TIME COURSE OF DEVELOPMENT OF OVAL CELL POPULATION INDUCED IN THE MOUSE LIVER BY DIPIN AND PARTIAL HEPATECTOMY

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In lesions of the rat liver due to most known carcinogens and certain hepatotoxins, small epithelial cells, described as oval cells, proliferate [4, 6, 10]. In recent years interest in this highly heterogeneous cell population, from the point of view of both morphology and properties, has risen sharply with development of ideas regarding the genesis of oval cells from hepatic stem cells [4], and their role as precursors of hepatocellular carcinomas [8, 10]. This phenomenon of oval-cell proliferation has been described and studied in detail on models of hepatocarcinogenesis in rats [10].

The aim of this investigation was to study the time course of development of the oval-cell population induced in mice by dipin (N,N'-*bis*(diaziridinylphosphinylidine piperazine) and by partial hepatectomy [2]. It was shown previously by methods of autoradiography and cytospectrophotometry that this schedule leads to irreversible damage to the genome of all original hepatocytes [3] and to subsequent complete renewal of the cell composition of the parenchyma [2].

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL6)F_1 mice weighing 21-23 g. Hepatocarcinogenesis was induced by the method in [2]. The animals were killed by cervical dislocation approximately once a week for 10 months after the procedure, at the rate of 10-12 mice during the first 2 months, but later 5-6 mice each time. The liver was fixed in parallel experiments in 10% formalin made up in 0.1 M phosphate buffer, pH 7.4, and then embedded in Histoplast ("Serva," West Germany) or Historesin ("LKB, Pharmacia," Sweden), or in a 2.5% solution of glutaraldehyde in phosphate buffer, followed by postfixation in 1% osmic acid solution in buffer, and embedding in Epon 812. Sections 5 M thick were cut from the liver embedded in Histoplast and stained with Mayer's hematoxylin or by Van Gieson's method [1]. Semithin sections 1-1.5 μ thick were cut from the liver embedded in Historesin or Epon, and stained with toluidine blue.

EXPERIMENTAL RESULTS

The principal stages of development of the oval-cell population were studied in ordinary histological preparations against the background of changes in the surrounding parenchyma. For a more detailed analysis, semithin sections were used, for they allow small oval cells, with no marked morphological distinguishing features, to be easily identified among inflammatory and connective-tissue cells.

The first oval cells appeared in the liver 10 days after induction of hepatocarcinogenesis in the region of several portal triads (PT). Most frequently they formed ducts consisting of a small number of cells, and they were located around the portal veins, in direct contact with them. The oval cells differed from those of the biliary epithelium in their distinct cuboidal shape and larger pale nuclei. By the 3rd week, definite oval-cell proliferation was recorded around each PT. After that time the oval cells penetrated into the depth of the hepatic trabeculae along the terminal branches of the portal vessels (Fig. 1a). Away from the

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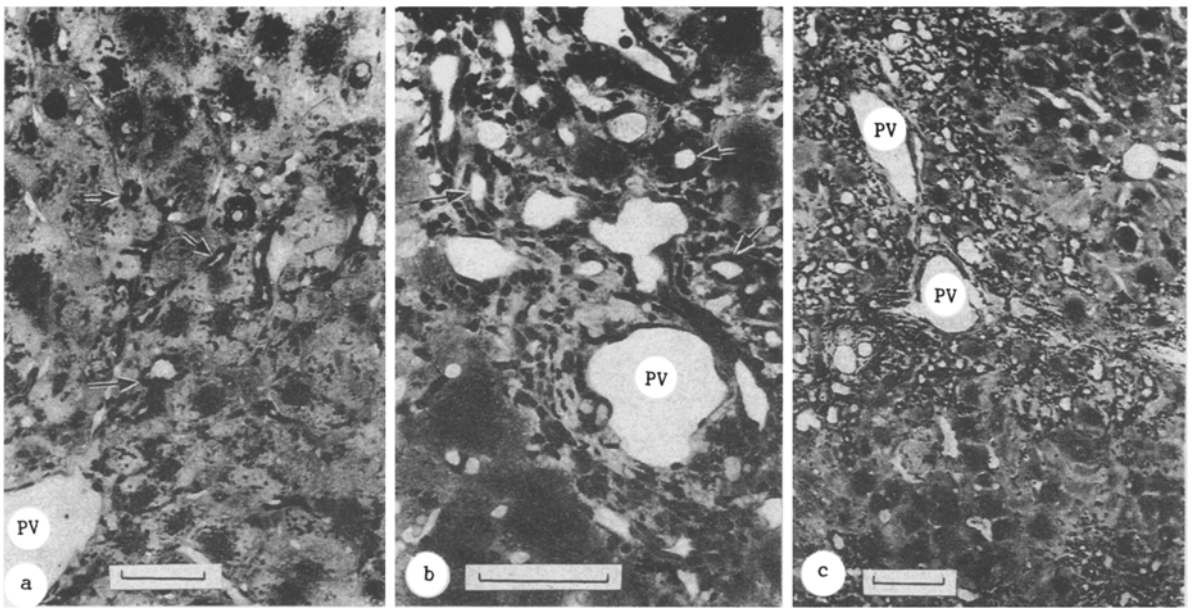

Fig. 1. Morphology of preneoplastic mouse liver at different stage of oval-cell proliferation. a) After 4 weeks, 250×, b) after 6 weeks, 400×, c) 8 weeks after induction, 200×. PV) Portal vein, CV) central vein. Arrows indicate size of ducts formed by oval cells. Here and in Fig. 2, stained with Mayer's hematoxylin.

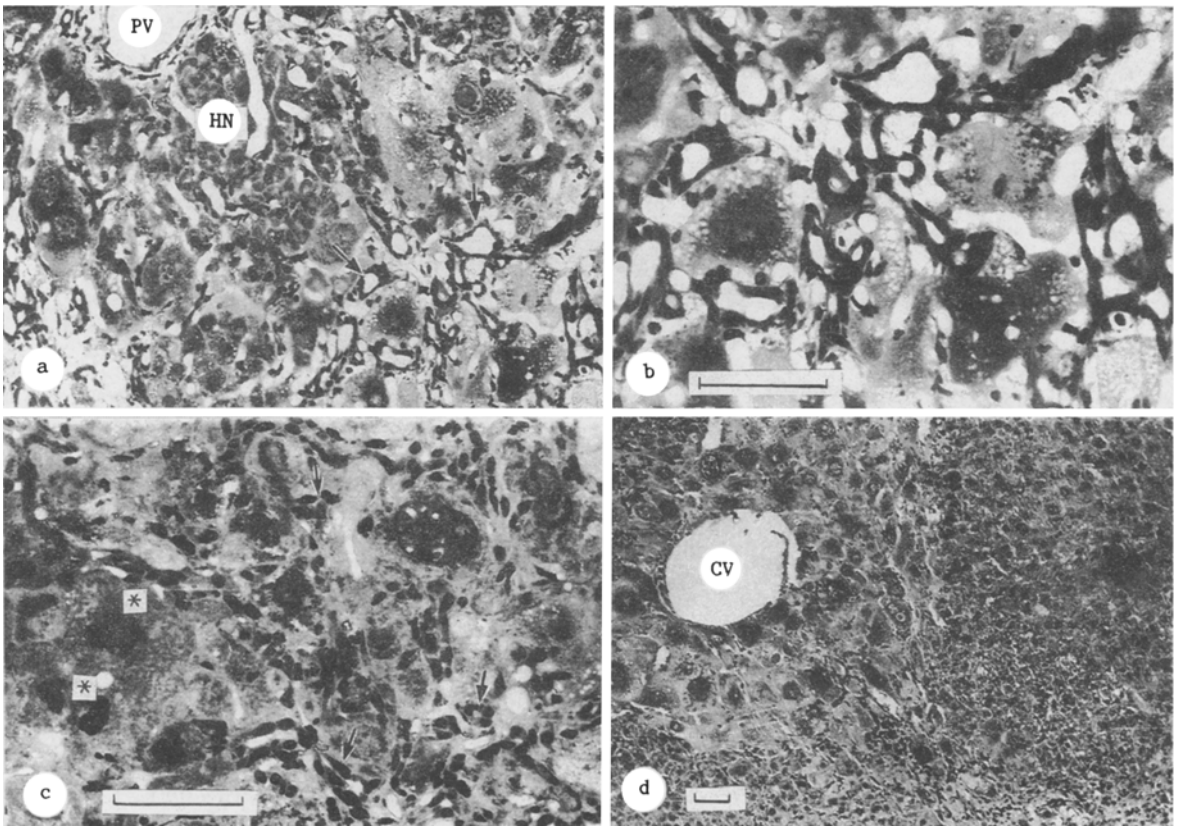

Fig. 2. Morphology of preneoplastic mouse liver during period of appearance (a-c) and enlargement of foci of growth of new hepatocytes (d). a-c) 10 weeks, d) 20 weeks after induction. Asterisk indicates mitoses of newly formed hepatocytes. Magnification: a) 200, b) fragment of photograph in a, c) 400, d) 100×.

center of their appearance, the ducts increased in size to 10-15 cells per transverse section (Fig. 1b). The shape of the ducts varied considerably and was evidently determined by the architectonics and pressure of the surrounding tissue. The oval cell population attained its peak of development after 8-10 weeks, when the oval cells occupied more than half of the hepatic lobule (Fig. 1c).

By this time the surrounding parenchyma showed marked changes. The hepatocytes were considerably enlarged, various forms of pathology of the cells and nuclei developed, with the appearance of single-cell and focal necrosis, most frequently at the periphery of the lobule. After 8-10 weeks, i.e., at the peak of oval-cell proliferation, the first foci of growth of newly formed hepatocytes were recorded in the region of PT (Fig. 2a). They consisted of small, intensively proliferating cells with basophilic cytoplasm and a large nucleus (Fig. 2c). In the same place, cells with intermediate morphology between definitely oval cells and young hepatocytes were frequently visible. With an increase in size of the hepatocyte nodules, the parenchyma damaged by dipin was displaced away from the portal tracts toward the central veins (Fig. 2d). In the course of 10-14 weeks, numerous ducts composed of oval cells penetrated into the central part of the hepatic lobule, coming into close contact with and surrounding the original giant hepatocytes. At this time the morphological signs of degeneration of hepatocytes damaged by dipin were most clearly defined (Fig. 2a-c). Complete replacement of the damaged parenchyma occurred after 22 weeks. However, the oval cells did not disappear completely from the tissue but remained in appreciable numbers in the region of PT. The normal structure of the liver, with its characteristic alternation of portal and central vessels and its single-cell trabecula, was not restored, however. As before, growth remained nodular and adenomatous in character, and led after 8-10 months to the development of multiple hepatocellular tumors.

Parallel to the oval-cell proliferation, fibrosis and inflammation developed. In most animals (on average 65%) mixed cellular infiltration was usually observed, consisting of oval cells, fibroblasts, and inflammatory cells, including lymphocytes, neutrophils, and macrophages (Fig. 1b, c). The first signs of excessive accumulation of collagen and fibroblasts in the region of PT were observed 1 week after the appearance of oval cells. The development of fibrosis and the inflammatory reaction reached their peak toward the 3rd-4th week, and remained at that level until the 10th week, corresponding in time to the stage of migration of oval cells to the middle of the hepatic lobule. After 12-14 weeks massive periportal fibrosis and inflammation disappeared, since they were displaced by the foci of growth of new hepatocytes developing in the region of PT. However, in the zones of distribution of oval cells, during their migration toward the center of the lobule, an increased content of collagen, fibroblasts, and macrophages was always preserved.

Oval cells were recorded during the first 2 months in 68% of mice, but later in 100%. The asynchronous origin of their appearance may have been due to differences in individual sensitivity to the damaging action of the hepatotoxic agent, dipin. The time course of development of the oval-cell population and the accompanying cellular changes in the dipin-damaged mouse liver are shown diagrammatically in Fig. 3. The main stages of the process were as follows: 1) origin in the portal region after 1-3 weeks; 2) migration into the depth of the parenchyma along terminal branches of the portal vessels after 3-8 weeks; 3) peak development of the oval-cell population corresponding to infiltration of half of the lobule after 8-10 weeks; 4) the appearance of hepatocytic foci of growth in the zone of the portal vessels, in close proximity to oval cells after 8-10 weeks; 5) gradual displacement of the oval cells into the central parts of the lobule and their involution together with dipin-damaged original hepatocytes after 10-22 weeks.

A leading role in the induction of new cell populations, successively replacing one another in the course of dipin-induced hepatocarcinogenesis, is played by the regions of PT, where the main structural changes take place in the tissue. This is evidently not by accident, for the portal region has the most highly organized and complex part of the parenchyma, in which maximal cellular diversity and a unique microenvironment are present. It is in the composition of the terminal bile ducts that the epithelial stem cells, capable of differentiating into both hepatocytes and cholangiocytes, are assumed to be found [5]. It can be tentatively suggested that the development of a fibrous reaction, revealing close topographic correlation with the oval cells, is a specific component of the process of induction of the oval cells. We know that in the zones of their distribution the absolute and relative concentrations of proteins of the extracellular matrix vary considerably, including those of collagen, fibronectin, and laminin [9], which play an active role in the choice of direction of differentiation of the liver cells [7]. Inflammation may perhaps be classed as a nonspecific side reaction, for it usually accompanies all lesions of the liver connected with necrosis and fibrosis of the parenchyma.

Comparison of the oval cells of mice and rats reveals a basic similarity in their cell structure, dynamics, and direction of development, evidence of the importance and universal nature of the phenomenon. Only the degree of their proliferation and maturation varies, and this may be connected with the level of cytotoxicity of the carcinogen used [6].

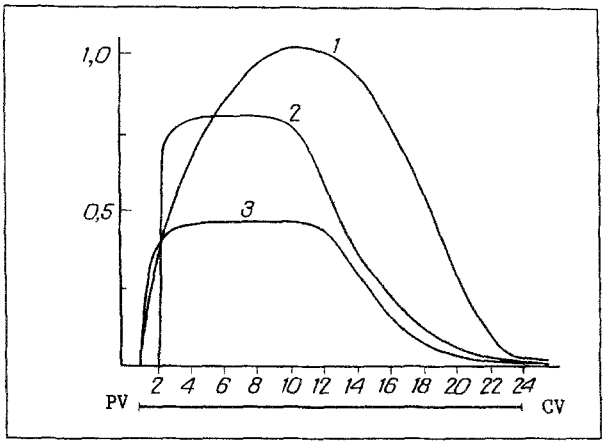

Fig. 3. Diagram of development of oval-cell population (1), fibrosis (2), and inflammation (3) in preneoplastic mouse liver depending on time and location in hepatic lobule. Abscissa, time after induction (weeks); below — conventional picture of hepatic lobule from portal (PV) to central (CV) vein; ordinate, intensity of reaction (in conventional units). Peak intensity of reaction, corresponding to infiltration of half of a hepatic lobule by oval cells, taken as 1.

A particular feature of the hepatocarcinogenesis induced by means of dipin is the powerful and undiminishing induction of proliferation of oval cells, accompanying the process of total renewal of the cell composition of the parenchyma, and even to the appearance of multiple hepatomas. Depending on the location in the structure of the hepatic lobule, the character of organization of the oval cells as well as the direction of their differentiation may vary.

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