DETECTION OF α_F-GLOBULIN IN LIVER SECTIONS FROM HUMAN EMBRYOS AND NEWBORN MICE BY MEANS OF FLUORESCENT ANTIBODIES

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The localization of α_F-globulin was studied by the fluorescent antibody method in liver sections from human embryos and newborn mice and in sections of the regenerating liver of adult mice. α_F-globulin was found only in the hepatocytes, which are heterogeneous in their content of this protein.

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The existence of embryo-specific proteins in the serum of animals and man is now firmly established; their physicochemical properties, their place of synthesis in the body, and the conditions for their formation are known [4, 6]. However, the problems of which cells form these proteins, of their morphological and physiological characteristics, and their fate in ontogenesis and in pathological conditions have received inadequate study. This is particularly true of α_F-globulin (α_F)—the most characteristic representative of the embryo-specific proteins. In the early stages of ontogenesis α_F is the chief component of embryonic serum, but in adults it is found only during regeneration of the liver (in mice) or during development of hepatomas and embryonic carcinoma [4]. Gitlin and co-workers have detected α_F in cryostat sections of rat embryos in the cells of the liver parenchyma and yolk sac [7].

The object of the present investigation was to detect α_F in paraffin sections of the human and mouse liver by immunohistochemical methods.

EXPERIMENTAL METHOD

The liver of a 6-week human embryo (from a case of abortion) and organs of mice of different ages (C_3H/Sh, BALB/c, and noninbred) were used in the experiments. The investigated tissues were fixed by the method suggested by Hamashima and co-workers for detection of albumin in human liver sections [9]. Immediately after removal of the liver from the body, pieces of tissue measuring not more than $3 \times 4 \times 5$ mm were placed for 1 h in a mixture of ethanol and acetic acid (1 vol. % glacial acetic acid in 96° alcohol), and cooled to 4°. After 1 h they were covered with a fresh batch of the same cold fixing solution and allowed to stand at 4° for 15-24 h. Subsequent treatment of the pieces and cutting of paraffin sections 2-3 μ in thickness were carried out by Sainte-Marie's method [9]. The indirect method of fluorescent antibodies [10] was used. Antisera against mouse α_F were obtained by immunization of a rabbit by injecting into the lymph glands a preparation isolated from the serum of newborn mice by electrophoresis in polyacrylamide gel. Antibodies against other serum proteins, existing as impurities, were completely exhausted by the addition of serum of adult mice, and the result verified by the agar diffusion test. The γ-globulin fraction was iso-

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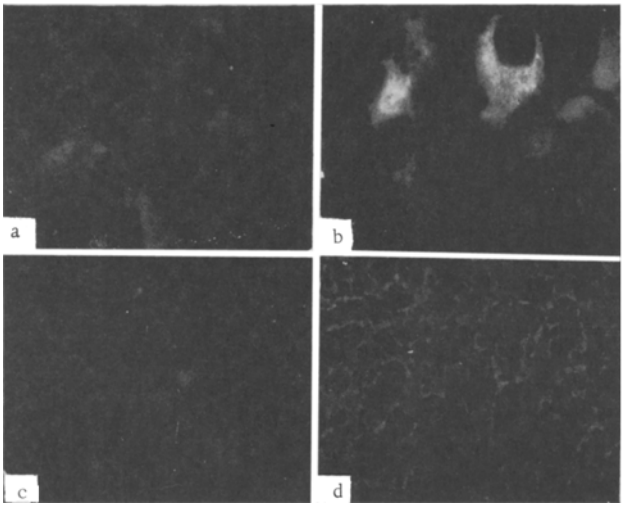

Fig. 1

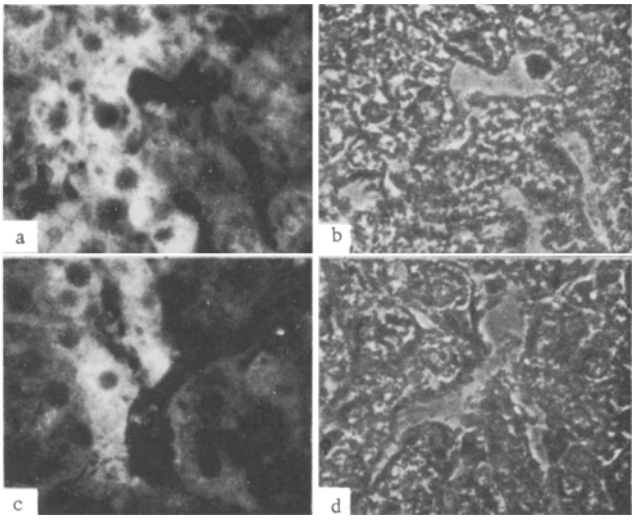

Fig. 2.

Fig. 1. Reaction of antiserum against α_F in sections of organs from adult mice: a) section of kidney b) section of spleen; c) section of liver of normal adult mouse; d) section of mouse liver 72 h after inhalation of CCl_4 vapor. Here and in other figures, magnification 90 ×.

Fig. 2. Reaction of antiserum against α_F in liver sections from 5- (a) and 20-day mice (c): b and d) the same fields of vision as in a and c, examined under phase contrast.

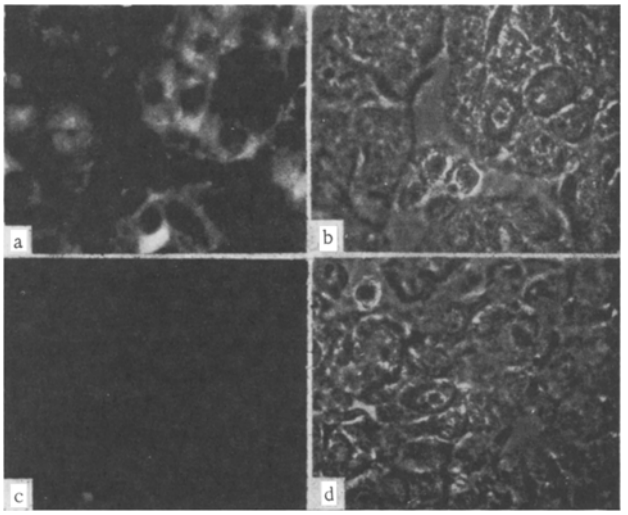

Fig. 3. Detection of α_F in liver sections of 6-week human embryo. a, b) Section treated with antibodies against α_F; a) luminescence; b) phase contrast; c, d) antibodies against α_F neutralized by donor's serum; c) luminescence; d) phase contrast.

lated from the exhausted monospecific antiserum against α_F on DÉAÉ-Sephadex A-50 by the method of Baumstart and co-workers [5]. To verify the immunologic specificity of the reaction, the same preparations of γ-globulin but completely neutralized with serum of newborn mice, and also nonimmune rabbit γ-globulin with a similar protein concentration were applied to the sections. Antiserum against human α_F was obtained and treated in a similar manner.

A preparation of monospecific ass antibodies against rabbit γ-globulin, labeled with isothiocyanate, was used as the labeled antiserum.

Treatment of the sections with antisera, preparation of the specimens, and recording of the results were carried out as described previously [3]. The agar diffusion reaction was carried out in the semimicromodification of Gusev and Tsvetkov [2].

EXPERIMENTAL RESULTS

The preparations of antibodies against human and mouse α_F used in the investigation were strictly monospecific: they formed only one precipitation line in agar with serum of human embryos or newborn mice, identical with the precipitation line of purified preparations. In sections of the liver, kidney, and spleen of adult mice, no α_F was detected (Fig. 1). An identical, dull background luminescence was observed in sections of these organs treated with γ-globulin against α_F, γ-globulin against α_F neutralized with the serum of newborn mice, and γ-globulin of normal rabbit serum.

Meanwhile, α_F was clearly detected in liver sections of newborn mice. In the first days after birth the cytoplasm of the overwhelming majority of liver cells was intensely fluorescent. By the 20th day the number of luminescent cells and the intensity of their fluorescence gradually diminished (Fig. 2). It is interesting to note that as a rule the luminescent cells were arranged in groups near the blood vessels. A few groups of luminescent liver cells were found also in the liver sections of two adult male BALB/c mice on the 3rd day after inhalation of CCl_4. Traces of α_F were found in the blood of these animals by the agar diffusion test. The appearance of α_F in the blood of mice inhaling CCl_4 vapor was described by Bakirov [1]. Dull and uniform luminescence of low intensity was observed in all control liver sections.

In sections of embryonic human liver treated with antibodies against α_F, luminescence of the cytoplasm was observed in most liver cells. Some cells, however, remained absolutely dark, and the hematopoietic cells likewise were not luminescent. Neutralization of antibodies against α_F-globulin completely abolished the reaction (Fig. 3).

The results obtained thus provide evidence in favor of the view that α_F is synthetized in newborn mice and human embryos by hepatocytes and not by other cells present in the composition of the liver. In sections treated with antibodies against α_F no luminescence of the hematopoietic cells or of the epithelial cells of the bile ducts was observed. This corresponds to the earlier observations of Gitlin and co-workers [7].

The hepatocytes were heterogeneous in their content of α_F-globulin, the heterogeneity increasing with an increase in age of the newborn mice, and it was well marked in the regenerating liver of adult mice after inhalation of CCl_4 vapor. The precise localization of α_F in paraffin sections of the liver fixed with a mixture of ethanol and acetic acid was noted in investigation of the liver both from newborn mice and from the human embryo; α_F was also found in crysotat sections of the liver from newborn mice fixed with acetone by the method of Gitlin and co-workers, but in this case the picture was less definite. It was generally impossible to detect α_F in cryostat sections of the liver of the same mice fixed with a mixture of ethanol and acetic acid. The reason for this complete disagreement with the results obtained by the use of paraffin derivates is not yet clear.

It can be hoped that the method of preparation of sections in the presence of monospecific antibodies used in these experiments will help to solve many of the problems connected with α_F production in human hepatomas and also to investigate the principles governing the cessation of its synthesis in adults at the level of cell populations.

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