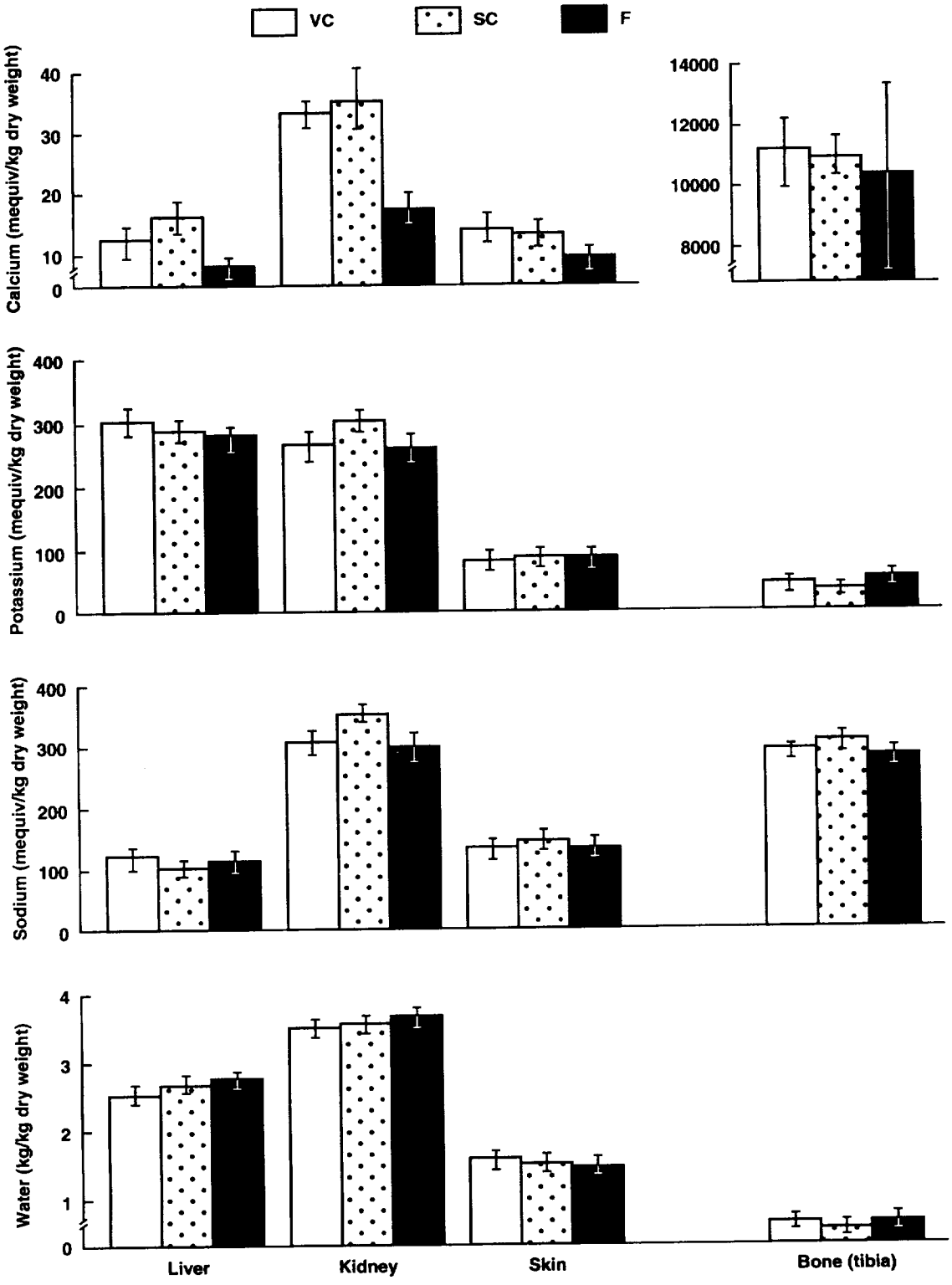


Figure 10. Levels of water and electrolytes in tissues of pregnant rats.

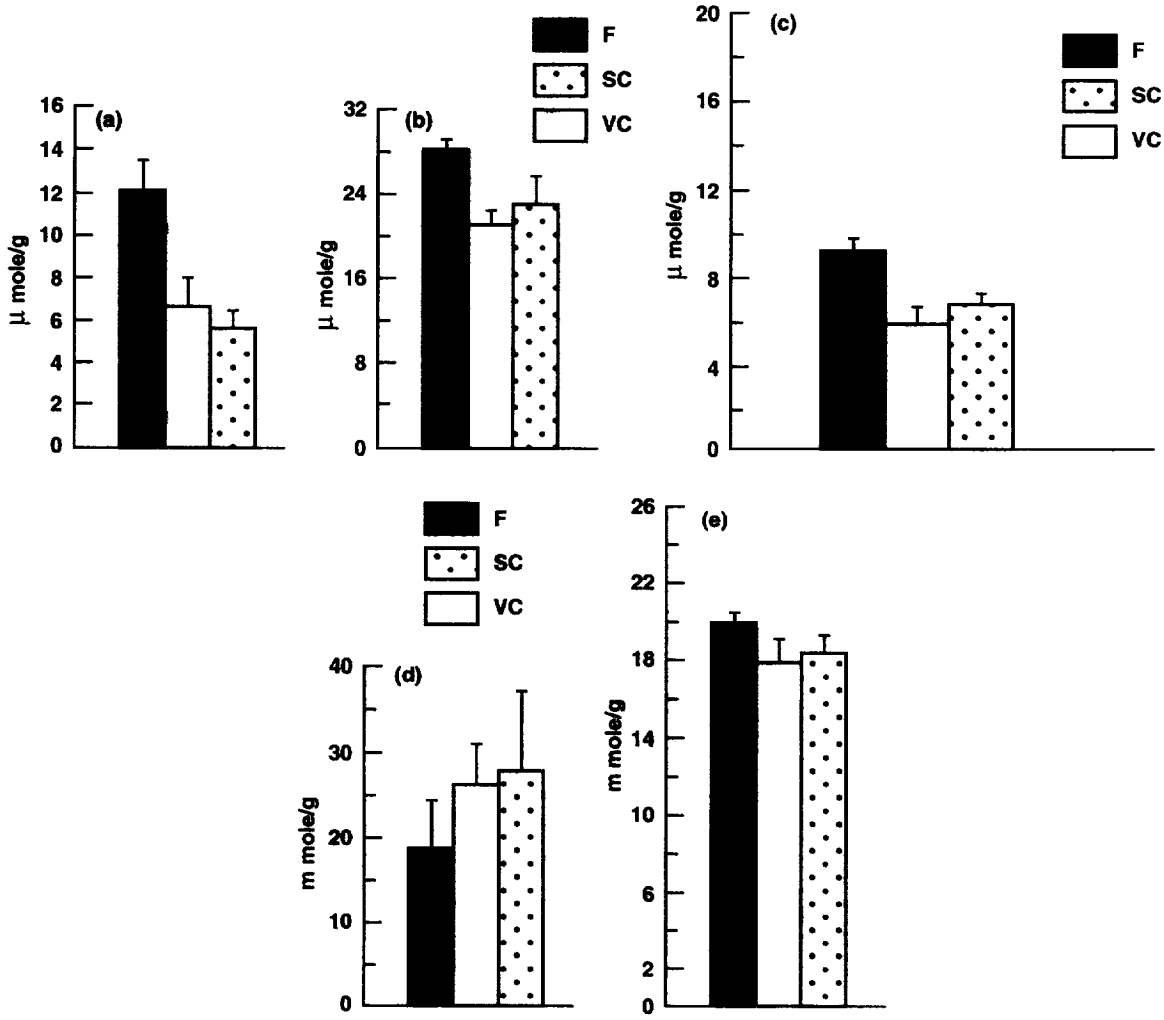
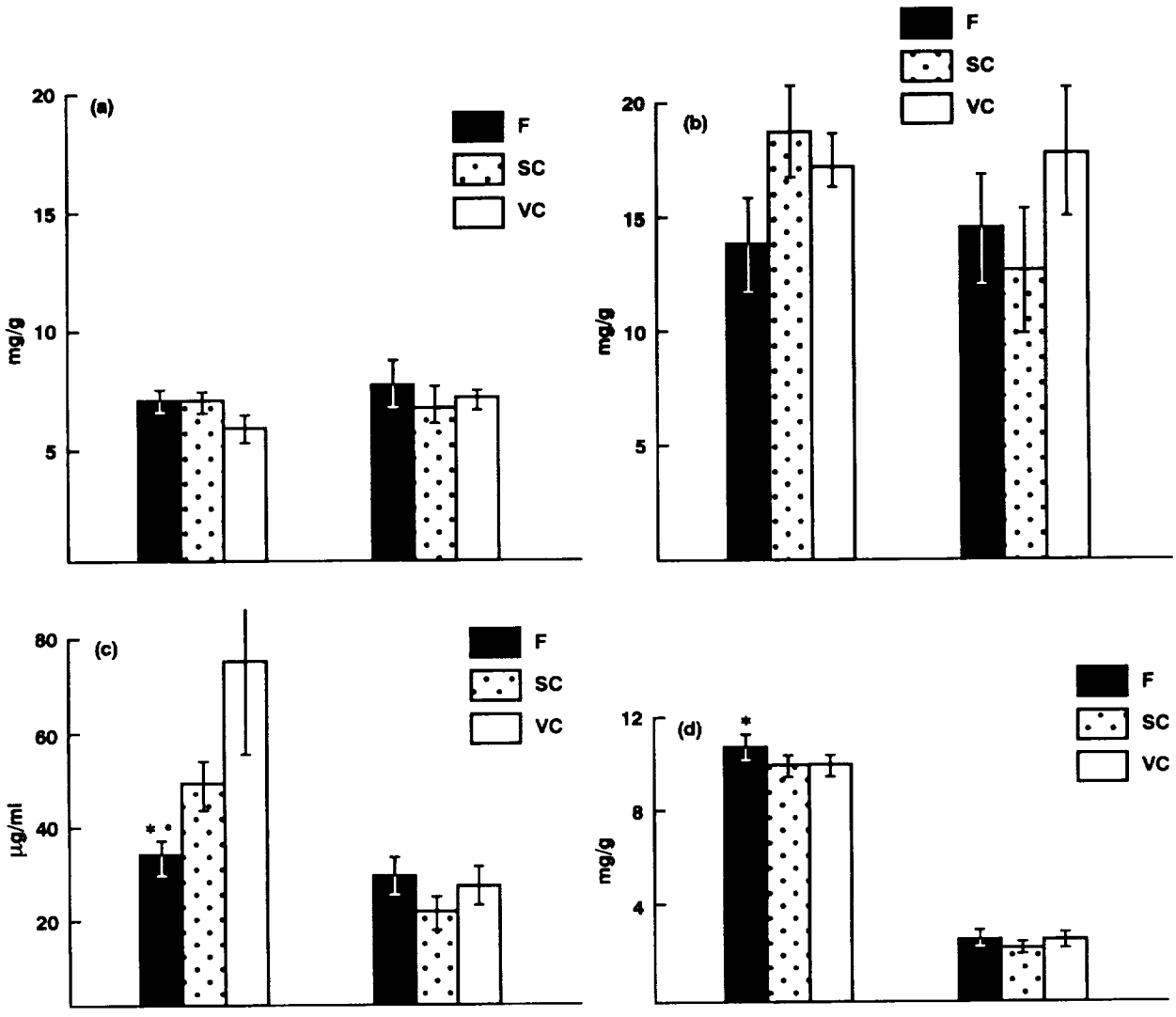
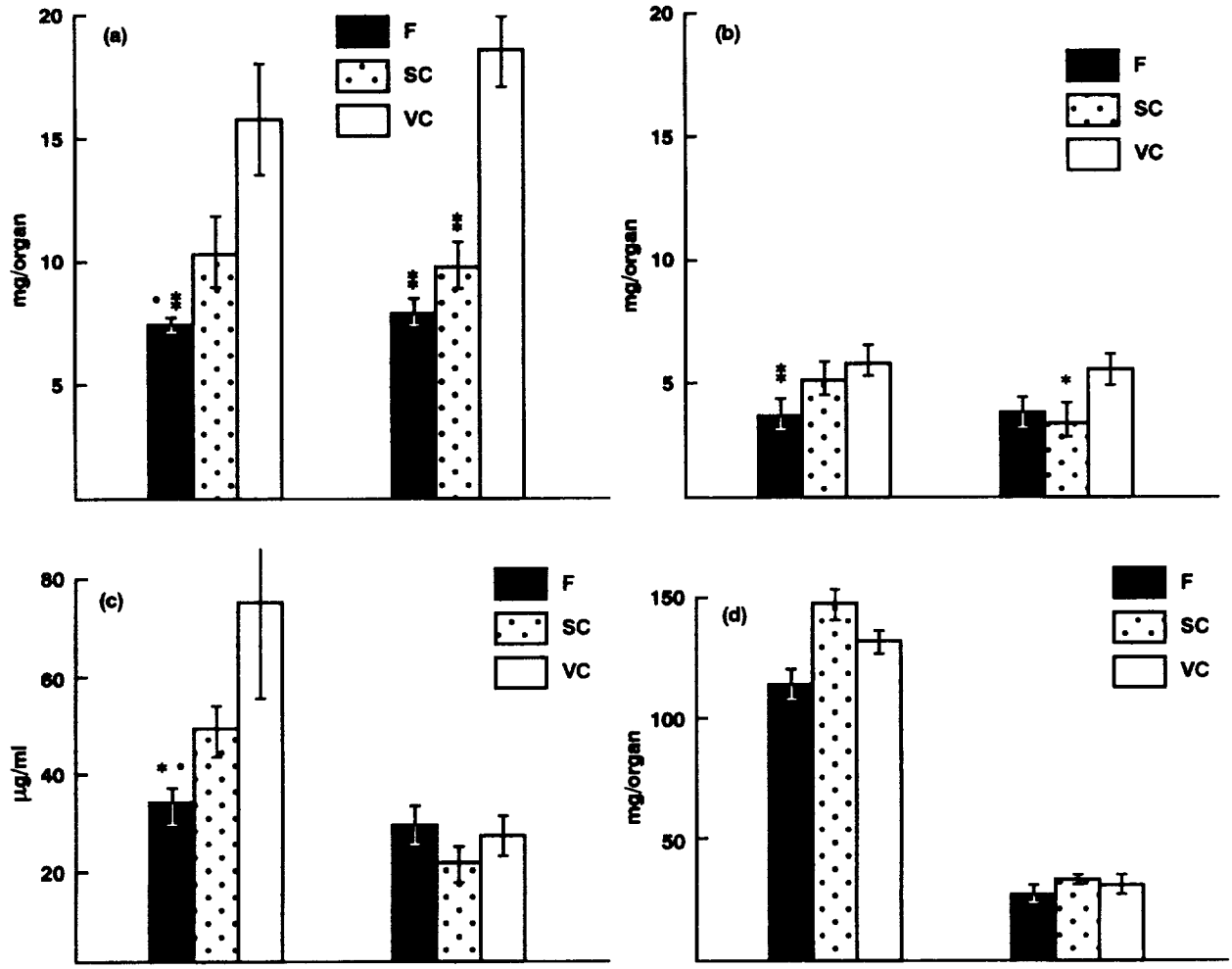


Figure 11. Lipid metabolism in female rats: nonesterified fatty acids in white (A) and brown (B) fat; triglycerides in the liver (C); and triglycerides (D) and phospholipids (E) in the thymus.



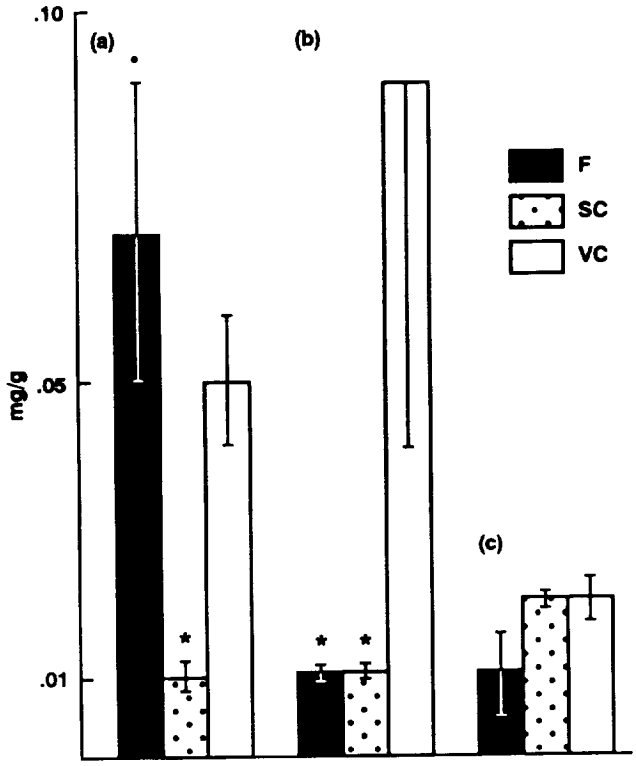
Asterisks and dots indicate a significant difference between flight and experimental controls.

Figure 12. RNA in various organs of female rats: spleen (A), thymus (B), blood sediment (C), and liver (D).



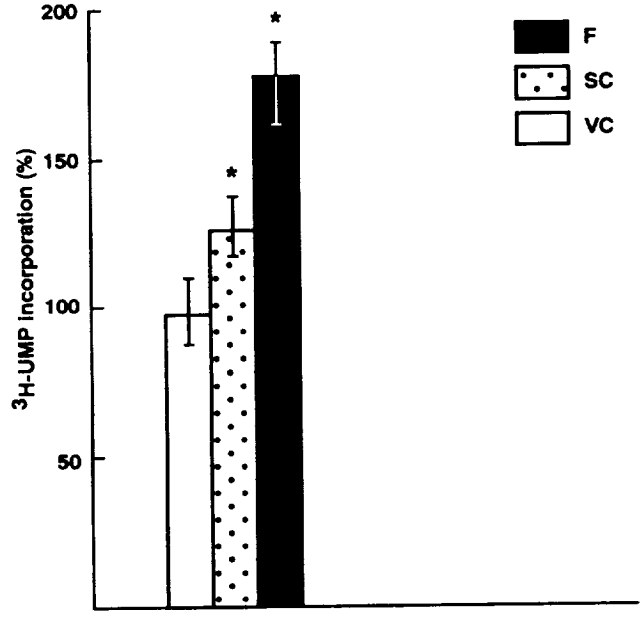
Asterisks and dots indicate a significant difference between flight and experimental controls.

Figure 13. DNA in various organs of female rats: spleen (A), thymus (B), blood sediment (C), and liver (D).



Asterisks and dots indicate a significant difference between flight and experimental controls.

Figure 14. Concentration of polydeoxyribonucleotides in various organs of female rats: spleen (A), thymus (B), and liver (C).



Asterisks indicate a significant difference between flight and experimental controls.

Figure 15. Endogenous synthesis of RNA in hepatocyte nuclei ^{3}H-UMP incorporation.

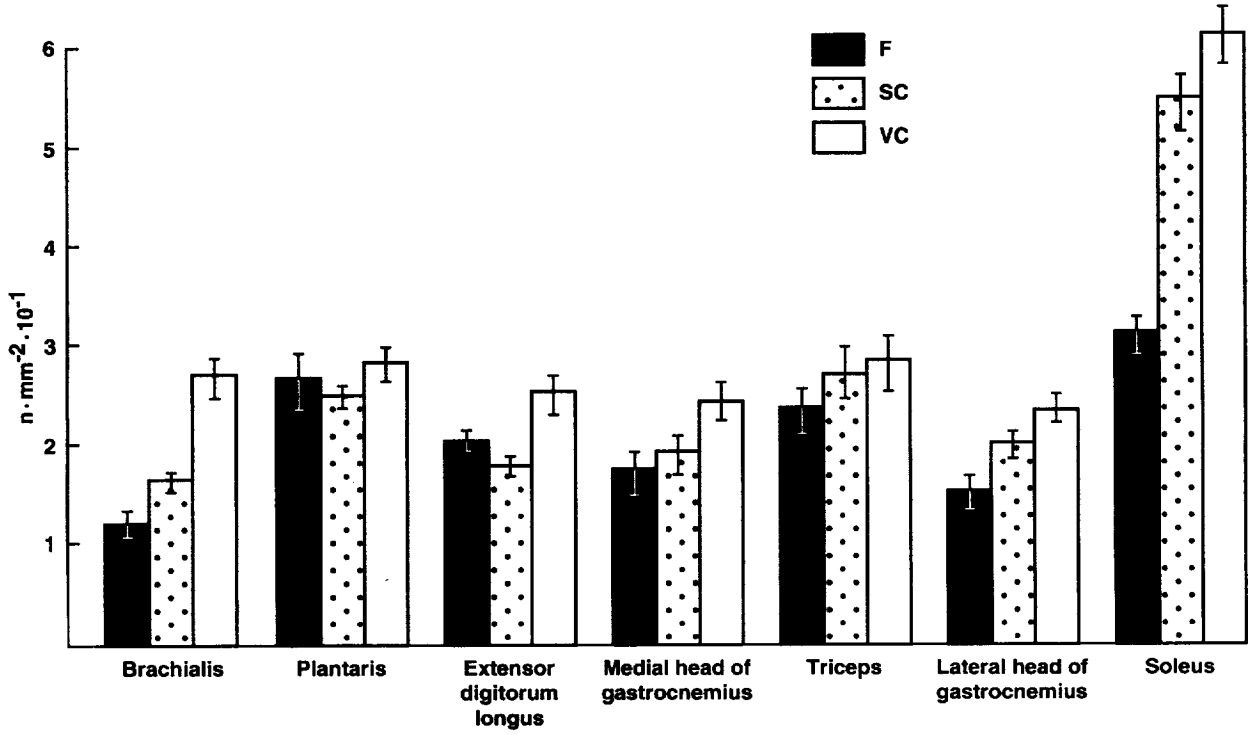


Figure 16. Changes in strength of contraction ($n \cdot mm^{-2} \cdot 10^{-1}$) of muscle fibers in female rats after space flight.

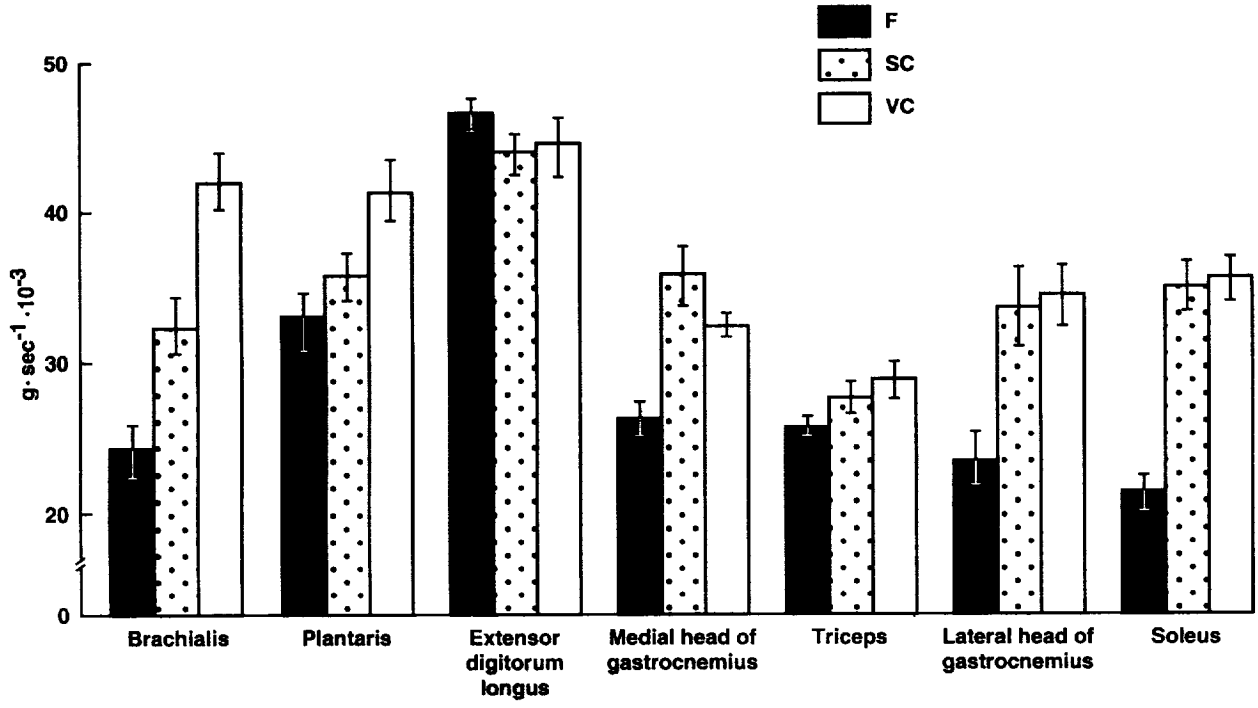


Figure 17. Change in the rate of contraction ($g\ sec^{-1} .10^{-3}$) of muscle fiber.

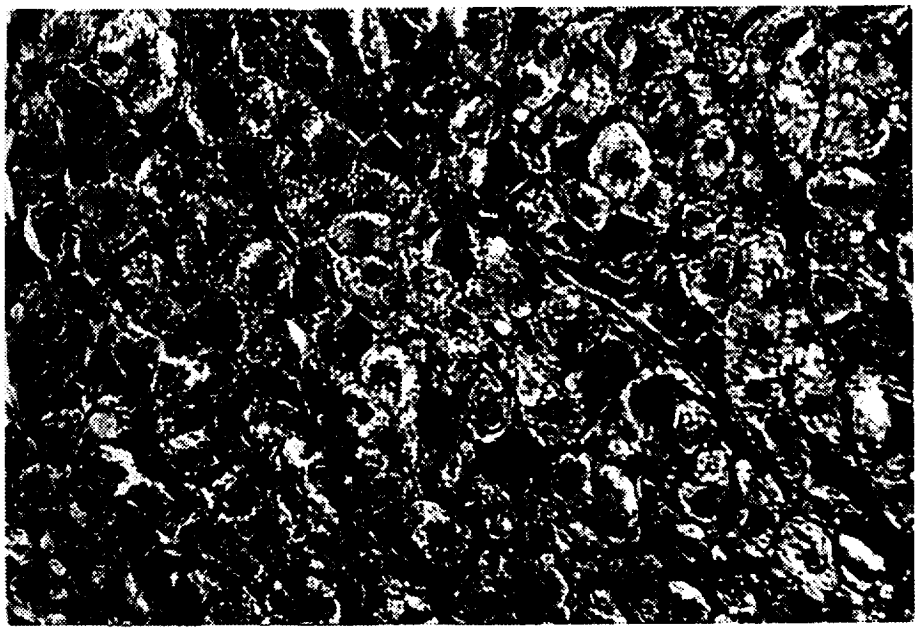


Figure 18. Fragment of the corpus luteum of a female rat of the flight group. X 350.

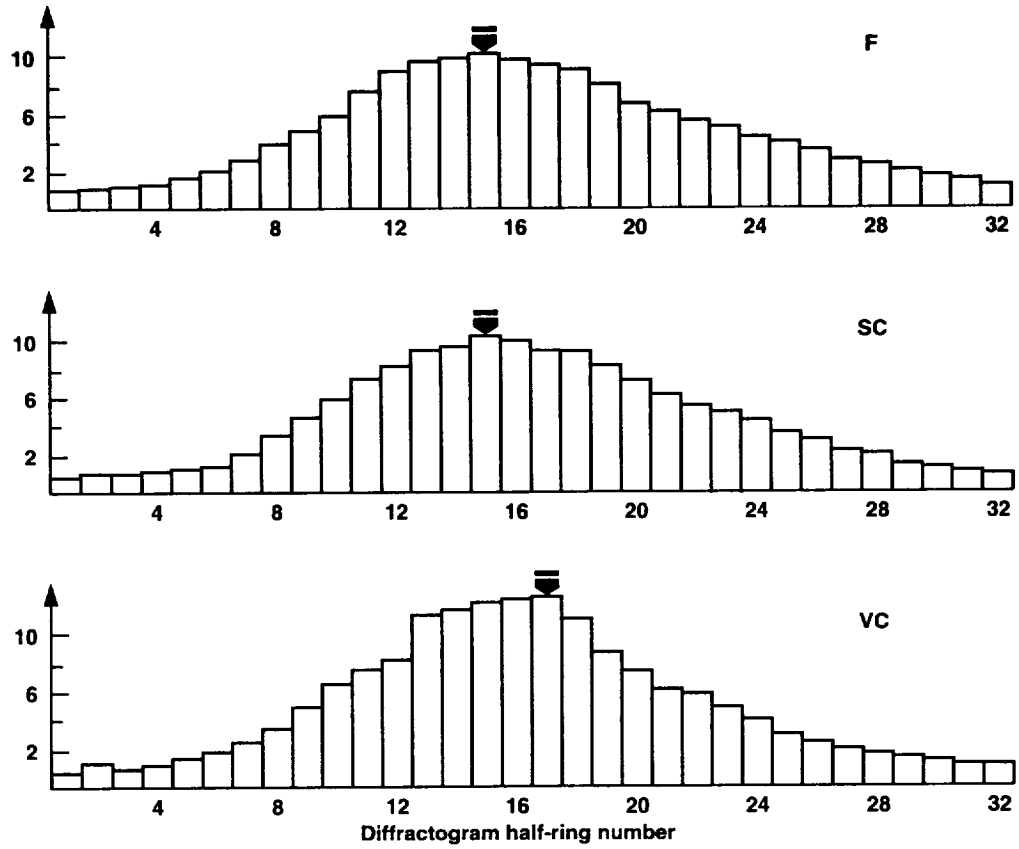


Figure 19. Radial analysis of elements of the corpus luteum. Distribution of light intensity among half-rings.

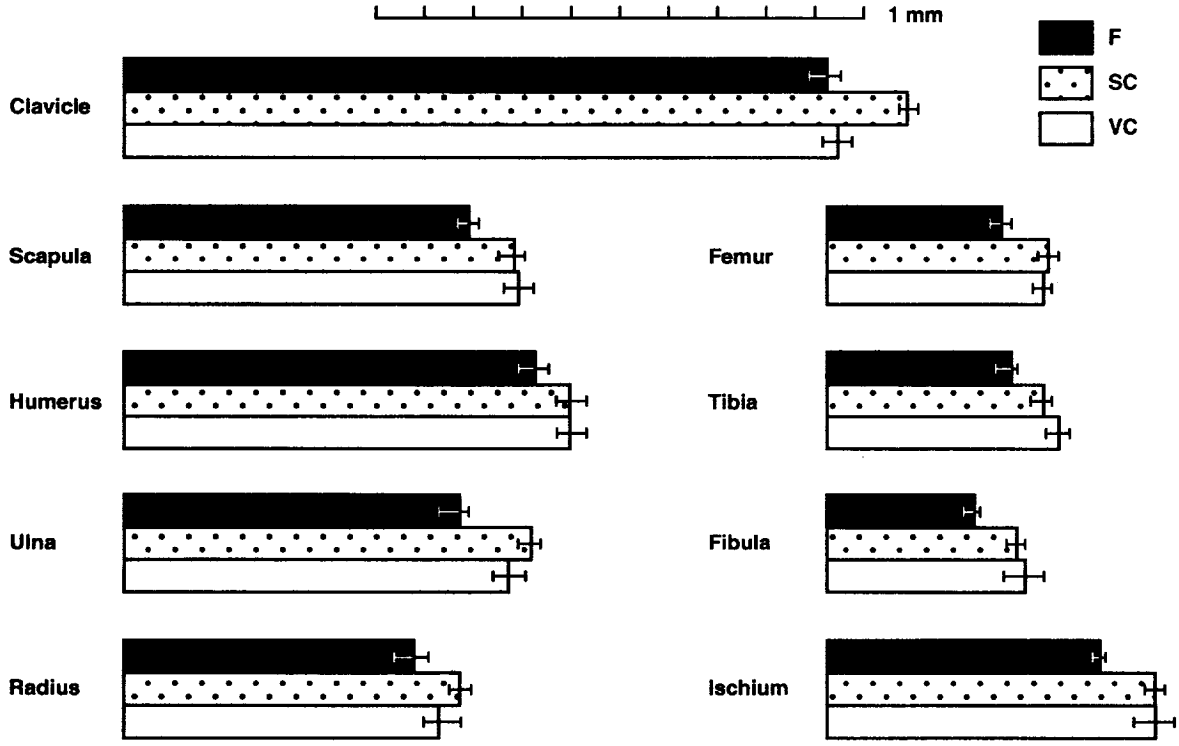


Figure 20. Size of ossification sites in the skeletons of 18-day-old fetuses.

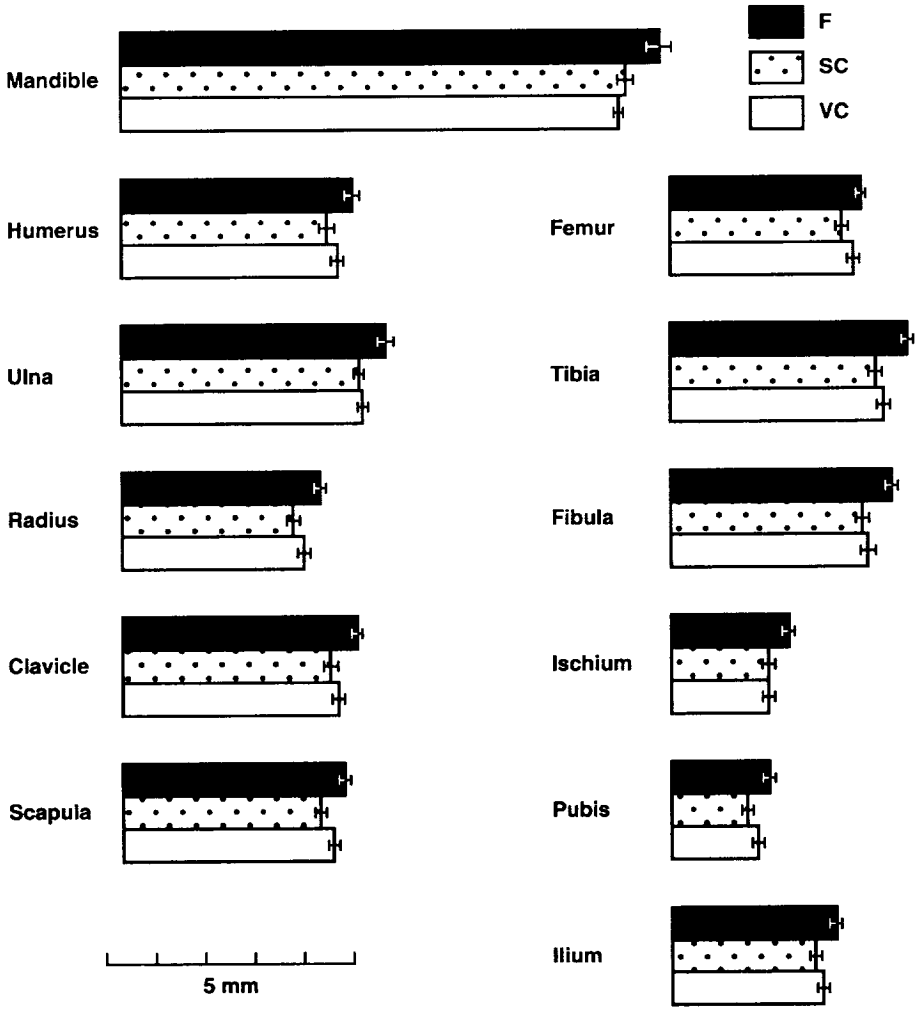


Figure 21. Size of ossification sites in the skeletons of neonate rats.

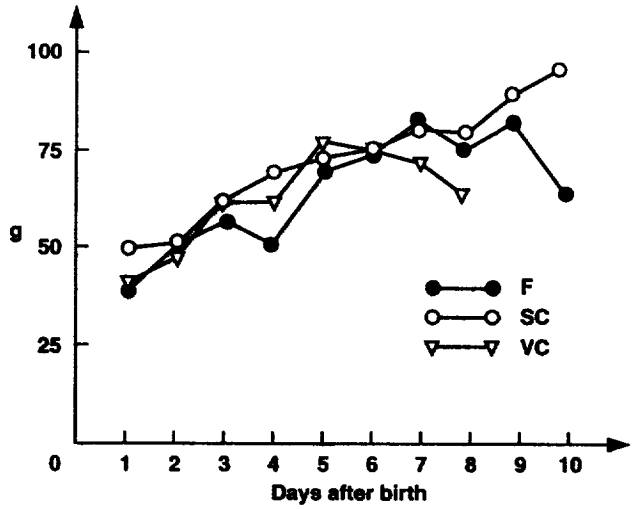


Figure 22. Food consumption by female rats during the initial nursing period.

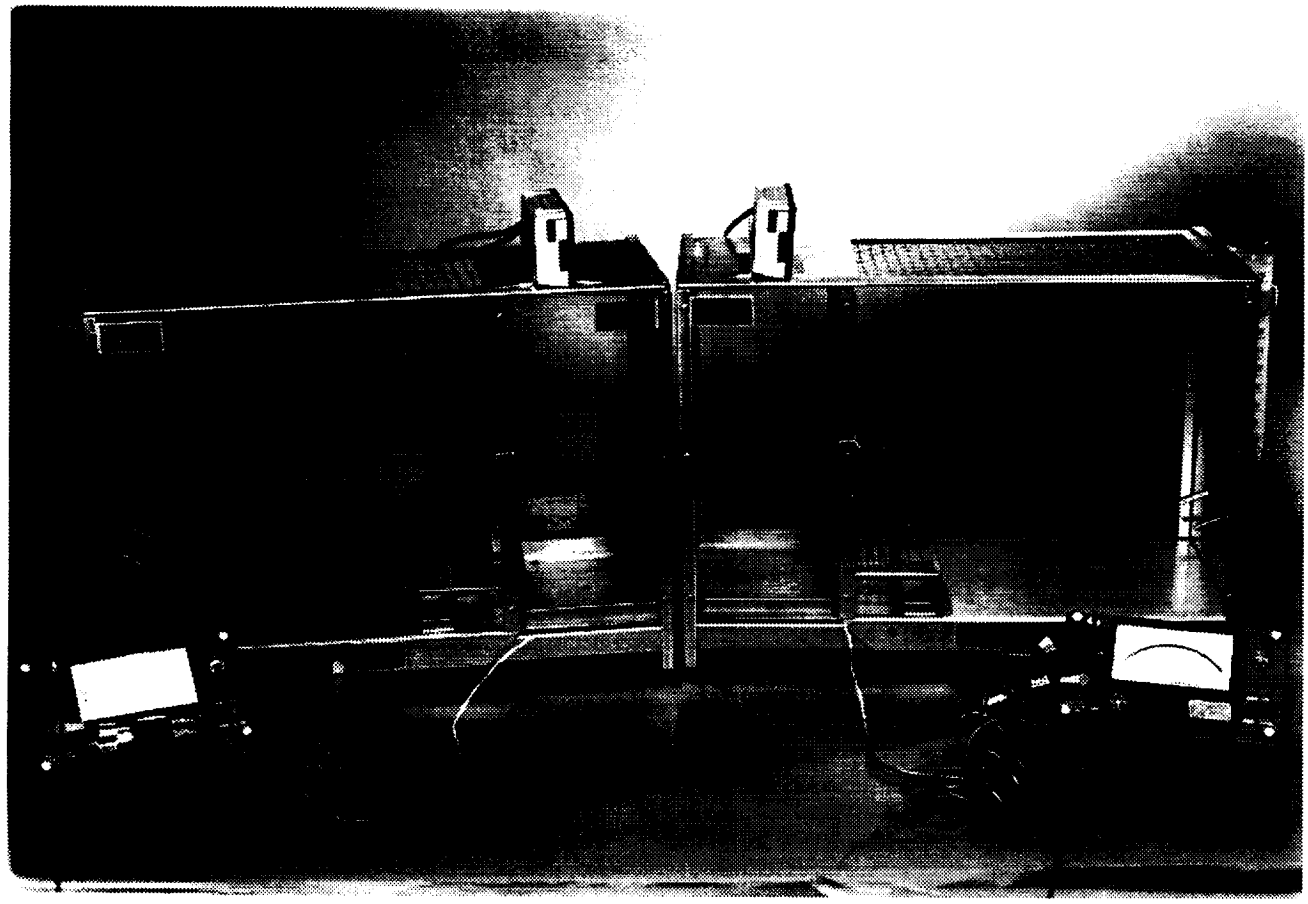


Figure 23. Cages for the study of maternal behavior in rats.

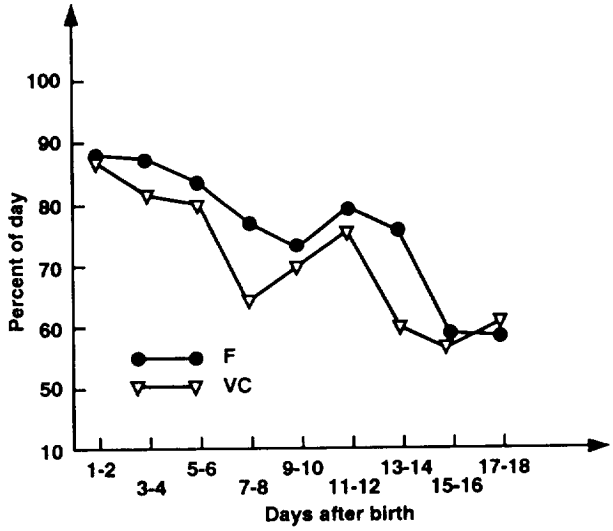


Figure 24. Portion of the day spent by mother rats in the nursery during the nursing period.

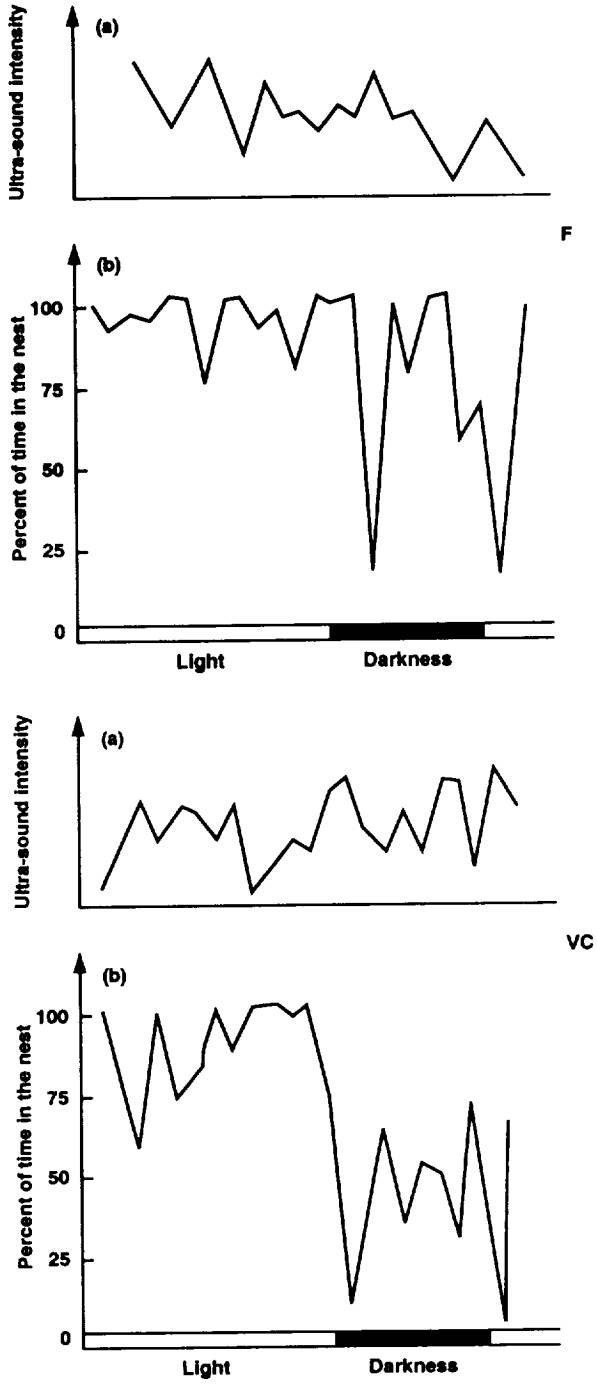


Figure 25. Day 6 of the postnatal period. Reactions of mother rats to sound made by the pups (A) and time spent in the nursery (B) in the flight and control groups.

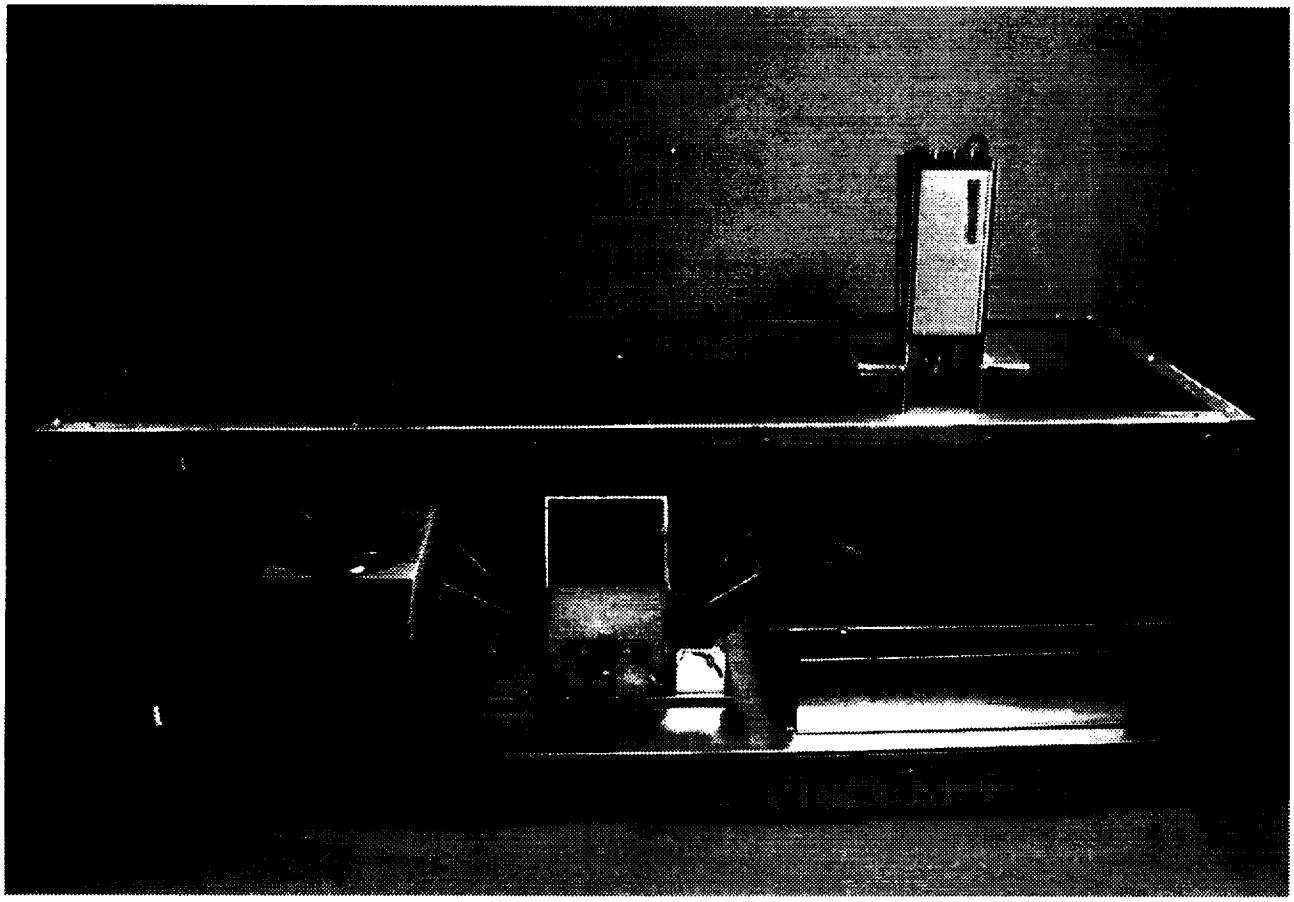


Figure 26. Incubator for work with neonate rats.

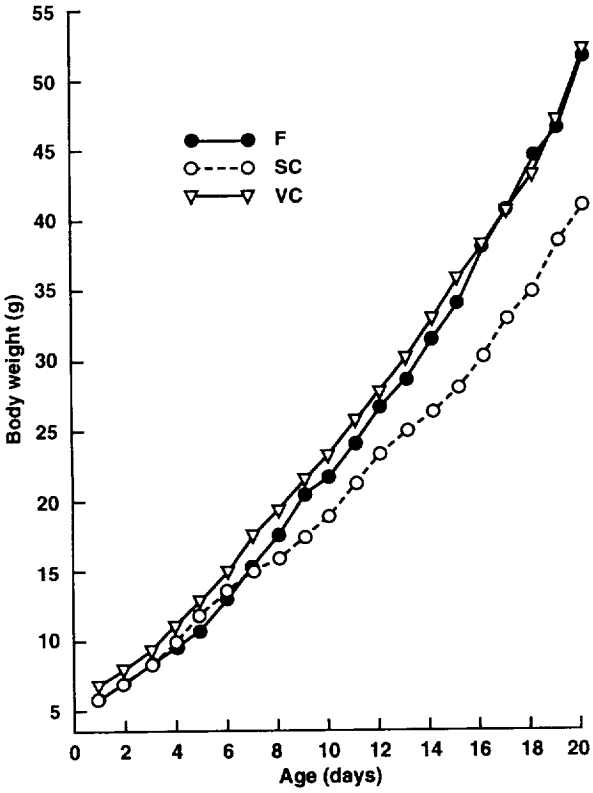


Figure 27. Body weight gain of pups during the first days of life.

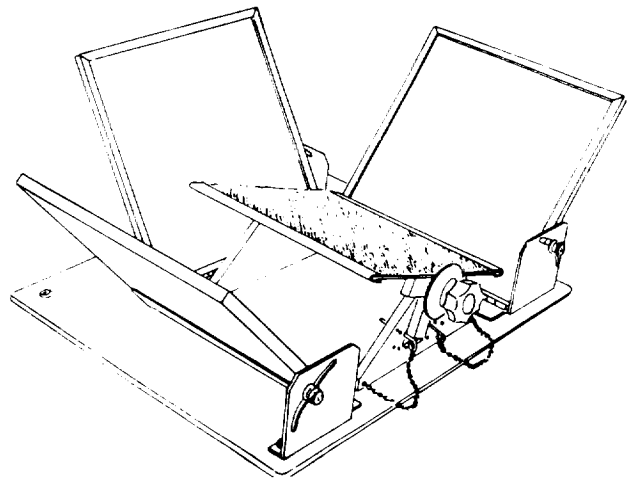


Figure 28. Platform for measuring motor activity of pups.

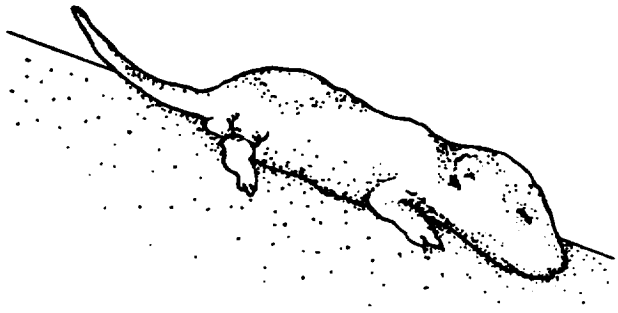


Figure 29. Position of a pup during a head-down tilt test.

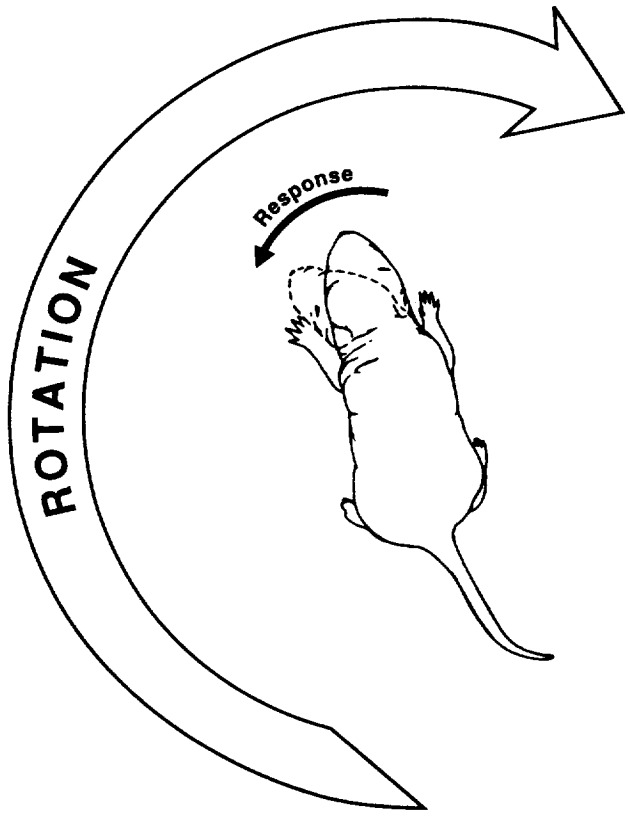


Figure 30. Position of the pup during a rotating platform test.

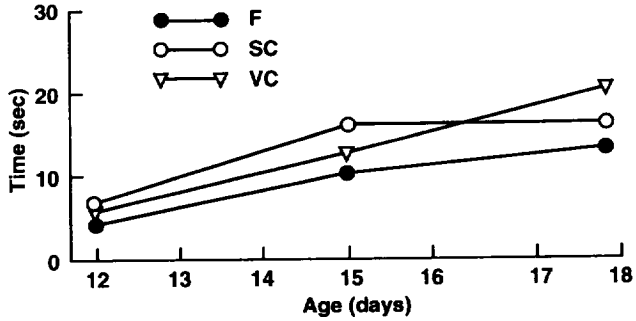


Figure 31. Time intervals during which pups were able to hold on to a cross bar.

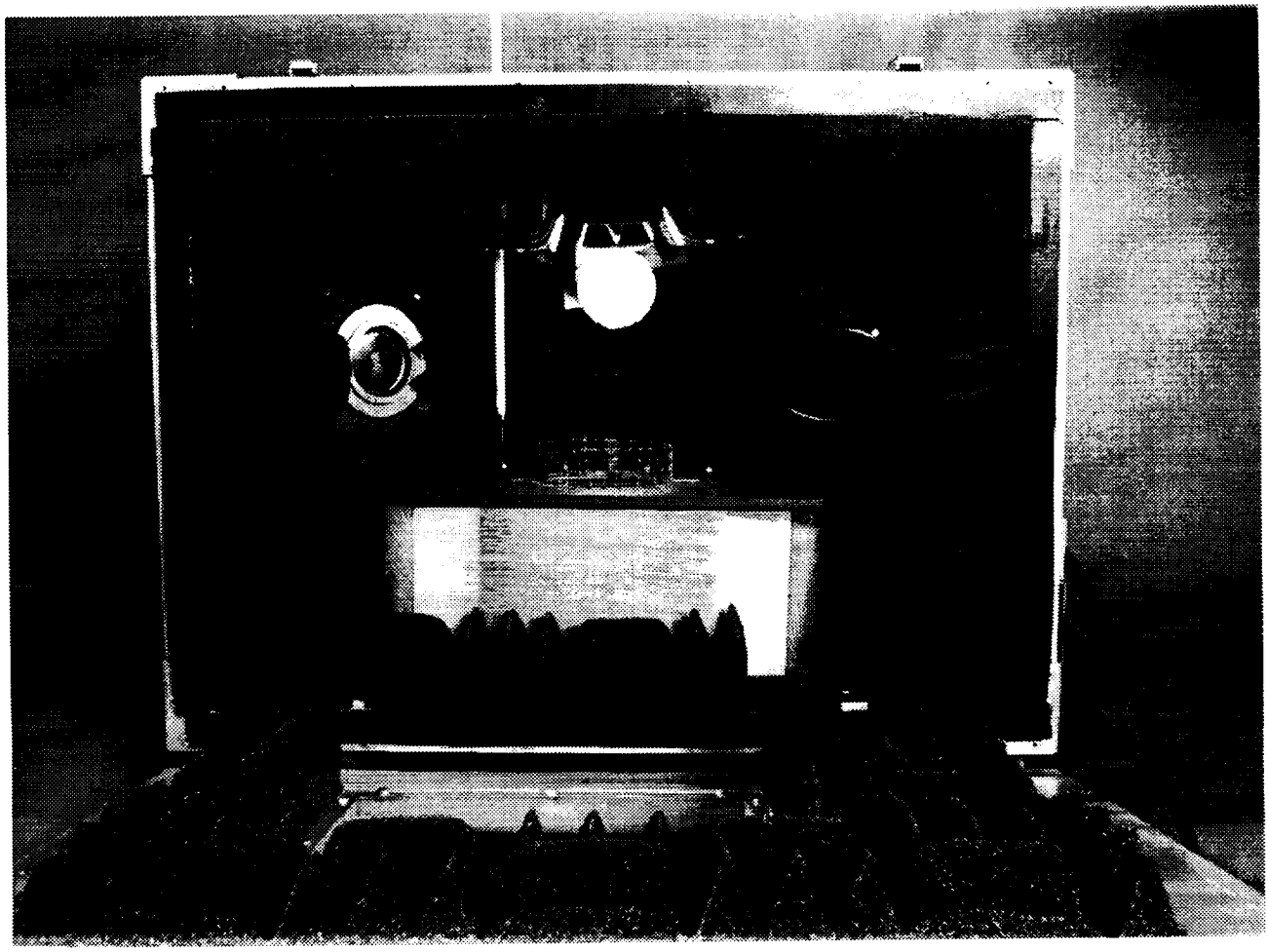


Figure 32. Chamber for studying hearing parameters of neonate rats.

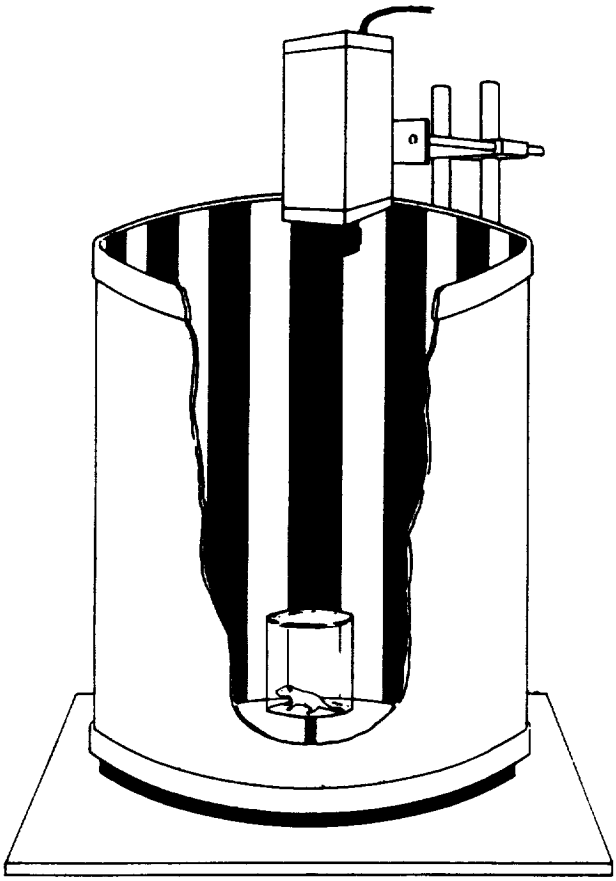


Figure 33. Apparatus for studying the vision of neonate rats.

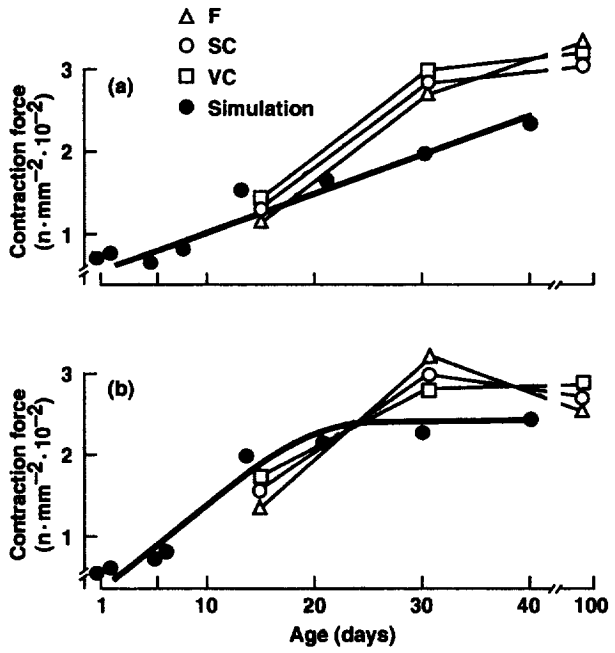


Figure 34. Time course variations in contraction force ($n \cdot mm^{-2} \cdot 10^{-2}$) of myofibers of the offspring of female rats exposed to space or to ground-based simulation experiments. A - soleus muscle; B - extensor digitorum longus.

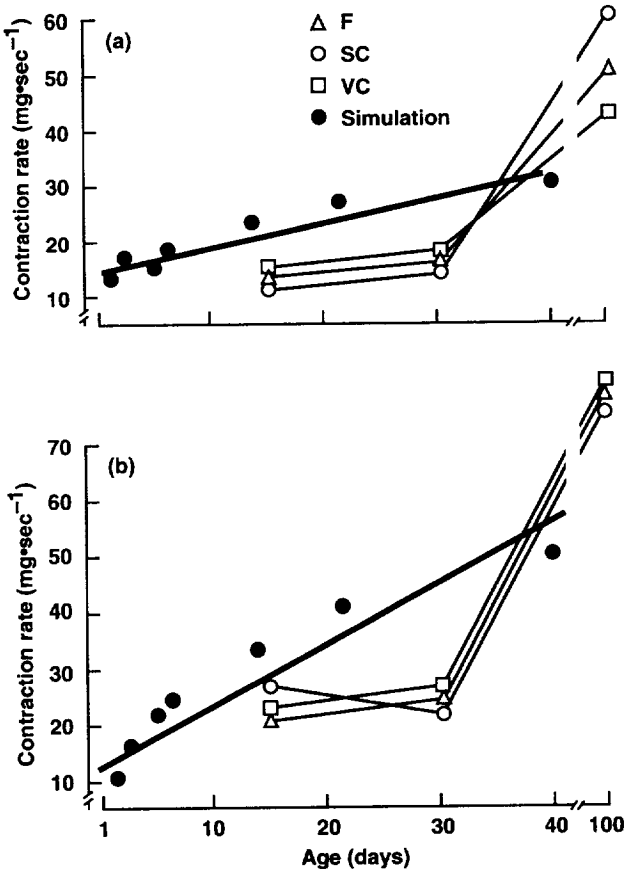


Figure 35. Time course variations in contraction rate (mg·sec^{-1}) of myofibers of the offspring of female rats exposed to space or to ground-based simulation experiments (for key: see fig. 34).

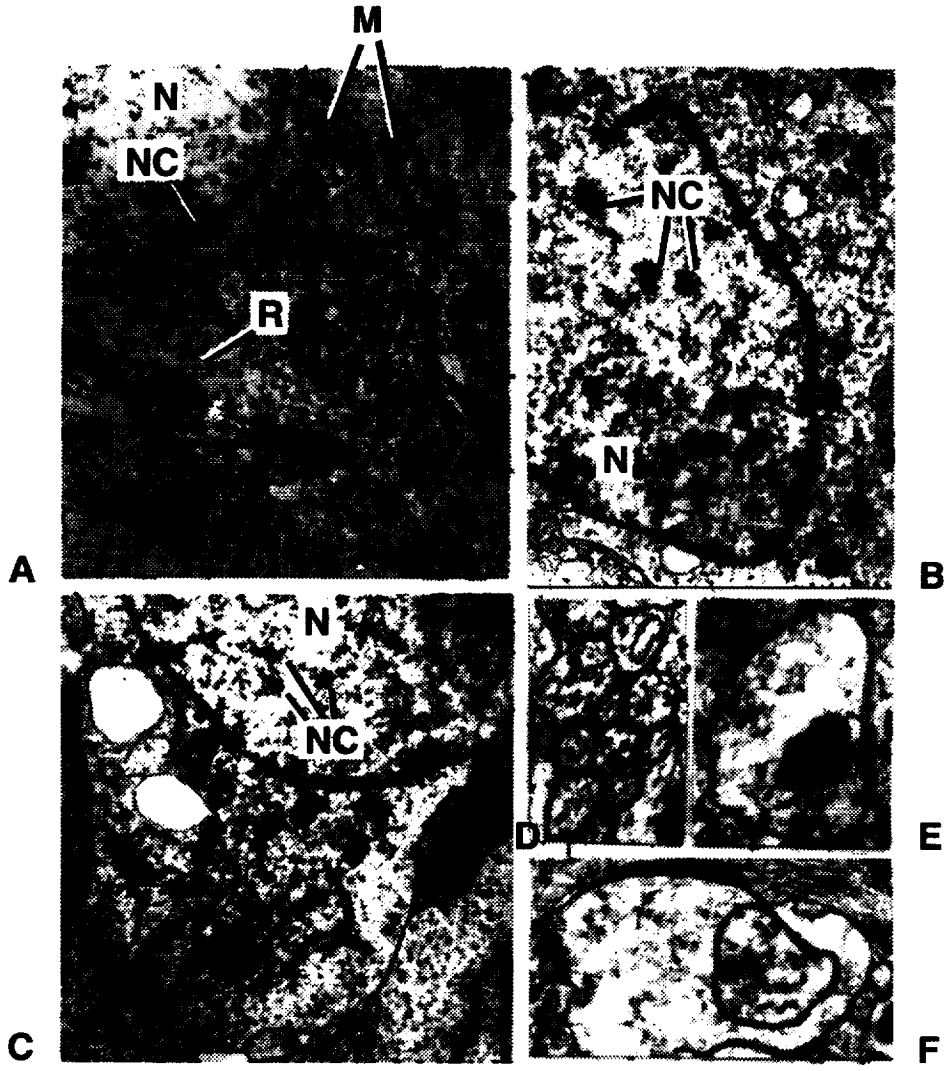


Figure 36. Ultrastucture of bodies and axonal terminals of neurosecretory neurons of supraoptic nuclei of the hypothalamus of 18-day-old rat fetuses. Neurosecretory neurons of supraoptic nuclei. A - vivarium control X 9,000; B - flight group, X 9,000; C -synchronous group, X 11,000; D - axons of neurosecretory cells in the posterior pituitary, flight group, X 24,000; E, F - axonal terminals of neurosecretory neuron of the supraoptic nucleus in the posterior pituitary, flight group, X 24,000; N - nucleus of neurosecretory neuron; NC - nuclear chromatin; M - mitochondria, R - ribosomes.

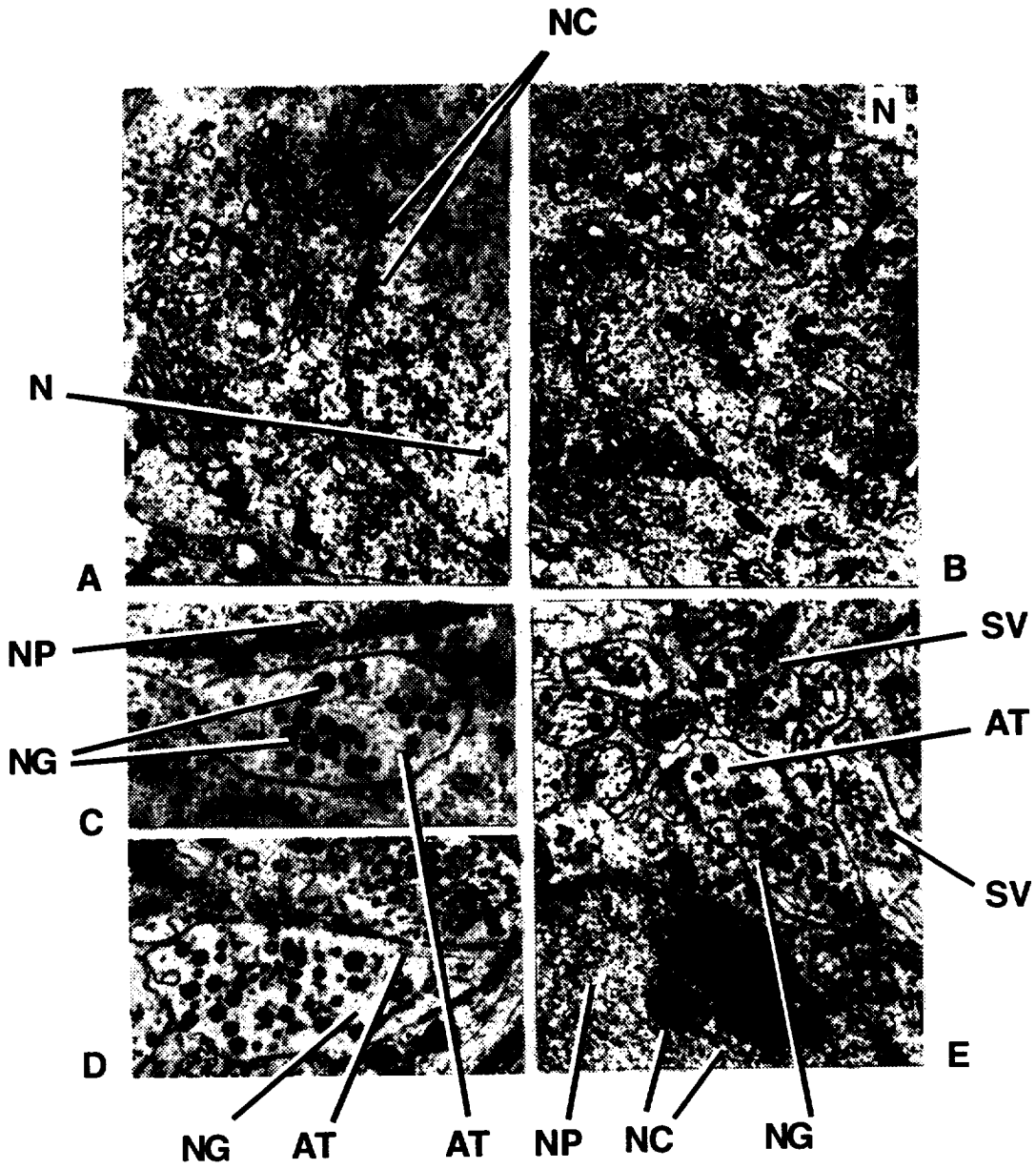


Figure 37. Ultrastructure of bodies and axonal terminals of neurosecretory neurons of supraoptic nuclei of the hypothalamus of neonate rats. Neurosecretory neurons of supraoptic nuclei. A - vivarium control, X 12,000; B - flight group, X 9,000; Axonal terminal of the neurosecretory nuclei in the posterior pituitary: C - vivarium control, X17,000, D - flight group, X16,000, E - synchronous control, X 18,000, N - nucleus of the neurosecretory neuron, NP - pituitary cell nucleus, NC - nuclear chromatin, AT - axonal terminal, SV - synaptic vesicles, NG - neurosecretory granules.

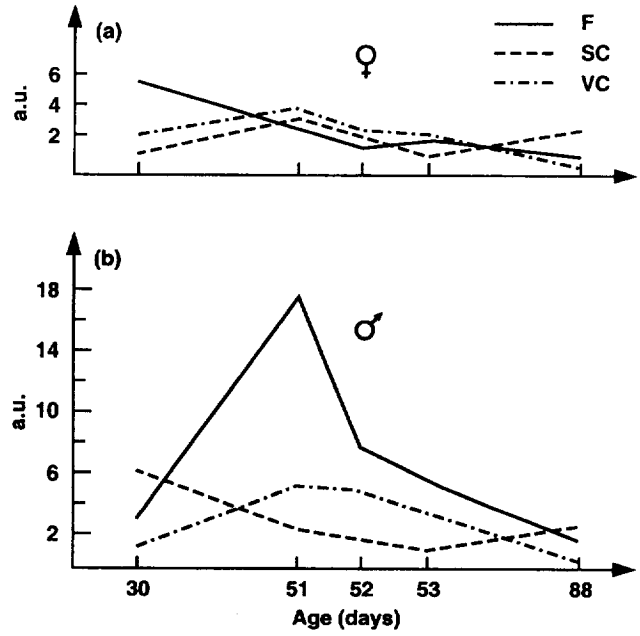


Figure 38. Amount of excrement in females (A) and males (B) in an "open field."

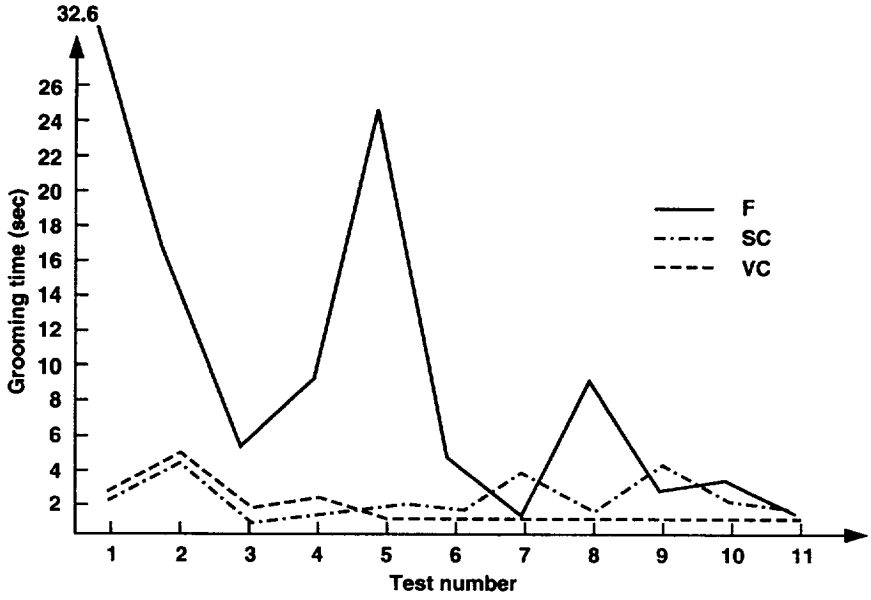


Figure 39. Grooming time spent by animals while running a Dombrovskaya maze.

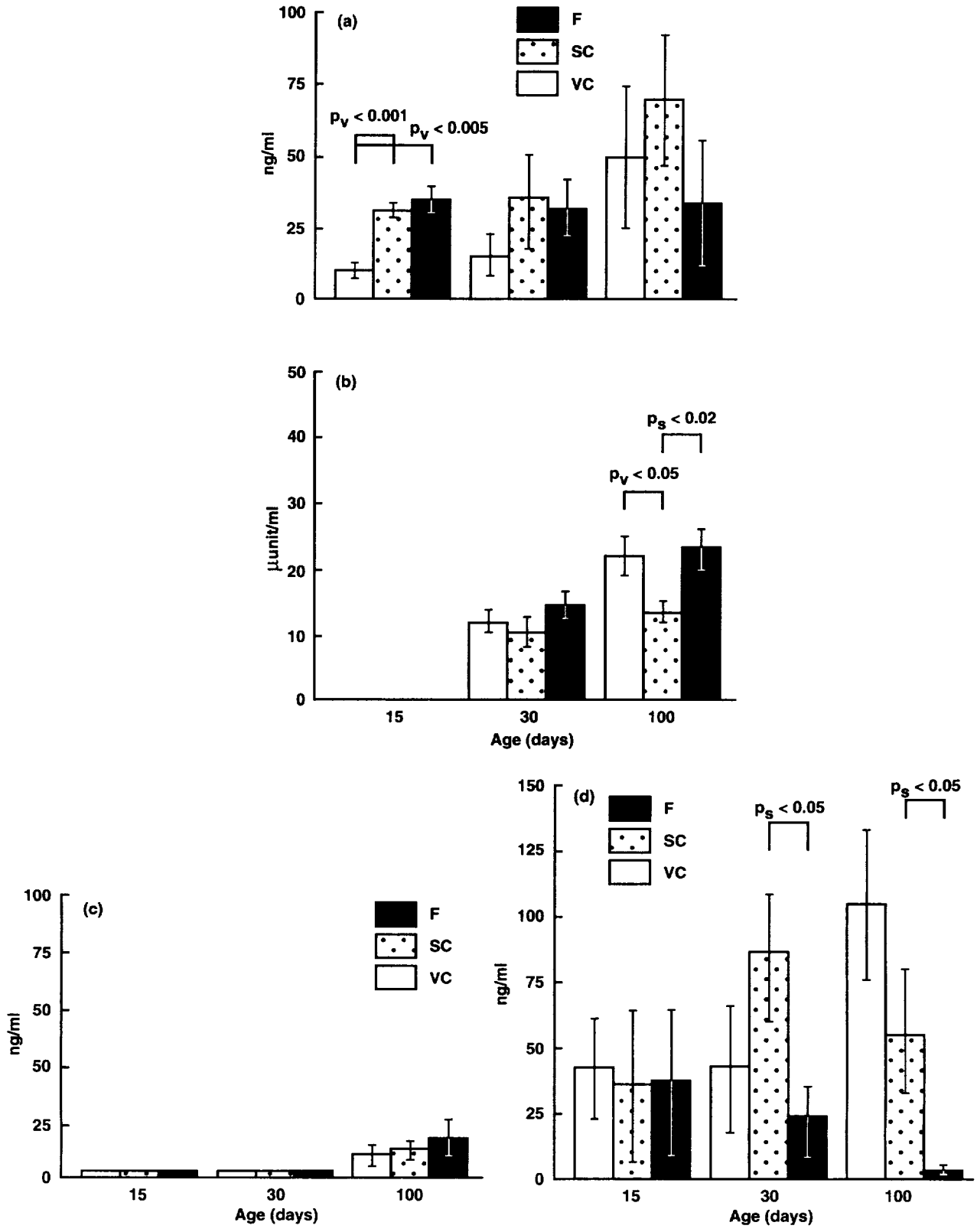
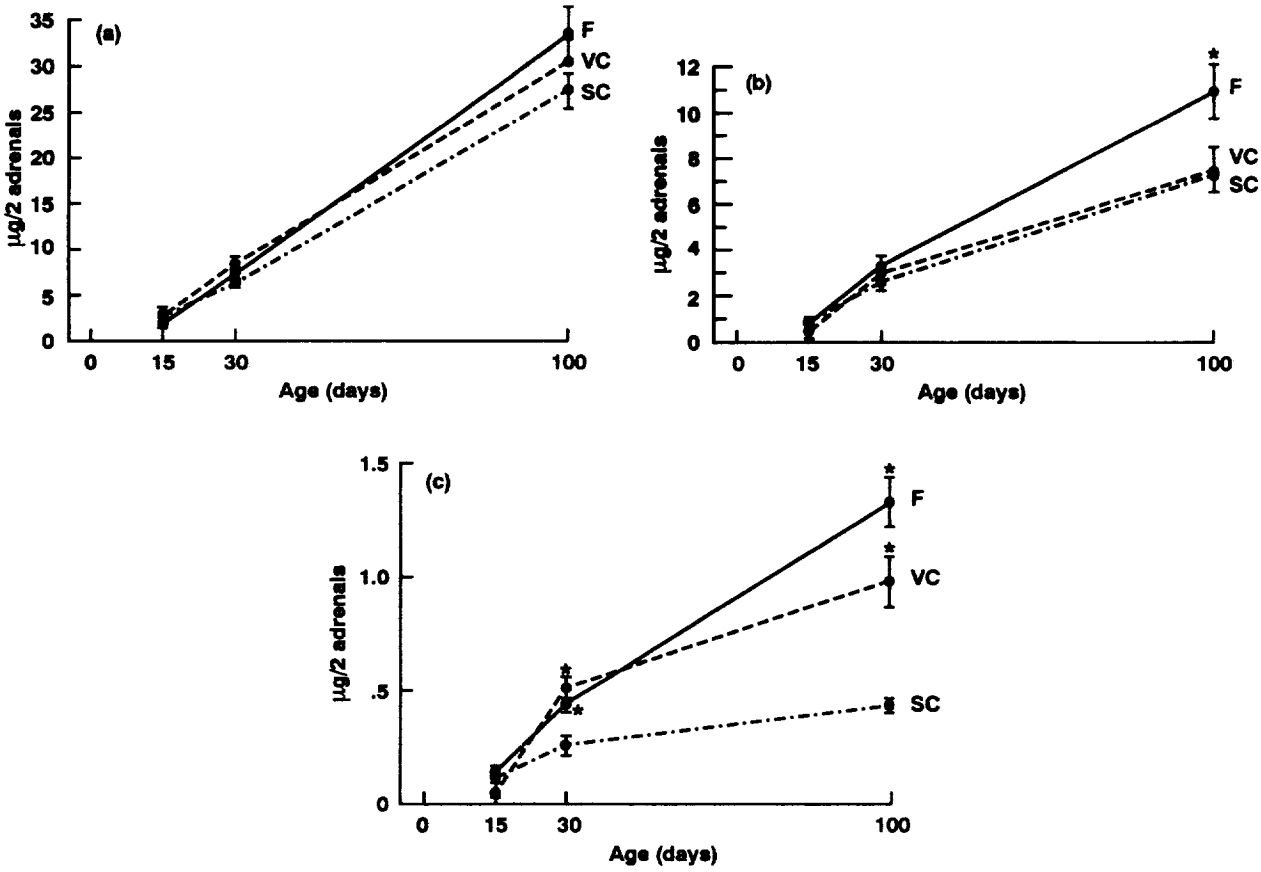


Figure 40. Postnatal ontogenesis. Concentrations of corticosterone (A), insulin (B), prolactin (C), and somatotropic hormone (D) in plasma.



Asterisks indicate a significant difference between flight and experimental controls.

Figure 41. Postnatal ontogenesis. Concentration of epinephrine (A), norepinephrine (B), and dopamine (C) in the adrenal glands.

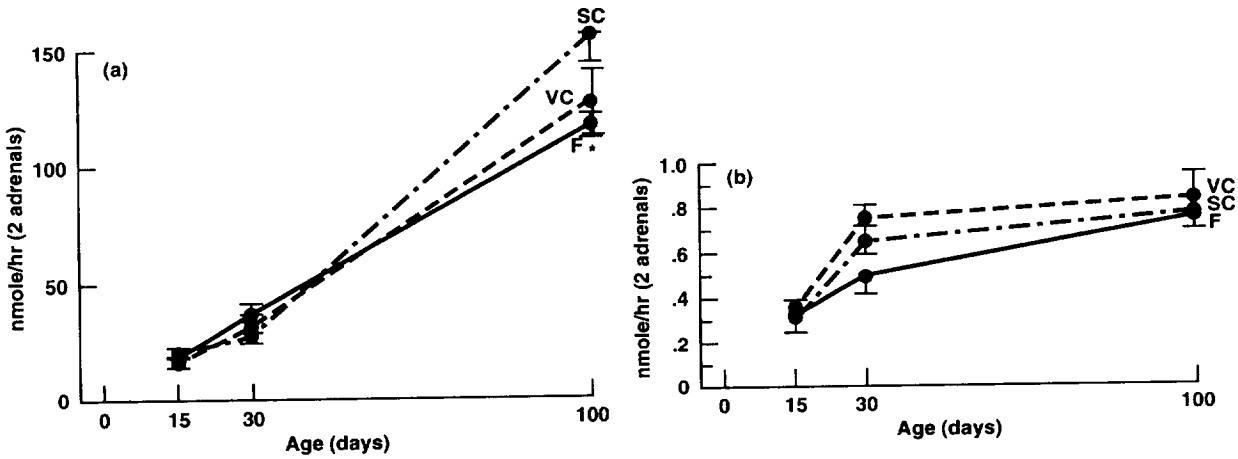


Figure 42. Postnatal ontogenesis. Activity of tyrosine-hydroxylase (A) and phenyl-ethanolamine-N-methyltransferase (B) in the adrenal glands.

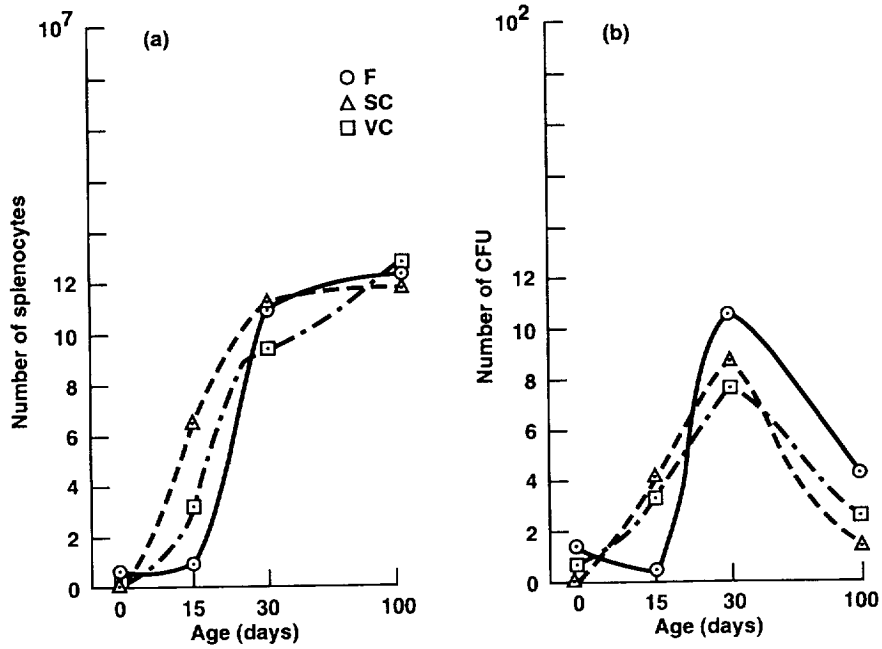


Figure 43. Postnatal ontogenesis: number of splenocytes (A) and CFUs (B) in the spleen.

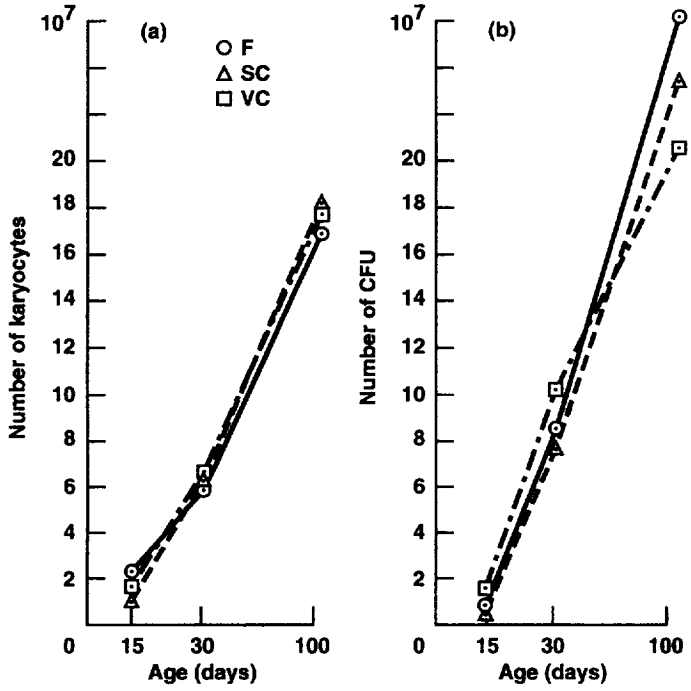


Figure 44. Postnatal ontogenesis: number of karyocytes (A) and CFUs (B) in bone marrow.

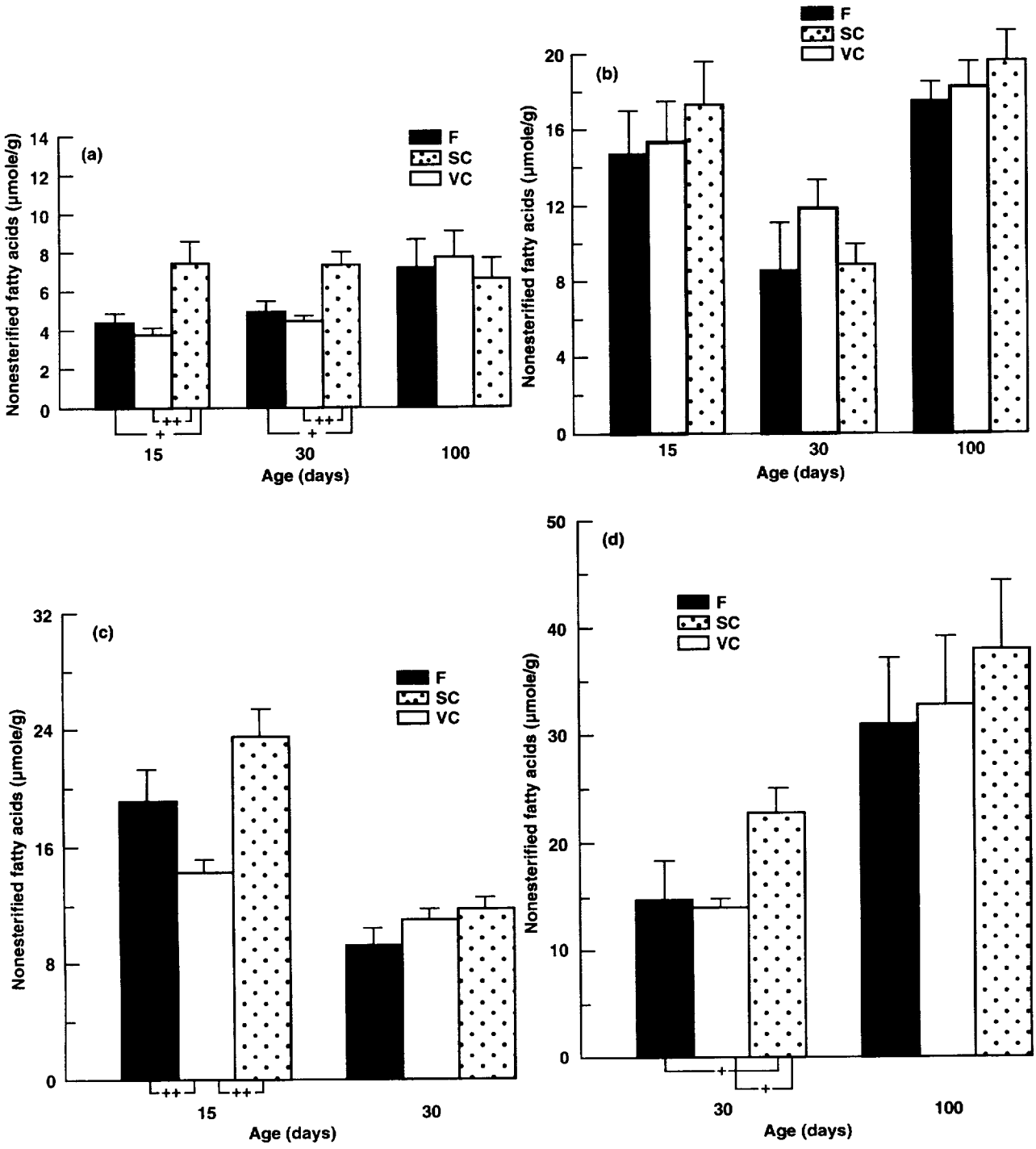


Figure 45. Postnatal ontogenesis: concentration of nonesterified fatty acids in white (A) and brown (B) fat; triglycerides in the liver (C) and thymus (D).

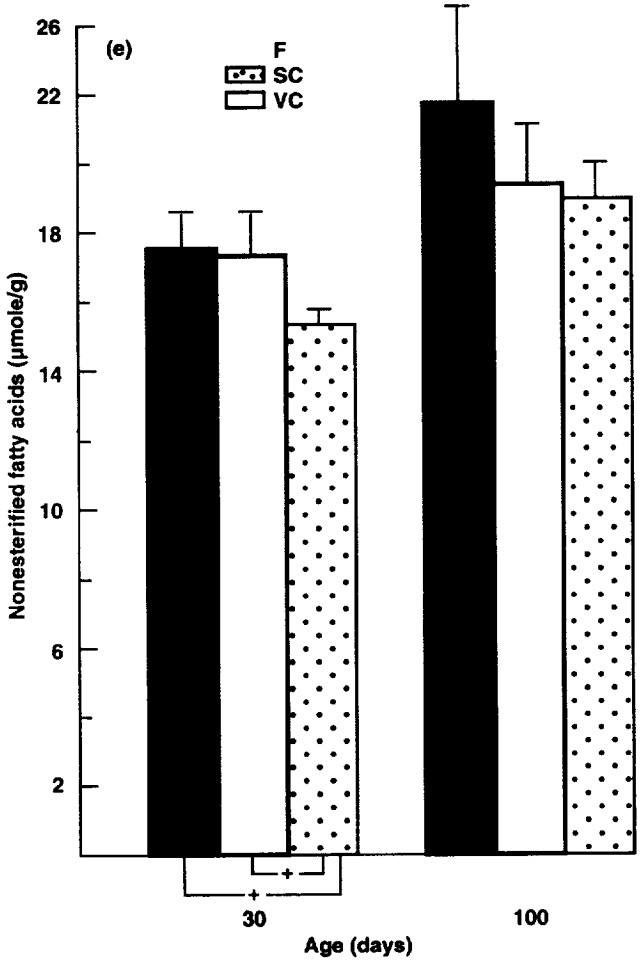


Figure 45. Concluded: phospholipids in the thymus (E).

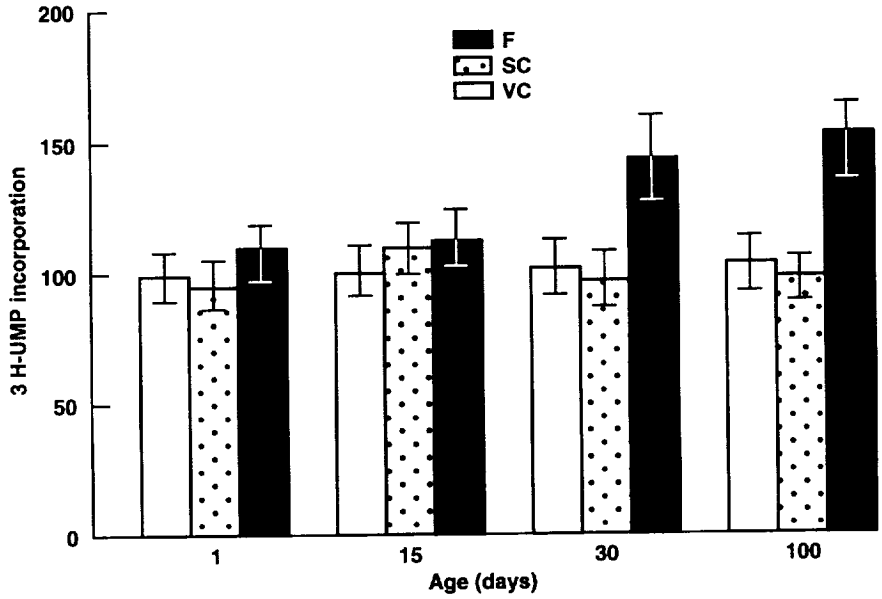


Figure 46. Postnatal ontogenesis: RNA-synthesizing activity in the liver.

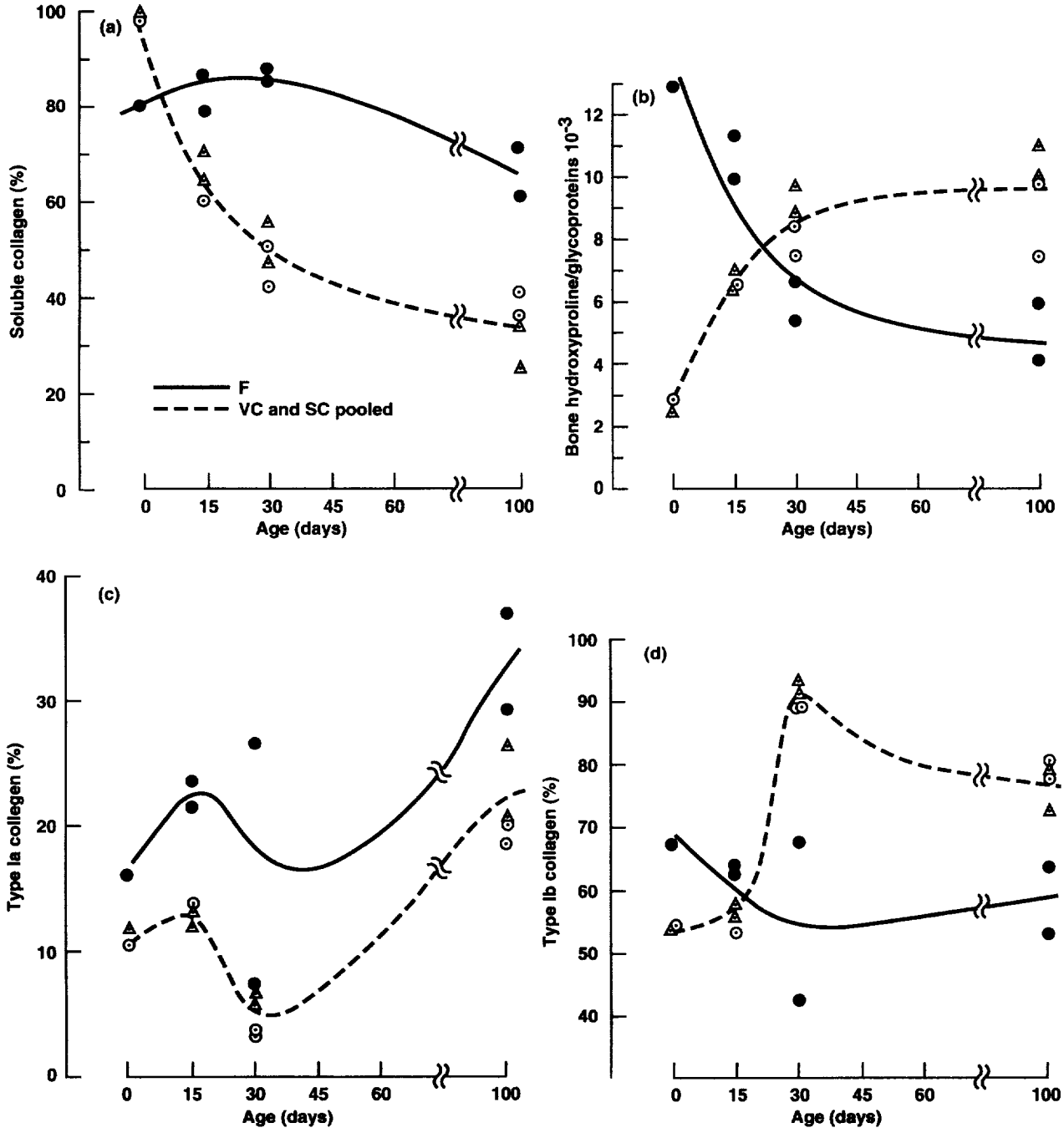


Figure 47. Postnatal ontogenesis: collagen metabolism in bone. Soluble collagen (A), ratio of hydroxyproline to glycoproteins (B), type IA collagen (C), type IB collagen (D).

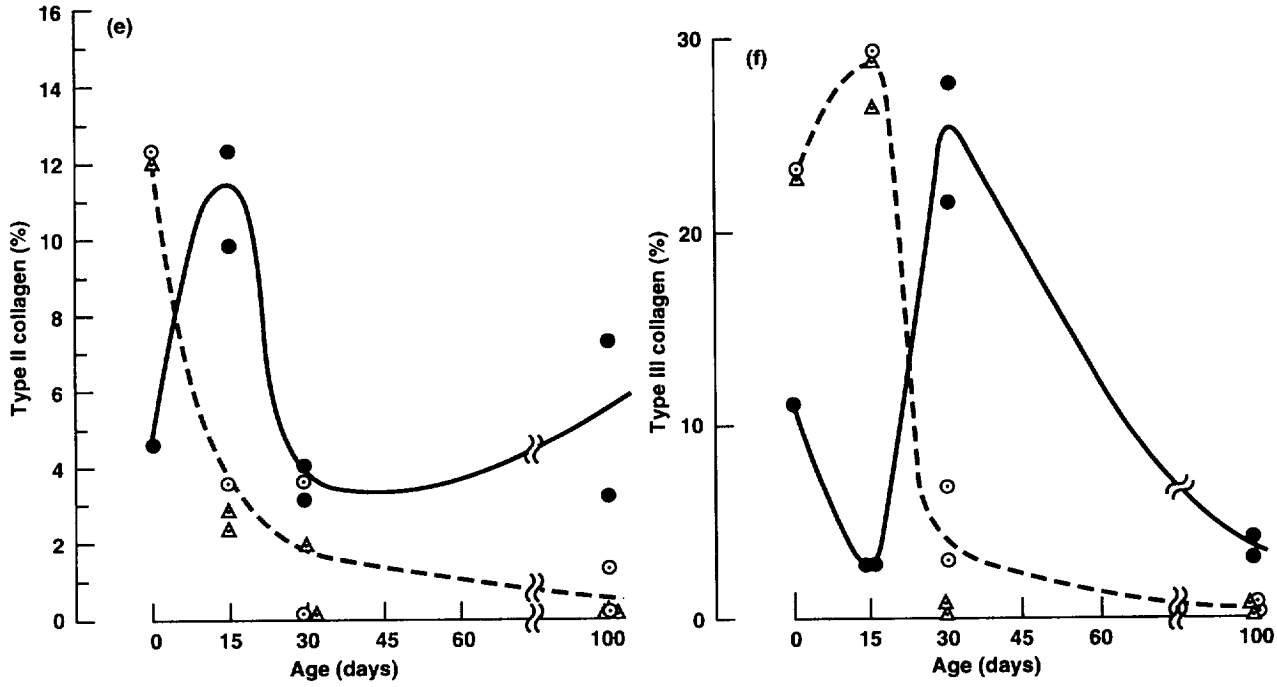


Figure 47. Concluded. type II collagen (E), type III collagen (F).

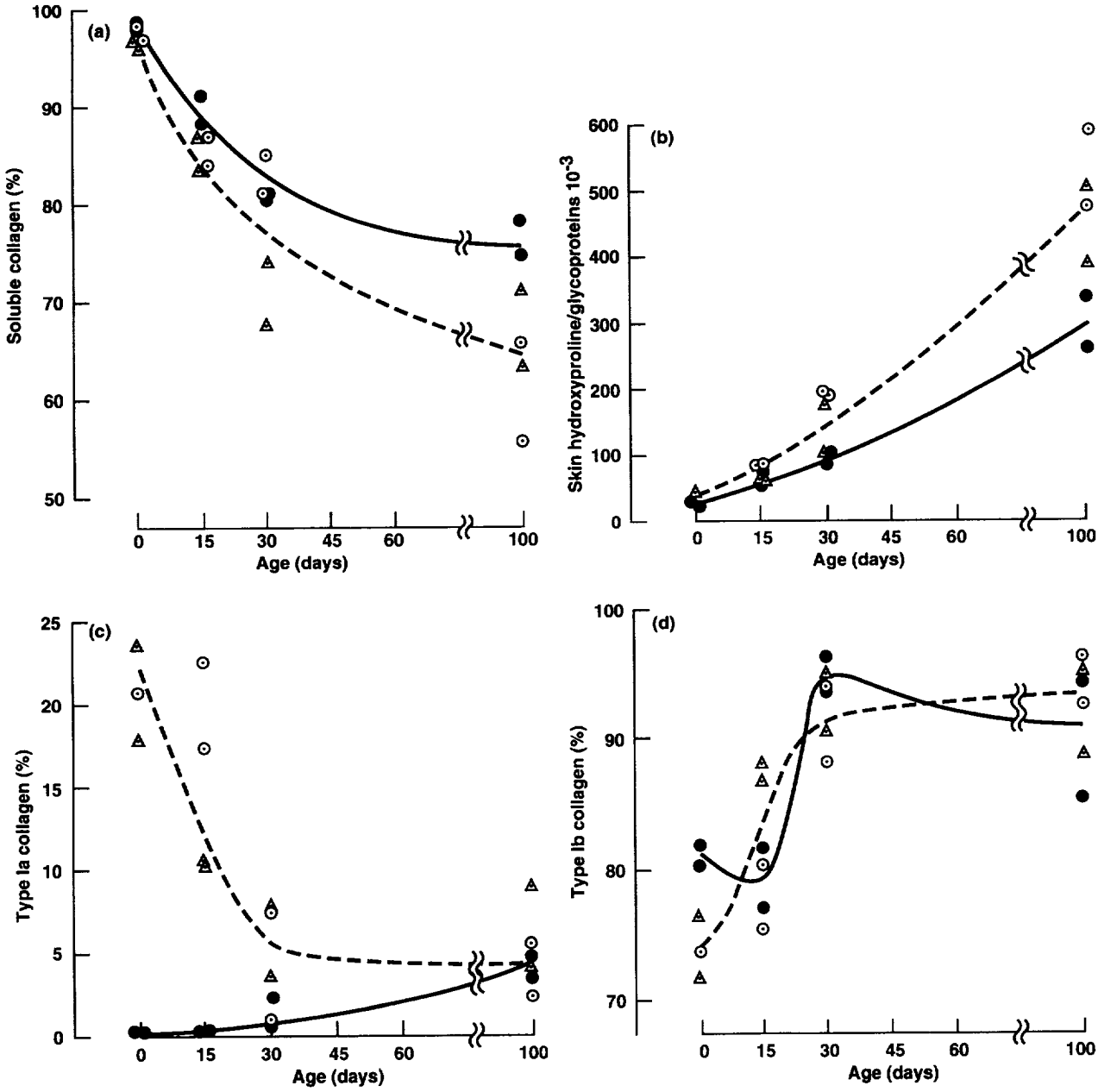


Figure 48. Postnatal ontogenesis: collagen metabolism in skin. Soluble collagen (A), ratio of hydroxyproline to glycoproteins (B), type IA collagen (C), type IB collagen (D).

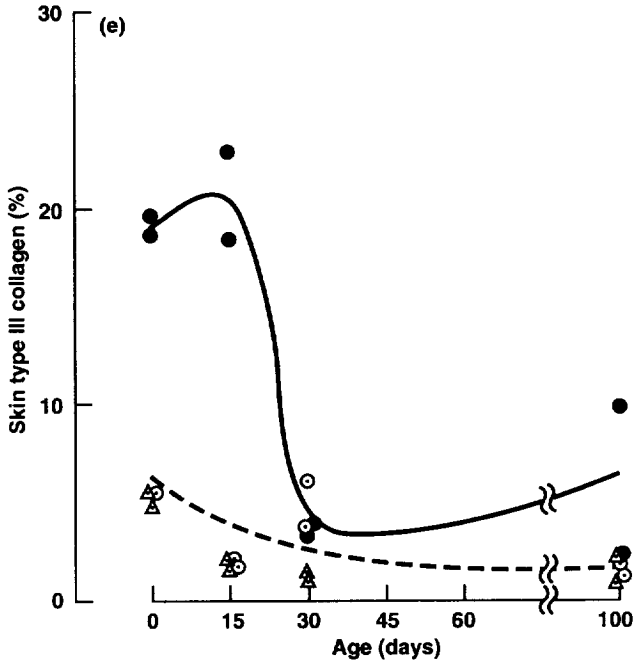


Figure 48. Concluded. type III collagen (E).

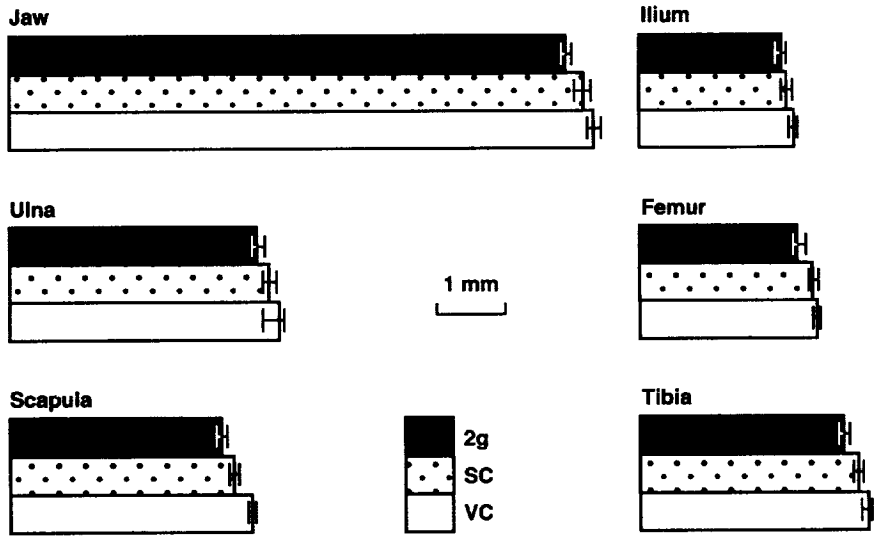


Figure 49. Size of ossification sites in 21-day fetuses that developed at 2 g from day 7 through day 21 of the prenatal period.

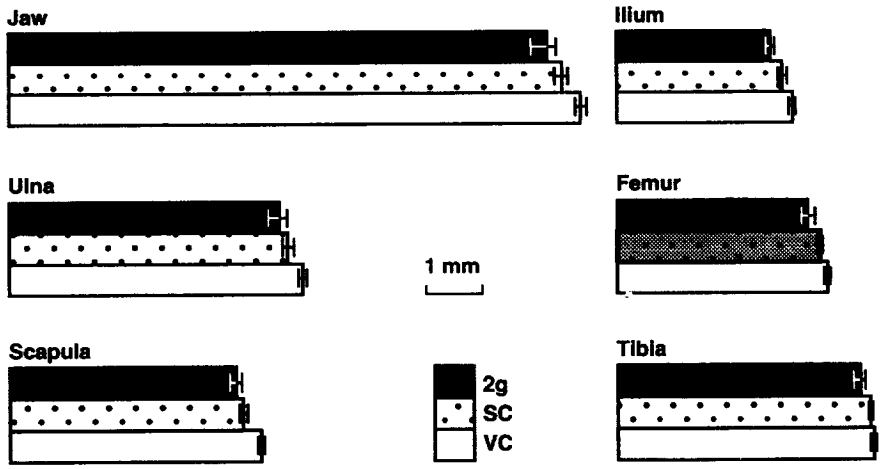


Figure 50. Size of ossification sites in newborns that developed at 2 g from prenatal day 7 through birth day.

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13. ABSTRACT *(Maximum 200 words)*

This report is an English translation of a Russian report, *Ontogenez Mlekopitayuschikh v Nevesomosti* (Moscow: Nauka Press, 1988), prepared by a group of authors from the USSR, Bulgaria, Hungary, the GDR, Poland, Czechoslovakia, France, and the USA. It presents results of the first microgravity experiment on mammalian embryology performed during the flight of the biosatellite Cosmos-1514 and in ground-based simulation studies. An overview is provided of the data available about the role of gravity in animal growth and development, and future studies into this problem are discussed. A new introduction has been provided for the English version.

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