LOCALIZATION OF ALBUMIN, TRANSFERRIN, AND α-FETOPROTEIN IN NORMAL AND REGENERATING MOUSE LIVER

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A technique of tissue fixation with a mixture of acetone and formalin followed by embedding in paraffin wax, enabling good detection of antigens, including serum proteins, is described. By means of this method the distribution of albumin, transferrin, and α-fetoprotein was described in normal and regenerating mouse liver. Both under normal conditions and during regeneration albumin and transferrin are contained by strictly the same hepatocytes. α-Fetoprotein is found in the regenerating liver independently of the other two proteins, although it is found in the same zones. Albumin and transferrin are found only in the perinecrotic zone in each cell containing α-fetoprotein.

KEY WORDS: *α-fetoprotein; albumin; transferrin; immunofluorescence; liver.*

Most of the plasma proteins in adult animals and embryos are synthesized by the liver. However, the distribution of synthesis of the various proteins among the cell population and the regulation of synthesis at the level of individual hepatocytes have received very little study. According to results obtained by immunofluorescence (IF), 10-15% of hepatocytes contain albumin [9], 10% contain transferrin [10], 10-20% contain prothrombin [7], and 1% fibrinogen [9]. It is not known, however, whether all hepatocytes are able to synthesize plasma proteins, which proteins are synthesized simultaneously by each given hepatocyte, and how their synthesis is regulated — whether together or separately. Hamashima et al. [9] showed that only 0.1% of hepatocytes in the rat liver contain albumin and fibrinogen at the same time. On the other hand, Éngel'gardt et al. [5] found that the localization of albumin coincides exactly with that of transferrin in the mouse liver. Cells containing these proteins accounted for 5-7% of the total number of hepatocytes [4].

The investigation described below was carried out in an attempt to discover whether coincidence of these two proteins is still found during regeneration of the liver, i.e., when processes of synthesis are intensified. Induction of synthesis of α-fetoprotein, a protein which is virtually absent in the adult animal, but which is synthesized by the embryonic liver and by hepatomas [1], takes place in the regenerating liver. In the mouse liver regenerating after CCl_4 poisoning, α-fetoprotein is localized in strictly definite zones [5]. It is not known how the synthesis of α-fetoprotein is connected with the synthesis of other proteins. To study the comparative localization of these three proteins the method of acetone fixation of the tissues described above was used in an improved modification [3].

EXPERIMENTAL METHODS

Experiments were carried out on male SWR and C3HA mice weighing 20-30 g. Regeneration of the liver was induced by poisoning with CCl_4 vapor [2]. Pieces of liver 1-2 mm thick were fixed in a mixture of acetone, formalin, and 0.03 M phosphate buffer, pH 6.1-6.2, in the ratio of 9:5:6, washed in 0.01 M buffer at the same pH for 18-24 h, dehydrated in 3 changes of anhydrous acetone (2 h altogether), transferred to petroleum ether for 1 h at room temperature, soaked in paraffin with 5% wax for 1 h at 56°C, and embedded in paraffin with wax. Fixation, washing, and dehydration were carried out at +4°C. During washing the volume of fluid was 500-1000 times greater than the volume of the fragments, but during other operations 50-100 times greater. Series of sections 3 μ thick were straightened out in water and glued to slides with egg albumin. The sections were treated with preparations of monospecific anti-

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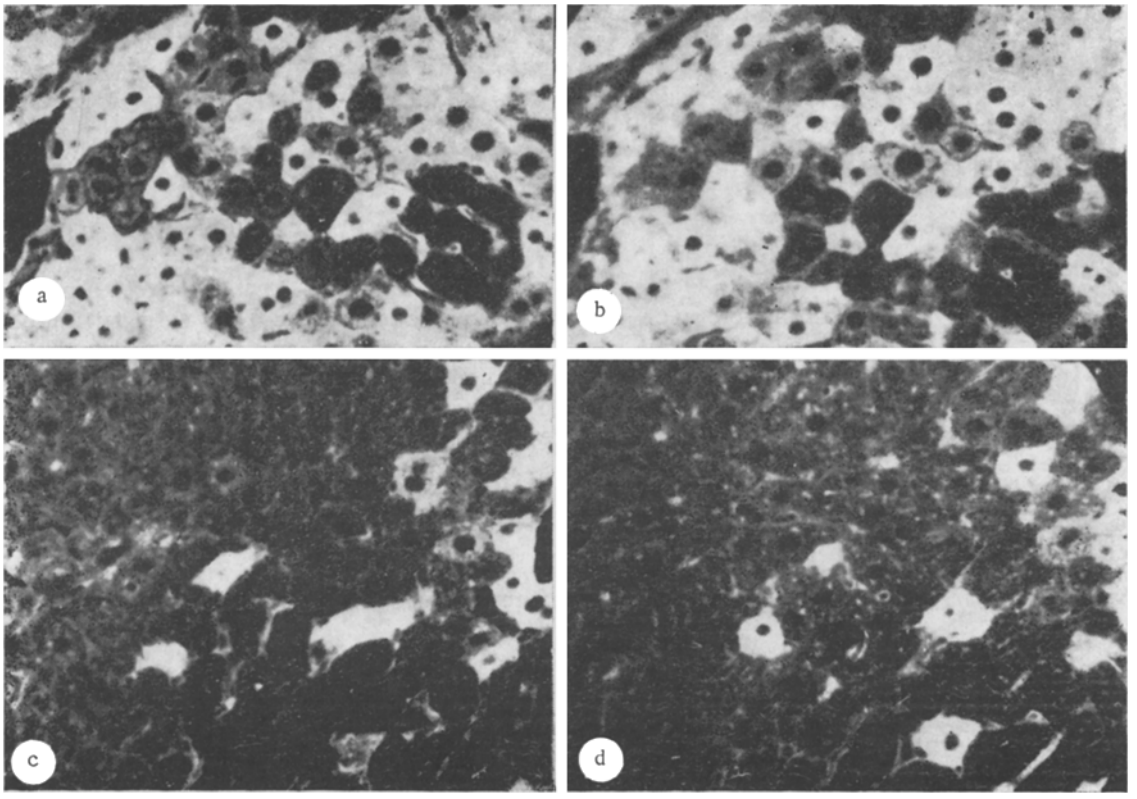

Fig. 1. Coincidence of localization of albumin and transferrin in normal mouse liver. Two successive serial sections were treated with antibodies against albumin (a, c) and transferrin (b, d). Magnification: objective 20×, homal 5×.

bodies against mouse albumin, transferrin, and α-fetoprotein and with monospecific rabbit antiserum against mouse γ-globulin.* The preparations of antibodies were obtained from the corresponding monospecific rabbit antisera on glutaraldehyde immunosorbents with pure preparations of the corresponding proteins. To the antibodies against albumin thus obtained, mouse transferrin and γ-globulin were added; to the antibodies against transferrin, mouse albumin and γ-globulin were added. Adult mouse serum was added to the antibodies against α-fetoprotein. Full details of the method of obtaining the antibodies are given elsewhere [4, 6]. The investigation was carried out by the indirect IF method [13] with luminescent donkey antiserum against rabbit γ-globulin, prepared at the Laboratory of Luminescent Sera of the Gamaleya Institute of Epidemiology and Microbiology. Sections for morphological study were stained with hematoxylin–azure–eosin and by Van Gieson's method. By treating the serial sections with antibodies against four antigens, these could be located in the same cell. Treatment of the sections with antibodies against mouse γ-globulin served as the control. Cells containing this protein were not taken into account in the subsequent analysis, for its presence in the hepatocyte is most probably due to disturbances of membrane permeability [6].

Choice of Fixation. Several different fixatives based on acetone were tested, for only acetone preserves some of the antigens to be studied [3], and also 10% formalin, 2.5 and 5% glutaraldehyde, and Bouin's fluid. Acetone was used mixed with picric, acetic, and sulfosalicylic acids, glutaraldehyde, and formalin. Acetone in concentrations of between 40 and 100% also was used for fixation. In all cases dehydration was carried out with acetone, and the sections were then taken through petroleum ether and embedded in paraffin. The suitability of the fixative was estimated by preservation of serum proteins, membrane antigens [3], and histological structures in the sections. Satisfactory results were obtained by treatment with two fixatives: 50% acetone and a mixture of acetone, formalin, and buffer, suggested by Mason et al. [11]. Subsequent tests showed that the first of these fixatives makes the outer membranes of the hepatocytes coarser and thicker. In addition, with this method of

*Antibodies against albumin, transferrin, and α-fetoprotein were obtained from N. V. Éngel'gardt and V. S. Poltoranina, antiserum against γ-globulin from O. M. Lezhneva.

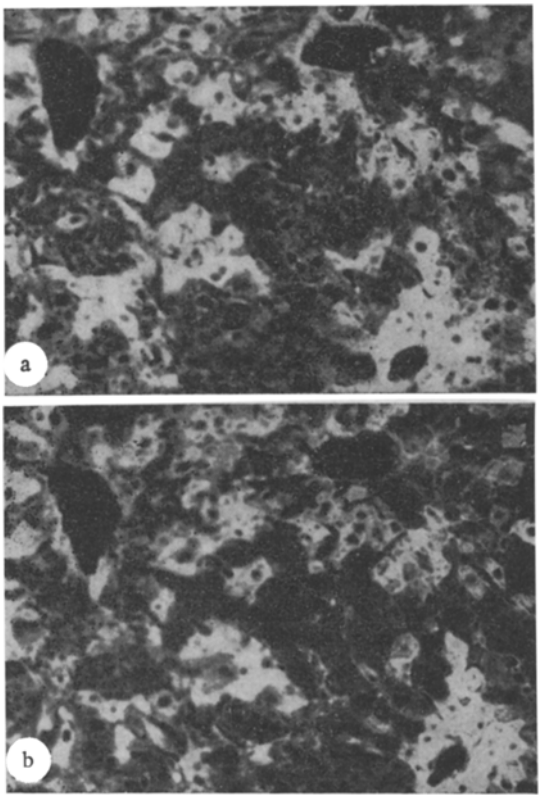

Fig. 2. Coincidence of localization of albumin and transferrin in regenerating mouse liver, 72 h after CCl_4 poisoning. Top) increase in necrosis; bottom right) portal tract. a) Albumin; b) transferrin. Magnification: objective 10×, homal 5×.

fixation the γ-globulin of rabbit nonimmune sera binds with the outer cell membrane. The second fixative preserves all the antigens to be studied and also the structure of the tissue and does not give nonspecific fluorescence. Contraction of the cells during this method of treatment is less marked than after fixation with ethanol or acetone. Subsequent work was undertaken with this fixative.

EXPERIMENTAL RESULTS

In most hepatocytes of the normal adult mouse liver a weak degree of granular fluorescence was found after treatment with antibodies against albumin and transferrin. In the remaining hepatocytes (not more than 10%) homogeneous cytoplasmic fluorescence of albumin and transferring simultaneously was discovered — these were the main sites of synthesis of these proteins. In future these cells will be called AT cells. No cells with homogeneous fluorescence of only one protein were found. This result agrees with those of Éngel'gardt et al [4]. AT cells were usually located near the blood vessels, but sometimes were far away from them (Fig. 1a-d). The number of AT cells in the lobules varied: Sometimes there were many, sometimes few.

In mice poisoned with CCl_4, 40-45% of the hepatocytes died, always around the central veins. The areas of necrosis reached the maximal size 18-24 h after poisoning. By 72-96 h the regions of centrilobular necrosis had been freed by cells of the reticuloendothelial system from dying cells. Processes of regeneration connected with replacement of the areas of necrosis by hepatocytes were usually complete by the 5th-6th day [12].

The granular fluorescence of albumin and transferrin was intensified in the regenerating liver, but contrast was preserved with the AT cells, which became more numerous than normally. Cells with homogeneous fluoresence of only 1 of the 2 proteins were not found, as under normal conditions also. The AT cells were grouped mainly near the portal tract and in the zone immediately adjacent to the area of necrosis. After 72 h their localization was still the same — the periportal zone and the infilling zone of necrosis (Fig. 2a, b). Cells containing α-fetoprotein appeared in the regenerating liver 24 h after CCl_4 poisoning. Usually they were located in the perinecrotic zone, but later they were found among the cells filling the affected areas. These cells were usually most numerous on the 3rd day of regeneration, but by the 5th to 6th day they disappeared. The dynamics of cells containing α-fetoprotein was

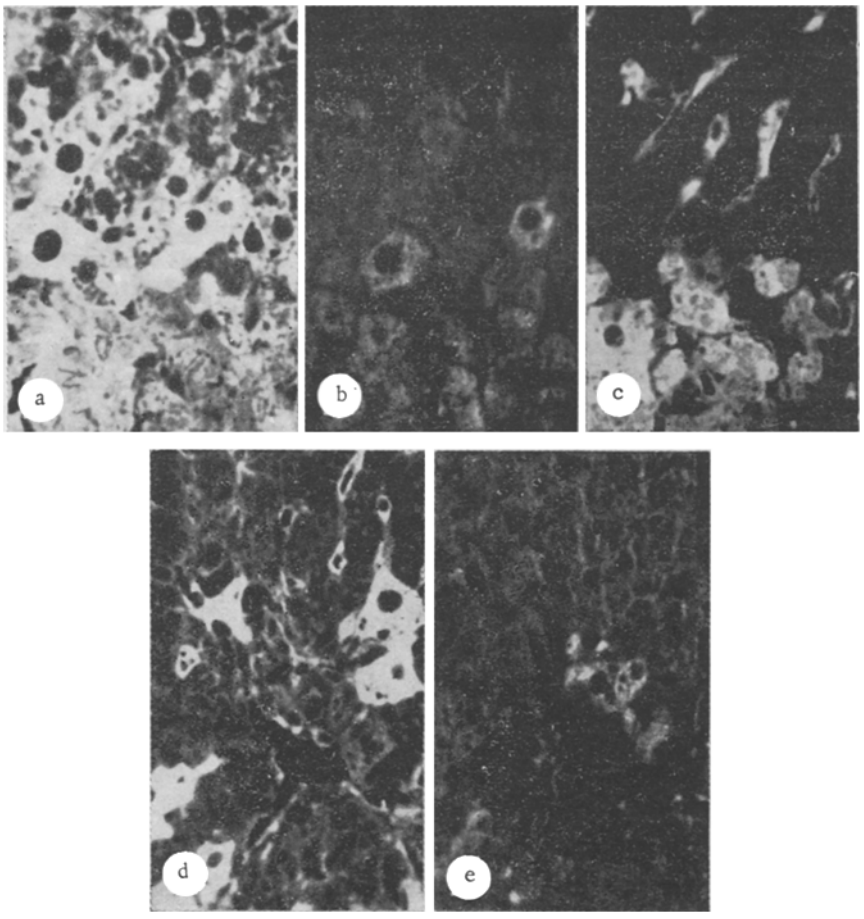

Fig. 3. Regenerating mouse liver: A) 48 h after CCl_4 poisoning. Three consecutive serial sections treated with antibodies against albumin (a) α-fetoprotein (b), and γ-globulin (c). Cells containing α-fetoprotein also contain albumin but not γ-globulin. γ-Globulin is found only in dying cells. B) 72 h after CCl_4 poisoning. Two consecutive serial sections treated with antibodies against albumin (d) and α-fetoprotein (e). Albumin not found in cells containing α-fetoprotein. Magnification: objective 20×, homal 5×.

studied in material fixed with a mixture of ethanol and acetic acid [5] and the results were completely reproduced in the present experiments. By 48 h after poisoning the layer of cells adjacent to the foci of necrosis consisted almost entirely of AT cells. Cells containing α-fetoprotein, which were present in this layer, also contained these two proteins (Fig. 3a, b). A few cells containing α-fetoprotein, but not in this layer, did not necessarily contain albumin and transferrin. On the 3rd day of regeneration, cells containing α-fetoprotein and AT cells were found among the hepatocytes filling in the affected areas. Some cells contained all three proteins) the fewer cells contained α-fetoprotein, the less frequently were cells with all three proteins found (Fig. 3d, e).

There is every reason to suppose that the localization of the proteins studied in this investigation coincides with the sites of their synthesis, because: a) Guillouzo et al. [8], using an immunoperoxidase method combined with light and electron microscopy, showed that these proteins are bound with polysomes in rat hepatocytes containing albumin and fibrinogen *in vitro*, i.e., they are actually synthesized; b) the mouse γ-globulin control ruled out any possible uptake of proteins from the blood. These results as regards the localization of proteins are in full agreement with those obtained on material treated by a different method [4, 5]. The need for developing a method of fixation is due to the fact that albumin and transferrin are not preserved over the whole area of the section in material fixed with a mixture of ethanol and acetic acid.

Regulation of α-fetoprotein synthesis at the tissue level is evidently connected with the number of cells taking part in this process, for positive correlation exists between the number of cells containing α-fetoprotein and the blood α-fetoprotein level [5], in the same way as the number of prothrombin-containing hepatocytes increases in response to administration of the corresponding stimulators [7].

The strict coincidence of the localization of albumin and transferrin in the normal and regenerating liver suggests the presence of a mechanism rigidly connecting the regulation of synthesis of these proteins. The fact that in the regenerating liver there are cells containing α-fetoprotein which do not contain albumin and transferrin and that by no means all AT cells contain α-fetoprotein suggests the absence of any close connection between the regulation of synthesis of α-fetoprotein and of the other two proteins. However, in some parts of the liver all three proteins are synthesized — the perinecrotic zone and the zone of infilling necrosis. The appearance of hepatocytes in these zones containing all three proteins can be explained by the activation of two independent processes of synthesis in response to the application of two different regulatory stimuli to the same cell.

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