STABILIZATION OF REACTION PRODUCTS OF HUMAN AND ANIMAL SERA WITH TISSUE EXTRACTS IN AGAR GEL

B. É. Chechik

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Sera of man and animals were found to contain two factors giving opposite effects on the formation of precipitation lines during reactions between sera and tissue extracts in agar gel: one factor precipitates tissue factor in agar, while the other, under certain conditions, prevents this process.

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The formation of precipitation lines and bands during investigation of the antigenic structure of tissues by Ouchterlony's double gel-diffusion method can take place not only by reaction between antibodies and antigens, but also as a result of nonimmunologic reactions. It has been shown [1], for instance, that during counter diffusion in agar gel of nucleic acids, histones, and certain other proteins, from one to six precipitation bands are formed.

Interaction between common globulin fractions of human and animal sera and extracts from their tissues and also some factors influencing this interaction were studied in the present investigation.

EXPERIMENTAL METHOD

Saline extracts were made up at the rate of 1 g tissue to 3 ml physiological saline. The tissues were triturated in a mortar with a glass pestle or in a blender. The homogenates were centrifuged at 6000 rpm for 20 min. The residues were discarded and the supernatant treated with merthiolate (1 : 10,000). Some of the extracts were lyophilized. Sera were obtained from immune and intact rabbits, adult Wistar rats, and healthy persons from a blood-transfusion station. Globulin fractions were isolated from the sera by semi-saturation with ammonium sulfate. The gel-diffusion reaction was carried out in a micromodification [2]. Agar for the reaction was made up in physiological saline (except where specially mentioned). Immunoelectrophoresis was carried out in an agar gel made up in phosphate buffer, pH 7.0, at a potential gradient of 5.5 V/cm.

EXPERIMENTAL RESULTS

A comparative study of antigens of human and mouse leukemic tissues showed that during counter-diffusion in agar gel of extracts from spleen, liver, kidney, or lung of persons dying from injury or from leukemia, against general globulin fractions from sera of rabbits immunized with tissue extracts from mice with leukemias, a precipitation line or band was formed. However, an identical line was also formed by reaction between globulin fractions of nonimmune rabbit, rat, and human sera and tissue extracts of rabbits, rats, mice, and man (Fig. 1).

The writer has previously shown that some antigens may have an inhibitory action in the gel-diffusion reaction on other antigens if they are placed in neighboring wells [3]. A similar phenomenon was found in the present investigation. When working with human sera and tissue extracts in neighboring wells, it was observed that the sera could inhibit the formation of precipitation lines due to reaction between the serum globulin fractions and tissue extracts. As shown in Fig. 2 the reaction was suppressed even if human tissue extracts were poured into the bottom well, physiological saline into the two lower side wells, and into the top well, and human serum into the two upper side wells. Inhibition of the reaction could also be produced by

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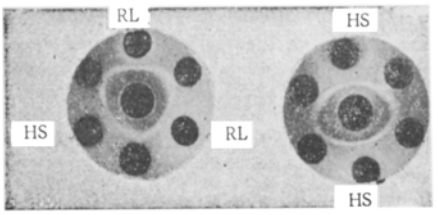

Fig. 1. Reaction between rabbit serum globulin and extracts of human and rat tissues. Central wells contain rabbit serum globulin; RL – rat liver extract; HS – human spleen extract.

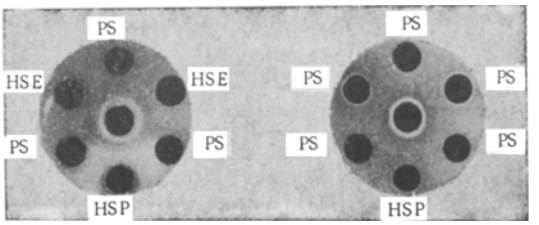

Fig. 2. Inhibitory action of whole sera on formation of precipitation band. Central wells contain rabbit serum globulin; PS – physiological saline; HSP – human spleen extract; HSE – human serum.

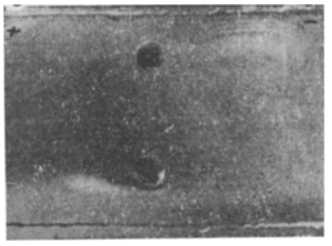

Fig. 3. Comparison of electrophoretic mobility of serum precipitating factor and rabbit immune globulin during immunoelectrophoresis. Top well contains rabbit antiserum against human serum proteins; bottom well contains rabbit serum globulins; top gutter contains human serum; bottom gutter contains human spleen extract.

treatment of the serum globulins with equal volumes of whole sera from which these globulins had been removed. The inhibitory activity of the sera depended on the concentration of tissue factor in the tissue extracts. If up to 25 mg lyophilized human spleen powder was dissolved in 1 ml distilled water, complete inhibition of the reaction by the sera was observed. With a further increase in concentration of tissue factor in the solutions, inhibition was either absent or incomplete. Whole sera, like their globulin fractions, started to react themselves with these extracts, forming a precipitation line or band. The results suggest that whole sera contain a stabilizing factor which, under certain conditions, can prevent the formation of precipitate in agar gel during counter-diffusion of tissue extracts and sera.

Precipitation lines or bands appearing during interaction between serum precipitating factor and tissue extracts differed from lines arising as a result of interaction between antibodies and antigens. This was seen most clearly when tissue extracts were poured into the top and bottom peripheral wells, the four side wells were left empty, and serum globulin was poured into the central well. With this arrangement of the reaction, one wide precipitation line was formed opposite each well with extracts, curving around the empty wells and joining together to form a complete elipse (Fig. 1).

A series of experiments was carried out to study the effect of individual buffer solutions on the course of this reaction. Four precipitation lines were found to be formed in agar made up in physiological saline or phosphate buffer, pH 7.0. A visible precipitate was absent or ill defined if the agar was made up in veronal-medinal buffer, such as is usually used in immunoelectrophoretic experiments (pH 8.6, ionic strength 0.025 or 0.05).

The electrophoretic mobility of the serum precipitating factor was studied in immunoelectrophoretic experiments. The agar for these experiments was made up in phosphate buffer, pH 7.0. The results showed that the zone of migration of this factor lies much nearer to the anode than that of immune γ-globulins (Fig. 3). These results indicated that the serum precipitating factor is not an antibody.

A study of the physicochemical properties of serum and tissue factors participating in precipitate formation was not among the objects of this investigation. All that was noted was that the serum precipitating factor retains its activity if kept in a refrigerator at 4° for several months; tissue factor is inactivated under these conditions within 5-7 days.

The results of this investigation can be summarized by saying that two factors were found in the sera of man and animals which had opposite effects on the process of formation of the precipitation line during interaction with tissue extracts: one factor can precipitate tissue factor in agar, the other prevents this process under certain conditions.

It may be that the physiological functions of the individual factors of this complex are not interconnected in vivo, although it can be postulated that the stabilization phenomenon as described above in agar gel also takes place in vivo. Under normal conditions this process is not accompanied by the formation of precipitates and it plays a definite role in the stabilization of serum proteins while they react with certain substances entering the blood stream after cell destruction. Under pathological conditions characterized by the entry of an excess of tissue factor into the blood stream, equilibrium between them and the serum factors is disturbed, leading to the formation of precipitates in the blood or on the surface of the cells.

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