INHIBITION OF GEL-DIFFUSION REACTION IF ANTIGENS ARE PLACED IN NEIGHBORING WELLS

B. É. Chechik

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Human blood serum contains a labile antigen detectable by the gel-diffusion reaction when tested with rabbit serum against human thymus. If the fresh sera and erythrocyte hemolysates to be tested are placed in neighboring wells, this antigen loses its serologic activity.

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The micromodification of Ouchterlony's gel-diffusion reaction is widely used for comparing the antigenic structure of tissues, bacteria, and viruses. However, the antigens to be compared are poured into neighboring wells. The well with antiserum lies opposite these wells. During migration of homologous antigen and antiserum toward each other, a precipitate is formed in the equivalent zone, visible as a precipitation line. The degree of identity of the antigens is estimated from the character of the precipitation lines formed.

In this paper an observation made during the study of the antigenic structure of human thymus is described: if the two antigens to be compared are placed in neighboring wells, one of them may inhibit the other's reaction with antiserum.

EXPERIMENTAL METHOD

Antigens for immunizing rabbits were prepared from a mixture of thymus glands from human fetuses aged 20-35 weeks dying as a result of premature birth. Thymus tissue, to which physiological saline was added in the proportion of 1:5, was homogenized in a blender. The homogenate was filtered through two layers of gauze; streptomycin and penicillin (2000 units of each antibiotic per ml antigen) were added to the filtrate. Rabbits were immunized in accordance with the usual scheme adopted in our laboratory [3]. Antisera obtained from 5 rabbits after the second cycle of immunization were pooled and absorbed by a mixture of 40 sera of healthy donors of blood groups O (I), A (II), and B (III) and with saline extracts of kidneys, lungs, and liver from the cadavers of two persons dying from accidental injuries. The donors' sera were kept in a refrigerator at 4° for 7 days. The total globulin fraction was isolated from the mixture of exhausted antisera by half-saturation with ammonium sulfate.

To obtain adult human hemolysates, blood was taken from the cubital vein, while neonatal blood was obtained from the umbilicus. The anticoagulant used was a 1.34% solution of sodium oxalate (1 part anticoagulant to 9 parts blood). The erythrocytes were washed in large volumes of physiological saline at 1000 rpm for 3-5 min. The residue after the third centrifugation was ground in a mortar with a glass pestle, during gradual addition of distilled water at the rate of 1 ml residue to 9 ml water. The homogenate was centrifuged at 6000 rpm for 20 min, the residue discarded, and the homoglobin concentration in grams % estimated in the hemolysates by means of the FEK M-1 photoelectric colorimeter [2]. The gel diffusion reaction was carried out in the micromodification [1]. Immunoelectrophoresis [4] was also carried out in a micromodification in an apparatus for high-voltage immunoelectrophoresis at a potential gradient of 8.3 V/cm and in a current of 20-40 mA.

EXPERIMENTAL RESULTS

The globulin fraction of the exhausted thymus antisera obtained after the second cycle of immunization, when tested with human thymus extract, formed 2 or 3 precipitation lines. When this antiserum re-

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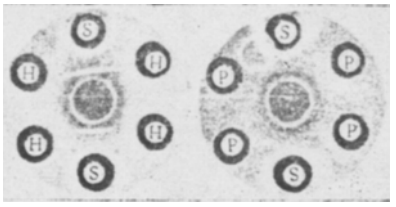

Fig. 1. Arrangement of gel-diffusion reaction with thymus antiserum. S) Fresh adult human serum; H) hemolysates of donors' erythrocytes; P) physiological saline; central wells contain exhausted thymus antiserum.

acted with fresh adult sera it formed one precipitation line. The antigen corresponding to this line was found in the sera of healthy donors and of patients with cancer and various diseases of the blood system. It could not be detected in hemolysates of neonatal and adult blood. It lost its antigenic activity when kept in a refrigerator at 4° for 4-5 days and when heated on a water bath at 56° for 30 min. In immunoelectrophoresis experiments it was detected in the zone of human serum α-globulin. A study of the properties has shown that it can no longer be detected in fresh human sera after they have reacted with hemolysates.

This effect could be obtained in two ways: first, if fresh human serum and hemolysates containing 1.5-3 g% hemoglobin were poured into neighboring wells. With this arrangement of the antigens, human serum did not react with thymus antiserum, although in the control, when physiological saline replaced hemolysates in the wells, a clear precipitation line was formed opposite the well with human serum (Fig. 1).

Second, the same effect was obtained by direct mixing of equal quantities of fresh human serum with successive dilutions of hemolysates. The mixtures were incubated at room temperature and then used in the gel-diffusion reaction, when they were poured into the peripheral wells while thymus antiserum was poured into the central well. When the experiment was set up in this manner the antiserum did not react with human sera to which dilutions of hemolysates starting from initial to dilutions of $1/8$-$1/16$ had been added, but it continued to form a precipitation line if hemolysates were added to the sera in higher dilutions. These results indicate that the hemolysates contain an enzyme which causes inactivation of labile serum antigen. Our purpose was not to discover the chemical nature of this antigen. Because of its lability it can be assumed to be a lipoprotein, and that the enzyme contained in the hemolysates is a lipoproteinase. This enzyme was either destroyed during preparation of the extracts used for immunization or was present in fetal tissues in quantities too small to cause complete inactivation of the substrate. If it is remembered that the antisera were exhausted with human sera kept for 7 days at 4°, this satisfactorily explains why the exhausted thymus antisera were able to detect labile serum antigen.

The formation of this antigen is evidently unconnected with the function of the thymus. We felt that certain of its properties should be investigated to enable us to describe this effect of inhibition of the gel-diffusion reaction when two different antigens are placed in neighboring wells. The possibility of this inhibition must always be borne in mind when the micromodification of the gel-diffusion reaction is performed.

LITERATURE CITED

1. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
2. G. V. Derviz and A. I. Vorob'ev, Lab. Delo, No. 3, 3 (1959).
3. B. É. Chechik and O. D. Ramonova-Tskhovrebova, Probl. Gematol., No. 8, 3 (1967).
4. P. Grabar and C. Williams, Biochim. Biophys. Acta, 10, 193 (1953).