effective, K_{50} corresponding to a concentration of $1.2 \cdot 10^{-8}$ M for enzymic LPO. The remaining ionol derivatives, in their half-maximal efficacy of antioxidative action in liver microsomes, occupy the concentration range from 10^{-5} to 10^{-7} M (Table 1). This character of the antioxiative action of ionol derivatives is not a specific features of liver microsomal membranes, but is recorded in other membranes also and, in particlar, in rat brain synaptomal membranes (Table 1).

The results indicate that correlation does not exist between the degree of inhibition of LPO in biomembranes and the value of K_7 for these compounds. It can be tentatively suggested that interaction of the reagents in isomembranes, which are microheterogeneous ordered systems, is largely determined by their orientation, their mutual accessibility, and their mobility [8]. Consequently, when choosing membrane antioxidants, guidance must be taken not only from their chemical characteristics, but also from their behavior in biomembranes.

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DETECTION OF NEUTROPHILIC MYELOPEROXIDASE IN RAT SKELETAL MUSCLES AFTER MUSCULAR ACTIVITY

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UDC 612(015.11+112.91+73/74(+613.731)

KEY WORDS: myeloperoxidase, neutrophil, skeletal muscles, muscular activity

Intensive muscular activity (MA) leads to the release of cytoplasmic and structural proteins of skeletal muscles into the blood stream, evidently as a result of disturbance of tissue integrity [6, 12]. Histologic and histochemical studies have revealed foci of necrosis of skeletal muscles after MA [3, 11]. Since an essential role in the development of tissue injury is ascribed to neutrophils [2, 14], it was decided to study the problem of the appearance of these cells in skeletal muscles after MA, by measuring the neutrophilic marker enzyme myeloperoxidase [10, 13] and its distribution in subcellular fractions.

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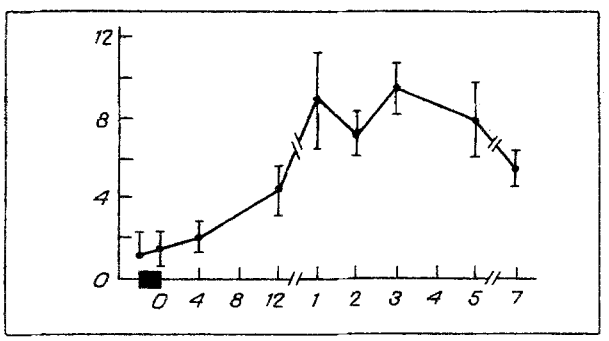

Fig. 1

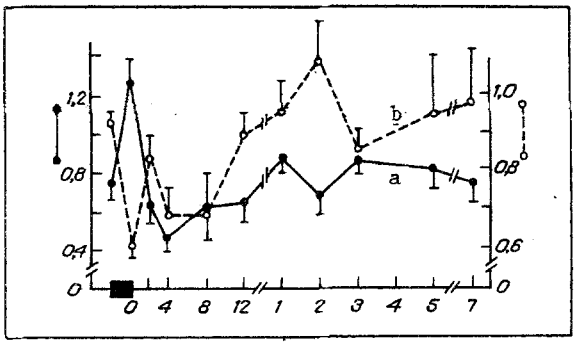

Fig. 2

Fig. 1. MPO concentration in skeletal muscles after MA. Abscissa, time after MA, in h (0-12) and days (1-7); ordinate, MPO concentration, in ng/mg protein.

Fig. 2. MPO concentration in blood plasma (A) and neutrophils (B) after MA. Abscissa, time after MA (Fig. 1); ordinate, MPO concentration, in μg/ml (a) and pg/cell (b).

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. MA consisted of swimming by the animals carrying a weight equivalent to 11% of their body weight [4]. The rats were decapitated immediately after MA and after various periods of rest. To study the trend of the different classes of leukocytes blood was collected from the tail. When neutrophils were obtained from the blood plasma, Na_2-EDTA was used as the anticoagulant. Granulocytes were separated by centrifugation on Ficoll–Paque ("Pharmacia," Sweden) after preliminary sedimentation of erythrocytes with gelatin [8]. The sedimented erythrocytes were hemolyzed with distilled water for 30 sec. The preparations thus obtained contained over 90% of neutrophils. Skeletal muscles from the hind limbs were cut into pieces with scissors and homogenized (1:5, w/v) in a solution containing 0.25 M sucrose, 2 mM Na_2-EDTA, 1 mM 2-mercaptoethanol (from "Serva," West Germany), 0.3% of cetyltrimethylammonium bromide ("Chemapol," Czechoslovakia), and 0.05 M Tris-HCl ("Sigma," USA), pH 7.4, and centrifuged (10,000 g, 30 min) to obtain the cytosol. The concentration of myeloperoxidase (MPO) was measured by radioimmunoassay [5]. The skeletal muscles were fractionated to obtain membrane fractions of the plasmalemma as in [14].

EXPERIMENTAL RESULTS

The MPO concentration in the total preparation of skeletal muscles from the hind limbs of resting rats was low (1.26 ± 1.00 ng/mg protein,* n = 9) and remained unchanged during MA (Fig. 1). While the animals rested after MA there was a gradual increase in the MPO concentration, and after 24 h it reached its maximum (8.93 ± 2.30 ng/mg protein, n = 8), at which level it remained for a further 2-3 days. The MPO concentration in the skeletal muscles then fell, but even 7 days after MA it was higher than initially. The possible causes of the increase in MPO concentration in the skeletal muscles under the influence of MA are either an increase in the number of neutrophils or the quantity of enzyme in the blood or an increase in the number of neutrophils in the skeletal muscle tissue, or these two processes together.

Determination of the plasma MPO concentration showed that MA caused it to rise sharply by 50% on account of release of the enzyme by the blood neutrophils (Fig. 2). However, later the MPO concentration fell quite quickly, and after 8-12 h it was the same as initially. Data on the effect of MA on the blood neutrophil count are given in Fig. 3. The peak content of these cells (181% of the control value) was found 8 h after MA, after which it fell, and after 3 days was significantly lower than initially. Monocytes also are known to contain MPO [13]. However, the contribution of these cells to the enzyme balance can hardly be signified, in view of their small number in the blood compared with neutrophils throughout the period of observation (Fig. 3). It can be concluded from the time course of MPO and of neutrophils and monocytes in the blood stream that none of these factors plays a significant role in the increase in the MPO concentration in skeletal muscles. The cause of that increase is the

*Here and subsequently, $\bar{X} \pm S_{\bar{X}}$.

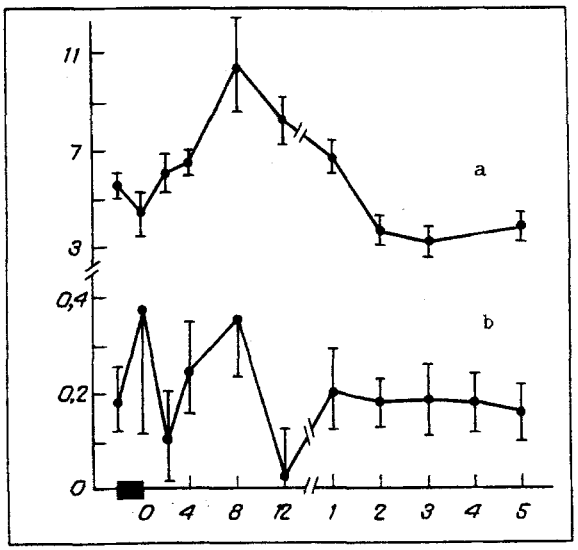

Fig. 3

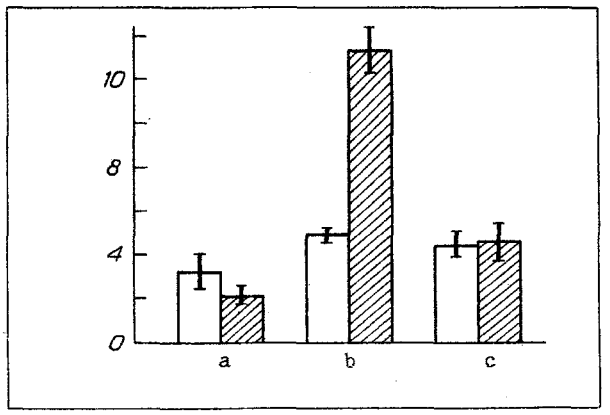

Fig. 4

Fig. 3. Number of neutrophils (A) and monocytes (B) in blood after MA. Abscissa, time after MA (Fig. 1); ordinate, cells, 10^{-9}/liter.

Fig. 4. MPO concentration (in ng/mg protein) in fractions of cytosol (a), plasmalemma membranes (b), and mitochondrial-nuclear residue (c) in control (unshaded columns) and 24 h after MA (shaded columns).

increase in the number of neutrophils in the skeletal muscles, evidently as a result of two consecutive processes: an increase in the marginal neutrophil pool in blood vessels of the skeletal muscles and an increase in the infiltration of muscle tissue by these cells.

Neutrophils infiltrating foci of injury of skeletal muscle, by secreting enzymes and cationic proteins with affinity for membranes [1, 7], can intensify the process of tissue damage. Evidence is support of this view is given by results of measurement of the MPO concentration in oxidative and glycolytic muscle fibers of m. quadriceps, of which the oxidative fibers do more work in this particular model of MA, with the result that they may undergo greater damage. The MPO concentration initially was higher in the oxidative fibers of this muscle (6.81 ± 1.32 ng/mg protein, n = 8) compared with the glycolytic fibers (2.53 ± 1.03 ng/mg protein, n = 7). These differences were much greater 24 h after MA (29.30 ± 6.08 and 5.05 ± 2.92 ng/mg protein, n = 6, respectively). MPO secreted by neutrophils may play a definte role in the mechanism of injury to muscle tissue, halogenating the membrane proteins in the same way as the membrane proteins of microorganisms. Starting from this hypothesis we studied the distribution of MPO In the various subcellular fractions. Whereas in the control the MPO concentration in the various fractions was small and did not differ, MA led to a significant increase (by 171%) in the concentration of the enzyme in the membrane fraction of the plasmalemma (Fig. 4).

We know that MPO catalyzes oxidative halogenation of proteins [2, 13]. With this in mind, we estimated halogenation of plasmalemma membranes (150 μg) in vitro in the presence of neutrophilic MPO (1 μg), $Na^{125}I$ ($5 \cdot 10^5$ cpm), and H_2O_2 (0.1 mM), the source of which in vivo could be activated neutrophils. The reaction proceeded for 30 min at 37°C in K, Na-phosphate buffer, pH 6.9. Under these conditions MPO catalyzed incorporation of ^{125}I (about 30%) in the acid-insoluble residue (about 10% in the control). Addition of 2-mercaptoethanol (1 mM) or NaN_3 (3 mM) to the system completely inhibited incorporation, indicating that it is oxidative in nature and dependent upon the dependence of MPO.

Thus the data given above provide a basis for the conclusion that intensive MA causes accumulation of neutrophils in the skeletal muscles. These cells, which secrete their own proteins, including MPO, can take part in damage to muscle tissue.

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DETERMINATION OF OXIDATION PHENOTYPE IN INBRED $C_{57}Bl/_6$ AND $BALB/_C$ MICE

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KEY WORDS: oxidative processes, inbred mice, antipyrin

An important achievement in pharmacogenetics has been the obtaining of evidence of inherited differences in ability to oxidize drugs, showing that the human population can be divided into strong and weak oxidizers, the ratio between which varies in different countries and in different racial and ethnic groups between quite wide limits [7, 10]. Genetic heterogeneity of oxidation in some cases may be the cause of the ineffectiveness of pharmacotherapy and the development of side effects [9]. It therefore was considered worthwhile to create an experimental model for use in studying dependence of the pharmacologic effect on the character of oxidation. The theoretical grounds for undertaking such a task, in our point of view, was N. I. Vavilov's general biological law of homologous series of inherited variation [1]. The experimental basis for the investigation consisted of interlinear differences established previously between the conversion of phenazepam into trihydroxyphenazepam and of synocarb into β-hydroxysydnocarb [5, 7].

The aim of this investigation was to study the oxidation phenotype of mice of strains $C_{57}Bl/_6(B_6)$ and $BALB/_C(C)$, used in previous investigations, in relation to the model drug antipyrin (AP).

EXPERIMENTAL METHOD

Experiments were carried out on male B_6 and C mice and on their F_1-hybrids (B × C), weighing 20-25 g.

AP and its metabolites were determined by gas chromatography on the basis of methods described in [8, 12]. The measurements were made on an "Varian Aerograph 2800" gas chromatograph with "Shimadzu" CR-1a integrator ("Chromatopal"). A spiral glass column 1.5 m long was used. The adsorbent was "Chromosorb G 100" (120 mesh) with OV phase of 17-3%. The

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