IMMUNOMORPHOLOGICAL STUDY OF LOCALIZATION OF AZOCARCINOGEN-BINDING PROTEIN IN RAT LIVER SECTIONS

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The distribution of a protein binding azocarcinogen in sections of normal rat liver was investigated by the indirect fluorescent antibody method. This protein was shown to be contained in the nucleus and cytoplasm of the hepatocytes in the region of the central veins but to be absent in the periportal zones of the hepatic lobule.

The rat liver contains a specific protein binding azocarcinogen (ABP) in vivo [8]. The possible role of this binding in malignant transformation accounts for interest in the study of the biochemical [5] and biological [4] properties of ABP. In a previous paper [1] the writers described a method of obtaining monospecific antibodies against this protein.

In the investigation described below the distribution of ABP was studied in sections of normal liver by the immunofluorescence method.

EXPERIMENTAL METHOD

Male noninbred albino rats and Wistar rats weighing 150-200 g were used in the experiments. Pieces of liver (measuring $3 \times 4 \times 5$ mm) were cut from the left lateral lobe of exsanguinated animals (six rats were investigated) and fixed in three portions of 1% glacial acetic acid solution in 100% ethanol for 1-24 h at 4°C. The pieces were passed through absolute alcohol and chloroform at the same temperature. The time which the material was kept in each solvent was 1 h. Embedding in paraffin wax was carried out in the usual way. Serial sections 2-3 μ in thickness were used in the experiments.

To detect the localization of ABP in the sections, the indirect method of Coons [10] was used. Monospecific antibodies against ABP were obtained by one of the methods described previously [1]. Ass serum against rabbit γ-globulins, labeled with fluorescein isothiocyanate (N. F. Gamaleya Institute of Epidemiology and Microbiology) was used as the fluorescent antibodies. Nonspecific fluorescence was abolished by absorption of the serum with mouse liver powder [2]. The dewaxed sections were incubated with antibodies against ABP for 30 min at room temperature. After washing in physiological saline (pH 7.4) the sections were incubated with labeled serum. The sections were again washed and mounted in a mixture of equal volumes of glycerol and phosphate buffer, pH 7.4. Details of the method were described by Engel'gardt [2, 3].

The immunological specificity of the fluoresecence was verified in sections: 1) Untreated with antibodies (autofluorescence); 2) incubated with labeled serum only; 3) incubated with nonimmune rabbit serum; 4) incubated with antibodies against ABP neutralized by a pure preparation of this protein.

In each experiment 7-8 serial sections through the liver were investigated, the third and fourth sections being stained with hematoxylin-eosin. The monospecificity of antibodies against ABP was tested in

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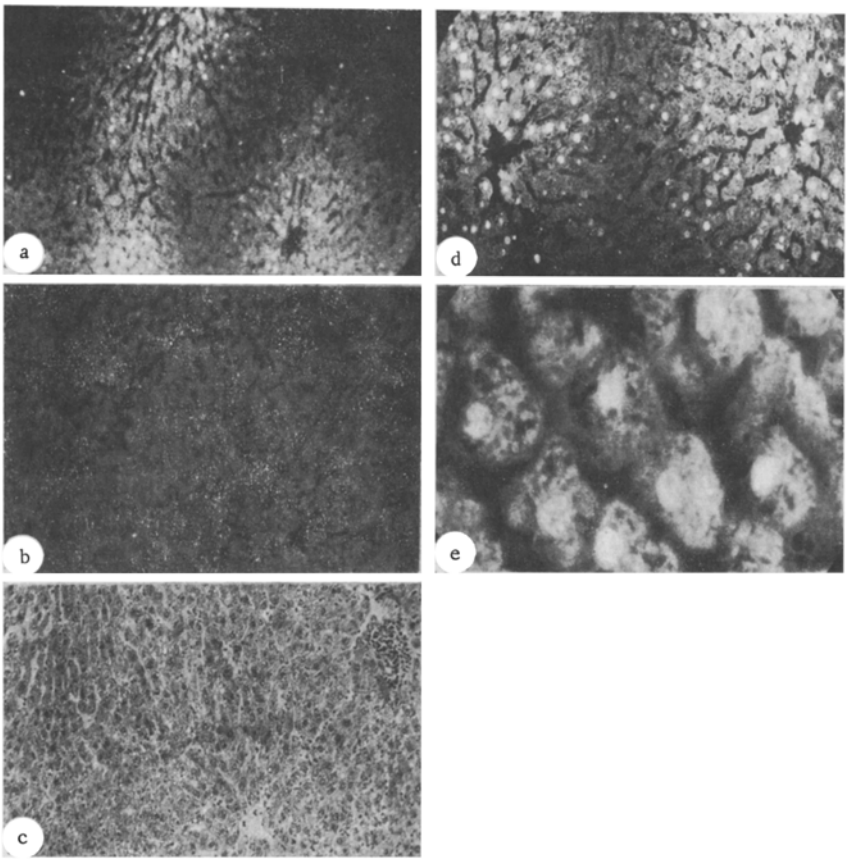

Fig. 1. ABP in sections of normal liver: a, d, e) incubation with antibodies against ABP; b) incubation with antibodies against ABP neutralized with a sample of this protein; c) stained with hematoxylin-eosin; a, b, c) serial sections. Magnifications: a, b, c) 30 ×; d) 60 ×; e) 450 ×.

sections through the spleen [1]. The specimens were examined in the ML-2 luminescence microscope in blue-violet light and with phase contrast, and were photographed on RF-3 film.

EXPERIMENTAL RESULTS

Sections of normal liver treated with antibodies against ABP showed a bright, clear fluorescence due to the presence of this protein in the cells (Fig. 1a); in all types of control listed above only a very slight fluorescence was observed, corresponding in its intensity to autofluorescence (Fig. 1b).

The obvious inequality of distribution of ABP within the hepatic lobule will be apparent. The strongest fluorescence was found in the central zones (Fig. 1a, c) but the hepatocytes of the periportal zones remained completely dark. The intensity of fluorescence changed steadily during transition from one zone to the other and was directly dependent on the closeness of the hepatocyte to the central vein (Fig. 1d). Fluorescence was absent in the epithelial cells of the bile ducts, the Kupffer cells, and the endothelium of the blood vessels.

Fluorescence in the cells forming the fluorescent zones was observed in both nucleus and cytoplasm (Fig. 1d, e). In most cells the fluorescence of the nucleus was brighter than that of the cytoplasm. The nuclei of these cells also appeared fluorescent if threshold dilutions of anti-ABP antibodies were present, when no fluorescence was seen in the cytoplasm. Cells with fluorescent cytoplasm and a dark nucleus were much less frequently found, although some could be seen in every section. Hepatocytes with more or less uniformly fluorescent cytoplasm and karyoplasm were the rule. The intracellular distribution of ABP is interesting in connection with the hypothesis [4] that this protein is a carrier of corticosteroid metabolites from the cytoplasm into the nucleus.

The presence of ABP only in the hepatocytes located in the central zones of the hepatic lobule is evidence that this protein is a product of differentiation, for cells surrounding the central veins are known to

perform a number of functions specific to the liver (synthesis of plasma proteins, of pigments, etc.) [7]. This hypothesis is also confirmed by the authors' previous findings [1].

The fact that the most highly differentiated cells are mainly affected after exposure to carcinogens [6] may be attributable to the fact that ABP is contained in precisely these hypatocytes.

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