ESTERASE SPECTRUM IN HEPATOCYTES AND KUPFFER CELLS OF THE REGENERATING LIVER

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The esterase spectrum in hepatocytes and Kupffer cells of the regenerating rat liver was determined by electrophoresis in starch gel. The relative number of Kupffer cells, the percentage of phagocytic Kupffer cells, and the frequency of mitosis in the hepatocytes were determined. The esterase spectrum of hepatocytes isolated from the intact liver consisted of six zones, and that of the Kupffer cells of five zones of enzyme activity. The spectrum of the hepatocytes was "simplified" 2.5 h after partial hepatectomy, additional bands of activity appeared toward 9 h, and these persisted until 24 h after the operation. The original esterase spectrum was restored 48 h after the operation. A similar reorganization of the esterase spectrum was observed in the Kupffer cells but this was not fully restored 72 h after partial hepatectomy.

KEY WORDS: *esterase spectrum; hepatocytes; Kupffer cells; regeneration of the liver.*

The many different forms of esterases and other simple enzymes can be regarded as sensitive integral markers of the level of differentiation of isolated cells and of homogeneous cell populations.

By electrophoresis in starch gel using α-naphthyl butyrate as the substrate ten bands of esterase activity have been found, whereas five bands were found with β-naphthyl acetate [4,5]. Kaneko [4] found two bands of activity in the hepatocyte fraction and five bands in the whole liver of adult animals, from which he concluded that three bands of esterase activity belong to cells of the reticuloendothelial system of the liver. No other investigations devoted to the comparative study of the esterase spectra in hepatocytes and Kupffer cells of the liver could be found in the literature.

In this investigation the various forms of esterases were studied in fractions of hepatocytes and phagocytic Kupffer cells of the liver after partial hepatectomy in order to assess the separate course of differentiation of the mesenchymal and epithelial branches in the early stages of reparative regeneration of the liver.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-280 g. Partial hepatectomy was performed under urethane anesthesia by the method of Higgins and Anderson [3]. Material for investigation was taken 2.5, 9, 24, 36, 48, and 72 h after the operation, using four or five rats at each time. The Kupffer cells were separated from hepatocytes in an electromagnetic field by the method of Davydov et al. [1]. For this purpose, 2 h before sacrifice the animals were given an intravenous injection of a suspension of mark R-100 iron carbonyl. The liver was perfused through the portal vein with an ice-cold solution of 0.25 M sucrose containing 0.1 M EDTA to remove blood. The viability of the cells was assessed by the trypan

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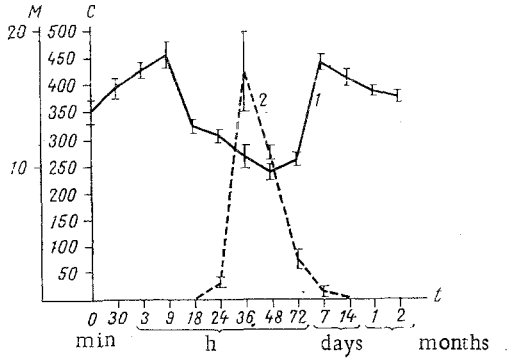

Fig. 1. Dynamics of number of Kupffer cells (1) and mitoses among hepatocytes (2) during reparative regeneration of the liver. Abscissa, time after partial hepatectomy; ordinate: M) number of mitoses in hepatocytes (in percent); C) number of Kupffer cells per 1000 hepatocytes.

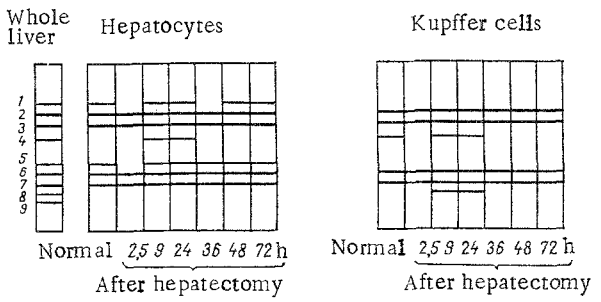

Fig. 2. Scheme of distribution of various forms of esterases of hepatocytes and Kupffer cells in regenerating rat liver on electrophoresis in starch gel.

blue test. From the fractions of hepatocytes and Kupffer cells 10% homogenate were prepared in 0.25 M sucrose, pH 7.4, containing 0.01 M EDTA. The homogenates were centrifuged for 1 h at 15,000 rpm. The supernatant was subjected to vertical electrophoresis in 14% starch gel, using 0.3 M sodium borate buffer, pH 8.6. The gel was made up in 0.5 M Tris—EDTA—borate buffer, pH 8.15. The conditions of electrophoresis were: 10,100 V, 50 mA, 4°C, 15 h. Esterase activity was demonstrated histochemically in slabs 2 mm thick cut from the starch block [6] using α- and β-naphthyl acetate as the substrate. The number of mitoses among hepatocytes was counted in 5000 cells in liver sections stained with hematoxylin and eosin, the relative number of Kupffer cells was counted, and their phagocytic activity determined.

EXPERIMENTAL RESULTS

Counting the relative number of Kupffer cells to 1000 hepatocytes showed a significant increase 2.5 h after the operation. After 36 h, i.e., at the peak of mitosis of the hepatocytes, it fell a little, and then returned to a high level at which it remained until two months after partial hepatectomy (Fig. 1). In all probability the early accumulation of Kupffer cells was due to their migration from outside, mainly from the bone marrow [7], and was not due to their increased proliferation in the liver itself, for the peak of mitosis of the Kupffer cells occurs 48 h after the operation.

On electrophoresis in starch gel nine bands of esterase activity were found in homogenates of whole liver of the normal animals. One zone of activity consisted of less mobile forms of esterases (the "anode" fraction, bands 1-4) and a second zone of less mobile forms (bands 5-9). Six forms of esterases, forming bands 1-3, 5-7, were found in the hepatocyte population of normal liver. Five bands (2-4, 6, 7) were found in the Kupffer cell population of normal liver (Fig. 2). During regeneration of the partial hepatectomy the spectra of multiple forms of esterases in the hepatocytes and Kupffer cells underwent characteristic changes (Fig. 2). Bands 1 and 5 disappeared from the esterase spectrum of the hepatocytes 2.5 h after partial hepatectomy, and band 4 disappeared from the esterase spectrum of the Kupffer cells. After 9 h the esterase spectra in the fractions of hepatocytes and Kupffer cells had a tendency to recover. It is curious that at this time band 4 appeared in the spectrum of the hepatocytes and band 8 in the spectrum of the Kupffer cells, whereas in cells isolated from normal liver these bands are usually not found. The spectra taken 24 h after partial hepatectomy continued to show the same characteristics, but by 36 h certain forms of esterases had disappeared unlike in the normal liver: band 1 in the hepatocyte fraction and band 4 in the fraction of Kupffer cells. The original esterase spectrum in the hepatocytes was restored by 48 h after the operation, whereas in the Kupffer cells it remained abnormal until the end of the period of observation (72 h).

Consequently, during reparative regeneration of the liver regular changes take place in the spectra of the many different forms of esterases, not only in the hepatocyte fraction, but also in the fraction of Kupffer cells. During the first 2-3 h after removal of two-thirds of the liver the esterase spectrum in the hepatocytes became "simplified." However, by 9 h after the operation, i.e., at the beginning of the S period [2], the spectra were "complicated"

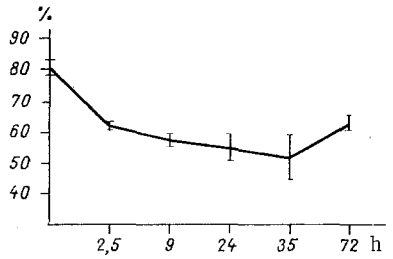

Fig. 3. Dynamics of changes in phagocytic activity of Kupffer cells during reparative regeneration of the liver. Abscissa, time after partial hepatectomy (in h); ordinate, number of phagocytic cells as a percentage of the total macrophage pool.

by additional bands of activity. The same picture continued to be recorded at the climax of the S period, i.e., 24 h after partial hepatectomy. It is interesting to note that, when the number of mitoses was at a maximum (in these experiments 36 h after the operation) the esterase spectrum was again "simplified," and it returned to its initial state 48 h after the beginning of reparative regeneration. The changes in the esterase spectra described above were recorded, it will be noted, in all rats without exception.

The esterase spectra in the fractions of phagocytic mesenchymal cells of the liver also were changed. The simplest explanation of this fact is deinhibition of their proliferative potential, as in the case of the hepatocytes. However, the first mitoses among Kupffer cells do not appear until 48 h after partial hepatectomy [2]. The increase in the relative number of Kupffer cells during the first few hours after the operation in the present experiments was most likely due to their inflow from outside, most probably through the bloodstream from the bone marrow, in the form of less differentiated precursors of the histiomonocytic series [7]. Such a situation could lead to the simplification of the esterase spectrum discovered in the fraction of Kupffer cells of the liver 2.5 h after the operation. It is interesting to note that this "simplification" was accompanied by a marked decrease in phagocytic activity of the Kupffer cells during the same period of reparative regeneration in the liver (Fig. 3). A second period of "simplification" of the esterase spectrum in the Kupffer cells was observed 36 h after partial hepatectomy, i.e., at a time when derepression of the proliferative potential of their precursors in situ could be postulated. It follows from these observations that spectra of the multiple forms of esterases can be used as a sensitive indicator of proliferative activity and of the level of differentiation of cells of the stromal and parenchymatous series during regeneration of the liver.

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