REGENERATION OF THE LIVER IN YOUNG RATS AFTER ALLYL ALCOHOL-INDUCED INJURY TO THE PERIPORTAL ZONES

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KEY WORDS: regeneration of the liver; allyl alcohol; mitochondria; dark cells; mitosis.

Several fundamentally important features of regeneration of liver tissue have now been discovered. In particular, it has been shown that the zone of greatest proliferative activity is located in the periportal third of the hepatic lobules [4]. An injured periportal zone loses its proliferative activity and its role is taken over by the middle zone [7]. However, the appearance of mitoses in the intermediate third takes place after a long time delay [8]. This delay, it has been suggested, is due to the fact that the time when intermediate cells enter the division cycle is strictly programmed.

It was therefore interesting to study the features of regeneration of the liver in the presence of weak injury to the hepatocytes in the periportal zone. A substance which selectively damages the cells of the periportal zone is allyl alcohol [3, 8, 9].

In the experiments described below a smaller dose of allyl alcohol was used than in the experiments of Rabes and Tuczek [7], and the dynamics of regenerative changes in the liver cells was analyzed. To improve

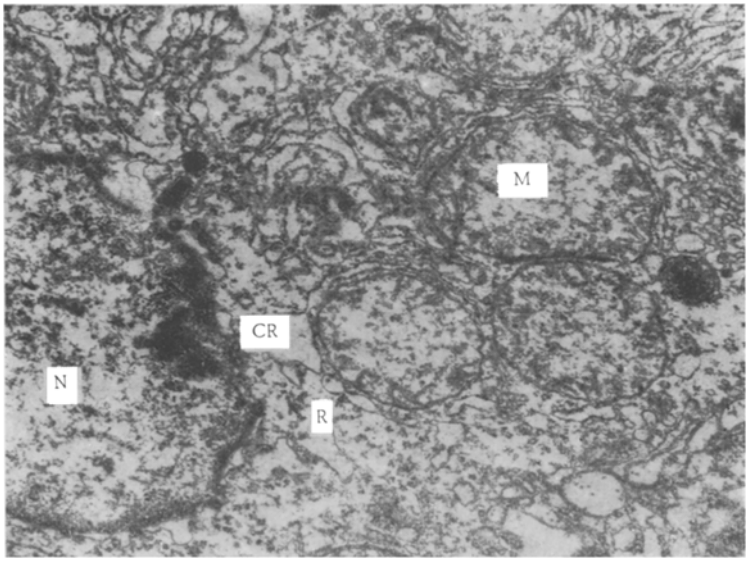

Fig. 1. Destructive changes in a hepatocyte in periportal zone of hepatic lobule 24 h after injection of allyl alcohol. Here and in Figs. 2 and 3: N) nucleus, M) mitochondrion, CR) cytoplasmic reticulum, R) ribosomes; 12,000×.

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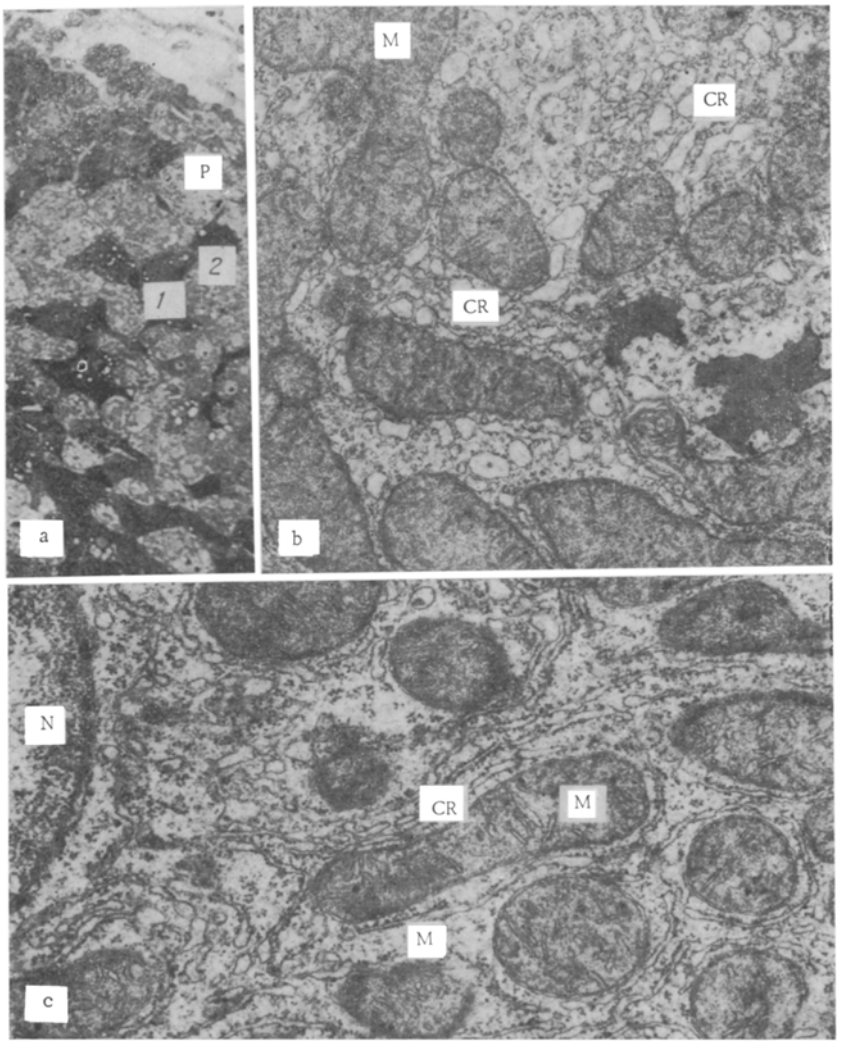

Fig. 2. Regenerative changes in periportal zone of hepatic lobules: a) different types of hepatocytes 96 h after injection of allyl alcohol: 1) dark type 1 hepatocytes, 2) dark type 2 hepatocytes, P) pale hepatocytes, 600×, b) dark type 1 hepatocytes 24 h after injection of allyl alcohol, 12,000×; c) dark type 2 hepatocyte 24 h after injection of allyl alcohol.

the regenerative response, the investigations were carried out on young animals, whose liver cells are in an intensive phase of proliferation.

EXPERIMENTAL METHOD

Male Wistar rats aged 2-3 weeks were used. A 3% aqueous solution of allyl alcohol was injected intraperitoneally in a dose of 0.03 ml/kg body weight. The animals were decapitated after 24, 48, 72, 96, 120, 144, 168, and 196 h. Four animals and one control were included in each group. Samples of liver were fixed in Carnoy's solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and by the PAS reaction. Some of the pieces of liver were fixed in OsO_4 solution or in a 2.5% solution of glutaraldehyde, postfixed in OsO_4, dehydrated, and embedded in Durcupan.

The sections were examined in the JEM-100B electron microscope. Hypertrophy and hyperplasia of the mitochondria were analyzed morphometrically. A grid with 5-mm scale was placed on an electron micrograph of the hepatocytes, with a magnification of 6000 ×, and intersections located on the cytoplasm and mitochondria were counted; on this basis the density in the cytoplasm and the size of the mitochondria were calculated in comparative units. These parameters were determined in the control animals and in experimental rats killed after 24, 96, and 120 h.

TABLE 1. Comparative Changes in Size and Number of Mitochondria in Periportal Hepatocytes Depending on Time of Investigation

Time after injection of allyl alcohol, h	Size of mitochondria, units		Density of content of mitochondria in cell	
	largest	average (M ± m)	largest	average (M ± m)
Control	26	20±1,2	1/3	1/5±0,05
24	44	24±2,0	1/2	1/4±0,02
96	20	18±0,7	1/2	1/2±0,13
120			1/3	1/3±0,5

The mitotic activity of the periportal zone was investigated under a magnification of 630 ×. The portal triad was positioned in the middle of the field of vision and dividing cells were counted. At the time of investigation, counting took place in 50 fields of vision for each animal. The percentage of fields of vision in which mitosis was observed was calculated. This method of investigation was chosen specially for analysis of the periportal zone.

EXPERIMENTAL RESULTS

Destructive changes were clearly seen at the light-optical level 24 h after administration of allyl alcohol in the periportal zone of the hepatic lobules. They were expressed as solitary localized areas of necrosis. In partially injured hepatocytes the nuclei were enlarged, chromatin was unevenly distributed, PAS-positive material had disappeared, and lipid drops were found in the cytoplasm.

Investigations in the electron microscope revealed fragmentation and dilatation of the tubules of the cytoplasmic reticulum, a decrease in the number of ribosomes, swelling of the mitochondria, and fragmentation and destruction of their cristae (Fig. 1).

The dose of allyl alcohol used thus produced degenerative changes similar to those observed after injection of a larger dose of allyl alcohol into adult animals [5, 6, 10]. The degenerative changes were manifested as injury to the mitochondria and endoplasmic reticulum in the periportal hepatocytes.

Besides degenerative changes, regenerative changes also were observed 24 h after injection of allyl alcohol. Investigation of semithin sections revealed small dark hepatocytes (type 1), polygonal in shape, in the periportal zone (Fig. 2a).

It was also found that the content of elements of the smooth and rough endoplasmic reticulum, and the number of free polysomes and mitochondria in these cells, were increased. Large mitochondria in sections through individual cells occupied half of the area of the cytoplasm and had an electron-dense matrix and narrow cristae. The large nucleus contained diffusely distributed chromatin and the tubules of the reticulum were dilated.

The degenerative and proliferative processes thus affected the same organelles. The greatest proliferative response was given by mitochondria, i.e., by organelles suffering primary injury following administration of allyl alcohol. This fact suggested that the increase in the number of mitochondria and in the content of endoplasmic reticulum in individual periportal hepatocytes was a manifestation of intracellular regeneration, subsequently replaced by compensatory hypertrophy, and not of true compensatory hypertrophy. This view is supported by the localization of the regenerating cells entirely in the periportal zone.

Dark cells of type 2 could be clearly seen 36-72 h after injection of the toxic compound, besides the dark hepatocytes already described above. These cells, in semithin sections, were star-shaped and their cytoplasm was darker than in the cells of type 1 (Fig. 2a). Hepatocytes of type 2 (Fig. 2c) also differed from type 1 by containing large numbers of densely packed tubules of the rough endoplasmic reticulum. The number of free ribosomes and polysomes and also of glycogen granules and lipid drops in the cytoplasm was increased. The mitochondria were enlarged and in some cells they were closely packed together. Comparative morphometric measurements showed that the mean size of the mitochondria was 24 units compared with 20 units in the control (Table 1). The total area of the mitochondria in individual cells was half of the area of the section of the whole cell (compared with only one-fifth in the control). The nucleus, with low optical density, contained compact, condensed bodies.

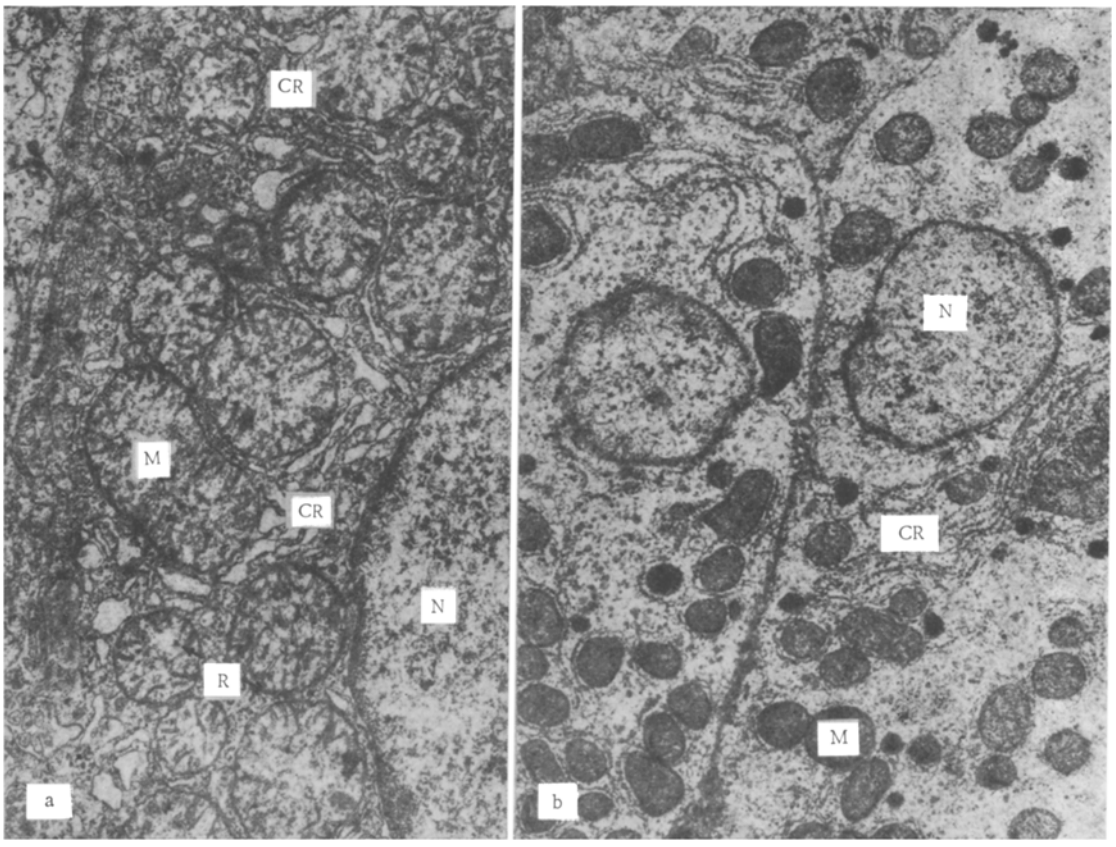

Fig. 3. Regenerative changes in hepatocytes of periportal zones of hepatic lobules 96 h after injection of allyl alcohol. a) Dark type 3 hepatocyte, 12,000×; b) pale hepatocyte, 6000×.

With an increase in the period of recovery the number of dark cells in the periportal region increased. After 96 h it reached a maximum. However, at this time significant changes were observed in the ultrastructure of the hepatocytes (Fig. 3a). The nucleus was of average size and dense, its chromatin was unevenly distributed, but its fibrils were sufficiently loosely packed and did not form condensed conglomerates. The rough endoplasmic reticulum was haphazardly arranged, and zones with a parallel arrangement of the tubules were absent. The mitochondria, tightly packed together, occupied half of the cytoplasm of the cells, so that the mean density of the mitochondria coincided with the greatest density, namely $\frac{1}{2}$ (Table 1). The increase in number of mitochondria was accompanied by a decrease in their size, which became more uniform. The greatest density of section of the mitochondria at this time was reduced to 20 conventional units, and their mean size to 18.

During development of the regenerative changes, three types of dark hepatocytes were thus observed, in which changes characteristic of the different periods of the cell cycle were seen together with intracellular regeneration. According to data in the literature [12], the ultrastructure of their chromatin and the particular features of organization and mutual relations of the organelles enable the three types of hepatocytes we dis-

TABLE 2. Changes in Number of Periportal Zones Containing Mitotic Figures, Depending on Time of Investigation

Time after administration of allyl alcohol, h	Number of zones containing mitotic figures	Number of zones without mitotic figures
Control	105 (37%)	168
24	39 (44,5%)	43
48	14 (28%)	36
120	155 (68%)	70
144	85 (76%)	29
168	81 (63%)	45

tinguished to be regarded as cells in the resting state and in the synthetic and postsynthetic periods of the cell cycle respectively. Single large pale cells (Fig. 2a), containing few organelles, began to appear in the periportal zone 96 h after injury, and these could be regarded as relatively dedifferentiated cells [1] (Fig. 3b).

No destructive changes were found 120-168 h after administration of allyl alcohol. The number of cells giving a positive PAS reaction was increased, a sign of regeneration. The dark cells also were shifted into the intermediate region between the periportal and middle zones, and the number of pale cells in the periportal zone was increased. The massive appearance of pale cells in the periportal zone coincided in time with an increase in the number of cells in mitosis (Table 2), and this confirmed the hypothesis of dedifferentiation of these cells.

Degenerative changes, followed by proliferative changes after injury to the liver by allyl alcohol, have been described by other workers also [9, 11]. However, the intensity of the changes was weaker in the present experiment because of the small doses, and this had an effect on the localization and kinetics of regeneration [7].

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EFFECT OF HUMAN BLOOD SERUM AND CIRRHOTIC LIVER EXTRACT ON REGENERATION OF THE MOUSE LIVER AFTER PARTIAL HEPATECTOMY

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KEY WORDS: chalones; regeneration of the liver; mitotic activity; cirrhosis of the liver.

The study of the regulatory mechanisms of cell division is a fundamental problem in both biology and medicine. However, the mechanisms regulating mitotic activity of the hepatocytes in patients with cirrhosis of the liver have been inadequately studied, although their understanding is of great importance to the discovery of the pathogenesis of cirrhosis.

The writers showed previously that liver extract and blood serum from patients with active cirrhosis of the liver stimulates mitotic activity of hepatocytes in the mouse liver regenerating after partial hepatectomy [2].

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