SYNCHRONIZATION OF CELL PROLIFERATION IN THE LIVER AND GASTRIC AND DUODENAL GLANDS

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The most effective method of inducing tumors in the liver and other organs is by administration of carcinogens to animals preceded by stimulation of target cell proliferation [4, 7, 8]. Cells have been shown to be most sensitive to transforming action in phases S, G_2, and M of the mitotic cycle (MC) [11]. However, according to other data [13], the highest yield of tumors is obtained when the carcinogen acts on cells in the late G_1 phase. The contradictory nature of the results can evidently be attributed to the fact that in most tissue systems studied the cells were only partially synchronized according to phases of MC. To study the role of cell proliferation in the initiation, promotion, and activation of carcinogenesis, the appropriate experiments must therefore be carried out on cell populations highly synchronized by the phases of MC.

These considerations determined the aim of the present investigation, which was to study the pattern of proliferation of cell populations of certain organs of the gastrointestinal tract, constantly undergoing renewal and stimulation to divide, and under conditions of synchronization induced by hydroxyurea (HU).

EXPERIMENTAL METHODS

Experiments were carried out on noninbred male rats weighing 110-130 g. Classical partial hepatectomy was performed between 9 a.m. and noon. HU was injected intraperitoneally into the animals 5 or 6 times in a dose of 500 mg/kg, 12 or 15 h after the operation with intervals of 3 h. At various times after partial hepatectomy, [^{3}H]thymidine was injected intraperitoneally into three to five rats in a dose of 0.037 MBq/g body weight, and the animals were decapitated under either anesthesial h later. Tissue from the liver, stomach, and duodenum was fixed in 10% formalin. After fixation of the liver tissue, isolated cells were obtained [1]. Sections 4-6 μ thick were cut from tissues embedded in paraffin wax. Histological sections and cytological films were covered with type M photographic emulsion. After development and staining, the index of labeled cells (labeling index — LI) was counted in 2000-3000 cells of the autoradiographs, and the mitotic index (MI) was determined among 5000-8000 cells. Nuclei were isolated from the rat liver and fractionated in a sucrose density gradient as described previously [3].

EXPERIMENTAL RESULTS

After partial hepatectomy two peaks of DNA synthesis were observed in the residual liver tissue of the control rats, made up of 32.2 ± 4.2 and 20.1 ± 2.9% of hepatocytes respectively (Fig. 1). Mitotic activity of the parenchymatous cells also was characterized by two peaks, each of which followed immediately after an increase in LI. During synchronization by HU the maximal number of DNA-synthesizing hepatocytes in the liver of the experimental animals was observed 4 h after the last injection of HU, and amounted to 62.5 ± 2.3% of the cells.

In view of data on the size of the proliferative pool of hepatocytes [5] it can be postulated that, if HU is administered in accordance with the scheme adopted, DNA synthesis is synchronized in 78% of cells participating in regeneration of the liver after partial hepatectomy.

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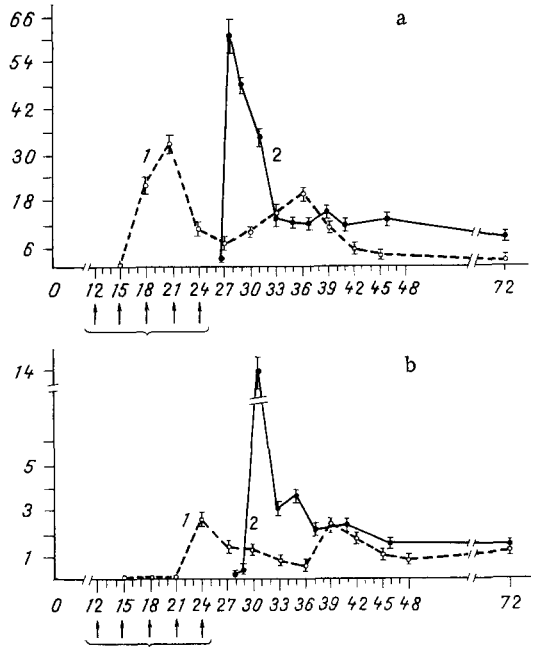

Fig. 1

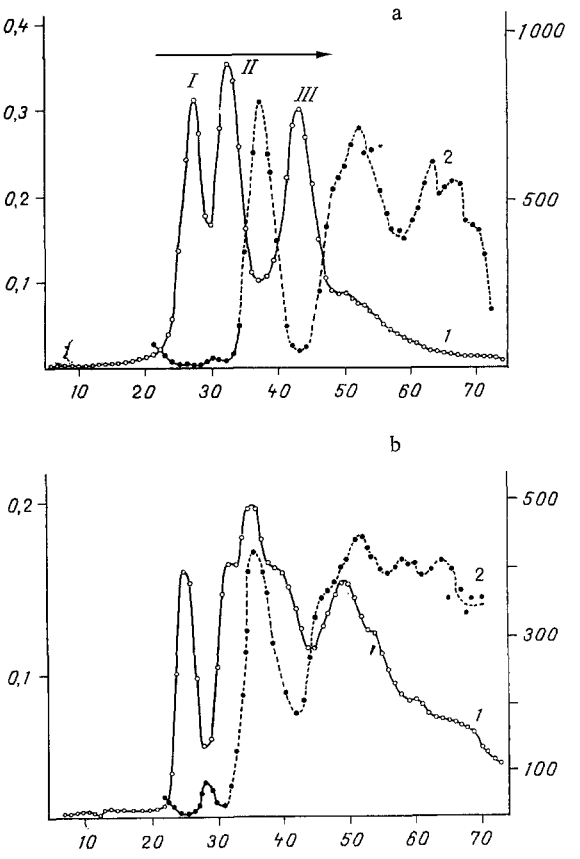

Fig. 2

Fig. 1. LI (a) and MI (b) of hepatocytes in regenerating rat liver during additional synchronization by HU. Abscissa, time after hepatectomy (in h); ordinate, LI (in %, a); MI (in %, b). 1) Control animals; 2) after injection of HU. Arrows indicate times of injection of HU.

Fig. 2. Sedimentation profile of liver cell nuclei. Abscissa, Numbers of fractions. 1) Extinction (ordinate on left, in relative units), determined by turbidimetric method; 2) specific radioactivity (ordinate on right, in cpm/optical density units· 10^{-3}); a) 21 h after hepatectomy; b) 28 h after operation and 4 h after last injection of HU. I, II, III) Peaks corresponding to location of diploid nuclei of stromal cells and of diploid and tetraploid hepatocyte nuclei.

The extremely high synchronization of entry of the hepatocytes into the S phase will be noted: as a rule 60 min before the peak of LI in the liver fewer than 2% of parenchymatous cells had labeled nuclei. Further evidence of the high level of synchronization of DNA synthesis in the hepatocyte population was given by sedimentation analysis of nuclei isolated from the liver at the time of the peak of LI (Fig. 2). The total duration of the S period of MC for synchronized hepatocytes was a little less than that for regenerating liver cells of the control animals, confirming the probable specificity of HU as an inhibitor strictly of elongation, but not of initiation of DNA synthesis [10]. No secondary rise in the number of DNA-synthesizing hepatocytes was observed in the regenerating liver after synchronization with HU, although the level of proliferative activity of the hepatocytes was higher than that in the control until 47-49 h after the operation.

According to data in the literature [12], during prolonged (for 26 h) intravenous infusion of HU practically 100% of cells of the proliferative pool of the regenerating liver accumulate in the late G_1 phase on the boundary with the S phase. However, the rate of entry of hepatocytes into the S phase, judging by the results of determination of incorporation of [^{3}H]thymidine into DNA, reaches a maximum during the 3 h after the end of infusion, i.e., the degree of synchronization was lower than in the present experiments.

After five injections of HU the number of cells entering the S phase synchronously in the epithellium of the pits of the pyloric glands of the gastric mucosa was 18.3 ± 3.9%, and

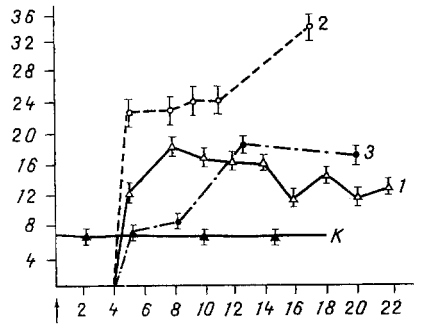

Fig. 3. LI of cells of gastric mucosa (1) and Brunner's glands (2, 3) during synchronization by HU. Abscissa, time (in h); ordinate, LI (in %). 1, 2) Six injections of HU; 3-5) five injections of HU. K) LI of normal gastric cells; LI of Brunner's glands under normal conditions was 0.29 ± 0.08%. Arrow indicates time of last injection of HU.

in the fundal glands 7.8 ± 1.5%, which was 2.7 and 1.7 times greater respectively than in the control (Fig. 3). In the fundal glands the first cells to enter the period of DNA synthesis were poorly differentiated cells in the neck of the glands (84.6 ± 4.6% of the labeled cells were from the neck of the glands and only 16% from the pits), whereas in the control animals 1 h after injection of [^{3}H]thymidine only 15% of labeled cells were poorly differentiated cells from the neck of the glands.

The first labeled cells appearing in the pyloric glands 4.5 h after injection of HU were located mainly within the four positions bordering on the body of the glands, i.e., in the zone where the least differentiated cells, possibly stem cells for all types of differentiated cells [2, 9], were located.

Meanwhile in the duodenum the first labeled cells appeared after repeated injections of HU along the whole length of the greatly shortened crypts, evidence that surviving, rapidly proliferating nonclonogenic enterocytes also take part in repopulation. For instance, after five and six injections of HU, a more than 100-fold increase in LI was observed (Fig. 3) in the Brunner's glands, which consist of very slowly renewed cells (LI in the control was 0.29 ± 0.08%). Considering that after a single injection of HU no cells with pycnotic nuclei were present in the Brunner's glands, the sudden activation of proliferation in them cannot be interpreted as compensatory. It may perhaps arise in response to death of the majority of enterocytes in the crypts. The possibility cannot be ruled out that the considerable cell losses under the influence of HU in different constantly renewed populations of the body cause increased production of the epidermal growth factor that is produced by Brunner's glands [6] and stimulates proliferation of the epithelium in various organs and in Brunner's glands themselves (autocrine stimulation).

By means of five or six injections of HU it is thus possible to effectively synchronize proliferating hepatocytes and the foveolar cells of the gastric glands and also to stimulate proliferation in the slowly renewed cells of Brunner's glands.

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