EFFECT OF X-RAY IRRADIATION ON CIRCADIAN RHYTHM OF MICROSOMAL HEMOPROTEIN LEVELS IN RAT LIVER

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Inconsistencies in information on postradiation changes in levels of cytochrome P-450 [6, 8, 11, 13, 16], which is responsible for the direct binding and oxidation of substances, and also the absence of data on the effect of irradiation on the circadian rhythm of activity not only of cytochromes of the P-450-dependent oxidation system, but also on the antioxic function of the liver as a whole, which greatly limit our ability to correct radiation pathology, motivated the present investigation. This paper gives the results of a study of circadian rhythms of microsomal concentrations of cytochromes P-450 and b_5 in the liver of rats with an acute form of radiation sickness.

EXPERIMENTAL METIIOD

Experiments were carried out on noninbred male albino rats weighing 160-190 g, kept on a standard diet. The investigations were conducted in the spring and summer, and the animals were kept under conditions of natural daylight and darkness. The rats were deprived of food for 20-24 h before the experiment, but were at lowed water ad lib. The rats were irradiated in a dose of 12 Gy on the RUM-17 apparatus under the following conditions: filter 0.5 mm Cu + 1 mm Al, tube voltage 20 kV, dose rate $2.15 \cdot 10^{-4}$ A/kg body weight. Irradiation lasting about 25 min was always carried out at the same time of day (8.30 a.m.). The rats were killed at 10 a.m., noon, 3 and 9 p.m., and 4 a.m. during the 1st and 2nd days after irradiation (6-8 animals at each time), and also on the day when most animals died (the 4th day). The liver (cut into pieces) was washed in 0.154 M KCl solution. The microsomal fraction was isolated by the method in [3] on a VAC-602 ultracentrifuge (105,000*g*) in 0.154 M KCl solution, pH 7.4. Concentrations of cytochromes P-450 and b_5 were determined on a "Specord UV VIS" recording spectrophotometer (East Germany), in the presence of dithionite and CO, and calculated allowing for coefficients of molar extinction of $91 \cdot 10^3$, $111 \cdot 10^3$, and $164 \cdot 10^3$ cm^2/mg microsomal protein respectively [15]. Protein was determined in the modification in [9]. The results were subjected to statistical analysis [2].

EXPERIMENTAL RESULTS

Determination of concentrations of cytochromes in the microsomal fraction of rat liver at the above-mentioned times of the 24-h period revealed the existence of definire biorhythms. At night and in the morning (4 and 10 a.m.) the cytochrome P-450 level was higher than its minimum, which occurred in the evening (9 p.m.), by 35 and 75% respectively, and reached a maximum between noon and 3 p.m., when it was increased by 2.5 times (Table 1). Circadian changes in cytochrome P-450 were accompanied by fluctuations of the level of its functionally inactive form, cytochrome P-420, which reached a maximum at 3 p.m. (Table 2). The cytochrome b_5 concentration was minimal at night, after which it shows a tendency to rise (10 a.m.), and by noon to 3 p.m. its level was more than doubled (Table 3). Thus circadian changes in concentrations of cytochromes P-450 and b_5 are characterized by a quite distinct acrophase during the day (noon 3 p.m.). A monophasic type of circadian rhythm also was observed for microsomal protein, but in this case the peak concentration was reached during the evening (9 p.m., Fig. 1). Evidently circadian rhythms of cytochrome concentrations, in view of the great complexity of the mechanisms of their regulation,

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TABLE 1. Cytochrome P-450 Concentration in Microsomal Fraction of Rat Liver

Time, h	Control	Irradiation, 12 Gy		
		1st day	2nd day	4th day
10	0,327±0,02 $p<0,001$	0,249±0,06 $p, p_1>0,05$	0,199±0,03 $p_1<0,001$	
12	0,480±0,038 $p<0,001$	0,224±0,03 $p_1<0,001$	0,162±0,02 $p_1<0,001$	0,111±0,02 $p_1<0,001$
15	0,470±0,45 $p<0,001$	0,308±0,05 $p<0,05$ $p_1<0,05$	0,260±0,04 $p<0,02$ $p_1<0,001$	
21	0,189±0,019	0,175±0,03	0,269±0,02 $p<0,02$ $p_1<0,02$	
4	0,257±0,019 $p<0,05$	0,191±0,02 $p_1>0,05$	0,188±0,03 $p_1>0,05$	

Legend. Here and in Tables 2 and 3, activity of cytochromes expressed in nanomoles/min/mg protein; p) index of significance within group relative to minimal value (underlined); p_1) index of significance relative to control.

TABLE 2. Cytochrome P-420 Concentration in Microsomal Fraction of Rat Liver

Time, h	Control	1st day	Irradiation, 12 Gy	
			2nd day	4th day
10	0,100±0,02 $p>0,05$	0,143±0,06 $p, p_1>0,05$	0,200±0,035 $p_1<0,01$	
12	0,086±0,02	0,104±0,01 $p, p_1>0,05$	0,222±0,04 $p_1<0,001$	Traces
15	0,185±0,019 $p<0,01$	0,197±0,056 $p, p_1>0,05$	0,161±0,02 $p>0,05$	
21	0,159±0,03 $p<0,01$	0,090±0,016 $p_1<0,05$	0,150±0,025 $p_1>0,05$	
4	0,099±0,012 $p>0,05$	0,129±0,029 $p_1>0,05$	0,165±0,029 $p_1>0,05$	

are linked with the rhythm of formation of endogenous substrates, which act as inhibitors or inducers of the cytochrome P-450 system, and are connected by negative feedback with the circadian rhythm of hormonal secretion and, in particular, of 11-hydroxycorticosteroids [14].

With the development of the acute form of radiation sickness (1st, 2nd, and 4th days) significant changes took place in the circadian rhythm and concentrations of the cytochromes. As early as 3-6 h after x-ray irradiation (corresponding to noon and 3 p.m.) the concentrations of cytochromes P-450 and b_5 were reduced by 1.5-2 times, the decrease in the cytochrome b_5 concentration lasting longer (Tables 1 and 3). The cytochrome P-450 concentration was unchanged, but a tendency was observed for it to increase (Table 2). The level of cytochrome P-450 on the 2nd day showed sharp changes compared with the control: it was reduced by almost two-thirds at noon, and increased by 1.6 times at 9 p.m. The reduction of its concentration was accompanied by an increase in that of cytochrome P-450 by 2-2.5 times, but no clear stoichiometry could be observed between the decrease in its concentration and the increase in that of P-420. The cytochrome b_5 concentration stabilized at the resting phase level (Table 2). On the 4th day (the day when most of the irradiated animals died) the concentrations of the principal hemoproteins fell to their minimal values. Thus the circadian rhythm of cytochrome P-450 behaves as a decremental process during irradiation, tot lowed by a shift of the phase dynamics; the circadian rhythm of cytochrome b_5 also is disturbed, but more significantly on the 1st day after irradiation. The trend of the microsomal protein level during the first 2 days was characterized by opposite changes (Fig. 1).

Available information in the literature on the effect of irradiation on the concentrations of cytochromes P-450 and b_5 in the rats' liver either is limited (for cytochrome b_5) and indicates no change in its level [11], or (for cytochrome P-450) extremely contradictory [1, 6, 8, 11, 13, 16]. The results of the present investigation confirm data revealing high sensitivity of cytochrome P-450 and of the processes of protein biosynthesis in the liver as a whole to irradiation [8, 12].

Cytochrome P-450 is the common name for a group of hemoproteins of the b-type, known as multiple forms of cytochrome P-450. It has been suggested that some of them exhibit increased sens itivity to irradiation, and to products of radiation-induced lipid peroxidation [6, 12]. Blocking of cytochrome P-450 biosynthesis, taking place under conditions of postradiation accumulation of lipid peroxides, the predominance of processes of its destruction, and intensification of heme

TABLE 3. Cytochrome b_5 Concentration in Microsomal Fraction of Rat Liver

Time, h	Control	Irradiation, 12 Gy		
		1st day	2nd day	4th day
10	0,319±0,033 $p>0,05$	0,319±0,036 $p<0,01$	0,307±0,016 $p_1>0,05$	
12	0,555±0,073 $p<0,01$	0,292±0,012 $p<0,01$ $p_1<0,001$	0,322±0,024 $p_1<0,01$	0,186±0,02 $p_1<0,001$
13	0,590±0,057 $p<0,001$	0,247±0,045 $p>0,05$ $p_1<0,01$	0,346±0,028 $p<0,01$	
21	0,344±0,017 $p>0,05$	0,172±0,023 $p_1<0,001$	0,319±0,017 $p_1>0,05$	
4	0,279±0,029	0,307±0,016 $p<0,001$ $p_1>0,05$	0,293±0,029 $p_1>0,05$	

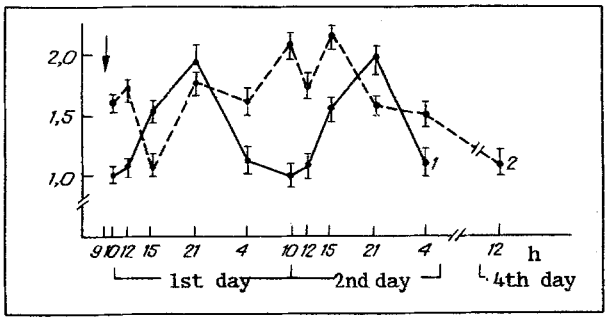

Fig. 1. Changes in protein concentration in liver microsomal fraction of intact (1) and irradiated (2) rats. Abscissa, time (in h); ordinate, protein concentration (in mg/ml). Arrow indicates end of irradiation procedure.

catabolism [1], are evidently associated with their predominant action on its less resistant multiple forms, and is accompanied by intensification of natural mechanisms of conversion of cytochrome P-450 into the functionally inactive cytochrome P-420. In that situation, the point of application of the converting action of irradiation is possibly the lipid, as is shown by the absence of stoichiometry between the lowering of the cytochrome P-450 concentration and the rise of P-420 [7]. Furthermore, a definite effect in postradiation inactivation of cytochrome P-450, especially on the 1st day, is exhibited by cytochrome b_5 the lowering of whose concentration limits the opportunities for stabilization of cytochrome P-450 [10]. However, disturbance of the cytochrome P-450 system may be due not only to the induction of lipid peroxidation, postulated above, but also to a change in the general humoral homeostasis. According to data in [4, 14], glucocorticoids have an inhibitory action on the activity and concentrations of these cytochromes and, consequently, there is reason to suppose that the lowering of the cytochrome levels which we observed as early as 3 h after irradiation was the result of their inactivation, mediated by the powerful release of these hormones into the blood stream [5] (similar experimental conditions). The hypercorticoid stress developing after irradiation is evidently involved both in disturbance of hemoprotein synthesis and in disturbance of the time course of detoxication at the endoplasmic reticulum membrane level in the liver of the irradiated animal [1, 14].

Thus on the basis of these chronobiological investigations it can be concluded that in acute radiation sickness the circadian rhythms of the microsomal hemoproteins cytochromes P-450 and b_5 are disturbed, with a distinct inhibition of the acrophase. Reduction of the concentrations of these cytochromes points to weakening of the cytochrome P-450 system, the development of toxic liver damage as early as with in a few hours of irradiation, and disturbance of the antiloxic function of the liver.

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SEASONAL CHANGES IN INTRALYSOSOMAL pH IN HEALTHY HUMAN PERIPHERAL BLOOD CELLS

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An important factor influencing the natural resistance of the body is seasonal changes in geophysical parameters. A seasonal rhythm of immune processes, influencing the time course of several diseases, has now been established [3]. An important contribution to the level of nonspecific resistance is due to the lysosomal bactericidal systems of the blood leukocytes and, in particular, of neutrophils and monocytes. A certain level of average pH of the granule population of these cells, being a necessary condition for the normal functioning of lysosomal enzyme systems, also may evidently vary in the course of the year. In this paper the terms "lysosome" and "granule" will be regarded as synonyms.

The aim of the investigation was to test the hypothesis relating to seasonal changes in pH of granules of leukocytes and platelets of healthy blood donors.

EXPERIMENTAL METHOD

Peripheral blood from 49 male and female blood donors aged from 18 to 38 years was studied. Blood was taken from a vein between 11 a.m. and noon in a volume of 10 ml into plastic tubes containing heparin (100 U) in 0.5 ml of physiological saline. Measurements were made 2-3 h after natural separation of the blood into fractions. Neutral red (NR), a vital stain accumulating in structures with acid pH and enabling lysosomes to be detected and their pH measured, was used as the pH-indicator [1].

The intralysosomal pH (pH_L) was measured after staining of the cells with NR by a microspectrophotometric method on the Univar instrument ("Reichert," Austria). Buffy coat (0.02 ml), isolated from the blood, was mixed on a slide with an equal volume of 0.025% NR, and incubated for 15 min, after which the preparation was covered with a coverslip, and the pH measured in each sample in 15-40 granules of neutrophils and in 5-10 granules of lymphocytes, monocytes, eosinophiis, an platelets. Incidentally, on contact of the blood cells with the surface of the slide, a so-called oxidative burst took place, so that the lysosomal pH measured by this method evidently reflected this metabolic state of the cells. On statistical analysis the mean value

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