By the end of the 2nd week of the postresuscitation period the ultrastructure of the heart muscle was largely restored to normal. As a result of intensive repair processes gradual regeneration of the T system was observed and its relative volume in the cardiomyocytes increased to 1.35 ± 0.12%, and the differences from the control were no longer significant.

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ULTRASTRUCTURAL LOCALIZATION OF α-FETOPROTEIN SYNTHESIS IN THE REGENERATING MOUSE LIVER

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KEY WORDS: liver; regeneration; α-fetoprotein; immunocytochemistry; ultrastructure.

After partial hepatectomy or the action of various hepatotoxins a sharp but temporary rise in the α-fetoprotein (AFP) level is observed in the animals' blood, and cells containing AFP are found in liver sections at these times [1]. Cells containing AFP have been shown by the ordinary immunomorphologic methods to be typical differentiated hepatocytes. In adult mice after the action of hepatotoxins as a rule these cells are few in number and they are located mainly on the boundary with the injured tissue [4, 6, 7]. These data suggest that during regeneration of the mouse liver temporary derepression of AFP synthesis takes place in mature differentiated hepatocytes [1]. However, only an immunoenzymic technique of antigen localization at the electron-microscopic level would enable the cells synthesizing this protein to be confidently differentiated from cells passively accumulating it as a result of toxic injury. Such a technique was used previously to detect AFP-synthesizing cells in human and mouse hepatomas, in human fetal liver, and in the liver of animals during chemical carcinogenesis [9, 10, 14, 15].

In the investigation described below an electron-microscopic immunoperoxidase technique [11] was used to identify and characterize AFP-synthesizing cells during regeneration of the mouse liver.

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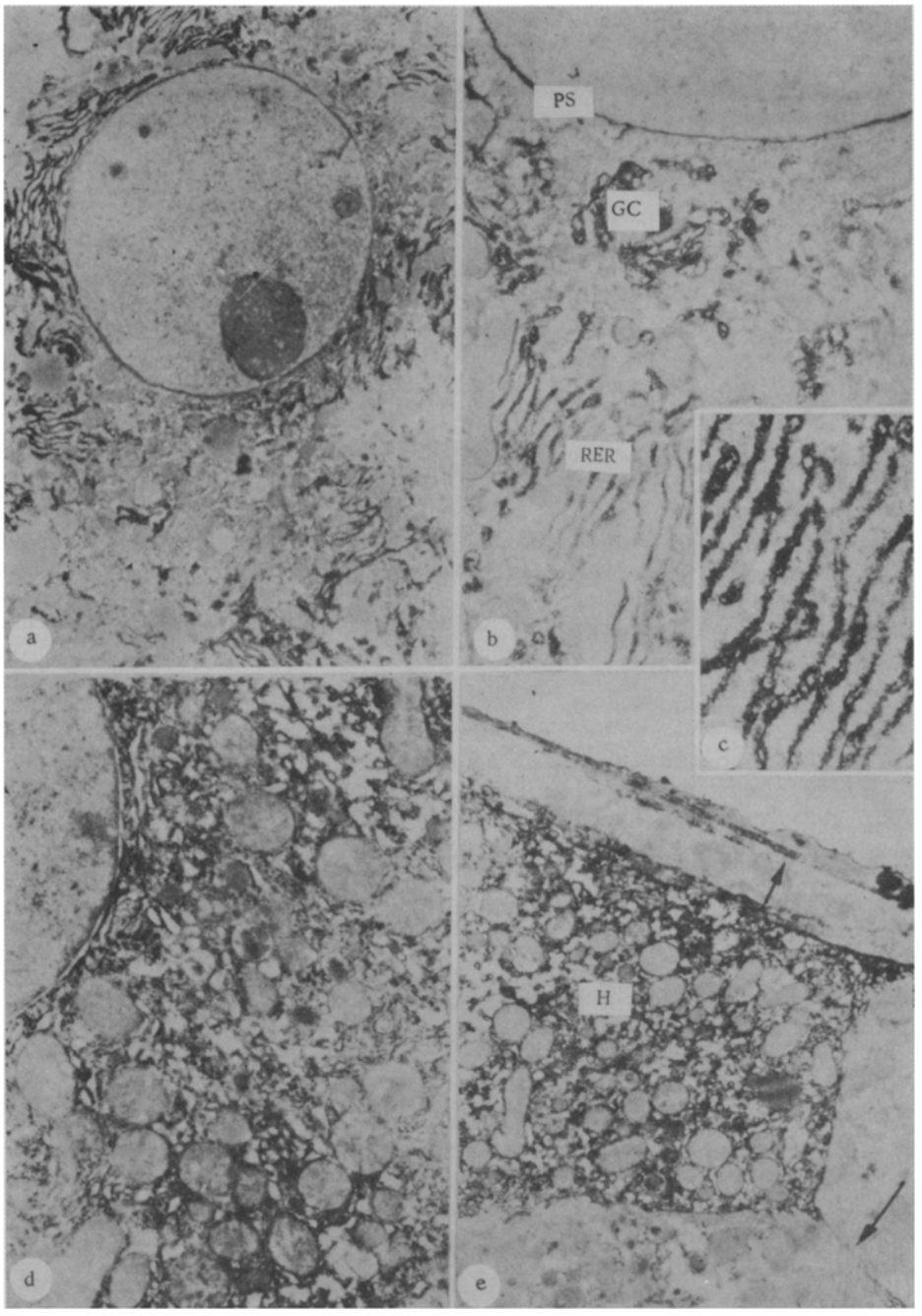

Fig. 1. Immune electron microscopy of mouse liver 72 h after CCl_4 poisoning: a) fragment of mature AFP-containing hepatocyte in perinecrotic zone, 5000×; b) localization of AFP in perinuclear space (PF), on ribosomes and membranes of rough endoplasmic reticulum (RER), in lumen of cisterns of RER and elements of Golgi complex (GC), 15,000×; c) location of AFP on membranes, ribosomes, and in lumen of RER, 30,000×; d) fragment of hepatocyte from perinecrotic zone with diffuse deposition of reaction product in cytosol, 12,500×; e) location of mouse IgG in Disse's space, intercellular spaces (arrow), and in cytosol of hepatocyte (H), 5000×.

EXPERIMENTAL METHOD

Regeneration of the liver was induced in SWR mice aged 2-3 months by poisoning with CCl_4 vapor [3]. The animals were killed 72 h after poisoning. Rabbit antisera (AS) against purified mouse AFP [9] were exhausted with a sorbent of normal mouse serum based on Sepharose 4B—CNBr, after which neonatal mouse sera on Sepharose sorbent distinguished monospecific antibodies (AB) against AFP. Some AB against AFP were neutralized with a small

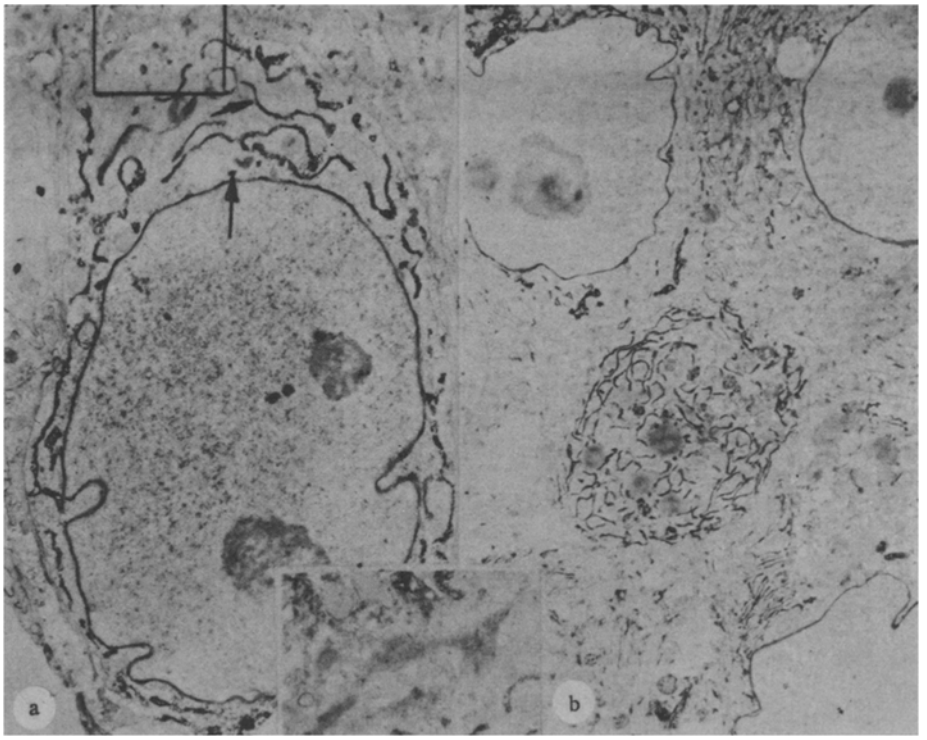

Fig. 2. AFP synthesis in "small hepatocytes" of perinecrotic zone: a) reaction product located in perinuclear space and cisterns of RER (arrow) of "small hepatocyte," 7500× (inset shows bile capillary; 15,000×); b) group of "small hepatocytes," 4000×.

excess of pure AFP [2]. Rabbit AF against mouse IgG was diluted 1:100 with buffered physiological saline (BPS). Goat AB against rabbit IgG were conjugated with horseradish peroxidase (RZ = 2.75; from Sigma, USA) by a periodate method [12].

Pieces of liver 1 mm^3 in volume were prefixed with a cold mixture of 8% paraformaldehyde and 0.05% glutaraldehyde for 20 min, then fixed in 8% paraformaldehyde for 16 h with constant mixing and at 4°C. The fixatives were made up in 0.1 M cacodylate buffer, pH 7.3, with the addition of 7% sucrose. After washing for 24 h with the same buffer the pieces of liver were frozen in liquid nitrogen and frozen sections were cut to a thickness of 15 μ; 20 to 25 freely floating sections were incubated in 0.5 ml of a solution of AB against mouse AFP for 2 h at room temperature with gentle stirring. As a control of the specificity of the reaction some sections were incubated either with neutralized AB against AFP or with AS against mouse IgG. After incubation the sections were rinsed 3 times with BPS, washed for 1 h in three changes of BPS, and incubated in 0.5 ml of AB against rabbit IgG, conjugated with peroxidase. The sections were then washed for 14-16 h in cold BPS, sprinkled with deionized water, and peroxidase activity was demonstrated with the aid of 3,3-diaminobenzidine [8]. After rinsing with water 3 times the sections were fixed for 1 h in 1.33% osmium tetroxide solution in 0.1 M phosphate buffer, pH 7.3, quickly dehydrated in acetone solution of increasing concentration, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on the LKB Ultrotome (Sweden). Areas of liver for subsequent electron-microscopic analysis were chosen in unstained semithin sections and blocks were trimmed accordingly. The ultrathin sections were examined without further staining in the JEM-100CX electron microscope.

EXPERIMENTAL RESULTS

A positive intracellular reaction for AFP was shown electron-microscopically as localized electron-dense precipitates of reaction product.

Most of the hepatocytes in the experimental material did not contain AFP. AFP was found in only a small percentage of hepatocytes, which as a rule were arranged in a narrow zone of parenchyma, containing two or three layers of cells, at the periphery of foci of necrosis in the center of the hepatic lobule. Cells containing AFP were either directly

adjoining foci of cell debris or were at a distance of one or at the most two hepatocytes from them. Sometimes hepatocytes with AFP were located beneath the endothelium of venous sinuses, whose wall was involved in a necrotic focus. Most hepatocytes containing AFP resembled in their ultrastructural features typical differentiated hepatocytes with large nuclei, with a fairly considerable volume of cytoplasm, and with a mean diameter of 20-25 μ (Fig. 1a). AFP was found in these cells in the perinuclear space, on membranes and ribosomes of the rough endoplasmic reticulum (RER), in the lumen of its cisterns, and in elements of the Golgi complex (Fig. 1b, c). However, the degree and extent of staining of these organelles with the reaction product varied from cell to cell. In some hepatocytes AFP was found mainly in cisterns of the RER, in others in the Golgi complex, where it was represented by five or six separate groups of structures. In some hepatocytes AFP was present only in the perinuclear space or, in very small quantities, on membranes and ribosomes of single narrow cisterns of the RER. It is evident that hepatocytes with this sort of trace reaction could not be found by light microscopy.

The presence of AFP in the test material was not limited to typical, mature hepatocytes. AFP also was found in the perinuclear space and RER cisterns of smaller cells, 10-15 μ in diameter, with a large oval nucleus and a small volume of cytoplasm (Fig. 2a). These cells had no definite location, they were usually single, but sometimes they formed groups of 3 or 4 cells (Fig. 2b). These small cells must evidently be regarded as "small hepatocytes," because typical bile capillaries were constantly found both between adjacent mature and "small hepatocytes" (Fig. 2, inset), and also in groups of the latter.

Besides the local deposits of reaction products described above, in individual hepatocytes in the perinecrotic zone large foci of deposition or diffuse precipitation of the product were observed in the cytoplasmic matrix (cytosol) and on membranes of organelles, although there was no reaction product in the lumen of these organelles (Fig. 1d). A similar diffuse distribution of the reaction product was found in the same zone in some of the hepatocytes when sections were incubated with AB against IgG (Fig. 1e), evidence in support of passive penetration of IgG and AFP into these cells from the blood as a result of their toxic injury. Outside the cells IgG was present on the surface of the endothelium of the venous sinuses, in Disse's spaces, and in the intercellular spaces between the hepatic trabeculae as far as the zone of tight junctions (Fig. 1e).

Sometimes hepatocytes containing AFP both in the cytosol and in the lumen of the RER cisterns were observed. These cells had no clear ultrastructural signs of injury. This type of localization of AFP could be connected either with necrobiotic changes in AFP-synthesizing cells or with their inadequate fixation.

In the control series of sections treated with AB against AFP, neutralized with pure antigen, the reaction product was found only in peroxisomes and lysosomes of hepatocytes (endogenous peroxidase) and in erythrocytes (peroxidase-like activity).

This investigation thus confirmed the results of light-optical observations [1, 4, 6, 7] and showed that derepression of AFP synthesized actually takes place in the regenerating mouse liver. The main site of localization of AFP is the cytoplasm of a few mature differentiated hepatocytes in the perinecrotic zone. The presence of AFP in the perinuclear space, on the membranes and ribosomes of RER, in the lumen of the RER cisterns and in elements of the Golgi complex indicates that these cells synthesize AFP. Moreover, smaller cells, which we called "small hepatocytes," also participate in AFP synthesis under these conditions. In their ultrastructure these cells resemble the oval cells described in the rat liver during chemical carcinogenesis [15]. The discovery of the role of the "small hepatocytes" in the process of liver regeneration and hepatocarcinogenesis is a very interesting problem which requires further study.

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CHANGES IN RELATIONS BETWEEN QUANTUM-VESICULAR PARAMETERS WHEN SECRETION OF SYNAPTIC TRANSMITTER IS DISTURBED IN MAMMALIAN NEUROMUSCULAR JUNCTIONS

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Data which evidently are opposed to the quantum-vesicular hypothesis of secretion have recently been obtained as a result of the development of new, notably biochemical approaches to the study of the mechanisms of secretion of synaptic transmitter [8, 10]. This has drawn added attention to its basic principles, especially in relation to exocytosis of transmitter from the vesicles, as the basis of the quantum character of secretion [7-10]. One of the most valid methods of testing these principles is assessment of the structural and functional correlation under the influence of factors causing oriented changes in the state of the secretion process. In the investigation described below this type of analysis was used to study the mammalian neuromuscular junction poisoned by tetanus toxin (TT), which inhibits the liberation of transmitter [1, 2, 4]. To test the hypothesis of formation of "quanta" of transmitter within the cytoplasm, in the synaptic vesicles (SV), some parameters of SV were compared in motor endings and in spontaneously arising "quantum" reactions — in miniature end-plate potentials (MEPPs).

EXPERIMENTAL METHOD

August rats weighing 100-120 g were used. The test objects were isolated preparations of the diaphragm and phrenic nerve, placed in a controlled-temperature (35°C) chamber through which was passed carbogenized (95% O_2 + 5% CO_2) Tyrode's solution of the following ionic composition (in mM): Na^+ 150; K^+ 2.7; Ca^{2+} 2.0; Mg^{2+} 1.0; Cl^- 145.7; HCO_3^- 12.0; $H_2PO_4^-$ 1.0; glucose 11.0. MEPPs were recorded intracellularly, using glass microelectrodes filled with KCl solution (2.5 M), with a resistance of 10-20 MΩ, selected for low noise level. Electrical activity was photographed from an oscilloscope screen. For electron-microscopic investigation the diaphragm was fixed successively in formaldehyde (4%) and osmium tetroxide and embedded in Araldite. TT in a dose of 2×10^5 MLD for mice was injected under ether anesthesia into the substance of the diaphragm 3-3.5 h before isolation of the preparation.

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