ANTIGENIC ANALYSIS OF HUMAN HEART, KIDNEY, LIVER, AND SPLEEN TISSUES BY ISOLATION OF "PURE" ANTIBODIES

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Antibodies reacting specifically only with antigens of the corresponding organ were isolated from antisera against human heart, kidney, liver and spleen tissues. Besides them, other antibodies reacting with extracts of all four organs, or with only two organs, were isolated.

A previous investigation [4], using the gel-diffusion reaction and the complement fixation test, showed that human heart, kidney, liver, and spleen tissues have the following antigenic composition (each letter denotes an antigen): heart, a, b, c; kidney, a, c, d, h; liver, a, c, e, g, h; spleen, a, c, e. The comparative serologic study of these four organs thus showed that three of them contain organ antigens (heart, b; kidney, d; and liver, g). They all contained serum antigens (a) and antigens common to or related to all these tissues (c). Antibodies against these antigens in the tissue antisera were designated ABC, ACDH, ACEGH, and ACE respectively.

The object of this investigation was to isolate organ-specific and other antibodies from cardiac, renal, hepatic and splenic antisera in order to confirm the scheme of their antigenic composition given above.

EXPERIMENTAL METHOD

Tissue antisera were obtained by intradermal immunization using Kashkin's scheme [2]. Tissue antigens were obtained by Witebsky [5]. The protein concentration in the tissue antigens was determined by the biuret reaction. Antibodies were isolated from the tissue antisera by the method of Sisenko et al. [3]. The sera were tested by the complement fixation test as described by Ioffe [1].

EXPERIMENTAL RESULTS

Antibodies against specific cardiac antigen b were isolated from cardiac antiserum (antibodies ABC) previously absorbed by splenic tissue extract (antigens a, c, e). After absorption in this manner, B antibodies remained in the serum, from which they were isolated by means of heart extract.

For preliminary absorption, 10 mg antigen was added to 0.1 ml of cardiac antiserum. The mixture was incubated for 2 h at 37° and 18-20 h at 4°. After the formation of a complex of this serum with antigens of splenic tissue (a and c; the e antigen does not take part in the reaction), the latter was removed by acidification of the previously dialyzed mixture with carbon dioxide followed by centrifugation. By this method, antibodies against the a and c antigens were removed.

The C antibodies were also easily isolated from cardiac antiserum (ABC) by means of splenic extract (antigens a, c, e). It was unnecessary to absorb the cardiac antiserum beforehand for this isolation.

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TABLE 1. Complement Fixation Test with Different Types of Antibodies Isolated from Tissue Antisera

Antibodies	Antigens (tissue extracts)			
	heart (a, b, c)	kidneys (a, c, d, k)	liver (a, c, e, g, h)	spleen (a, c, e)
B (from cardiac antiserum)	1:200[1]	–	–	–
C (from cardiac antiserum)	1:100	1:100	1:200	1:100
D (from renal antiserum)	–	1:200	–	–
E (from splenic antiserum)	–	–	1:200	1:100
G (from hepatic antiserum)	–	–	1:200	–
H (from hepatic antiserum)	–	1:100	1:200	–

[1]Titer of antibodies equivalent to orginal serum is shown. Dilution indicated for complete inhibition of hemolysis with 1, 1.5, and 2 units complement.

Antibodies against organ-specific kidney antigen g were isolated from renal antiserum previously absorbed with liver. Since renal antiserum contains antibodies ACDH, and liver contains antigens a, c, e, g, h, after absorption the renal antiserum contained only D antibodies, and these were isolated with kidney antigen.

Antibodies of group E were present in antisera against liver and spleen tissues. To isolate them, the splenic antiserum was absorbed, as described above, initially with extract of heart tissue. After the formation of a complex of the antibodies of this serum with heart antigens a and c (b does not participate in the reaction because no antibodies against it are present), only E antibodies were left in the splenic antiserum, and these were isolated by the use of splenic extract.

Organ-specific G antibodies were isolated from hepatic antiserum after its preliminary absorption with splenic (a, c, e) and renal (a, c, d, h) antigens. Since hepatic antiserum contains antibodies ACEGH, after absorption in this way only the G antibodies were left, and these were isolated with liver extract in the usual way.

Finally, H antibodies present in hepatic and renal antisera could be isolated as follows. In the first place, hepatic antiserum (antibodies A, C, E, G, H) was absorbed (with removal of the immune complex as described above) with splenic extract, thereby removing antibodies ACE. The H antibodies could be isolated by adding kidney extract to the absorbed serum. Since the absorbed serum contained only GH antibodies, the renal antigen could react only with the H antibodies, for they contain the h group of antigens which also were isolated from this complex.

The results of investigation of the isolated antibodies in the complement fixation test are given in Table 1. The isolated antibodies reacted strictly specifically only with extract of the organ containing the corresponding antigens. The so-called organ-specific antibodies from cardiac (B), renal (D), and hepatic (G) sera reacted only with extract of the same organ.

The C antibodies reacted with all extracts, for each of them contain the corresponding antigen. The E antibodies reacted only with extract of the liver and spleen, the H antibodies only with extracts of the kidney and liver, since these organs contain the corresponding antigens.

The isolated antibodies are thus narrowly specific, and react only with strictly limited antigens, an important factor during their isolation from the mixture of antibodies comprising the tissue antiserum.

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