con in their molecule. However, the much stronger activity of I and III than of IV is evidence that the most important role in the effective manifestation of the function of silicon belongs to its introduction into the silatrane group $\overset{\downarrow}{Si}(OCH_2CH_2)_3\overset{\downarrow}{N}$. Organosilicon compounds with this structure are much more active than acyclic compounds.

The results confirm that an essential component in the mechanism of the stimulating action of silatranes on the course of repair processes and, in particular, on wound healing, is their effect on the proliferative-reparative function of connective tissue. According to the principal biochemical parameter of "maturity" of developing GT, namely the collagen content, the best effect was caused by liniments containing 0.5% of compounds I and II. This indicates that the local application of excessive doses of silatranes is contraindicated.

ROLE OF PHOTOPERIODICITY AND THE CIRCADIAN RHYTHM OF GLUCOCORTICOIDS IN SYNCHRONIZATION OF FREE-RADICAL OXIDATION FLUCTUATIONS IN RATS

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KEY WORDS: free-radical oxidation of lipids; antioxidative activity; circadian rhythms; glucocorticoids; light-darkness cycle.

The antioxidative activity (AOA) and content of products of free-radical oxidation (FRO) of lipids significantly affect many parameters of cell metabolism: the permeability of cytoplasmic membranes [4], activity of enzymes bound with them [2], and so on. Circadian fluctuations in FRO of lipids and AOA have been described in man [6] and rats [1]. However, the role of various factors in the synchronization of these rhythms remains undecided.

There is information in the literature on the connection between rhythm of AOA and FRO of lipids with mitotic activity of cells [1] and the state of the multipurpose oxidase system of the endoplasmic reticulum of the liver [5]. Among other factors influencing these rhythms and possibly under their control are corticosteroids [7], the distinct circadian rhythm of which has been described many times [3].

The object of this investigation was to study artifical modification of the circadian rhythm of glucocorticoids and the light-darkness cycle on AOA and the content of products of FRO of lipids in the liver and erythrocytes of rats.

EXPERIMENTAL METHOD

The role of photoperiodicity in the synchronization of the above-mentioned rhythms was investigated in experiments with reversal of the light-darkness cycle. Adult male Wistar rats were used. The conditions of illumination were 12 h of light:12 h of darkness in one chamber and 12 h of darkness(12 h of light in the other, regulated automatically with simulation of dawn and dusk for 3 h. Transitions to total darkness and light occurred at 6 a.m. and 6 p.m. The experiments were carried out before and 14 days after reversal.

In another experiment noninbred female albino rats were given a single daily intramuscular injection of hydrocortisone acetate in a dose of 250 µg/100 g for 16 days in the morning or evening. The time of the injections was synchronized with the time of switching the light on (8 a.m.) and off (8 p.m.). Control animals were given an injection of physiological saline at these same times. In this particular experiment a sudden change of illumination was provided during the transition from darkness to light and vice versa. The experiments were carried out in June and July.

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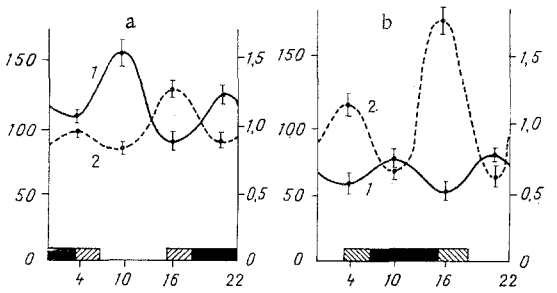

Fig. 1. Circadian rhythms of AOA and content of products of FRO of lipids in rat liver with normal (a) and reversed (b) light-darkness cycle. 1) AOA of lipids, 2) content of diene conjugates. Obliquely shaded regions — periods of twilight. Abscissa, time of day (in h); ordinate, on left — AOA (in h/ml/g lipids); right — content of diene conjugates (in optical density units/ml of lipids).

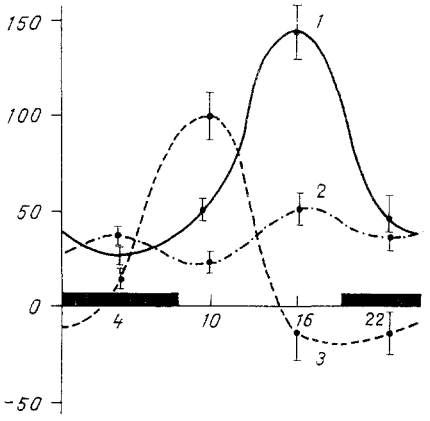

Fig. 2. Antioxidative activity of lipids in liver of female rats receiving cortisol at different times of 24-h period (16 daily intramuscular injections each of 250 μg/100 g). 1) Control, injections of physiological saline; 2) injection of cortisol in the morning (8 a.m.); 3) injection of cortisol in the evening (8 p.m.). Abscissa, time of 24-h period (in h); ordinate, AOA (in h/ml/g lipids).

The rats were kept five to a cage at a temperature of 24 ± 1°C and with food and water ad lib. The experiments began not earlier than 2 weeks after the beginning of adaptation of the animals to the conditions of keeping. Animals were killed 4 times during the 24-h period. AOA of lipids [2] and the content of products of FRO of lipids were determined in the liver and erythrocytes by studying the UV-spectrum of diene conjugates [9]. The concentration of 11-hydroxycorticosteroids (11-HCS) in the blood plasma and urine was determined by a fluorometric method in the modification in [8].

EXPERIMENTAL RESULTS

In both experiments a significant circadian rhythm of AOA of lipids was found in the liver of the control animals with a rise during the daytime. When the conditions of illumination simulated dawn and dusk, a second peak also was observed at night (Fig. 1a). The rhythm of AOA of lipids in erythrocytes showed a similar phase structure and it correlated significantly ($r = 0.73$) with the rhythm of AOA in the liver. Curves showing the content of products of FRO of lipids in the liver and erythrocytes are opposite in phase to the rhythms of AOA.

After reversal of the light-darkness cycle the mean diurnal level and amplitude of the rhythm of AOA of lipids in the liver fell (Fig. 1b). Against this background the amplitude and mean diurnal level of FRO products increased sharply. The phase relations between the rhythms were the same as before: The maximum of AOA corresponded to the lowest content of products of FRO of lipids in the liver. Analysis of biorhythms thus confirms the known reciprocity of relations between AOA and the intensity of FRO, which was preserved after reversal of the conditions of illumination.

The increase in the mean diurnal level and amplitude of the rhythm of FRO of lipids during a change in the photoperiodic transducer is evidence of stress accompanying the process of temporal reorganization. After a 12-hour shift of the time transducer the phase structure of the rhythm of FRO, which has a 12-hourly component, was unchanged so that the position of twilight and the time axis after reversal remained the same as before. Peaks of concentration of FRO products, just as in the control group, were observed during the twilight hours (Fig. 1b).

These results are evidence that an increase in the intensity of free-radical oxidative processes is characteristic not only of actively metabolizing tissues [6], but also of phases of increased metabolism — the transition periods in the light-darkness cycle when in rats, which are twilight animals, peaks of motor activity and other manifestations of vital activity are observed. Similar results were obtained previously in a study of the content of lipid hydroperoxides in human erythrocytes in the polar region. Both during a normal light-darkness cycle and during the polar day and polar night, the acrophase of the rhythm occurred during daytime and coincided with the maximum of oxygen consumption, i.e., with the period of the greatest functional load on the red blood system [6].

The highest 11-HCS concentration in the plasma of the female rats was observed at 10 p.m., which was the time of peak motor activity, measured as the number of vibrations of the cell during the animal's movements. The raised blood corticosteroid level was followed by an increase in their excretion with the urine, which was maximal in the portions collected from 10 p.m. to 2 a.m.

The rhythm of the plasma 11-HCS concentration in males kept under conditions of illumination of 12 h daylight:12 h darkness with twilight periods had a biphasic structure with a very small rise at 10 a.m. and a larger rise at 10 p.m. Reversal of the light-darkness cycle led to a prolonged increase in the corticosteroid content in the period from 4 to 10 a.m., and at 10 p.m. minimal concentrations were observed, evidence of reversal of the circadian rhythm of these hormones.

The question accordingly arose: What led to the changes found in the rhythms of FRO and AOA of lipids — a shift of the photoperiodic transducer or changes in corticosteroid rhythms, which are regularly connected with it? The use of an experimental model of an artifical glucocorticoid rhythm in rats gave the following results.

Injection of cortisol during the evening, at a time of increased endogenous corticosterone secretion, caused an increase in amplitude of the circadian rhythm — a considerable evening-night peak of 11-HCS excretion. This was accompanied by a shift of the maximum of lipid AOA from 4 p.m. to 10 a.m. (Fig. 2).

Morning injection of hydrocortisone caused the appearance of a daytime rise in the 11-HCS concentration together with a decrease in the noctural maximum. In this connection the amplitude and significance of the circadian rhythm were considerably reduced and the representation of the 12-h component was increased. Injections by this program were accompanied by similar changes in fluctuations of AOA of lipids: a decrease in amplitude of the 24-hourly harmonic and the appearance of an ill-defined biphasic pattern (Fig. 2).

Injection of hydrocortisone both at the end and at the beginning of the light period, causing hypercortisonemia in both cases, led to a decrease in the mean diurnal level of lipid AOA in the liver by 46% in the morning and by 71% in the evening group. The experiments are thus evidence that external interference with the glucocorticoid rhythm without any change in the conditions of illumination itself can modify the rhythm of AOA of lipids.

Analysis of these results relating to levels and amplitude-phase relations between the rhythms of the parameters studied leads to the conclusion that glucocorticoids play the role of modulators of circadian fluctuations in AOA and FRO of lipids and may be responsible for changes in these fluctuations observed during reversal of the light-darkness cycle.

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GENERATION AGE AS A FACTOR DETERMINING THE USE OF HEMATOPOIETIC STEM CELLS

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The population of hematopoietic stem cells (HSC) is heterogeneous as regards its degree of self-renewal [2]. It is considered that this heterogeneity is due to differences in the generation age of the stem cells, i.e., differences in the number of divisions through which they have passed. It has been postulated that the older stem cells, i.e., those which have passed through mitosis more often, undergo differentiation first [3, 4]. This hypothesis, which has now become almost canonical, regarding the organization of release of stem cells into differentiation depending on their generation age, is in fact based only on the results of the study of self-renewal of HSC after exposure to cytostatics. In particular, repeated exposure to hydroxyurea, which kills cells in the synthetic period of the cell cycle, leads to selection of the youngest stem cells, with a high degree of self-renewal; the older stem cells are mobilized more easily into the cycle of depopulation of the hematopoietic system caused by the first injections of hydroxyurea, and die when subjected to its action during subsequent injections [3, 4]. These data are of fundamental importance for the understanding of the mechanisms of regulation of HSC, more especially because the conclusion that the proliferative potential of stem cells is directly connected with their generation age is not immune from criticism.

For the foregoing reasons it was decided to undertake an experimental verification of the generation-age hypothesis of the use of HSC.

EXPERIMENTAL METHOD

Female C57BL/6 and hybrid (CBA × C57BL/6)F_1 (abbreviated hereafter to F_1) mice aged 8-12 weeks were used. HSC were determined by cloning in the spleen of mice [5] irradiated in doses of 10 Gy (C57BL/6) or 13 Gy (F_1). Under these conditions the number of endogenous colonies did not exceed 0.2 per spleen. Self-renewal of HSC was characterized by the number of CFUs in the pool of splenic colonies and their number in individual 11-day colonies were determined as described previously [1]. Hydroxyurea in a dose of 1 mg/g body weight was injected intraperitoneally into the mice either strictly in accordance with the scheme [3], i.e., five times altogether 32, 26, 10, 7, and 2 h before removal of bone marrow, or six times every 12 h or every 15 h, followed by removal of bone marrow 2 h after the last injection.

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