ULTRASTRUCTURE OF HEPATOCYTES AFTER MEASURED LOCAL COOLING OF THE LIVER

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KEY WORDS: liver; electron microscopy; low temperatures.

The use of low temperatures in surgical hepatology is based on the destructive action of this physical factor [1, 4, 8]. Meanwhile, when cryosurgical methods are used certain difficulties arise, in connection with the precise prediction of the volume and degree of tissue cell destruction [7]. Data on this subject are very contradictory, so that a detailed study of the fine mechanisms of the destructive action of local cooling is imperative. Elec-

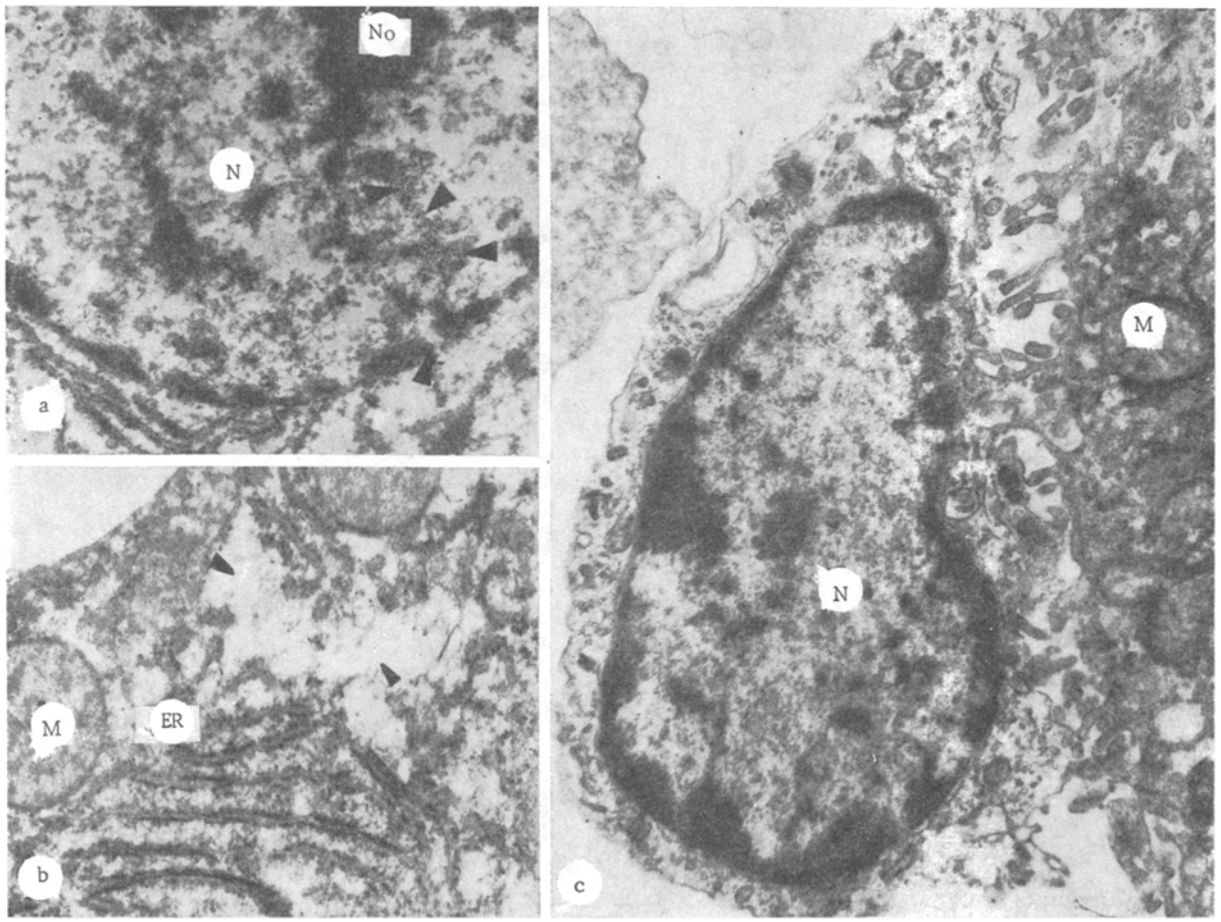

Fig. 1. Ultrastructure of liver cells after cooling (—30°C). a) Hepatocyte nucleus with ribosomal complexes (arrows). Compact chromatin forms canals (arrows). 20,000 ×; b) empty spaces and cavities (arrows) present in cytoplasm of a hepatocyte immediately after rewarming. 30,000 ×; c) area of hepatocyte with Kupffer cell immediately after cooling to —30°C. 20,000 ×. N) Nucleus, M) mitochondira, No) Nucleolus, ER) endoplasmic reticulum.

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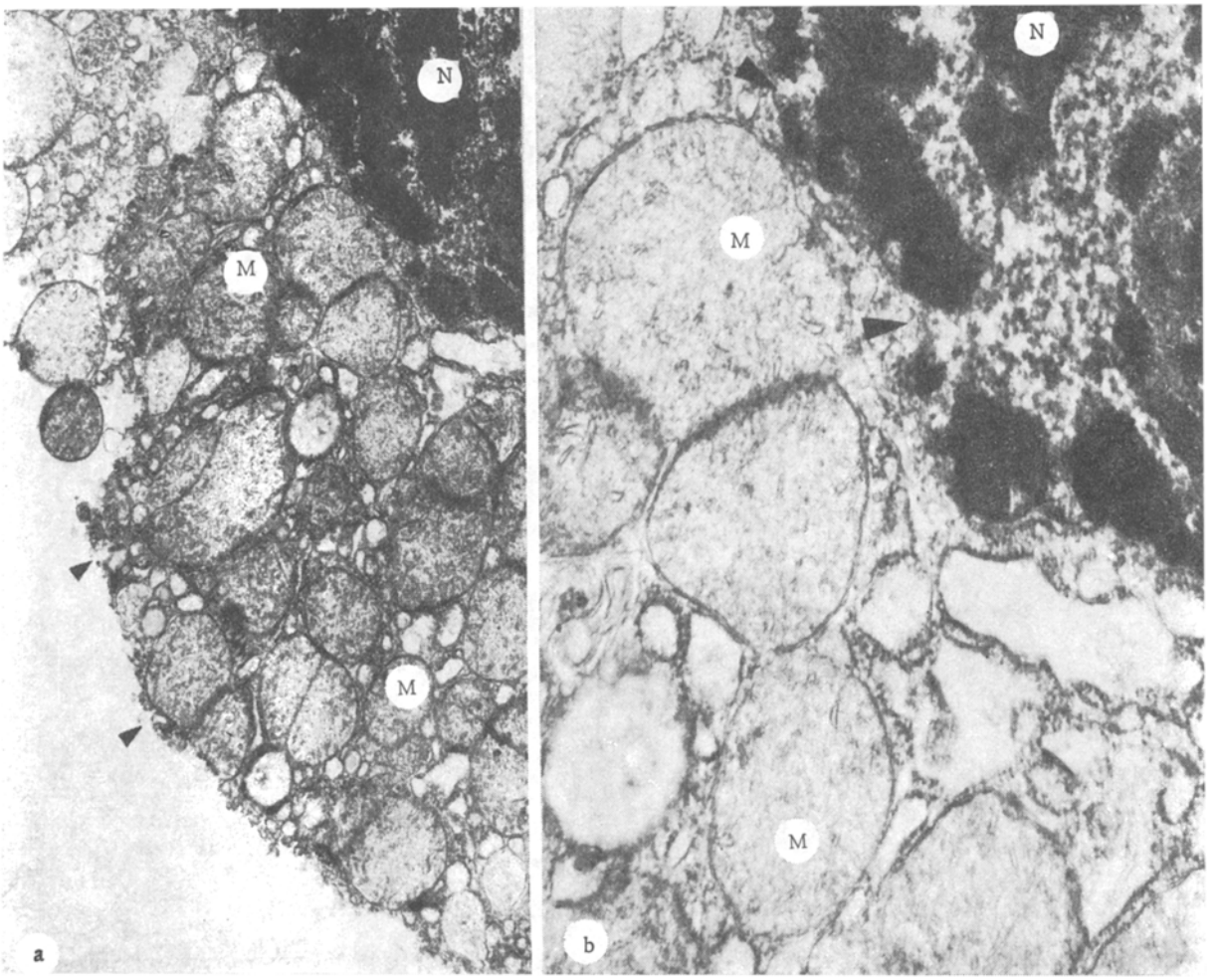

Fig. 2. Endothelial cell of heaptic sinus after cooling (—30°C). a) Disturbance of integrity of plasma membrane (arrows), many mitochondria in cytoplasm located in immediate vicinity of nucleus. 20,000 ×. b) Area of same cell under high power (60,000 ×). Legend as to Fig. 1.

tron microscopy has justified itself as the most informative method for studying the action of low temperatures on biological objects [5, 6].

The aim of this investigation was to study the qualitative and quantitative characteristics of hepatocyte ultrastructure during local cooling (—30°C) *in vivo*.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 200-250 g. A midline laparotomy was performed on the anesthetized animals (thiopental sodium, 4 mg/100 g body weight, intramuscularly) and cold was applied to the liver of the experimental rats by means of a specially designed and produced automatic flat cryoprobe with a tip 10 mm in diameter of the working part of —30°C, with thermographic monitoring of the zone of cooling. The duration of application of cold was 120 sec. After rewarming in air, the liver was fixed intravitally by perfusion through the portal vein with 2% glutaraldehyde solution in cacodylate buffer.

Material for investigation was taken from the region immediately beneath the applicator, cut into small pieces and fixed in glutaraldehyde and osmium fixatives in cacodylate buffer, dehydrated in acetone, and embedded in a mixture of Epon and Araldite. Sections were stained with uranyl acetate and lead citrate and examined in the EM-10 electron microscope (Opton, West Germany) under an accelerating voltage of 60 kV.

For stereometric analysis of the hepatocytes by the method in [2] a test system containing 357 points, and with a constant length of 160 cm, was used. The relative volume and density of the surfaces of the nuclei, the diffuse and compact chromatin, mitochondria

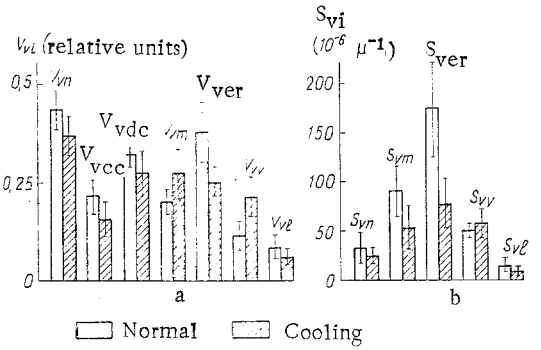

Fig. 3. Stereometric parameters of principal ultrastructural components of hepatocyte under normal conditions and after cooling to −30°C: a) relative volume of nucleus V_{vn}, of compact chromatin V_{vcc}, of diffuse chromatin V_{vdc}, of mitochondria V_{vm}, of vacuoles V_{vv}, of lysosomes V_{vl}; surface density of nuclei S_{vn}, of mitochondria S_{vm}, of ER S_{ver}, of vacuoles S_{vv}, of lysosomes S_{vl}.

endoplasmic reticulum, lysosomes, and vacuoles were calculated. The numerical results were subjected to statistical analysis by Student's method for small samples, on the SM-1 computer.

EXPERIMENTAL RESULTS

Analysis of the ultrastructure of the cooled hepatocytes showed that their round nuclei contained one nucleolus in the center or at the periphery, in the immediate vicinity of the nuclear membrane. The presence of ribosomal complexes was clearly visible in the nucleus (Fig. 1a). Compact chromatin, located at the periphery of the nucleus, formed channels connected with the rather dilated nuclear pores. The nuclear membrane had its usual structure. Migration of the nucleolus to the periphery of the nucleus, toward its membrane, and the presence of ribosomal complexes in the nucleus could be evidence of an increase in the nucleo-cytoplasmic ratios, with strenghening of the regulatory effect of the nucleus on the cytoplasm [9]. The rough endoplasmic reticulum (RER), the cisterns of which had lost their integrity in some places, was located around the nucleus. Empty spaces, evidently connected with focal lysis of the content, were observed in the cytoplasm (Fig. 1b). Round mitochondria were mainly normal in structure, but absence of the outer membrane was noted in some of them.

Electron-microscopic analysis of the hepatic sinus showed that the ultrastructure of the cytoplasm of hepatocytes facing the perisinusoidal space had the usual structure after cooling (Fig. 1c). Their cell membrane had characteristic microvilli making contact with the plasma membrane of the Kupffer cells. The latter were elongated in shape and formed microvilli on their surface. Organoids in the cytoplasm and nucleus of some Kupffer cells were unchanged. However, there were other cells with many mitochondria in their cytoplasm, and whose plasma membrane could not be defined throughout its length (Fig. 2a). The mitochondria were round, with the normal structure of their cristae and matrix. Clarity of outline of the outer membrane of some mitochondria was disturbed and their cristae were thickened. Mitochondria were present in the immediate vicinity of the nucleus, which was elongated in shape, with the nucleoli located at the poles. Some mitochondria were in contact with each other, and at the site of contact formed a single boundary which had the appearance of a blurred electron-optically dense line (Fig. 2b). Alongside the nucleus and mitochondria were dilated cisterns, round and elongated in shape, of the rough and smooth endoplasmic reticulum, (ER; Fig. 2b). The nuclear membranes could not be defined everywhere and the nuclear pores were a little widened. Canals in the compact chromatin were dilated and filled with electron-optically dense polysome-like granules.

Stereometric analysis of the ultrastructure of native and cooled hepatocytes showed changes in certain parameters (Fig. 3a, b). A tendency was noted for the relative volume of the nucleus and ER to decrease. The relative volume of the vacuoles and mitochondria, on the other hand, was increased somewhat after cooling. The density of the surfaces of the

nuclei and lysosomes remained at the same level, whereas the density of the surface of ER and the mitochondria showed a tendency to decline. The surface density of the vacuoles increased.

It can thus be concluded from vital fixation of liver tissue and ultrastructural stereometry that local cooling, under the experimental conditions used, is accompanied by destructive changes in the hepatocytes, expressed as translucency of part of the hyaloplasm, the formation of empty spaces and cavities in the cytoplasm, disturbance of the integrity of mitochondrial membranes, and changes in ER. These qualitative data are supplemented and confirmed by values for the volume and surface density of these structures. Meanwhile the character of changes in the nuclear apparatus of the hepatocytes and Kupffer cells (widening of the nuclear pores and of canals in the condensed chromatin, a peripheral location of the nucleolus, the presence of ribosomal complexes in the nucleus, concentration of mitochondria near the nucleus) suggests strengthening of the regulatory influence of the nucleus on cytoplasmic structures, associated, evidently, with the commencement of repair processes.

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QUANTITATIVE ANALYSIS OF DENDRITIC BRANCHING PATTERNS IN STRIATAL NEURONS BY THE LEITZ ASM SYSTEM

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KEY WORDS: quantitative analysis; dendritic system; long-axon neurons; striatum.

The neuronal organization of the mammalian striatum has been the subject of much research [10-12, 14]. However, the facts so far obtained are very contradictory. According to our own data [4, 5] the striatum contains two classes of long-axon cells: densely branched arborescent spinous neurons (small and medium-sized cells, accounting for about 96% of the total number of cells) and sparsely branched reticular neurons (large cells, accounting for about 1% of all neurons). Until now spinous cells in many publications have been regarded as short-axon neurons, even though investigations by the horseradish perioxidase method have demonstrated their long-axon nature [7, 9, 13].

The dendritic system of long-axon striatal neurons was studied in the investigation described below in a comparative series of mammals, using quantitative methods of analysis.

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