# DAMAGE TO CELLS OF THE HEPATIC MACROPHAGAL SYSTEM BY STRESS AND ITS PREVENTION BY ADAPTATION TO PERIODIC HYPOXIA

**A. A. Nikonorov, G. N. Smagin, B. A. Frolov, and F. Z. Meerson**

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It was shown previously that under the influence of stress considerable damage is observed to hepatocytes, as shown by the release of fructose-1-phosphate aldolase, a marker enzyme of cell damage, into the blood stream, and the mechanism of this damage has been discovered to be activation of lipid peroxidation (LPO) in the organ [7, 8]. It was found at the same time that preliminary adaptation of animals to periodic hypoxia led to limitation of stress-induced damage to liver cells [4]. However, we know that a large part of the cell population of the liver consists of stellate endotheliocytes (hepatic macrophages), with an important role in the maintenance of homeostasis. The hypothesis of stress-induced damage of this important component of nonspecific defense, and also the possible elimination of this damage by adaptation to hypoxia, rests on solid evidence. The aim of the investigation described below was to study these two problems.

## EXPERIMENTAL METHOD

Experiments were carried out on 137 male Wistar rats weighing 160-180 g. Stress-induced liver damage took the form of the creation of an "anxiety neurosis" in the animals by the method in [14]. Some of the animals were adapted to hypoxia by lifting them daily in a pressure chamber to an altitude of 5000 m for a period of 6 h for 40-50 days [6]. Activity of cells of the hepatic macrophagal system (MPS) was assessed as the rate of clearance of the blood from intravenously injected ink [12], for this population of mononuclear phagocytes is known to be responsible for the elimination of most ink (up to 90%) from the blood stream [12]. For this purpose the animals were anesthetized by intraperitoneal injection of urethane in a dose of 1 g/kg body weight [1]. To 3 ml of a 3% solution of gelatin 2 ml of ink was added and the resulting mixture was injected in a dose of 0.26 ml/100 g body weight into the femoral vein. Blood for investigation was taken from the jugular vein in a volume of 0.1 ml 15 sec after injection of ink, and during the next 3 min for 24 min, into tubes containing 3 ml of physiological saline with heparin (5 U/ml). The presence of ink in the samples was determined after centrifugation at 1000 rpm for 15 min, spectrophotometrically at a wavelength of 650 nm. The result was expressed in percentages of the initial quantity, taking the concentration of ink in the blood 15 sec after its injection to be zero. During investigation of the effect of stress on the ingestive function of the hepatic macrophages, three groups of stressed animals in which clearance of ink was determined immediately after exposure to stress and 24 and 48 h after the end of exposure, were investigated.

LPO activity was assessed as the content of TBA-reagents [15] and of diene conjugates [11] in liver homogenates at the above time intervals after exposure to emotional-painful stress (EPS).

## EXPERIMENTAL RESULTS

Absorption of intravenously injected ink took place in accordance with an exponential law. By the 9th minute, in unstressed animals more than 70% of the injected ink had been ingested, and by the 24th minute, virtually no ink could be

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found in the blood. In animals subjected to EPS, clearance of the blood was disturbed immediately after exposure to stress. In particular, during the first 9 min only 40% of the injected ink was ingested, and not until the 24th minute was the level of ingestion corresponding to that at the 9th minute in intact rats achieved. A tendency for ink clearance to return to normal was observed 24 h after the end of exposure to stress, although differences between the experimental and control groups still remained, and in certain cases (the 12th minute after injection of ink) reached the significant level ($p < 0.05$). By the 48th hour the ink clearance curve of the stressed animals coincided completely with the ink clearance curve of the intact rats.

Thus this series of experiments demonstrated the inhibitory effect of EPS on the ingestive function of cells of the MPS in the liver, manifested during the first 24 h after exposure to stress.

Adaptation of the animals to the periodic action of hypoxia led to enhancement of the ingestive function of the phagocytic cells in the adapted animals compared with the unadapted, as shown by more rapid clearance of ink throughout the period of investigation (24 min), up to the level of significant differences ($p < 0.05$) between them (after 3 and 12-21 min).

This important fact is in some agreement with existing data on ability of adaptation to hypoxia to cause activation of immunologic processes [2, 3, 5, 9], and is probably based on the pattern of reorganization of metabolism in the adapted animals, which is connected primarily with the intensification of protein biosynthesis [5]. This intensification may lead both to an increase in the blood level of nonspecific proteins ($\alpha_2$SB), potentiating the phagocytic activity of the stellate reticuloendotheliocytes of the liver and of the system of mononuclear phagocytes as a whole [10], and to the ability to restore components of cell membranes, required for phagocytosis, more rapidly [13]. The effect of adaptation, connected with intensification of protein biosynthesis, may perhaps also be mediated through activation of energy metabolism, although it has been shown that "nonimmune" phagocytosis is energy-independent [13].

EPS, to which animals adapted to periodic hypoxia, and also unadapted fats, were exposed led to depression of the phagocytic activity of cells of the hepatic MPS immediately after exposure to stress. For instance, by 3 and 6 min after injection of ink, the hepatic macrophages of the adapted rats after EPS ingested 20% less of it than macrophages of adapted animals not exposed to EPS. This level of differences was preserved until the end of the observations. The ink clearance curve of the adapted, stressed animals 24 h after the end of exposure to stress, was virtually coincident with the clearance curve of the adapted rats not exposed to EPS. This pattern was fully preserved 48 h after stress.

Comparison of results reflecting the effect of stress on the ingestive ability of the hepatic macrophages in adapted and unadapted rats leads to the distinguishing of three factors. The first is that stress-induced disturbance of the ingestive power of macrophages of adapted rats is much weaker than of unadapted rats (the level of differences with the corresponding control was 20 and 50%). Second, despite the reduction of the ingestive power of the macrophages taking place in adapted animals, its quantitative parameters were within the limits of values determined for intact rats. Third, disturbance of the ingestive power of the macrophages of adapted animals exposed to EPS was shorter in duration than in unadapted, Stressed rats. For instance, whereas in unadapted animals disturbance of the ingestive power of the hepatic macrophages was discovered 24 h after EPS, in the adapted rats it was found only immediately after exposure to stress.

The results are thus unequivocal evidence that preliminary adaptation to periodic hypoxia has a marked protective effect against stress-induced disturbances of the hepatic MPS.

It is important to emphasize that this effect correlates directly with data on the ability of adaptation to periodic hypoxia to prevent stress-induced activation of free-radical oxidation. EPS caused accumulation of primary (DC) and secondary (MDA) lipid peroxidation products in the liver tissue. For instance, immediately after the end of exposure to stress the increase in the concentration of LPO products in the stress-control group was 47% DC and 52% MDA ($p < 0.01$). These values 24 h after the end of exposure to EPS were 27 and 96% respectively. No significant differences could be observed 48 h after exposure to stress between the control and experimental groups.

No such marked activation of LPO processes took place in the group of stressed rats adapted beforehand to hypoxia. For instance, immediately after the end of EPS the increase in DC was 16% and in MDA 62. These values 24 h after the end of exposure to stress were 4 and 20% respectively. After 48 h no difference was observed between the experimental and control groups. Since it has been shown that products of free-radical oxidation, reactive metabolites of oxygen ($O_2^{\cdot}$; $H_2O_2$, OH) damage biological membranes [6], disturb the functions of Fc- and $C_3$b-receptors, and thus depress the ingestive capacity of the macrophages [16], there is strong evidence in support of the pathogenetic role of LPO in the realization of stress-induced damage to this important component of nonspecific defense.

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