addition of Ca^{2+} and due to the Ca^{2+} influx through the membrane Ca^{2+} channels.

The addition of different concentrations of HC (0.5-5.0 μM) to the cell suspension showed that the glucocorticoid exerted a weak but reliable effect on the basal $[Ca^{2+}]_i$ (Fig. 3). The subsequent addition of AA (3 μM) resulted in a rise of $[Ca^{2+}]_i$ by 102±30 nM, which was similar to the Ca^{2+} response in the Ca^{2+}-free medium (Figs. 1, 2).

The data suggest that hydrocortisone in a therapeutic range of concentrations blocks the membrane Ca^{2+} channels and inhibits Ca^{2+} influx into JW cells. At the same time, it has virtually no effect on AA-induced Ca^{2+} release from the intracellular stores.

On the basis of these data we concluded that both arachidonic acid and hydrocortisone possess membranotropic activity in JW plasmacytoma cells. However, their effects are oppositely directed: arachidonic acid increases, while hydrocortisone decreases the permeability of the plasma membranes.

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Effect of Nitrobenzene and its Chloro-Substituted Derivatives on Parameters of Antioxidant Homeostasis in Rat Tissues

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Nitrobenzene and its chloro-substituted derivatives are widely used in industry for the manufacture of dyes, pharmacological drugs, and other compounds. These substances are very harmful for workers coming in contact with them due to their volatility and ready ability to penetrate into the organism. The toxic properties of these substances are well known [3,12], but their metabolic pathways in the organism are little understood. General systems of xenobiotic decontamination [1,2,5,13] as well as lipid peroxidation (LPO) processes [6-8] are known to participate in nitrobenzene detoxication. However, there are practically no data on the involvement of antioxidation (AO) systems and lipid-soluble vitamins in the maintenance of the normal functioning of the organism's systems un-

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TABLE 1. Effect of Nitrobenzene, o-Chloro-Nitrobenzene, and p-Chloro-Nitrobenzene on Accumulation of Primary and Secondary LPO Products in Tissues

Exp.	DC, nmol/mg lipids	TC, nmol/mg lipids	ODS, nmol/mg lipids	Tetraenes, rel.units/mg	MDA, nmol/mg lipids
			Liver, 5 exposures		
Control	53±3	9±0.7	19±1	0.47±0.02	4.24±0.50
NB	53±6	10±0.3	19±0.7	0.47±0.02	3.12±0.28*
oCNB	51±5	8±0.7	18±2	0.48±0.06	3.58±0.72
pCNB	35±6**	10±2	18±2	0.38±0.06	4.70±0.24
			Liver, 30 exposures		
Control	52±4	12±0.2	23±2	0.48±0.10	3.80±0.46
NB	60±4	16±2*	31±5	0.66±0.09	4.20±0.62
oCNB	39±3**	8±1***	18±2*	0.38±0.06	2.26±0.44**
pCNB	48±5	11±1	24±2	0.54±0.04	2.07±0.20**
			Spleen, 5 exposures		
Control	49±5	13±2	24±2	0.38±0.06	7.56±0.44
NB	89±7***	19±1**	36±2	0.70±0.06***	9.50±0.66***
oCNB	68±9	14±1	28±3	0.48±0.04	7.80±0.96
pCNB	64±3**	17±0.6*	42±9	0.56±0.03**	11.26±1.42**
			Spleen, 30 exposures		
Control	46±2	12±1	25±2	0.50±0.06	4.60±0.98
NB	45±4	10±1	20±2		0.40±0.002
7.42±0.58**					
oCNB	46±6	10±0.6	20±1	0.42±0.02	4.60±0.64
pCNB	54±3	14±1	24±2	0.49±0.06	6.34±1.14

Note. Here and in Table 2: $n=10$; *: $p<0.01$; **: $p<0.05$; ***: $p<0.01$.

der conditions of acute and subchronic exposure to nitrobenzene and its chloro-substituted derivatives. Yet, this is the path toward effective diet-based preventive measures against occupational diseases.

In light of this, the objective of the present study was to investigate the dependence of the LPO level, antioxidant activity (AOA), and the content of vitamin E in rat liver and spleen tissue on the duration of exposure to nitrobenzene (NB), o-chloro-nitrobenzene (OCNB), and p-chloro-nitrobenzene (PCNB).

MATERIALS AND METHODS

Experiments were carried out on 250 white male rats weighing 200-300 g. The animals were maintained on the usual balanced vivarium ration. For toxicity testing we chose a subacute intragastric poisoning in a dose of 1/10 LD_{50}, which constituted 70 mg/kg body weight for NB, 51 mg/kg for OCNB, and 83 mg/kg for PCNB [3]. The substances, dissolved in 1% sunflower oil, were administered through a tube daily during 5 or 30 days. The control group received the same volume of vehicle. The rats were then decapitated, and the liver and spleen were removed, frozen in liquid nitrogen, and stored until analyzed. After cryopounding and thawing procedures, the specimens were homogenized in phosphate buffer saline (pH 7.4) and a 20% homogenate was used for further analysis. The content of primary LPO products: diene (DC), triene (TC), oxodiene (ODC), and tetraene conjugates was determined spectrophotometrically [4], and the content of secondary LPO products, thiobarbituric acid-reactive substances (TBARS), was determined colorimetrically [11]. Total AOA was measured using a model of oleate thermoautooxidation [9]. The content of vitamin E (TP) was determined after alkaline hydrolysis of the homogenate in an unsaponifiable fraction [11]. The content of the substances tested was calculated using the following coefficients of molar extinction: $\varepsilon_{232}=27{,}000$ mol$^{-1}\times$cm^{-1} for DC, $\varepsilon_{268}=43{,}000$ mol$^{-1}\times\times$cm^{-1} for TC, $\varepsilon_{276}=22{,}000$ mol$^{-1}\times$cm^{-1} for ODC [15], $\varepsilon_{282}=3170$ mol$^{-1}\times$cm^{-1}and for TF [14]. The content of tetraene conjugates was expressed in relative extinction units. The content of TBARS was determined by a calibration curve constructed using 1,1,3,3-tetraethoxypropane (Merck, Germany) as a standard. AOA was expressed in relative units. The data were processed using the Student t test [10].

RESULTS

Short-term (5 poisonings) exposure to PCNB reduced the content of DC in the liver, while a 30-day administration of OCNB led to a drop in almost all LPO products, which was reliable for DC, TC, and malonic dialdehyde (MDA). PCNB also lowered the MDA level (Table 1). Nitrobenzene

TABLE 2. Effect of Nitrobenzene Derivatives on α-Tocopherol (TP) Content (nmol/mg lipids) in Rat Tissues and Total Antioxidative Activity (AOA, rel.units)

Parameter	Control	Substances		
		NB	oCNB	pCNB
Liver, 5 exposures				
TP	4±0.7	2±0.2**	4±0.7	3±0.1
AOA	0.98±0.08	2.28±0.50**	1.66±0.1***	1.28±0.22
Liver, 30 exposures				
TP 1±0.4	1±0.4	5±1***	2±0.8	
AOA	4.08±1.55	2.52±0.73	2.68±0.62	0.94±0.16*
Spleen, exposures				
TP	5±0.6	2±0.4***	2±0.4***	3±0.6**
AOA	1.75±0.45	1.24±0.29	1.15±0.08	1.20±0.1
Spleen, 30 exposures				
TP	2±0.6	3±0.6	5±1.4*	4±1.6
AOA	2.65±1.13	3.76±1.54	1.42±0.28	1.16±0.1

itself somewhat raised (unreliably) the level of all LPO products. This demonstrates that under conditions of subacute exposure the detoxication mechanisms, localized, in particular, in the liver, are able to handle detoxication of both xenobiotics and their metabolites. Moreover, some metabolites (for instance, OCNB and, probably, products of its transformation) may be utilized in reactions with LPO products (DC, TC, and MDA), which is evidenced by their decreased tissue content. Hence, the well-known detoxication pathways are probably bolstered by recombination reactions which greatly facilitate biotransformation of these xenobiotics.

A different pattern was observed in the spleen. The 5-day administration of NB led to a reliable accumulation of all LPO products. PCNB caused an elevation of DC, tetraene conjugates, and MDA, while OCNB unreliably increased these parameters. With the 30-day administration, a reliable increase of the MDA content was observed solely for NB, whereas other substances did not affect these parameters. This suggests a mobilization of adaptive mechanisms promoting a more effective utilization of the xenobiotics. In order to confirm this assumption an additional 5-day administration of nitrobenzenes against the background of phenobarbital, a well-known stimulator of the monooxygenase system, was performed. Such treatment was shown to abolish completely all the effects of the test substances on the LPO processes, thus proving that adaptive systems are indeed mobilized for xenobiotic detoxication. This adaptation sets in, moreover, as soon as the 30th exposure.

The toxicity of the substances studied is well known and manifests itself, specifically, in functional changes of the hemopoietic organs (hemopoiesis tension), and in a changed level of reduced thiols [3,6,8]. Comparison of our results with published data [1,2,7] drew us to the conclusion that antioxidant systems may play an important role in the processes of adaptation to these xenobiotics. In this context we studied the total AOA and the content of α-tocopherol in the tissues. It was shown (Table 2) that a 5-day NB administration led to a reliable drop of the TP content in both the liver and the spleen, with its subsequent restoration by the 30th day. Other substances (PCNB and OCNB) decreased the TP content in the liver only, the initial level also being attained by the 30th day. Moreover, OCNB caused even some accumulation of TP by this time. This suggests the active participation of TP in the stabilization of the adaptive mechanisms. In addition to the above-mentioned changes, we observed a reliable rise of the total AOA following the 5-day administration of NB and OCNB, while in all other cases this parameter remained unchanged. Thus, for stimulated AOA, the organism's reserves, both enzymatic and nonenzymatic, are sufficient for the maintenance of AO homeostasis. The elevated blood level of TP and AOA may indicate activation of physiological mechanisms of redistribution of these important element from other tissues, as well as intensified work of the existing AO enzymes. For instance, after a 5-day administration all the substances induced a rise of the blood catalase activity, together with a drop of reduced glutathione. From the analysis and comparison of the data it may be surmised that NB and its chloro-substituted derivatives stimulate the general AO system of the organism, which stabilizes in the course of time and maintains AO homeostasis.

Thus, on the basis of our experiments, NB and its chloro-substituted derivatives markedly af-

fect the LPO intensity. The spleen was found to be the most sensitive tissue, especially at an early stage of poisoning. The long-term influence of NB and its chloro-substituted derivatives mobilizes the adaptive systems of the organism, which brings the LPO processes under control, although their capacity is practically exhausted, judging from the AOA decline. Administration of the test substances results in an intensive redistribution of vitamin E reserves in the organism, which implies its efficiency as a prophylactic.

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Ability of Low-Molecular Heparin (Fraxiparine) to Counter the Action of Exogenous Coagulases: a Fundamental Difference from Nonfractionated Heparin

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Snake venoms are known to contain proteases inducing blood coagulation by activating the hemocoagulation cascade on various levels [1-9,12,16,19, 21]. For example, in our previous studies, as well as in some reports from other laboratories, it was established that *Vipera lebetina turanica* venom contains a coagulase activating factor X, *Echis carinatus* and *Echis multisquamatus* venoms contain the enzyme directly activating prothrombin, factor II [5,7,9,10-13,17,20], while *Agkistrodon halys halys* venom includes a protease which con-

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