Time Course of Changes in Macrophagal Activity, Metabolic Activity of the Liver, and Hydrocortisone Level in Mouse Serum following Injections of Bacterial Immunostimulants

S. V. Sibiryak, S. A. Sergeeva, S. A. Kryzhanovskii, and I. L. Krasilova

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A relationship is revealed between macrophage activation, suppression of cytochrome P-450-dependent liver monoxygenases, and the hydrocortisone level in the sera of mice in response to bacterial lipopolysaccharides and muramyldipeptide.

Key Words: *macrophages; microsomal oxidation; corticosteroids; immunostimulants*

Numerous data have now been reported indicating the depression of cytochrome P-450-dependent monoxygenases of the liver following the administration of various immunostimulants, similarly as in acute bacterial or viral infections [1,2]. This effect is explained by macrophage activation [3,16]. The factors acting to suppress hepatocyte metabolic activity are presumably interferon [10,16], interleukin-1 [15], or another protein factor [13]. Suppression of the metabolic activity of the liver interferes with the biotransformation of many xenobiotics and, hence, alters their pharmaco- and toxicodynamics. This problem has been widely discussed in the literature [1]. On the other hand, the metabolism of numerous endogenous compounds (prostaglandins, steroid hormones, etc.) is mediated by the monoxygenase system of the liver. The majority of these compounds are characterized by a polymodal activity and participate in the intricate system of neuroendocrine-immune relationships, in which the key role is played by corticosteroids.

This research was aimed at investigating the time course of macrophagal activity, the metabolic activity of the liver, and the hydrocortisone level of the serum in response to the administration of bacterial immunostimulants.

Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow (Presented by M. D. Mashkovskii, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Outbred albino mice of both sexes weighing 18-20 g were used in the study. The animals were kept in plastic cages with a 12-hour light cycle and fed a standard diet.

The absorption activity of Kupffer's cells and splenic macrophages was assessed from the rate of elimination of intravenously injected colloid carbon (160 mg/kg) spectrophotometrically at 650 nm. The result was expressed as a corrected value of phagocytosis [3].

The metabolic function of the liver was assessed from the duration of sleep induced by hexenal injection (60 mg/kg). The duration of sleep was counted as the time elapsing between the loss and acquisition of the "turn over reflex". During sleep the animals were kept in an incubator at 26±1°C.

Serum hydrocortisone was radioimmunoassayed using STERON-K-^{125}I-M diagnostic kits.

The following agents were used as macrophage activators: *Serratia marcescens* lipopolysaccharide (LP) prodigiosan (Moskhimfarmpreparaty), muramyl-

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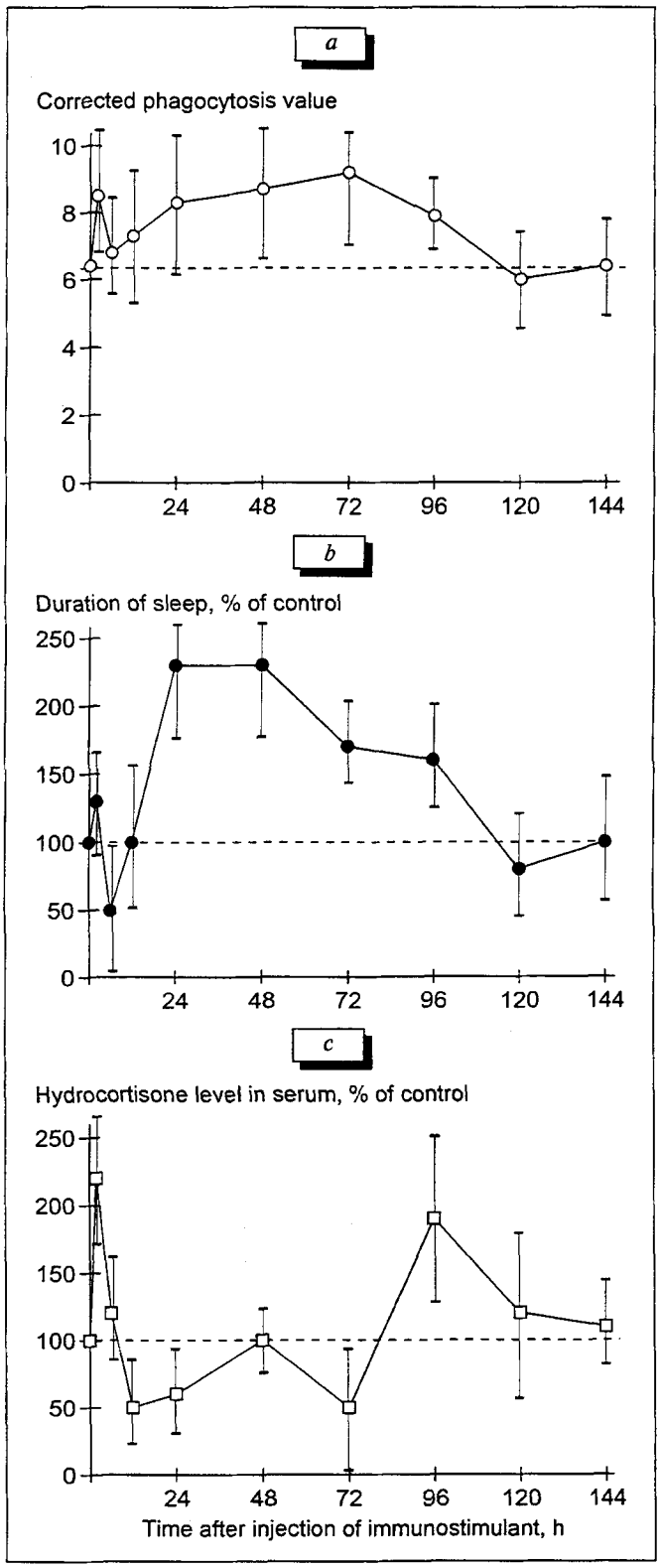

Fig. 1. Effect of prodigiosan (0.5 mg/kg) on the absorption capacity of macrophages (*a*), duration of hexenal sleep (*b*), and hydrocortisone level in the serum (*c*) of mice. Here and on Figs. 2 and 3: the dashed line is the control.

dipeptide (MDP, Sigma), and LP of *E. coli* serotype 0111:B4 (Sigma). The immunostimulants were injected intraperitoneally: prodigiosan in a dose of 0.5 mg/kg, MDP in a dose of 2 mg/kg, and *E. coli* LP in a dose of 1.5 mg/kg.

The absorptive activity of macrophages, the duration of hexenal sleep, and the hydrocortisone level were assessed 2 to 144 h after the injection of the immunostimulants in three parallel groups of mice, each group consisting of 6-7 animals, taken at random from the total group of animals injected immunostimulants. In parallel with this, the above parameters were measured in control groups of mice injected normal saline. The values of glucocorticoid levels and the duration of hexenal sleep in the experimental groups were expressed in percent of the control values, with due consideration for the diurnal fluctuations in these parameters. A total of 8 experiments on 3000 mice were carried out. The hydrocortisone level in controls (in all experiments) varied from 10 to 40 nmol/liter, and the duration of hexenal sleep from 30 to 60 min.

The results were statistically processed using Student's *t* test.

RESULTS

Prodigiosan caused similar changes in the parameters of interest in all experiments (Fig. 1). No sex-specific differences in the type of reaction were observed. Three phases may be arbitrarily distinguished in the time course of the response to LP. The duration of the first phase is 12 hours; the absorptive activity of macrophages increases for a short time and then tapers off, with the metabolic activity of the liver remaining virtually the same. The level of hydrocortisone in the serum at first sharply increases and then drops below the control values. It is known that after just 2 to 4 hours of macrophage contact with bacterial endotoxins, a potent emission of interleukin-1 and tumor necrosis factor starts, both of these, in turn, being inductors of corticosteroid release [4-6,12].

The second phase of the reaction occurs between the 12th and 72nd hours after prodigiosan injection. The absorptive activity of macrophages again increases, to reach the maximum during this period. A pronounced decrease of microsomal oxidation in the liver and prolongation of hexenal sleep are observed. A latent period is known to exist between the injection of macrophagal stimulants and interferon inductors and the depression of cytochrome P-450 [16]. The depressant effect of cytokines on cytochrome P-450 is not caused by direct inactivation of the enzyme (as is typical of the majority of synthetic low-molecular inhibitors), but is realized through inhibition of the synthesis and activation of the degradation of the apoenzymatic part of cytochrome [10,11]. The level of hydrocortisone in this phase of the reaction is boosted (the feedback

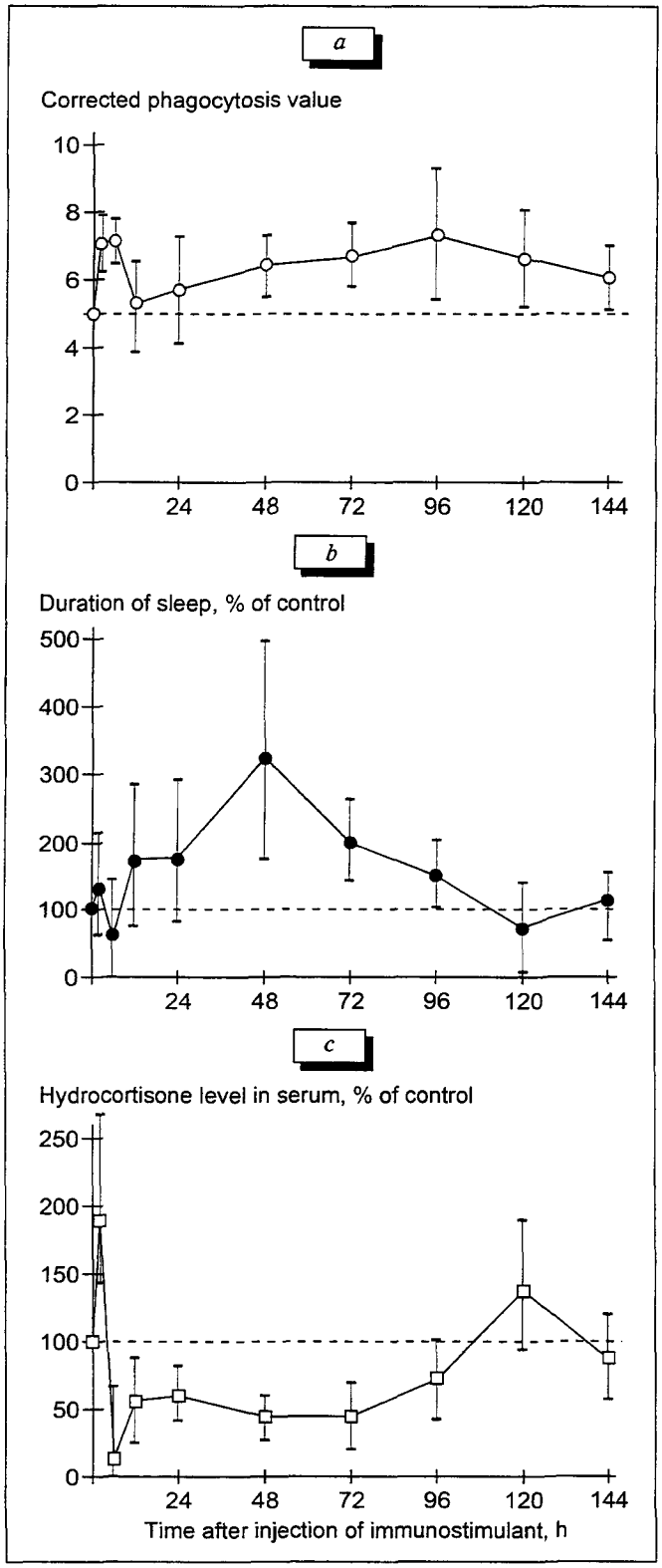

Fig. 2. Effect of MDP in a dose of 2 mg/kg on the absorption capacity of macrophages (*a*), duration of hexenal sleep (*b*), and hydrocortisone level in the serum (*c*) of mice.

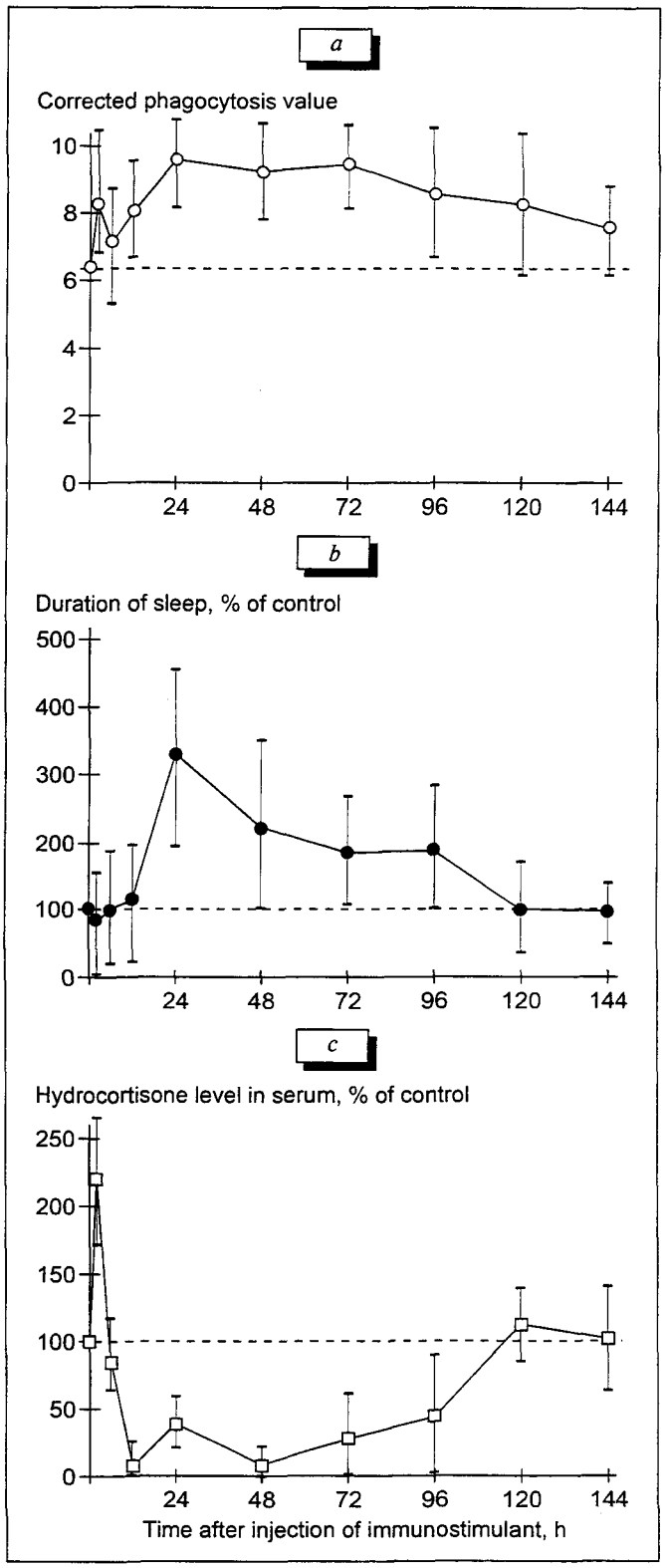

Fig. 3. Effect of *E. coli* serotype 0111:B4 (1.5 mg/kg) on the absorption capacity of macrophages (*a*), duration of hexenal sleep (*b*), and hydrocortisone level in the serum (*c*) of mice.

system "kicks in": hydrocortisone level drops → ACTH → hydrocortisone level rises), and then again falls below its level in intact mice.

The next phase (days 3-6 after injection of LP) is characterized by a gradual reduction of macrophage activation, normalization of the metabolic func-

tion of the liver, and a new peak of serum hydrocortisone concentration, the level of which then drops to control values.

A similar regularity in the time course of the parameters was observed after injection of MDP and *E. coli* LP: the curves differed only in the degree of changes and the time constant (Figs. 2 and 3). Hence, the observed effect was nonspecific and typical of all the immunostimulants.

Macrophages are known to metabolize steroids by restoring the double bond of the A ring with 3-ketosteroid dehydrogenase, and in the case of macrophage activation the intensive biotransformation of steroids in these cells appreciably contributes to the elimination of corticosteroids [9,14]. On the other hand, in physiological and pharmacological concentrations corticosteroids have a protective effect in endotoxic shock and limit the production of acute-phase cytokines [7,8]. Through inhibition of protein synthesis, corticosteroids can lower the sensitivity of cytochrome P-450 to the inhibitory action of cytokines [10]. Suppression of microsomal oxidation under conditions of macrophagal stimulation may be regarded from this viewpoint as a mechanism limiting the metabolism of corticosteroids in hepatocytes in the presence of increased "intramacrophagal" biotransformation. Later a gradual rise of the level of corticosteroids with their metabolism in hepatocytes suppressed leads to a reduction of both macrophage activation (including the secretory function) and the sensitivity of cytochrome P-450 to the inhibitory action of cytokines, thus providing the feedback in the described process.

Bearing in mind the nonspecific pattern of action of different immunostimulants [16] on microsomal oxidation, we may propose, along with other factors, the presence of a defense "metabolic" system of immune function regulation under conditions of macrophagal activation during an infectious process.

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