α-FETOPROTEIN PRODUCTION IN REGENERATING MOUSE LIVER SYNCHRONIZED *IN VIVO*

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Liver cells regenerating after CCl_4 poisoning were synchronized *in vivo* by continuous administration of hydroxyurea. Accumulation of hepatocytes at the G_1-S phase boundary or in the S-phase did not affect the course of changes in the blood α-fetoprotein (α-FP) levels or the characteristic location of α-FP for regenerating liver for 2 or 3 days after poisoning. α-FP production began in the hepatocytes before their entry into the S-phase, and α-FP was found in the cells also at different times after they had ended their mitotic cycle. No dependence of α-FP synthesis on any particular phase of the mitotic cycle could be observed.

KEY WORDS: α-fetoprotein; synchronization; regeneration; mitotic cycle.

In the mouse liver regenerating after carbon tetrachloride (CCl_4) poisoning α-fetoprotein (α-FP) has been found in single hepatocytes, chiefly at the borders of necrotic foci [4]. Since hepatocytes in regenerating liver pass through the mitotic cycle asynchronously, it might be supposed that the fact that α-FP is localized only in certain cells is connected with its production in a particular phase of the cycle, as was postulated previously on the basis of analysis of hepatoma cultures [10].

On the other hand, the characteristic location of cells containing α-FP in the perinecrotic zone suggests that α-FP synthesis is determined by disturbance of contacts between the cells in the column [1].

To investigate dependence of α-FP synthesis on the mitotic cycle, synchronization of the regenerating liver was carried out *in vivo* by means of hydroxyurea (HU), by the method suggested by Rabes et al. [7]. The synchronizing effect of HU is due to the fact that it prevents conversion of ribonucleotides into deoxyribonucleotides, and so blocks DNA synthesis [6, 11].

EXPERIMENTAL METHOD

Experiments were carried out on CC57BR mice, male and female, aged 2-3 months. Regeneration of the liver was induced by poisoning the mice with CCl_4 vapor or by parenteral injection of an 8% solution of CCl_4 in oil in a dose of 0.1 ml solution/20 g body weight.

HU (from Serva, West Germany) was injected intraperitoneally into the animals in physiological saline hourly by means of the apparatus suggested by Rovenskii and Moizhess [2].

DNA synthesis was analyzed by injecting radioactive thymidine-5-methyl-^{3}H (USSR, 21.4 Ci/mmole) body weight. During saturation with thymidine-^{3}H the mouse received 0.3 μCi/g body weight hourly. The labeling index was counted by examining 1500-2000 hepatocyte nuclei.

On the 2nd-3rd days after CCl_4 poisoning blood samples were taken from the retro-orbital sinus of the mice. The animals were then decapitated. The blood α-FP concentration was determined by the precipitation test with a test system [3]. Pieces of liver were fixed with a cold mixture of ethanol and acetic acid, and α-FP was localized in paraffin sections of the liver by the indirect peroxidase reaction, using a complex of rabbit antibodies with peroxidase (PAP, from Daco, Denmark) [9].

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TABLE 1. Choice of Optimal Dose of HU

Index	Of HU, mg/g						
	0,5	0,25	0,1	0,05	0,02	0,01	0,005
No. of mice	2	3	7	4	6	2	2
Presence of label	—	—	—	±	±	+	+

TABLE 2. Synchronization of Liver by Means of HU

Experimental conditions	Labeling index, %				
- Pulse labeling of thymidine-^{3}H 75 h after CCl_4 administration	0,4	0,6	1	2,1	0,5
- Saturation labeling with thymidine-^{3}H until 75 h after CCl_4 administration	66				
- Saturation with HU from 24 to 74 h after administration of CCl_4+pulse labeling with thymidine-^{3}H 75 h aft. CCl_4 adm.	0	72	50	63	

TABLE 3. Localization of α-FP in Hepatocytes Completing Mitotic Cycle

Index	Experiment No.									
	1					2	3			
Labeling index, %	30	34	36	54	35	75	15	85	26	16
Labeling index in cells containing α-FP, %	44	70	60	56	53	80	70	80	60	82
No. of nuclei of cells containing α-FP examined	93	48	96	95	49	57	39	131	110	39

For simultaneous analysis of α-FP production and participation of the cells in the mitotic cycle, liver sections in which α-FP was found were coated with type A photosensitive emulsion (Ilford, England). The autoradiographs, developed 2-4 weeks later, were stained with Mayer's hematoxylin.

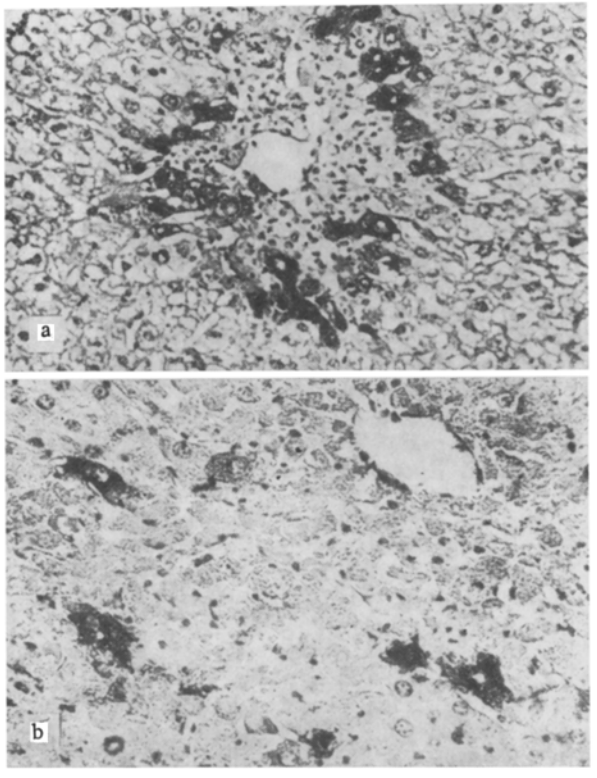
EXPERIMENTAL RESULTS

To reduce the mortality among the animals receiving HU, the minimal dose of HU completely suppressing incorporation of labeled thymidine into DNA (0.1 mg/g body weight/h) was chosen (Table 1). In all subsequent experiments this dose of HU was used.

DNA synthesis was blocked from 24 to 72 h after poisoning. During that period the overwhelming majority of hepatocytes normally pass through the S-phase [8]. Despite the HU-block, blood samples taken from the retro-orbital sinus showed the usual increase in α-FP levels in the period between the 2nd and 3rd days after poisoning. On the 3rd day after CCl_4 poisoning α-FP was found in liver sections, just as in the absence of HU, in single hepatocytes, mainly at the boundary with the focus of necrosis (Fig. 1).

The rules governing α-FP production were thus not disturbed because the hepatocytes were fixed at the G_1-S boundary and could not pass through the mitotic cycle. These results also agree with earlier observations showing that mature hepatocytes in the regenerating mouse liver can produce α-FP before passing through the S-phase [5].

Soon after the end of continuous HU administration its blocking action ceased and the cells entered upon the S-phase synchronously [7]. In animals receiving thymidine-^{3}H 3-3.5 h after removal of the HU-block, which continued until the 3rd day after poisoning, a high degree of synchronization comparable with the size of the proliferative pool was observed (Table 2, Fig. 2a, b). As a result of the transition of hepatocytes from the G_1-S boundary to the S-phase no change was observed in the blood α-FP levels or in the localization of α-FP in the sections. α-FP present in some hepatocytes in the S-phase of the liver with cells synchronized with respect to the S-phase, and also in unlabeled cells (Fig. 2).



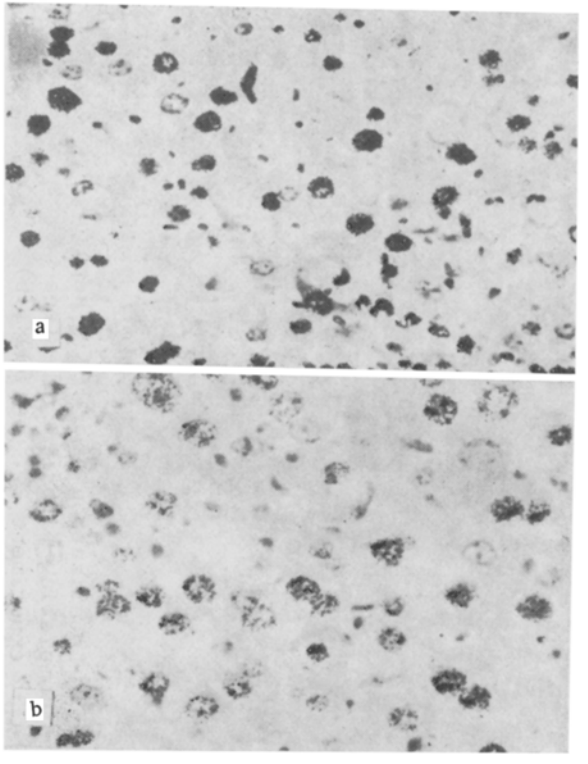


Fig. 1

Fig. 2

Fig. 1. Localization of α-FP in mouse liver on 3rd day after CCl_4 poisoning. a) Section through regenerating liver, not treated with HU; b) section through liver after synchronization by HU. 312×.

Fig. 2. α-FP in mouse liver cells synchronized with respect to S-phase (3rd day after CCl_4 poisoning). a) Section through mouse liver after saturation labeling with thymidine-^3H, without HU, 200×; b) section through liver with synchronized cells, 200×.

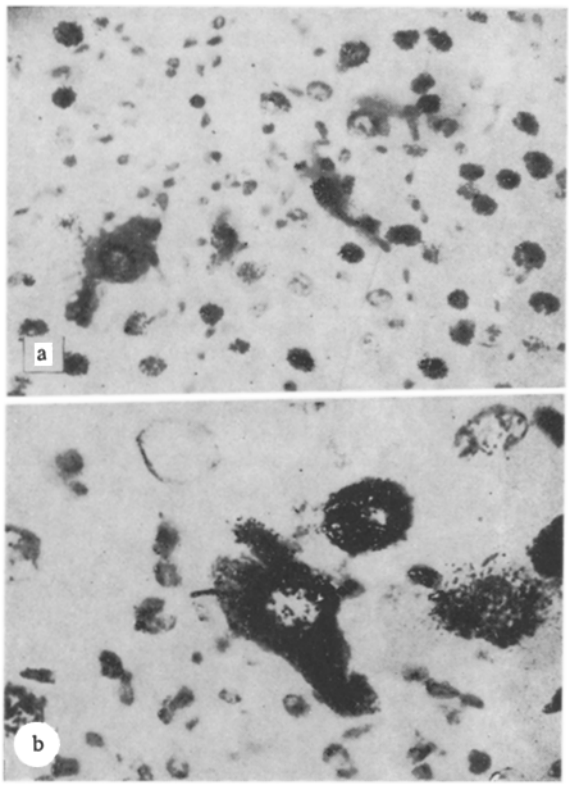


Fig. 3. Localization of α-FP in hepatocytes completing mitotic cycle (see Table 3, experiment 2). a) 500×; b) 1250×.

The α-FP content in hepatocytes which had already completed the mitotic cycle was next determined. After CCl_4 poisoning regeneration of the liver took place mainly on account of a single passage of the hepatocytes through the mitotic cycle [8]. Cells in the S-phase after 12 h must therefore have reached the resting state, for $S + G_2 + M = 12$ h.

Mice receiving thymidine-^3H on the 2nd day after CCl_4 poisoning were killed 1 day later. Autoradiographs showed labeled hepatocytes containing α-FP (Table 3, experiment 1). These cells had completed mitosis about 12 h beforehand, but despite this fact they had not lost their ability to produce α-FP. Similar experiments were set up on the synchronized liver. Cells synchronously entering into the S-phase after HU-block were labeled with thymidine-^3H and the mice were killed 12.5 and 20 h after injection of the isotope (Table 3, experiments 2 and 3). Labeled cells with α-FP were found in all mice in this case also.

It can thus be concluded from these results that there is no direct dependence of α-FP production in the regenerating mouse liver on any particular phase of the mitotic cycle.

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HEMATOPOIESIS IN RATS AFTER DESTRUCTION OF THE POSTERIOR HYPOTHALAMIC NUCLEI ASSESSED BY BONE MARROW TOTAL CELL COUNT

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The effect of bilateral electrocoagulation of nuclei of the mammillary body and the posterior hypothalamic nucleus on the total cell count in the bone marrow was studied in rats. Destruction of the posterior hypothalamic nuclei led to inhibition of erythropoiesis. The number of mitoses and the total number of erythroid cells were reduced. Granulocytopoiesis, however, was stimulated. Proliferative activity and the total number of immature granulocytes in the bone marrow of the rats were increased.

KEY WORDS: hypothalamus; mammillary body; posterior hypothalamic nucleus; erythropoiesis; granulocytopoiesis.

According to data in the literature destruction of the hypothalamus (especially the nuclei of its posterior part) affects the composition of the blood erythrocytes [2, 5-7]. The present writers have shown in recent investigations [3, 4] that destruction of the posterior hypothalamic nuclei leads to inhibition of erythropoiesis and stimulation of granulocytopoiesis. A decrease in proliferative activity and in the relative percentage of erythroid cells together with an increase in the number of mitoses and in the relative percentage of cells of the granulocyte series were observed in the bone marrow of animals with injured posterior hypothalamic nuclei. Maximal changes were observed on the 3rd day after the operation. It was accordingly decided to study the absolute composition of bone marrow cells of animals at this period.

EXPERIMENTAL METHOD

Experiments were carried out on 78 male rats weighing 250-350 g (18 experimental, 17 control, and 43 intact animals). By means of a stereotaxic apparatus bilateral electrical coagulation of the nuclei of the mammillary body and of the posterior hypothalamic nucleus was carried out with a direct current of 2 mA for 5 sec. A platinum electrode 100 μ in diameter was used for this purpose.

On the 3rd day after the operation the blood vessels of the brain were perfused initially with physiological saline, later with a 10% solution of neutral formalin. The brain was fixed in a 10% solution of neutral formalin for 2-4 weeks, after which it was mounted in gelatin solutions of increasing concentration (from 12 to 24%). To determine the location and volume of the focus of destruction, serial sections through the brain were cut to a thickness of 25-30 μ in the frontal plane and stained by Nissl's method.

Bone marrow for counting the total number of myelokaryocytes and the myelograms was taken from the femur. The total number of myelokaryocytes was counted in 1 mm^3 bone marrow. The experimental results were compared with values for bone marrow taken from two or three intact rats.

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