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STRUCTURAL CHANGES IN THE CYTOSKELETON IN REGENERATING MOUSE LIVER CELLS

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KEY WORDS: intermediate filaments; actin cytoskeleton; regeneration of the liver.

Synthesis of the embryo-specific serum protein a-fetoprotein (AFP) is observed during regeneration of the liver induced, in particular, by the action of CCl_4 vapor [1, 2]. In most lines of mice AFP synthesis is carried out after exposure to CCl_4 by only a small proportion of hepatocytes (not more than 10%), which are characterized by a mainly perinecrotic distribution [5]. Redistribution of membrane antigens is observed under these circumstances on the surface of perinecrotic hepatocytes [4]. This redistribution, which correlates well with expression of AFP synthesis, has led the "embryonalization" of the perinecrotic cells to be linked with local structural changes in junctions between hepatocytes. The system of prekeratin intermediate filaments (tonogilaments) is, as we know [6, 7], in close connection with interhepatocytic junction structures, and evidently plays a role in their creation and stability. Accordingly the reorganization of intercellular interactions arising in regenerating parts of the liver must somehow or other be reflected in the tonofilament system and also, probably, in other structures of the hepatocyte cytoskeleton.

The aim of this investigation was to study the connection between the above-mentioned phenomena by indirect immunofluorescence with specific antibodies.

EXPERIMENTAL METHOD

AKR and SWR mice of both sexes, aged 3-4 months, were used. Liver damage was induced by poisoning with CCl_4 vapor [2]. The mice were killed 1-4 days after poisoning. Pieces of liver either were frozen in 7% gelatin at the temperature of liquid nitrogen, after which sections 5 μ thick were cut in a cryostat and then fixed for 5 min in 4% formaldehyde in 0.1M phosphate buffer, pH 7.4, or were fixed by perfusion with 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4, with the addition of 0.05% of saponin, and were then embedded in paraffin wax. Frozen sections revealed only water-insoluble antigens, including cytokeratins and actin. Both water-soluble AFP antigen and cytokeratins could be demonstrated in paraffin sections. To demonstrate the latter it was necessary to incubate the dewaxed sections, before treatment with antibodies, for 30-60 min at room temperature in 0.05% trypsin solution in physiological saline. The distribution of AFP, cytokeratin, actin, and biliary capillary antigen (AGI) were investigated by the indirect immunofluorescence method. Monospecific rabbit antibodies against AFT were provided by A. I. Gusev and V. S. Poltoranina, and antibodies against action by A. D. Bershadskii. Rabbit antibodies against Poltoranina, and antibodies against actin by A. D. Bershadskii. Rabbit antibodies against AGI were provided by N. I. Kuprina and T. D. Rudinskaya [4]. Preliminary experiments showed that monoclonal antibodies against rat liver cytokeratin interact equally effectively with mouse cytokeratin. FITC-Labeled pig antirabbit antiserum (from "Daco," Denmark) and FITC-labeled rabbit antimouse serum (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were used as the second antibodies.

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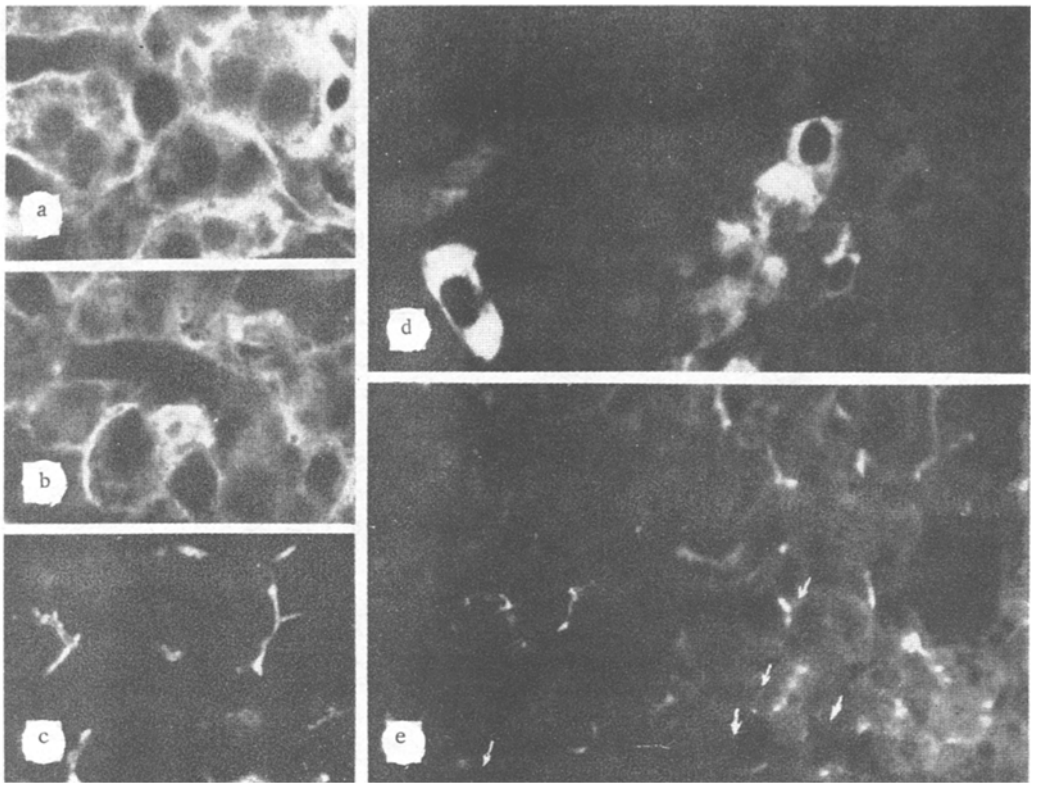

Fig. 1. Localization of prekeratin (a), actin (b), and AGI (c) in paraffin sections through normal mouse liver. d, e) Serial sections through mouse liver on 3rd day after poisoning with CCl_4 vapor, treated with antibodies against AFP (d) and AGI (e): decrease in content of AGI and its disappearance in perinecrotic hepatocytes, some of which contain AFP. Arrows in e denote AFP-positive cells.

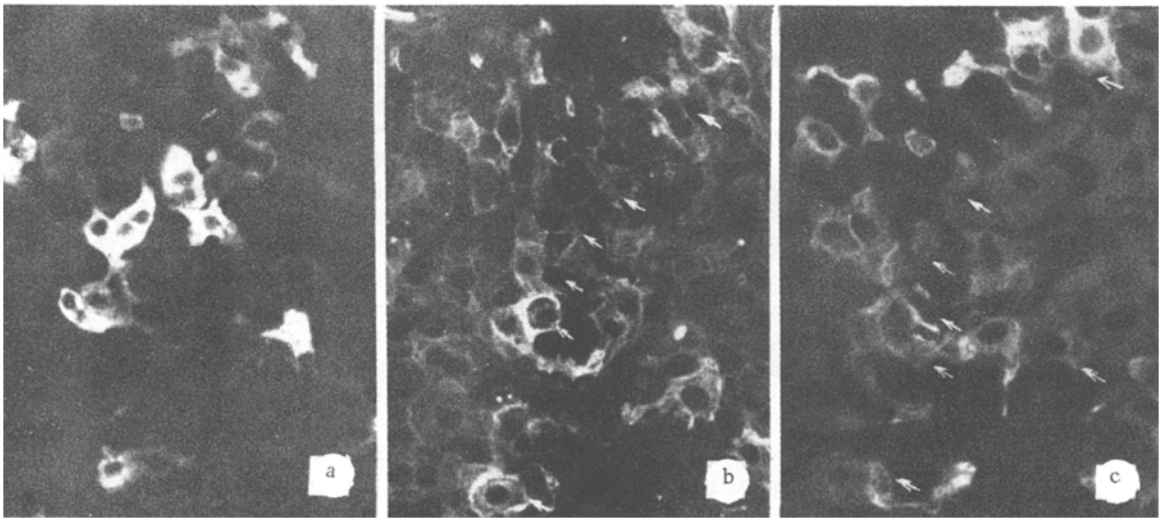

Fig. 2. Localization of AFP (a), prekeratin (b), and actin (c) in serial sections of mouse liver on 2nd day after poisoning with CCl_4 vapor. Intensification of staining for actin and prekeratin in perinecrotic hepatocytes, some of which contain AFP. Arrows in b and c denote AFP-positive cells.

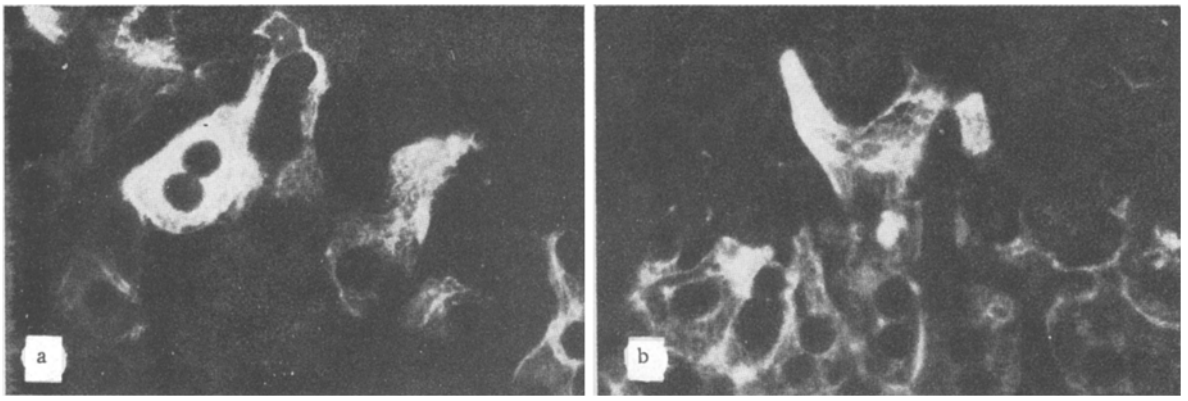

Fig. 3. Typical localization of prekeratin (a) and actin (b) in perinecrotic hepatocytes.

EXPERIMENTAL RESULTS

AGI, marking biliary capillaries, was found on the surface of all hepatocytes in the liver of adult AKR and SWR mice (Fig. 1c). Prekeratin and actin were located mainly beneath the membrane, ornamenting its inner surfaces, which faced both sinusoids and regions of biliary capillaries (Fig. 1a, b). No AFP was found in the cells. The same topography of these antigens was described previously for the adult mouse liver [4, 6].

After CCl_4 poisoning centrilobular necroses formed in the liver during the next 24 h. Single AFP-containing cells appeared on the 2nd day of regeneration. By the end of the 2nd day a perinecrotic layer of cells containing AFP was formed. Until this moment no appreciable changes were found in the localization of the remaining antigens. It will be noted that the time required for the appearance of changes in the localization of antigens could vary a little from one experiment to another, but their order always remained the same.

The maximal number of AFP-containing cells after CCl_4 poisoning was observed as a rule on the 3rd-4th day (Fig. 1d). Loss of AGI in the perinecrotic region (Fig. 1e) was observed at the same time. Considerable structural changes in the cytoskeleton were found at this same stage in cells located in the region of necrosis. These differences were most clearly visible in the layer of hepatocytes immediately adjacent to the affected regions (Figs. 2 and 3). The hepatocytes in this layer were elongated. They stained significantly brighter than the remaining cells with antibodies against prekeratin and actin. Clearly visible internal bundles of actin and prekeratin appeared in them. The prekeratin bundles formed a branched inner network, whereas actin bundles were mainly oriented parallel to the long axis of the cells. No such bundles were found in hepatocytes of the normal liver lobule.

In CCl_4 poisoning during regeneration of areas of the hepatic lobule not only does a change take place in the hepatocytes participating in regeneration, with respect to several markers, from the "adult" phenotype to the "embryonic," but considerable structural changes also take place to the cytoskeleton and structures connected with it. The causes of these changes are not yet clear and, in particular, we do not know whether they are interconnected by any direct mechanism. The largest number of cells containing AFP following the action of CCl_4 was observed as a rule when the damaged areas of the liver were cleared of fragments of dying hepatocytes and replenished with cells of macrophage type. At that moment local reorganization of the definitive trabecular structure was taking place in the perinecrotic region, and assumed the following form. Hepatocytes of this region changed their shape and migrated into the necrotic focus. Their characteristic packing was disturbed under these circumstances. This phase of regeneration, as was shown previously, is characterized by changes in morphology of the cells and loss of AGI, marker of biliary capillaries. The present investigation showed that these same cells, at the same phase of regeneration, have a reorganized cytoskeleton. In perinecrotic cells long bundles of actin microfilaments, oriented along the long axis of the cells are formed, together with prekeratin bundles, forming a dense internal network. Probably these changes in the hepatocyte cytoskeleton are necessary for active movement. Hepatocytes with altered morphology are not completely separated from the remaining mass of cells, although no AGI is found on their surface. A variant of migration of the epithelial sheet of cells probably takes place in this case, when the whole sheet

migrates immediately after the leading active cells [3, 8]. Unfortunately, it is impossible at present to reproduce the precise sequence of events leading to migration. It is not clear, for instance, whether the initial stage of this process is a change in the intercellular junctions (loss of AGI) or whether these changes arise through active movement of peripheral cells.

The main conclusion from this investigation can be taken to be the discovery of definite correlation between loss of AGI, the appearance of bundles of prekeratin and actin, and expression of AFP synthesis. It is therefore possible to include all these features in a single marker complex of "embryonalization" of the hepatocyte.

For a more detailed study of relations between all these features and processes of reorganization of the structure of the liver, simpler systems reproducing this process in tissue culture are probably necessary.

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ULTRASTRUCTURE OF NEURONS AND INTERNEURONAL CONNECTIONS IN THE SENSOMOTOR CORTEX OF PROGENY OF ALCOHOL-ADDICTED RATS

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KEY WORDS: neurons; interneuronal connections; alcohol intoxication; progeny.

There is clinical evidence of the high degree of risk that parents who are chronic alcoholics will produce physically and mentally defective children. However, the morphological basis of alcohol-induced brain damage in the progeny has not been adequately studied. Experiments on animals have demonstrated the negative effects of simultaneous alcoholic intoxication of females and males on physical development and structure of the higher levels of the motor system in the progeny [9, 10] and have revealed significant disturbances of the ultrastructure of neurons and interneuronal connections of the caudate nucleus under these conditions [14]. There are no data in the literature on changes in ultrastructure of the sensomotor cortex of the progeny of animals following simultaneous alcohol intoxication of females and males.

The aim of this investigation was to study the ultrastructure of neurons and interneuronal connections in the sensomotor cortex of the progeny of alcohol-addicted rats.

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