11. F. Halberg, E. Haus, and L. E. Scheving, in: Biomathematics and Cell Kinetics, Amsterdam (1978), p. 175.
12. F. Halberg and H. B. Visscher, Proc. Soc. Exp. Biol. (New York), 75, 846 (1950).
13. D. T. Krieger, in: Endocrine Rhythms, New York (1979), p. 123.
14. L. G. Lajtha, R. Oliver, and F. Ellis, Br. J. Cancer, 8, 367 (1954).
15. F. Levi, F. Halberg, M. Nesbit, et al., in: Neoplasms: Comparative Pathology of Growth in Animals, Plants, and Man, Baltimore (1981), p. 267.
16. A. M. Mauer, Blood, 26, 1 (1965).
17. E. Nir, P. Efrati, and D. Danon, Exp. Hematol., 4, 161 (1976).

DEREPRESSION OF HEPATOCYTE PROLIFERATION BY CHANGES IN RETICULO-ENDOTHELIAL SYSTEM FUNCTION

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Hepatic stromal cells ensure an adequate microenvironment for hepatocyte proliferation [2]. The most reactive component of the stroma is the Kupffer cell (KC). In the early stages of stimulation a combination of mediators passes from it into the microenvironment: prostaglandins [9], lysosomal proteases and glycosidases, stimulators of lymphocytes and fibroblasts [5, 11, 13]. As a result of activation of KC mononuclear infiltration into the hepatic stroma takes place [6, 8].

It is not only the state of the hepatic stroma that depends on KC function. By changing the functional state of KC it is possible, a priori, to modify substantially the course of hepatocyte regeneration after partial resection of the liver [3, 4]. Preliminary stimulation of KC by the bacterial stimulator from *Serratia marcescens* (prodigiosan) 24 h before the operation created conditions for more rapid realization of the early stages of regeneration of the resected liver. Meanwhile, if the KC were loaded with colloidal iron 2 h before or 3 h after the operation, the course of reparative regeneration of the liver was delayed almost to half the control rate. This suggested that KC can become the source of factors inducing hepatocyte proliferation.

Further confirmation of this hypothesis was obtained in the present investigation. In experiments on mice and rats the effect of different agents causing primary modification of KC function on the DNA-synthesizing ability of the hepatocytes was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 240-280 g and on male (CBA × C57BL)F_1 mice weighing 18-22 g. Colloidal iron particles (brand R-100F) measuring 0.8-1.5 μ, in a dose of 100 mg, suspended in 5% isotonic starch solution, were injected into the femoral vein of some rats, and latex microspheres 0.2-0.5 in diameter, suspended in 0.85% NaCl, were injected into other rats in a dose of 10 mg. To stimulate KC, 2 mg zymosan, 10 μg prodigiosan, and 3 mg BCG in 0.5 ml 0.85% NaCl was injected into the caudal vein of the mice. The animals were killed 4, 8, 16, 24, 32, 48, and 72 h, 5, 7, 9, 12, 15, and 21 days, and 1 month later. [^{3}H]Thymidine was injected in a dose of 1 μCi/g body weight (specific activity 12.8 Ci/mmole). Liver sections stained with hematoxylin and eosin were coated with liquid photographic emulsion (Photographic Chemical Research Institute) and exposed in darkness at 4°C for 3 weeks. To determine the mitotic index (MI) 5000 hepatocytes were counted in liver sections stained with hematoxylin and eosin; to determine the index of labeled nuclei (ILN) of the hepatocytes and sinusoidal cells 3000 nuclei were counted in autoradiographs of liver sections. The numerical data were subjected to statistical analysis by Student's t test.

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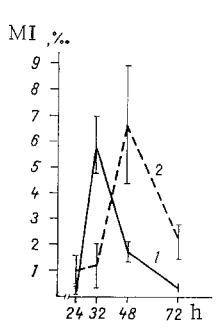

Fig. 1

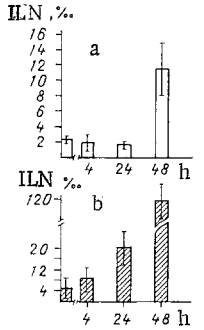

Fig. 2

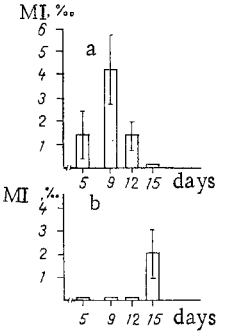

Fig. 3

Fig. 1. Mitotic activity of hepatocytes after injection of colloidal iron (1) and latex (2). Here and in Figs. 2 and 3: abscissa, time after injection of modulating agents.

Fig. 2. ILN of parenchymatous (a) and sinusoidal (b) cells.

Fig. 3. Mitotic activity of hepatocytes after injection of zymosan (a) and prodigiosan (b).

EXPERIMENTAL RESULTS

After intravenous injection of a suspension of R-100F colloidal iron particles they were ingested by KC, for particles of such a large size virtually do not pass through the endothelium of the hepatic sinusoids. Two hours after loading about 80% of the hepatic macrophages were filled with iron granules. The first mitoses were recorded in hepatocytes 24 h after loading (Fig. 1). The number of mitoses was increased by 32 h, but after 48 and 72 h it was reduced although still higher than in the control liver. After loading of KC with latex microspheres mitoses also appeared in the hepatocytes after 24 h, their number increased until 48 h, and then decreased (Fig. 1).

After intravenous injection zymosan particles were ingested by cells of the hepatic reticuloendothelial system (RES). Electron-microscopic investigation showed them to be present regularly in the Kupffer and endothelial cells of the hepatic sinusoids [6], but not in hepatocytes. In response to administration of zymosan foci of infiltration consisting of lymphocytes, monocytes, and their derivatives, began to form around the activated KC after 48 h. Later, toward the 5th and, in particular, the 9th day the volume of mononuclear infiltration of the liver increased progressively. Individual foci not only increased in size, but showed a tendency to merge also. The infiltration became diffuse-focal in character. After injection of zymosan DNA synthesis in the liver was disinhibited. The intensity of incorporation of [^{3}H]thymidine into nuclei of sinuosidal cells (endothelial + Kupffer) was increased as early as 4 h after stimulation. Later, toward 24 and 48 h, ILN of the sinusoidal cells increased to 2 and 12% respectively, whereas in the intact mice it reached only 0.4%; 7.5% of cells of the foci of infiltration also incorporated [^{3}H]thymidine. The number of labeled hepatocyte nuclei increased, but at a slower rate than incorporation of [^{3}H]thymidine into nuclei of sinuosidal cells: ILN of the hepatocytes was significantly increased 48 h after stimulation (Fig. 2). The first mitoses of hepatocytes appeared 5 days after stimulation by zymosan, and this was followed by an increase until the 9th day and a decrease on the 12th day (Fig. 3). Numerous mitoses also were observed in the sinuosidal cells and in cells forming the zones of infiltration. The weight of the liver was increased by 1.5 times 9 days after injection of zymosan.

After injection of BCG vaccine morphological transformations of the hepatic stroma were observed, resembling in many ways the effect of zymosan stimulation. The weight of the liver was doubled after 2-3 weeks. During the period of intensive mononuclear infiltration of the stroma, 9 days after bacterial stimulation, 2.65 ± 0.68 ‰ of hepatocytes were in mitosis. In precisely the same way, mitotic activity of the hepatocytes was disinhibited after stimulation of the macrophages by prodigiosan. In this case mononuclear infiltration of the liver combined with foci of hematopoiesis appeared later than after stimulation by zymosan and BCG — not earlier than 2 weeks after injection. An increase in the number of mitoses in hepatocytes was recorded at that same moment (Fig. 3).

In the different versions of stimulation of KC reactive changes thus appeared in the liver, manifested not only by transformation of the stroma, but also by disinhibition of proliferative activity of the parenchyma. Hepatocyte proliferation reached its peak 1.5-2 days after injection of the inert particles (colloidal iron granules and latex microspheres). In these cases the time course of the relationship between stimulation of the hepatic RES and disinhibition of hepatocyte proliferation could be distinguished particularly clearly. What is the mechanism of this disinhibition? In our view it is connected with activation of the secretory function of the hepatic macrophages, linked with ingestion of the inert particles. It is known, for instance, that latex microspheres initiate the secretion of lysosomal enzymes of peritoneal mononuclear phagocytes [7]. In all probability this effect is not limited to the latter, but also extends to other classes of mononuclear phagocytes, including KC. Lysosomal proteases are universal regulators of proliferative processes [1]. Proteases of phagocytic cells are no exception [12]. It is thus realistic to conclude that disinhibition of hepatocyte proliferation was connected in these experiments with the lysosomal proteases of KC. The same mechanism is activated after stimulation of the system of mononuclear phagocytes of the liver by zymosan. In that case, however, just as after stimulation by bacterial polysaccharide and BCG, hepatocyte proliferation depends on the degree of mononuclear infiltration of the liver. This suggests that cells of the zones of mononuclear infiltration produce substances which regulate proliferation of parenchymatous cells. Their identification will be the object of future investigations. The possibility cannot be ruled out that these factors are related to substances of macrophagal nature which disinhibit hematopoiesis [10, 14].

LITERATURE CITED

1. N. E. Isanin and A. Yu. Yakovlev, Tsitologiya, No. 6, 575 (1977).
2. V. P. Kaznacheev, M. Ya. Subbotin, and N. I. Tsirel'nikov, in: Proceedings of the 4th All-Union Symposium on the Histophysiology of Connective Tissue [in Russian], Vol. 1, Novosibirsk (1972), pp. 39-49.
3. D. N. Mayanskii, V. I. Shcherbakov, and Yu. M. Mirokhanov, Byull. Éksp. Biol. Med., No. 11, 616 (1977).
4. D. N. Mayanskii and V. I. Shcherbakov, Byull. Éksp. Biol. Med., No. 7, 69 (1978).
5. D. N. Mayanskii, The Kupffer Cell and System of Mononuclear Phagocytes [in Russian], Novosibirsk (1981).
6. V. I. Shcherbakov, D. N. Mayanskii, and G. V. Pravotorov, Byull. Éksp. Biol. Med., No. 12, 731 (1981).
7. M. Berit, J. Reticuloend. Soc., 26, 749 (1979).
8. J. Ellegard, H. Starklint, K. Kragballe, et al., Acta Pathol. Microbiol. Scand., Sect. C, 86, 79 (1978).
9. H. Leser and D. Gemsa, in: Abstracts of the 9th International RES Congress, Switzerland (1982), p. 18.
10. M. Moore, J. Reticuloend. Soc., 20, 89 (1976).
11. R. C. Page, P. Davies, and A. Allison, Int. Rev. Cytol., 52, 119 (1978).
12. E. R. Unanue, Am. J. Path., 83, 396 (1976).
13. D. Van Waarde, E. Hulsing-Hesselink, and E. Van Furth, Blood, 50, 727 (1977).