EFFECT OF STIMULATION OF KUPFFER CELLS BY PRODIGIOSAN ON REGENERATION OF HEPATOCYTES

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The effect of the polysaccharide of Bacillus prodigiosus (prodigiosan) on DNA synthesis and mitotic activity of the hepatocytes was studied in the regenerating liver of male Wistar rats. After administration of the stimulator in a dose of 50 μg per rat 24 h before partial hepatectomy the indices of labeled nuclei and mitotic indices were higher and reached their maximum sooner in the hepatocytes in the experimental animals than in the controls. It is suggested that the stimulating effect of prodigiosan is connected with activation of the Kupffer cells of the liver.

KEY WORDS: polysaccharide prodigiosan; regenerating liver; DNA synthesis; mitotic index.

The writers showed previously that regeneration of the liver is largely dependent on the functional state of the lysosomal apparatus of the Kupffer cells, which form part of the single system of mononuclear phagocytes (SMP) [7]. Within the first few hours after partial hepatectomy the relative number of Kupffer cells in the rat liver was increased. Biochemical investigation of the Kupffer cell fraction showed that 2.5 h and, in particular, 9 h after the operation (i.e., in the prereplicative period of proliferation of the hepatocytes) the free activity of the lysosomal enzymes [2], which can be regarded as inducers of cell growth [5], rose sharply in the liver macrophages. Proliferation of the hepatocytes was appreciably inhibited if the Kupffer cells were blocked by an inert colloid [2]. To continue the study of the role of the Kupffer cells in reparative regeneration of the liver it was important to study the course of proliferation of the hepatocytes after stimulation of the Kupffer cells of the liver. Prodigiosan, a preparation of polysaccharide nature obtained from the nonpathogenic organism Bacillus prodigiosus, was chosen as the factor stimulating the macrophages of the liver. Special investigations showed that prodigiosan has a powerful and selective stimulating action on the activity of lysosomal enzymes and on the phagocytic power of the tissue macrophages [1].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 140-180 g. The experimental animals were given an intraperitoneal injection of 50 μg prodigiosan (batch 50976, Experimental Product Division, Central Postgraduate Medical Institute, Moscow) in 0.85% NaCl solution 24 h before resection of the liver by the method of Higgins and Anderson [4], performed under ether anesthesia between 9 and 10 a.m. Instead of prodigiosan the control animals received an injection of 1 ml of 0.85% NaCl solution. The animals were killed 5 at a time 8, 12, 16, 20, 24, 30-32, 48, and 72 h after the operation. The relative number of Kupffer cells was counted in 1000 hepatocytes in liver sections stained with hematoxylin and eosin. Meanwhile the indices of labeled nuclei (ILN) of the hepatocytes were determined in autoradiographs of liver sections from the same animals. To obtain autoradiographs, 1 μCi thymidine-^{3}H/g body weight (specific activity 12.8 Ci/mmole) was injected intraperitoneally into the animal 1 h before sacrifice. The liver sections, stained with hematoxylin and eosin, were coated with liquid photographic emulsion (Photographic Chemical Research Institute Project) and exposed in darkness at 4°C for 10 days. The numerical results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

After injection of prodigiosan in a dose of 50 μg a marked increase was found in the relative number of Kupffer cells, with a maximum 48 h after injection. In the control this number was 35.0 ± 3.7, rising after 24 h to 41.2 ± 0.9, after 36 h to 42.5 ± 1.0, and after 48 h to 44.1 ± 1.7, and falling after 72 h to 40.9 ± 1.2. In all cases differences from the control were significant. The mitotic indices of the hepatocytes in the control in-

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TABLE 1. Mitotic Indices of Hepatocytes (in ‰) in Control and after Injection of Prodigiosan in Hepatectomized Rats (M ± m)

Time after partial hepatectomy, h	Control	Experiment
16	Infrequent mitoses	1.4±0,17
20	» »	9,4±0,62
24	3,1±0,28	26,2±0,97†
30—32	20,7±0,71	13,2±0,71†
36	15,7±1,02	6,7±0.52†
48	6,8±1,01	4.7±0,33*
72	1,8±0,17	0.9±0,38*

*P < 0.05.
†P < 0.01.

TABLE 2. ILN of Hepatocytes (in %) in Control and after Injection of Prodigiosan in Hepatectomized Rats (M ± m)

Time after partial hepatectomy, h	Control	Experiment
8	0,4±0,03	1,2±0,12†
12	1,1±0,12	5,2±0,65*
16	4,1±0,33	49,6±2,50†
20	36,9±1.84	31,9±1,62†
24	29,5±1,59	20,1±2,01†
32	15.3±1.57	11,5±1,49†

*P < 0.05.
†P < 0.01.

creased with effect from 20 h after the operation, to reach a maximum 30-32 h after the operation. A high frequency of mitosis also was found 36 h after resection, i.e., the period of maximal mitotic activity was somewhat protracted. In the experimental animals 16 h after the operation the frequency of mitosis among the hepatocytes was 1.4 ‰, and 20 h after the operation 9.4 ‰. The peak of mitoses occurred 24 h after hepatectomy. It was followed by a rapid decline in the frequency of mitosis until 30-32 h after the operation (Table 1).

In the control animals ILN of the hepatocytes rose gradually to 0.4% 8 h after the operation and to 36.7 and 29.5% 20 and 24 h after the operation respectively. By 32 h, i.e., by the time of the peak of mitoses among the hepatocytes, ILN showed a significant decrease. In the experimental animals 8 h after the operation ILN was significantly higher than the control. Later ILN rose rapidly to reach a maximum 15 h after the operation (49.6%). ILN still remained high 20 h after the operation, but then fell rapidly, by 60% and 78% respectively 24 and 32 h after the operation (Table 2).

After injection of the bacterial polysaccharide prodigiosan, proliferation of hepatocytes in the rat liver after resection of the organ was thus sharply intensified. The stimulating action of prodigiosan could have been due to functional reorganization of the SMP, primarily of the system of Kupffer cells of the liver. Stimulation of macrophages is known to be accompanied as a rule by an increase in the activity of their lysosomal apparatus. In the modern view macrophages, stimulated in various ways, begin to secrete increased quantities of substances of lysosomal origin [6] which act as mediators of intercellular interaction in the connective-tissue system. The possibility likewise cannot be ruled out that these substances in certain situations may play the role of growth-regulating factors within the system of stromal-parenchymatous interaction in the regenerating organ. According to data not included in this paper, after injection of prodigiosan there was virtually no change in the total activity of acid phosphatase, the marker enzyme of lysosomes in the intact liver. However, it increased significantly 24 h after resection of the liver in homogenates of the organ obtained from animals stimulated with prodigiosan compared with the unstimulated control. Consequently, under the influence of prodigiosan activation of the lysosomal apparatus of the Kupffer cells was apparently potentiated, as the writers found previously during the first few hours after resection [2]. Besides activation of the lysosomal apparatus of the Kupffer cells, the increased proliferation of the hepatocytes in the present experiments could also have been due to more active metabolism of glucocorticoids by Kupffer cells stimulated by prodigiosan [1]. Meanwhile, the duration of delay of synchronized liver cells in the G_1 phase in rats after resection of the liver depends on the free blood glucocorticoid level [3].

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LITERATURE CITED

1. Z. V. Ermol'eva and G. E. Vaisberg, Stimulation of the Nonspecific Resistance of the Organism and Bacterial Polysaccharides [in Russian], Moscow (1976).
2. N. N. Mayanskaya, V. I. Shcherbakov. L. E. Panin, et al., in: Structure and Function of the Lysosomes. Abstracts of Proceedings [in Russian], Moscow (1976), pp. 96-98.
3. L. Desser-Wiest, Cell Tissue Kinet., 8, 1 (1975).
4. G. Higgins and H. Anderson, Arch. Path., 12, 186 (1931).
5. F. Seelich, Oster. Z. Onkol., 2, 31 (1975).
6. E. P. Unanue, Am. J. Path., 83, 396 (1976).
7. R. Van Furth, Agents and Actions, 6, 91 (1976).

ALPHA-FETOPROTEIN SYNTHESIS IN DIFFERENT LINES OF ADULT MICE DURING REGENERATION OF THE LIVER

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The concentration of alpha-fetoprotein (AFP) in the sera of adult mice of 12 different lines and F_1 hybrids of lines SWR and B10D2 was determined by radioimmunodiffusion in agar during regeneration of the liver after poisoning with CCl_4. In the mice of 6 of 10 lines differences in the AFP concentration between females and males were statistically significant. Interlinear differences also were found: The mean AFP concentrations in the sera of inbred C57BL/6 and B10D2 mice were significantly lower than the corresponding values for mice of most other lines. The F_1 hybrids were intermediate as regards their AFP concentration between the two parental lines. Small but statistically significant differences were found between groups of male F_1 hybrids in direct and reciprocal crosses. It is suggested that induction of AFP synthesis during regeneration of the liver in adult mice is under polygenic control.

KEY WORDS: alpha-fetoprotein; regeneration of the liver; interlinear differences; genetic control.

Alpha-Fetoprotein (AFP), the principal protein of embryonic serum is present in adult animals and man in very low (nanogram) concentrations [2, 10, 13]. During regeneration of the liver after partial hepatectomy or poisoning by various hepatotoxins, there is a sharp but transient increase in the AFP level in the animals' blood [3, 4, 6, 12, 15].

In a few cases differences in the intensity of AFP synthesis have been found in mice of different lines during regeneration of the liver [1, 12]. A detailed analysis of such differences could provide an approach to the study of the genetic control of induction of AFP synthesis in the adult.

In the investigation described below AFP was determined quantitatively during regeneration of the liver in the sera of mice of different genotypes.

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