5. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
6. A. J. Barrett and M. F. Heath, in: Lysosomes. A Laboratory Handbook, Amsterdam (1977), p. 19.
7. J. S. Fruton and M. J. Mycek, Arch. Biochem., 65, 11 (1956).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
9. T. G. Petters, M. Müller, and C. De Duve, J. Exp. Med., 136, 1117 (1972).

EFFECT OF COLD STRESS ON CONTENT AND ACTIVITY OF MICROSOMAL CYTOCHROME P-450 IN RAT LIVER

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KEY WORDS: cold stress; cytochromes P-450 and b_5; demethylase activity.

Under the influence of various stressors long-term changes in enzyme activity in the blood and digestive system take place in living organisms [2, 11]. The effect of stressor agents on enzymes located in the endoplasmic reticulum of the animal liver has received less study. It has been shown that in acute (pain) stress the content of microsomal cytochrome P-450 – an enzyme playing an important role in detoxication and metabolism of a wide range of endogenous compounds and xenobiotics [8] – in the rat liver falls sharply. Data on the formation of toxic metabolites in animal tissues during stress have been reported in the literature [1, 4, 5]. The study of the effect of various stressor agents on the level and activity of enzymes of the endoplasmic reticulum of the animal liver is accordingly very interesting.

This paper describes a study of possible changes in the content of microsomal cytochromes P-450 and b_5 and in the aminopyrine-demethylase activity of the liver microsomes of rats after short-term cooling of the animals. To elucidate one of the possible mechanisms of the changes discovered, the effect of cold stress was studied on the ratio between the forms of cytochrome P-450 which differ in their resistance to the destructive action of linoleic acid hydroperoxides.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-180 g were used. Ice baths (0°C) lasting 5 min were used as the stressor agent [7]. The microsomal fraction of the liver was isolated by gel filtration on Sepharose 2B [15]. Protein was determined by Lowry's method. The content of cytochromes P-450 and b_5 in the microsomes was determined spectrophotometrically [13, 14], using extinction coefficients of $E_{450-500} = 91$ $mM^{-1}\,cm^{-1}$ for cytochrome P-450 and $E_{424-409} = 185$ $mM^{-1}\,cm^{-1}$ for cytochrome b_5. The ratio between the two forms of cytochrome P-450, differing in their resistance to linoleic acid hydroperoxides, was determined by the method described previously [10].

The demethylase activity of rat liver microsomes was estimated from the rate of formaldehyde formation during demethylation of aminopyrine. The incubation mixture contained, in a volume of 1.3 ml, 2 mg/ml of microsomal protein, 0.1 M Na,K-phosphate buffer (pH 7.5), 5 mM $MgCl_2$, 3 mM NADPH, and 10 mM aminopyrene. After incubation for 10 min at 37°C the quantity of formaldehyde formed was determined [12].

The experimental results were subjected to statistical analysis. The significance of differences was estimated by Student's t-test.

EXPERIMENTAL RESULTS

The results showed that cold stress causes phasic changes in the content of cytochromes P-450 and b_5 in rat liver microsomes (Fig. 1). The levels of cytochromes P-450 and b_5, which normally are 0.768 ± 0.033 and 0.304 ± 0.016 nmole/mg protein, respectively, remained virtually unchanged during the first 4 h after stress, although a tendency was observed for the content of both cytochromes to increase.

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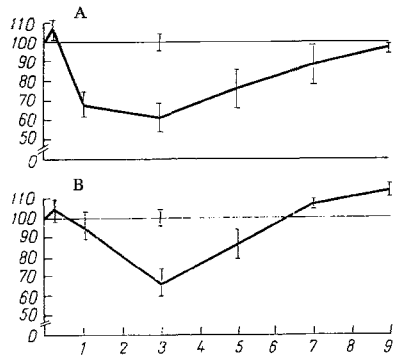

Fig. 1. Changes in content of cytochromes P-450 (A) and b_5 (B) in rat liver microsomes after cold stress. Abscissa, days after exposure to stress; ordinate, content of cytochromes (in %). Level of corresponding cytochromes in control taken as 100%.

TABLE 1. Effect of Cold Stress on Demethylase Activity and Content of Stable* (S) and Labile* (L) Forms of Cytochrome P-450 of Rat Liver Microsomes

Experimental conditions	Demethylase activity, nmoles/mg/mg protein	Ratio S/L
Control	7,01±0,23 (8)	1,55±0,11 (8)
After stress		
4 h	8,48±0,41 (4) $P<0,05$†	1,58±0,08 (4)
24 h	5,54±0,15 (4) $P<0,001$	1,91±0,07 (8) $P<0,05$

*Relative to linoleic acid hydroperoxides.
†P compared with control.
Legend. Number of experiments shown in parentheses.

The short and small increase in the cytochrome P-450 level was followed by a long and significant decrease in its content in the microsomes. During 1-3 days after cold stress the content of cytochrome P-450 in the microsomes of the experimental rats fell by almost 40% (Fig. 1). An increase was observed in the fraction of the form of cytochrome P-450 more resistant to linoleic acid hydroperoxides (the stable form) in the residual pool of the hemoprotein (Table 1). During the 4-9 days of the experiment the content of cytochrome P-450 rose steadily and on the 9th day it reached the normal level. The decrease in the cytochrome b_5 content was shorter in duration, smaller in amplitude, and began later than the decrease in the cytochrome P-450 level (Fig. 1).

During the first day after cold stress, besides changes in the cytochrome content, changes also were observed in aminopyrine demethylase activity of the microsomal fraction of rat liver, which were almost equal in amplitude but opposite in direction. For instance, during the first 4 h after stress the aminopyrine-demethylase activity of the microsomes increased by more than 20%, after which it fell, so that 24 h after stress it was about 80% of the normal level.

The changes observed in aminopyrine-demethylase activity of rat liver microsomes after cold stress were more complex in character than the more simple type of changes described previously (an increase or decrease followed by a return to normal) in other enzyme systems of animals exposed to various stressor agents [2, 11]. The concrete causes of the short-term increase in aminopyrine-demethylase activity during the first 4 h after cold stress, against the background of an unchanged level of cytochrome P-450 (the enzyme determining demethylase activity) are not yet clear, whereas the decrease in enzyme activity after 24 h was evidently due to the synchronous reduction in the content of this hemoprotein.

When the causes of the fall in the cytochrome P-450 level in rat liver microsomes after cold stress are examined, besides a possible change in the rate of synthesis of the hemoprotein and in heme oxygenase activity, attention must also be paid to the probability of destruction of cytochrome P-450 during intensification of lipid peroxidation in the tissues of stressed animals. It has been shown, for instance, that during exposure to various stressors, including that used in the present investigation, the lipid peroxide level in rat tissues rises [1, 4, 5]. Meanwhile cytochrome P-450 is known to be highly sensitive to the destructive action of lipid peroxides both *in vitro* and *in vivo* [3, 6]; in particular, it has been found that administration of lipid peroxides to animals causes a fall in the cytochrome P-450 level similar to the change described above in the content of this hemoprotein after cold stress [3].

Indirect confirmation of the possible connection between the fall in the cytochrome P-450 level after cold stress and intensification of lipid peroxidation in the tissues may be given by the greater decrease in the concentration of the form of cytochrome P-450 which is labile with respect to linoleic acid hydroperoxides, and the later change in the content of cytochrome b_5, which is relatively resistant to the destructive action of lipid peroxides [9], compared with cytochrome P-450.

The above hypothesis does not contradict the current view of the leading role of the hypothalamic–hypophyseal–adrenal system in the changes in the enzyme spectrum after stress [2], for intensification of lipid peroxidation in the tissues of animals exposed to stress is linked with an increase in the catecholamine and glucocorticoid concentrations [4].

LITERATURE CITED

1. L. I. Deev and É. A. Goida, Byull. Mosk. Ova. Ispyt. Prir., Otd. Biol., No. 4, 157 (1967).
2. M. K. Kurbanov and Ch. R. Alimkhodzhaeva, Byull. Éksp. Biol. Med., No. 5, 412 (1979).
3. V. Z. Lankin, V. M. Polyakov, and S. M. Gurevich, in: Lipids. Structure, Biosynthesis, Conversions, and Functions [in Russian], Moscow (1977), p. 93.
4. F. Z. Meerson, V. A. Kagan, L. L. Prilipko, et al., Byull. Éksp. Biol. Med., No. 10, 404 (1979).
5. M. M. Melkonyan, É. A. Araratyan, É. M. Mikaélyan, et al., Zh. Éksp. Klin. Med., No. 4, 25 (1978).
6. A. G. Platonov, L. I. Deev, O. V. Vasilenko, et al., in: Role and Mechanisms of Action of Biologically Active Compounds in the Gamma-Irradiated Organism [in Russian], Pushchino (1978), p. 38.
7. H. Selye, Chemoprophylaxis of Cardiac Necrosis [Russian translation], Moscow (1961).
8. Chung Ho and D. R. Brown, Toxicol. Appl. Pharmacol., 37, 2 (1976).
9. E. G. Hrycay and P. J. O'Brien, Arch. Biochem., 147, 14 (1971).
10. E. H. Jeffrey, D. Nerland, R. El-Arhary, et al., Physiol. Chem., 357, 1032 (1976).
11. J. M. McVeigh, P. V. Tarrant, and M. G. Harington, Biochem. Soc. Trans., 7, 539 (1979).
12. T. Nash, Biochem. J., 55, 416 (1953).
13. T. Omura and R. Sato, J. Biol. Chem., 239, 2370 (1964).
14. B. Schone, R. A. Fleischmann, and H. Remmer, Eur. J. Clin. Pharmacol., 4, 65 (1972).
15. O. Tangen, J. Jonsson, and S. Orrenius, Anal. Biochem., 54, 597 (1973).

CHANGES IN ERYTHROCYTE MEMBRANE PROTEINS DURING PROLONGED ELEVATION OF BLOOD CHOLINERGIC ACTIVITY IN ANIMALS

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In the modern view cholinergic mechanisms play an exceptionally important role in metabolism and in transmission of nervous impulses [1, 2, 4]. Erythrocytes are known to participate in the regulation of cholinergic processes, and these processes themselves influence the vital activity of the red blood cells [3, 5].

The object of this investigation was to study relations of cholinergic processes with the polypeptide composition of the erythrocyte membrane and the sensitivity of the chemoreceptor system of erythrocytes to plasma components.

EXPERIMENTAL METHOD

The influence of cholinergic factors on erythrocyte membranes was studied in systems *in vivo* (dogs) and *in vitro* (action of acetylcholine on healthy human erythrocytes).

Erythrocytes, washed in physiological saline, were incubated (n = 10) with acetylcholine *in vitro* under sterile conditions for 6 h. For this purpose a 50% erythrocyte suspension was mixed with equal volumes of 0.006% solution of acetylcholine, made up in buffered physiological saline, pH 7.4 (final acetylcholine concentration $1.7 \cdot 10^{-4}$ M), and for incubation of the acetylcholinesterase (AChE) of the erythrocytes neostigimine solution was added to the incubation medium in a final concentration of $2.5 \cdot 10^{-5}$ M. After incubation the erythrocytes were washed 5 times with buffered physiological saline (pH 7.4).

High blood cholinergic activity was created in dogs (10 mongrel animals) *in vivo* by parental injection of acetylcholine (0.9 mg/kg) and neostigmine (0.008 mg/kg) into the animals for 3 months. Blood was taken from the animals' saphenous vein and sodium citrate was used as anticoagulant. The erythrocytes were washed 3 times with buffered physiological saline (pH 7.4). The membranes were isolated from erythrocytes treated with acetylcholine in both *in vivo* and *in vitro* systems by gradual osmotic hemolysis [6].

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