sue of the disease) the content of cytochrome P-450, a heme-containing protein, changes markedly in rats with HDR, whereas the activity of NADPH-cytochrome P-450 reductase does not differ from that in healthy animals. However, it should be mentioned that these alterations manifest themselves far later than changes in the heme-free components of the microsomal membrane and are evidently associated with the activation of compensatory processes in the damaged tissues. In the rats with HDR the content of hemoglobin, a soluble heme-containing protein, is unchanged throughout the studied postnatal stages. On the whole, our findings corroborate our previous data that in this disease it is precisely the microsomal fraction of target tissues where changes in iron-containing proteins occur.

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Hypolipidemic Effect of 6-Oxa-D-Homo-Isoestrone and Its Methyl Ester

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Administration of methyl ester to ovariectomized rats is shown to lower cholesterol and normalize the serum lipoprotein spectrum. 6-Oxa-D-homo-8-isoestrone also reduces the serum cholesterol level, but does not normalize the lipoprotein spectrum. Neither the cholesterol content nor the serum lipoprotein spectrum is affected by estradiol.

Key Words: *cholesterol; lipoproteins; ovariectomy; estrogen analogs*

There are published data indicating a marked decrease in total serum cholesterol (Ch) and, correspondingly, low-density lipoproteins (LDL) with a simultaneous increase in α-Ch and, accordingly, high-density lipoproteins (HDL) in postmenopausal women and women who have undergone an ovariectomy in accordance with medical indications [8]. At the same time, the concentration of serum triglycerides (TG) rises in such patients, posing an independent risk factor of the development of atherosclerosis and coronary artery damage [3]. In view with this, several studies are underway to seek

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estrogen analogs which possess sufficient hormonal activity and at the same time have an ameliorative effect on serum lipids, notably without stimulating the production of TG-rich very-low-density lipoproteins (VLDL).

A number of estrogen analogs are synthesized at the Department of Chemistry of Natural Compounds of St.Petersburg University, among them 6-oxa-D-homo-8-isoestrone (compound I) and its methyl ester (compound II).

The objective of the present investigation was to study the effect of the above compounds on the spectrum of serum lipoproteins (LP) and on the content of Ch and TG in blood serum and in the liver of intact rats during the development of postovariectomy hyperlipidemia (HLP).

MATERIALS AND METHODS

Compounds I and II were synthesized after Torgov [6] and their structure was verified by UV-, IR-, and PMR-spectroscopy. The experiments were carried out on male rats weighing 180-200 g and female rats weighing 35-45 g. In female rats HLP was induced by bilateral ovariectomy [2]. The test compounds were administered daily 45 days postoperation during 10 days either intramuscularly or per os in 0.1-0.2 ml vegetable oil. In experiments on male rats the compounds were administered daily during 10 days per os in 0.2 ml vegetable oil. The content of total Ch, α-Ch, and TG in blood serum was determined using an AA-II Technicon analyzer (USA). The content of Ch in the liver was determined after the lipids were extracted after Folch [7]. The content of TG was measured as described previously [10]. The data were processed using methods of variational statistics [1]. The serum LP spectrum was studied by analytical ultracentrifugation, which was carried out at 26°C and 52,000 rpm in a Beckman centrifuge (model E) equipped with a standard optical system. The flotation rate distribution of LP $F^{\circ}_{1.20}$ and S_f was evaluated from the results of correction of the schlieren pattern recorded after 8, 14, and 52 min of analytical ultracentrifugation of the total LP fraction [2]. For determination of the acute toxicity compounds I and II were administered per os to male mice weighing 18-20 g. However, we were unable to determine LD_{50} for these compounds, since they exhibited no toxic effect in a dose of 200 mg/kg (which surpassed the dose used in further experiments 100-fold) [4].

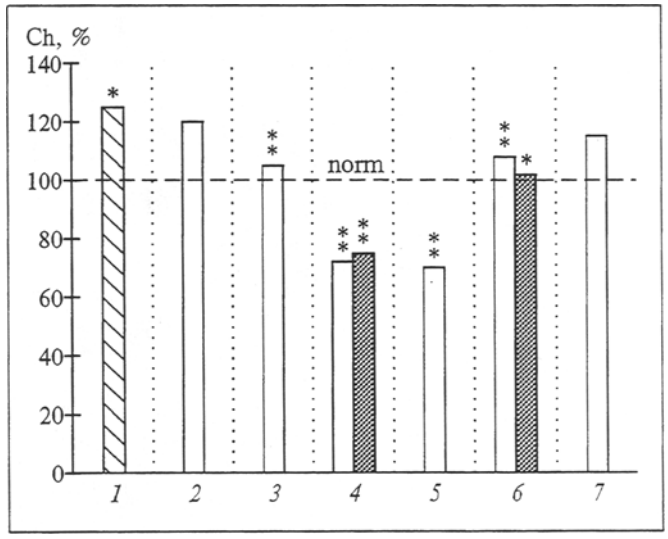

Fig. 1. Effect of compounds I and II on the content of Ch in blood serum of ovariectomized rats. *1*) ovariectomy; *2*) compound II (0.05 mg/kg); *3*) compound II (0.1 mg/kg); *4*) compound II (0.2 mg/kg); *5*) compound II (0.5 mg/kg); *6*) compound I (0.05 mg/kg); *7*) estradiol (0.01 mg/kg); dark bars: administration per os; open bars: intramuscular injection. * – $p<0.05$, ** – $p<0.01$.

RESULTS

Table 1 and Fig. 1 summarize the data on the effect of the test isoestrone analogs (compounds I and II) on the serum Ch concentration in ovariectomized rats.

Compound II in a dose of 0.1 mg/kg reduced the level of serum Ch virtually to the normal value (Fig. 1). It should be noted that compound I exhibited a more pronounced hypocholesterolemic

TABLE 1. Content of Ch and TG in Blood Serum and in the Liver of Ovariectomized Rats Treated with Test Compounds

Group of animals, dose	Serum lipids, mmol/liter		Liver lipids, mg/g	
	Ch	TG	Ch	TG
Intact rats ($n=8$)	1.72±0.16	0.62±0.03	2.2±0.2	9.2±1.0
Ovariectomy ($n=11$)	2.09±0.07*	0.59±0.03	2.7±0.1	7.6±1.0
Ovariectomy + compound I (0.05 mg/kg) ($n=11$)	1075±0.07**	0.55±0.07	3.1±0.2	7.2±0.6
Ovariectomy + compound II (0.1 mg/kg) ($n=11$)	1.67±0.07**	0.41±0.04**	2.7±0.1	5.2±0.05***
Ovariectomy + estradiol (0.01 mg/kg) ($n=11$)	1091±0.06	0.46±0.06	3.0±0.1	6.8±0.7

Note. * – $p<0.05$ in comparison with the control, ** – $p<0.01$, and *** – $p<0.05$ in comparison with ovariectomy.

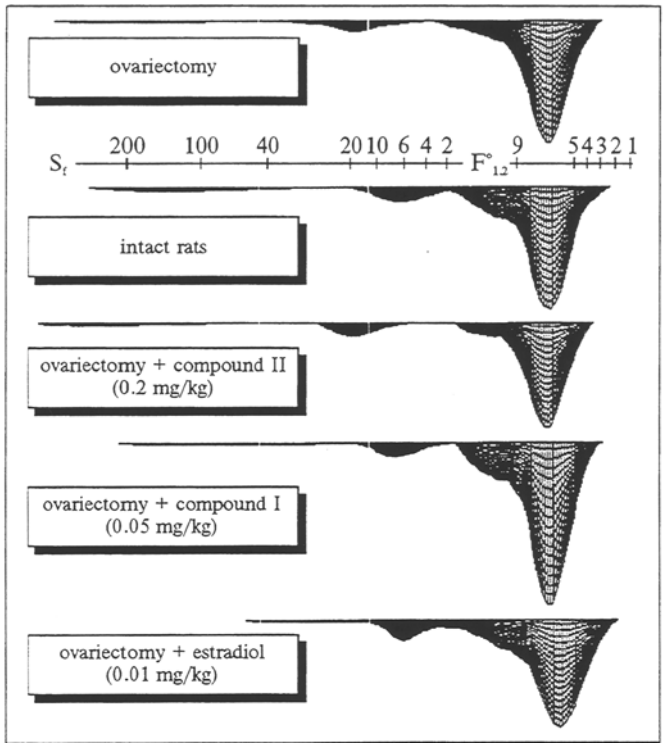

Fig. 2. Distribution of total fraction of blood serum LP of ovariectomized rats during analytical ultracentrifugation. *1*) ovariectomy; *2*) intact rats; *3*) ovariectomy + compound II (0.1 mg/kg); *4*) ovariectomy + compound I (0.05 mg/kg); *5*) ovariectomy + estradiol (0.01 mg/kg).

effect than compound II; in a dose of 0.05 mg/kg it reduced the level of serum Ch 16% ($p<0.01$) (Table 1), while compound II in this dose had no hypocholesterolemic effect (Fig. 1). The mode of administration did not affect the activity of the preparation. In a dose of 0.5 mg/kg compound II led to a considerable reduction of the level of total serum Ch (Fig. 1). The optimal dose for compound II is evidently 0.1 mg/kg, since it lowered the concentration of total serum Ch to the normal value. Figure 2 illustrates the results of analytical ultracentrifugation of blood serum from animals in this experimental series. As is seen from the figure, ovariectomy resulted in an increased concentration of lipoprotein particles in the flotation interval corresponding to nascent HDL or HDLc [9]. Estradiol in a dose of 0.01 mg/kg did not normalize the spectrum of serum LP. While compound I reduced the level of total serum Ch and elevated the concentration of HDL, it did not normalize the serum LP spectrum (Fig. 2). Moreover, the number of particles in the flotation interval corresponding to nascent HDL was even higher in animals treated with compound I in comparison with ovariectomized animals. Administration of compound II in a dose of 0.1 mg/kg resulted in both a reduction of total serum Ch and normalization of the serum LP spectrum.

The second experimental series was performed on male rats receiving the test compounds orally in 0.2 ml vegetable oil. In intact animals estradiol considerably changed the LP spectrum: an increased concentration of HDL and its accumulation in the flotation area corresponding to nascent HDL (Fig. 3). At the same time the content of total serum Ch was elevated 30% ($p<0.01$), probably due to a 35% rise of α-Ch ($p<0.01$) (Table 2). Compounds I and II lowered the concentration of the total fraction of LP, particularly in flotation intervals corresponding to VLDL and LDL (Fig. 3), however without the appearance of na-

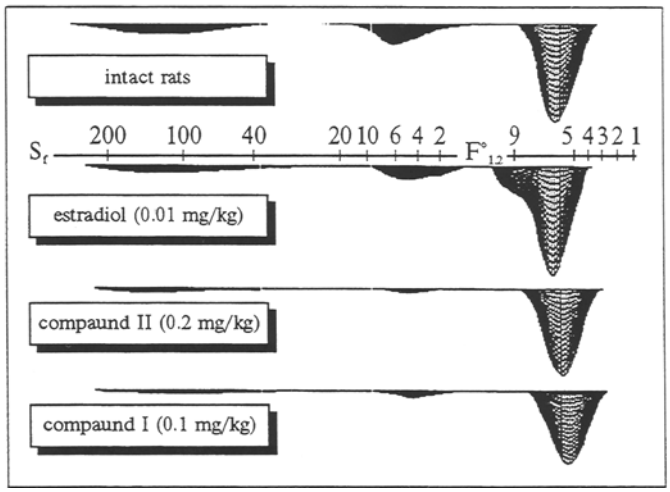

Fig. 3. Distribution of total fraction of blood serum LP of ovariectomized rats treated with estradiol and compounds I and II. *1*) intact rats; *2*) estradiol (0.01 mg/kg); *3*) compound II (0.2 mg/kg); *4*) compound I (0.1 mg/kg).

TABLE 2. Content of Ch and TG in Blood Serum and in the Liver of Intact Rats Treated with Test Compounds

Group of animals, dose	Serum lipids, mmol/liter			Liver lipids, mg/g	
	Ch	α-Ch	TG	Ch	TG
Intact rats ($n=9$)	1.43±0.05	0.97±0.12	0.68±0.03	2.0±0.1**	5.1±0.2*
Compound I (0.1 mg/kg) ($n=9$)	0.84±0.06*	0.64±0.06**	0.66±0.03	2.3±0.05**	9.0±1.2
Compound II (0.2 mg/kg) ($n=9$)	0.88±0.09*	0.90±0.06	0.83±0.06	2.5±0.1	5.6±0.1
Estradiol (0.01 mg/kg) ($n=8$)	1.87±0.16**	0.90±0.09	1.07±0.12*	2.4±0.1*	9.9±1.1*

Note. * – $p<0.01$, ** – $p<0.02$, and *** – $p<0.05$ in comparison with the control.

scent HDL. The content of total serum Ch dropped by 38% ($p<0.01$) and by 41% ($p<0.01$) after the administration of compound II and compound I, respectively. However, the level of α-Ch did not drop, and in the case of compound II it even rose 22%, i.e., the drop of total Ch occurred due to LDL and VLDL Ch. Both test compounds increased the content of Ch in the liver, whereas accumulation of TG was observed only in the case of compound I (Table 2).

Thus, the effect of compounds I and II on serum LP and on the content of Ch in blood serum and in the liver surpasses that of estradiol, which does not normalize the LP spectrum in ovariectomized animals and even alters it adversely in experimental animals. Compound II possesses a less pronounced hypolipidemic activity in comparison with compound I; however, its effect on the serum LP spectrum is more favorable.

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Effect of Tuftsin on the Functional Activity and Intracellular pH of Murine Peritoneal Macrophages

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The effect of tuftsin and its tripeptide analog in various concentrations (from 0.001 to 10.0 μg/ml) on phagocytosis and on the intracellular pH is studied in murine peritoneal macrophages. Tuftsin causes a uniform dose-dependent increase of these two parameters in the cells. This effect is maximally pronounced at concentrations of the peptide close to its physiological level (about 0.3 μg/ml) and gradually decreases as its content in the incubation medium is lowered or raised. On the other hand, the tripeptide analog of tuftsin does not exhibit such an effect on the cells and under the same conditions suppresses phagocytosis and acidifies their intracellular medium.

Key Words: *tuftsin; phagocytosis; intracellular pH; macrophages*

Recently, increasing attention has been paid to the mechanism of action of the biologically active peptide regulators. Tuftsin, an endogenous tetrapeptide with a pronounced phagocytosis-stimulating activity, is among these. Despite a rather long history of studies, the molecular mechanism of its action upon the cell is far from clear [8]. A powerful tool for solving this problem is an analysis of the effect of the peptide on the major regulatory sys-

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