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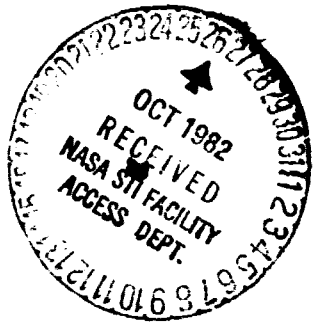

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OBJECTIVES

A unique change occurring only in space flights is the absence of gravity. Agravity affects several physiological functions and several organ systems. The absence of gravity has a profound effect on cardiovascular performance. In order to elucidate the effects of altered gravity on circulation, we have studied circulating mechanisms in head-down hypokinetic rats. Antiorthostatic hypokinesia mimics many of the circulatory changes and circulatory adaptations observed in man during space flights. Adequate experimental techniques already developed in our laboratory were used in the work. Cardiovascular studies were performed on unrestrained, unanesthetized rats and on the same animals in head-down hypokinetic conditions as well as during readaptation of the same animals to free activity. An already developed rat suspension system (Morey-Musacchia) was used to identify possible circulatory mechanisms that evolved in mammals during long-lasting gravity exposure. These mechanisms are likely to be affected during exposure to 0-g forces. The last part of this work will be done during the exposure of chronically cannulated rats to the conditions of Space Lab IV environment (October, 1985).

Circulatory adaptations occur during exposure of man to weightlessness (see "Background"). New circulatory readaptations have been observed after the return of a man to earth. We have shown that antiorthostatic hypokinesia induces cardiovascular changes similar to those observed in space flights. We have further shown that orthostatic hypokinesia is not an adequate model to study 0-g induced circulatory changes. We have also demonstrated that both Morey suspension model and Musacchia suspension model induce similar circulatory adaptations and give similar results. We have studied some of humoral mechanisms that occur during exposure to antiorthostatic hypokinesia as well as

during readaptation of rats to control conditions (after hypokinetic exposure). We believe that the study contributes better understanding of mammalian circulatory mechanisms that operate under 1-g and 0-g forces.

Surgery and anesthesia drastically decrease cardiac output and other circulatory parameters in rats. Therefore, only unanesthetized rats were used in the experiments. The aorta and right atrium of the animals were permanently cannulated fifteen days before experiments. Arterial and right atrial pressures, cardiac output, and other cardiovascular parameters were measured with techniques routinely used in our laboratory.

METHODS AND PROCEDURES

Animals and procedures. 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 the proposed work.

Rats have been used more 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 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 evaluation) can be used in ground-based experiments, in hypokinetic conditions, and in Space Shuttle/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. After permanent cannulation of the aorta and right ventricle of the heart, the same rat can serve as its own control, before, during, and after a space flight or exposure to hypokinesia. Rats have been chosen by NASA to be the experimental animals for the first Space Lab (Space Lab IV in 1985) scientific project and the Principal Investigator's proposal was tentatively selected for this flight.

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

All rats are brought to our animal rooms from the breeding colony (Harlan-Sprague-Dawley, Madison, Wisconsin) at an early age, with a body weight of 40-60 g. They 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 rat strain are eliminated. The lighting cycle in the rooms is 8:00 a.m. - 8:00 p.m. The temperature is controlled at 24°C ± 1°C.

Chronic cannulation of the aorta and of the right ventricle of the heart. A polyethylene PE10 cannula is placed into the aorta through the left common carotid artery. This procedure is being done when the body weight of the rats reaches 180 g. One tip of the cannula is located in the aortic arch while the opposite end is exteriorized at the back of the animal's neck. Implantation is done under light Halothane anesthesia. Once implanted, the aortic cannula stays patent for extended periods and if necessary for the life-span of the animals (1, 2).* Cannulation of the right atrium of the heart is done simultaneously with the aortic cannulation, i.e. 15 days prior to an experiment. Until now, over 16,000 rats have been cannulated in our laboratory. Adverse effects of cannulation of the aorta, right ventricle (or atrium) of the heart have never been described, nor has published criticism of this procedure been offered. Our own studies have shown that there is no regurgitation of blood between right ventricle and right atrium (2). Histologically, the walls of the blood vessels and the right heart are unchanged. The cannulation technique was found useful for cardiovascular studies in rats and in other small laboratory animals (3-21). 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 right

*Number refers to the corresponding number of reference (see References)

ventricle) has shown no changes in the blood vessels, brain tissue and other tissue that were examined. The 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 and intraventricular ECG. Were monitored through intravascular implanted cannulas ("intraventricular ECG"), thus the movements and electrical activity of the exercising muscles do not distort the ECG tracings.

Arterial blood pressure recordings and right ventricular pressure. Were measured through the chronically implanted cannulas (2, 3). Right arterial pressure was measured by direct techniques in unanesthetized animals, through chronically implanted cannulas.

Cardiac output. Cardiac output was measured on the basis of the Fick principle. We have adapted the Hamilton-Kety technique (Landau et al., 1955; Hamilton, 1962; Reivich et al., 1969; Goldman and Sapirstein, 1973; Goldman et al., 1973; etc.) for our chronically cannulated rats. The technique of cardiac output determinations consists of injection of 2.5 μCi of ^{14}C-antipyrine into the right ventricle of the heart (through chronically implanted micro-cannula) and collection of the arterial blood into a small nonheparinized hematocrit tube or on a moving absorbent paper. The tube is immediately frozen and then cut into several parts each representing filling lasting one second. Blood from each cut piece is placed in a counting vial to which 15 ml of liquid scintillation solvent (Bray's) is added after weighing and the radioactivity is counted in a Packard Tri-Carb counter. The cardiac output values are calculated on the basis of second-by-second measured radioactivity. The results obtained with this technique were compared with another Fick principle technique (also routinely used in our laboratory) in which the cardiac output is

calculated after determining O_2 consumption of cannulated, unanesthetized resting rats while sampling the mixed venous blood from the right ventricle of the heart and from the aorta.

Our cardiac output values obtained from individual animals show good reproducibility. Two measurements done on the same day at one hour invervals 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 minutes 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%).

A new technique (electromagnetic flow probe technique) was used to measure cardiac output in unanesthetized rats in our laboratory. This was done because of our involvement in the work of Space Lab IV. Carolina Medical Electronics (King, NC) was the supplier for the chronic electromagnetic flow probes. The standard EP400 probe was used or a modified probe (EP100 series) depending on the diameter of the aorta on which the probe was implanted. We were using the probes that are 7 mm or 8 mm in lumenal circumference. Prior to the experiment, all probes were calibrated. Calibration was performed with rat aorta and whole blood. Nembutal was surgical anesthesia for all implantation procedures. Flow-probe implantation was via an intercostal thoracotomy at approximately the third right intercostal space. A rodent respiratory set was used to maintain ventilation during the thoracotomy. The entire recording system for aortic flow had a frequency response of approximately 80 Hz. The

hemodynamic variables measured are pulsatile and mean ascending aortic flow and mean aortic pressure. We are recording these at a paper speed of 10 mm/sec to obtain heart rates and then at 250 mm/sec to obtain accurate values of peak aortic flow velocity. Hemodynamic variables derived from the above data are cardiac output (ml/min, considered equal to ascending aortic flow), cardiac index (cardiac output/100g body weight), stroke volume (ml/beat) as cardiac output x heart rate^{-1}, stroke index (cardiac index x heart rate^{-1}), stroke work (mean arterial pressure x stroke volume), minute work (mean aortic pressure x cardiac output), normalized minute work (mean aortic pressure x cardiac index), and total peripheral resistance (mean arterial pressure x cardiac index^{-1}).

Blood volume determinations. Many of the techniques of blood volume determination were developed before the 1950s. Blood volume and extracellular space of rats have been determined a number of times. The often used method is ^{51}Cr obtained in form of $Na_2{}^{51}CrO_4$. Radioactive Chromium (Cr^{51}) was introduced first by Sterling and Grey in 1950. In the hexavalent anion form, 90% of the chromium binds to the globin moiety of hemoglobin. The binding is stable with a half life of 27.8 days and when the tagged red cells hemolyze, unlabelled red cells do not pick up the Cr^{51}. Labelling is done in vitro and the labelled red cells injected back into the donor or the experimental animal. The rate of Cr^{51} disappearance from the blood is exponential. This makes it suitable for use in repeated sampling experiments where label remains. After samplings of blood and slight centrifugation the erythrocyte sediment is suspended in a physiological solution to 1 ml. The mixture is stored for one hour after 15 μ Ci ^{51}R is added (at room temperature). After another centrifugation the erythrocytes are washed twice with saline, the plasma is added and the suspension serves as the radioactivity standard. The aortic blood samples are taken 60 minutes past administration of ^{51}Cr erythrocytes. Hematocrit ratio is measured at the same

time (1500 x g for thirty minutes and corrected by a factor of 0.96).

An accurately measured volume of ^{51}Cr labelled cells prepared from a donor rat and ^{125}I albumin (for Red Blood cell volume determination) is administered in the same syringe. A 1:1000 dilution of the standard is prepared to determine the quality of tracer administered. A background blood sample is also drawn for analysis. Plasma volume is calculated according to the equations:

$$DV = \frac{(\text{Std ct/ml/min} - \text{Bkg1}) \times \text{Dil Factor} \times \text{Vol injected}}{\text{Zero-time ct/ml/min} - \text{Bkg2/ml/min}}$$

where Bkg1 = atmostpheric background unit time

Bkg2 = patient background/unit volume/unit time

Plasma volume = DV x (1 - LVH)

Similarly, red cell volume is calculated from the equations:

$$DV = \frac{(\text{Std ct/ml/min} - \text{Bdg1}) \times \text{Dil Factor} \times \text{Vol injected}}{\text{Equilibrated dil ct/ml/min} - \text{Bkg2}}$$

Red Cell Volume = DV x LVH
Total Blood Volume = Plasma Volume + Red Cell Volume

Oxygen consumption was measured continuously by an open system (Depocas and Hart, 1957) 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.

Hematocrit ratio was determined in sampled aortic blood. (In a few specific cases, both aortic as well as right ventricular hematocrit ratios were determined.)

Treadmill exercise. In order to study the effect of weightlessness on work performance, cardiac output and other circulatory parameters were measured in the exercising rats before and after hypokinesia. Two levels of exercise (10 m/min and 20 m/min) were used to test the animals.

Induction of head-down hypokinesia. Holton's model system (1979) was used in our experiments to induce hypokinesia in rats. The system includes 1) the ability for 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 those presently existing. The animal is free to move about a 360° arc at will. The rear limbs are totally unloaded, but unrestrained. We are presently using denim harness, a modification of the Holton system. The rat is tilted -20°, but the animal's free movement of 360° is restricted to 180° arc.

Introduction of orthostatic hypokinesia. The same system as described above will be used, but without head-down tilt.

Determination of "stress" hormones in blood. To assay five blood hormones the radioimmunoassay method was employed. This method was developed by Berson and Yalow in 1960 and is based on the principle of isotopic dilution in the presence of specific antibodies. The radioimmunoassay of hormones in body fluids depends 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 specifically measures quantities of protein or a small hapten in the nanogram or picogram range and allows the simultaneous determination of many 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 fluids. The radioimmunoassay principle has been used successfully for a number of years for the measurement and study of polypeptides, hormones and steroids. Because of their simplicity, these radioimmunoassays are applicable to the analysis of a large number of samples. they are also rapid, sensitive, specific, reliable and require a minimum quantity of blood.

ACTH. Rat plasma ACTH is biologically and immunologically very similar to human ACTH, especially in the excellent cross reaction that occurs between rat plasma ACTH and antiserum to human ACTH. This provides a very sensitive method for rat ACTH determination with human ACTH antiserum, human 1251-ACTH and human ACTH standards, reagents which are available from commercial sources (Immunonuclear Corporation. Stillwater, MN). The interassay coefficient of variation of a pool containing 80 pg/ml ATH ACTH was 9% and the interassay was 12%. The sensitivity is 30 pg/ml with 25 µl of the undiluted serum (the manufacturer's specifications require 100 µl of plasma for ACTH determination). The required amount of plasma is only 25 µl.

The main corticosteroid in the rat plasma is corticosterone. We use a direct corticosterone radioimmunoassay which requires only 10 µl of rat plasma. The method is similar to the method of Donohue and Sgoutas (23) for the direct radioimmunoassay of cortisol in human plasma. 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 purification is not necessary since cortisol is not secreted in the rat. Different volumes of diluted serum (5, 10, 25, and 50 µl) were assayed and showed similar

results provided they fell in the span of the standard curve. The intra-assay coefficient of variation of a pool containing 10.35 µg/dl was 5.9% (n=9) and a pool containing 31.9 µg/dl was 6.6% (n=9). The inter-assay variability of the latter pool was 12.0% (n=8). Serum extracted with charcoal showed a value of 1.07 + 0.2µ g/dl (n=4). The sensitivity of the standard curve was 6+ µg/dl when using 4 µl of the diluted serum of 1 µl of undiluted serum. Accuracy was examined by adding corticosterone, 1.25, 2.5, 5, 10, 20, and 40, µg/dl, to charcoal-treated serum and assaying the serum. The regression line was y=1.0009 x +0-.49 indicating that no systematic error is present.

Prolactin levels in plasma were determined by a double antibody and radioimmunoassay method developed by Neill (24). The method was modified so that we use only 25 µl of serum. All reagents, except 125I-rat prolactin, were obtained from the National Pituitary Agency. Rat prolactin radioiodination is carried out in our laboratory as needed. Different volumes of serum were assayed and showed similar results provided they were not less than 50 µl of plasma. The intra-assay coefficient of variation of a pool containing 60 ng/ml rat prolactin was 10% and the interassay 13.5%. The sensitivity is 3.5 ng/ml when using 50 µl aliquot.

BACKGROUND

Previous personal work. Several years ago we developed a rat model with chronically implanted aortic and right atrial cannulas that has been used extensively in cardiovascular, hematologic, pharmacologic, and some other studies. The technique permits adequate, reproducible measurements of the cardiovascular parameters in rats. Until the present time more than 16,000 animals have been cannulated in our laboratory. We are now able to detect and precisely evaluate even small cardiovascular changes, changes that are induced by cold or heat exposure, exercise or exercise training, circadian rhythms, temperature adaptation or other environmental or drug-induced changes.

Hypokinetic antiorthostatic rat model. We use in our work the Holton-Mussacchia (1979-1980) system (20° negative tilt). Each rat is cannulated 15 days prior to the experiment. Only the rats that had anormal growth before and after cannulation were used in the experiments. The rats that had an abnormal WBC more than 8,000 granulocytes/mm, or an increased resting arterial blood pressure were eliminated,* approximating the space shuttle-space lab requirements. Each rat served as its own control (normokinesia for 10 days, hypokinesia for 7 days, and back to normokinesia for 7 to 30 days).

Body weight of the eight experimental animals at the momemt of suspension was 180-200. The body weight of experimental animals during hypokinetic conditions decreased during the initial two days and then returned slowly toward the normal value (Fig. 1). At the end of hypokinetic exposure all animals had somewhat higher body weight tyan in the beginning of the exposure. After the return to free activity, the rats continued to grow as control rats of the same body weight.

Water intake, food consumption and body temperature in antiorthostatic rats are shown in Figs. 2, 3, and 4.

Heart rate of the rats was determined in resting animals only. The heart rate was measured at the same time each day, 9-11 a.m. Each measurement lasted 5 minutes. The heart rate had a very stable value of 390-400 beats per min, before suspension. The heart rate was slightly elevated (no statistically significant differences) the first and second day of hypokinetic exposure Twenty-four hours after return to free activity, the heart rate was increased (significant difference). The resting heart rate was still increased on the 3rd and 7th day after return from hypokinesia. Seven days after hypokinetic exposure the heart rate was back to normal (Figs. 5 and 6).

Right atrial pressure of the resting normokinetic rats were measured twice per day (at 9-11 a.m. and at 4-5 p.m. for 5 to 10 mins.). The obtained value was 0 mm Hg. The negative tilt (-20°) induced an increase in the right atrial pressure that lasted one to two days. From day 3 the right atrial pressure was back to "normal values." (Fig. 5).

The mean arterial blood pressure was measured twice per day between 9 and 11 a.m. and 4 and 5 p.m. The resting arterial pressure was very stable before hypokinesia but decreased slightly from an average value of 118 mm Hg

*Approximating the Space Shuttle-Space Lab requirements.

to 100 mm Hg during the initial hours of the anti-orthostatic hypokinesia. The full return of the normal pressure was observed after two days of hypokinetic exposure. During readaptation to free activity the mean arterial blood pressure of the rats was practically unchanged, with a slight decrease observed on the first day of the readaptation process (Fig.5).

Cardiac output of the resting rats significantly increased during early exposure to hypokinesia. The increase was probably brought forth by an increased stroke but also by an increased metabolic rate volume. The stroke volume was also decreased. From the third day of exposure cardiac output was continuously decreasing, even after seven days of negative tilt hypokinesia. After the return to normal free activity, the value of the cardiac output was somewhat lower than in the prehypokinetic conditions (Fig.6). The cardiac output of the rats returned to the normal value on the third day of posthypokinetic activity.

Heart rate during exercise or during cold exposure after hypokinetic exposure. After seven days of orthostatic hypokinesia, resting heart rate in the rats was increased. Even during the slow exercise (10 m/min) the heart rate of the animals was much increased and the animals refused to run more than 2-5 min. The animals were not able to stand a more strenuous exercise (20 m/min). The exercising heart rate was still high even seven days after cessation of hypokinesia. During the cold exposure (10°C), heart rate was increased and colonic temperature -2°C to -5°C lower than in the control rats. The spontaneous hypothermia that developed at an external temperature of 0°C was so profound that these experiments were termineated before 30 minutes (Fig. 7).

Cardiac output during exercise or during cold exposure after head-down hypokinesia. Cardiac output (ml/min/kg) was measured in resting conditions or during exercise on a treadmill (10 m/min; temperature 20°C) or in animals placed in a cold room (10°C temperature) for 30-60 minutes. Eight animals were used in these experiments. After seven days of head-down hypokinesia the cardiac output of resting, exercising or cold-exposed rats was decreased (statistically significant difference). (Fig. 8).

Levodopa and norepinephrine induced pressor responses after hypokinetic exposure. The pressor responses of rats to administered levodopa after hypokinetic exposure, was the same as in control animals but it was much decreased after norepinephrine administration. (Fig. 9 and 10).

Blood volume. The plasma portion of the blood is a continuous medium (across the capillary membrane) with the interstitial fluid and it forms part of the extra-cellular pool of body water. Its regulation is by the same mechanisms which regulate body fluid volume. The red cell mass, on the other hand, which constitutes the bulk of the cellular component of blood, is regulated as a function of O_2 demand and O_2 supply and it is mediated by erythropoietin. Body fluid regulation is a multifaceted process with involvement of body peripheral and central components. The variables to be controlled are body water and Na^+. On the one hand, blood volume changes are sensed by the atrial stretch receptors which engage the renin-angiotensin-aldosterone system that controls Na^+ retention by the kidney and the hypothalamic ADH system which controls

water reabsorption in the distal and collecting tubules of the kidney. On the other hand, there is evidence for the role of osmoreceptors in the brain distributed along the cerebroventricular system. These receptors seem to be sodium sensitive and to be influenced by the CSF Na^+ concentration. They participate in regulating ADH secretion, water intake, and renal Na^+ excretion. Studies on astronauts undergoing prolonged periods of weightlessness in space show that there is a decrease in red blood cell volume as well. The initial decrease does not seem to be due to intravascular hemolysis. Splenic trapping of red cells may be responsible. However, the reduced red cell volume persists for about 30 days indicating more an inhibition of bone marrow activity. Dunn (28) and Dunn and Smith (29) suggest that on the basis of reevaluation of the data from dehydrated mice that the principal cause of the erythrosuppression was the concomitant reduction of food intake, rather than hemoconcentration.

In our work (hypokinetic head-down animals) we observed a blood volume decrease during the first two days of exposure of hypokinesia from 56.0 ± 1.2 ml/kg to 43.1 ± 2.3 ml/kg. The blood volume stayed at this level for two more days and then returned slowly toward the prehypokinetic level. Red cell mass (estimated on the basis of values of blood volume and hematocrit ratio) appeared to decrease sharply in the early part of hypokinesia. The return to control values was observed only when the blood volume returned to control values as well. Thus it seems that the rat model is more adequate than the bed rest in man to study the effect of weightlessness. Blood volume seems to decrease abruptly in both astronauts and in antiorthostatic hypokinesia and then to stay, in the case of men, at the new equilibrium for weeks or months (Fig. 11).

"Stress hormones" in antiorthostatic hypokinesia. Determination of "stress hormones" represents one way to measure stress to the rats imposed by placement of the harness and induced by head-down position, as well as to measure the level of adaptation of the animall to the new situation during seven days exposure to hypokinesia. 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, and prolactin were determined in the antiorthostatic hypokinetic animals (radioimmunoassays). The blood (0.3 ml) was sampled in resting rats from the aortic cannual three times prior to antiorthostatic hypokinesia. Selection of these hormones is based on their common link to the existence of exogenous stressors. The primary physiologic role for ACTH is to stimulate the secretion and synthesis of corticosterone and of cortisol by the adrenal cortex. In the rat, corticosterone is the more sensitive responder of the two hormones to exogenous stress. ACTH also acts as a trophic substance maintaining the size and blood flow to the adrenal cortex and it exhibits extraadrenal physiologic effects on cyclic AMP-mediated systems. Both ACTH and corticosterone are readily affected by stress (30). By assaying both ACTH and corticosterone and multiple input pathways (anterior hypothalmic and posterior

mid-brain/pons pathway, hippocampal pathway and different sites of feedback control) are studied. Catecholamine levels are important because of the known stimulatory effects of stress on the sympathetic nervous system (30). Plasma concentrations of growth hormone vary widely in response to a variety of stimuli and bursts of growth hormone secretion occur in response to a stressful stimuli (31, 32). Thus, growth hormone is also an indicator of general non-specific stress. It is known that a variety of stressful stimuli evoke the stimulation of prolactin (33).

The sampline was performed four times during head-down hypokinesia in harnessed rats (on the first, second, 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 ACTH and corticosterone levels were elevated on the day 1 and less on day 2 and day 3 of the anti-orthostatic exposure. The plasma prolactin level was elevated but proportionally less than that of the other two stress hormones. On the day 7 of the exposure all of stress hormones were of the low resting (pre-exposure) level. After the release from the harness (and return to their own cages) the plasma stress hormone levels were again elevated and the elevation lasted at least five days (Fig. 12-14). Plasma catecholamine levels and plasma growth hormone levels have been determined and are being analyzed at this moment. These results will be presented at the Gravitational Meeting in San Diego, October 1982.

Discussion of personal work. We have continued during the last three years to work on the hypokinetic rat model. The necessary techniques have been developed and the study of circulatory characteristics of antiorthostatic rats has been initiated. The hypokinetic system (Holton-Mussachia) used in our laboratory is, we believe, the least stressful of all the systems used in this country, in Western or in Eastern Europe. Three years ago the Principal Investigator spent several months in Eastern Europe as a part of the USA-Polish scientific exchange and visited many laboratories in Poland, Czechoslovakia and Yugoslavia. At that time we initiated a joint program with Dr. Koslowsky (Polish Academy of Sciences) to study certain aspects of hypokinesia in rats, using two different restraining systems. Even "very gentle" immobilization systems (34-36) appear rather stressful to this investigator when compared to our system.

Body fluid regulation and compartmentalization, renal and hormonal regulation of blood and of blood pressure levels--are some of the circulatory functions that are gravity dependent. Dehydration occurs during space flights (37), plasma and red cell blood volume appear decreased (38) and there is a redistribution of body fluids. The redistribution of the blood volume leads to thoracic and cranial vascular engorgement (39) activation of atrial stretch receptors, and inhibition of ADH and probably aldosterone-angiotension II secretion. Experimentally induced atrial stretching also inhibits ADH release and leads to polyuria and to saluresis. However, experimental atrial stretching (induced by balloons) leads to polyuria but seldom to saluresis (40, 41). Furthermore, in nonhuman primates experimental atrial

stretchins has no effect on water excretion. It is known also that ADH administration interrupts diuresis in dogs. Thus ADH inhibition appears to be the main mechanism of the diuresis induced by left atrial distension in dogs and some other animals. How close these changes are to those observed in weightless man is not clear because of absence of measured data from the space flights. It should be added that most experimental data (in conditions of 1 g) have been obtained on anesthetized dogs. In unanesthetized dogs an increase of left atrial pressure leads to an increased arterial blood pressure in some animals (41) but not in others (42). The rat model of antiorthostatic hypokinesis that was used in our experiments demonstrated earlier that the rat responds with diuresis, increased excretion of nitrogenous waste products and muscle atrophy (43-46) and circulatory changes similar to those described in astronauts (47, 48). During the first few days of suspension, the antiorthostatic rats have a reduced water intake. Hypokinesis alone without negative tilt does not change the circulating plasma volume (45, personal observations) and does not lead to diuresis (44, 45) or circulatory changes (47, 48).

We have shown that the antiorthostatic rat model is an adequate model mimic and to study circulatory changes obserbed in man during space flights. Headward and chestward movements of body fluids observed in weightless man has been observed and measured in our rat model as an increased central venous pressure. The increase lasted less than 48 hours. The strething of atria induced by the fluid movement induced probably stimulation of stretch receptors and humoral changes, especially a decreased release of ADH. These experiments (to determine plasma ADH angiotensin II and aldosterone levels) are now in progress.

Heart rate of antiorthostatic rats was increased. The heart rate was slightly increased; similar results were reported by Boger and Aakoshi, 1973. We did not observe irregularities in cardiac activity reported in hypokinetic conditions by other researchers (49). Similar findings were reported in astronauts during flights (50, 51). The mean arterial blood pressure was decreased during the early exposure of our rats to antiorthostatic hypokinesia. The decrease was observed immediately after change in the position of the studied subjects. In a recent study Katkow et al. (52, 53) confirmed that there is a decrease of the arterial blood pressure after a 20° head-down tilt in man. However, the decrease was first observed 2-3 hours after the beginning of the exposure. Kolpakov et al. (54) and Pavlik and Frenkl (55) found a similar decrease in arterial blood pressure in dogs during hypokinetic exposure. But contrary to these findings some investigators observed during orthostatic hypokinesia a slightly increased arterial blood pressure (56). Yaremenk (56) has shown some disturbances of reflex regulation (baroreceptor involvement) in dogs after hypokinesia. Longer lasting hypokinesia led to hypertensive state (57, 58). There are reports, also, that arterial blood pressure does not change during hypokinesia (59). It is important to note that repeated, daily immobilization lasting 2 hours daily for 4 weeks leads to hypertension, both in unanesthetized and in ether anesthetized rats (60). Spontaneously hypertensive rats that did not

develop hypertension develop it immediately after immobilization (61). That is why it is important, we believe, to eliminate all rats with even slightly increased resting arterial blood pressure before exposure to hypokinesia.

Cardiac output was increased in our experiments in the early stages of antiorthostatic kinetic exposure but decreased continuously during the next seven days. The increase was mainly due to an increase in the stroke volume. Similar findings were reported for cardiac output in orthostatic hypokinetic dogs. Their cardiac output was decreased (62).

The ability of rats to exercise after hypokinesia was decreased. Similar results were described after space flights or after prolonged bed rest in man. During exercise the heart rate was increased as described after bed rest, especially after bed rest with negative tilt. Free activity brings the changed circulatory parameters back to the control levels, both after a prolonged bed rest (63, 64), after antiorthostatic hypokinesia (47, 48) or after a space flight.

Blood volume was profoundly decreased in our experiments with a parallel decrease of red cell mass (judged on the basis of the hematocrit ratio).

It is clear that in all hypokinetic models motor activity of the rats is much increased the first day of the exposure when the rats try to escape from the cages (65). Bonfils et al. (66) studied in detail the motor behavior of rats during the first 24 hours of restraint. Svitsunov (67) describes this kind of behavior as rapid adaptation to confinement. Use of different strains also poses a problem for comparison of the obtained result. Nikityuk et al. (68) have shown, for instance, that the "August" Russian rats recover faster from hypokinesia than Wiston rats. Furthermore, many laboratories in USSR are mixed breed (mongrel) rats.

As it might be expected, there are some physiological differences between the effects and the mechanisms of weightlessness and of hypokinesia. While in weightlessness deficit drinking might lead to decreased circulating blood volume, an excess urinary excretion and deficit drinking bring a similar circulatory change in hypokinesia. Furthermore, it is possible that in weightless man increased capillary filtration is limited to the cephalad region of the body only, while horizontal hypokinesia brings forth an increased filtration throughout the whole body. Hypokinesia with negative tilt eliminates at least partially this difference.

Thus it seems that our hypokinetic rat model appears to be predictive of circulatory changes observed during weightlessness in man and that it can be used to study circulatory mechanisms in weightless man. Would the model be predictive of circulatory changes in rats or other animals exposed to weightlessness one will learn soon enough after the Space Lab IV experiments.

We can conclude with Heaney (69) that, despite some of the differences, it is "surprising how much similar" the effects of hypokinesis are to the physiological effects of weightlessness.

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, to ask proper questions and to employ adequate techniques in learning more from future space flights, not only about effects of a space flight, but about effects of gravity on earth as well. Thus, we need more answers and, happily, hypokinetic models and then Shuttle experiments will be able to provide them. 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 for the Space Lab comes and the new vistas for future work. New theoretical and technical approaches, advanced and better techniques, and a deeper understanding of involved mechanisms obtained in group-based experiments and in hypokinetic conditions will make the choice of circulatory studies in space more selective, more meaningful, and less costly, limiting hopefully future Space studies to those experiments that are not only relevant to extended space flights but also that answer the question: What is the effect of gravity on circulation in man and in animals?

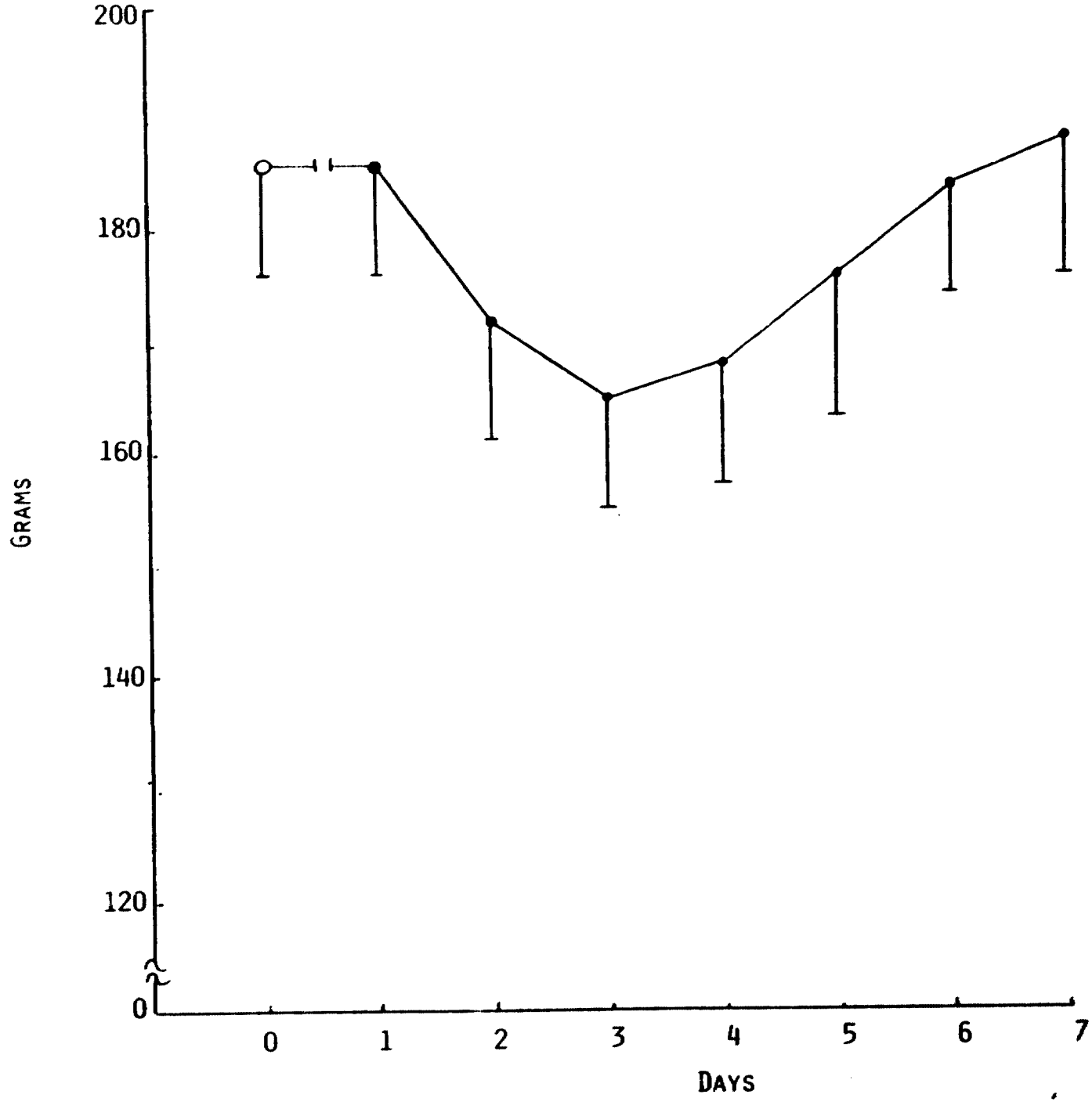

Fig. 1. Body weight during exposure of rats to antiorthostatic hypokinesia.

FOOD CONSUMED

GROUP SIZE
N = 50

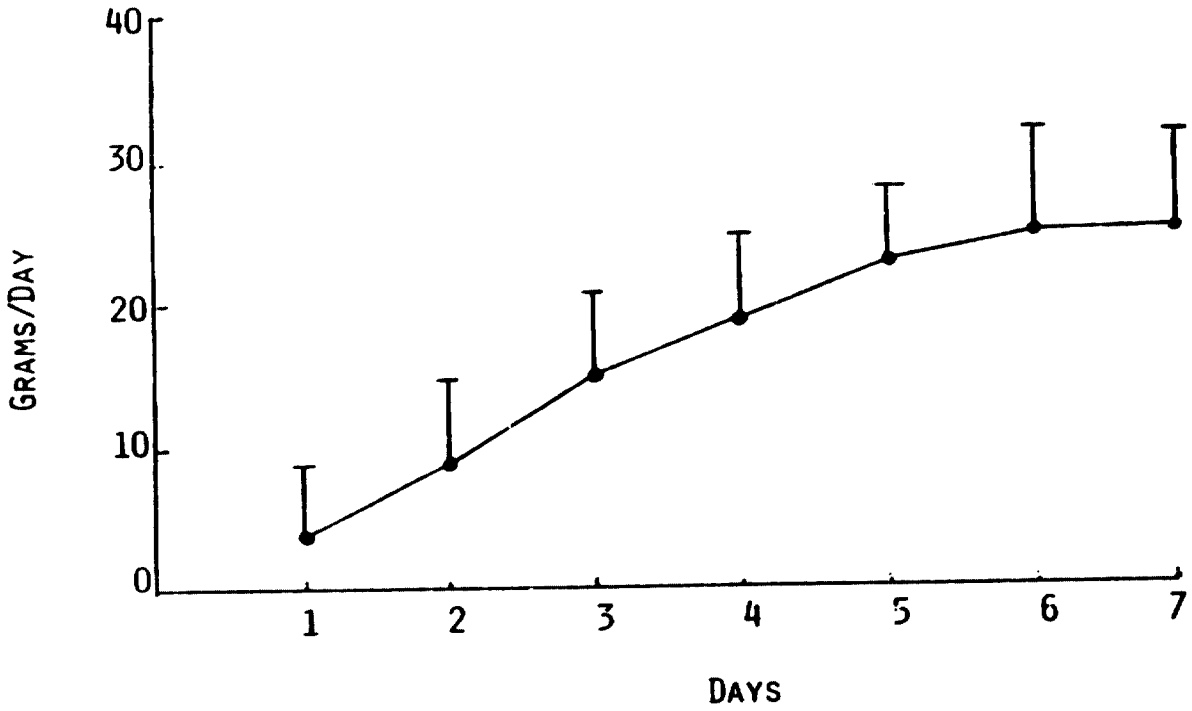

Fig. 2. Food consumption during antiorthostatic hypokinesia.

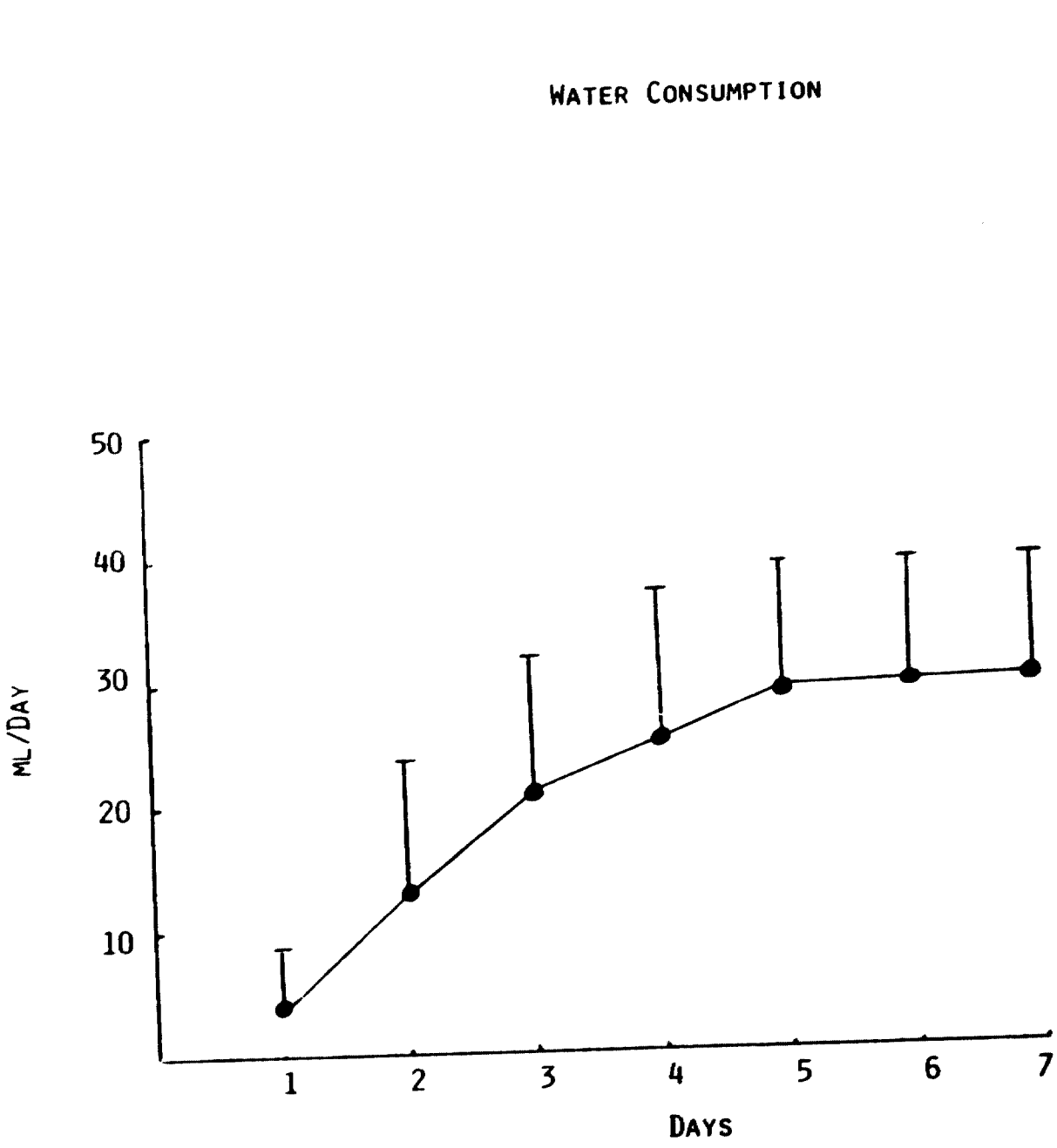

Fig. 3a. Water consumption during antiorthostatic hypokinesia.

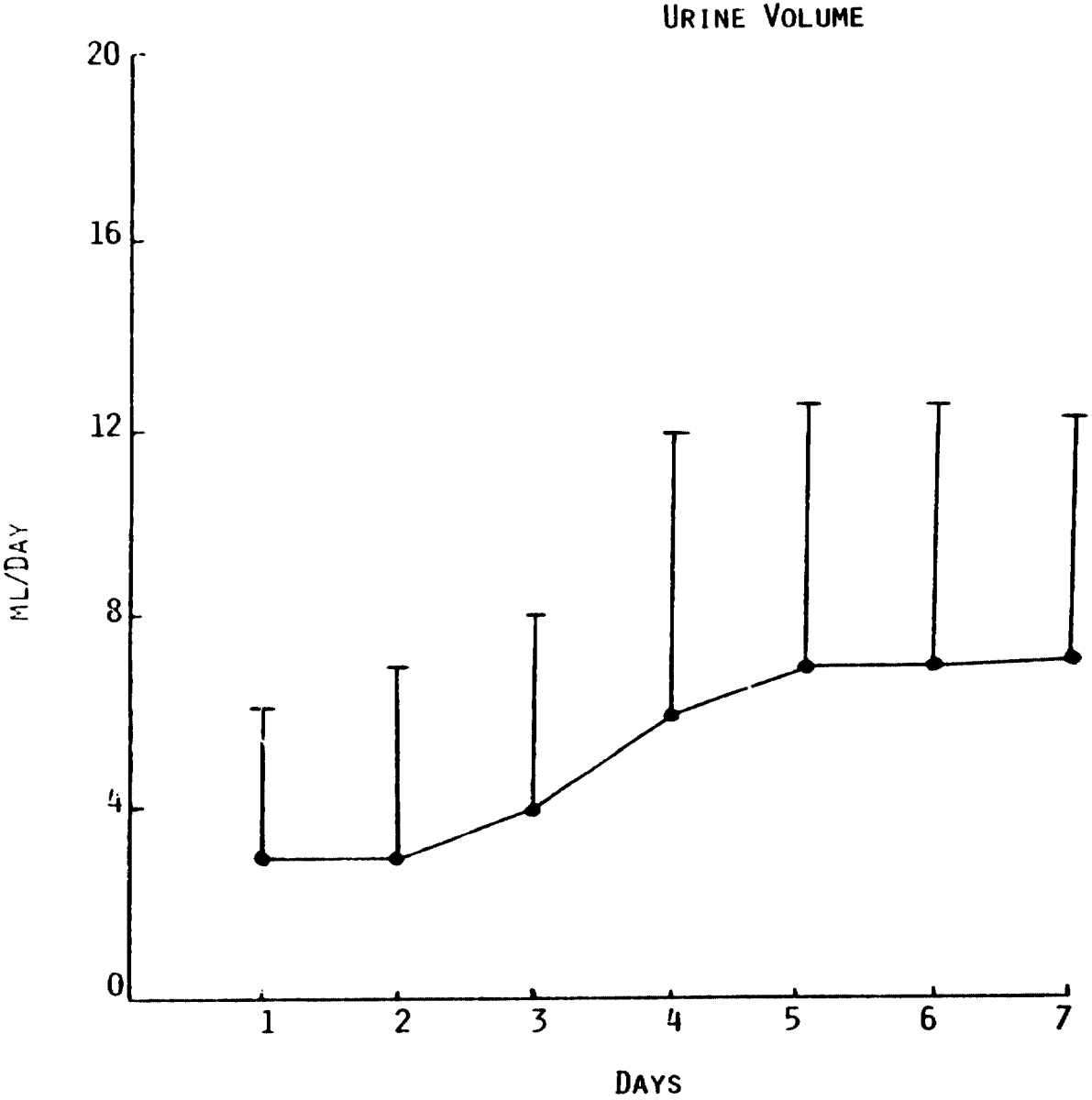

Fig. 3b. Urine volume during antiorthostatic hypokinesia.

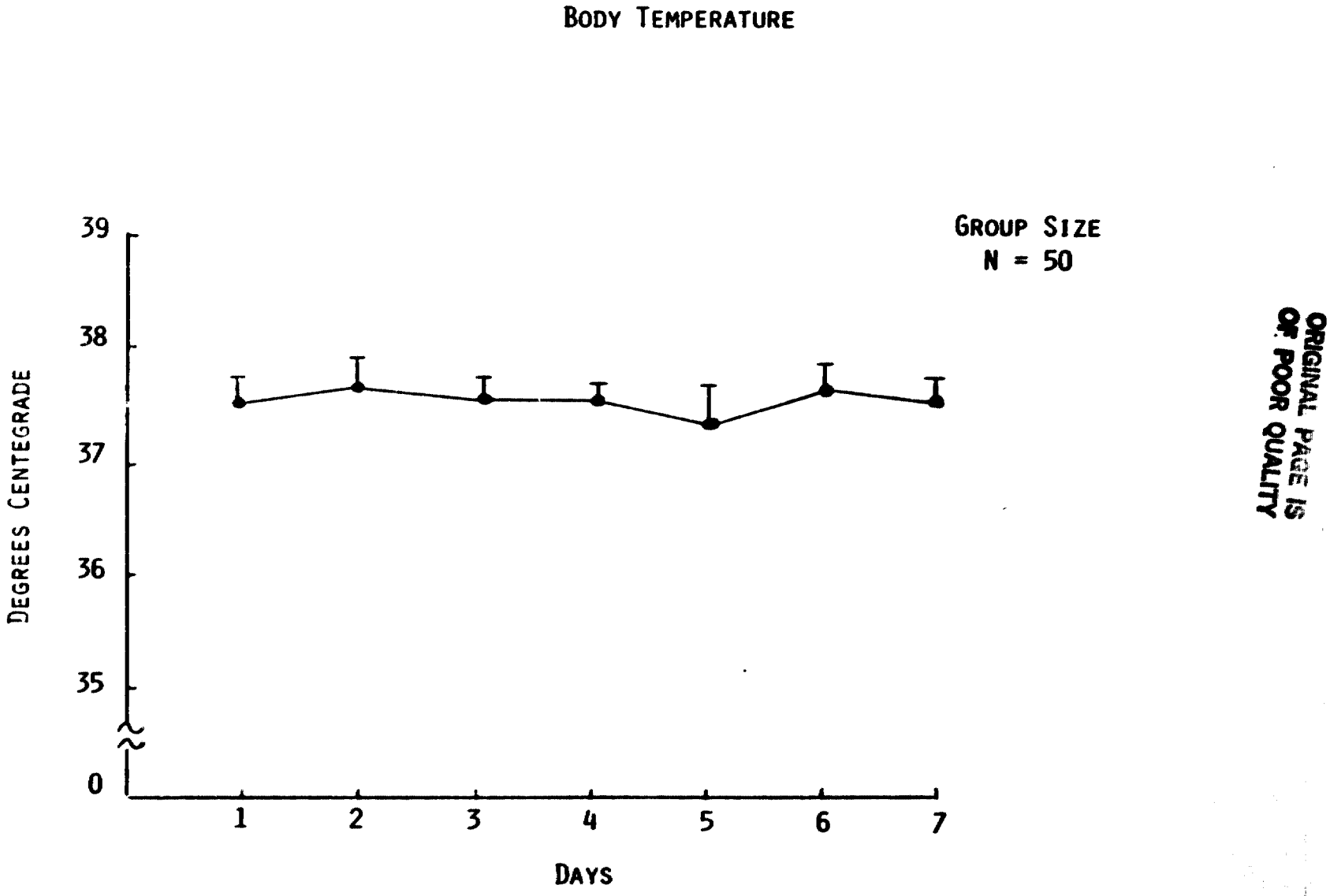

Fig. 4. Deep colonic temperature during antiorthostatic hypokinesia.

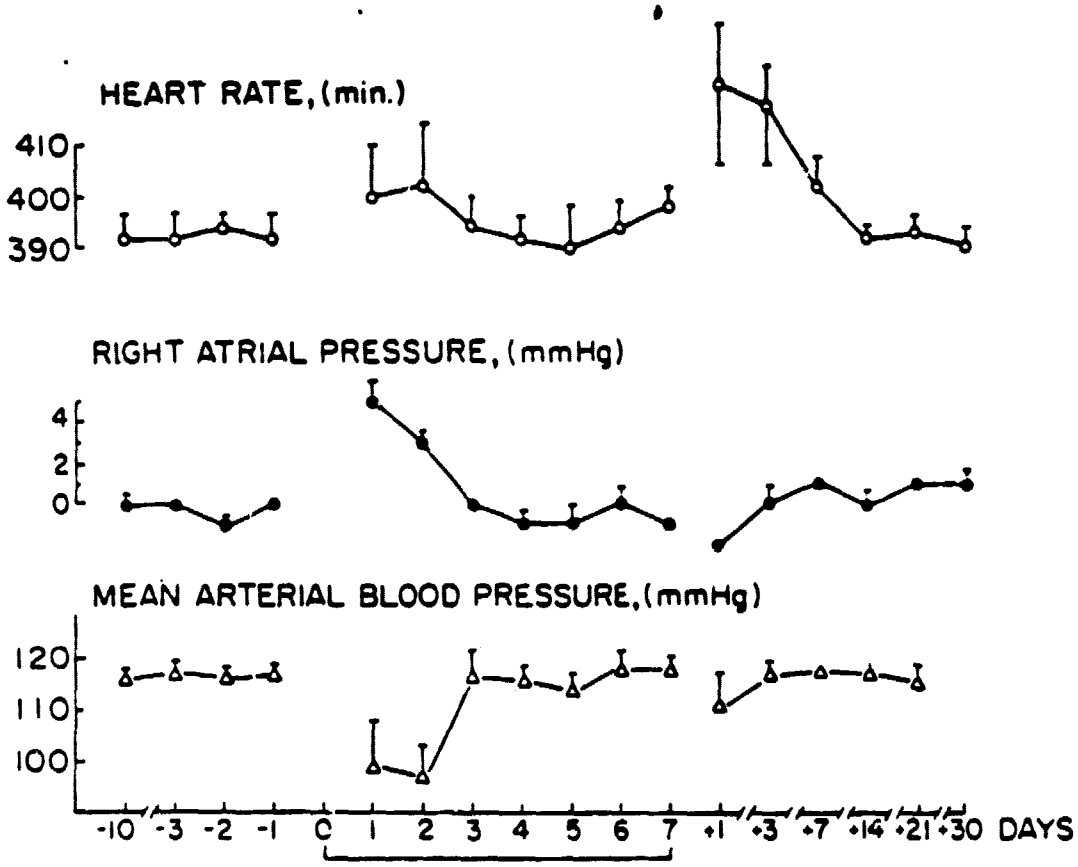

Fig. 5. Heart rate, right atrial pressure and mean arterial blood pressure (± SE) of eight rats prior, during and after exposure to hypokinesia with -30° tilt.

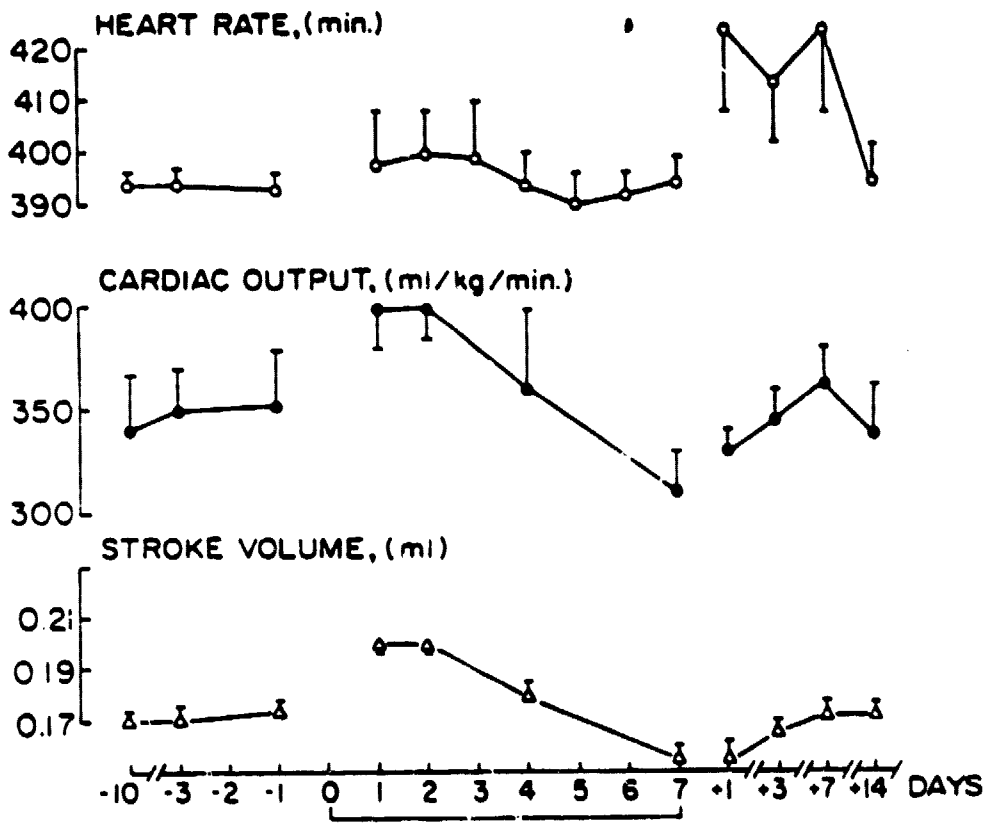

Fig. 6. Heart rate, cardiac output, and stroke volume during and after seven days long hypokinesia.

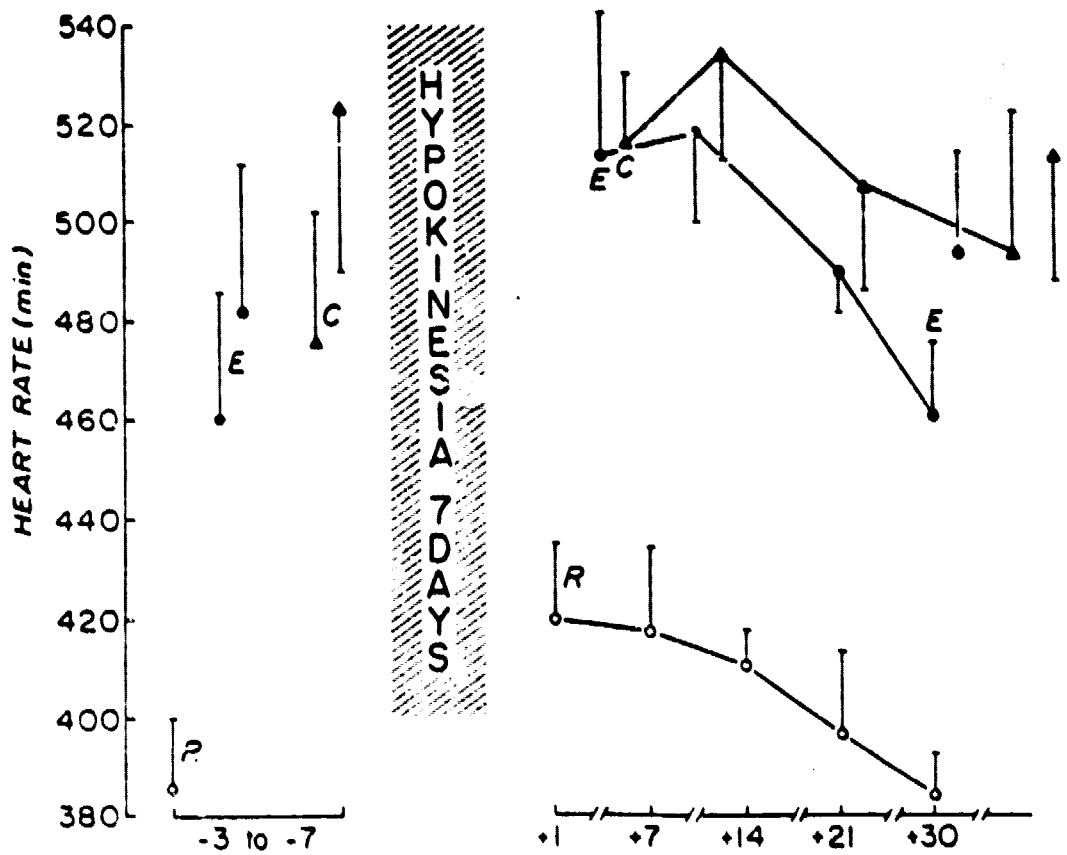

Fig. 7. The heart rate during rest (R, open circles) and during light or heavy exercise (E, closed circles) or during two levels of cold exposure (C, triangles) of rats prior (seven to three days) to head-down hypokinesia that lasted seven days and after hypokinesia (1,7,14,21 and 30 days).

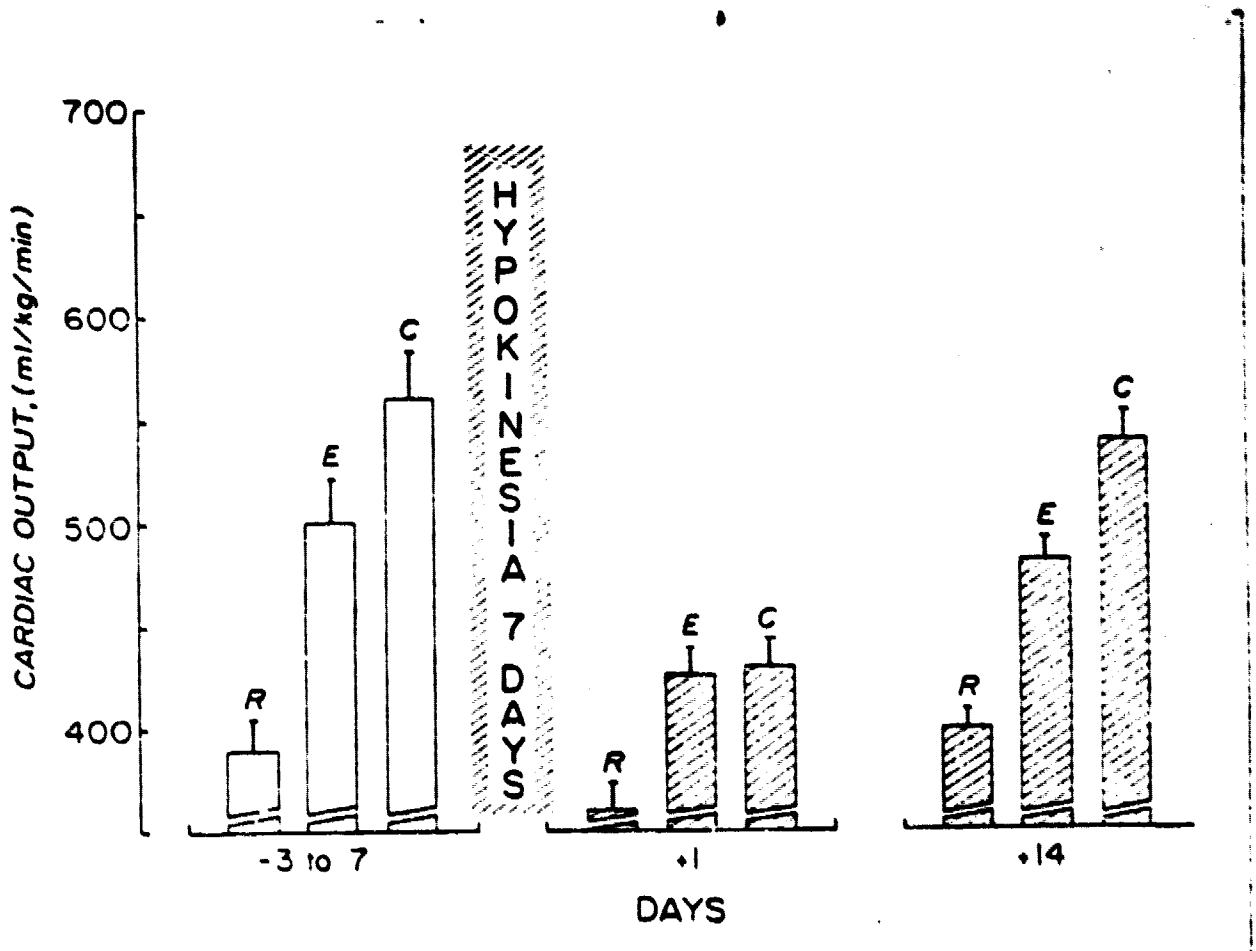

Fig. 8. Cardiac output (ml/min/kg) of resting (R), exercising on a treadmill at 10 m/min (E), or cold (C) exposed (10°C room temperature) rats prior (seven to three days) and after seven days hypokinesia.

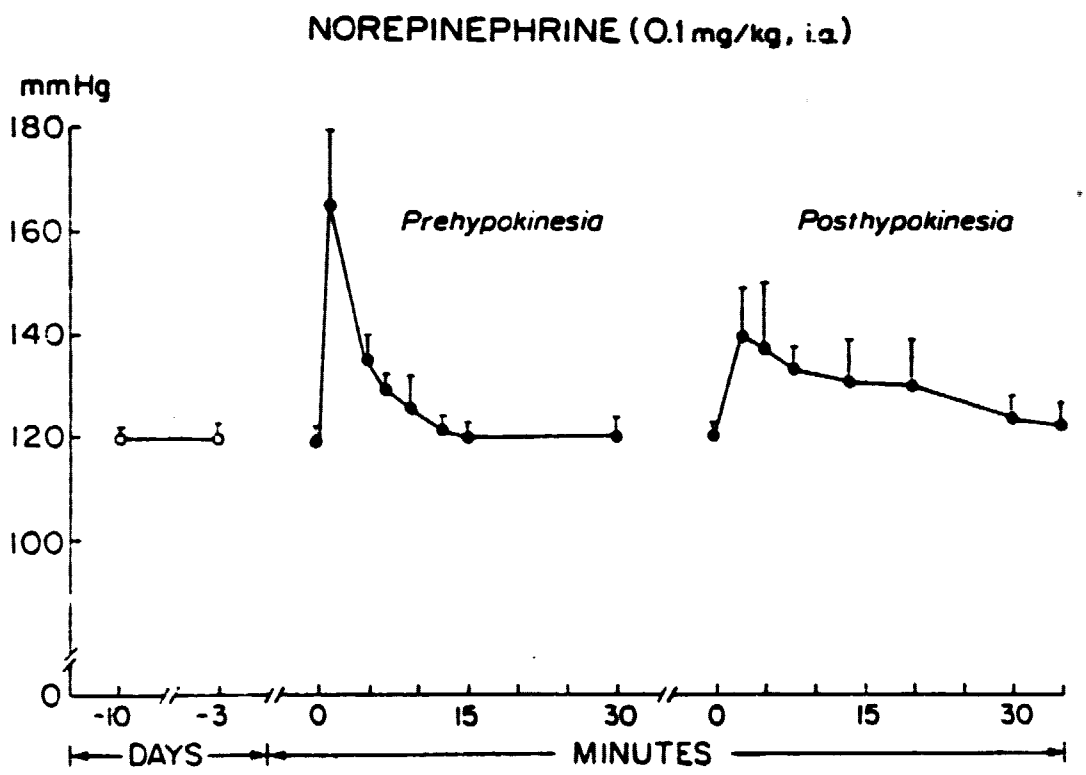

Fig. 9. Mean arterial blood pressure in the resting rats (10 and 3 days prior to hypokinesia) and hypertensive responses after administration of norepinephrine one day before and one day after seven day long hypokinesia.

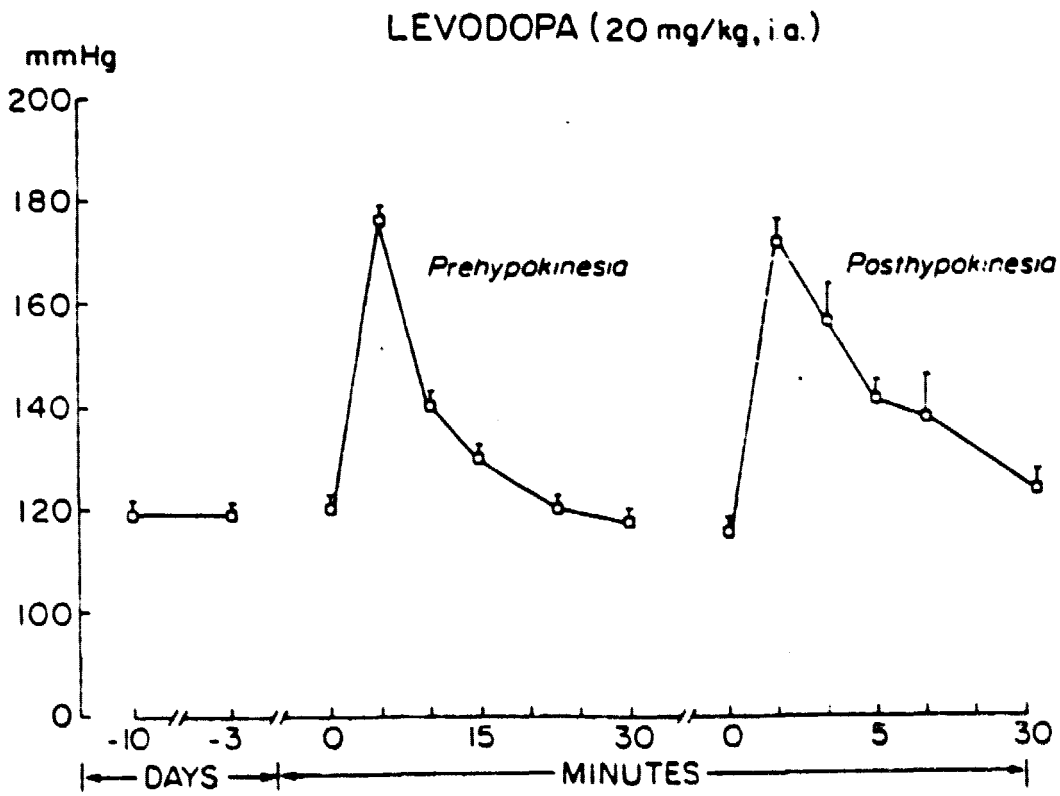

Fig. 10. Levodopa-induced mean arterial blood pressure of rats before and after seven days long hypokinesia with -30° tilt. The mean arterial blood pressure was measured 10 and 3 days before administration of levodopa in the resting rats.

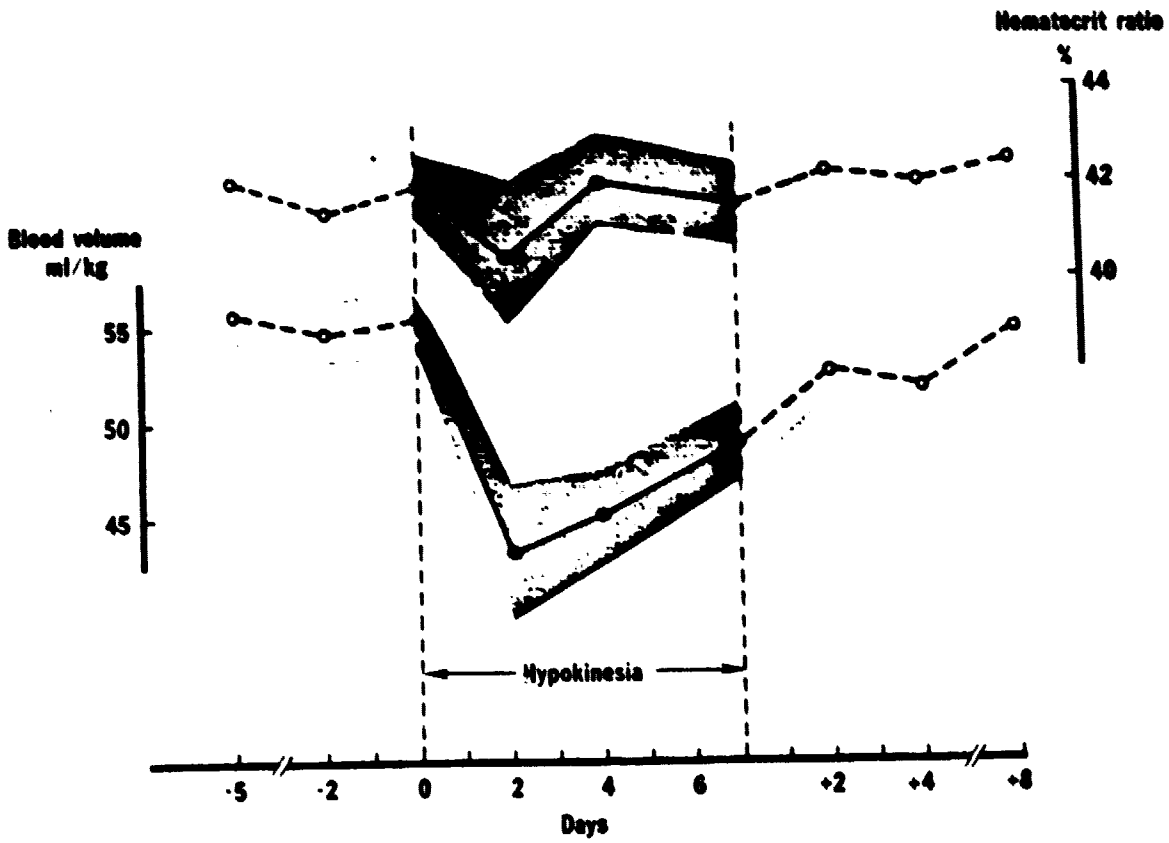

Fig. 11. Blood volume and hematocrit ratio of antiorthostatic rats.

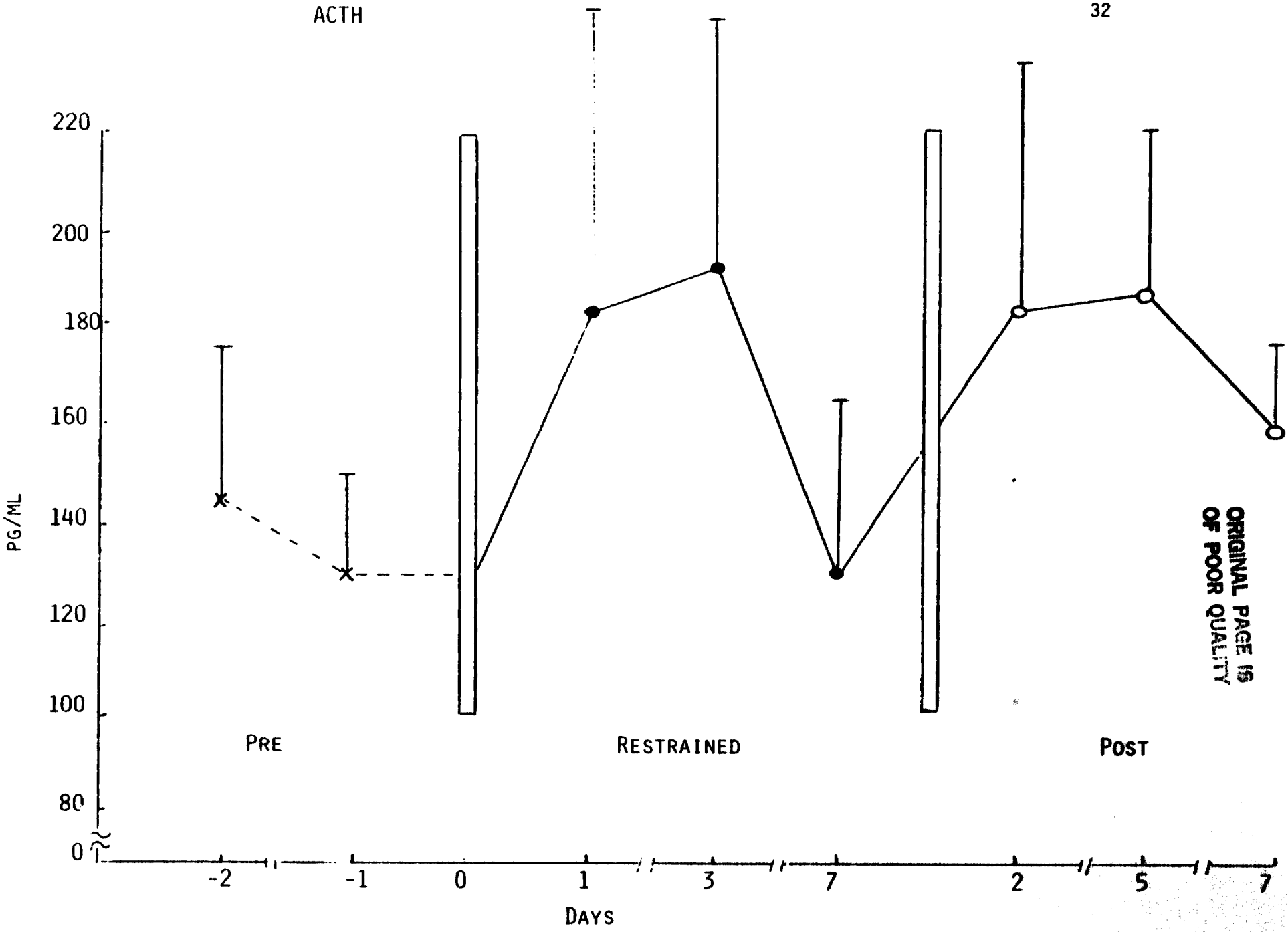

FIG. 12. PLASMA ACTH DURING ANTIORTHOSTATIC HYPOKINETIC EXPOSURE IN RATS.

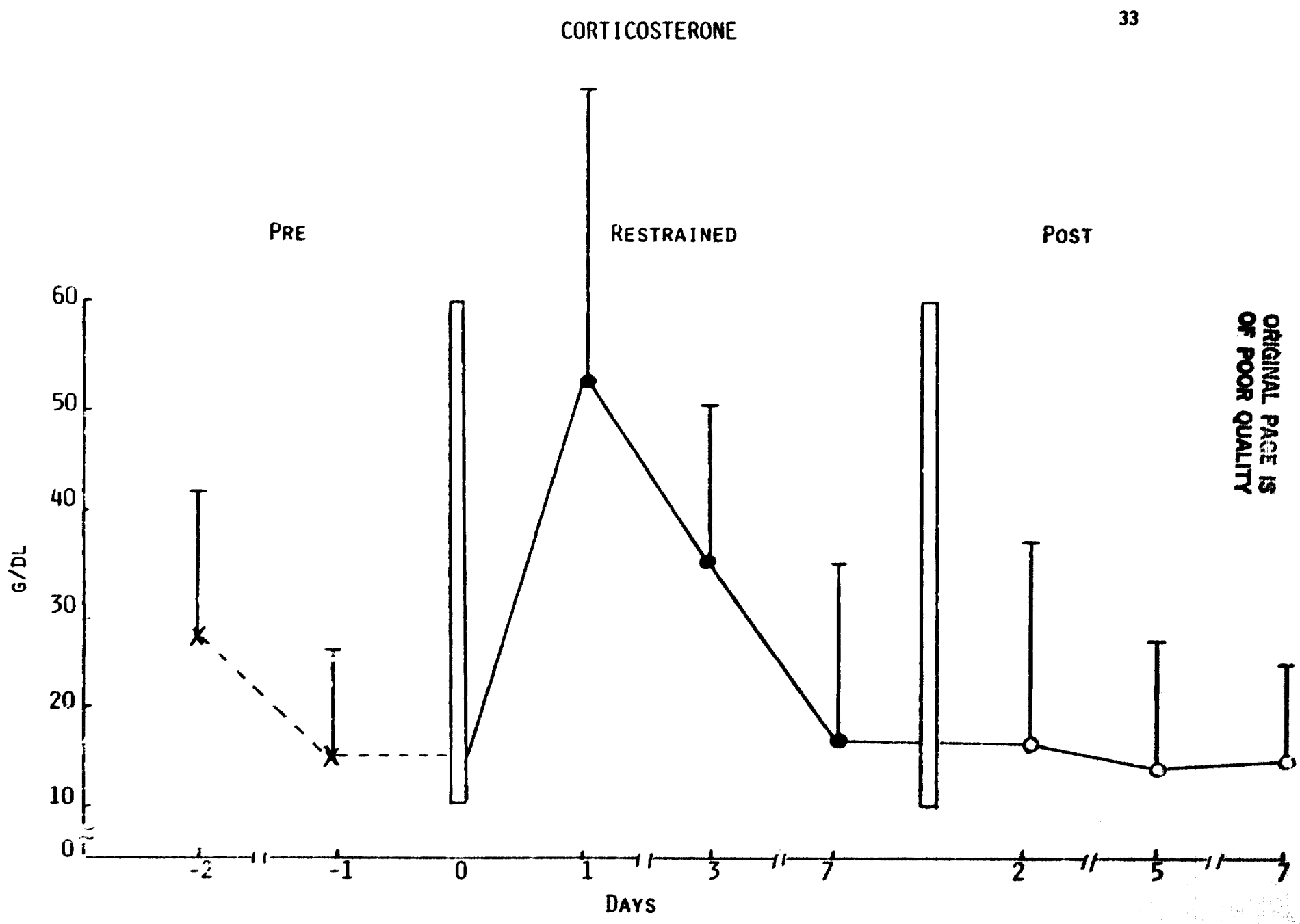

FIG. 13. PLASMA CORTICOSTERONE DURING ANTIORTHOSTATIC HYPOKINETIC EXPOSURE IN RATS.

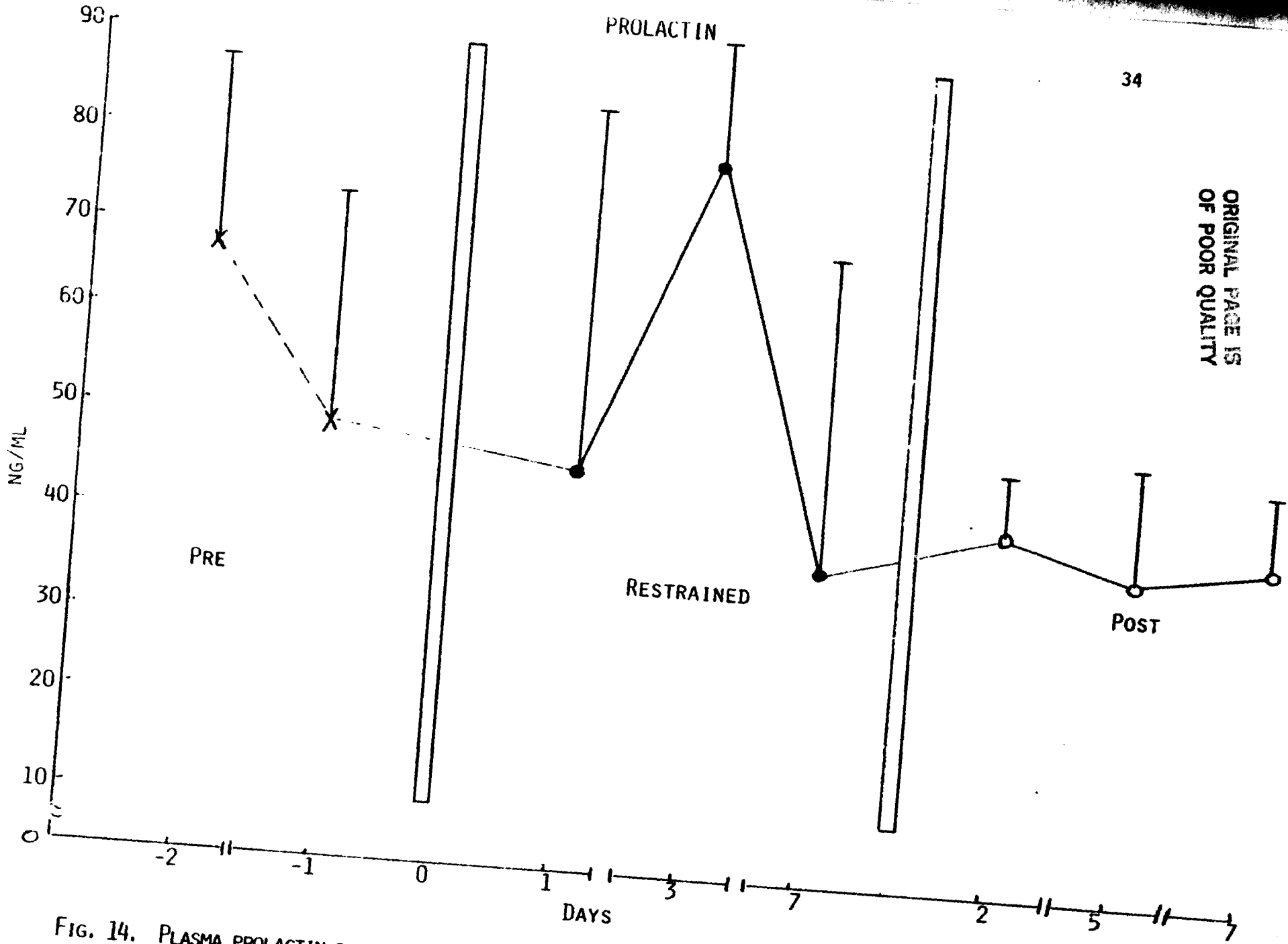

FIG. 14. PLASMA PROLACTIN DURING ANTIORTHOSTATIC HYPOKINETIC EXPOSURE IN RATS.

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