AUTORADIOGRAPHIC AND ELECTRON-MICROSCOPIC INVESTIGATION OF DARK CELLS OF THE LIVER

L. D. Krymskii, B. V. Vtyurin, K. V. Botsmanov, A. K. Badikova, and K. I. Dzarakhokhov

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The appearance of dark cells in the liver evidently reflects increased function of the liver tissue, the cells themselves being centers of regeneration. The appearance of the largest number of labeled dark hepatic cells after administration of thymidine-H^3 indicates that DNA synthesis is most intensive in their nuclei. Dark cells capable of DNA synthesis, i.e., of regeneration, accumulate in foci of injury in the liver.

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There is no general agreement as yet regarding the sources of regeneration of liver tissue. A. A. Zavarzin and A. V. Rumyantsev [6] and Yu. N. Darkshevich [3] consider that the cambial elements of the liver are the so-called dark cells, with basophilic cytoplasm. The appearance of dark cells in the regenerating liver after partial hepatectomy and in newborn rats are also considered a sign of increased proliferation of the cells [12, 14, 15]. In the stage of regeneration after burn injury, B. V. Vtyurin [2] observed an increase in the number of membranes of the granular endoplasmic reticulum, mitochondria, and ribosomes in some liver cells, and he concluded that these were dark cells. Some authors [5, 8, 11], however, consider that the dark cells are not cambial in nature, but on the contrary, old and worn-out structures.

We considered that autoradiography, supported by electron-microscopic investigations, would supply a reliable answer to the question of the functional significance of dark cells.

EXPERIMENTAL METHOD

Experiments were carried out on 13 albino mice of both sexes weighing 18-20 g, divided into two groups. The experimental mice all received an injection of 0.2 ml 40% CCl_4 solution. The mice of group 1 received CCl_4 in 10 injections at intervals of one day, and those of group 2 received 10 injections at intervals of 14 days. Three animals from each group received an intraperitoneal injection of thymidine-H^3 in a dose of 1.5 μCi/g body weight on the 3rd day after the last injection of CCl_4.

The animals were sacrificed 1.5 h after injection of the isotope. Pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. Autoradiographs were prepared from sections 6-8 μ in thickness. The sections were freed from paraffin and coated with NIKFI emulsion (type M), and exposed in a refrigerator at 4-6° for 34-45 days in lightproof containers.

Development was carried out by the formula recommended by the Motion Picture Research Institute (metol-hydroquinone developer, fixation in 30% hyposulfite solution), sections stained with metatoxylin and eosin.

Autoradiographs of the liver were studied under the optical microscope with magnification of 1000×. Cells were regarded as labeled if their nuclei contained not less than five grains (tracks). Approximately 3000 cells were counted in each section, light and dark (labeled and unlabeled) heptaic cells, and Kupffer cells being counted separately.

Pieces of liver for electron-microscopic investigation were fixed in 2% OsO_4 solution and embedded in a butyl-methyl methacrylate mixture. The sections were examined in the ÉM-7 electron microscope.

Laboratory of Histochemistry and Electron Microscopy, Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR A. A. Vishnevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 9, pp. 105-108, September, 1968. Original article submitted December 28, 1967.

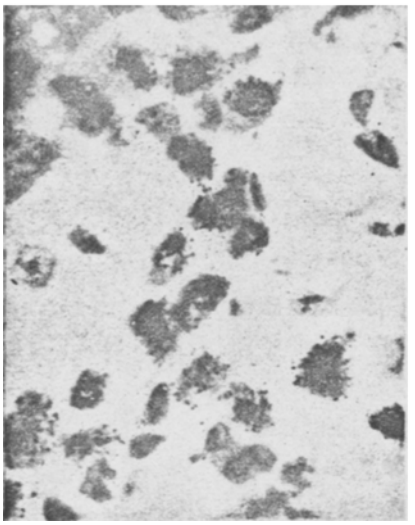

Fig. 1. Autoradiograph of regenerating mouse liver after ten injections of CCl_4 (0.2 ml of 40% solution each time). Numerous dark hepatic cells labeled with thymidine-H^3 can be seen.

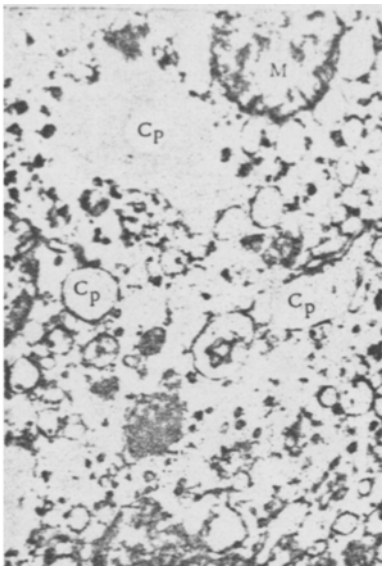

Fig. 2. Enlargement of cavities and tubules of endoplasmic reticulum (Cp) of a light liver cell of a mouse after injections of CCl_4. M denotes a mitochondrion. 25,000×.

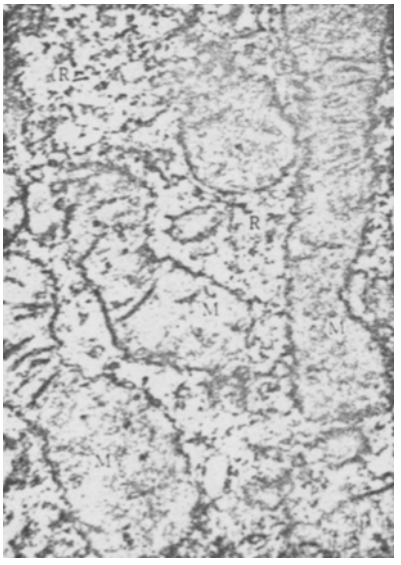

Fig. 3. Numerous ribosomes (R) and mitochondria (M) with dense matrix in a dark liver cell of a mouse after injections of CCl_4. 35,000×.

EXPERIMENTAL RESULTS

With an increase in intensity of reparative regeneration (experiments of series I, injection of CCl_4 at intervals of one day) the number of dark cells in the liver of the mice increased considerably. In the animals of the control group, for instance, the liver contained 8.13% of dark hepatic cells. In animals receiving CCl_4 once every 14 days and in animals receiving CCl_4 in the same dose, but on alternative days (10 injections altogether), the number of dark hepatic cells increased to 17.34 and 30.36% respectively (relative to the total number of hepatic cells counted).

A similar tendency was observed when labeled hepatic cells were counted (Fig. 1). The percentage of labeled dark hepatic cells are much greater in animals receiving CCl_4 injections on alternative days than in those receiving CCl_4 once every 14 days and animals of the control group. The percentage of labeled cells in the animals of these groups was 16.68, 8.82, and 2.84 respectively.

The total number of dark cells and also the number of dark labeled cells were therefore distinctly higher in the liver of mice in a phase of active reparative degeneration than in intact control animals.

The index of labeled Kupffer cells (i.e., the number of labeled cells per hundred cells counted) was 3.7% in the animals of group 1 and 3% in those of group 2, while in the control animals no labeled Kupffer cells could be discovered.

The number of labeled light cells in the intact control animals was negligible, being only 0.18%. As the intensity of reparative regeneration increased the percentage of labeled light hepatic cells increased to reach 2.13 in the animals of group 2 and 6.88 in those of group 1. The number of labeled light hepatic cells thus increased with an increase in intensity of reparative regeneration.

The impression was gained that light hepatic cells are much less able than dark cells to synthesize DNA. The increase in number of labeled light hepatic cells in the actively regenerating liver was associated with the conversion of dark cells into light, frequently after DNA synthesis had taken place in the dark cells.

The difference between light and dark cells in density was also clearly apparent from electron micrographs. The mitochondria in the light cells were swollen, with a translucent matrix, their cristae were disorganized, the cavities and tubules of the endoplasmic reticulum were dilated, and in some places vacuolated. Their cytoplasm possessed very low electron density, and contained few membranes of the granular endoplasmic reticulum (Fig. 2). The dark cells were distinguished by high electron density, numerous mitochondria with a dark granular matrix, a rich endoplasmic reticulum, and also by the presence of numerous free ribosomes (Fig. 3). Many lipid inclusions and lysosomes of different sizes and shapes could be seen in the cytoplasm. Characteristically, the number of dark hepatic cells was particularly large after the end of exposure to the toxic action of CCl_4.

The study of dark liver cells under the electron microscope thus showed that their appearance is evidently a sign of increased functional activity of the liver tissue. Morphological signs of this functional activity are expressed by hypertrophy of these cells, based on hyperplasia of the specific organelles – the mitochondria, endoplasmic reticulum, and ribosomes. The sharp increase in number of these ultrastructures within the cells is responsible for their conversion into the dark cells of the liver, and this process can be seen under the optical microscope using normal staining methods.

Autoradiographic and electron-microscopic investigations revealed not only qualitative, but also the quantitative characteristics of reparative regeneration of hepatic cells in the experimental animals. From these investigations we concluded that the appearance of dark cells is a morphological sign of increased functional activity of the liver tissue, and that the cells themselves are apparently centers of regeneration.

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