LOCALIZATION OF ALBUMIN AND TRANSFERRIN IN THE MOUSE LIVER

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In the course of localization of albumin and transferrin in successive serial sections through the mouse liver the two proteins were found in the same parenchymatous cells, constituting a very small proportion of the population. In sections through transplanted hepatomas they were found in the same zones, but their localization in individual cells did not coincide exactly.

The liver is the site of synthesis of most serum proteins, including albumin, transferrin, fibrinogen, prothrombin, and haptoglobin [19], but it is still not sufficiently clear which liver cells produce which proteins, whether all or only some cells participate in their synthesis, and whether specialization among the cells exists or each cell is potentially capable of synthesizing several serum proteins simultaneously. The localization of the individual proteins in liver sections of various species of animals and man has made it possible to obtain certain information regarding albumin [12, 16], prothrombin [7, 9, 10], and fibrinogen [11, 16].

In this investigation the distribution of two proteins (serum albumin and transferrin) was studied simultaneously in sections through the mouse liver.

EXPERIMENTAL METHOD

Male mice (C3HA) aged 2-6 months, from the "Stolbovaya" nursery of the Academy of Medical Sciences of the USSR were used. Transplantable strains 22a, 48, 60, and 61 of mouse hepatomas also were studied [1].

The animals were decapitated and pieces of tissue measuring 2 × 4 × 4 mm were immediately fixed at 4°C in a mixture of 96° ethanol and glacial acetic acid (99:1) overnight [16]. The pieces were taken through alcohols and xylol at 4°C and embedded in paraffin wax at 56°C [20]. Serial sections were cut to a thickness of 3μ on a Reichert microtome [6, 13].

A preparation of albumin (A) was obtained from the serum of adult mice (MS) by preparative electrophoresis in agarose. No contamination by foreign serum proteins could be detected in this preparation by agar diffusion and immunoelectrophoresis. Pure transferrin (Tr) was isolated from MS by double electrophoresis in polyacrylamide gel [3].

Antisera (AS) against Tr and against γ-globulin (GG) were obtained by immunizing rabbits with preparations of pure Tr and GG into the popliteal lymph glands [4, 15]. AS against MS were obtained by the same method.

Monospecific antibodies (AB) were obtained by means of glutaraldehyde immunosorbents [8]. To isolate AB against A, AS against MS were used together with immunosorbent from purified A; to obtain anti-Tr-AB monospecific AS against Tr and immunosorbents from MS were used.

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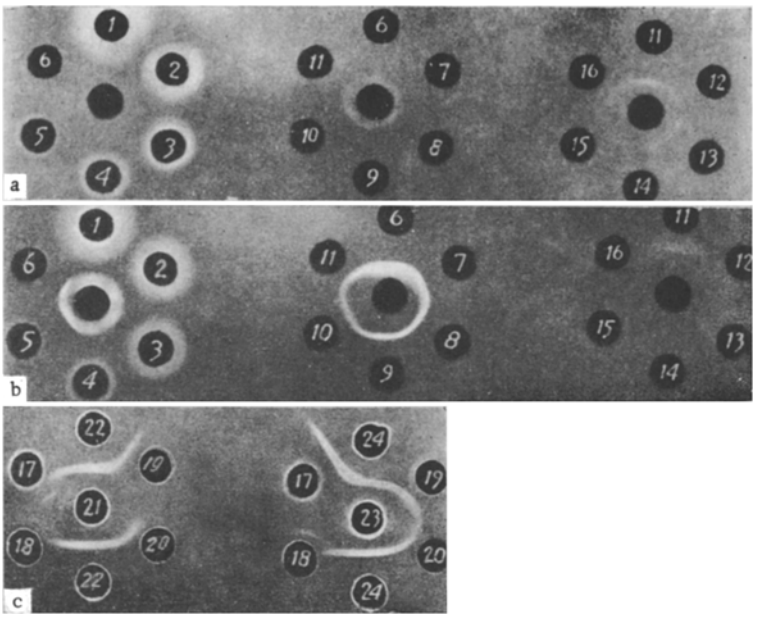

Fig. 1. Characteristics of antibodies against albumin and transferrin in agar diffusion test: 1-16) serial double dilutions of MS from undiluted to 1:32,000; central wells contain: a) anti-A-AB; b) anti-Tr-AB; c: 17) anti-Tr-AB + A, 18) anti-Tr-AB + A neutralized by Tr, 19) anti-A-AB + Tr, 20) anti-A-AB + Tr, neutralized by A; test system for A: 21) AS, 22) AG; test system for Tr: 23) AS, 24) AG.

The AB were neutralized with a small excess of pure antigens (AG) under the control of the agar diffusion test [13] which was carried out in a semimicro-modification [2]. The localization of AG in the sections was determined by the indirect method of fluorescent AB [21] using AB against rabbit GG labeled with fluorescein isothiocyanate [5, 13].

EXPERIMENTAL RESULTS

Specificity of the AB used to localize AG in the sections was first verified by the agar diffusion test. Both anti-A-AB and anti-Tr-AB were monospecific; they each formed one precipitation line with dilutions of MS from undiluted to 1:32,000 (Fig. 1). As an additional guarantee of their monospecificity, Tr was added to the anti-A-AB and A was added to the anti-Tr-AB in the quantity detected in the agar diffusion test (Fig. 1c).

The working dilution of AB was selected under the control of the reaction in the sections [13]. To detect A, the optimum concentration was five times less than that shown in Fig. 1, while to localize Tr, undiluted AB were used. The localization of A and Tr was studied in the liver sections of five normal C3HA males. Pieces were taken from different areas and from different lobes of the liver. In sections treated with AB neutralized by homologous pure AG, no specific fluorescence was observed.

The distribution of A, Tr, and GG was determined in consecutive serial sections (Fig. 2). Since it is known that GG is not synthesized in the liver, it was regarded as an indicator of the nonspecific presence of blood serum [13]. As a rule, GG was found in connective-tissue structures – in the walls of blood vessels and capillaries, Kupffer cells, and blood cells. In some cases GG was also present in the parenchymatous cells of the liver, evidently because of their injury, accompanied by a disturbance of the permeability of the cell membranes.

In sections treated with AB against A the total intensity of fluorescence the parenchymatous cells of the liver was increased. Against this background some cells showed up with much brighter fluorescence in the cytoplasm. If in the serial sections these cells contain no GG, they were regarded as the specific sites of localization of A ("A+" cells). Such cells were found more often in groups of two-to-five at a time, and

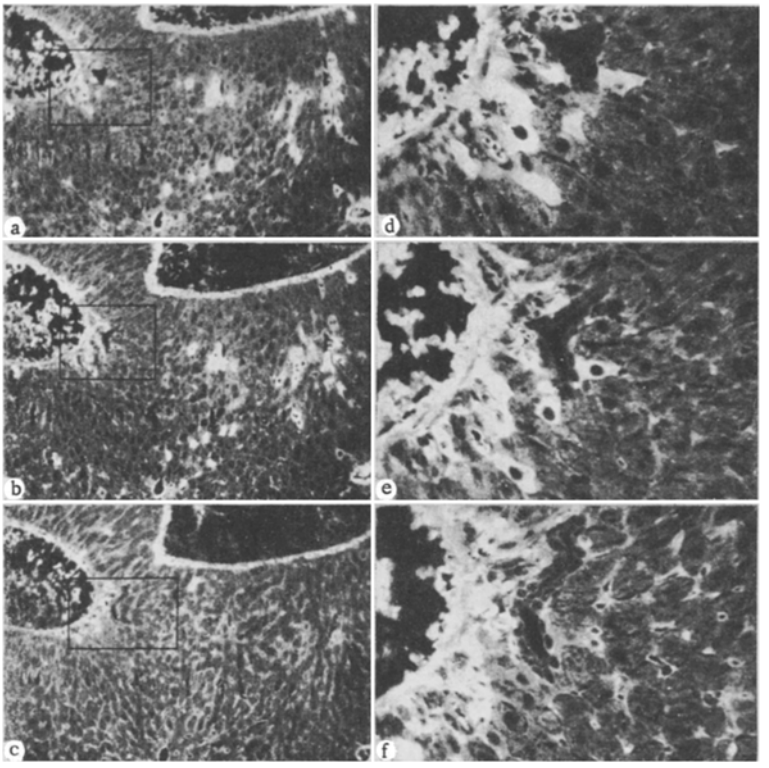

Fig. 2. Albumin, transferrin, and γ-globulin in sections through mouse liver. Sections treated with: anti-A-AB + Tr (a, d), anti-Tr-AB + A (b, e), anti-GG (c, f). Magnification in a, b, c 36 ×, in d, e, f 140 ×. The rectangle drawn in a, b, and c corresponds to the areas in d, e, and f.

sometimes in whole zones, although they could exist singly. No definite regularity of their distribution in the lobule could be found. The number of "A+" cells and the intensity of their fluorescence varied considerably and irregularly in the sections from pieces taken from different lobes of the liver, from different animals, and from different parts of the same section; on the average they accounted for 3-7% of the parenchymatous cells. A also was found in the connective tissue, in the blood cells, the periportal zones, some of the Kupffer cells, and in the walls of blood vessels. Usually GG was found in the same structures, and for this reason the writers are inclined to attribute this localization to the nonspecific presence of blood serum.

During the detection of Tr, the total intensity of fluorescence of the parenchymatous cells was much lower than in the case of A. Certain "Tr+" cells with specific fluorescence in the cytoplasm, coinciding exactly with the "A+" cells in neighboring serial sections, could be distinguished among them (Fig. 2). The connective-tissue structures stood out more sharply and resembled the distribution of GG. Neither A nor Tr could be reliably detected in the epithelium of the bile ducts.

It was shown previously that hepatoma 22a retains the power of synthesizing A and Tr even in vitro [17]. Both proteins were found in the sections of the transplantable hepatomas studied, but absolute coincidence between the "A+" and "Tr+" cells was not obtained. The reaction with anti-A-AB was more intensive and covered larger areas, and the number of "Tr+" cells was smaller, and they were more contrasted, but they were found in zones with an increased content of A (Fig. 3).

A decisive factor in the localization of individual substances by the immunofluorescence method is the use of strictly monospecific AS and of an adequate tissue fixative which does not modify the antigenicity of the substance tested and which preserves its natural distribution in the tissues. The specificity of the AB used was verified sufficiently strictly. The fixative used, according to the literature, was one of the best – it was with its aid that A was first detected in the parenchymatous cells of the liver [16], and not only in the sinusoids, the portal vein, and the adventitial connective tissue [14]. Moreover, when this method of fixation

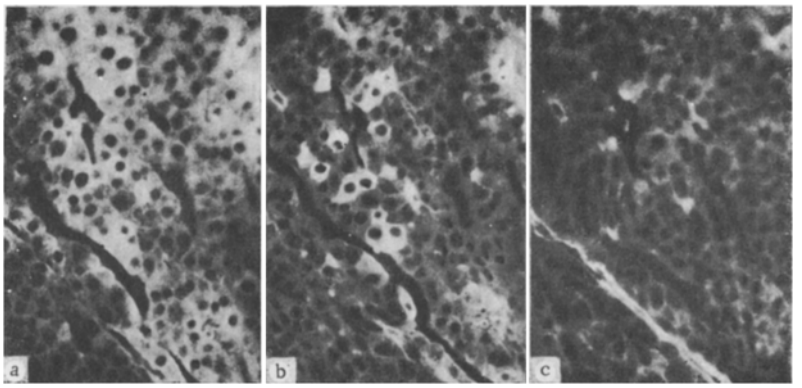

Fig. 3. Albumin, transferrin, and γ-globulin in sections through transplanted hepatoma 60. Sections treated with: anti-A-AB + Tr (a), anti-Tr-AB + A (b), anti-GG-AB (c), 140 ×.

was used, increased synthesis of A during regeneration of the liver and nephrosis in rats was shown to be accompanied by an increase in the number of "A+" cells in the sections [18].

The results of this study of the distribution of A and Tr agree with those published earlier to the effect that under normal conditions only relatively few parenchymatous cells of the liver contain A (10% [16]), fibrinogen (1% [16], 5% [11]), and prothrombin (10% [9, 10, 11]). However, the writers consider that the number of "A+" and "Tr+" cells found in the present investigation (3-7%) is an underestimate because of the inadequacy of fixation. As a rule these cells were at the edge of the section or close to large blood vessels. In the center of the overwhelming majority of sections, however, only an indistinct fluorescence not localized in individual cells was observed. Brozman [12], who obtained similar results after fixing pieces of liver in 10% neutral formalin, suggests that under normal conditions most hepatacytes contain A, and he questions the quality of fixation. The choice of a more adequate fixative for A and Tr is essential for subsequent investigations.

Of all types of cells in which A and Tr were found, the most probable site of their synthesis is in the parenchymatous cells of the liver. Even after malignant transformation of these cells in the culture of mouse hepatoma 22a they still remain capable of producing A and Tr [17]. Besides the work of Peters et al. [18], the consistent discovery of A and Tr in the parenchymatous cells, together with the absence of GG, by contrast with other structures, is further support for the view that synthesis of A takes place in these cells.

During a simultaneous study of the localization of A and fibrinogen, the percentage of cells containing both proteins was extremely low [16]. On the contrary, in the present experiments clear coincidence of the localizations of A and Tr was observed in the same cells of the liver parenchyma, and the same tendency persisted in hepatomas. Very probably this reflects the ability of the cells to produce several serum proteins simultaneously. To verify this hypothesis it would be interesting to study the cellular localization of A and Tr in different physiological states accompanied by marked shifts in their synthesis [10, 11]. The writers' attempt to influence the production and localization of A and Tr by artificial blood loss proved unsuccessful. Admittedly, even in the case of fibrinogen, blood loss did not cause a shift in the synthesis and distribution of fibrinogen in the sections [11]. More powerful and specific agents are evidently required for this purpose.

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