Physiol. Res. 42: 397–402, 1993

Suspension Hypokinesia in Rats: Effects of the Time of Day on Metabolic and Hormonal Changes

I. AHLERS, E. AHLERSOVÁ, P. NOSKOVIČ, G. TOTH, Ĺ. RAČEK, B. ŠMAJDA, J. SMOLNICKÁ

Institute of Animal Physiology, Faculty of Science, Šafárik University, Košice, Slovak Republic

Received April 20, 1993
Accepted July 8, 1993

Summary

Suspension hypokinesia is a new model which can simulate some effects of microgravity on the organism of laboratory animals. Two groups of male SPF-bred Wistar rats were suspended for 24 h. In the first group hypokinesia began in the morning (M) (1 h after light onset, 0800 h), whereas the other group was subjected to this treatment from the evening (E) (1 h after dark onset, 2000 h). In the serum, there was a statistically significant increase in non-esterified fatty acids, triacylglycerols (TG) and glucose and a decrease in triiodothyronine concentration in the M group, while only a significant increase in phospholipids (PL) was found in the E group. The serum corticosterone level was increased in both groups, more markedly in the M group. There was an increase in TG and PL in the liver in M rats. In the bone marrow (femur), an increase of triglycerols in E rats and an increase of phospholipids in M rats were found. The concentration of glycogen in the heart muscle, m. quadriceps femoris and m. soleus rose in the M group only. The changes in the analyzed parameters predominate in the rats subjected to hypokinesia in the morning period. This fact confirmed the hypothesis about a higher sensitivity of rats to the stressor acting in the period of inactivity.

Key words

Suspension hypokinesia – Rats – Metabolism – Day-time effects

Introduction

The spectrum of ground-based model experiments in space biology was extended by the use of so-called "suspension hypokinesia". In this model, a laboratory rat is suspended in such a manner that the hind part of the body is lifted and the body axis forms an angle of about 30 degrees with the horizontal plane. The suspension may be carried out in various ways. The common principle is that the animal can move on the plane using only its forelimbs having easy access to food and water.

Hypokinesia represents psychoemotional stress associated with activation of the neurohumoral system resulting in a marked alteration of metabolic pathways. The hormonal and metabolic effects of the hypokinesia (immobilization) depend on the time of its onset and realization (Ahlersová *et al.* 1988).

Any immobilization (short-term or prolonged) is an efficacious psychoemotional stress, with a consequent marked increase in hypothalamic-hypophyseal-adrenocortical axis activity, an increase in the synthesis and secretion of catecholamines and other substances. This reaction is accompanied by enhancement of lipolytic processes, and, consequently, by an increased concentration of lipids in the serum and liver. The concentration of TG in the bone marrow increases, whereas the content of TG in the thymus declines. Besides the reduction of thymus mass, the decrease of the PL content is a good marker of tissue lipid changes (Ahlers 1984). The short-term immobilization evokes an increase in blood glucose, which depends on the nutritional status, and also an increase in skeletal and heart muscle glycogen (Ahlersová and Ahlers 1976). Considering the classical scheme of the stress reaction, a decrease in thyrotropin and thyroid hormones should be expected. However, due to some additional factors such as changes in thermoregulation, the situation seems to be far more complicated (Langer *et al.* 1981).

In the present experiments we have tried to analyze the appearance and the extent of metabolic hormonal changes in rats, immobilized for 24 hours in a nyctohemeral arrangement using the suspension technique.

Material and Methods

Male SPF-bred Wistar rats (Velaz Praha) weighing about 200 g were adapted to a light-dark (12:12 h) cycle (light on 0700 h to 1900 h) for three weeks. They were housed singly in a cage in a special room at 23±2 °C, relative humidity 70–80 %. They were fed LD pellets (Velaz Praha) and drank tap water *ad libitum*. Then the animals were suspended for 24 h using the fixation in the region of overtail fold by the method of Noskovič *et al.* (1990). The body axis formed an angle of about 30 degrees with the ground. The onset of hypokinesia was 1 h after light onset, i.e. at 0800 h ("morning" group) or 1 h after the onset of darkness, i.e. at 2000 h ("evening" group). The rest of the animals served as the control (non-restrained) group. After a 24 h period of hypokinesia, the rats were killed by rapid decapitation and the following parameters were determined: the concentration of non-esterified fatty acids (Dole and Meinertz 1960) and glucose (Hugget and Nixon 1957) in the serum, the concentration of triacylglycerols (Eggstein and Kreutz 1966) and phospholipids from lipid phosphorus (Bartlett 1959) in the serum, liver, bone marrow (femur) and thymus, the concentration of total cholesterol (Zlatkis *et al.* 1953) in the serum and liver, the concentration of glycogen (Roe and Dailey 1966) in the liver, heart muscle, quadriceps femoris and soleus muscles and the concentration of lipid peroxides (Placer and Slabochová 1962) in the liver, bone marrow and thymus. The concentrations of corticosterone (Guillemin *et al.* 1958), thyroxine, triiodothyronine and insulin were estimated in the serum by radioimmunoassay (kits from Institute of Radioecology and Applied Nuclear Techniques, Košice, Slovak Republic and Radioisotope Centre, Swierk, Poland). Each group consisted of 8 animals. The significance of differences between restrained and non-restrained animals in both parts of the experiment was evaluated by the t-test for non-paired data.

Results

The data obtained are plotted in diagrams. In both morning and evening parts of the experiment, only the differences between hypokinetic and control animals were evaluated; the well-known differences in the course of the day (morning vs evening values) are given without special comment.

Serum

The concentration of non-esterified fatty acids was higher in hypokinetic rats but significantly only in the "morning" group; the same was true for the triacylglycerol (TG) concentration. The level of total cholesterol tended to be higher in both groups of hypokinetic animals. On the other hand, the concentration of phospholipids (PL) which was higher in suspended rats, was significant only in the "evening" group (Fig. 1).

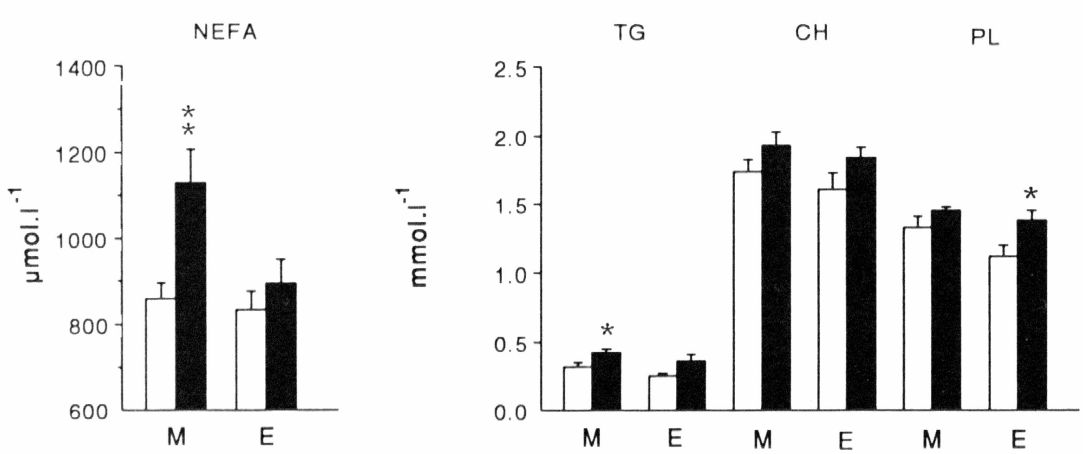

Fig. 1
The concentration of non-esterified fatty acids (NEFA), triacylglycerols (TG), total cholesterol (CH) and phospholipids (PL) in the serum of control (open columns, C) rats and of rats subjected to suspension hypokinesia (black columns, R) in the morning (M) or in the evening (E). Values given as mean±S.E.M. Significance of differences between C and R groups denoted as * ($P<0.05$) or ** ($P<0.01$).

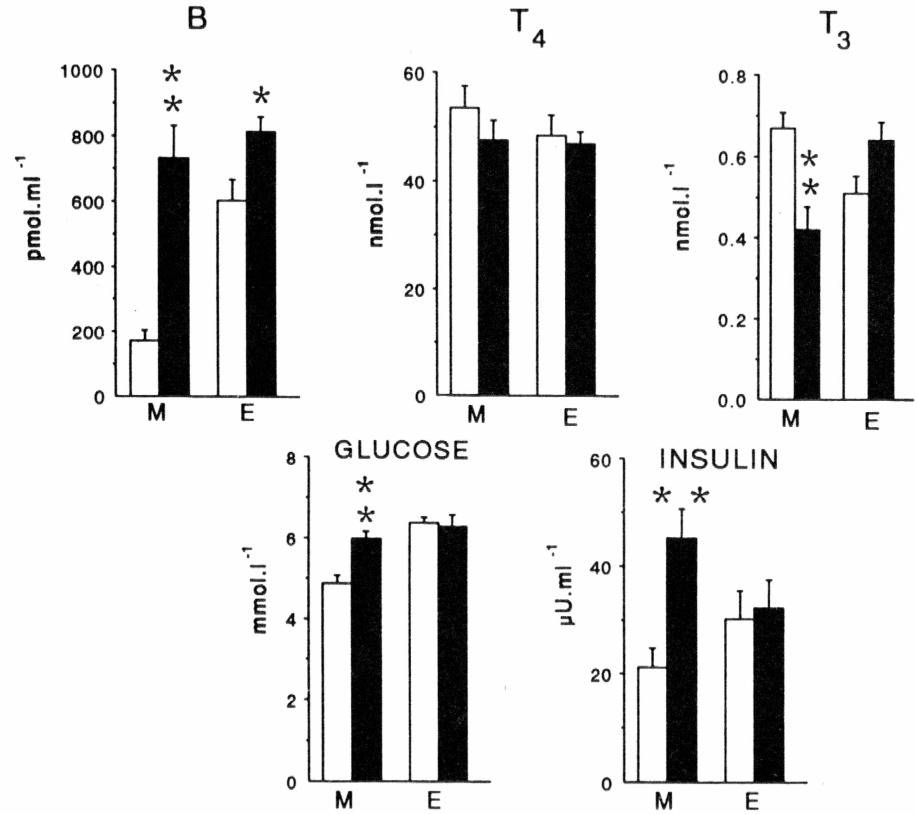

Fig. 2
The concentration of corticosterone (B), thyroxine (T_4), triiodothyronine (T_3), glucose and insulin in the serum of control and restrained rats. For details see Fig. 1.

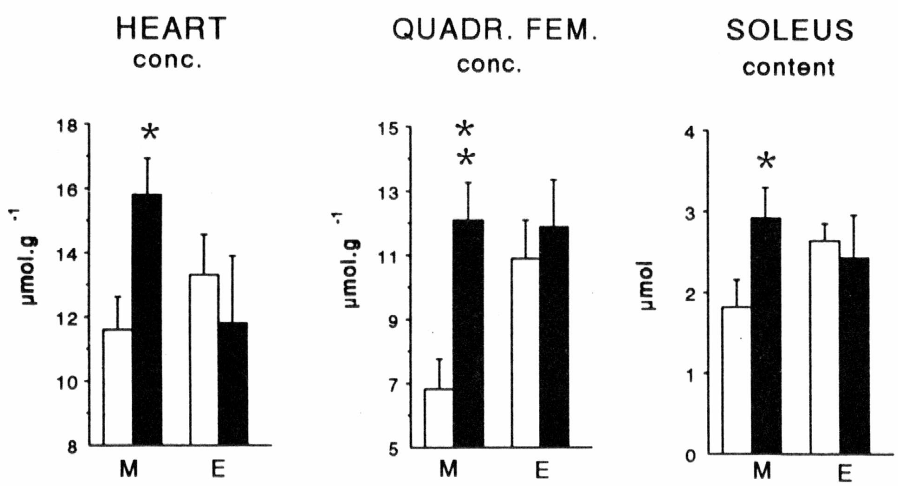

Fig. 3
The concentration or the content of glycogen in the heart muscle, quadriceps femoris and soleus muscles in control and restrained rats. For details see Fig. 1.

The concentration of corticosterone (B) was elevated in hypokinetic rats in both groups. Thyroxine (T_4) levels declined insignificantly in both hypokinetic groups, while that of triiodothyronine (T_3) decreased only in the morning group. The concentration of glucose and insulin increased in the morning group only (Fig. 2).

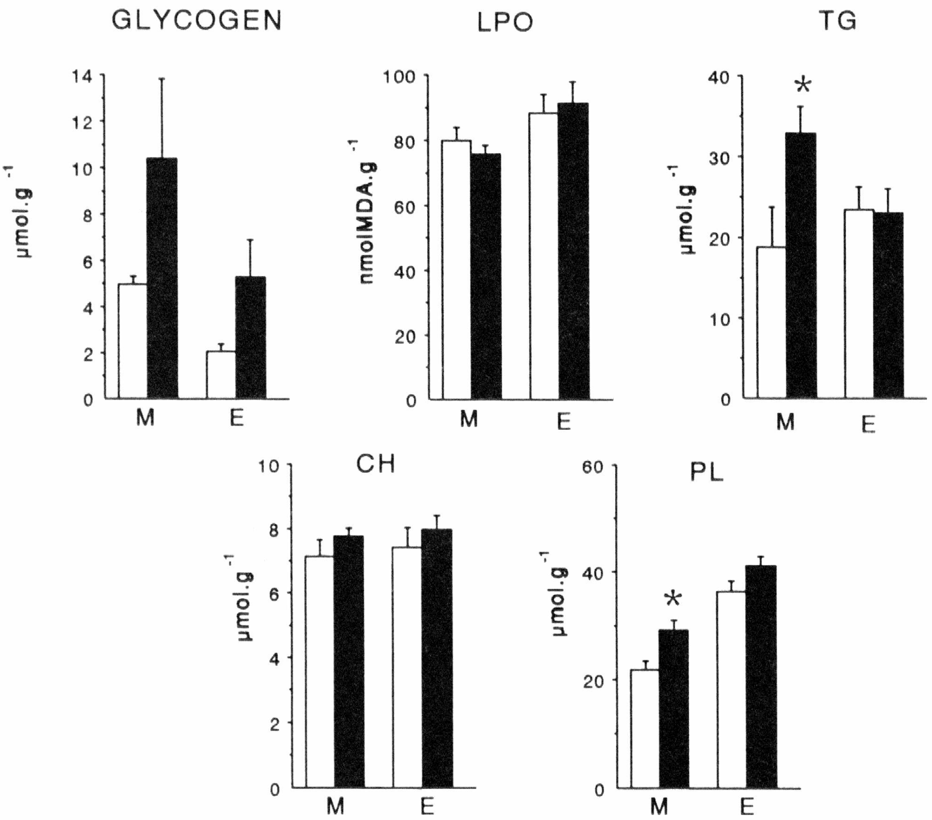

Fig. 4
The concentration of glycogen, lipid peroxides (LPO), triacylglycerols (TG), total cholesterol (CH) and phospholipids (PL) in the liver of control and restrained rats. For details see Fig. 1.

Muscles

The concentration of myocardial glycogen only increased in the "morning" group, the same was noted in the quadriceps nuscle, or in the soleus muscle (here the changes are expressed as the glycogen content, Fig. 3).

Liver

Glycogen concentrations were elevated in both hypokinetic groups but, the results were not significant because of the great variability in the values of individual rats. The cholesterol and lipoperoxide levels (LPO) were not substantially changed and whereas TG and PL concentrations rose in the "morning" group of hypokinetic rats only (Fig. 4).

Bone marrow, thymus

Triacylglycerol concentrations in the bone marrow increased in the "evening" group of suspended rats; on the contrary, PL concentrations increased in the "morning" group only. The concentration of lipid peroxides declined in both parts of the day nonsignificantly. There were no changes in the thymus lipid or lipoperoxide content/concentration (Fig. 5).

Discussion

The effect of the stressor depends on the time of day in which it is applied and/or on its duration. From the standpoint of the law of initial values a higher increase could be expected in the morning in these indices, which have low values at this time, and *vice versa*. The response of corticosterone confirms such a hypothesis. The "morning" immobilization produced a higher increase as compared to the "evening" variant (Zimmermann and Critchlow 1967, Dunn *et al.* 1972, Torrellas *et al.* 1981). Short-term restraint of the rats at the onset of light also increased the levels of adrenocorticotropin, prolactin, beta-endorphin and beta-lipotropin more markedly, as well as the content of cyclic adenosine monophosphate in the hypophysis (Kant *et al.* 1986). However, the production of corticosterone in the adrenals following the evening administration of adrenocorticotropin ($ACTH_{1-24}$) was higher than after the morning administration (Nicholson *et al.* 1985).

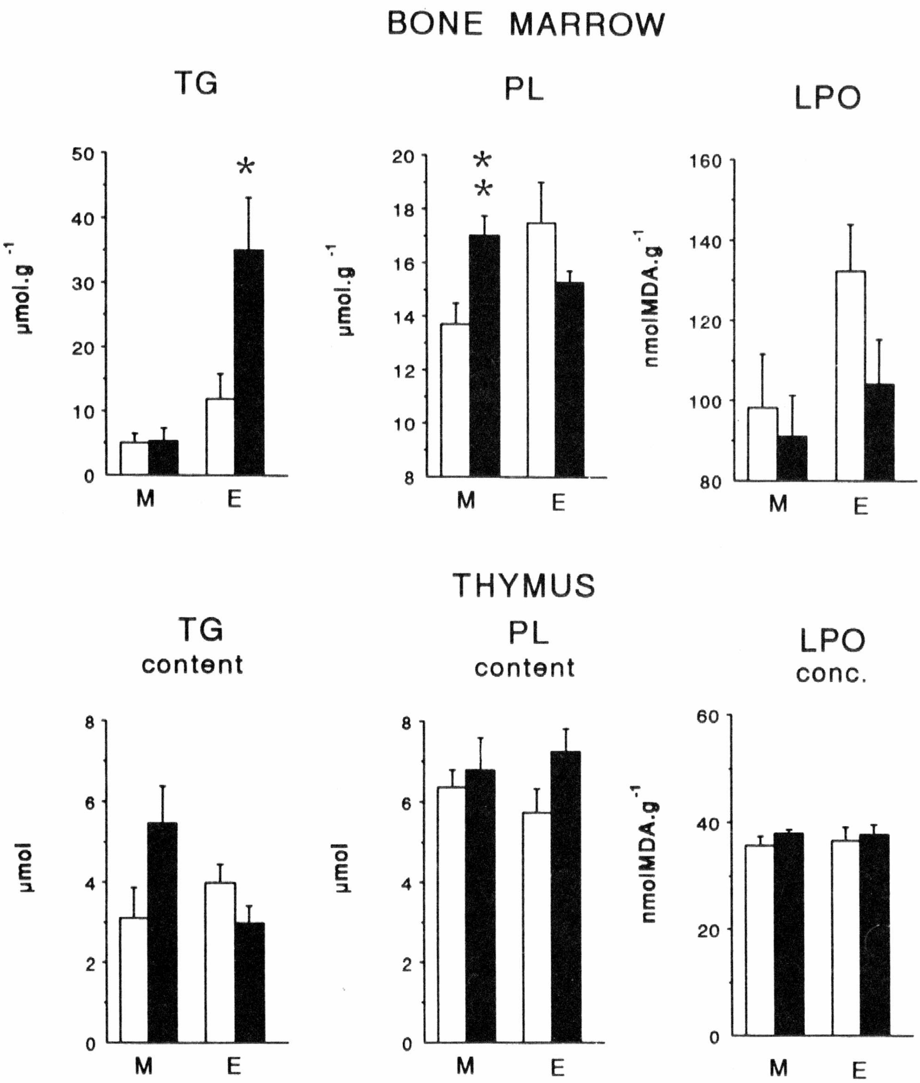

Fig. 5
The concentration or content of triacylglycerols (TG), phospholipids (PL) and lipid peroxides (LPO) in the bone marrow (femur) and thymus of control and restrained rats. For details see Fig. 1.

In general, the 24-h suspension hypokinesia, elevated the serum, liver and bone marrow lipids as well as corticosterone, declined the thyroid hormones in the serum, increased the liver and muscle glycogen; only thymus lipids did not change substantially. Most of these changes appeared only when hypokinesia was applied in the morning, as in the case of the increase in non-esterified fatty acids, TG, glucose and insulin in the serum, the decrease of serum T_3 concentration, increase of TG and PL in the liver, and increase of glycogen in the heart and skeletal muscles. Only an increase in the serum PL and bone marrow TG was seen when hypokinesia was started in the evening. The concentration of corticosterone increased in both times of the day, being higher after morning hypokinesia.

We previously reported results from 24-h immobilization in boxes using the same nyctohemeral arrangement of the experiment (Ahlersová *et al.* 1988). In this model during the evening immobilization, the following parameters involved in the mechanisms of food intake and locomotor activity which are higher in the dark part of the day, were changed: TG in the serum, liver and bone marrow, glycogen in the liver, T_3 in the serum. The changes of parameters closely related to the patterns of the stress reaction (corticosterone in the serum, triacylglycerols in the thymus) changed largely during morning immobilization. The concentration of serum glucose and liver cholesterol changed uniformly in both experiments. Comparing the results of the previous and present experiments, considerable differences were noted in the circadian sensitivity of rats. The immobilization in boxes with accompanying fasting and thirst appeared to be a more potent and qualitatively different type of stress. The suspension hypokinesia represents a milder type of stress as may be documented by the absence of thymus mass changes and thymus biochemical changes, both clearly manifested in rats after immobilization in boxes.

The predominancy of changes in the morning type of hypokinesia may be explained by a greater sensitivity to the stimuli within the inactivity period. Thus, in the case of a nocturnal animal, such as the rat, the more sensitive period should be the light part of the day. The inability to fall asleep could partly contribute to these changes.

In conclusion, attention should be drawn to the day-time dependence of the stressor action. The specificity of the stressor's action with respect to the time of its induction and duration must be taken into account in the evaluation of biological experiments.

References

AHLERS I.: The Effect of Space Flight on Tissue Lipids in Rats. (in Slovak), Veda, Bratislava, 1984, 129 pp.

AHLERSOVÁ E., AHLERS I.: The effect of adrenergic blocking agents and nicotinic acid on lipid and carbohydrate metabolism in immobilized rat. In: *Catecholamines and Stress*. E. USDIN, R. KVETŇANSKÝ (eds), Pergamon Press, London 1976, pp. 491–497.

AHLERSOVÁ E., AHLERS I., MOLČANOVÁ A.: Metabolic and hormonal changes in rats immobilized at various times of day. *Physiologist* **31**(Suppl. 1): S132–S133, 1988.

BARTLETT G.R.: Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466–468, 1959.

DOLE V.P., MEINERTZ H.: Microdetermination of long-chain fatty acids in plasma and tissue. *J. Biol. Chem.* **235**: 2595–2599, 1960.

DUNN J., SCHEVING L.E., MILOT P.: Circadian variation in stress-evoked increase in plasma corticosterone. *Am. J. Physiol.* **223**: 402–408, 1972.

EGGSTEIN M., KREUTZ F.H.: Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe. *Klin. Wschr.* **44**: 262–267, 1966.

GUILLEMIN R., CLAYTON H.W., SMITH J.D., LIPSCOMB K.S.: Measurement of free corticosteroids in rat plasma. Physiological validation of a method. *Endocrinology* **63**: 349–355, 1958.

HUGGET A., NIXON D.A.: Enzymatic determination of blood glucose. *Biochem. J.* **66**: 12, 1957.

KANT G.J., MUGGEY E.H., MEYERHOFF J.L.: Diurnal variation in neuroendocrine response to stress in rats: plasma ACTH, beta-endorphin, beta-LPH, corticosterone, prolactin and pituitary cyclic AMP responses. *Neuroendocrinology* **43**: 389–390, 1986.

LANGER P., FÖLDES O., MACHO L., KVETŇANSKÝ R.: Changes of iodothyronine levels after acute and long-term hypokinesis (unforced restriction) in rats. *Endocrinol. Exp.* : 139–144, 1981.

NICHOLSON S., LIN J.H., MAHMOUD S., CAMPBELL E., GILHAM B., JONES M.: Diurnal variations in responsiveness of the hypothalamo-pituitary-adrenocortical axis of the rat. *Neuroendocrinology* **40**: 217–224, 1985.

NOSKOVIČ P., AHLERS I., RAČEK Ĺ.: New modification of suspension hypokinesia in the rat. *Physiol. Bohemoslov.* **39**: 471–474, 1990.

PLACER Z., SLABOCHOVÁ Z.: The colorimetric estimation of polyenoic fatty acids in the biological material. *Čs. fysiol.* **11**: 543–547, 1962 (in Czech).

ROE J.H., DAILEY R.E.: The determinanation of glycogen with anthrone reagent. *Anal. Biochem.* **15**: 245–250, 1966.

TORRELLAS A., GUAZA C., BORRELL J., BORRELL S.: Adrenal hormones and brain catecholamines responses to morning and afternoon immobilization stress in rats. *Physiol. Behav.* **26**: 129–133, 1981.

ZIMMERMANN E., CRITCHLOW V.: Effect of diurnal variation in plasma corticosterone levels on adrenocortical response to stress. *Proc. Soc. Exp. Biol. Med.* **125**: 658–663, 1967.

ZLATKIS A., ZAK B., BOYLE A.J.: A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.* **41**: 486–490, 1953.

Reprint Requests

Dr. I. Ahlers, Institute of Animal Physiology, Faculty of Science, Šafárik University, 041 67 Košice, Moyzesova 11, Slovak Republic.