# IMMUNOLOGICAL HYSTOCHEMICAL CHARACTERISTICS OF ONE OF THE ORGAN-SPECIFIC ANTIGENS OF MOUSE LIVER

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Translated from Byulletin' Esperimental'noi Biologii i Meditsiny, Vol. 56, No. 11, pp. 97-101, November, 1963
Original article submitted February 8, 1963

By precipitation in agar and by immuno-electrophoresis five or six specific organ antigens have been found in an extract of mouse liver, and have been numbered from I to V according to the reduction of their electrophoretic mobility [2]. Monospecific antibodies to most of them have been obtained and have been used to study the distribution of these antigens in the liver, in the normal organs, and in four strains of grafted hepatoma [8, 9].

Here we present the results of the study of yet one more antigen additional group (IV') made by precipitation in agar, and by the indirect method of fluorescent antibodies.

## EXPERIMENTAL METHOD

We used mice of the lines C3HA, CC57BR, BALB/C, C57BL, C3H/SN, as well as impure lines. As antigens for precipitation in agar we used extract of mouse organs prepared in veronal buffer at pH 8.6 dilution 1 : 3 [2].

The fractions of liver extract containing the different organ-specific antigens were obtained by electrophoresis in agar [2, 4].

We used serum of rabbits obtained after several cycles of immunization with the mitochondrial fraction (65), and a mouse-liver homogenate (193).

The scheme of immunization has been described previously [2].

Antibodies to antigen IV' were separated by separating in an acid medium the specific precipitate formed by the fraction of the liver extract containing this antigen, by the antiserum of the exhausted organ-specific antigen V of mouse liver, by normal mouse serum, and by extracts of heterologous organs of mice. The method of obtaining monospecific antibodies has been described previously [1, 8].

The micro-modification [3] of the method of precipitation in agar [4] was used.

The content of antigen IV' in the different preparations was determined by titration by means of precipitation in agar by Feinberg's method [13]. To obtain comparable results we used a standard test system consisting of monospecific antibodies to antigen IV' and a liver extract in optimum dilution which give a sharp precipitation line with these antibodies. The sensitivity of the determination was increased through use of a diluted test system [7].

The antigen was localized on the sections by the indirect method of fluorescent antibodies [18]. Ass antibodies to rabbit gamma-globulin were labelled with isothiocyanate fluorescein [15]. Sections of the unfixed frozen tissue 3-5 μk were placed in a cryostat fixed with 96% alcohol for 15 min at 37° after which the alcohol was removed for 30 min at 37°. The method of fluorescent antibodies has been described previously [10]; it does not differ in principle from the generally accepted method [11].

## EXPERIMENTAL RESULTS

Obtaining Antibodies to Antigen IV'. It has been shown previously that after separation of the mouse liver extract by preliminary electrophoresis in agar, in the fraction having the mobility of gamma-globulin the organ-

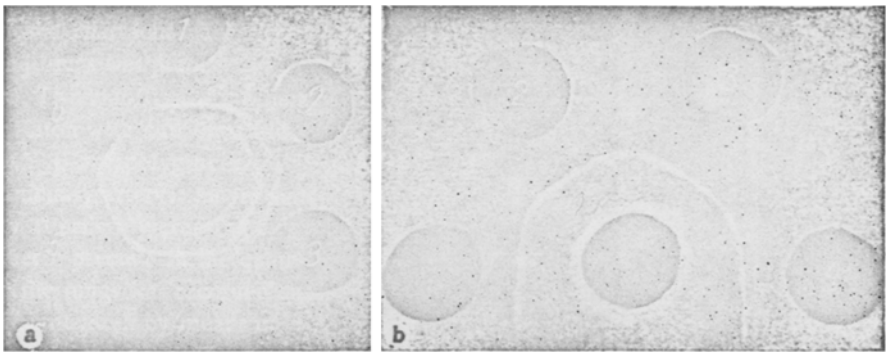

Fig. 1. Characteristics of antibodies to the organ-specific antibodies IV and IV' of mouse liver in the precipitation in agar reaction. A) Mixture of antibodies to antigens IV and IV' from the antiserum 193: 1-6) two-fold dilution of the liver extract from its original strength to 1/32; B) liver extract dilutes 1 : 16; 1,4) second sample of antibodies II; 2) antibodies II diluted 1 : 16; 3) antibodies I, undiluted.

specific antigen IV is present together with an admixture of the slow-moving antigen V [8]. Further study has shown that another component is present belonging to the group of organ-specific antigens; it was subsequently identified as antigen AO [4].

Antibodies to this antigen were not found in all the anti-liver antisera. In many cases of the specific precipitates of the partially exhausted anti-liver antisera and of the fractions of liver extract containing antigen IV we obtained antibodies reacting only with antigen IV. From other antisera, including serum 193 from those used in the present work, by the same method we obtained organ-specific anti-liver antibodies which gave two lines of precipitation in agar with liver extract. With the help of monospecific antibodies to antigen IV it was shown that the precipitation line which was most clearly defined with a liver dilution of 1 : 8 corresponded to it. The second precipitation line, which was the clearest in reaction with the original liver extract corresponded to the antigen designated as antigen IV' (Fig. 1, a).

A sharp precipitation line is formed when the antigen and antibody are present in equivalent amounts. For antibodies to antigen IV this relationship was achieved at for higher dilutions of the liver extract than was required for the antibodies to antigen IV'. Therefore, having obtained from antiserum 193 a mixture of antibodies to antigen IV and antibodies to antigen IV' we attempted to neutralize the former by the addition of a small amount of liver extract. As a result antibodies to antigen IV were completely neutralized, while antibodies to antigen IV', despite a marked diminution of their activity, continued to react with liver extract, and were used for further work.

When antiserum 65 was used we were able to select a fraction of the liver extract which reacted with antibodies to antigen IV; and for the precipitate we immediately obtained a monospecific eluate from antibodies to antigen IV'; with repeated elution of precipitates with an acid buffer an active preparation of antibodies was also obtained.

Antibodies separated from antiserum 193 (antibodies I) and from antiserum 65 (antibodies II) were identical in the precipitation in agar reaction, but the latter were approximately 16 times as active as the former (Fig. 1, b). These antibodies reacted with the antigen of the liver extract, which differed both from the organ-specific antigen IV and from antigen V.

Amount of Antigen IV' in the Liver and in the Normal Organs of Mice. All results on the amount of antigen IV' in different preparations were obtained by the use of the standard test system consisting of a single liver extract and antibodies II with diluted 1 : 8. In most tests of the liver preparations (10 out of 13) the titre of antigen IV' was 1 : 64, but in three preparations it was 1 : 32, in another three it was 1 : 128 and in two it was 1 : 256, and these variations did not depend either on the age or the line of the mice.

No antigen IV' was found in a single one of the six spleen or lung preparations of mice or in the three samples of normal mouse serum tested. In one of the six investigations of liver preparations it was present to the extent of 1.5% of the total liver content. Consequently from the evidence of precipitation in the agar this antigen must belong to the group of organ-specific liver antigens.

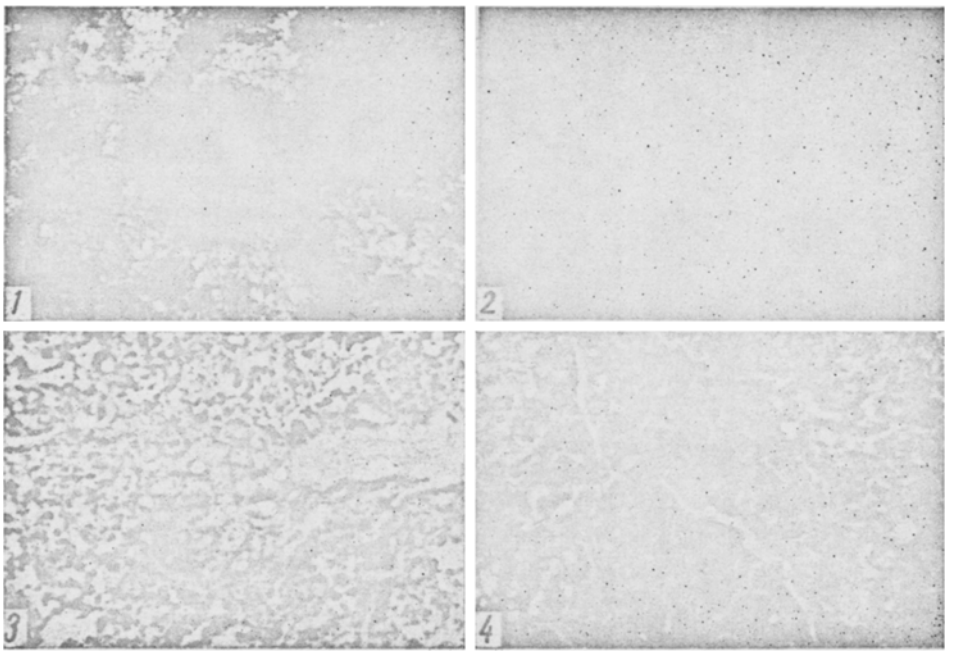

Fig. 2. Antigen IV' in mouse liver sections. 1,2: Portion of a section of mouse liver treated with antibodies I to antigen IV' and then by labelled antibodies to rabbit gamma-globulin; 3,4:control: portion of a parallel section of liver treated not with antibodies to antigens IV' but by nonimmune rabbit gamma-globulin; 1,3: in ultraviolet light; 2,4; same portions of the sections seen by phase contrast. Magnification: objective ×90, ip ×10.

A Discovery of Antigen IV' in Sections of Normal Organs and of Mouse Liver. We usdd the original antibodies I and II in dilutions 1 : 2 and 1 : 8.

Sections of the spleen, kidney, and small intestine of mice treated with antibodies to antigen IV' and then with antibodies to rabbit gamma-globulin were labelled with isothiocyanate fluorescein, and in ultraviolet light looked exactly the same as parallel control sections of these organs which were treated not with antibodies to antigen IV' but with non-immune rabbit gamma-globulin. At the same time similar sections were quite well stained after treatment with the original antiserum 193.

In seven experiments we investigated the liver of five mice of different lines and of different ages; we protected them with both samples of antibodies to antigen IV' and obtained similar results. In all the experiments the liver sections were stained weakly but very characteristically.

The walls of the blood vessels, the Kupfer cells, and the epithelium of the bile ducts were completely unstained, but a characteristic granular luminescence of the parenchymal cells appeared (Fig. 2). The nuclei and the hyaloplasm of the parenchymatous cells as a rule remained completely dark. Sometimes one had the impression that the luminous granules outlined the membranes of the cell and the nuclei, while in the cytoplasm they were regularly distributed. However, we were unable to identify these granules or to relate them to a definite cell structure by means of phase contrast or by staining the sections with hematoxylin.

The size of the luminous granules varies; it was found that it depended very much on the concentration of antibodies. Thus, the original antibodies II stained the liver section very much more strongly than did antibodies I, which, as we showed above, were 16 times weaker. In this case the luminous structures in the cells of the liver parenchyma appeared as large agglomerations of particles. When the antibodies II were diluted two-fold the intensity of the luminescence showed a slight decrease, but it could be seen that the smaller particles were stained. Dilution with antibodies II four or eight-fold led to a considerable fall in the intensity of the luminescence while at the same time the sharp contrast of the stains increased. The picture obtained more nearly resembled a section treated by antibodies I.

Thus, although a very high concentration of antibodies made for a high intensity of luminescence, it also caused a reduction in the sharpness of staining, and even some abnormality.

Despite the fact that in many organs of various animals including man characteristic antigenic substances have been found [2, 5, 6, 12, 16, 17], in most cases the nature, histological localization and the biological function of the organ specific antigen remain unknown. The development of new methods and new approaches to immunological investigations gives hope of explaining many unsolved problems. In particular great promise is shown by the use not of original antisera but of monospecific antibodies to different antigens, as have been used in the study of several organ-specific antigens of mouse liver [8, 9]. The monospecific antibodies to the organ-specific antigen IV' have also made it possible to show that the content of this antigen in the mouse liver must be 70-100 times higher than in the other normal mouse organs investigated. It has been shown that in the liver its concentration may vary over wide limits; the reason for these variations has not been explained.

Great possibilities have been opened up for the study of organ-specific antigens by the method of fluorescent antibodies applied in conjunction with monospecific antibodies to different antigens. Thus the organ-specific antigen IV' of the liver was found only in the cytoplasmic granules of the cells of the liver parenchyma; the antigen V previously investigated appears only in cells of the parechyma but is also present in the nuclei and in the cytoplasm [10]. But the distribution of the organ-specific antigens may be determined not only within the tissue but within the cells, which will help to determine the biological roll of these substances. However, some care is required in evaluating the results obtained, and the possibility of artifacts due to wrong treatment of the sections, the use of unsuitable concentrations of antibodies, etc. must be borne in mind. Similar investigations should be carried out by histochemical and other methods, and by making appropriate preparations.

## SUMMARY

A method is described for obtaining monospecific antibodies to one of the organ-specific antigens of mouse liver. With the aid of these antibodies the antigen content of the liver and of normal mouse organs was determined by means of double diffusion in gel. The fluorescent antibody method was used to locate this antigen in the cytoplasmic granules of hepatic parenchyma cells.

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