USE OF AN IMMUNOSORBENT FOR PREPARING TISSUE ANTIBODIES

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By means of a multicomponent immunosorbent obtained by fixing serum proteins on diazotized p-aminobenzylcellulose, antibodies against blood proteins can be completely removed from a tissue precipitating antiserum. The serum prepared in this manner is not contaminated by antigen proteins. The method described can be used for obtaining tissue antisera of a high degree of purity.

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In experimental work and medical practice it is often necessary to obtain monospecific sera. They can be prepared by immunization of animals with purified antigen, although in the case of multicomponent systems the homogeneity of such an antigen cannot always be guaranteed. Purification of the antiserum from nonspecific antibodies is a no less complicated task, although it can be performed comparatively easily by exhausting the sera with nonspecific antigens.

Exhaustion of the sera is most successful if the antigen is insoluble in water or if fixed on a water-insoluble basis, for in that case, during treatment of the sera contamination of the antibodies with foreign admixtures can be avoided. Campbell and co-workers [5] suggested purifying antibodies by absorbing them on antigen fixed on diazotized p-aminobenzylcellulose, with subsequent dissociation of the immune complex thus formed at pH 3.5. A. E. Gurvich and co-workers [1] used haloid alkylate-cellulose for this same purpose, improved the method considerably, and used it for quantitative estimation of antibodies. The possibility of fixation of multicomponent antigens on an immunosorbent and of the use of such complexes for exhaustion of sera has not yet been studied.

In this paper we describe the results of an attempt to obtain tissue antisera completely freed from antibodies against serum proteins by means of an insoluble antigen–immunosorbent complex.

EXPERIMENTAL METHOD

Antisera against water-soluble proteins of the hyaloplasm, mitochondria, and microsomes of August rat liver cells were obtained by repeated injection of the proteins of the fractions mixed with Freund's complete adjuvant into rabbits [2]. At the height of immunization the animals were exsanguinated and the sera partially purified and concentrated by salting out the globulin fractions with ammonium sulfate. Such sera in immunoelectrophoresis experiments detected 28–30 antigens among the cell sap proteins and 12–16 antigens among the cytoplasmic granules of liver cells. Antisera against serum proteins treated in this manner detected up to 24 antigens.

Preparation of the immunosorbent. A highly dispersed p-aminobenzylcellulose (PABC) was obtained diazotized, and proteins were fixed on it by a method which did not differ in principle from that which we described earlier [3]. During fixation of the serum proteins on PABC the following proportions were observed: 3.5 ml rat blood serum to 0.1 g residue of diazotized PABC. The antigen–PABC precipitate was separated by centrifugation, washed three times with distilled water, suspended in a known volume of water, and kept in a refrigerator. The amount of protein fixed by 1 g PABC under our conditions varied from 200–400 mg.

Exhaustion of the sera. A certain quantity of antigen–PABC suspension was centrifuged (2000 g, 10 min), the liquid was poured off, and exhausted antiserum was added to the residue. The mixture was car-

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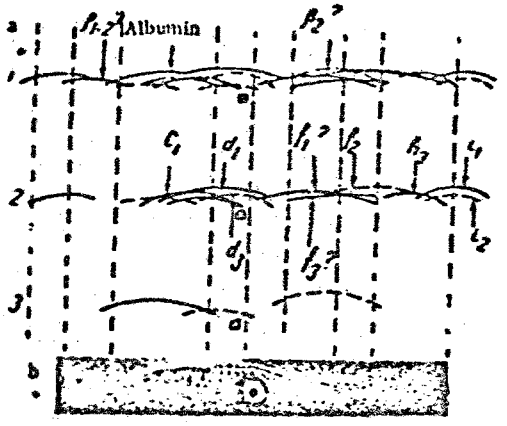

Fig. 1. Immunoelectrophoresis of water-soluble mitochondrial proteins of rat liver cells (a) and (photograph of preparation) immunoelectrophoresis of rat blood serum using eluate of antibodies from immunosorbent exhausted with antimitochondrial serum as developer (b). Sera used for immuno-development: unexhausted antimitochondrial serum (1); antimitochondrial serum exhausted with rat blood serum proteins (2) serum against rat blood serum (3).

fully stirred, uncubated at 37° for 1 h, and again centrifuged (2000g. 15 min). The serum was poured off and tested for the presence of antibodies against serum proteins by the ring-precipitation test and by Ouchterlony's immunodiffusion method. The residue was washed with a minimal volume of physiological saline and the supernatant was concentrated and added to the main serum.

To elute antibodies absorbed on the antigen the residue was treated with a small volume of acidified physiological saline (pH 3.5), the suspension was thoroughly mixed, and if necessary acidified with 0.1 N HCl to pH 3.5, kept in a refrigerator for 10-12 h, and centrifuged (2000 g, 10 min). The resulting supernatant containing eluted antibodies was alkalified to pH 7.5 and concentrated. The residue was washed with acidified physiological saline and again with water. This residue could be used repeatedly for exhaustion, but its capacity gradually diminished. For comparison analogous antisera were completely exhausted from antibodies to rat blood serum. For exhaustion both with water-insoluble antigen and with antigen powders, the amount of antigen needed to produce exhaustion of the sera was chosen empirically by adding the antigen successively and testing the antibody content.

EXPERIMENTAL RESULTS

Unexhausted serum against liver cell hyaloplasm (anti-HP serum) detected 8-9 antigens among the serum proteins, consisting of albumin, γ-globulin, α_2-haptoglobin (haptoglobin was detected by its peroxidase activity), three antigens with mobility of γ_1-globulins, and one component each of the prealbumins and α_2- and β_2-globulins. Unexhausted sera against proteins of the cytoplasmic granules (antimitochrondial and antimicrosomal sera) detected one or two antigens more in the rat blood serum than in anti-HP serum.

Sera exhausted with water-insoluble antigen and sera exhausted with lyophilized powder of rat serum when used in immunoelectrophoresis experiments detected the same number of antigens among proteins of the hyaloplasm, mitochondria, and microsomes of the liver (23-24, 10-12, and 7-8, respectively), but sera exhausted with water-insoluble antigen gave clearer precipitation lines. Antibodies eluted from the immunosorbent distinguished the same antigens as unexhausted sera in rat blood serum (Figs. 1 and 2).

To investigate the completeness of fixation of serum antigens on diazotized PABC and also the completeness of absorption of antibodies on these antigens, we exhausted a rabbit serum against rat serum proteins with water-insoluble antigen (serum proteins-PABC). This showed that this particular method completely removed all precipitating antibodies against blood serum antigens revealed in the immunoelectrophoresis experiments. Hence, with an immunosorbent prepared on the basis of diazotized PABC all 24 discovered antigens were fixed in adequate amount for exhaustion of highly active precipitating sera.

It must be assumed that stable fixation of proteins on diazotized PABC takes place by the formation of azo bonds with histidine, tyrosine, and phenylalanine, and also by reaction of free amino groups and other groups of the protein molecule with the diazo derivatives [3, 4]. This probably also accounts for the absence

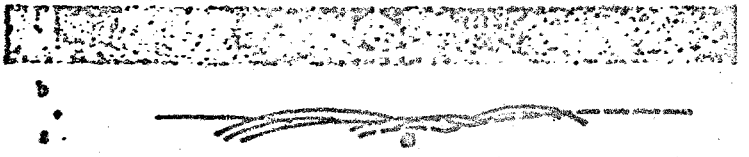

Fig. 2. Immunoelectrophoresis of rat blood serum using immune serum against rat liver mitochondria as developer. a) Scheme; b) photograph of preparation.

of any clear selectivity in the fixation of individual serum proteins on diazotized PABC, making it possible to obtain a polyantigenic immunosorbent. At the same time, we were unable to prepare a complete immunosorbent from water-soluble proteins of the hyaloplasm and cytoplasmic granules of rat liver and kidney cells. Experiments using electrophoresis and immunoelectrophoresis showed that tissue protein fractions with lower electrophoretic mobility than serum γ-globulins (separation in agar gel with medinal buffer, μ 0.05 and pH 8.2), i.e., proteins with a low negative electric charge, are only weakly fixed on diazotized PABC. This can be understood because of the reacting groups of PACB ($-N \equiv N^+Cl^-$) are positively charged, and other conditions being equal, proteins with a low negative charge are not fixed so well on PABC, thus not ensuring an adequate content of antigen on the immunosorbent for exhaustion of antisera.

Immune sera against tissue proteins exhausted by the method described above have been successfully used in the laboratory of Radiation Immunology at the Institute of Medical Radiology, Academy of Medical Sciences of the USSR, in immunoluminescence experiments by Coon's method to study the localization of water soluble antigens in the cells of a number of animal organs, thereby freeing the experimenters from the need to remove nonspecific protein admixtures and soluble immune complexes from the antisera.

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