DISTRIBUTION AND ACTIVITY OF GLUTATHIONE REDUCTASE IN THE LIVER OF ADULT RATS FOLLOWING EMBRYONIC EXPOSURE TO PARAQUAT

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The late effects of exposure to toxic substances are currently giving rise to increased interest among research workers in various specialties. This is particularly true of the late effects that may be induced at critical periods of individual development [1]. Whereas macroscopically distinguishable morphological injuries (severe teratogenic effects) have been sufficiently well described in the literature [8], functional effects of teratogens at the cellular and biochemical level and also the mechanisms of their development still require detailed study. The aim of this investigation was to study late consequences of exposure during the embryonic period to paraquat, a herbicide used on a worldwide scale. The reaction of glutathione reductase (EC1.6.4.2), a key enzyme of defence against poisoning by this compound, was used as a sensitive marker of the action of paraquat on the liver.

EXPERIMENTAL METHOD

On the 10th-12th days of pregnancy female rats were given paraquat (1,1-dimethyl-4,4-dipyridyl) intraperitoneally in a total dose of 24 mg/kg body weight. Control animals at the same times of pregnancy were given intraperitoneal injections of physiological saline. Glutathione levels in the liver, initially and induced by the herbicide (24 mg/kg body weight, intraperitoneally, one injection 2 days before sacrifice) were assessed in the mature progeny (males).

Glutathione reductase activity of hepatocyte cytosol was assessed spectrophotometrically, by measuring the decrease in NADPH concentration in the reaction of reduction of oxidized glutathione [6].

The localization of the enzyme in the liver cells was determined by means of a method developed by the writers, based on interaction of oxidized glutathione, tagged with colloidal gold, with the aid of glutathione reductase present in the sections [2].

Rat liver tissue was placed in a 4% solution of paraformaldehyde, fixed overnight, and embedded in paraffin wax after the usual treatment. Sections 4 μm thick were mounted on albumin-coated glass and, after preincubation for 1 h in 0.01% albumin solution (from human serum) the reagent thus obtained [2] was diluted with distilled water (1:5) and layered above the specimen. Incubation was carried out at room temperature in a humid chamber overnight.

EXPERIMENTAL RESULTS

Under normal conditions, in the liver of intact animals, staining corresponding to glutathione reductase is distributed mainly centrilobularly, at the periphery of the hepatocyte cytoplasm and in sinusoidal cells. Some degree of staining also was found in erythrocytes. The hepatocyte nuclei did not stain (Fig. 1a). Glutathione reductase activity in the hepatocyte cytosol from animals of this group amounted to 33.4 ± 3.29 nmoles · min^{-1} · mg $protein^{-1}$. In acute paraquat poisoning, the intensity of the stain increased sharply. The distribution of the enzyme lost its zonal character over the hepatic lobule (Fig. 1b). Distinct staining appeared in the nuclei of individual hepatocytes. Zones of destruction appeared, which were extremely brightly stained but had

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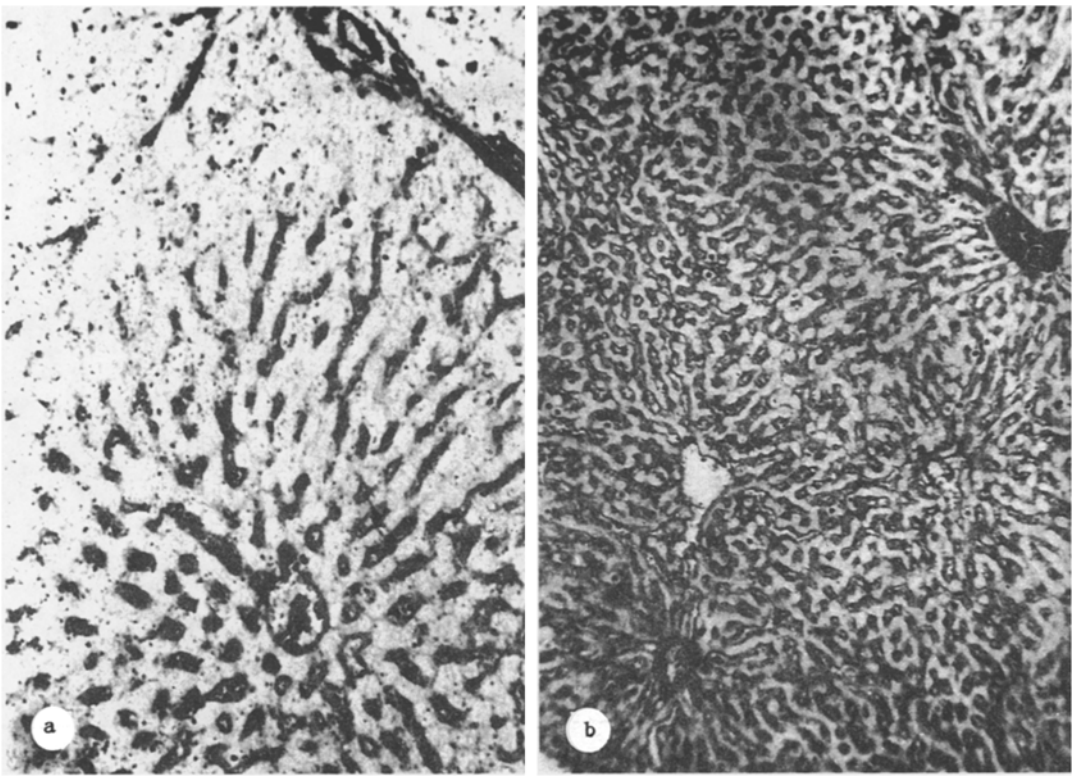

Fig. 1. Centrilobular arrangement of marker for glutathione reductase in control. Animals aged 2 months. Staining with oxidized glutathione and colloidal gold, without counterstaining. 200×.

Fig. 1b. Intensification and loss of zonal character of staining for glutathione reductase in hepatic lobule. Acute paraquat poisoning. Rats aged 2 months. Staining as in Fig. 1a. 100×.

no cell boundaries. Foci appeared in which deeply stained hepatocytes were next to cells with a low content of the enzyme. In these cases a significant ($p < 0.05$) increase in the glutathione reductase activity of the cytosol was observed (44.9 ± 2.89 nmoles · min^{-1} · mg $protein^{-1}$) compared with intact animals.

This structural-metabolic response may provide a good illustration of the active involvement of the glutathione system in protection of the cell against damage by free radicals, induced by paraquat, whose cytotoxicity is manifested as oxidation of cytosol GSH [9]. The increase in the content and activity of glutathione reductase under these conditions most probably reflects an adaptive reaction of the hepatocytes to reduction of glutathione disulfide, formed in large quantities. Intensive staining of the hepatocyte nuclei may be due to the special role of the glutathione regeneration system in protection of genetic material against free-radical products of oxygen metabolism [5].

In animals exposed to paraquat in the embryonic period the intensity and distribution of the stain resembled that in rats with acute poisoning by this herbicide (Fig. 2a). For instance, rather more intensive staining of the hepatocytes than normally and the distribution of the enzyme in them likewise had no intralobular differences, and many nuclei and Kupffer cells were stained. The glutathione reductase level in the hepatocyte cytosol was quite high and close to that in the acutely poisoned animals, although it was not significantly higher than values in intact rats (41.4 ± 2.53 nmoles · min^{-1} · mg $protein^{-1}$). In acute paraquat poisoning of animals of this group the structure of the liver was mosaic: foci of bright staining of hepatocytes was observed next to foci of cells with almost complete absence of the enzyme. Nuclei (Fig. 2b) and sinusoidal cells were deeply stained. No increase in glutathione reductase activity (induction) of the hepatocytes was found compared with the previous group (43.8 ± 4.24 nmoles · min^{-1} · mg $protein^{-1}$).

On the whole, if the structural and metabolic features of the liver tissue in animals exposed prenatally to paraquat are characterized, the permanent changes in the intensity and distribution of staining for glutathione reductase must be emphasized, both within the same hepatocyte and also within the extent of the lobule. This is in good agreement with modern experimental data indicating that an important role in the mechanism of the cytotoxicity of paraquat is played by damage to the cell skeleton

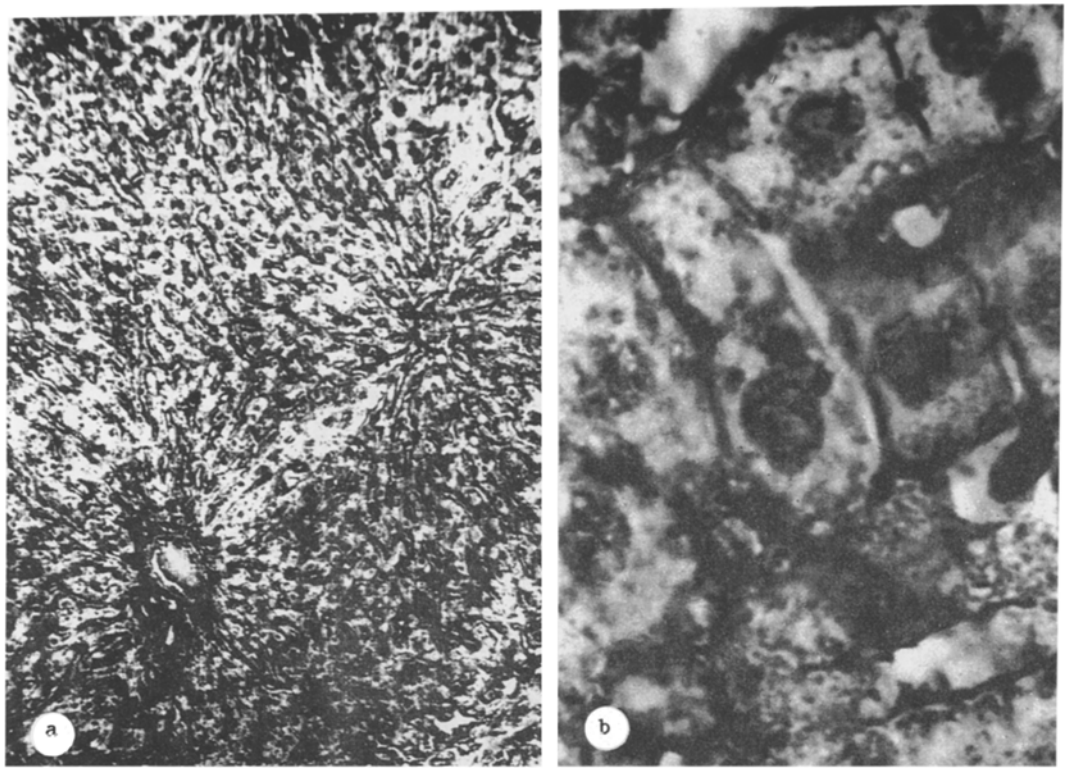

Fig. 2. Intensified reaction and universal character of glutathione reductase distribution in liver tissue of animals aged 2 months, exposed to paraquat in the embryonic period. Staining as to Fig. 1a, 100×.

Fig. 2b. Intensively stained nuclei and heterogeneity of staining of hepatocytes for glutathione reductase. Acute poisoning of animals exposed prenatally to paraquat. The same staining as before. 900×.

[7], and also by inhibition of intercellular connections between hepatocytes [10], leading to disturbance of their metabolic cooperation. One result of this is evidently loss of inducibility of glutathione reductase in the liver cells, of importance in adaptation, in animals with acute paraquat poisoning exposed to this same compound in utero.

Thus administration of paraquat to experimental animals in the period of embryogenesis leads to marked disturbances of the detoxication system, which persist throughout postnatal development. This phenomenon of a persistent change in the activity of neonatally induced enzymes has been described by Soviet workers, who have called in enzymic imprinting [3, 4]. The combined approach to the study of the mechanism of enzymic imprinting in embryogenesis, used in the present study, enabled us to demonstrate both changes in general activity (inducibility) of an enzyme (glutathione reductase), and also its visualized redistribution within tissues and cells.

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