STIMULATION OF PROLIFERATION AND METABOLISM IN THE LIVER AFTER INJECTION OF ALLOGENEIC HEPATOCYTES

K. G. Kavtiashvili and U. A. Gabuniya

UDC 616.36-008.64:577.213.3.7.216.3

KEY WORDS: acute hepatic failure; hepatocytes; DNA and RNA synthesis.

In recent years, the most promising method of temporary organ replacement in acute hepatic failure (AHF) has been considered to be the use of a suspension of hepatocytes from a healthy donor [1, 2, 7, 8]. Investigations by several workers [1, 3, 6, 8, 9] have shown that hepatocytes in suspension possess all the organ-specific functions and, under certain conditions, can also take over detoxication, anabolic, and catabolic functions of the damaged liver.

This paper describes an attempt, using autoradiography, to determine precisely how compatible are degenerative changes in liver tissue with its intracellular regeneration and ability of the cells to reproduce; to establish correlation between morphological and functional changes in the liver and the ability of its cells to synthesize RNA and DNA in patients with AHF and after injection of allogeneic hepatocytes (AH).

EXPERIMENTAL METHOD

A model of AHF was created in 46 mature mongrel dogs, male and female, by total ischemia of the liver for 1 h followed by restoration of the arterial blood supply. Immediately after creation of the AHF model 36 of the animals were given an intraperitoneal injection of a suspension of AH obtained by combined enzymic and mechanical treatment of the liver of newborn dogs. The control animals (10 dogs) did not receive an injection of hepatocytes after the same procedures. Material was studied 1, 2, 4, 6, 8, 10, 14, 30, and 60 days after the operation. Autoradiography was carried out on pieces of liver by incubation of the specimens with radioactive precursors of DNA and RNA synthesis [4, 5]. Semithin sections 0.5 μ thick were cut from blocks prepared for investigation under the electron microscope, covered with type M photographic emulsion, exposed for 3 days, and stained with toluidine blue.

EXPERIMENTAL RESULTS

Analysis of autoradiographs of the semithin sections showed that after ischemia for 1 h the hepatocytes located in the zones of necrosis were completely without label in sections incubated with ^{3}H-thymidine or ^{3}H-uridine. The number of grains of silver in sections incubated with ^{3}H-thymidine above the hepatocytes with marked degenerative changes was usually smaller than above nuclei in cells showing less marked changes. DNA synthesis was observed in hepatocytes in whose cytoplasm many tiny lipid droplets could be seen.

Single large hepatocytes with hypertrophied nuclei and nucleoli, above which many grains of silver could be seen, were found in the liver together with a marked decrease in incorporation of ^{3}H-thymidine. Granules of silver in these cells were concentrated mainly above the hypertrophied nucleolus. Degenerative changes, vacuolated cells with nuclei of the ordinary size, and intensively labeled, and also cells with hypertrophied nuclei and nucleoli without any signs of incorporation of ^{3}H-uridine, also were found. On the whole, no evident connection could often be established between the concentration of label and the structural features of the hepatocytes (Fig. 1).

Department of Pathological Morphology, A. N. Natishvili Institute of Experimental Morphology, Georgian Academy of Sciences, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 315-317, September, 1991. Original article submitted December 20, 1990.

0007-4888/91/0009-1343$12.50

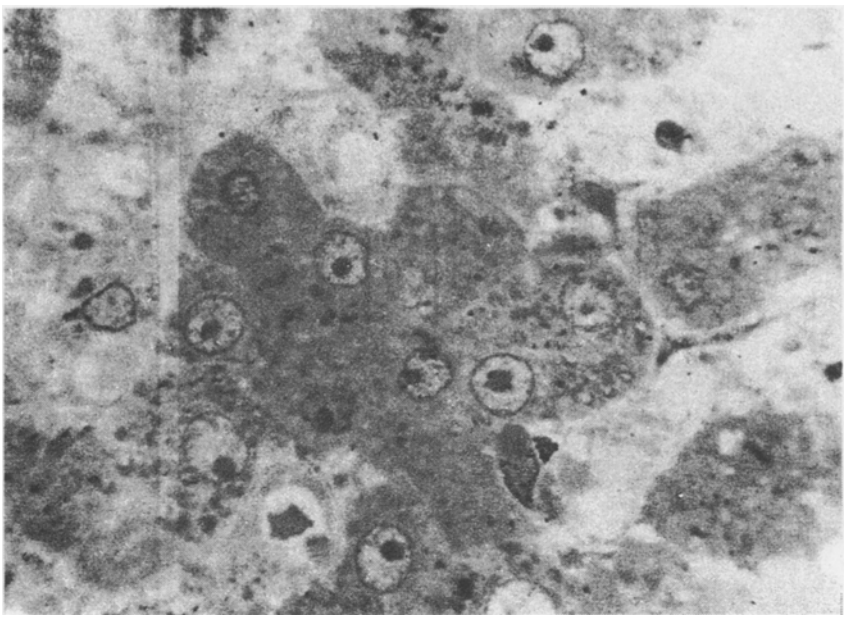

Fig. 1. Single grains of silver (^{3}H-uridine) above nuclei and cytoplasm of some preserved hepatocytes. Animals after ischemia of the liver (1 h). Here and in Figs. 2 and 3: semithin section, stained with toluidine blue. 1000×.

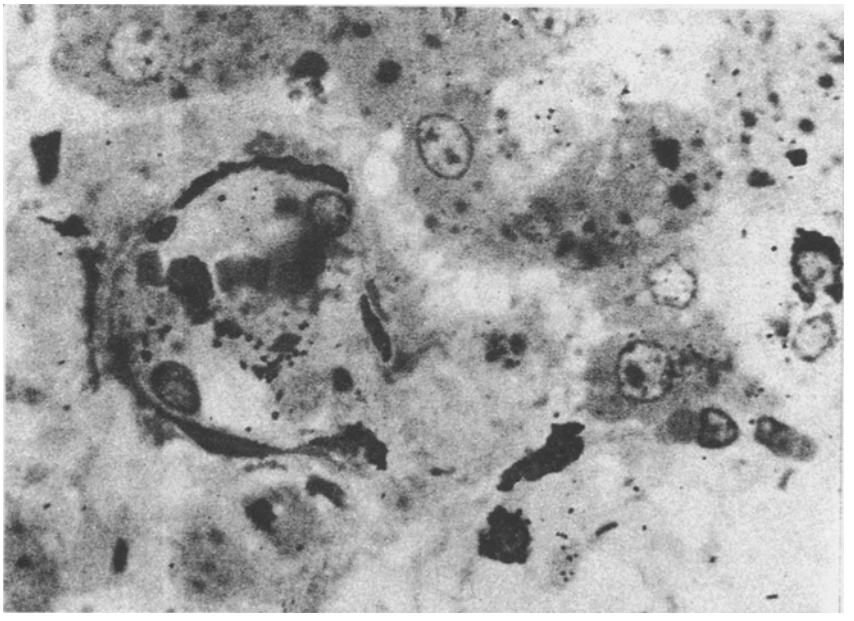

Fig. 2. High concentration of grains of silver (^{3}H-uridine) in reticuloendothelial cells of the liver 2 days after injection of AH.

Stromal cells which, though damaged, were preserved were usually able to synthesize DNA and RNA. Fibroblasts and Kupffer cells incorporated ^{3}H-thymidine and ^{3}H-uridine to different degrees, but much less intensively than macrophages. Lymphocyte-like cells, often arranged side by side and morphologically similar to one another, incorporated different numbers of grains of silver.

Thus in AHF, certain hepatocytes showing a varied degree of damage and, in particular, cells of the reticuloendothelial series, remained viable, as was shown by their ability to incorporate ^{3}H-uridine and, to some extent, ^{3}H-thymidine.

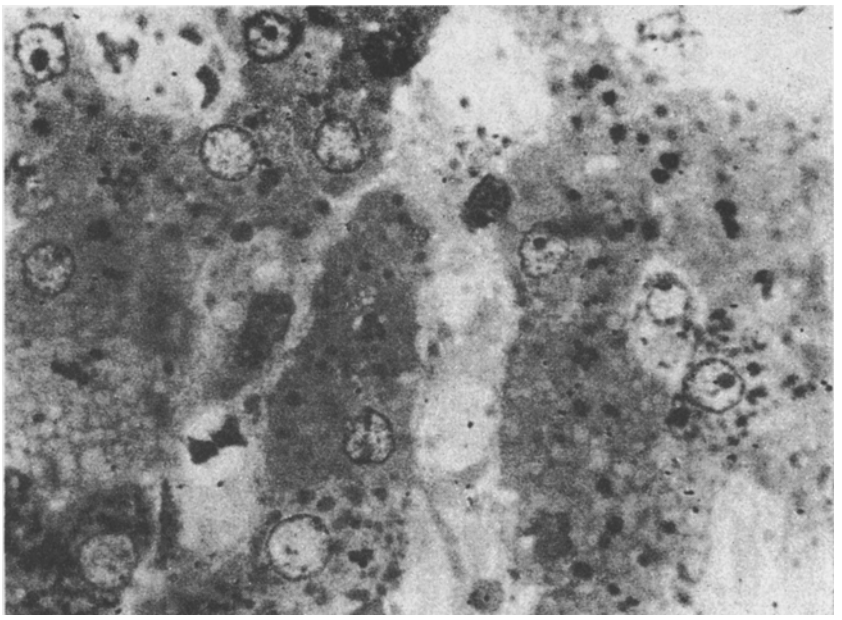

Fig. 3. ^{3}H-Thymidine label above nuclei and in cytoplasm of hepatocytes 4 days after injection of AH.

Autoradiographic study of the liver in AHF, during treatment with AH, gave the most complete idea of the time course of the tissue and cell changes and the possibility of reparative regeneration with restoration of organ-specificity.

The increase in the number of cells synthesizing DNA and RNA and providing for reparative regeneration of the liver, depends directly on the time after AH treatment. Investigations 24-48 h after AH treatment showed that hepatocytes which had undergone marked structural changes were able to synthesize DNA. Numerous enlarged and irregularly distributed hepatocytes were found with hypertrophied nuclei and nucleoli. In these cells incubation with ^{3}H-uridine revealed numerous grains of silver, especially above the nucleoplasm. They were usually not present in the center of the lobule, but at the periphery small groups of them were seen, often together with binuclear cells. Displacement of the label from nucleus into cytoplasm, indicating acceleration of RNA synthesis, was observed in the hypertrophied cells.

High proliferation and metabolic activity was found in the reticuloendothelial cells. In the Kupffer cells and fibroblasts the most active DNA synthesis was found. Nuclei of fibroblasts, macrophages, lymphocytes, and certain mast cells were intensively labeled with ^{3}H-uridine (Fig. 2). Some results gave indirect evidence that mitotic division of hepatocytes with a varied degree of damage was preceded by hypertrophy and intensive RNA synthesis. According to our findings, RNA synthesis is an indicator of an earlier and more intensive regeneration reaction of the cell after injection of AH.

In the later stages of observation (4-8 days after injection of AH) proliferative activity of the hepatocytes in the reticuloendothelial cells showed some decrease. At the periphery of the lobule, a relatively uniform distribution of a small number of grains of silver (^{3}H-thymidine) was observed in most hepatocytes, among the karyoplasm. Cells (more often stromal cells) with a massive concentration of grains of silver over the whole nucleus, and hepatocytes with concentration of grains of silver in the peripheral zone of the nucleus, at sites of heterochromatin, and also cells totally without label, were occasionally seen. Thus marked heterogeneity of the distribution of the thymidine label was observed (Fig. 3). For successful survival of a damaged cell and its subsequent division, preservation of a certain volume of cytoplasm, necessary for reparative DNA synthesis and proliferation, is evidently important. It must be pointed out that the cellular form of regeneration described above, expressed as DNA replication, subsequently turns into an intracellular form and creates the material basis for compensation of the injury. After incubation with ^{3}H-uridine, accumulation of label in both the nucleus and cytoplasm was observed in the hepatocytes, although some of them synthesized RNA weakly, as shown by marked condensation of the chromatin and a small number of grains in the nucleus. Intensified RNA synthesis in considerably altered cells, virtually without any cytoplasmic structures, appears paradoxical. It must be emphasized that incorporation of the labeled RNA precursor was found only in hepatocytes at the center of the hepatic lobule. RNA synthesis proceeded normally in endotheliocytes located in this zone. Increased RNA synthesis in the hepatocytes located at the periphery of the

lobule continued until 4 days after injection of AH. Incorporation of ^{3}H-uridine in this zone, just as in the center of the lobule, was definitely reduced after 4 days.

At later times of observation (after 8-10 days and later) definite parenchymatous-stromal correlation began to appear in the metabolic and proliferative reactions of the hepatocytes and connective-tissue cells of the liver: intensification of metabolic activity in the structural elements named above was accompanied by activation of proliferative reactions of the hepatocytes and stromal cells.

RNA synthesis in AHF in the hepatocytes thus ceases, although it remains capable of resumption and, after injection of AH, resumed RNA synthesis may take place at higher or lower rates depending on local conditions and times of observation.

In AHF some necrobiotically changed hepatocytes evidently maintain their viability and, after injection of AH, a certain proportion of them can proliferate. Intracellular regeneration precedes cell proliferation, and that determines the outcome of the pathological process. AH of the newborn donor evidently possess biologically active properties and facilitate restoration of the structure of the liver. It can be concluded from a general review of the results of this investigation that injection of AH accelerates normalization of morphological and functional disturbances in the liver in AHF and increases the metabolic and proliferative activity both of hepatocytes and of cells of the reticuloendothelial series.

LITERATURE CITED

1. E. I. Gal'perin, S. R. Karagulyan, O. Yu. Abakumova, et al., Khirurgiya, No. 4, 82 (1985).
2. M. S. Margulis, E. A. Erukhimov, and L. A. Andreiman, Anest. Reanimatol., No. 3, 29 (1986).
3. G. E. Ostroverkhov, V. G. Bruslik, S. M. Chechel'nitskaya, et al., Transplantation of Organs and Tissues [in Russian], Tbilisi (1982), p. 140.
4. D. S. Sarkisov, A. A. Pal'tysn, and B. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
5. D. S. Sarkisov, A. A. Pal'tysn, and E. G. Kolokol'chikova, Arkh. Patol., No. 9, 16 (1984).
6. S. Cienfuegos and A. Javier, Rev. Esp. Enferm. Apar. Dig., **70**, 536 (1986).
7. A. A. Demitriou, A. B. A. Whiting, and S. M. Levenson, Ann. Surg., **208**, 259 (1986).
8. M. Kusano and M. Mito, Gastroenterology, **82**, 616 (1982).
9. L. Makowka, L. E. Rotstein, R. E. Falk, et al., Transplant Proc., **13**, 855 (1981).