ACTIVITY OF NONSPECIFIC ESTERASES AND α-GLYCEROPHOSPHATE DEHYDROGENASE AND FAT CONTENT IN THE REGENERATING CHICKEN LIVER

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The results of a histochemical study of changes in the activity of nonspecific esterases and α-glycerophosphate dehydrogenase (α-GPD) and the fat content in the regenerating liver of domestic chickens are given. Accumulation of fat in the hepatocytes in the early states of regeneration (1st-5th days of the experiment) is due partly to a decrease in nonspecific esterase activity. Maximal steatosis in the liver is also accompanied by an increase in α-GPD activity, evidence of intensification of glycolysis and of primary synthesis of triacylglycerides – the reserve lipids of the liver – in the period of regeneration.

KEY WORDS: regeneration of the liver; nonspecific esterases; α-glycerophosphate dehydrogenase.

Biochemical and histochemical studies have shown that after partial hepatectomy in mammals fatty infiltration develops in the remainder of the organ [7, 11, 13]. Maximal accumulation of fat after hepatectomy coincides with the time of maximal mitotic activity. This suggests that the steatogenic response of the liver reflects the mobilization of the energy-yielding resources of the cells for regeneration [2, 12]. The data given in these publications show that the quantity and distribution of lipid inclusions return to normal on the 7th day of regeneration [6, 8, 15].

There are few data in the literature obtained by histochemical study of changes in the activity of enzymes catalyzing particular reactions in the lipid metabolism of the liver. For instance, histochemical investigations have shown a reduction in esterase activity during the period of increase in weight of the regenerating mouse liver on the 3rd-8th day of the experiment [16]. However, no changes in nonspecific esterase activity were found in the dog liver at any time during the experiment [4], although the number of fat droplets in the hepatocytes increased 3-7 days after hepatectomy. Observations on the regenerating pigeon liver showed [5] that the beginning of cell divisions close to the damaged part of the parenchyma is preceded by disappearance of neutral fats from this zone and an increase in lipase and esterase activity in it.

Because of the lack of study of enzyme activity in the course of regeneration and the contradictory data for animals of different species it was decided to undertake a histochemical study of the activity of nonspecific esterases and α-glycerophosphate dehydrogenase (α-GPD) and the content of neutral fats in the regenerating chicken liver.

EXPERIMENTAL METHOD

Between 1/5 and 1/4 of the liver parenchyma was removed from chickens aged 5-6 months. Material for investigation was taken 1, 3, 5, 10, 20, 30, and 60 days after the operation. Fats were stained with Sudan III in frozen sections, and activity of α-GPD and nonspecific esterases was revealed in freshly frozen cryostat sections, stained by Nachlas' method and with naphthyl acetate respectively. The enzyme activity was expressed in conventional units.

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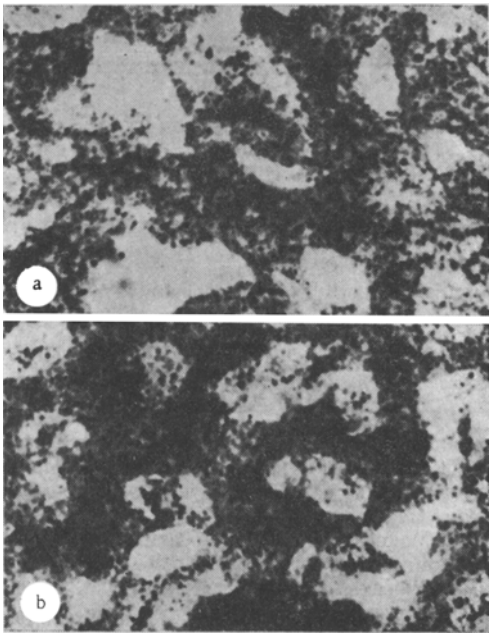

Fig. 1. α-GPD activity in domestic chicken liver cells. a) Control; b) 5th day after hepatectomy. Freshly frozen sections. Nitro-BT reaction after Nachlas'. Objective 63, ocular 12.5.

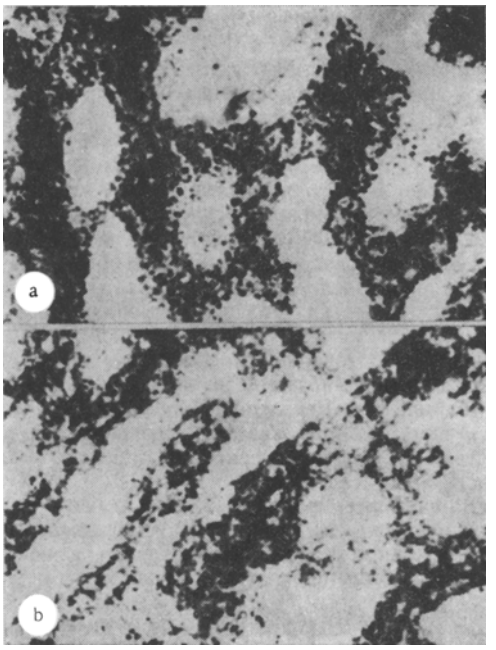

Fig. 2. Nonspecific esterase activity in domestic chicken liver cells. a) Control; b) 5th day after hepatectomy. Freshly frozen sections. Reaction with α-naphthyl acetate. Objective 63, ocular 12.5.

EXPERIMENTAL RESULTS

In the intact domestic chicken liver, α-GPD activity in the hepatocytes was revealed mainly in the form of monoformazan, although large granules of diformazan were also present in the cytoplasm, and frequently formed a rim around the nucleus (Fig. 1a). Enzyme activity was uniform throughout the organ and could be assessed as 1 point.

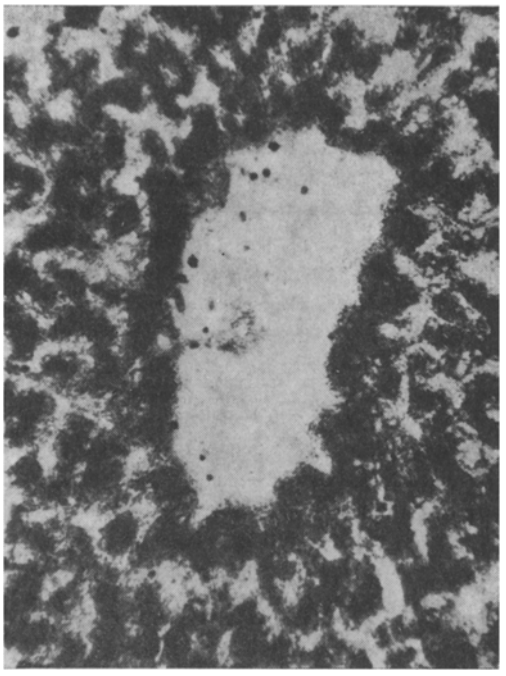

Fig. 3. Increased nonspecific esterase activity in hepatocytes around central vein. Freshly frozen section. Reaction with α-naphthyl acetate. Objective 25, ocular 12.5.

Nonspecific esterase activity in the hepatocytes of the intact liver was revealed as large granules, more concentrated around the nucleus (Fig. 2a). Activity of the enzyme was a little higher in the hepatocytes around the central veins (Fig. 3). In the endothelium of the capillary walls and in the Kupffer cells the granules were less densely distributed, and the interlobular connective tissue and vessel walls showed only very weak nonspecific esterase activity.

In the early stages after hepatectomy (days 1-3 of the experiment) α-GPD activity in the liver increased. In areas of parenchyma adjacent to the zone of resection and a short distance from it, the number of diformazan grains, corresponding to α-GPD activity, was considerably increased (Fig. 1b). Activity of the enzyme in these areas of the parenchyma could be assessed at 2 points. In the injured lobules the quantity of monoformazan was increased.

At these times of the experiment, nonspecific esterase activity was appreciably reduced in the extensive area of liver parenchyma adjacent to the zone of resection (Fig. 2b). Meanwhile zones with considerably increased activity of these enzymes could be seen in the sections. Increased esterase activity in the hepatocytes was observed at the periphery of the lobules, especially close to the wide bands of interlobular connective tissue and on the boundary with the necrotic tissues. Often in the zone of resection lobules still functioning, and with very high esterase activity, were preserved between areas of necrotic parenchyma. Nonspecific esterase activity also was increased in the walls of the blood vessels and in the interlobular connective tissue. In the liver parenchyma at a distance from the zone of resection esterase activity was almost at the control level, but the granules corresponding to it were coarser and less densely distributed.

At these times of regeneration the number of fatty inclusions in the parenchyma of the liver was increased, especially in areas adjacent to the zone of resection. Most cells of the undamaged parenchyma were filled with large granules of lipids, often merging to form larger drops. It was in these areas of the parenchyma that α-GPD activity was increased and esterase activity sharply reduced. Conversely, lipids were not found in cells with very high esterase activity.

Increased α-GPD activity persisted in the liver 5 days after hepatectomy. However, activity of the enzyme at this time was irregularly distributed. Hepatic tubules with relatively high enzyme activity (2 points) and tubules with reduced α-GPD activity compared with the control could be seen in the section. Mainly monoformazans were found in the cells. At this period the distribution of nonspecific esterase activity still remained irregular. It was reduced in extensive areas of parenchyma adjacent to the zone of resection but was

very greatly increased in cells bounding the developing granulation tissue, and also in newly formed epithelial tubules, growing into the granulations. In the differentiating cells of these tissues lipids as a rule were absent, although by this time the number and size of the fat droplets in the cells of the parenchyma adjacent to the zone of resection had reached a maximum.

On the 10th-20th days of the experiment α-GPD activity in the liver parenchyma fell rather below the control level, and the irregularity of distribution of nonspecific esterase activity described above still remained. The fat content of the liver was almost back to normal, and it remained high only in the hepatocytes around the central veins.

Activity of the enzymes studied began to return to normal after the 30th day of the experiments, although in the zone directly bordering the developing scar tissue and around the blood vessels increased nonspecific esterase activity still remained. Esterase activity also was high in the vessel walls and in the interlobular connective tissue. In the rare cases when fatty degeneration developed in the liver, nonspecific esterase activity fell sharply below normal.

In the last stage of the observations, 2 months after the operation, the activity of the various enzymes studied and the neutral fat content in the hepatocytes of the experimental chickens returned to normal.

In the early stages of regeneration of the liver in domestic chickens, parallel with an increase in the number of lipid inclusions in the parenchyma of the organ, α-GPD activity rises and nonspecific esterase activity falls. In the newly formed epithelial tubules in zones bordering on developing granulation tissue and on necrotic areas, where regeneration takes place more intensively, no lipid inclusions are present and esterase activity is considerably increased. In the course of regeneration an increase in nonspecific esterase activity also takes place in the hepatocytes around the blood vessels and at the periphery of the lobules.

The fall in esterase activity in the parenchyma adjacent to the zone of resection facilitates active accumulation of fat. The rise in esterase activity, on the other hand, in areas with more marked signs of regeneration and in dividing cells promotes utilization of this energy resource of the liver cells.

The rise in activity of α-GPD, an enzyme linking carbohydrate and lipid metabolism in the liver, is evidence of intensification of glycolysis and the utilization of its products for the synthesis of triethylglycerides, the reserve lipids of the liver, and for oxidative phosphorylation of new ADP molecules, during regeneration of the liver in domestic chickens.

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