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Circadian and Circaannual Oscillations of Tissue Lipoperoxides in Rats

P. SOLÁR, G. TOTH, B. ŠMAJDA, I. AHLERS, E. AHLERSOVÁ

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

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Summary

Circadian and circaannual oscillations of tissue lipid peroxides (LPO) were studied in young male Wistar rats. The concentration of malondialdehyde, one of LPO degradation products, was measured at 3-h intervals during 24 hours in rats, adapted to light:dark 12:12 h regimen in the course of the year. LPO in the liver, thymus and bone marrow oscillated rhythmically in the course of the day and year. Circadian oscillations in all tissues were two-peaked, with zeniths at various times of the light and dark parts of the day. In the liver and thymus, the highest mesors were found during the winter, in the bone marrow during the spring. The same holds for amplitude values, with the exception of the bone marrow which exhibited the highest values during the summer. The reason for the LPO oscillations is probably resulting from the changing ratio of pro- and anti-oxidative capacities in various tissues during the day and the year.

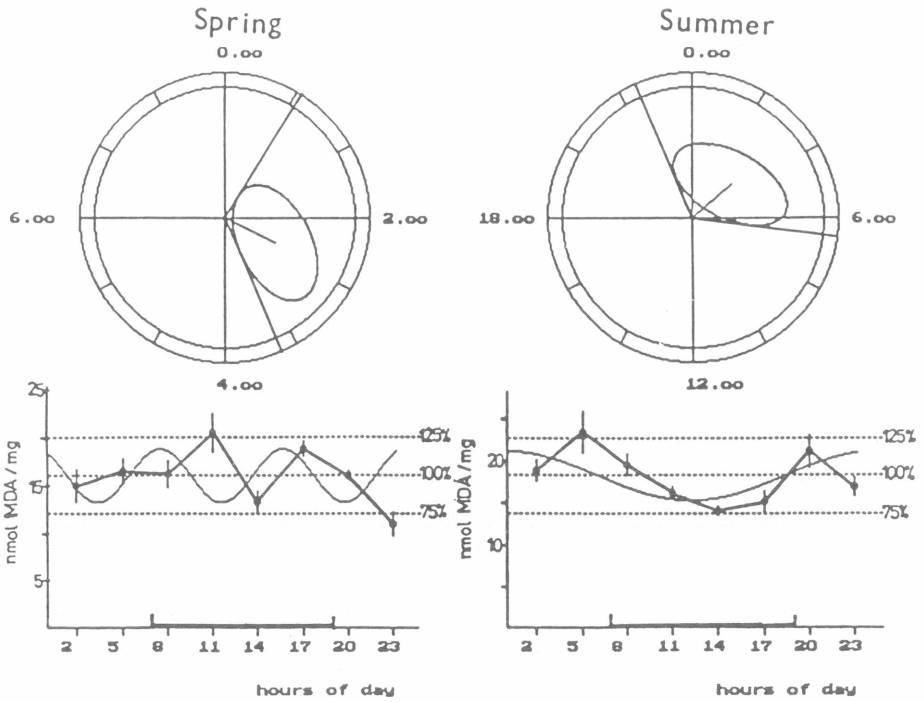
Key words

Rat tissues – Lipoperoxides – Day and year – Oscillations

Introduction

Peroxidation of lipids is a set of reactions taking place in biological systems under both normal and pathological conditions. Lipoperoxidation may be activated by various factors, e.g. radiation, excess or release of peroxidative activators (reactive products of oxygen), and decrease or inhibition of the action of antioxidative factors. An intensified form of lipoperoxidation occurs during the aging process, during degenerative changes (Petkau 1982), but also in diseases such as atherosclerosis, hepatic necrosis and others (McCay 1981, Matsumoto *et al.* 1981). The reactive products of oxygen are supposed to participate in the activation of oncogenes and to be involved in gene mutation, malignant transformation or cell death (Bergendi 1988, Jackson 1994). The hydroxyl radical, singlet oxygen and superoxide anion (reactive products of oxygen) induce peroxidation of unsaturated fatty acids that causes functional and structural changes in biological systems (especially in biomembranes). On peroxidation, unsaturated fatty acids are released from membranes, and a number of degradation products are formed (e.g. hydroperoxides, ketones of fatty acids, malondialdehyde and short-chained alkanes) (Tappel 1980). Many of lipoperoxidation products are toxic. It seems that malondialdehyde and 4-hydroxy-2,3-transnonenale, which may form cross-bonds between polypeptides and polynucleotide chains, belong to the most reactive ones (Marnett and Tuttle 1980). The organism protects itself from the noxious effect of oxygen reactive products (radicals) by a system of antioxidative agents that decrease the radical effect and in this way also diminish lipid peroxidation (Lippman 1983). Some enzymes (superoxide dismutase, catalase, the glutathione redox system), low-molecular traps and quenchers of free-radicals such as L-ascorbic acid, mannitol, alpha-tocopherol, bilirubin, histidine and others, belong to these antioxidative agents.

At present, only little information is available concerning the natural variation of animal lipid peroxides in tissue homogenates or in cellular organelles. The seasonal changes in lipoperoxides are even absent in the literature. Therefore, the aim of our work was to find out the circadian changes in lipoperoxides in the liver, thymus and bone marrow of rats during four seasons of the year. These organs were selected as representatives of low-proliferative (liver) and high-proliferative (thymus, bone marrow) tissues in relation to our previous studies in radiobiology.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0.00
6.00
2.00
4.00
Summer
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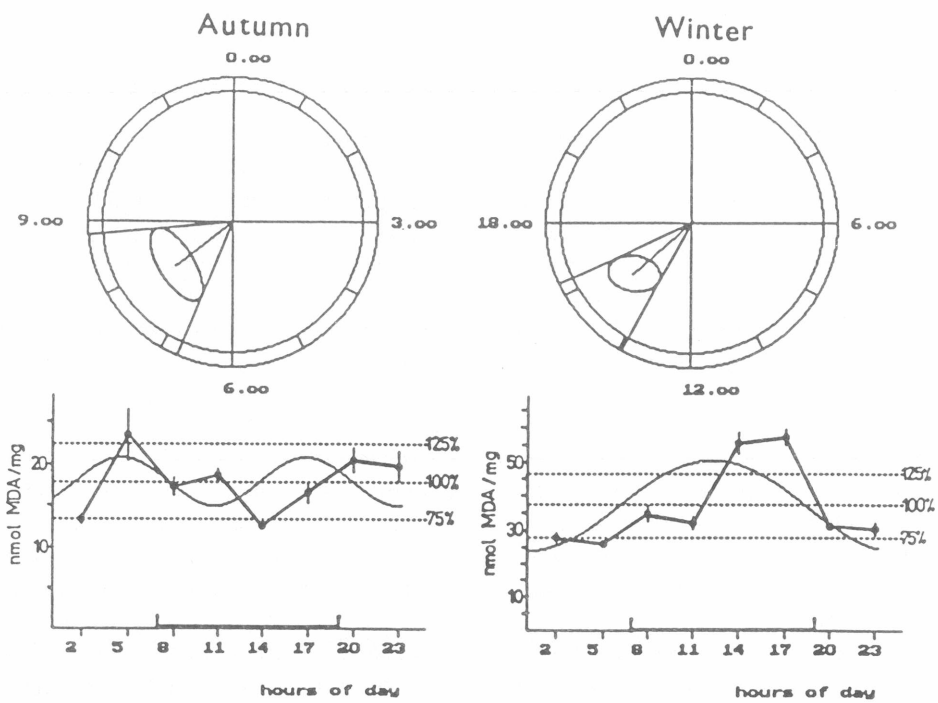
6.00
12.00
nmol MDA/mg
125%
100%
75%
2 5 8 11 14 17 20 23
hours of day

Liver


Autumn
0.00
9.00
3.00
6.00
Winter
0.00
18.00
6.00
12.00
nmol MDA/mg
125%
100%
75%
2 5 8 11 14 17 20 23
hours of day

Material and Methods

The circadian oscillations of lipid peroxides were studied in the liver, thymus and bone marrow of 3-month-old male Wistar SPF rats (Velaz, Prague). Rats were adapted to a light regimen (LD 12:12, light with an intensity of 150 lux in the cage from 0700 to 1900 h) for 4 weeks. All animals were fed (LD feed, Velaz, Prague) and received water *ad libitum*. Eight groups of animals, each consisting of 7–8 rats, were analyzed within 24 hours at 3-h intervals. The animals were killed by fast decapitation at 0800 h, 1100 h, 1400 h, 1700 h, 2000 h, 2300 h, 0200h and 0500 h approximately at the time of spring and autumn equinox and the summer and winter solistice.

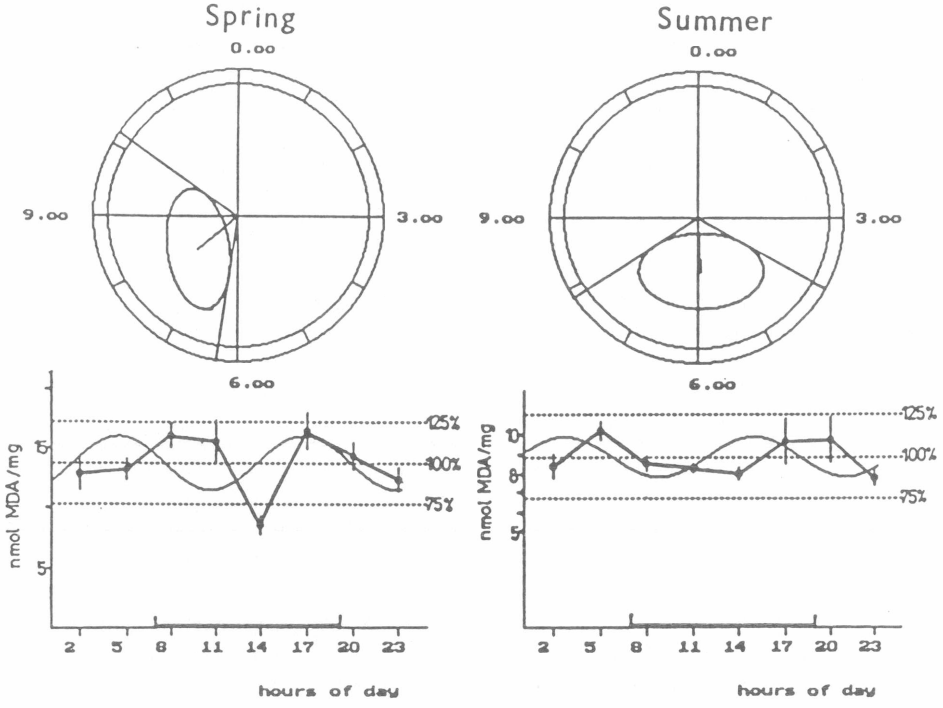
The concentrations of lipid peroxides were determined according to Satch (1978) using the reaction of malondialdehyde (MDA) with thiobarbituric acid. Briefly: 100 mg of liver, thymus and bone marrow were removed into 0.5 ml of isotonic saline solution. After homogenization of tissues 1 ml of 20 % trichloroacetic acid (Lachema, Czech Republic) and 0.5 ml of 0.67 % thiobarbituric acid (Merck) in 2 M sodium sulfate were added. The mixture was heated in a water bath (90 °C for 30 min) and the resulting chromogen was extracted with 2 ml of n-butyl alcohol (Lachema). The absorbance of the organic phase was determined at the wavelength of 535 nm. MDA formation was quantified using a molar extinction coefficient of 1.49×10^5 $l.mol^{-1}.cm^{-1}$. The values are given in nanomoles of malondialdehyde per gram of the tissue. The intra-assay precision of the method for the specimen with lipid peroxide value of 3.5 nmol/ml was 3.1 % (n=10) and the limit of detection was 0.03 nmol/test tube. The results were evaluated using the Peritz' F-test (Harper 1984) and population-mean cosinor analysis (Halberg *et al.* 1967).

Results

In the liver, the circadian oscillations of lipid peroxides were rhythmic during the 24 h period in summer and winter, and the 12 h period in autumn and 8 h period in spring. In summer and autumn, the maximal concentrations of lipoperoxides were found at 2000 h and 0500 h. In spring, the maximal value was observed at 1100 h and 1700 h, and in winter at 0800 h and 1700 h. In spring, summer and winter the amplitudes ranged within 2.80–1.93 nmol MDA/g; the amplitude increased to a value of 13.21 nmol MDA/g in winter only. The acrophase was localized in the spring and summer before the beginning (at 0235 h and 0313 h) and in autumn and winter after the beginning (at 0745 h and 1515 h) of the light part of the day. The highest mesor was recorded in winter (37.12 nmol MDA/g) and the lowest one in spring (15.99 nmol MDA/g), while the difference between the winter and spring mesor, and the summer and autumn mesor was significant (Figs. 1 and 4, Table 1).

In the thymus, the lipid peroxidation oscillated rhythmically at the selected 12 h period in spring, summer and autumn. Only in winter, lipoperoxidation oscillated rhythmically with the 24 h period. The highest concentrations of lipoperoxides were observed in this organ at 0800 h and 1700 h in the spring, autumn and winter. In summer, the maximum was found out at 0800 h and 0500 h. The amplitude reached its highest value (13.21 nmol MDA/g) in winter, while in summer its value was the lowest (1.05 nmol MDA/g). In autumn and summer the acrophase was localized before the beginning of the light part of the day (at 0642 h and 0533 h, respectively), in spring it was close after the beginning of the light part of the day (at 0743 h), and in winter at 1545 h before the beginning of the dark part of the day. The lowest mesor was observed in summer (8.85 nmol MDA/g); this was approximately twice as high in autumn (16.51 nmol MDA/g) and about four times higher in winter (37.12 nmol MDA/g). All differences between the mesors during individual seasons, except the difference between spring and autumn, were significantly different (Figs 2 and 4, Table 1).

Fig. 1
Circadian oscillations of lipid peroxides in the liver according to the four seasons of the year. The basal characteristics of oscillations are illustrated on the cosinor diagrams (circular plots). The vector originating from the centre of the circular system of coordinates represents the amplitude of oscillations. The orientation of the vector indicates the acrophase on the circular scale (in hours). The ellipse drawn with the centre at the end of the vector represents the confidence area ($P<0.05$) for the acrophase and amplitude. The tangents to the ellipse delimitate the confidence interval for the acrophase ($P<0.05$). If the ellipse does not overlap the origin, the rhythm with the chosen period is present. Only results for significant rhythmic components are given. The thick line represent the experimental values given as means ± S.E.M. and the thin line represents the approximated model function. The values are expressed as MDA concentrations in nmol/mg (on the left) and as % of the overall mean (on the right). Broken line indicates the light part of the day.



Thymus

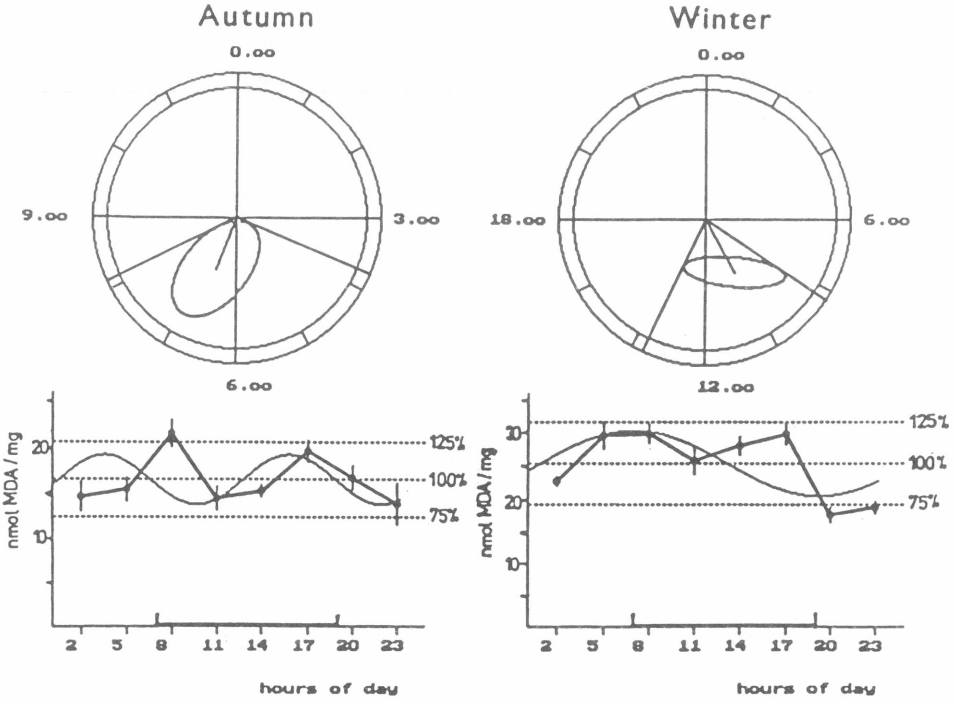


Fig.2
Circadian oscillations of lipid peroxides in the thymus in four seasons of the year. For other details see Fig. 1.

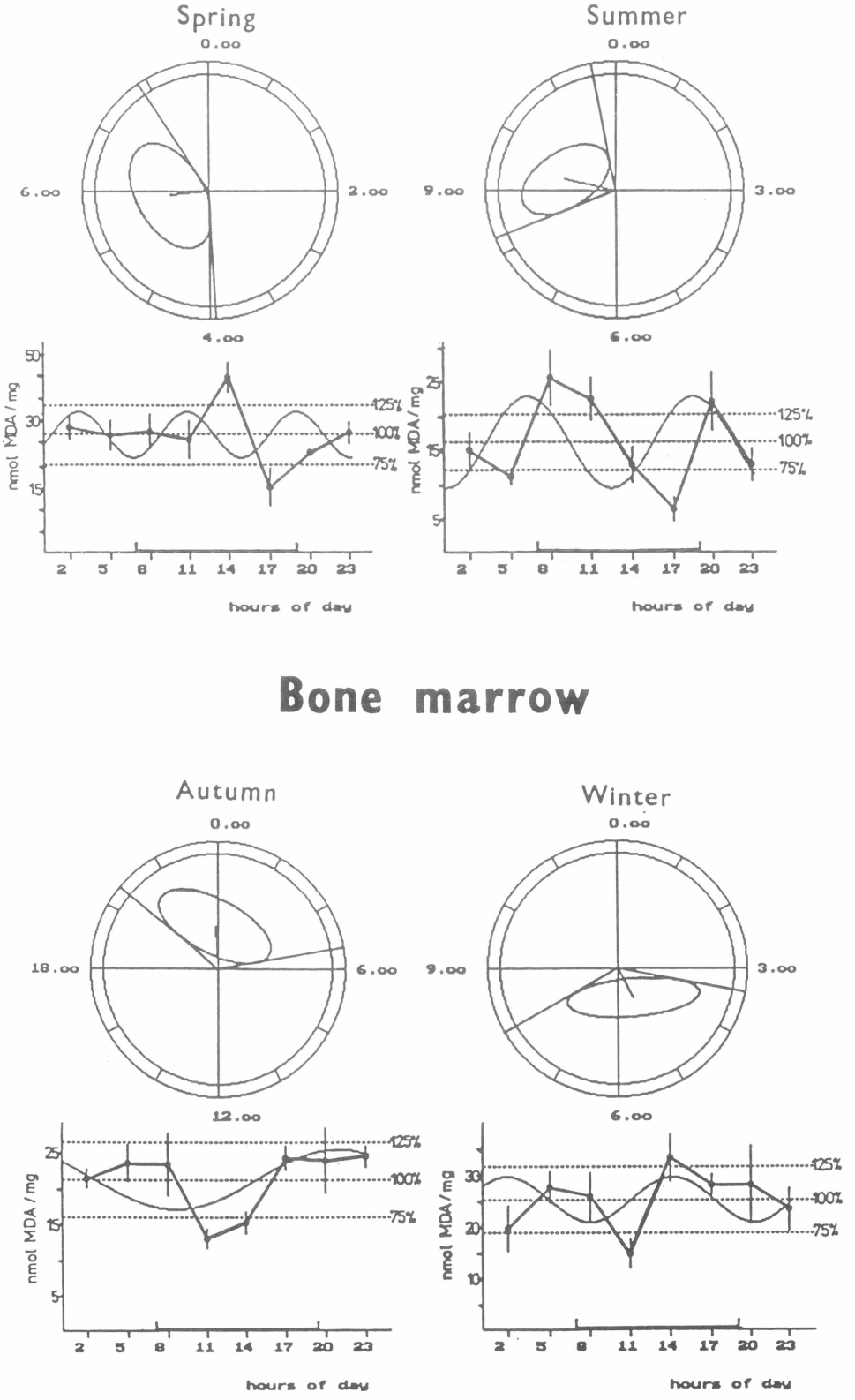


Fig. 3
Circadian oscillations of lipid peroxides in the bone marrow in four seasons of the year. Other details as in Fig. 1.

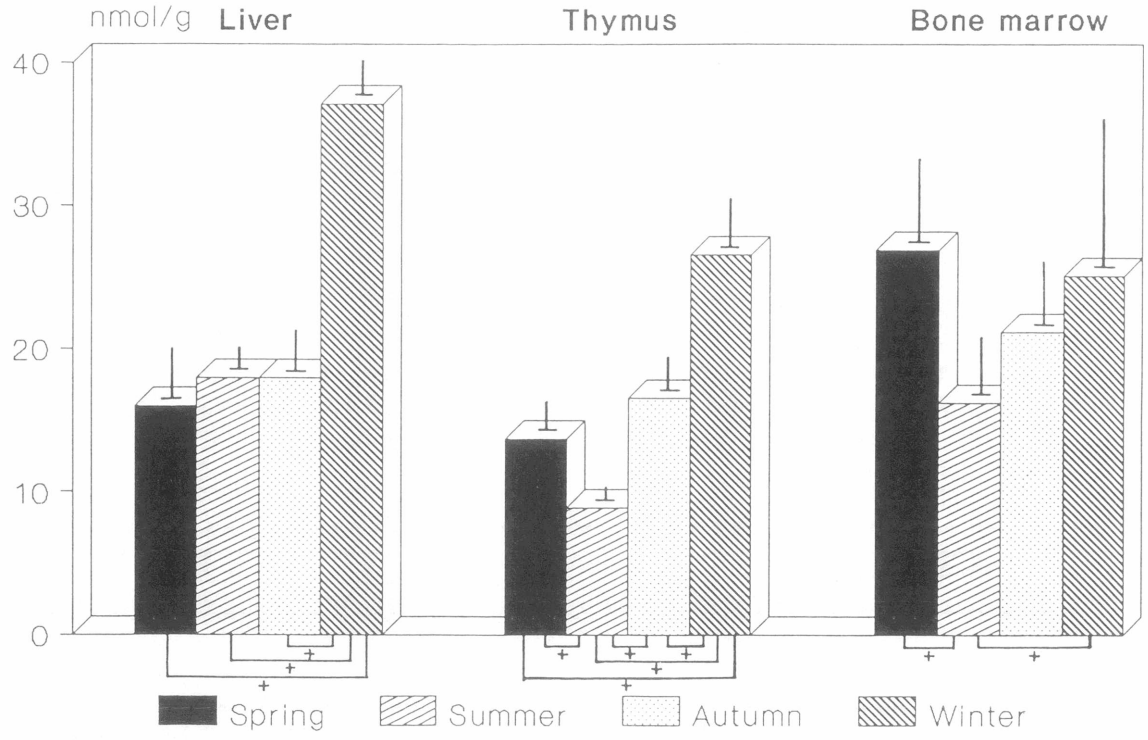


Fig. 4
Mesors (mean of adjusted values ± S.E.M.) of lipid peroxides in the liver, thymus and bone marrow in individual seasons of the year. The significance of differences between individual seasons are at the probability level of $P<0.05$.

Table 1
Characteristics of the cosinor test.

Tissue	Season	Period of the rhythm	Mesor ± S.E.M. nmol MDA/mg	Amplitude ± CI (95 %) nmol MDA/mg	Acrophase ± CI (95 %) hours
LIVER	Spring	8	15.99±0.83	2.80 (0.42; 5.03)	0052 (0015; 0116)
	Summer	24	17.97±0.32	2.89 (0.87; 5.14)	0313 (2248; 0653)
	Autumn	12	17.93±0.67	2.93 (2.15; 3.54)	0352 (0323; 0425)
	Winter	24	37.12±0.54	13.21 (9.19;16.08)	1515 (1356; 1637)
THYMUS	Spring	12	13.65±0.45	2.25 (0.73; 3.63)	0351 (0313; 0508)
	Summer	12	8.85±0.21	1.05 (0.29; 1.69)	0256 (0200; 0356)
	Autumn	12	16.51±0.58	2.73 (0.35; 7.23)	0321 (0153; 0406)
	Winter	24	26.53±0.82	5.23 (3.63; 6.54)	1006 (0826; 1351)
BONE MARROW	Spring	8	26.87±1.40	5.21 (0.38; 9.96)	0157 (0121; 0228)
	Summer	12	16.17±0.95	6.67 (2.10;10.67)	0442 (0414; 0549)
	Autumn	24	21.13±1.04	4.20 (1.20; 7.20)	2344 (2038; 0537)
	Winter	12	25.04±2.56	4.38 (1.19; 4.78)	0233 (0148; 0400)

The mesor (the mean value of the fitted curve) and amplitude are given in nmol MDA/mg; the acrophase is given in relation to time. CI – confidence interval; its limits are given in brackets.

The lipid peroxidation in the bone marrow oscillated rhythmically with the selected 24 h period in autumn, with the 12 h period in summer and winter, and with the 8 h period in spring. Concentrations of lipoperoxides reached their highest values at 1400 h and 0200 h in spring, at 0800 h and 2000 h in summer, at 0800 h and 2300 h in autumn, and at 1400 h and 0500 h in winter. The amplitudes ranged over a narrow interval from 4.2 (in autumn) to 6.67 nmol MDA/g (in summer). However, acrophases, except for those in spring and winter (0552 h and 0506 h), differed considerably, The highest values were observed in spring and winter (26.87 and 25.04 nmol MDA/g, respectively), but the difference between them was not significant. Significant differences were recorded only between the summer and winter mesors and summer and spring mesors. In summer, the mesor was the lowest (16.17 nmol MDA/g)(Figs. 3 and 4, Tab. 1).

Discussion

The concentration of lipoperoxides in the liver, thymus and bone marrow oscillated during the day and year. The circadian oscillations were double-peaked, with peaks in various light and dark parts of the day. In the liver and thymus the highest values of mesor and amplitude were observed in winter, the lowest ones in spring or summer. In the bone marrow, the values were more balanced in the individual parts of the year, with their lowest value in summer. Oscillations were rhythmic at the selected 24 h period or the period shorter than 24 h. The acrophase of the rhythm in individual seasons "wandered" in various light and dark parts of the day.

The interpretation of the circadian rhythm of tissue lipid peroxides is difficult. The peaks are divided into both parts of the day without unequivocal culmination only in the dark part of the day. This indicates a low probability of its relationship to food intake, which, in rats, takes place in the dark part. The circadian patterns of oscillations of the main lipid fractions are known in the rat serum and liver (Ahlers *et al.* 1980), in the thymus and bone marrow (Ahlers *et al.* 1983). It is difficult to search for, at least, certain analogies with circadian oscillations of lipid peroxides. The formation of lipoperoxides is modified by the representation of pro- and anti-oxidative capacities in individual tissues and it also depends on the type of cellular proliferation in the given tissue. Diaz-Muňoz *et al.* (1987) reported the circadian variations of lipoperoxides in the cerebral cortex of adult rats. Their report indicated that there is a relationship of lipoperoxide oscillations to the metabolic pathways of glutathione and to the activity of superoxide dismutase. The increase in lipoperoxidation was observed simultaneously with a decrease in glutathione reductase and an increase in superoxide dismutase activity (Diaz-Muňoz *et al.* 1985). The relationship of circadian rhythms of lipoperoxidation to the glutathione reductase activity was also observed in various tissues in rats by Farroqui *et al.* (1984). They also confirmed the reversed ratio between the concentration of lipoperoxides and the activity of glutathione reductase.

Seasonal changes in the oscillations of lipids were expressed only little in the serum of rats (Ahlers *et al.* 1982). It is necessary to consider the fact that the seasonal effect in circadian rhythms is evident in laboratory rats despite their maintenance under standard laboratory conditions during the year (Ahlersová *et al.* 1991, 1992) The annual cycle in rats has its important period at the end of winter and at the beginning of spring. At this time, the level of food intake increases (Rietfeld *et al.* 1980) and the circadian oscillations of thyroid hormones are more pronounced (Ahlersová *et al.* 1984). In spring, the maximum of adrenocortical (corticosterone) response to the stress stimulus was formed in young male Wistar rats; in females the reactivity culminated in winter (Ahlers *et al.* 1990).

In conclusion, we have stated the necessity to evaluate the results of rat tissue lipoperoxides in the dependence on the time of day and year.

References

AHLERS I., AHLERSOVÁ E., ŠMAJDA B., SEDLÁKOVÁ A.: Circadian rhythm of serum and lipids in fed and fasted rats. *Physiol. Bohemoslov.* **29:** 525–553, 1980.

AHLERS I., AHLERSOVÁ E., DAXNEROVÁ Z., TOROPILA M., ŠMAJDA B.: Influence of the season on the circadian rhythm of serum lipids in male Wistar rats. *Physiol. Bohemoslov.* **31:** 65–73, 1982.

AHLERS I., AHLERSOVÁ E., ĎATELINKA I., TOROPILA M.: Circadian rhythm of bone marrow and thymus lipids in rats. *Physiol. Bohemoslov.* **32:** 476–480, 1983.

AHLERS I., AHLERSOVÁ E., HALÁTOVÁ K., ŠMAJDA B., TOROPILA M.: The circadian and circaannual response of rat serum corticosterone to different stressors. *Physiol. Bohemoslov.* **39:** 371–378, 1990.

AHLERSOVÁ E., AHLERS I., MILAROVÁ R., ĎATELINKA I., TOROPILA M.: Circadian oscillations of thyroid hormones, insulin and glucagon in the blood of laboratory rats in the course of the year. *Physiol. Bohemoslov.* **33:** 309–319, 1984.

AHLERSOVÁ E., AHLERS I., ŠMAJDA B.: Influence of light regimen and time of year on circadian oscillations of thyroid hormones in rats. *Physiol. Res.* **40:** 305–315, 1991.

AHLERSOVÁ E., AHLERS I., ŠMAJDA B.: Influence of light regimen and time of year on circadian oscillations of insulin and corticosterone in rats. *Physiol. Res.* **41:** 307–314, 1992.

BERGENDI Ľ.: Superoxide and Other Bioactive Oxygen Forms (in Slovak), Publishing House of the Slovak Academy of Sciences, Bratislava, 1988.

DIAZ-MUŇOZ M., HERNANDEZ-MUŇOZ R., SUAREZ J., CHAGOYA DE SANCHEZ V.: Day-night cycle of lipid peroxidation in rat cerebral cortex and their relationship to the glutathione cycle and superoxide dismutase activity. *Neuroscience* **16:** 859–863, 1985.

DIAZ-MUŇOZ M., SUAREZ J., HERNANDEZ-MUŇOZ R., CHAGOYA DE SANCHEZ V.: Day-night cycle of lipidic composition in rat cerebral cortex. *Neurochem. Res.* **4:** 315–321, 1987.

FARROQUI M.Y.N., AHMED A.E.: Circadian periodicity of tissue glutathione and its relationship with lipid peroxidation in rats. *Life Sci.* **34:** 2413–2418, 1984.

HALBERG F., TONG Y.L., JOHNSON E.A.: Circadian system phase – an aspect of temporal morphology, procedures and illustrative examples. In: *The Cellular Aspects of Biorhythms.* H.V. MAYERSBACH (ed.), Springer, Berlin, 1967, pp. 20–48.

HARPER J.F.: Peritz' F test: basic program of a robust multiple comparison test for statistical analysis of all differences among group means. *Comput. Biol. Med.* **14:** 437–445, 1984.

JACKSON J.H.: Potential molecular mechanisms of oxidant-induced carcinogenesis. *Environ. Health Persp.* **102** (Suppl 10): 155–158, 1994.

LIPPMAN R.D.: Lipid peroxidation and metabolism in aging: a biological and medical approach. In: *Review of Biological Research in Aging.* Vol. 1., M. ROTHSTEIN, W. ADLER, V. CRISTOFALO, C.E. FINCH, J. FLORINI, G. MARTIN (eds), Alan R. Liss, New York, 1983, pp. 315–342.

MARNETT L.J., TUTTLE M.A.: Comparison of the mutagenicities of malondialdehyde and the side products formed during its chemical synthesis. *Cancer Res.* **40:** 276–282, 1980.

MATSUMOTO M., WAKASUGI H., IBAYASHI H.: Serum vitamin E, lipid peroxide and glutathione peroxidase in patients with chronic pancreatitis. *Clin. Chim. Acta* **110:** 121–125, 1981.

McCAY P.B.: Introduction: physiological significance of lipid peroxidation. *Fed. Proc.* **40:** 173–177, 1981.

PETKAU A.: Concluding remarks: a prospective view of active oxygen in medicine. *Can. J. Physiol. Pharmacol.* **69:** 1425–1429, 1982.

RIETFELD W.J., FLORY W., KOOIJ M., TEN HOOR F.: Observations on total food intake and 24 h food intake patterns during long-term registration of food intake behaviour in rats. *Z. Versuchstierk.* 22: 156–160, 1980.

SATCH K: Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta* **90:** 37–43, 1978.

TAPPEL A.: Measurement of and protection from in vivo lipid peroxidation. In: *Free Radicals in Biology.* Vol. 4., W.A PRYOR (ed.), Academic Press, New York, 1980, pp. 1–47.

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Mgr. P. Solár, Institute of Animal Physiology, Faculty of Science, Šafárik University, Moyzesova 11, 041 67 Košice, Slovak Republic