During AS of rats receiving a normal diet, a decrease in immune reactivity was thus observed, accompanied by marked activation of the lysosomal system of the liver. The decrease in immune reactivity under conditions of an acute nutritional deficiency may be linked to a certain degree with a decrease in the intensity of antibody synthesis. The increase in total activity of lysosomal hydrolases, aimed during endogenous nutrition at redistributing the intracellular reserves of biopolymers of different kinds for replenishing the cellular reserves of the corresponding components, essential for meeting both structural and functional requirements [2, 3], is logical in this connection. It must be recalled that injection of an antigen against the background of an activated liposomal enzyme system may be the cause of changes in the character of transformation of the antigen and depression of its immunogenic properties. The sharp increase in the level of nonsedimented activity may be both the result of irreversible structural disturbances of the lysosomal membranes, caused by starvation [4, 7, 8], and the result of their oriented transformation and labilization, due to the need to release lysosomal hydrolases into the systemic circulation [9]. The combined action of factors of widely different physiological character probably causes mutual induction of the lysosomal apparatus, activation of which is compensatory in character when the immune response is depressed, and is aimed at ensuring the most complete realization of their protective function by the lysosomes.

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HYDROXYLATION PRODUCTS OF HYDROPHOBIC XENOBIOTICS AND CYTOCHROME P-450 STABILIZERS IN HEPATOCYTES

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KEY WORDS: hepatocytes; lipid peroxidation; cytochrome P-450.

When hepatocytes are cultured the terminal component of the mixed-function mono-oxygenase system, cytochrome P-450, undergoes spontaneous destruction [3, 10, 11]. The essential mechanism of its degradation is lipid peroxidation (LPO) [5, 6]; inhibitors of free-radical oxidation (4-methyl-2,6-di-tert-butylphenol and 2-ethyl-6-methyl-3-hydroxypyridine) prevent degradation of cytochrome P-450 in a primary hepatocyte culture [3]. Experiments on the microsomal fraction of the liver showed that during hydroxylation of certain xenobiotics, phenolic products are formed which behave as antioxidants, with ability to reduce the intensity of LPO [1, 2] and to protect cytochrome P-450 against destruction. In the investigation described below the validity of this hypothesis

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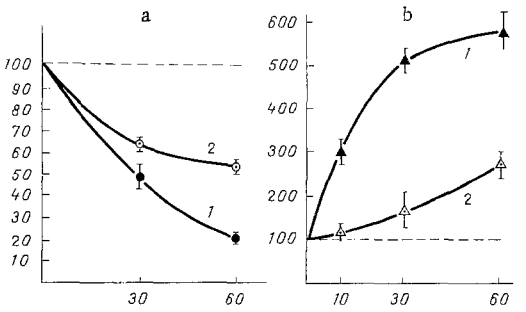

Fig. 1. Dynamics of cytochrome P-450 degradation (a) and MDA accumulation (b) in rat hepatocytes. Abscissa, time (in min); ordinate, content of cytochrome P-450 and MDA (in % of initial value). 1) Cytochrome P-450 and MDA respectively in presence of Fe^{++}–ADP + NADPH, 2) the same, in the presence of 1,2,3-trihydroxybenzene.

was tested for hepatocytes, in which a complete series of reactions of metabolic activation of xenobiotics takes place, not merely their first (hydroxylation) phase, as in the microsomal fractions of the liver. The action of hydroxylation substrate 3,4-benz(a)pyrene and of antioxidant 1,2,3-trihydroxybenzene on degradation of cytochrome P-450, induced in rat hepatocytes by an Fe^{++}–ADP + NADPH system, was investigated.

EXPERIMENTAL METHOD

Intact noninbred albino rats weighing 150-180 g were used. Hepatocytes were isolated by the method in [12] with certain modifications. To isolate the cells, Krebs–Ringer–Henseleit bicarbonate buffer of the following composition was used (in mM): NaCl 120, KCl 4.8, KH_2PO_4 1.0, $MgCl_2$ 1.2, $NaHCO_3$ 24 (pH 7.4; 37°C). The liver was perfused with this calcium-free solution with the addition of 0.5 mM EGTA, and the perfusate was aerated with carbogen (O_2:CO_2 = 95:5). Recirculating perfusion was carried out with a solution containing 2 mM $CaCl_2$ and 0.03% of collagenase (from Sigma, USA). After dispersion of the liver tissue a suspension of hepatocytes was obtained by triple centrifugation (100 g, 1-2 min) in the same calcium-free solution with the addition of 1.5% of albumin purified from fatty acids. The hepatocyte suspension in buffer (40 mM HEPES), containing all the above-mentioned salts, with 1 mM $CaCl_2$ and 1.5% albumin, was incubated at 37°C on a shaker (one shake per second). The number of cells was counted in a Goryaev's shaker with trypan blue to assess viability of the hepatocytes. The content of cytochrome P-450 in the hepatocyte suspension was determined by the method in [9]. LPO was induced by a system of Fe^{++}–ADP ($5 \cdot 10^{-5}$ M) + NADPH (10^{-4} M). Accumulation of malonic dialdehyde (MDA) was recorded in the hepatocyte suspension by the reaction with 2-thiobarbituric acid [7]. The 3,4-benz-(a)pyrene was added to a suspension of hepatocytes in acetone (the final concentration of acetone did not exceed 0.5%), and 1,2,3-trihydroxybenzene was added in alcohol, so that its concentration did not exceed 1%. The final concentration of 3,4-benz(a)pyrene in the incubation medium was 10^{-4} M, and of 1,2,3-trihydroxybenzene $5 \cdot 10^{-5}$M.

EXPERIMENTAL RESULTS

The standard hepatocyte suspension contained $(2\text{-}4) \cdot 10^6$ cells/ml. Suspensions containing at least 70-75% of intact cells were used in the experiments. The cytochrome P-450 content in native hepatocytes was 0.109 ± 0.021 nmoles/10^6 cells, in agreement with data in the literature [4, 8]. During induction of LPO with the Fe^{++}–ADP + NADPH system, MDA accumulated in the hepatocyte suspension and cytochrome P-450 was destroyed (Fig. 1; coefficient of negative correlation $r = -0.97$). It can be tentatively suggested that degradation of cytochrome P-450 was due to activation of LPO. The rate of destruction of cytochrome P-450 varied only a little during 1 h of incubation, whereas the rate of MDA accumulation was much greater in the first 30 min than in the next (Table 1).

On addition of 1,2,3-trihydroxybenzene to the incubation medium stabilization of cytochrome P-450 took place (Fig. 1a), accompanied by a decrease in the rate of accumulation of MDA and the quantity formed (Table 1; Fig. 1b). After incubation for 60 min in the presence of antioxidant, more than twice the amount of cytochrome P-450 remained in the hepatocytes than in suspension without the antioxidant (Fig. 1; Table 1). During the same time interval the quantity of MDA accumulating during incubation in the presence of 1,2,3-trihydroxybenzene also

TABLE 1. Rate of Cytochrome P-450 Degradation and of MDA Accumulation (in nanomoles/10^6 cells/30 min) in Rat Hepatocytes (M ± m)

Experimental conditions	Rate of cytochrome P-450 degradation		Rate of MDA accumulation	
	0-30 min of incubation	30-60 min of incubation	0-30 min of incubation	30-60 min of incubation
Fe^{++}–ADP + NADPH	0,038±0,010	0,034±0,005	1,300±0,075	0,230±0,120
Fe^{++}–ADP + NADPH + 1,2,3-trihydroxybenzene	0,024±0,001	0,009±0,004	0,500±0,095	0,140±0,095
Fe^{++}–ADP + NADPH + 3,4-benz(a)pyrene	0,029±0,004	0,006±0,002	0,450±0,170	0,060±0,020

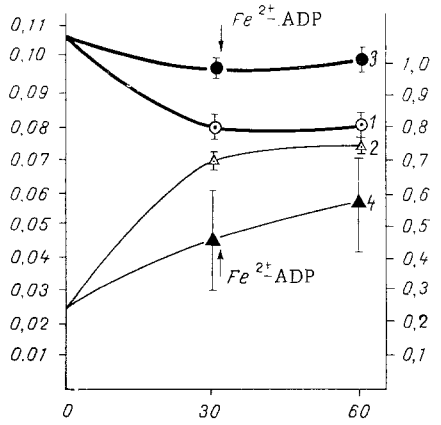

Fig. 2. Dynamics of cytochrome P-450 degradation (1, 3) and MDA accumulation (2, 4) in hepatocytes in presence of 3,4-benz(a)pyrene. Abscissa, time (in min); ordinate, content (in nmoles/10^6 cells) of cytochrome P-450 (left) and MDA (right). 1) Cytochrome P-450 + NADPH + Fe^{++}–ADP, 2) MDA + NADPH + Fe^{++}–ADP, 3) cytochrome P-450 + NADPH; Fe^{++}–ADP added 30 min later, 4) MDA + NADPH; Fe^{++}–ADP added 30 min later.

was only half of that found in its absence. The rate of cytochrome P-450 degradation and MDA accumulation was much less in the presence of antioxidant (Table 1).

The results showed that 3,4-benz(a)pyrene also prevented degradation of cytochrome P-450 due to LPO activation (Fig. 2). During the first 30 min there was a very small decrease in the rate of destruction of cytochrome P-450 compared with the control, in the absence of benz(a)pyrene (Table 1), but during the next 30 min this rate fell sharply and practically no degradation of cytochrome P-450 took place (Fig. 2). Meanwhile, in the presence of benz(a)pyrene the rate of MDA accumulation during the first 30 min (Fig. 2) was 3 times slower than in the control, and in the next 30 min no MDA accumulation was observed (Table 1).

The equal ability of the antioxidant (1,2,3-trihydroxybenzene) and of the oxygenase substrate 3,4-benz(a)pyrene to inhibit LPO (50% inhibition of MDA accumulation during 1 h of incubation of the hepatocytes), incidentally, was matched by their equal effectiveness as stabilizers of cytochrome P-450, and in conjunction with previous data showing the stabilizing action of other antioxidants (4-methyl-2,5-di-tert-butylphenol and 2-ethyl-6-methyl-3-hydroxypyridine) on cytochrome P-450 during LPO induction in hepatocytes [3], is evidence of the interconnection between cytochrome P-450 degradation and accumulation of LPO products in these cells.

The virtually complete stabilization of cytochrome P-450 and prevention of MDA accumulation after 30 min of incubation of the hepatocytes in the presence of 3,4-benz(a)pyrene were evidently determined mainly by the fact that during this time hydroxylation products of benz(a)pyrene were formed, including phenolic derivatives, which are antioxidants and able, as was shown previously on the microsomal fraction of liver [1], to prevent de-

struction of cytochrome P-450. This protective effect of 3,4-benz(a)pyrene is actually due to the antioxidant properties of its hydroxylation products, and not to competition for sources of reducing equivalents in the NADPH-dependent electron-transport chain. This conclusion can be drawn from the following experiments. On incubation of hepatocytes in the absence of Fe^{++}–ADP, but in the presence of NADPH (10^{-4} M) very little accumulation of MDA was observed in the cells (0.20 ± 0.11 nmoles/10^6 cells), and cytochrome P-450 was correspondingly stabilized (Fig. 2). After addition of Fe^{++}–ADP ($5 \cdot 10^{-5}$ M) to the hepatocyte suspension, neither accumulation of MDA nor destruction of cytochrome P-450 took place during the next 30 min (Fig. 2). In suspension without benz(a)pyrene and without Fe^{++}–ADP, but with NADPH, MDA accumulation during the first 30 min was the same as in the system with addition of benz(a)pyrene, but in the 30 min after addition of Fe^{++}–ADP the MDA content increased by 1.5 times under these conditions.

Thus antioxidants of phenolic type, both exogenous and hydroxylation products formed as a result of oxidative metabolism of hydrophobic substrates, are effective protectors of cytochrome P-450 in liver cells against destruction due to lipid peroxidation.

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AFTEREFFECTS OF SHORT-TERM EXPOSURES TO HEAT OR COLD ON MONOAMINE OXIDASE ACTIVITY

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Exposure for short periods to high or low temperatures is followed by the development of trace reactions [6]. There is evidence of lasting morphological and physiological changes in animals after exposure to hypo- and hyperthermia [1, 4]. Profound and lasting changes after exposure to brief but extreme cold have been described also at the level of the effector mechanisms of chemical temperature regulation [7]. However, it is not yet known whether aftereffects of exposure to high or low temperatures are preserved at the level of central mechanisms of temperature regulation. An important role in the regulation of body temperature is played by the serotoninergic system of the brain [8]. The principal enzyme participating in serotonin catabolism is monoamine oxidase (MAO), whose activity determines the intensity of serotonin destruction and can regulate its level in synaptic endings [2].

The aim of this investigation was to study the effects of exposure to different temperatures on the activity and catalytic properties of MAO.

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