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ULTRASTRUCTURAL DETECTION OF CHOLESTEROL IN THE LIVER OF ALCOHOLIC RATS

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Key Words: hepatocyte ultrastructure; cholesterol; alcohol

Disturbance of lipid metabolism is one of the main features of liver disease. At the hepatocyte level this is manifested as accumulation of lipids and changes in the intracellular organelles [1, 7, 8]. Deposition of cholesterol in the liver is one form of disturbed lipid metabolism. There are few reports in the literature on this subject [2, 5, 6]. The aim of this investigation was to study the distribution of cholesterol in hepatocytes and to determine its effect on the state of the intracellular ultrastructures in the liver during alcohol poisoning.

EXPERIMENTAL METHOD

Cholesterol deposition was studied in 35 male albino rats weighing 180-220 g, receiving alcohol in a dose of 2 g/kg body weight by the intragasric route. The liver was studied electron-microscopically 1, 7, 14, 30, and 45 days after the beginning of alcohol administration. The control group consisted of 15 intact animals. For a general electron-microscopic survey the liver tissue was treated by the usual method. Electron-cytochemical demonstration of free cholesterol was undertaken by a reaction based on the formation of a cholesterol–digitonin complex [9, 10]. We developed a perfusion method of fixing the liver and incubating it in situ: under ether anesthesia laparotomy was performed and the liver perfused through the portal vein. To maintain the necessary pressure in the system, a perfusion apparatus [4] was used. The indicator of successful perfusion was a uniform pale yellow color of all the lobes of the liver. For prefixation, a 4% glutaraldehyde-formaldehyde fixing solution, made up in Hanks' buffer, pH 7.2, at 20°C was used for 5 min (70-150 ml of solution, or on average 5 ml/min/100 g body weight). Immediately after prefixation, the liver was perfused for 5 min with incubation medium (50-70 ml) containing pH 7.2, at 20°C (full details of the method of preparing the solutions are given by Loginov et al. [3]). After incubation the liver tissue, cut up into pieces measuring 1 mm^3, was transferred to a fresh portion of 0.2% digitonin for 4 h or allowed to stand overnight at 4°C. The specimens were then washed with 0.1 M cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4 in cacodylate buffer for 2 h. The following schedule of dehydration and embedding in Araldite was used: 30-70° alcohol 5 min, 96° alcohol (three changes, 5 min each), saturation with a working mixture of M Araldite: N Araldite (1:1) with 2% catalyst for 2 h at room temperature. In the final stage the fragments were transferred into a fresh portion of the working mixture for 2 h at 37°C, after which they were embedded in Araldite and kept for 2 days at 60°C. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-1200 EX electron microscope. The instrumental magnification was 5000-50,000.

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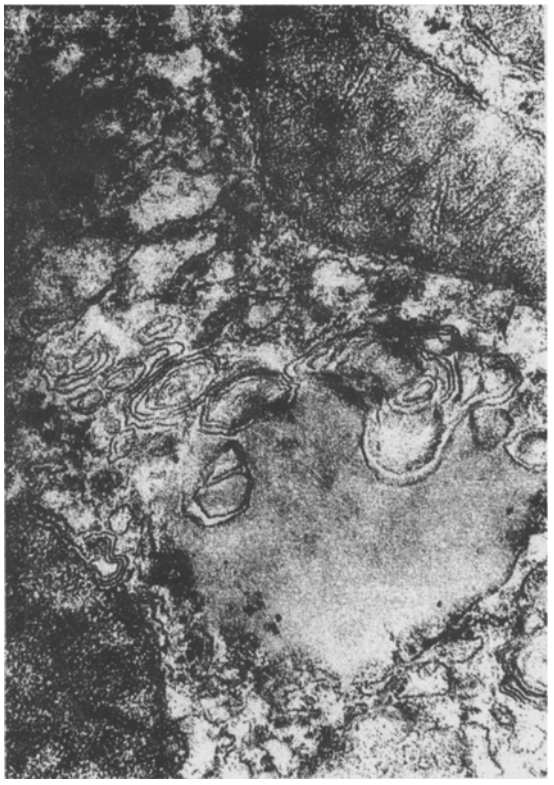

Fig. 1. Hepatocyte of rat after 1.5 months of alcohol poisoning. Deposition of cholesterol as laminated structures in hyaloplasm, close to mitochondria and in lipid drop. Reaction of formation of cholesterol—digitonin complex. 50,000×.

EXPERIMENTAL RESULTS

Lipid inclusions in the control animals were found in individual hepatocytes in the form of small drops consisting of triglycerides. Cholesterol (henceforward this term will be used instead of "cholesterol—digitonin complex," because the cytochemical reaction product reflects the location of free cholesterol), in the form of single coils, cylinders, and laminated formations, was located in the hyaloplasm, close to the organoids and lipid drops, and very rarely in the intercellular spaces.

Alcohol poisoning led to marked changes in the liver. An increase in the content of lipids, including cholesterol, was observed in the hepatocytes 24 h after administration of alcohol to the animals. Triglycerides were located most frequently at the sinusoidal pole in the form of drops of different sizes. Where they were in contact with mitochondria and the rough endoplasmic reticulum, the membranes of the latter were often destroyed. Cholesterol was located near the nucleus and around the periphery of the cell. It could be unconnected with the intracellular organoids, and distributed freely in the hyaloplasm in the form of small concentrations of coils amd cylinders, and also of laminated formations with dense or loose packing of their component elements. Sometimes the laminated formations were found inside the rough endoplasmic reticulum, when the membranes of the tubules were largely destroyed. The very small quantity of cholesterol located around the periphery of individual lipid drops will be noted. Besides its intracellular accumulation, cholesterol also was deposited in the extracellular space. Although the intercellular spaces were not widened, small areas of destruction of the plasma membranes of two adjacent cells were found, where cholesterol could be seen in the form of coils and cylinders.

After alcohol poisoning for 7-14 days the lipid content in the hepatocytes increased. Accumulations of cholesterol in the hyaloplasm were found most frequently in the form of coils and cylinders. Where cholesterol was closely linked with mitochondria, the membranes of the latter were destroyed. Deposition of cholesterol also was found around the periphery and inside the lipid drops in the form of coils and cylinders. Cholesterol was deposited extracellularly in the intercellular spaces. In this case regions of destroyed plasma membranes were very extensive. The largest amount of cholesterol was found at the points of transi-

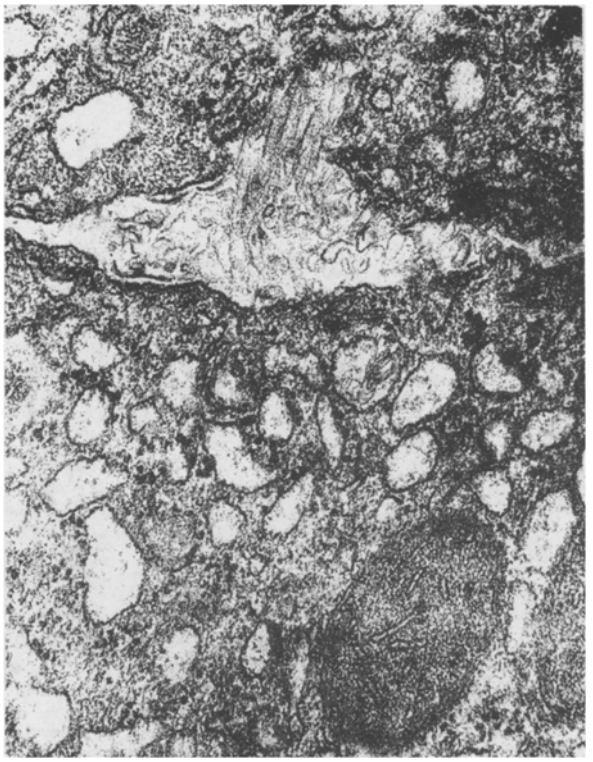

Fig. 2. Hepatocytes of rat after 1.5 months of alcohol poisoning. Deposition of cholesterol as coils and cylinders in widened intercellular space. Cytoplasmic membranes of two hepatocytes in this area are destroyed. Reaction of formation of cholesterol–digitonin complex. 65,000×.

tion from intercellular spaces into Disse's space. Single bundles of collagen fibrils were distributed in the neighborhood of these regions.

After exposure to alcohol for 1-1.5 months the hepatocytes differed greatly from one another in lipid deposition. In cells showing the most marked changes lipid drops were arranged in groups. The cholesterol appeared much more frequently in this case as lamellar bodies with loose packing of their components, and mainly located near the biliary pole and in the intercellular spaces. Sometimes lamellar bodies, but with dense packing, were found inside the mitochondria and in tubules of the rough endoplasmic reticulum. Laminated formations located inside lipid drops partly or completely replaced them (Fig. 1). Extracellular deposition of cholesterol in the form of laminated formations or, less frequently, of coils and cylinders was accompanied by widening of the intercellular spaces (Fig. 2) and destruction of plasma membranes of neighboring cells over a wide area. Well-developed bundles of collagen fibrils were located in Disse's spaces close to cholesterol deposits.

Thus alcohol poisoning causes profound disturbances of lipid metabolism in the rat liver, expressed as the appearance of lipid drops and cholesterol deposits in the hepatocytes. Two structural forms of cholesterol–digitonin complex were discovered: in the form of coils and cylinders, and in the form of laminated structures, differing in the packing density of their component elements. Cholesterol deposition inside the cell is accompanied by destruction of organoids. The extracellular cholesterol deposits cause widening of the intercellular spaces and destruction of the cytoplasmic membranes, leading to disturbance of intercellular junctions. All the changes noted above increase with an increase in the duration of alcohol poisoning, and they may lead to death of the hepatocytes.

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AGE CHANGES IN ENZYMES OF NEURONAL AND MICROVASCULAR ENERGY METABOLISM IN THE BRAIN OF SPONTANEOUSLY HYPERTENSIVE RATS

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Key Words: arterial hypertension; neuronal energy metabolism; microvessels

One of the most important risk factors of cerebrovascular disturbances is arterial hypertension (AH), as epidemiologic studies in man [7, 12] and experimental models [14] have demonstrated. The brain of hypertensive animals is more vulnerable to hypoxia and ischemia [2, 9]. The traditional explanation of these facts is to attribute them to disturbances of regulation of the cerebral circulation under conditions of AH [4, 10]. Yet there have been few investigations into the metabolism of brain tissue and of the microvessels, although there is evidence [1, 13] that they may play a decisively important role in the severity of brain damage when the circulation in them is disturbed.

The aim of this investigation was to compare some aspects of metabolism of neurons and microvessels in the brain of spontaneously hypertensive (SH) rats at different age periods.

EXPERIMENTAL METHOD

Experiments were carried out on SH rats and on control normotensive Wistar—Kyoto rats. The experimental animals were obtained from the Experimental Biological Models Group (Director, Yu. S. Dmitriev) of the I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR. Three groups of control animals and three of SH rats aged 1.5, 3, and 6 months were studied (six, eight, and seven animals in each group respectively). All the animals were kept under identical conditions: the systolic blood pressure (SBP) was measured in the caudal vessels by an indirect method in rats of groups 2 and 3 several times in the course of 3 weeks. Material for investigation was obtained after decapitation of the animals. A piece was excised through a parasagittal incision from the right cerebral hemisphere, measuring 0.6 × 0.4 × 0.3 cm, corresponding to the sensomotor cortex,

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