of EDTA (in the presence only of Fe^{3+}), and correspondingly, decomposition of 15-HPA also ceases much sooner. This probably can also be explained by the chelating action of oxidation products of ascorbate formed rapidly.

If it is accepted that Fe^{2+}-induced decomposition of hydroperoxides is an essential stage in the development of the LPO process [9, 13], the results demonstrate that one cause of inhibition of LPO by high concentrations of ascorbate is the chelation of the iron by oxidation products of ascorbate. Incidentally, this effect of oxidation products of ascorbate can inhibit Fenton's reaction.

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EFFECT OF MELATONIN AND PINEALECTOMY ON THE STATE OF THE RAT LIVER MONO-OXYGENASE SYSTEM

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Melatonin, the principal pineal hormone, possesses varied pharmacologic properties, including psychotropic activity [1, 13]. Meanwhile, when melatonin is combined with the use of certain neurotropic substances with a central action (apomorphine, antidepressants), it modifies their effects [2, 3]. This latter feature of melatonin may be pharmacokinetic in nature and, in particular, it may be determined by a change in the biotransformation of the drugs. It was accordingly decided to assess the state of microsomal oxidation in the rat liver during administration of melatonin and also after pinealectomy.

EXPERIMENTAL METHOD

Experiments were carried out on 44 noninbred male albino rats weighing 140-200 g.

Activity of enzymes of the mono-oxygenase system was evaluated in liver microsomes, isolated by differential centrifugation [6]. The concentrations of cytochromes P-450 and b_5 in the microsomal suspension were measured by means of the dual-beam SF-18 spectrophotometer by the method in [11], taking the value of ε to be 91,000 and 165,000 $M^{-1} \cdot cm^{-1}$, respectively. Activity of NADPH-cytochrome c-reductase was determined at 30°C on an SF-26 spectrophotometer at 550 nm. The incubation mixture, in a volume of 3 ml, contained 330 μM NaCN, 50 μM

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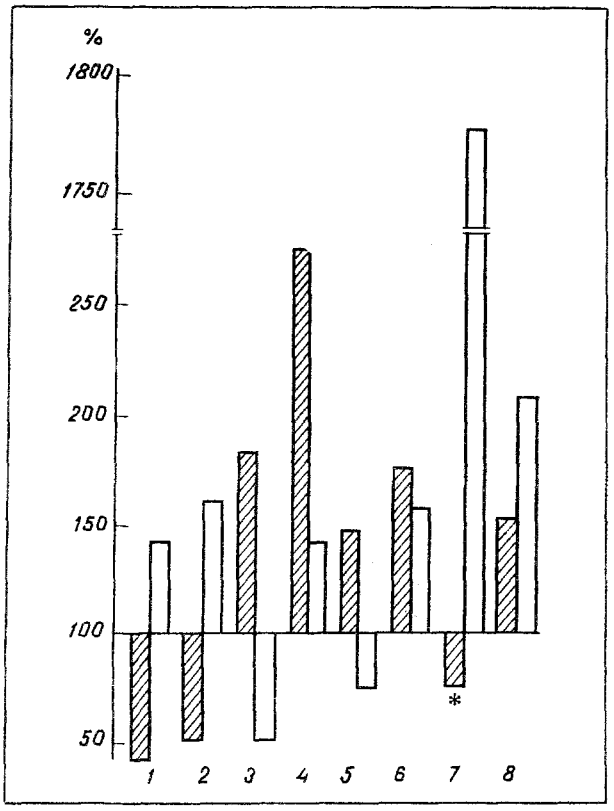

Fig. 1

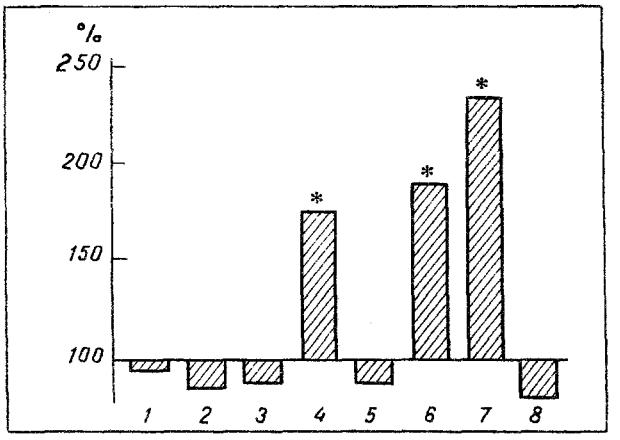

Fig. 2

Fig. 1. Content and activity of various components of the microsomal oxidation system of rat liver during chronic administration of melatonin and after pinealectomy. Columns denote concentration and activity of enzymes relative to rats undergoing mock operation (in percent): unshaded) effect of melatonin; obliquely shaded) pinealectomy. 1) Cytochrome P-450; 2) cytochrome b_5; 3) NADPH-N-demethylase; 4) NADH-N-demethylase; 5) NADPH-p-hydroxylase; 6) NADH-p-hydroxylase; 7) NADPH-cytochrome c-reductase; 8) NADH-ferricyanide-reductase. Changes in all cases (unless marked with asterisk) are statistically significant (at the $p < 0.05$ level).

Fig. 2. Effect of melatonin on content and activity of enzymes of the microsomal oxidation system in penealectomized rats. Columns denote relative (in percent) changes compared with pinealectomized rats. Asterisks indicate statistically significant changes ($p < 0.05$). Remainder of legend as to Fig. 1.

cytochrome c, 100 μM NADPH, 100 mM Tris-HCl buffer (pH 7.5), and 0.01 mg or microsomal protein. Activity of NADH-ferricyanide-reductase was determined under the same conditions: the incubation mixture contained (in a volume of 3 ml) 330 μM potassium ferricyanide, 100 μM NADH, 100 mM Tris-HCl buffer (pH 7.5), and 0.005 mg of microsomal protein. For the calculations, values of ε were taken to be 18,500 and 1020 $M^{-1} \cdot cm^{-1}$ for cytochrome c and for oxidized ferricyanide, respectively [9].

The velocity of N-demethylation of dimethylaniline (DMA) and of p-hydroxylation of aniline (AN) was measured in the presence of 3 mM NADPH or 6 mM NADH [5]. Besides the above, the incubation mixture contained (in a volume of 1 ml) 80 mM Tris-HCl buffer (pH 7.6), 16 mM $MgCl_2$, 1-2 mg microsomal protein, and 6 mM DMA or 3 mM AN. Incubation was conducted at 37°C (using a water thermostat) with vigorous shaking. The velocity of formaldehyde formation during oxidation of DMA and p-aminophenol and during hydroxylation of AN was determined by the method of [5], and microsomal protein was estimated as in [10].

These determinations were made on several groups of animals: intact, undergoing a mock operation (trephining the skull anteriorly to the coronal suture), undergoing the mock operation and receiving melatonin in a dose of 1 mg/kg daily for 24 days (intraperitoneally), pinealectomized animals, and also groups of pinealectomized rats receiving melatonin for the above period of time. The rats were kept under animal house conditions with natural lighting

TABLE 1. Content and Activity of Components of Mono-Oxygenase System of Rat Liver in Intact Animals (I), Animals undergoing Mock Operation (II), after Administration of Melatonin to Animals after Mock Operation (III), after pinealectomy (IV) and Pinealectomy followed by Administration of Melatonin (V; M ± m)

Type of procedure	Cytochrome, nmoles·mg^{-1} protein		Hydroxylase activity				Reductase activity, nmoles·mg^{-1} protein·min^{-1}	
			N-demethylase	DMA	AN p-hydroxylase			
	P-450	b_5	NADPH	NADH	NADPH	NADH	NADH-cytochrome c-reductase	NADH-ferricyanide-reductase
I	1,33±0,09	0,91±0,05	15,34±2,36	3,14±0,50	0,74±0,11	0,09±0,01	163,8±16,1	7598,4±1206,0
II	1,60±0,14	0,99±0,05	13,79±1,18	1,89±0,22	0,43±0,04	0,07±0,01	120,7±17,8	8081,2±1386,0
III	2,22±0,31*	1,55±0,22*	7,51±1,58*	2,69±0,17*	0,32±0,02*	0,11±0,01*	2175,2±180,4*	16466,0±1953,6*
IV	0,67±0,13*	0,50±0,07*	24,72±3,62*	5,07±0,61*	0,63±0,05*	0,12±0,01*	92,9±7,1	12285,5±44,5*
V	0,63±0,12	0,42±0,06	21,36±4.43	8,96±1,43**	0,53±0,12	0,23±0,07**	221,9±16.3**	9424,9±1114,1

Note. *) Statistically significant changes relative to rats undergoing mock operation ($p < 0.05$); **) the same, compared with pinealectomized rats; each group contained 5-8 animals.

and standard temperature and dietary conditions. The animals were deprived of food for 24 h before the investigation, and received only water. The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The content and activity of various components of the liver mono-oxygenase system of the intact animals agreed with data in the literature. There was no change in these parameters in animals undergoing the mock operation (Table 1).

Chronic administration of melatonin led to a considerable (by 1.4-1.6 times) increase in concentrations of microsomal cytochromes P-450 and b_5. There was a correspondingly large increase in NADH-dependent hydroxylation, and activity of NADH-ferricyanide-reductase was doubled. An unusually strong inducing action of the hormone was observed on activity of NADPH-dependent flavoprotein (NADPH-cytochrome c-reductase), which was increased by more than 18 times (Table 1, Fig. 1). This was evidently a case of significant activation of mono-oxygenase activity in the liver, if it is recalled that one molecule of the enzyme can service from 16 to 20 molecules of cytochrome P-450 [4].

Meanwhile, under the influence of melatonin a significant decrease was observed in NADPH-dependent N-demethylase and p-hydroxylase activities (Table 1, Fig. 1). A combination of this inhibitory effect with activation of reductase and induction of cytochrome P-450 was probably due to the fact that the stimulating action of the hormone extends to isoforms of P-450, responsible for the manifestation of other mono-oxygenase activities.

Biochemical changes after pinealectomy were largely opposite to the effects of melatonin, thus confirming their specific character. In particular, the decrease in concentrations of cytochromes P-450 and b_5 compared with the situation in rats undergoing the mock operation, was significant. After pinealectomy not only was the activation of NADPH-cytochrome c-reductase typical of the hormone absent, but its activity was actually reduced somewhat. Conversely, the velocity of the N-demethylase and the p-hydroxylase reactions was significantly increased (Table 1, Fig. 1).

Diametrically opposite changes were found for all parameters except NADH-dependent reactions, which were enhanced as a result of both melatonin and pinealectomy. This can be taken as evidence that biologically active factors, with a modifying effect on the microsomal oxidative system of the animals' liver, are present in the pineal gland and certain other organs.

On the basis of these results it is reasonable to suggest that the pineal gland, through melatonin, exerts control over the state of microsomal oxidation in the liver. Thanks to hormonal induction of important enzyme systems involved in the biotransformation of xenobiotics, iron must evidently play an active role in the formation of drug resistance. This conclusion is in agreement with ideas relating to the adaptogenic properties of pineal factors [7].

Meanwhile, we know that some properties of melatonin, including its antigonadal properties, as realized with the participation of the pineal gland [12]. With this in mind, the effect of melatonin on the state of the mono-oxygenase system was studied in pinealectomized

rats in a separate series of experiments. The results showed that pinealectomy weakened or prevented the appearance of all effects characteristic of melatonin: there was no increase in the concentrations of cytochromes P-450 and b_5, the activating effect on NADPH-cytochrome c-reductase was substantially weakened (Fig. 2).

In this situation also, NADH-controlled reactions were again the exception, for as before their velocity increased by amounts comparable with the control values. Normally, the NADH-dependent redox chain is responsible for the manifestation of only 10-15% of mono-oxygenase activity. Both in rats undergoing the mock operation and in pinealectomized rats, after administration of melatonin activity of the NADH-dependent chain increased up to 35-45% of the total (Table 1). Thus the adaptogenic properties of melatonin are probably realized through accessory mechanisms of activation of the liver mono-oxygenase system.

It can be postulated on the basis of these data that the biochemical effects of exogeneous melatonin which were found are due to triggering of the secretion of the endogenous hormone or of other pineal factors (possibly of peptide nature) through melatonin receptors. The latter are present in sufficiently large numbers on membranes of pinealocytes [8].

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