Apoptosis: Decrease of Hepatocyte Population in Mice after Hyperthermia

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Hyperthermia caused hemodynamic disorders in the liver and degenerative and necrobiotic changes in hepatocytes of CBA mice. Total hepatocyte count decreased during restitution, this decrease being most pronounced 30 min after exposure. The number of binucleated cells also markedly decreased. The absence of necrotic changes in hepatocytes during the entire restitution period indicated their apoptotic death and elimination by macrophagal resorption. Under these conditions liver regeneration at the cellular level occured mainly via division of binucleated hepatocytes. On the other hand, proliferation of oval cells in the portal zones and their differentiation into hepatocytes were observed at certain stages of reparative regeneration of the liver.

Key Words: *whole-body hyperthermia; hepatocytes; alkaline dissociation of tissues; apoptosis*

Structural and functional disorders in the liver are often paralleled by impaired recovery and plastic repair of other organs and systems, which attracts special attention to alteration and regeneration of the liver [5,7,9]. Moreover, the outcome of adaptive compensatory processes in the organism largely depends on the state of detoxifying and synthetic systems of the liver [3,6]. In parallel with regeneration and proliferation hepatocytes perform all main functions essential for homeostasis. However massive loss of hepatocytes, which normally almost do not proliferate (being in the G_0 phase of cell cycle), but possess high capacity for proliferation and clonal growth [11,13] under conditions of prolonged or intensive exposure to damaging factors, can lead to hepatic failure.

All this necessitates more detailed evaluation of regeneratory strategies of the liver during exposure to various damaging factors. It is however noteworthy that structural and metabolic reactions of the liver and its regeneratory potential during exposure to subextreme and extreme ecological factors are little studied.

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We studied liver regeneration in inbred mice exposed to hyperthermia by evaluating the hepatocyte population at various terms of postthermal restitution (PR).

MATERIALS AND METHODS

Thirty-six male CBA mice aged 3 months were exposed to single whole-body hyperthermia (43°C) for 35 min; 8 animals served as controls. The duration of exposure corresponded to the threshold level after which all animals died. Liver samples were taken from animals decapitated 30 min after exposure and on days 3, 7, and 14 of PR. Controls for all experimental series were kept under standard vivarium conditions on a standard diet with free access to water.

The absolute number of hepatocytes was estimated after alkaline dissociation of fixed tissues [1,8]. After fixation in 10% formalin for at least 10 days, 1-mm plates were cut from the large lobe, weighed, and put in 50% KOH for 20-24 h, after which they were treated as described previously [8]. Methods for hepatocyte counting, formula for evaluating cell concentration and estimation of their absolute count were

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described previously [4]. The significance of differences was evaluated using Student's t test [10].

RESULTS

Single whole-body hyperthermia of CBA mice did not affect body weight or liver weight during the entire experiment (Table 1). However the relative weight of the liver tended to decrease on day 14 of PR.

Photooptic examination 30 min after hyperthermia showed circulatory disorders in the liver: plethoric sinusoids and central veins were seen. On day 3 of PR hemodynamic disorders were still seen in the lever, hepatocyte staining was mosaic, which was due to different functional activity of the cells and damage to hepatocytes (lytic and necrobiotic changes, Fig. 1, *a*). Regeneratory reactions of hepatocytes were enhanced: mitotically active binucleated hepatocytes (BNH) appeared. Enlarged basophilic nucleoli containing micronucleoli indicated changes in their functional activity (Fig. 1, *b*). Some nuclei contained large lipid incorporations. Small aggregations of macrophagal cells resorbing hepatocyte fragments were seen in the periportal zones.

Heterogeneous lesions of hepatocytes and hemodynamic disorders (uneven hyperemia) were still observed in the liver on days 7-14 of PR (Fig. 2, *a*). Pronounced degenerative changes were seen in the majority of cells: large "devastated" spaces in the cytoplasm represented sites of accumulation of glycogen fractions with modified tinctorial properties (Fig. 2, *b*). There were mononuclear cells with giant nuclei and large nucleoli, often with lipid incorporations. The number of mononuclears surrounding degenerative hepatocytes increased in the periportal zone. Regenerative activity of BNH increased (formation of chromatin threads and approximation of nuclei).

Aggregations of oval cell, often arranged in chains appeared in the portal zones. This is a special type of hepatocyte regeneration associated with activation of the stem reserve (Fig. 2, *a*). Small hepatocytes, morphologically belonging to young cells, were seen near these chains. This type of regeneration was previously described for liver tumors and irreversible damage to hepatocyte genome [11]. Our findings indicate that subextreme and extreme ecological factors can also lead to activation of the stem reserve in the liver.

Quantitative analysis revealed a decrease in hepatocyte absolute count. The most pronounced decrease was observed 30 min after single whole-body hyperthermia (by 13%, $p<0.05$). By day 3 of PR the total cell count almost returned to the baseline, but after this term the absolute count of hepatocytes constantly tended to decrease: by day 14 their number decreased by 12% compared to the control (Table 1).

The decrease in the total hepatocyte count 30 min after hyperthermia was due to a 22% decrease in their concentration per mg tissue. Hepatocyte concentration remained decreased over the entire experiment, but the most pronounced decrease was observed 30 min, 7 and 14 days after hyperthermia. Quantitative analysis of hepatocyte population showed that single whole-body hyperthermia reduced the number of BNH, this effect being most pronounced 30 min and 14 days after hyperthermia (by 24 and 26%, respectively, $p<0.01$).

The absence of pronounced necrotic changes in hepatocytes during the entire PR period indicates their predominant apoptotic death and resorption by macrophages. This type of hepatocyte death is described under normal conditions and in many hepatobiliary diseases (acute and chronic hepatitis, alcohol-induced damage, liver cirrhosis and atrophy, ligation of the choledochus and portal vein [12,14-16]).

Regeneration of the liver after its damage normally proceeds via proliferation of intact mature hepatocytes. This process can be paralleled by an increase in hepatocyte polyploidy and formation of BNH. During liver regeneration, a clone of mononuclear cells can originate from a binucleated cell due to regular alteration of polyploid mitoses [11]. None the less, the number of BNH is an indicator of regeneratory processes in the liver.

Considerable decrease in the number of BNH 30 min and 14 days after hyperthermia indicates their im-

TABLE 1. Quantitative Assessment of Hepatocyte Population in the Liver of CBA Mice after Whole-Body Hyperthermia ($M\pm m$)

Parameter	Control	Time after hyperthermia			
		30 min	day 3	day 7	day 14
Liver weight, mg	1150.0±84.2	1287.5±37.5	1180.0±25.5	1116.7±71.3	1150.0±73.6
Hepatocyte concentration, 10^3/mg tissue	73.9±3.8	57.9±1.3**	69.9±4.5	69.7±7.9	64.2±4.6
Absolute hepatocyte count, 10^5	878.3±25.9	764.3±20.8**	856.6±47.2	777.6±35.2	771.6±52.3
Number of binucleated cells, %	49.6±3.1	37.5±2.9*	41.7±1.9	52.8±1.4	36.6±1.7*

Note. *$p<0.01$, **$p<0.05$ *vs.* the control.

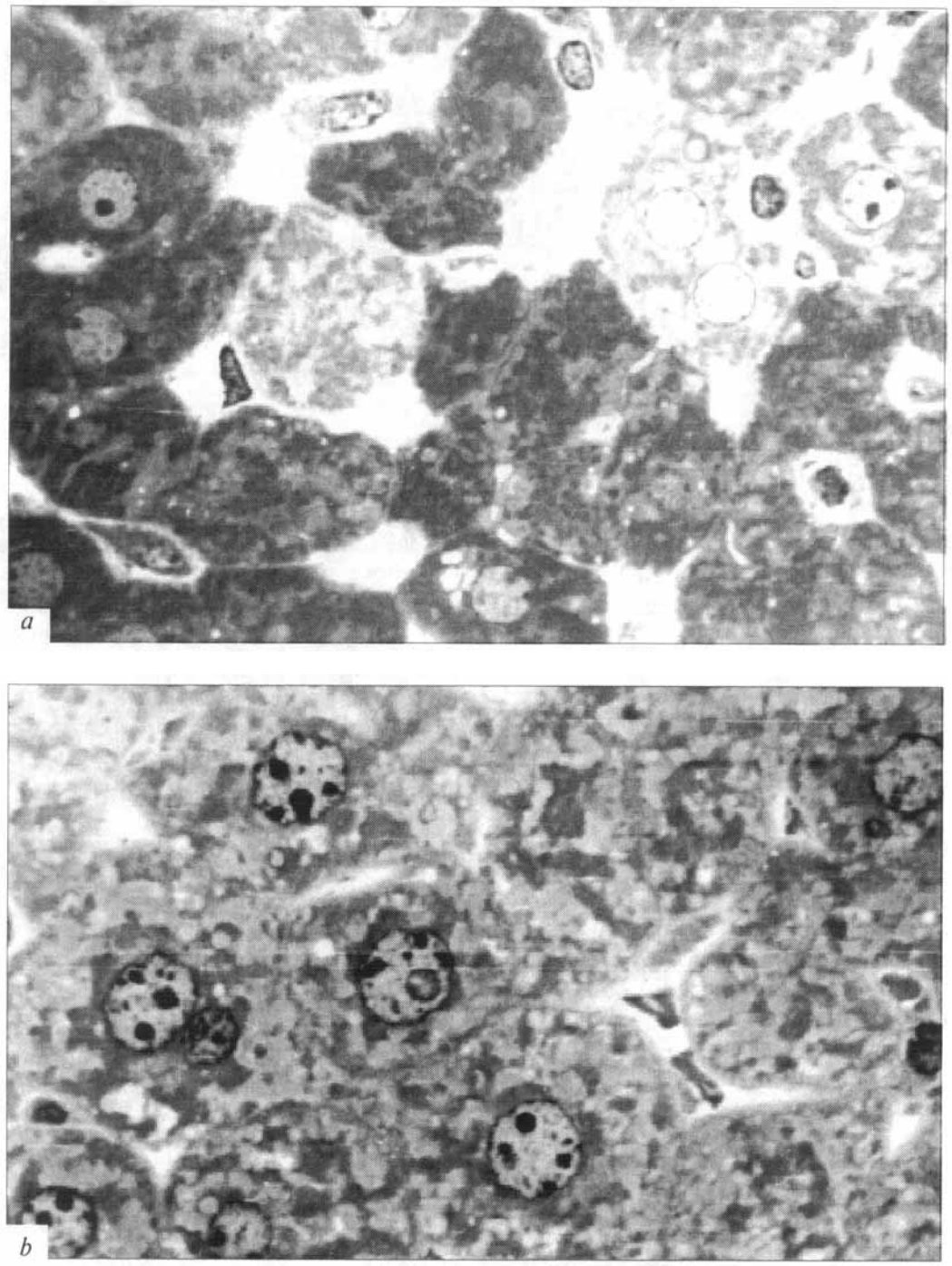

Fig. 1. Structural changes in the liver of CBA mice 3 days after whole-body hyperthermia. *a*) heterogeneous changes in hepatocytes; *b*) enlarged nucleoli and lipid incorporations in the nucleus. Here and in Fig. 2: semithin sections, azure II staining, ×1000.

portant role in the maintenance of the population of parenchymatous cells after exposure to extreme ecological factors. Our findings indicate wave-form fluctuations of BNH count. The decrease in BNH count and simultaneous increase in the mean ploidy are the main features of reparative regeneration of the liver distinguishing it from liver growth during ontogeny [2]. Mitosis in BNH is the first regeneratory reaction of the liver at the cellular level aimed at the maintenance of the total population of parenchymatous cells. The proliferative potential of BNH is gradually exhausted during PR and their total number decreases.

Hence, whole-body hyperthermia caused hemodynamic disorders in the liver associated with degenerative and necrotic changes in hepatocytes. Hepatocyte population decreased due to their death and elimination, but not necrosis. Under these conditions repara-

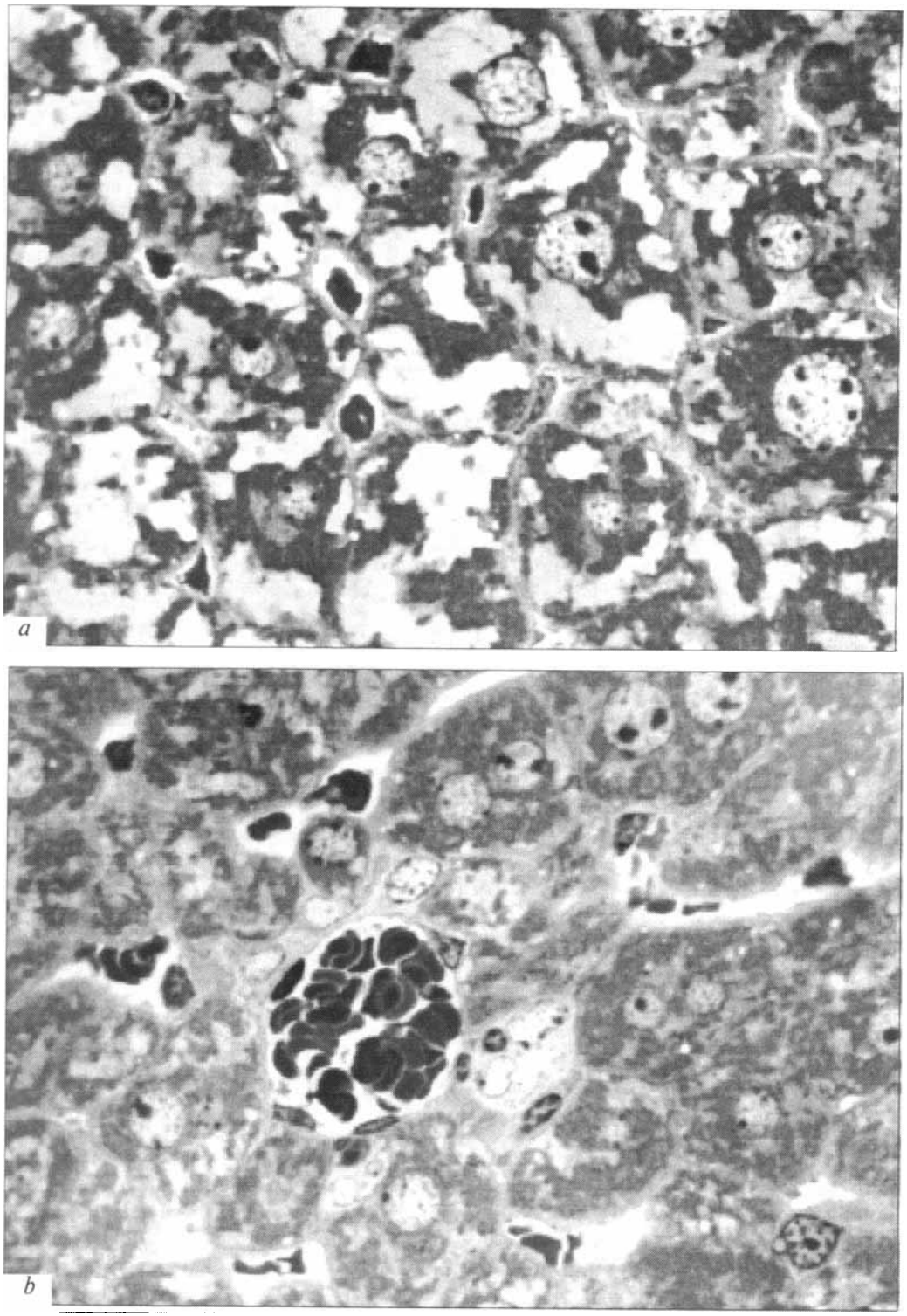

Fig. 2. Structural changes in the liver of CBA mice 7 (*a*) and 14 (*b*) days after whole-body hyperthermia. *a*) plethoric vessels and aggregations of oval cells in the portal zone; *b*) degenerative changes in hepatocytes presented as large "devastated" zones in the cytoplasm.

tive regeneration of the liver at the cellular level was realized mainly via division of BNH and gradually exhausted the BNH pool. On the other hand, proliferation with differentiation of oval cells into hepatocytes was observed at some stages of PR.

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