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Effect of Decreased Gravity on Circulation in the Rat

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SUMMARY

Gravity has a profound effect on mammalian organisms. Some organs and organ systems are especially affected by gravity, for instance, bone, muscle and circulation. In a standing man, gravity induces a large hydrostatic pressure especially in blood vessels of the legs. The values of the hydrostatic pressure is about 100 mm Hg. In the prone man this pressure almost disappears. The increase in blood pressure in a standing man represents a tremendous obstacle to the flow of blood through the body. Cardiovascular mechanisms and cardiovascular adaptations developed during many millenia of man's biological history have overcome this obstacle, but in the weightless state of a space flight, these mechanisms disappear. During space flight, shifts of blood toward the chest and head were observed, and it is speculated that this overloading of the heart leads to neurohumoral stimulation and to an excessive water loss, profound blood volume decrease and other circulatory changes. After returning to earth and to 1g force, astrounauts show profound circulatory (orthostatic) intolerance and other circulatory malfunctions together with a decreased work ability lasting several days.

In order to be prepared for the time when adequate circulatory studies will be performed in man and in animals in the Space Lab IV (LSI) as well as to understand better the effect of gravity on circulatory mechanisms we have studied circulatory changes and their physiological

mechanisms in hypokinesic rats. These conditions mimic circulatory effects of weightlessness. We employed rats because direct measurements and various advanced experimental manipulations can be used, something that cannot be done in man. Most of the experimental techniques for this work have been developed in our laboratory. The cardiovascular measurements were performed in control experiments (in earth) in unanesthetized, unrestrained rats and in the same animals in hypokinetic condition (semi-restrained animals, a condition similar to bed rest in man, with the head of the animals tilted 20^{o} down to induce blood shift toward the head as observed in astronauts). To avoid the use of anesthesia, aorta and right ventricle of the heart of the animals were permanently cannulated fifteen days before the experiments and an electromagnetic blood flow probe implanted around the aorta. The implanted cannulas stay patent during the whole lifespan of the animals and are used to measure heart rate, arterial and right ventricular pressure, hematocrit ratio, blood volume and other cardiovascular parameters. The elctromagnetic flowmeter is used to measure cardiac output. The hypokenetic rat is free to move the front legs (mimicking the work of the astronauts, who use extensively their arms), but the legs are not in use (in the astronauts, the legs are "unloaded" because of the lack of gravity). Seven-day-long experiments correspond to the future seven-day-long Space Lab flights. Our experiments have shown that the

hypokinetic rat has initially an increased right atrial pressure due to the congestion of blood in the chest, a lowered atrial pressure (consequence of overstretching of the heart), an increased stroke volume and an increased cardiac output. After two days of hypokinesia, all cardiovascular parameters are back to normal except cardiac output which continues to decrease, continuously. Thus, during hypokinetic exposure, a rat behaves like an astronaut during a space flight and appears to represent an excellent model for studying the effect of space flights on circulation. After 7 days of hypokinesia, the rats were released from the harnesses and the circulatory study continued. The rats showed a decreased ability to "work" (exercise on a treadmill) due to circulatory maladjustments. Their heart rate was elevated and the cardiac output decreased during moderate exercise, while their body temperature was increased. The rats were not able to sustain strenuous exercise. The same rats during rest, on the other hand, had a tendency to develop hypothermia. The rats' reaction to noreipinephrine and to levodopa was changed. Oxygen consumption was decreased during exercise as well as during exposure to cold and epinephrine-induced increases in O_2 consumption was absent. Most of these changes were observed even 14-30 days after the hypokinetic exposure (which lasted only 7 days).

OBJECTIVES

It is known that gravity has a profound effect on mammalian organisms. The absence of gravity (weightlessness) is the unique change occurring only in space flights. The effect of decreased gravity on the cardiovascular system is especially great. In order to study the effects of altered gravity on the cardiovascular system, we used in this work white rats and adequate techniques to study cardiovascular parameters in control experiments in unrestrained, unanesthetized rats and in the same animals in hypokinetic conditions. Hypokinesia was studied because this condition mimics to a high degree the effects of weightlessness, especially the effects associated with the cardiovascular system. These studies served to develop an adequate ground based model system and to identify possible circulatory mechanisms that evolved in mammals during long lasting gravity exposure, mechanisms that are affected by hypokinesia and that are likely to be affected during exposure of organisms to weightlessness.

Some circulatory adaptations occur during exposure of man to weightlessness; shifts in blood distribution, overloading of atria, neurohumoral stimulation leading to excessive water loss, profound blood volume loss concomitant and other circulatory changes occur and have been reported in man. After returning to earth, new circulatory readaptations have been observed. The circulatory changes that occur during space flights lead to orthostatic

intolerance and a decreased work ability after returning to earth.

It has been reported that hypokinesia and bed rest induce cardiovascular changes similar to those observed in space flights. Using an animal model that was developed in our laboratory and that was extensively studied and characterized in our laboratory and other laboratories in this country and abroad, we have studied circulatory mechanisms that occur during exposure to hypokinesia (with or without negative tilt) as well as during readaptation to control conditions (free activity). We believe that this study contributes to better understanding of mammalian circulatory mechanisms that operate under 1-g force and will serve to provide control data to be compared with cardiovascular data obtained in conditions of a Space lab. Because surgery and anesthesia drastically decrease cardiac output and other circulatory parameters in rats (Popovic and Kent, 1964; Popovic, Popovic, Schafer, and McKinney, 1977), only unanesthetized rats were used in experiments. Aorta and right atrium of the animals were permanently cannulated fifteen to twenty days before experiments. Arterial and right ventricular blood pressures, cardiac output, cerebral and other regional blood flow, ECG, other cardiovascular parameters, and oxygen consumption were measured with techniques routinely used in our laboratory.

Specifically, we undertook the following investigations during the period of three years:

1. A study of circulatory changes (right ventricular pressure, arterial blood pressure, heart rate, cardiac output) during exposure of rats to hypokinetic conditions. Used the head down tilted Holton-Musacchia system in order to compare the results with the results already obtained on unrestrained rats.

2. Humoral changes were investigated in animals exposed to hypokinesia; also, lymphocyte and neutrophil levels in hypokinetic animals (with or without tilt) were determined to ascertain the level of induced stress and possible changes observed in weightless animals.

3. Circulating blood volume was determined during and after hypokinesia.

HISTORICAL

Cardiovascular changes induced by weightlessness. Profound changes of the cardiovascular system are observed even after space flights of short duration. Orthostatic hypotension has been described in the crew members of Project Mercury (34 hour long space flight). A more profound decrease in orthostatic tolerance (lasting up to 2 days after return to earth) was described after Gemini flights and longer exposures to decreased g forces. Reduction in orthostatic tolerance appears to be a consequence of space flight exposure itself (Hoffler and Johnson, 1975) as the Apollo Program has shown. The same applies to a decreased cardiovascular responsiveness observed after a space flight (Hoffler and Johnson, 1975). Skylab experiments confirmed these findings (Bergman et al, 1976). Both American as well as Russian researchers have described changes of the resting heart rate during flights (Johnson et al, 1974), a decrease in circulating blood volume (Lamb and Rambout, 1974), shifts in blood distribution (Johnson et al, 1974), a decrease in the erythrocyte mass (Degtyarev et al, 1979) but not in crew members of Solyuit 6 accompanied with headward congestion (Thornton and Ord, 1974) as well as a decreased orthostatic tolerance during space flights (Kerwin and Ross, 1974). Other changes that might affect cardiovascular performance of animals have been reported as well (Durnova et al, 1979). It seems that both stroke volume and cardiac output were

decreased in Skylab astronauts. Changes in body fluid volume and circulatory changes that are observed during early stages of exposure to weightlessness occur probably during the first few hours of a space flight. The venous return from the lower part of the body to the right heart is increased due to the absence of the hydrostatic pressure. Though in all likelihood the pulmonary vessels accommodate some of the excess blood, there is overloading of both atria. Atrial stretching leads to neurohumoral stimulation and a profound loss of fluid through diuresis (Berry, 1974). It appears also that there is an increased activation of barorecptors, leading to vasodilatation and bradycardia. Redistribution of cardiac output occurring during weightlessness is probably not only a simple headward shift of the circulating blood volume but includes other changes as well. The headward fluid shift appears to be very large. The anthropometric measurements show a loss of more than 2 liters of fluid from calf and thigh of the astronauts (Thornton et al., 1974). Despite this tremendous change, an exercise stress up to 75% of maximal aerobic capacity is well tolerated during space flights, though with a decreased physiological effectiveness (Sawin et at., 1975). Elevated heart rate, a decreased stroke volume, and a somewhat smaller cardiac output for the same level of an increased O_2 consumption are observed during inflight exercise. Thus, something, still uncovered, happens during the space flights with the cardiovascular system, indicating that other

investigative approaches (besides the present ones) are needed especially now when the time of the Space Shuttle/Space Lab has come.

The postflight circulatory changes led to a decreased exercise ability as well. A decreased red blood cell mass has been described in astronauts during and after Skylab flights (Johnson et al., 1974).

Circulatory changes in animals during or after space flights have been less studied. One of the most recent work deals with the experiments of Soviet Kosmos type satellites. The obtained data should be evalulated with caution for many reasons. From 46 rats that flew Kosmos-605, only 35 were recovered alive. The rest of them died during the 22-day long space flight probably because of malfunctioning of supporting systems. Concerning cardiac physiology, one of the more important findings was that enzymatic activity of the protein fractions of the myocardium was changed. After a 22-day space flight, it was reported that in six out of seven rats examined one day after the return to earth, the ATPase activity of the myocardial myosin was increased. Twenty-six days after the flight, the myosin ATPase activity in four out of five rats was back to normal. Furthermore, nitrogen level of the blood was significantly decreased. Hypoplesia of the spleen, thymus, and lymphoid organs was described (Durnova et al., 1976; Durnova et al., 1979). Fatique (at least partially due to cardiovascular deconditioning) was observed after the flight in Kosmos-690

rats (Livshits et al., 1978). Number of open ("functioning") capillaries of the gastrocnemius muscles of Kosmos-936 rats was decreased by 30% (Kaplanskiy, 1978). The lifespan of red blood cells of Kosmos-782 rats was decreased (Leon et al., 1978) while erythropoesis of Kosmos-605 rats was suppressed concomitant with increased hemolysis (Illyin et al., 1975). The hemopoetic system of Kosmos-690 rats was more sensitive to irradiation than it was in control animals (Kalandarova et al., 1978). The inhibition of erythropoiesis (Shvets, 1977) and adrenal cortex changes with speedy return to normal after return to earth (both in Kosmos-605 and Kosmos-782 rats) were also described (Savina, 1978). It has been further reported that the O_2 consumption was increased after weightlessness (Golov and Illyin, 1977). In early Soviet experiments, two dogs showed marked fluid loss which is in accordance with data obtained in man. The U.S. primate (Meehan and Rader, 1971), overinstrumented and overrestrained, demonstrated an important increase in the central venous pressure during the space flight, a fact that fits other information obtained in weightless conditions, but it could also be associated, with accidental hypothermia. It has been speculated that many other physiological changes occur during a space flight that might indirectly influence and change circulatory parameters. Such changes include probable appearance of a new "set point" brought forth by modifications in the body temperature regulation (Kluger, 1980), possible metabolic

changes (Pace, 1980), consequences of metabolic loading (Pace et al.,1979; Pace, 1980), changes in red cell production (Leon, 1980), loss of body mass (Leach et al., 1979), and possible renal changes (Musacchia and Halstead, 1979).

Readaptation to 1-g (after a space flight) begins in man very early. Although the body is able to adapt successfully to zero-gravity, readaptation to earth gravity presents problems. Impairment of venous return, probably at least partially due to changes of the myocardial system is observed. The general impression is that the cardiovascular system readapts fast to 1-g forces (Nicogossian et al., 1974) though not as fast as it adapts itself to 0-g conditions. Cardiac output is probably decreased. Orthostatic stresses (Rummel et al., 1973), and a significant change in ejection time index and of pre-ejection period (Bergmen et al., 1974) of the heart were observed during 1-g readaptation process. It appears that cardiovascular values reach normal levels within 30-60 days after return to earth, seldom earlier. A somewhat reduced end-diastolic volume (Henry et al., 1974) and a reduced stroke volume (Rummel et al., 1975) with concomitant decrease of ventricular volumes are also observed (Nicogossian et al., 1974). The last finding points toward a reduction of cardiac contractility and/or a decreased Starling effect, an important factor in keeping cardiac output decreased after the return to earth. After Skylab,

the astronauts had a tachycardia, a decreased stroke volume of 50%, and a decreased cardiac output of 30% lasting at least 5 days. The changes observed immediately after return to earth are sometimes so drastic that some of the investigators were obliged to write: "The systolic time intervals obtained on the Apollo 17 crewmen during lower body negative pressure exposures were similar to those noted in patients with significant heart disease" (Bergman et al., 1974).

In summary, cardiovascular changes induced by weightlessness are brought forth during early hours or during early days of a space flight. The cardiovascular mechanisms involved in adaptation to weightlessness are unknown. Furthermore, the time sequence of any potential fluid shifts is unknown (Stone, 1980). They might be the consequence of the sudden disappearance of the hydrostatic pressure in man and to a lesser degree the consequence of muscular hypokinesia. It is likely that besides this simplified picture of space flight induced changes, there are some other physiological mechanisms that are brought forth during exposure to weightlessness and that these mechanisms might be evaluated more easily and with more adequate techniques in animal models. However, little is known about effect of a space flight on circulation in animals. Whatever these possible--still unknown--space induced changes and mechanisms are, it appears (at least for man) that after some time they stabilize and become self-

limiting ("full adaptation to weightless state"). Readaptation to 1-g in man occurs more slowly, lasting at least 30-60 days. Thus the process of readaptation might be easier to study and the physiological mechanisms of the process easier to identify.

Cardiovascular changes during "induced weightlessnes." Hypokinesia (prolonged bedrest, immobilization, hypokinetic states) and weightlessness during space flights appear to have many similar physiological consequences (Gauer et al., 1970), including a significant redistribution of circulating blood volumes, a decreased blood volume (Dickey et al., 1979), an increased central venous pressure, appreciable diuresis, natriuresis and potassium loss. Thus, studies of hypodynamic states are relevant to a better understanding of circulatory, fluid and electrolyte changes that accompany manned space flights. This fact has been recognized in the U.S.S.R. and other Eastern European countries where the scientific interest has been concentrated much more on hypokinesia (Portugalov and Petrova, 1976; Chernov, 1978; Nikityuk et al., 1978; Tikhanova et al., 1979) than in the Western world. Seventy five percent of the work done in this field has been performed on rats as experimental animals (Nikityuk, et al., 1978).

Twenty years ago a NASA publication summarized ground based experiments that have attempted to simulate short or long-term weightlessness by using techniques of immobilization or sometimes of water immersion (Busby,

1967). Today it is clear that though these conditions (bedrest, immobilization) mimic some of the effects and some of the physiological changes induced by 0-g force, they do not completely eliminate the effects of gravity on the cardiovascular system.

Hypokinesia. Interest in gravitational physiology has prompted the development of various Earth based man or animal experimental approaches (Musacchia and Holstead, 1979; Kirsch et al.,1979; Guell et al.,, 1979). Prolonged bedrest has been used as a model system for study of circulatory changes induced by weightlessness (Wilkins et al.,1950; Miller et al., 1964; Fisher et al., 1967; Melada et al., 1975 Chobanian et al., 1974; Hyatt, 1971; Hyatt et al., 1969, 1970, 1975, 1977; Legenkov et al., 1973; Volicer et al., 1976; Katkov et al., 1979; and Chestukhin et al., 1979). Twenty or more days of bedrest in man leads to profound metabolic and cardiovascular changes that Russian authors call "myocardial hypodynamic syndrome" (Panferova et al., 1967). Researchers in this country found less striking but still profound changes that hypokinesia induces: a decrease in plasma volume observed even after one (Johnson et al., 1971) or more days of bedrest (Taylor et al., 1945; Vogt and Johnson, 1967; Van Beaumont et al., 1971; Greenleaf et al., 1973; Chobanian et al., 1974; Dickey et al., 1979), a decrease that is even more pronounced after a prolonged bedrest (Lamb and Stevens, 1965). After 6 days of bedrest, the decrease in plasma blood volume is

approximately 10% (Lamb and Stevens, 1965; Stevens et al., 1966; Vogt and Johnson, 1967; Vogt et al., 1967; Johnson et al., 1971) and after several weeks of bedrest the decrease reaches 20% (Miller et al., 1964; Vogt et al., 1966). The decrease in blood volume is induced by an increased diuresis observed during recumbency (Smith, 1957; Hyatt, 1971). Longer bedrest seems to decrease further the plasma blood volume. The central venous pressure during bedrest (Leach et al., 1972; Greenleat et al., 1973) is increased due to the headward shift in body fluids. However, resting heart rate, blood pressures, and cardiac output appear to be unchanged (Saltin et al.,1968; Malada et al., 1975). The bedrest induced orthostatic intolerance can be ameliorated by negative pressure or saline administration (Hyatt and West, 1977).

Stroke volume in man decreases from 70 to 60 ml during the initial 8-10 days of bedrest, while cardiac output slightly decreases. The decrease is more pronounced in the persons who develop more cardiovascular deconditioning as measured during readaptation to free activity (Dorofeyev, 1978). These persons have a high lactic and a high pyruvic acid level during bedrest. A decrease in arterial blood pressure and in cardiac output is observed during bedrest with immobilization of limbs in man. The central venous pressure decreases also in horizontally placed man (Zakatayeva, 1978).

After prolonged bedrest, pooling of blood in extremities of a standing man decreases volume blood flow to the brain bringing forth dizziness and sometimes fainting and, of course, leads to orthostatic intolerance (Hyatt et al., 1969; Miller et al., 1964; Taylor et al., 1945; Vogt and Johnson, 1967), but not in exercise trained personnel (Saiki et al., 1979). The same situation in untrained persons leads to a decreased ability to exercise (Cardus, 1966; Hyatt et al., 1969; Miller et al., 1965; Raab et al., 1960; Saltin et al., 1968). There is changes and resetting of postganglionic norepinephrine release especially during a prolonged bedrest (Pestov et al., 1969; Leach et al., 1972). This change is probably brought forth by a decreased vasomotor tone in the absence of peripheral (g-induced) blood pooling.

Studies of Hyatt and co-workers (1970-1975) show that the changes induced by bedrest (and by implication, weightlessness) occur during initial 24-48 hours. The prolonged bedrest (hypokinesia and decreased hydrostatic blood pressure) induces deconditioning of the cardiovascular system. Some researchers believe hypokinesia is the main cause of deconditioning (Georgiyevskiy and Mirhaylov, 1978).

Prolonged bedrest (up to 49 days) in man leads to some immuno changes. It is important to mention here that physical immobilization was used in animals (espically in rats) as a stressor to produce gastric ulceration (Buchanan and Cane, 1974). Thus, animal hypokinetic studies should

include morphologic gastric studies to ascertain that gastric ulceration was not induced during partial immobilization.

In rabbits, long lasting hypokinesia causes a decrease in left ventricular contraction phase and induces vasodilatation. Stroke volume and cardiac output are unchanged. However, when heart rate is increased, the cardiac output does not increase proportionally (Meyerson et al., 1978).

Hypokinesia with negative tilt. Early research (Wilkins et al., 1950) of American investigators in this field was mainly associated with cranial decongestion in patients. Negative tilt was used by Soviet authors to simulate in man the effect of weightlessness on the cardiovascular system, i.e., to induce the headward movement of blood (Georgiyevsriy et al., 1971; Voskresenskiy et al., 1972; Katkov et al., 1979). The investigators found smaller orthostatic hypotension in bed rested men with negative tilt than without tilt (Zhernavkov, 1979), maybe a consequence of cerebral circulatory adaptations. They believe that such a model is more adequate to simulate the effects of weightlesness than horizontal models. Even a -5^{o} tilt lasting 24 hours leads to a striking decrease in plasma volume in man (Katkov et al., 1977), an increase in sodium excretion (Volicer et al., 1976) and total protein level in plasma (Nikityik et al.,, 1978), as well as changed intracardiac hemodynamics (Katkov et al., 1978; Chestukhin

et al., 1979). Plasma renin activity and plasma aldosterone activity are increased after 24 hours of 5^{o} negative tilt. Increased renin secretion probably represents a compensatory response to sodium depletion and to the increased aldosterone secretion, and it is probably responsible for the sodium retention observed in the post-tilting period. Increased aldosterone excretion has been observed in Skylab crewmen during space flights, although plasma aldosterone levels were not significantly elevated. In contrast to these findings with head-down tilt, previous studies (Volicer et al., 1976) have indicated that plasma renin and aldosterone levels are not increased during prolonged bedrest. Both cosmonauts and -4^{o} hypokinetic volunteers experienced changes in energy metabolism of erythrocytes (Ushakov et al., 1977) as well as circulatory changes (Gazenko et al., 1980). The head-down tilt, therefore, appears to be a better model for inducing metabolic and cardiovascular changes similar to those induced by weightlessness than it is the case of bedrest in the horizontal position (Morey and Baylink, 1978). Energy metabolism (Jordan et al., 1979), bone turnover (Morey et al., 1979), and electrolyte balance (Musacchia et al., 1980) change during rat hypokinesia (with 20^{o}-30^{o} negative head tilt) and return to normal value after readaptation to free activity. A five weeks hypokinetic exposure leads to increased excretion of K^{+}, a consequence of increased aldosterone activity and maybe some other physiological

changes (Saiki et al., 1976). Thus, it has been demonstrated that hypokinesia with negative tilt in animals is an excellent model system for the study of changes induced by weightlessness (Musacchia et al., 1980; Morey and Baylink, 1978).

METHODS AND PROCEDURES

Animals. Before describing in detail the animals and the techniques that were used, the following paragraph should explain, in general terms, why the rat was chosen as the experimental animal in this work.

Rats have been used more often than any other animal species in the space research (Kosmos series). Furthermore, there is an abundance of hypokinetic data in the literature dealing with rats, especially from the Eastern European countries. Recent advances in methodology permit that most cardiovascular studies can now be done on rats. The physiology and pathology of rats are well understood. Use of rats is less expensive. Less food, less space, less care, and more animals (permitting adequate statistical evalulation) can be used in ground-based experiments, in hypokinetic conditions, and in Space Lab conditions. All the techniques proposed for our work are simple and can be performed by personnel after several weeks of training. Ground-based data (circulatory parameters in rats) have been collected in our laboratory for 15 years.

Animals. Adult male Sprague-Dawley rats, weighing 200 q 10 g (S.D.) housed in separate, individual cages, were used in the experiments. Close matching of the body weight and age of rats appears to be very important for high reproducibility of results. Each animal is housed in a separate cage and it is given food (Purina Chow) and water ad libitum.

All rats are brought to our animal rooms from the breeding colony (Camm) to the animal quarters of our laboratory at any early age, with a body weight of 40-60 g. The animals are carefully screened for any apparent respiratory problems while they are growing to a body weight of 170-180 g. The body weight of the animals is measured twice per week. The animals that do not follow normal body weight curves established in our laboratory for this strain of rats are eliminated. The lighting cycle in the rooms is 8 AM - 8 PM. The temperature is controlled at $24^{o}C \pm 1^{o}C$.

Chronic cannulation of aorta and of right ventricle of the heart. A polyethylene PE10 cannula is placed into the aorta through the left common carotid artery when the body weight of the rats reaches 180 gm. One tip of the cannula is located in the aortic arch while the opposite end is exteriorized at the back of the animals's neck. Implantation is done under light anesthesia. Once implanted, the aortic cannulas stay patent for the lifespan of the animal (Popovic and Popovic, 1960; Popovic et al., 1963). Cannulation of the right ventricle of the heart is done simultaneously with the aortic cannulation , i.e. 15 days prior to the experiment (Popovic et al., 1963; Popovic et al., 1969).

After implantation, both cannulas stay patent for extended periods of time, often for the duration of the entire lifespan of the animal (Fig. 1). Until now, over 16,000 rats have been cannulated in our laboratory. Adverse

effects of cannulation of the right ventricle or the atrium, or the aorta have never been described, nor has published criticism of this procedure ever been offered. Our own studies have shown that there is no regurgitation of blood between right ventricle and right atrium and that, histologically, the walls of the blood vessels and the right heart are unchanged. The cannulation technique was found useful for cardiovascular studies in other small laboratory animals (Popovic, 1964; Popovic and Berger, 1968; Popovic et al., 1969), and in physiological states other than BMR conditions such as exercise (Popovic et al., 1969), hypothermia (Popovic and Kent, 1965; Popovic et al., 1967; 1968; and 1971; Horecky et al., 1971), induction of granulocytosis (Popovic et al., 1976), in experimental tumor treatment (Popovic et al, 1976), effect of levodopa on blood pressure (Popovic et al., 1977), levodopa treatment in induced paraplegia (Popovic, 1976), and glycerol treatment in experimental cerebral infarction (Popovic et al., 1978). An extensive study of the brains of cannulated rats (histological, histochemical, and electromicroscopic investigations 1,7, and 21 days after cannulation of the aorta and the right ventricle) has shown no changes in the blood vessels, brain tissue and other tissues that were examined. This study gives additional information to the studies performed earlier, in which acquisition processes and performance (learning and retention) were studied and

which proved also that there is no difference between cannulated and uncannulated animals.

Heart rate. Heart rate was monitored through implanted aortic cannula.

Arterial blood pressure recordings. Arterial blood pressure recordings were done in unanesthetized, unrestrained rats after full recovery from surgery and from anesthesia, i.e. 15-21 days after implantation of the aortic cannula (Popovic and Kent, 1964; Popovic et al., 1969; Popovic et al., 1977).

Right ventricular pressure. Right ventricular pressure was measured by direct techniques in unanesthetized animals, through chronically implanted cannulas (Popovic and Kent, 1964).

Cardiac output. Cardiac output was measured on the basis of the Fick principle (Popovic and Kent, 1964; Popovic et al, 1969).

Our results obtained from individual animals show good reproducibility. Two measurements done on the same day at one hour intervals differ very little. Good reproducibility of the results is attributed to the precision of the procedures as well as to the fact that the metabolic rate of rats is a true resting metabolic rate. The stable resting metabolic rate is observed only if the animal is placed in the metabolism chamber at least 10 min before the measurement. If the O_2 consumption and the cardiac outputs are measured immediately after placing the animal into the

chamber, they are considerably higher (even in the resting state). After the resting metabolic rate was achieved, the standard error of the mean of the cardiac output was only 2.6% (0.4 - 4.8%).

Hematocrit ratio. was determined in aortic blood.

Oxygen consumption was measured continuously by an open system in which the CO_2-free outlet air is passed through a Beckman oxygen analyzer (Model C3) and metered terminally. The airflow is adjusted to give a pO_2 difference of approximately 7 mm Hg. The analyzer is calibrated before each run with gas mixtures of known composition.

Treadmill exercise. In order to study the effect of weightlessness on work performance, cardiac output and other circulatory parameters was measured in the rats before and after hypokinesia. Two levels of exercise (10 m/min and 20 m/min) were used to test the animals. The results in ground-based experiments have already been obtained (Popovic et al., 1969) and all the necessary techniques developed.

Induction of hypokinesia. The Morey-Musacchia model system was used in our experiments to induce hypokinesia in rats. This system includes: 1) the ability of the animal to exercise using only front limbs, 2) a fluid shift (negative tilt), 3) total unloading of the rear limbs without restraining, 4) the ability to eat and drink ad libitum and to groom at least one part of the body, and 5) a less stressful system than that presently existing.

° Hormonal determination. The first radioimmunoassay was developed and introduced by Berson and Yalow in 1960 and is based on the principle of isotopic dilution in the presence of specific antibodies. Although many variants exist, classical radioimmunoassays of hormones depend upon the competition between unknown levels of an unlabeled hormone being measured and a fixed amount of labeled hormone for a limited number of binding sites of specific antibodies. Known quantities of unlabeled hormone are varied in order to obtain a standard curve and unknown amounts of the analyzed hormone are determined by interpolation with the generated standard curve. The procedure is specifically designed to measure quantities of protein or small haptens in the nanogram or picogram range and allows the simultaneous determination of several samples. Antibodies are usually produced in rabbits, goats, or guinea pigs by repeated injections of antigen (immunogen). These raised antibodies are then used in the immunoassay to determine small amounts of substances in biological number of years for the measurement and study of polypeptides and steroids.

Radioimmunoassays are rapid, sensitive, specific, reliable and require a minimum quantity of blood. The last feature is very important, especially when repetitive sampling is required in a particular study or when small laboratory animals are used.

A radiometric technique will be employed for determining catecholamines in plasma. This technique is

more sensitive and accurate than flourescent or other (bioassay) techniques which were used previously for the determination of catecholamines.

Corticosteroid determination. Sgoutas and his group have developed a direct radioimmunoassay of corticosterone which requires only 10 ul of rat plasma. The method is similar to the direct radioimmunoassay of cortisol in human plasma, as described by Donohue and Sqoutas (45). After heat inactivation of corticosterone binding proteins in rat plasma, plasma corticosterone is assayed with a specific antibody raised against corticosterone 21-hemisuccinate. While this antibody has a significant cross-reactivity with cortisol, prior separation of corticosteroids is not necessary since cortisol is not produced in the rat. Different volumes of diluted serum (5, 10, 25 and 50 ul) were assayed and showed good linearity provided the values fell in the span of the standard curve.

The intra-assay coefficient of variation of a pool containing 10.35 ug/dl was 5.9%) (n = 9) and of a pool contianing 31.9 ug/dl was 6.6% (n = 9). The interassay variability of the latter pool was 12.0% (n = 8). Serum extrated with charcoal showed a value of 1.07 $\pm$ 0.2 ug/dl (n = 4). The sensitivity of the standard curve was 0.7 ug/dl when using 4 ul of the diluted serum or 1 ul of undiluted serum.

Accuracy ws examined by adding corticosterone, 1.25, 2.50, 5, 10, 20, and 40 ug/dl, to charcoal-treated serum and

assaying the serum. The regression line was y = 1.009 x + 0.49, indicating no systematic error.

ACTH determinations. It has been reported previously that stressed rat plasma ACTH is immunologically very similar to human ACTH, especially in the excellent cross reaction that occurs between rat plasma ACTH and antiserum of human ACTH. This provides a very sensitive method for measuring rat ACTH with human ACTH antiserum, human ^{125}I-ACTH and human ACTH standards, reagents which are readily available from commercial sources (Immunonuclear Corporation, Stillwater, MN 55082). Different volumes of serum (10, 25, and 50 ul) were assayed and satisfactory linearity was obtained when human serum low in ACTH as diluent was used for the 25 and 50 ul aliquots. The inter-assay coefficient of variation of a pool containing 80 pg/ml ACTH was 9% and the interassay was 10%. The sensitivity is less than 8 pg/ml when using 100 ul of plasma for ACTH determination. The method was modified with the result that the required amount of plasma was decreased to 25 ul.

Prolactin determination. Prolactin levels in plasma will be determined by a double antibody radioimmunoassay method developed by Neil. The method was scaled down and modified so that only 25 ul of serum was used. All reagents, except ^{125}I rat prolactin, were obtained from the N.I.H. (National Pituitary Agency). Rat prolactin radio-iodination was carried out in the laboratory as needed. Different volumes of serum were assayed and showed similar

results--provided they were not less than 50 ul plasma. The intra-assay coefficient of variation of a pool containing 60 ng/ml rat prolactin was 10% and the inter-assay 13.5%. The sensitivity is 4 ng/ml when using 50 ul aliquot.

RESULTS

During the last several years, we have developed a rat model that has been used extensively in cardiovascular, hematologic, pharmacologic and other studies in this country and abroad. Two PE 10 micro cannulas are chronically implanted into the rat's aorta and the right ventricle of the heart. After surgery and anesthesia, the animal recovers completely. Ten to fifteen days after chronic implantation, experiments are performed in which cardiac output and other circulatory parameters, hematological, endocrine and other changes are measured in unrestrained, unanesthetized animals using their chronically implanted cannulas. The same rat model has been used to study the effects of hypokinesia (with or without negative tilt) on circulation in the rats.

Development of an unanesthetized, unrestrained rat model. Surgery and anesthesia drastically decrease cardiac output and other circulatory parameters in rats (Popovic and Popovic, 1969; Popovic and Kent, 1964 and 1965; Popovic et al., 1969 and 1977). In order to conduct circulatory and other investigations in unanesthetized, unrestrained rats, the aorta of the animals is permanently cannulated ten to fifteen days before an experiment (Popovic and Popovic, 1960 and 1974). The technique permits arterial blood pressure measurements, intravascular administration of various drugs, and arterial blood sampling from unanesthetized and unrestrained animals. When an additional cannula is

implanted in the right ventricle of the heart (Popovic et al., 1963), other circulatory parameters such as cardiac output (Fick principle, Popovic, 1964), intraventricular ECG, and organ blood flow are measured.

The technique permits adequate, reproducible measurements of the cardiovascular parameters in rats. Until the present time more than 16,000 animals have been routinely cannulated in our laboratory. We are now able to precisely evaluate even small cardiovascular changes, changes that are induced by exercise (Popovic et al., 1969), cold or heat exposure, exercise training, circadian rhythm, temperature adaptation or by other environmental or drug-induced changes. The chronically cannulated animals stand exposure to increased g forces well.

Hypokinetic rat model We have used in our work the Morey system (20^{o} negative tilt) slightly modified system (denim harness). Each rat is cannulated 15-20 days prior to experiment. Only the rats that had a normal growth before and after cannulation were used in the experiments. The rats that had an abnormal WBC and more than 8,000 granulocytes/mm were also eliminated (Popovic et al., 1976 and 1977). Each rat served as its own control (unrestrained for 10 days, hypokinesia for 7 days and unrestrained for 7 to 30 days).

Circulation in head-down hypokinetic rat. In the early stages of head-down tilt, hypokinetic exposure, cardiac output was increased mainly due to an increase in stoke

volume (see figures -). The values of cardiac output (ml/min) were expressed per kg of the body weight because the effect of a constant body growth in the rat on the cardiac output was eliminated due to an early decrease (lasting two days and then a small increase (the last 3-4 days) in the body weight brought forth by antiorthostatic hypokinesia. The ability of rats to exercise after hypokinesia was decreased. Similar results were described after space flights or after prolonged bed rest. The heart rate was increased during exercise more than before hypokinesia while the exercising cardiac output decreased. The recovery of the entry was slow (lasting more than 14 days) as judged by cold exposure. The hypertensive response after levodopa administration was not changed in post-hypokinetic animals but it was decreased profoundly after administration of norepinephrine, indicating important circulatory changes and new adaptive characteristics. Muscles reacted less in post-hypokinesia to the administration of epinephrine than was observed in control animals. The surprising fact was that circulation and other readaptations to free activity lasted much longer than adaptation to negative tilt hypokinesia. Similar long-lasting changes were observed after space flights as well.

"Stress hormones" in antiorthostatic hypokinesia. Determination of "stress hormones" measures the stress to the rats imposed by placement of the harness and induced by head-down position. We have shown that early exposure to

hypokinesia leads to an increase in the cardiac output. The increase of the cardiac output (and of the stroke volume) during hypokinesia is probably the consequence of a blood volume shift toward the chest and head of the animal brought about by head-down position. However, struggling of the animals to escape from the harness and an increased metabolic rate might also contribute to the observed increase of the cardiac output. In order to study the level of stress imposed by the placement of the harness and by the exposure to uncomfortable head-down position, the levels of ACTH , corticosterone, prolactin, growth hormone and catecholamines were determined in the antiorthostatic hypokinetic animals (radioimmunoassays). The blood (0.3 ml) was sampled in resting rats from the aortic cannula three times prior to antiorthostatic hypokinesia.

The sampling was performed three times during head-down hypokinesia in harnessed rats (on the first, third, and seventh day) and three times after release of the animals from the harness (second, fifth and tenth day). The results indicate that plasma levels of stress hormones were elevated on the day 1 (plasma growth hormone was decreased) and less on day 3 of the anti-orthostatic exposure. On the seventh day of the exposure, all stress hormones were of the low resting (pre-exposure) level. After being released from the harness (and returned to their own cages) the plasma stress hormone levels were slightly elevated (see figures).

DISCUSSION

Cardiovascular consequences of exposure to weightlessness are little understood, though it is clear that the effects of exposure (even a short one) is profound and long lasting. The cardiovascular changes after a space flight can be summarized as follows: one observes in man a shift in circulating blood volume together with overloading of the heart. As a consequence, there is neurohumorally induced excessive water and possibly salt excretion leading to a decreased circulating blood volume. After return to 1-g forces, one witnesses a decreased orthostatic tolerance and to a decreased ability to exercise that lasts 30 days or more.

Similar circulatory changes (a decrease and shift of blood volume, overloading of atria, and increased central venous pressure) have been described during bed rest, especially bed rest with negative tilt. These circulatory changes lead to distension of the heart and(through neurohumoral adjustments) to a decreased circulating blood volume. Free activity after a prolonged bed rest (Miller et al., 1964; Vogt et al., 1966) brings the changed circulatory parameters back to the control (pre-exposure) levels.

However, there are some physiological differences between effects and mechanisms of weightlessnes and hypokinesia. While in weightlessness, possible deficit drinking leads to a decreased circulating blood volume, hypokinesia brings forth an excess urinary excretion, both

conditions leading to a decreased blood volume. There might be some other differences: it is poosible that weightlessness in man increases capillary filtration only in the cephalad region of the body while hypokinesia brings forth probably an increased filtration throughout the whole body. Hypokinesia with negative tilt eliminates (at least partially) this difference. The circulatory changes in rats during hypokinesia with negative tilt (increased central venous pressure, decreased mean arterial blood pressure, decreased stroke volume and a decreased cardiac output) as well as circulatory changes during readaptation to free activity (slow increase of the cardiac output, striking tachycardia during exercise) are similar to the changes observed in man during a space flight. Thus, our hypokinetic rat model appears to be predictive of circulatory changes observed during weightlessness in man. Would the model be predictive of circulatory changes in rats or other animals? One will know only after Space Shuttle experiments.

Thus, we can conclude with Heaney (1974) that, despite some of the differences, it is "surprising how much similar" the effects of hypokinesia are to physiological effects of weightlesness.

Of course, the final question is "Where do we go from here?" Obviously, the answer is "Back to Space." What we need now is to be discriminative in our scientific approach, in asking proper questions and in employing adequate

techniques to learn more from future space flights, not only about effects of a space flight but about effects of gravity on earth. Thus, we need more answers, and happily both bed rest (hypokinetic) model and future shuttle experiments will be able to provide them. Furthermore, one cannot but agree with Heaney (1974) that "because of the long lag between experimental design and results, very careful advance planning is vital so as to insure that we obtain maximum useful information from a set of (hypokinetic) experiments. We badly need numbers in this game. Ground-based experiments constitute our only reasonable hope until the time Space Shuttle brings us new exciting data and new vistas for future work.

Thus, new theoretical and technical approaches, advanced and more adequate techniques, and a better understanding of involved mechanisms obtained in ground-based experiments and in hypokinetic conditions will make the choice of physiological studies dealing with circulation in space more selective, more meaningful, less costly, and hopefully limit future Space studies to those experiments that are not only relevant to extended space flight, but that are also answering the question: What is the effect of gravity on circulation in man and in animals.

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Fig. 1 A rat with the chronic aortic and right ventricular cannulas.

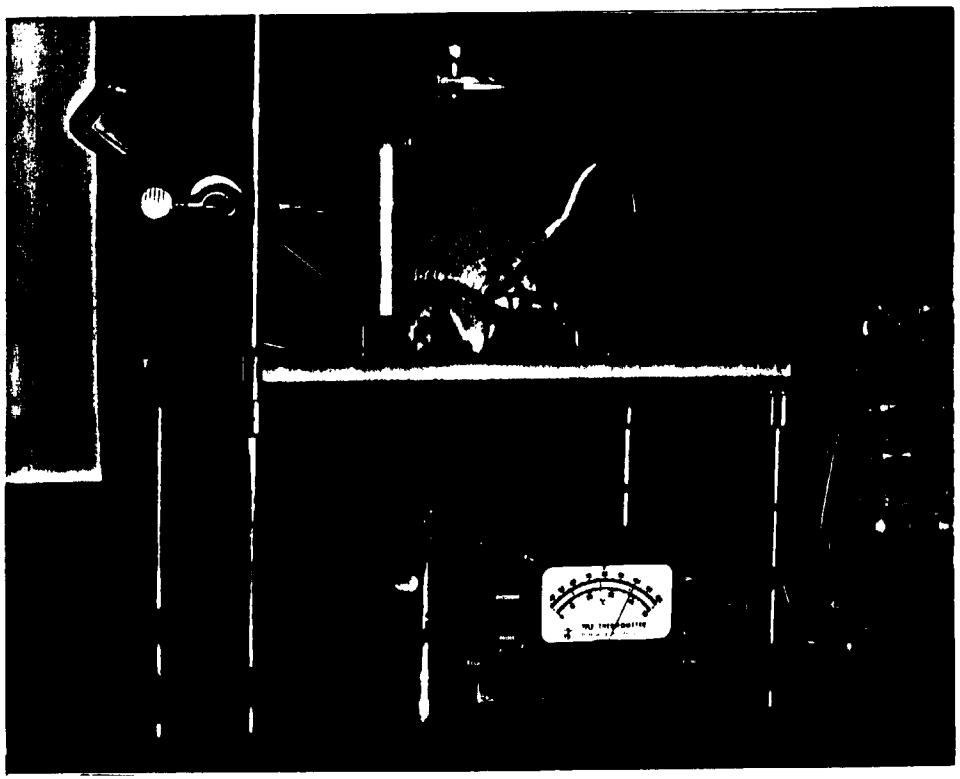


Fig. 2 A head-down hypokinetic rat

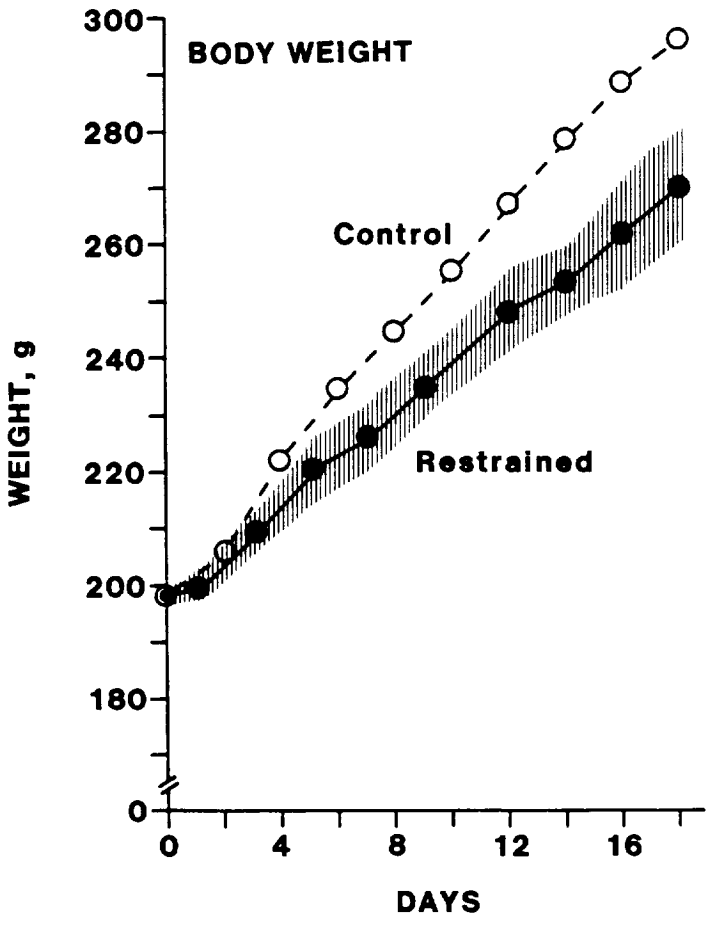


Fig. 3 Body weight of restrained rats during a period of 18 days.

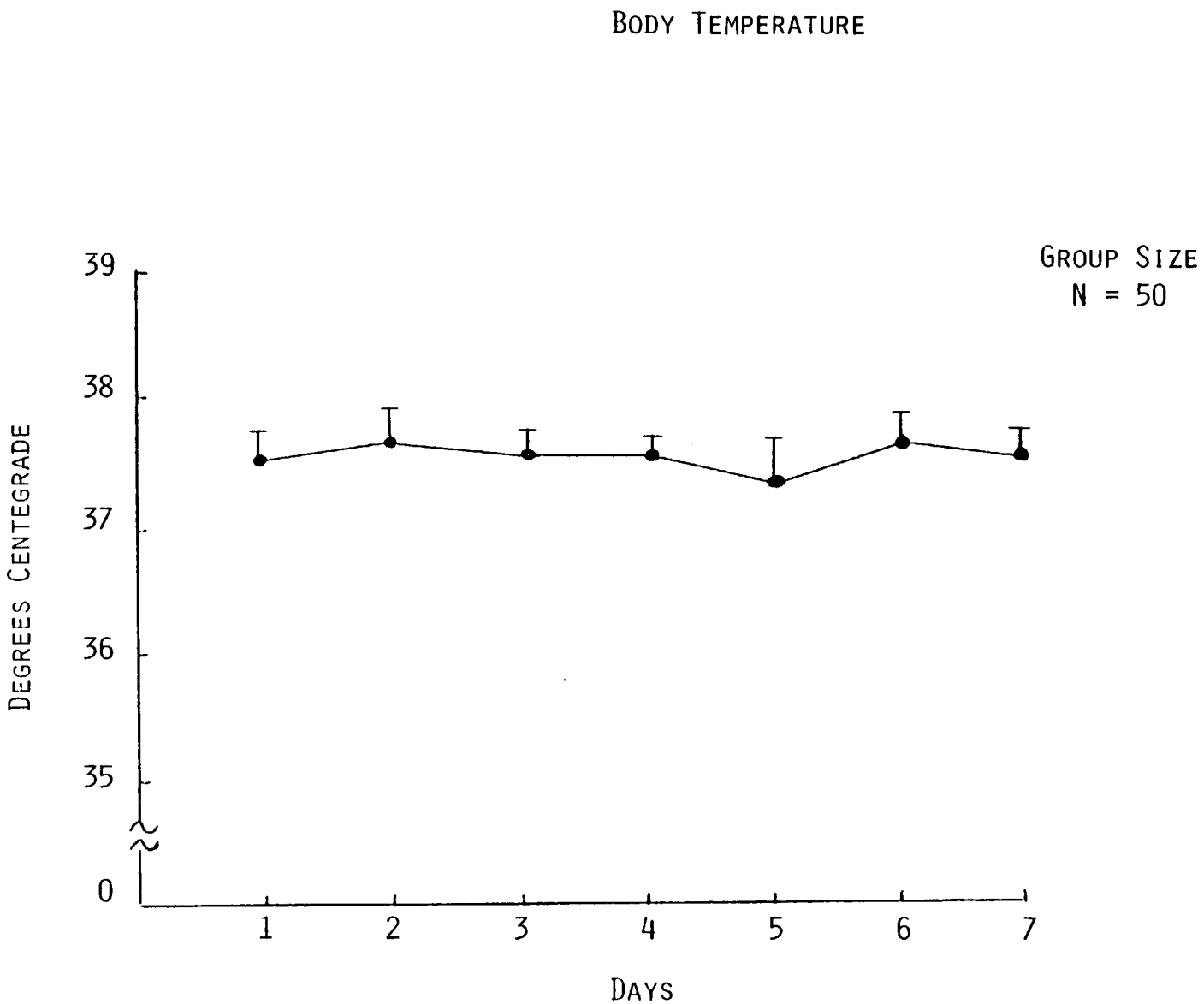


Fig. 4. Deep colonic temperature during antiorthostatic hypokinesia.

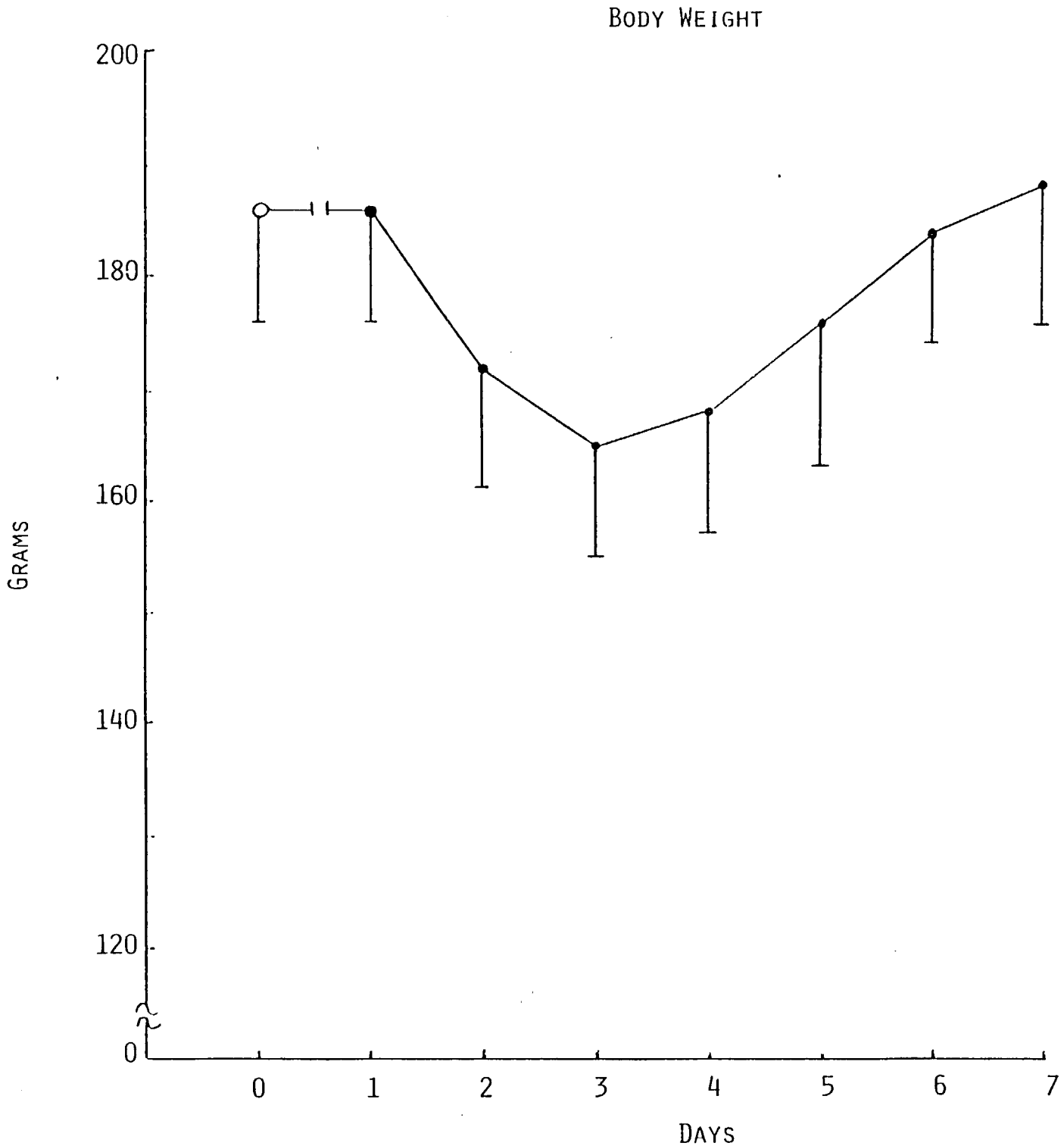


Fig. 5. Body weight during exposure of rats to antiorthostatic hypokinesia.

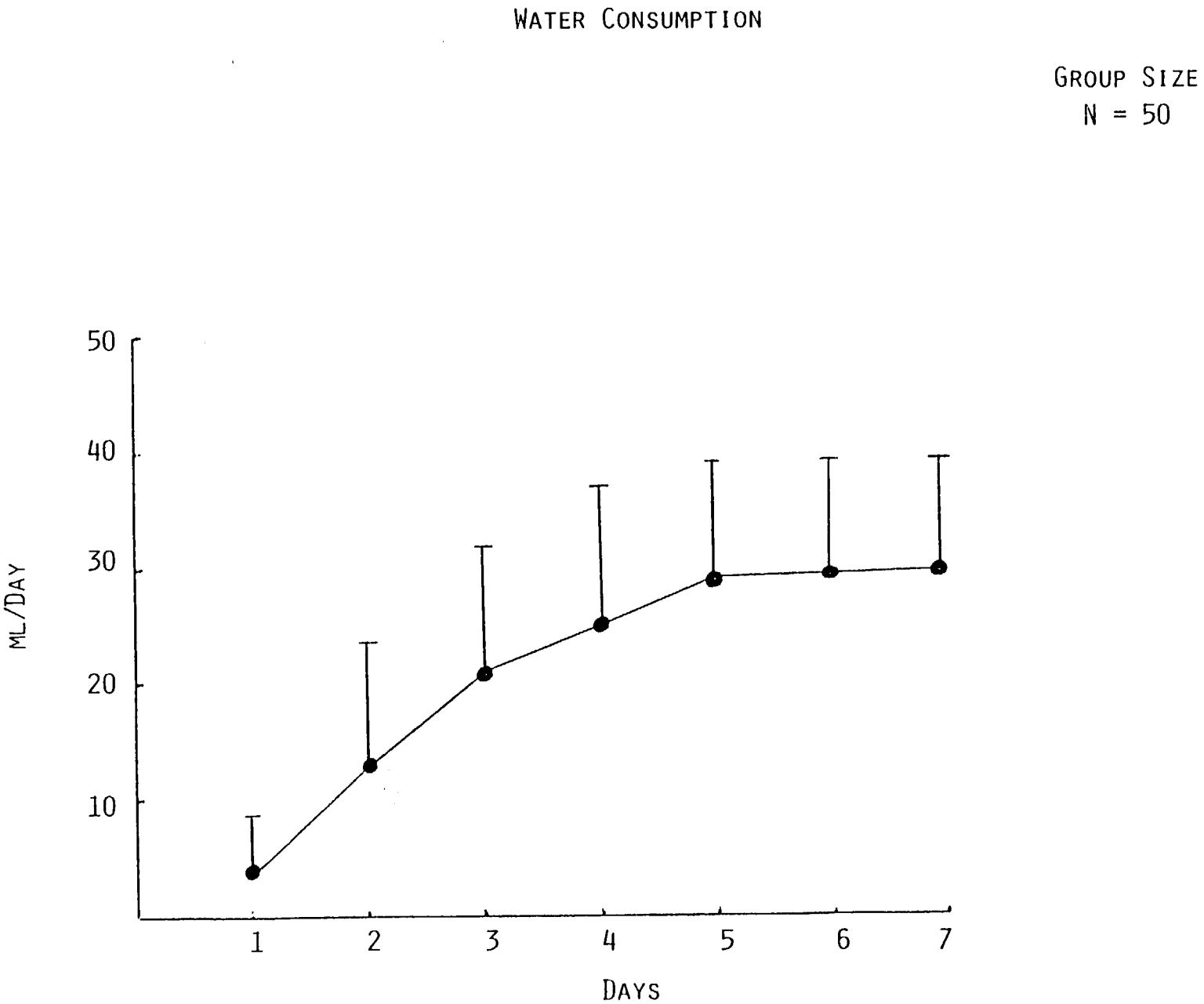


Fig. 6. Water consumption during antiorthostatic hypokinesia.

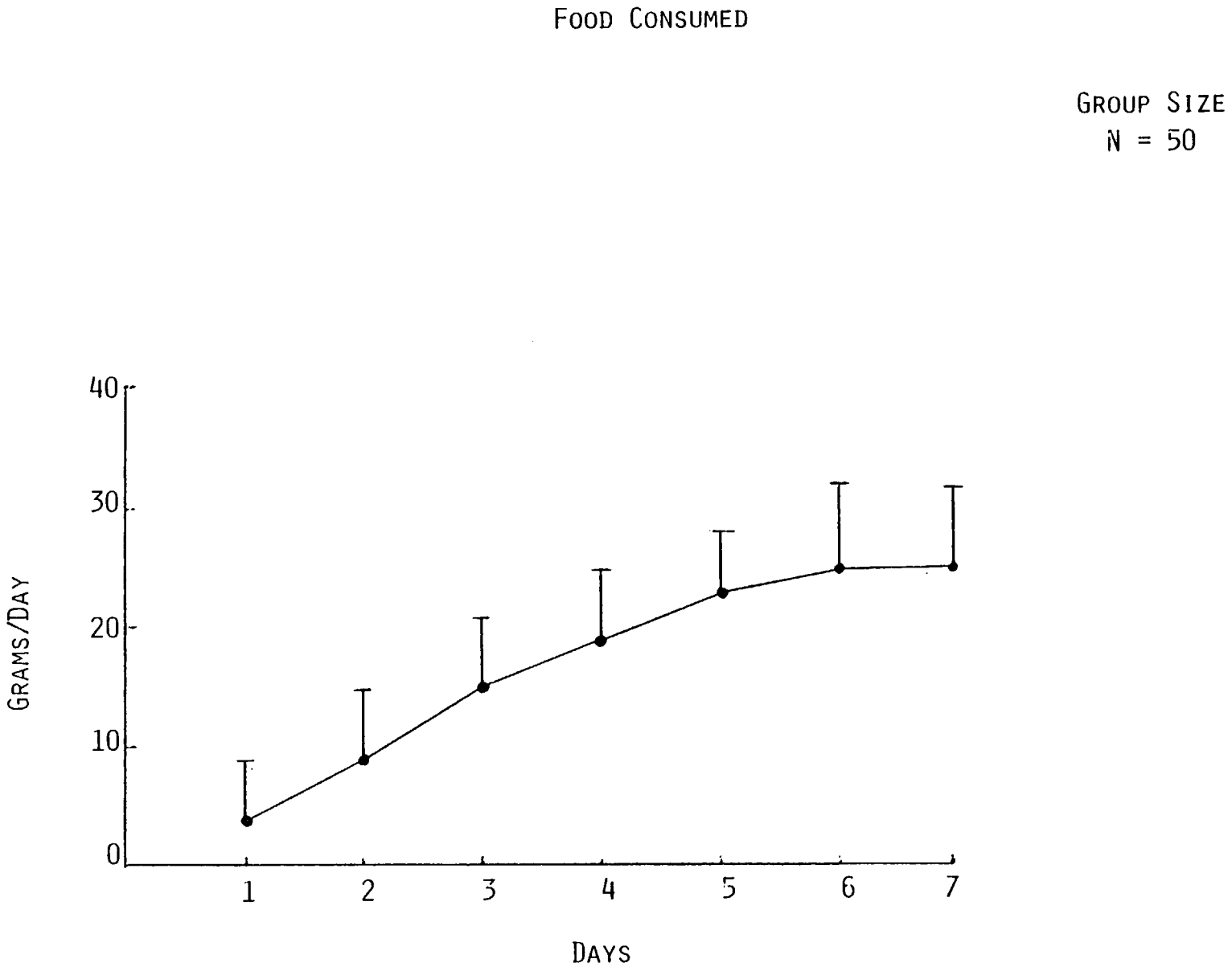


Fig. 7. Food consumption during antiorthostatic hypokinesia.

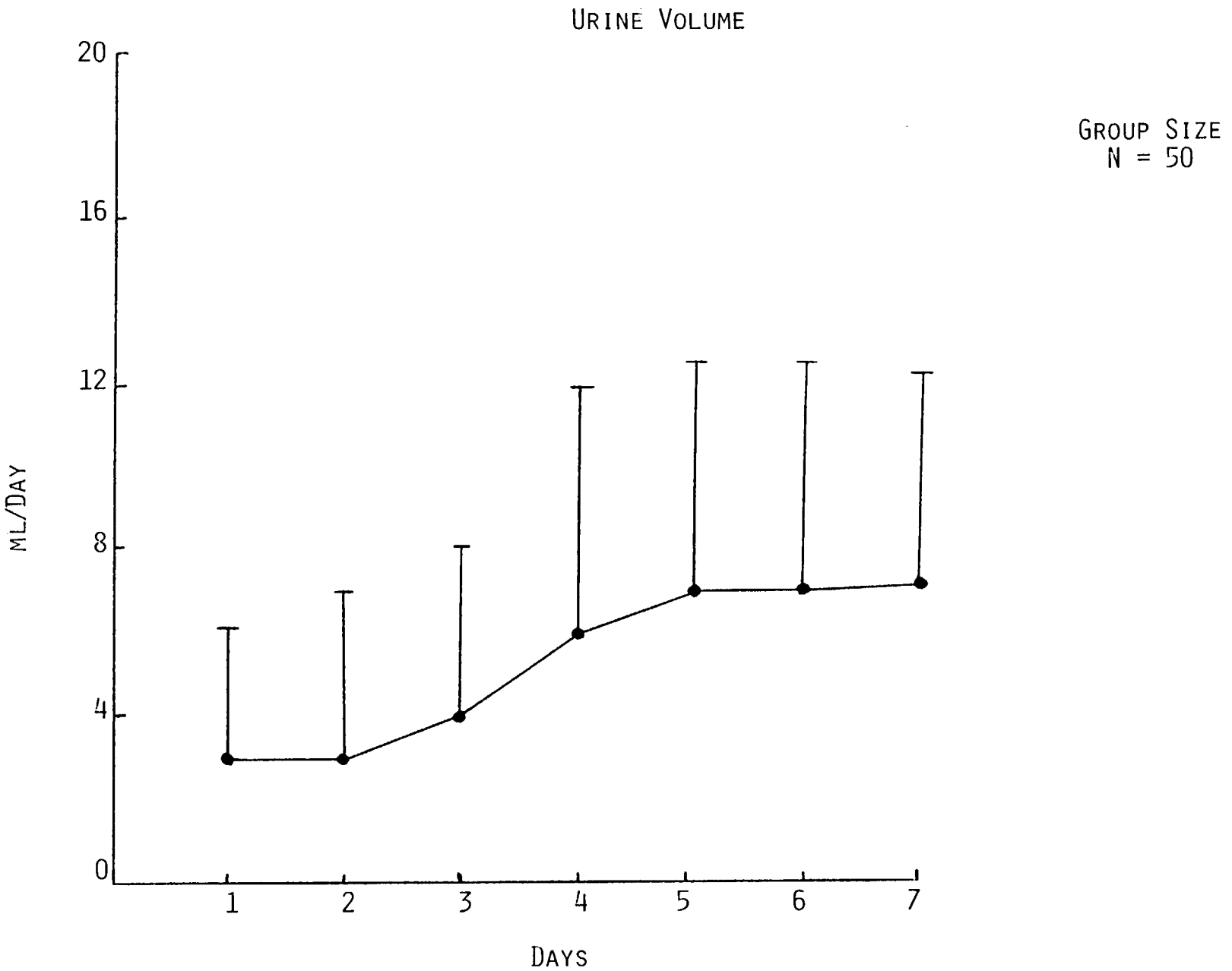


Fig. 8. Urine volume during antiorthostatic hypokinesia.

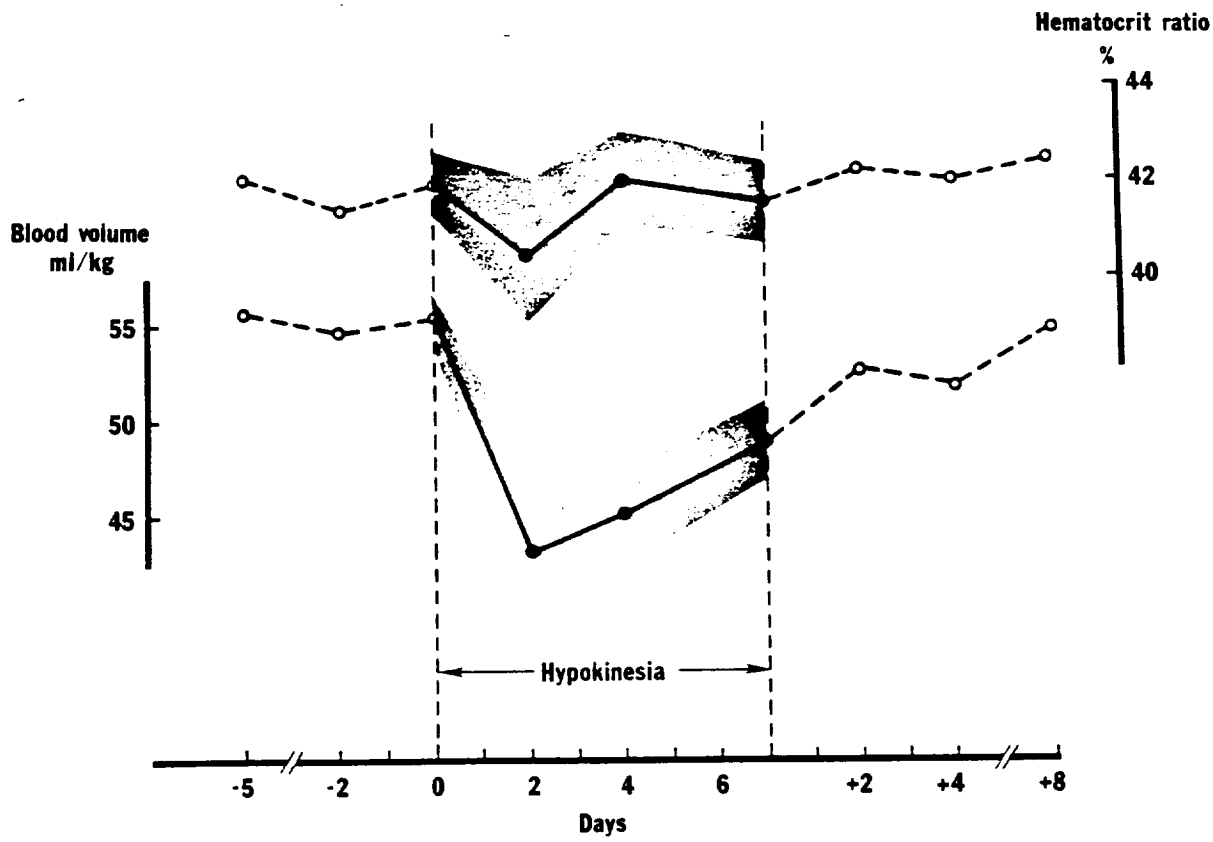


Fig. 9 Blood volume and hematocrit ratio of antiorthostatic rats.

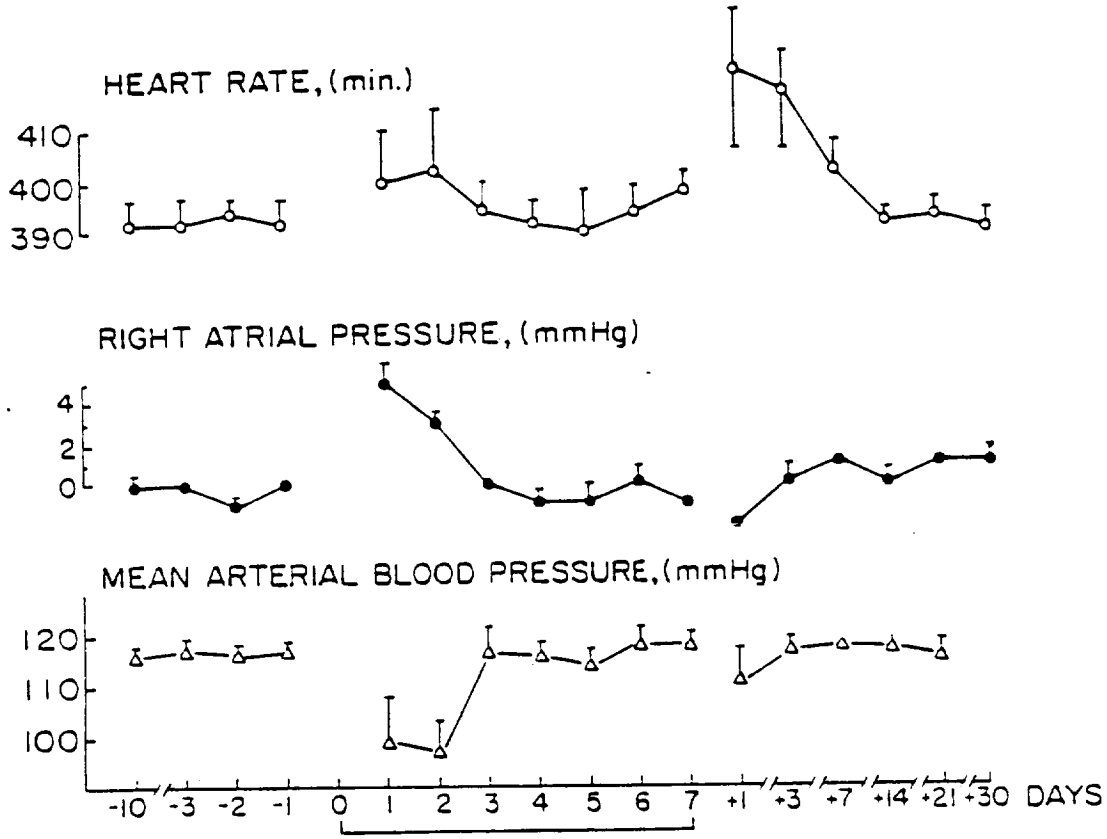
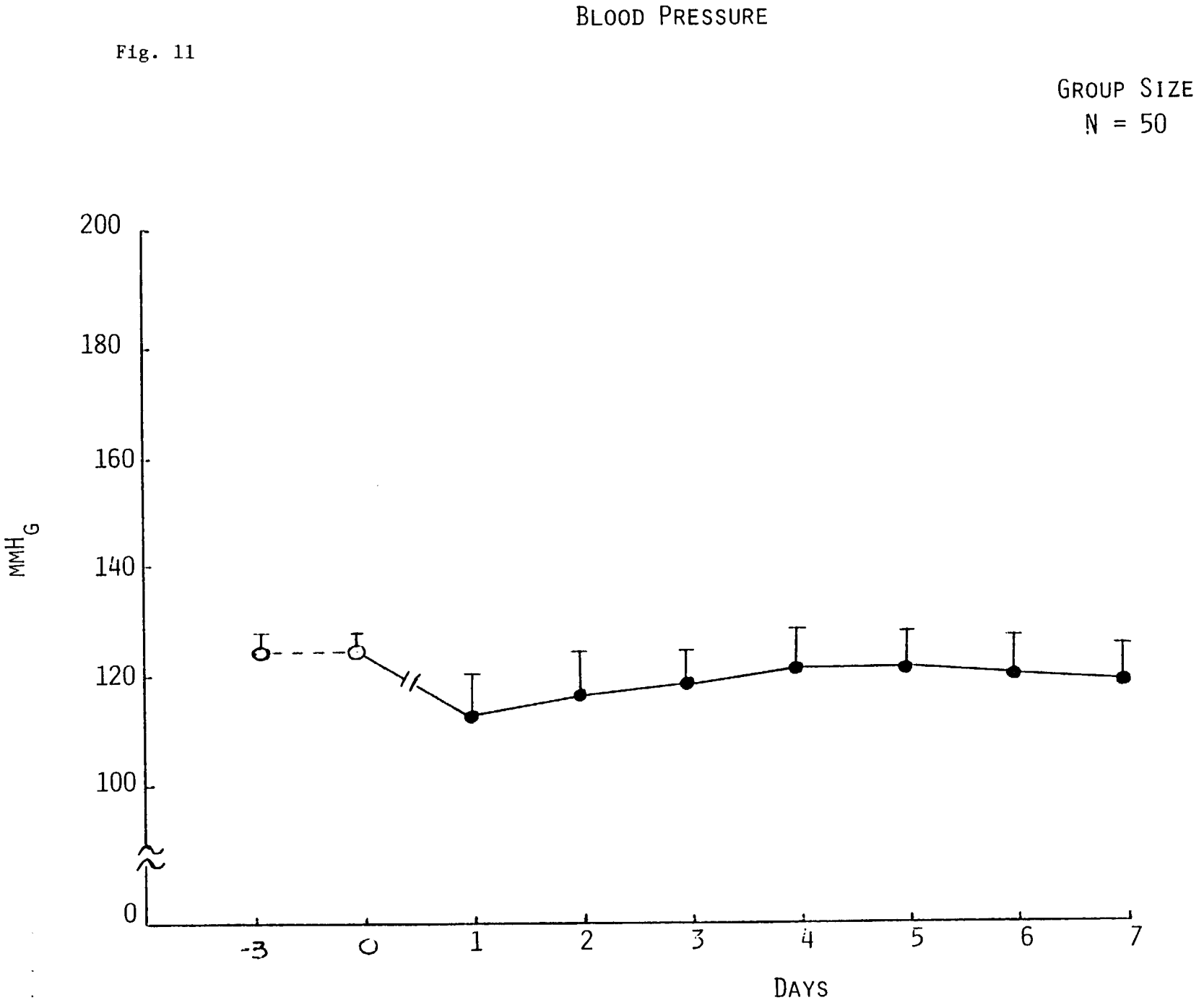


Fig. 10 Heart rate, right atrial pressure and mean arterial blood pressure (± SD) of eight rats prior, during and after exposure to hypokinesia with -30° tilt.

Fig. 11


BLOOD PRESSURE
GROUP SIZE
N = 50
200
180
160
140
120
100
0
MMH$_G$
-3
0
1
2
3
4
5
6
7
DAYS

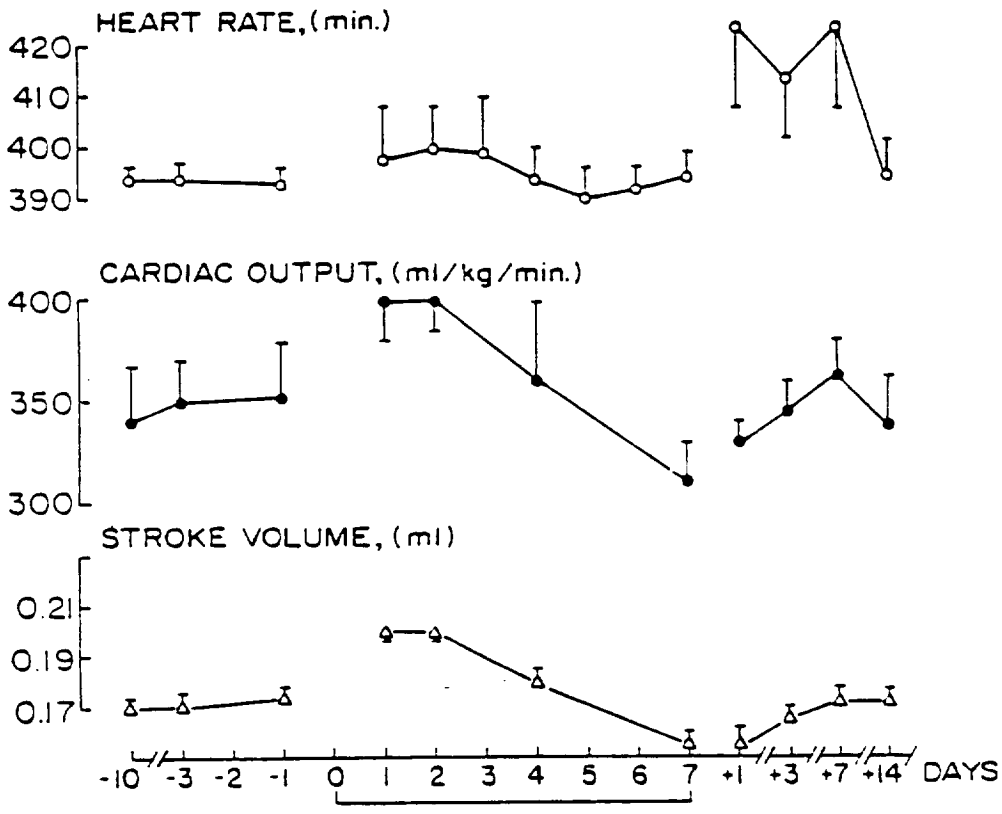


Fig. 12 Heart rate, cardiac output, and stroke volume during and after seven days long hypokinesia.

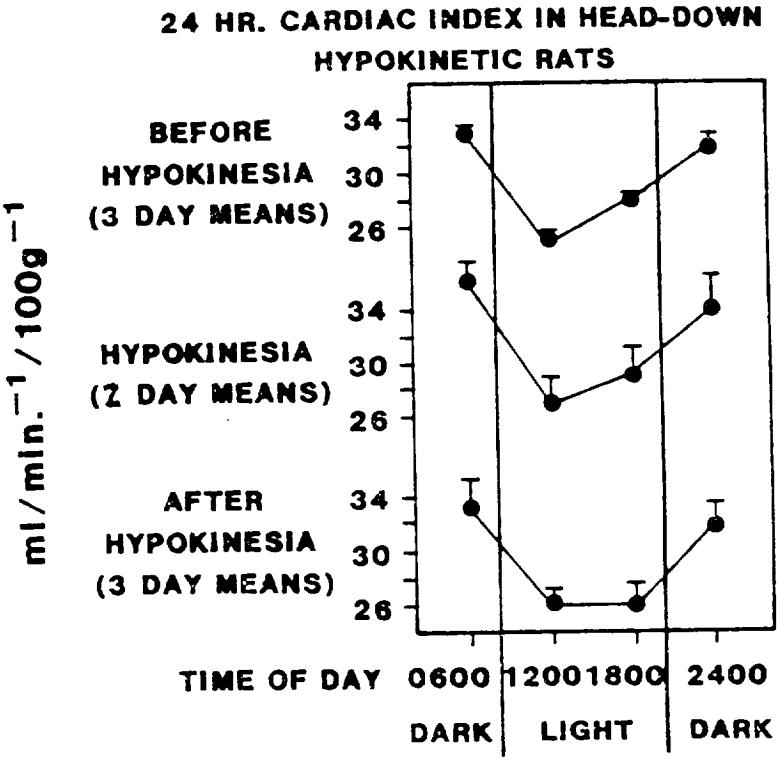


Fig. 13 Circadian rhythm and cardiac index.

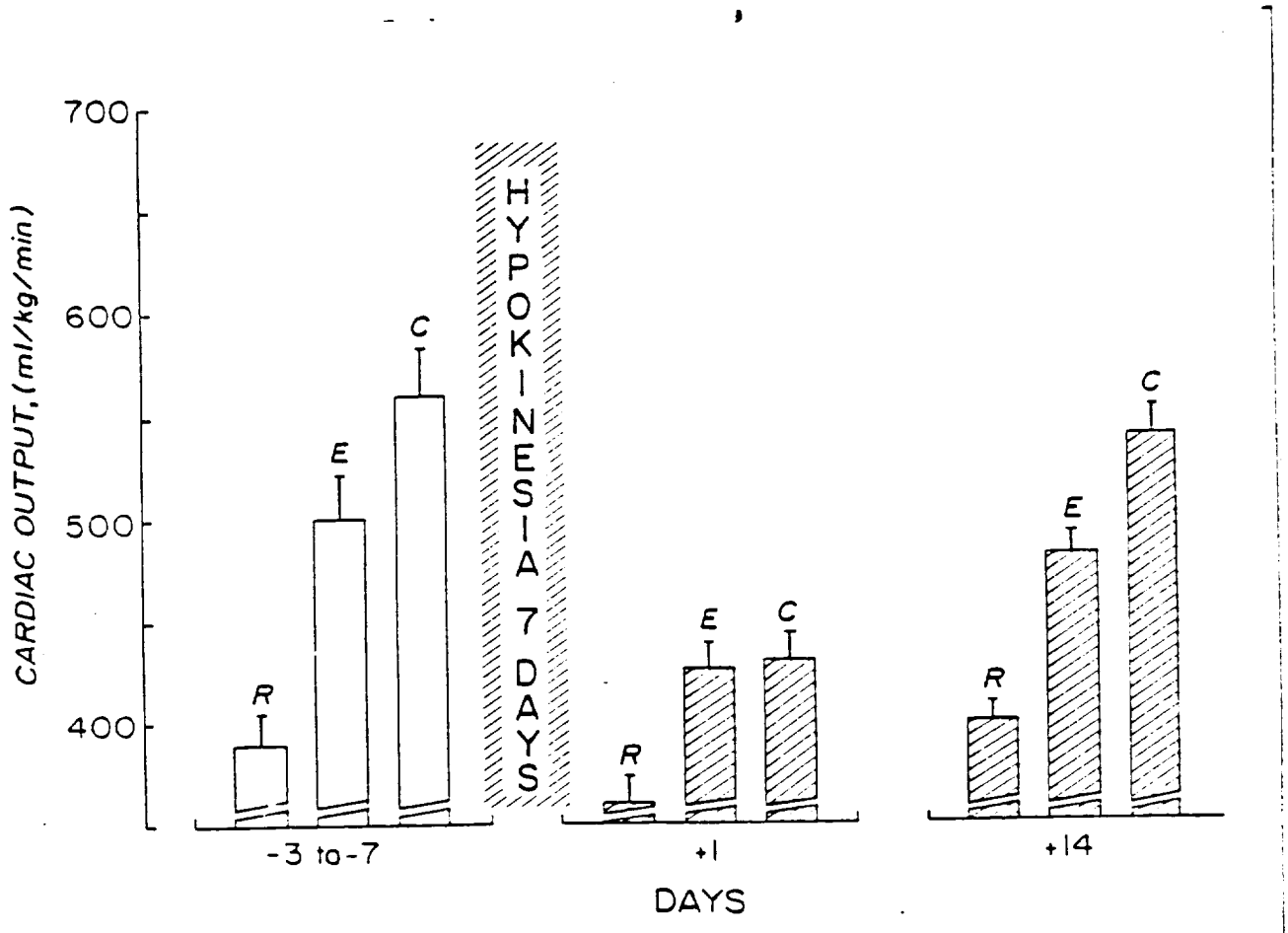


Fig. 14 Cardiac output (ml/min/kg) of resting (R), exercising on a treadmill at 10 m/min (E), or cold (C) exposed (10°C room temperature) rats prior (seven to three days) and after seven days hypokinesia.

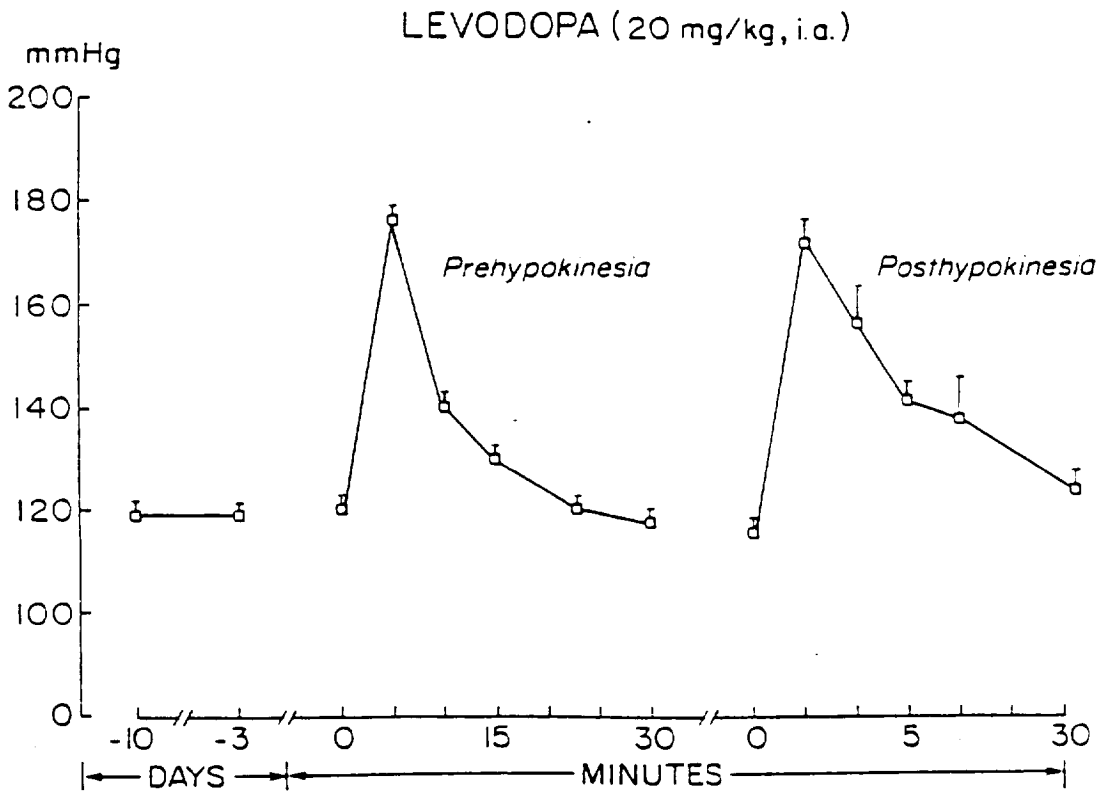


Fig. 15. Levodopa-induced mean arterial blood pressure of rats before and after seven days long hypokinesia with -30° tilt. The mean arterial blood pressure was measured 10 and 3 days before administration of levodopa in the resting rats.

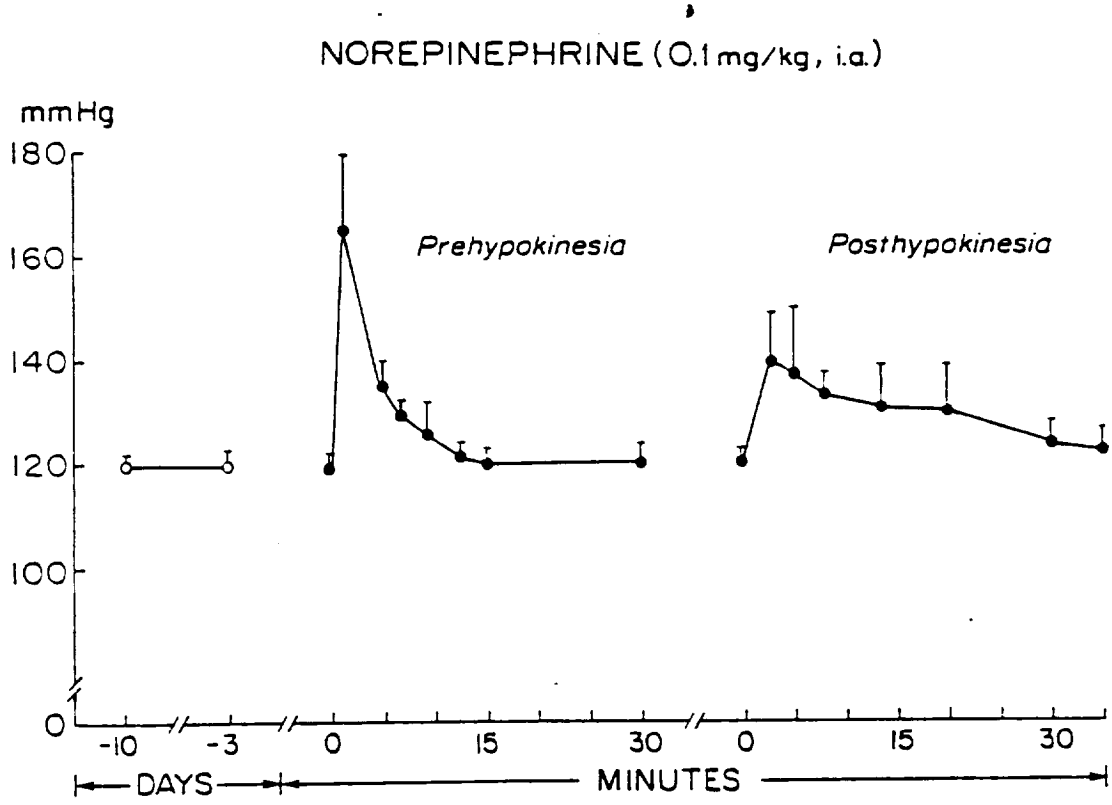


Fig. 16. Mean arterial blood pressure in the resting rats (10 and 3 days prior to hypokinesia) and hypertensive responses after administration of norepinephrine one day before and one day after seven day long hypokinesia.

EFFECT OF HEAD-DOWN HYPOKINESIA ON CIRCULATION IN THE RAT

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Popovic, Vojin. Effect of head-down hypokinesia on circulation in in the rat. J. Appl. Physiol. Respirat. Environ. Exercise Physiol.

Circulatory changes during exposure of rats to 30^{o} head-down (anti-orthostatic) hypokinesia have been studied using a recently developed suspension rat model. Head-down hypokinesia was used because it mimics some of the physiological effects of weightlessness. Unanesthetized Sprague-Dawley rats with chronically cannulated aorta and right atrium of the heart were used in the experiments. Circulatory parameters of resting rats were measured before the animals were suspended, during suspension and after the release from the harnesses. In the early part of the seven day long exposure heart rate, right atrial pressure cardiac output and stroke volume were increased while mean arterial blood pressure remained unchanged. The circulatory parameters returned to control values on day three of the antiorthostatic hypokinesia while cardiac output decreased until the end of hypokinetic exposure. The results indicate that the antiorthostatic hypokinesia in rats, useful for investigations of possible physiological changes induced by weightlessness, might be a good model to study circulation as well.

Antiorthostatic hypokinesia, unanesthetized rats, heart rate, right atrial pressure, mean arterial blood pressure, cardiac output, stroke volume.

INTRODUCTION

Gravity has a profound effect on some physiological processes as, for instance, distribution of body fluids, regulation of blood volume, water balance and electrolyte metabolism. Bed rest in man, without or with head-down tilt (3, 5, 6, 15), or head-down hypokinesia in animals (1, 10-14, 18, 19, 21) mimic some of the physiological consequences of weightlessness. In order to study the effects of head-down hypokinesia, a rat model was recently developed (1, 10-14). The hind legs are "unloaded" and the rat is made hypokinetic, while the head is tilted down, inducing a shift of body fluids toward the chest and the head. The term "antiorthostatic hypokinesia" was used for this model, while orthostasis was used to describe the normal horizontal position in the animal. This animal model was used in our experiments to measure right atrial pressure, heart rate, mean arterial pressure, cardiac output and stroke volume in rats before and during seven day long antiorthostatic hypokinesia (duration of a Space Lab mission), and during readaptation of the same animals to free activity.

MATERIALS AND METHODS

Animals. Thirty six adult male Sprague-Dawley rats were housed individually and given food (Purina Chow) and water ad libitum. The animals were brought to the animal facilities from the breeding colony (Camm, New Jersey) with a body weight of 50-60 g. The lighting cycle in the animal rooms was 8:00 a.m. to 8:00 p.m. and the temperature was controlled at $24^{o}C \pm 1^{o}C$. After growing to a body weight of 160 g, a polyethylene cannula (PE10) was implanted into aorta of each animal via the left common carotid artery (16, 17). The opposite end of the cannula was exteriorized at the back of the animal's neck. Another PE10 cannula was implanted at the same time in the right atrium of the heart (16, 17). Implantation of both cannulas lasted 8-10 minutes and it was done under light Halothane * anesthesia. Once implanted the cannulas stay patent for months, often for the lifespan of the animals (17). The circulatory measurements were initiated after body weight of the rats reached 200 ± 10g.

Antiorthostatic hypokinetic animals. After control measurements eighteen cannulated rats were suspended in the denim harnesses. The head-down suspension (30^{o}) lasted seven days. The suspension technique of Morey-Musacchia (1, 10-14) was used.

Control animals. Eighteen rats with the cannulas were used in this group.

Measurements. All of the circulatory values were obtained in rats resting for at least 8 minutes. Right atrial pressure, heart rate, and mean arterial blood pressure were measured through the chronically implanted aortic cannula. Each measurement lasted five to six minutes. The right atrial pressure, heart rate and mean arterial pressure were measured two times before

* Kindly supplied by Ayers Laboratories.

exposure to hypokinesia, during hypokinetic exposure and three times after the exposure. The hypokinetic measurements were initiated 2 hours after placement of the animals in the harness. Cardiac output was measured using the indicator dilution technique. After injection of 2.5 u Ci of ^{14}C-antipyrine into the right atrium of the heart, the blood from the aortic cannula was collected for 12-15 seconds on a moving absorbent paper (approximately 0.1 ml). The paper was dried and cut into 10-15 pieces (each piece representing one second of absorption). After weighing, the pieces were placed in counting vials and 15 ml of liquid scintillation solvent (Bray's) was added. The radioactivity was counted in a scintillation counter. The cardiac output value was calculated on the basis of a second-by-second measured radioactivity. Stroke volume was calculated.

Body weight, and deep colonic temperature were recorded daily.

All measurements were done between 9:00 a.m. and 12:00 a.m. to avoid the effects of circadian variations.

RESULTS

Antiorthostatic hypokinetic animals. Body weight of the rats decreased during the first 48 hours of antiorthostatic hypokinetic exposure. After that body weight increased and returned to the prehypokinetic value at the end of the 7 day long suspension. The deep colonic temperature, measured with thermocouples was unchanged (Figure 1).

Right atrial pressure, heart rate, cardiac output and stroke volume of the rats were increased in the early part of antiorthostatic hypokinesia while mean arterial blood pressure was unchanged (Figure 2). The right atrial pressure rose from 0 to 6 mm Hg and the heart rate from 380 to 405/minute.

On the third day of antiorthostatic hypokinesia, the circulatory parameters returned to prehypokinetic values. During the subsequent days, the measured values were similar to the values observed before exposure. The initially increased cardiac output began falling from the second day of antiorthostatic hypokinesia (Figure 2) reaching on the seventh day value twenty percent lower than before hypokinesia (280 instead of 328 ml/kg/min).

During readaptation to free activity, the heart rate was initially elevated, the right atrial pressure and mean arterial pressure were unchanged while the cardiac output was somewhat lower than in the same animals before exposure to antiorthostatic hypokinesia.

DISCUSSION

In man, weightlessness induces physiological changes that lead eventually to adaptation to the new environment. The effect of weightlessness on the cardiovascular system appears to be especially profound. In the early beginning of a space flight, there is a redistribution of body fluids, a shift toward the chest and head (20). The central venous pressure is probably increased and the increase endures 12 hrs (7). Stretching of the atria of the heart occurs with consequent neurohumoral adjustments and a profound decrease in the blood volume. Because of the obvious technical difficulties, the suspected circulatory changes have not yet been studied during actual space flights. Postflight measurements suggest that cardiovascular performance of the astronauts is decreased for some time and that the decrease is induced by the circulatory mechanisms acquired during weightlessness.

Accepted methods to simulate and to study physiological adaptation to decreased gravity are bed rest in man (2, 3, 5, 6, 15, 23) and hypokinesia in animals. Head-down bed rest in man (5, 6, 9, 15, 23) and head-down hypo-

kinesia in animals (1, 10-14, 18, 19, 21) appear to be models that best mimic physiological effects of weightlessness. Though increase from 5^{o} to 12^{o} in head-down tilt intensifies and accelerates circulatory changes and seems to mimic better blood volume distribution in weightless man (4-6), a 5^{o} head-down position seems more comfortable and therefore used more often in human studies. A 24 hour long 5 head-down tilt leads in man to a decrease in extracellular fluids while intracellular fluid compartment does not change. To induce similar cardiovascular changes in laboratory animals a larger head-down tilt is necessary, 20^{o} or 30^{o}. The difference in the degree of the head-down tilt might be the consequence of a smaller hydrostatic pressure change induced in laboratory animals by the tilt.

The antiorthostatic rats lost body weight during the initial 2 to 3 days of hypokinesia in our experiments and in experiments of other investigators (1). Body weight losses were also reported in astronauts during space flights. Body weight loss is induced by a decreased water and food intake, and by an increased water excretion. In our rat model, the body weight loss might have been also induced by a high metabolic rate brought forth by hyper-activity of the animals struggling to escape from the harnesses and head-down position.

In the early part of the antiorthostatic exposure, cardiac output of the rats was increased indicating a larger filling of the heart but also an increased metabolic rate as well. The initially increased cardiac output of the rats decreased continuously during the duration of exposure to head-down hypokinesia. At the end of the seven days of exposure the cardiac output was below the prehypokinetic values. There was no change of cardiac output in head down resting man (15) while other investigators found an increased cardiac output in the same position (4). The right atrial pressure of the

antiorthostatic rats was increased in early head-down hypokinesia demonstrating a fluid shift toward the chest. In our earlier work (18) measurements of the central venous pressure were initiated 18 hours after the rats were made antiorthostatic. In the present experiments the first measurements were made after 2 hours. The increased central venous pressure in head-down position seems to return to normal value after 12 hours in man (15) or after 1-2 days in the rat (18, 21). The difference might be explained by a somewhat smaller increase of the central venous pressure observed in the 5^{0} head-down tilted man (15).

The mean arterial blood pressure was unchanged in the antiorthostatic rats though in previous experiments we found a slight decrease (18). Other authors found an increased arterial blood pressure during the early exposure (14). It is possible that conditions under which the measurements of the arterial blood pressure were done in our experiments, e.c. (only between 9 a.m. and 12 a.m. and only at rest) contribute to the observed difference. In the head-down tilted man, some authors found a temporarily decreased arterial blood pressure and an increased cardiac output (4) while others did not (15).

In conclusion, it seems that the antiorthostatic hypokinetic rat model is predictive of circulatory changes seen in man during weightlessness and that the model can be used to study circulatory mechanisms in man during space flights. Whether the same model might serve to study circulatory changes in rats and in other animals during weightlessness will be known after the Space Lab IV experiments in which cardiac output and blood pressures will be measured.

(Supported by NASA Grant NAG-2-87)

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Legends

Fig. 1. Deep colonic temperature of head-down hypokinetic rats

Fig. 2. Circulatory parameters in head-down hypokinetic rats.

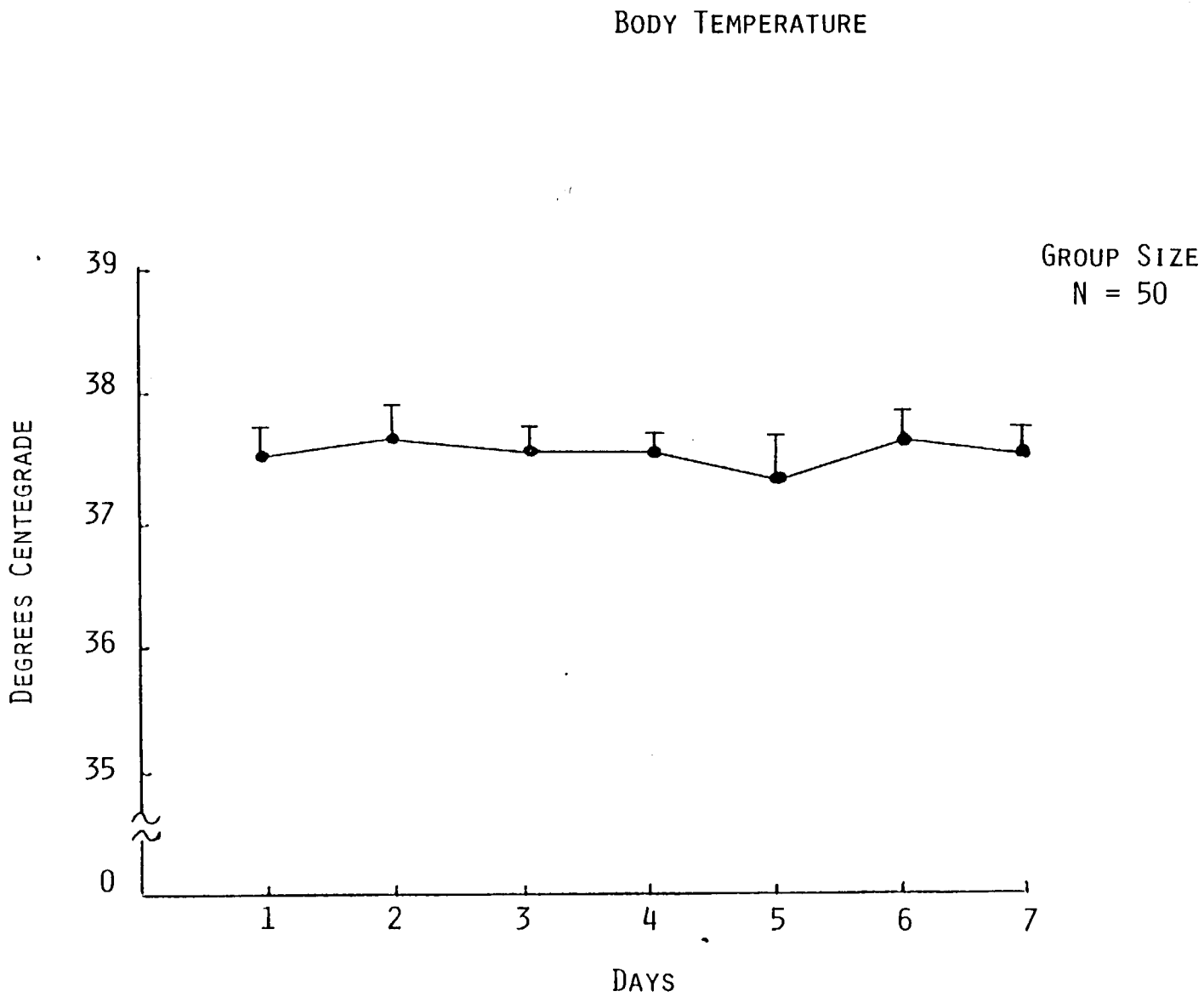


Fig. 1. Deep colonic temperature during antiorthostatic hypokinesia.

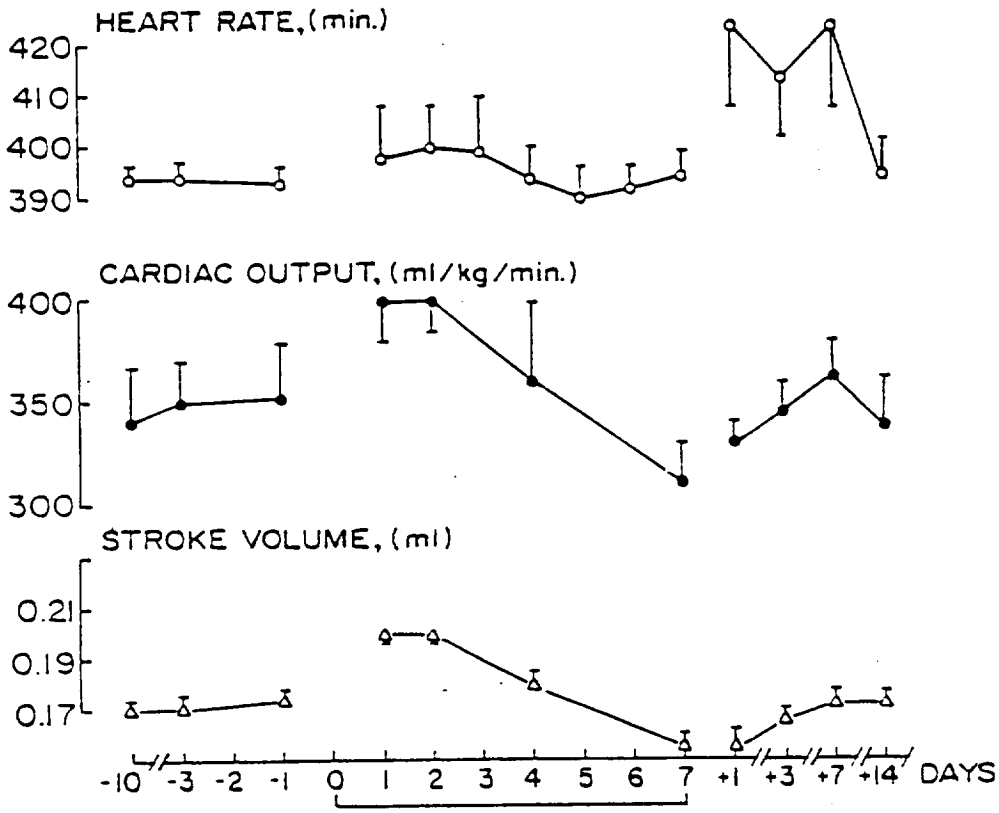
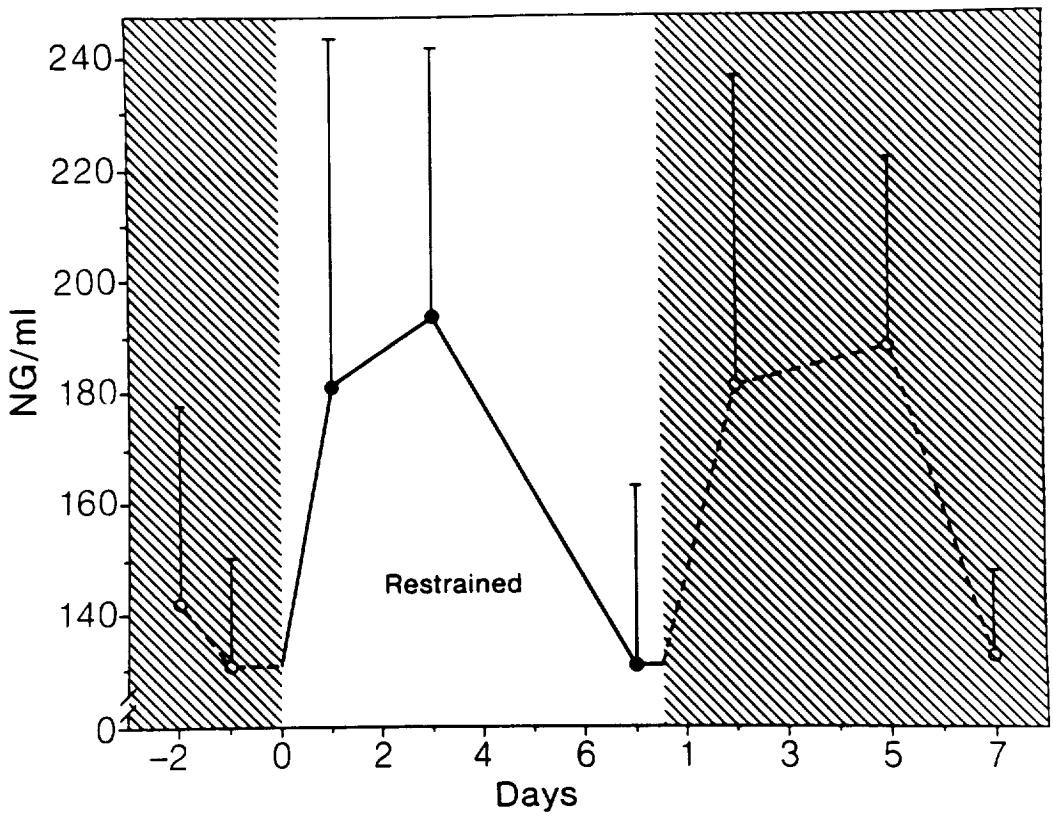


Fig. 12 Heart rate, cardiac output, and stroke volume during and after seven days long hypokinesia.

Fig. 1 Plasma ACTH in rats before, during and after head-down hypokinetic



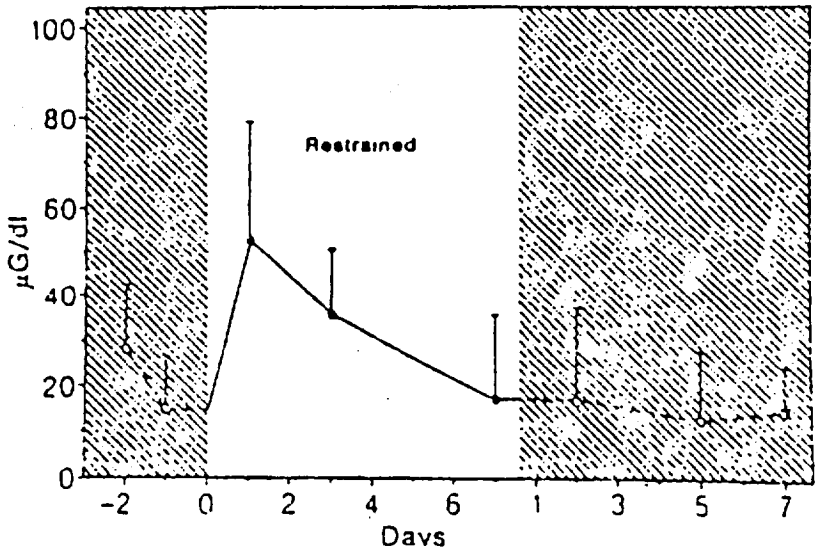


Fig 2. Plasma corticosterone

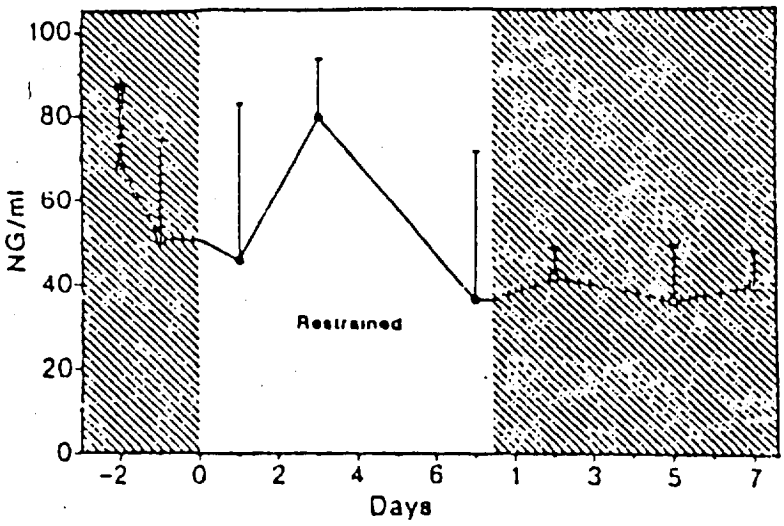


Fig 3. Plasma prolactin before, during and after head-down hypokinesia.

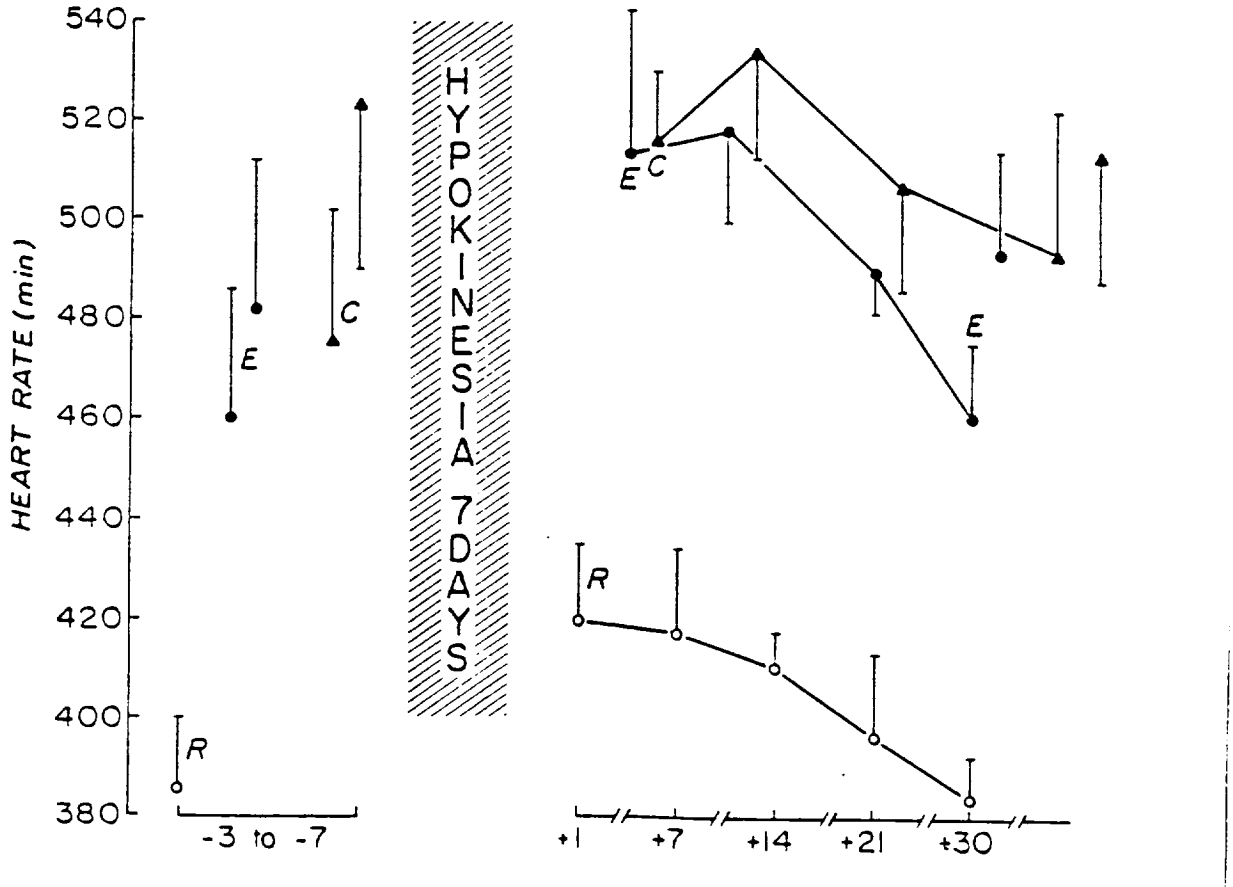


Fig. 17 The heart rate during rest (R, open circles) and during light or heavy exercise (E, closed circles) or during two levels of cold exposure (C, triangles) of rats prior (seven to three days) to head-down hypokinesia that lasted seven days and after hypokinesia (1,7,14,21 and 30 days).

STRESS HORMONES IN ANTIORTHOSTATIC RATS

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Popovic, V., Popovic, P., & Honeycutt, C. Stress hormones in antiorthostatic rats. J. Appl. Physiol. Respirat. Environ. Exercise Physiol., (1988).

Hypokinesia, especially head-down (antiorthostatic) hypokinesia, mimics some of the physiological effects of weightlessness. This has been shown in human and in animal experiments for bone turnover, muscle and cardiovascular system. Using antiorthostatic rat model, we have already shown that hypokinesia leads to circulatory changes. In the present work, plasma "stress hormones" (corticosterone, ACTH and prolactin) were determined in rats during a seven day long antiorthostatic hypokinetic exposure. The stress hormones were increased during the early hypokinesia. Toward the end of seven day exposure, plasma hormones were back at the control level indicating adaptation of animals to the harness hypokinesia and head-down position.

Head-down hypokinesia, unanesthetized rats, chronic aortic cannula, ACTH, corticosterone, prolactin.

INTRODUCTION

Exposure to weightlessness induces certain physiological changes. Hormonal synthesis and hormonal release are altered during space flights. Some hormones in Skylab inflight measurements were found increased, as for instance level of plasma cortisol, but others decreased, for instance plasma ACTH. Hormonal changes are especially likely to occur during early exposure to weightlessness. Hormones that are under 1 g condition play a role in postural adaptations are likely to be affected more than others. In weightless man hydrostatic pressure disappears and renin-aldosterone-vasopressin axis is activated leading to a modified synthesis and release of hormones and to consequent circulatory alterations. Furthermore, stress increases plasma corticosterine, ACTH, and prolactin (Kvetnansky et al 1980). These stress hormones are likely to increase in the early part of space flights.

In order to study possible hormonal changes that occur during exposure to weightlessness, antiorthostatic hypokinesia in rats was used in our experiments. Antiorthostatic hypokinesia mimics some physiological effects of weightlessness (Morey, 1979; Musacchia et al., 1980; Popovic, 1981). We have shown that circulation in head-down hypokinetic animals is altered (Popovic, 1981). In the present experiments levels of three "stress hormones" (ACTH, corticosterone and prolactin) were determined in plasma of rats with chronic aortic cannulas before, during seven-day long head-down hypokinesia, and after.

METHODS

Thirty-three adult male Sprague-Dawley rats (200 ± 10g) with chronically implanted aortic cannulas (Popovic and Popovic, 1960) were used in the experiments. The aortic cannulas were implanted fifteen days before the beginning of the experiments. Use of cannulated rats permitted the sampling of mixed arterial blood from the same unanesthesized resting rats before, during and after exposure to antiorthostatic hypokinesia. The Holton-Musacchia system (7, 8) was used to induce the antiorthostatic hypokinesia. After being placed in a denim harness, the head of the animal was tilted down 30^{0} and hind legs of the animal thus unloaded. The antiorthostatic hypokinesia lasted seven days, the expected duration of the Spacelab IV flight. Plasma hormone levels were determined by radioimmunoassays (YALOW, 1987). The blood (0.3 ml) was sampled from the chronic aortic cannula two times prior exposure to the antiorthostatic hypokinesia, three times during antiorthostatic hypokinesia (days one, three and seven) and three times after release of the animals from the harness (days two, five and seven). The blood was withdrawn from the animals between 9 a.m. and 12 a.m. to avoid any circadian effects.

RESULTS

Antiorthostatic hypokinesia brought forth an increase in plasma ACTH on day one and day three. The plasma ACTH level returned to normal value before or on day seven of the exposure. (Fig 1) Plasma corticosterone and plasma prolactin concentration were increased on day one of the antiorthostatic exposure but returned to control values on day three (Figs 2 and 3)

After removal from the harness, the plasma corticosterone and plasma prolactin remained unchanged, near the control level, but plasma ACTH level was elevated. The plasma ACTH returned to control value on day ten.

DISCUSSION

Gravity has a profound effect on mammalian organisms. It seems that the effect of gravity on the cardiovascular system is especially great. It has been reported that certain circulatory adaptations occur during man's exposure to weightlessness . After return to earth, new circulatory readaptations have been observed. The main circulatory changes include a cephalad shift of blood and of body fluids and overloading of both atria of the heart. This leads to neurohumoral stimulation, to excessive water loss and blood volume decrease. As a consequence of these adaptive changes, orthostatic tolerance and work ability in men are decreased after return to earth.

Using the rat model that mimics circulatory consequences of weightlessness (Morey, 1979; Musacchia et al., 1980), we have shown that antiorthostatic hypokinetic rats have increased stroke volume and increased cardiac output as well as an increased central venous pressure (Popovic, 1981). The increased cardiac output and increased stroke volume might have been induced by the headward blood volume shift, but they might have been also induced by an increased metabolic rate due to struggling of the animals that try to escape from the harnesses. In order to study this hypothesis, stress hormone levels were measured in the arterial blood of the antiorthostatic hypokinetic rats. Thus the determination of hormone levels represents an attempt to quantify the magnitude of stresses imposed by the head-down position and by placement of the animals in the harness.

Concentration of plasma stress hormones in head-down hypokinetic rats was increased during the first few days of exposure indicating that the early exposure to antiorthostatic hypokinesia represents clearly a stress. The metabolic rate of the rats was probably increased. But the animals adapt rapidly to the new situation. The concentrations of stress hormones returned to the resting level at the days three to seven of the antiorthostatic hypokinesia. It has been reported that space rats (Cosmos series) after being 18-22 days weightless had also an increased plasma corticosterone (Gazenko et al., 1984). the basis of these and some other histological and histochemical findings Gazenko and his coworkers conclude that space flight induced stress reactions remain at a moderate level. On the other hand Kvetnansky et al. (1981) found unchanged plasma catecholamine levels in the rats after space flights.

Our results show that plasma concentration of ACTH, corticosterone and prolactin is increased during the early exposure of rats to antiorthostatic hypokinesia and that the increased stress hormonal levels are probably one of the factors leading to elevation of the cardiac output observed in the early part of antiorthostasis. The antiorthostatic hypokinetic animals adapt to the new situation very fast. After a few days in antiorthostesis, the plasma stress hormone levels returned to control values.

CONCLUSION

Early exposure to antiorthostatic hypokinesia induces stress. The stress is one of the factors that leads to elevation of cardiac output during early exposure to orthostatic hypokinesia. The animals, however, adapt to the new situation quickly. Seven days later stress induced plasma hormonal changes disappear.

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