THE USE OF THE PAPER PRECIPITATION METHOD FOR EVALUATING THE IMMUNE REACTIONS OF THE SERUM PROTEINS IN SYPHILIS

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Of great value in serological investigations are immunochemical methods, which enable the results of examinations to be expressed not as crosses or titers, but as precise units of protein entering into the composition of the antigen-antibody complex. Recently certain methods have been described, comparitively simple in performance and reasonably accurate, for determining the content of antigen and antibodies by the amount of protein or nitrogen combined as a result of the paper precipitation reaction [1, 3, 5].

In the present communication are given the results of investigations of the immune reactions of the proteins of Wassermann-positive sera with lipoid antigen on paper. As we know, the lipoid particles of the antigen form an ultraprecipitate with the Wassermann "reagin", visible only under the microscope with dark-ground illumination. In our experiments the quantitative estimation of the reaction was carried out by determining the content of protein remaining after paper chromatography at the site of successive application of antigen and serum or, conversely, of serum and antigen. According to A. E. Gurvich's findings [3], the flow of the buffer solution during chromatography removes from the site of application of the antigen and immune serum all the proteins not deposited as precipitate.

EXPERIMENTAL METHOD AND RESULTS

Variant 1. Lipoid antigen for the Wassermann reaction was diluted with physiological saline (1:10) immediately before the experiment. The antigen solution was applied to a strip of chromatographic paper B (rapidly absorbing paper from the Volodarskii paper factory, Leningrad). The strips of paper were than placed in an incubator for drying at 45° for 20 minutes. Next, the inactivated syphilitic and control sera were carefully applied, in volumes of 0.02 ml, to the same places where the antigen had previously been applied, and incubated in a humid chamber at 37° for 10 minutes. After incubation was completed, the strips of paper were placed in a chamber for descending chromatography. As solvents we used a veronal-medinal or phosphate buffer (pH = 9.2). Chromatography was continued for 5-6 hours, after which the chromatograms, dried at 100°, were stained with a solution of bromphenol blue, prepared from the usual formula. The quantity of protein in the precipitate was calculated from the quantity of dye combined with the protein. The difference between the extinction of the eluted solution in the experimental and control tests showed the increase in the protein content at the site of application of the serum, and was interpreted as a positive reaction (expressed in mg% of specifically reacting proteins of the serum tested). The calculation of the absolute content of antibodies in the precipitate was made from a calibration curve for crystalline albumin, derived from data in the literature [6].

By way of illustration we give a chromatogram (Fig. 1). As may be seen from Fig. 1, sera giving complete arrest of hemolysis in the Wassermann reaction (++++) showed a varying ability of their proteins to combine with antigen on paper. In some patients with progressive paralysis and cerebral syphilis, for instance, their sera contained from 15 to 253 mg% of protein, firmly combining with antigen. Simultaneous testing of

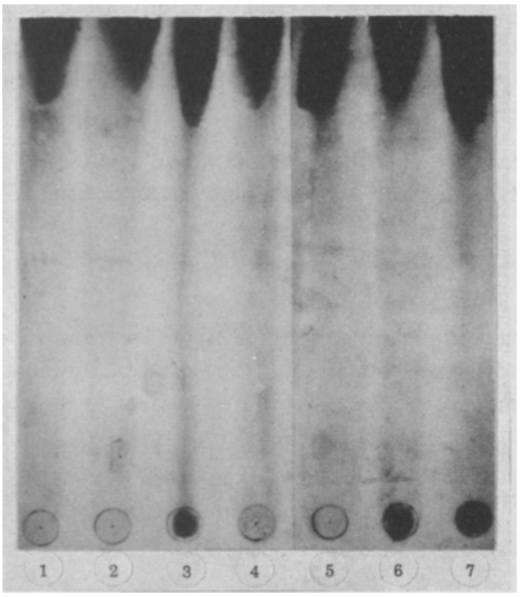

Fig. 1. Precipitation reaction of the serum proteins with antigen absorbed on paper (diluted lipoid antigen for the Wassermann reaction — 0.02 ml, serum — 0.02 ml). 1) Antigen + donor's serum; 2) the same + serum (leprosy); 3) the same + serum (cerebral syphilis; all syphilitic sera were W.R. positive [++++]); 4) the same + serum (cancer); 5) the same + serum (tuberculosis); 6) the same + serum (progressive paralysis after a course of treatment); 7) the same + serum (progressive paralysis before treatment).

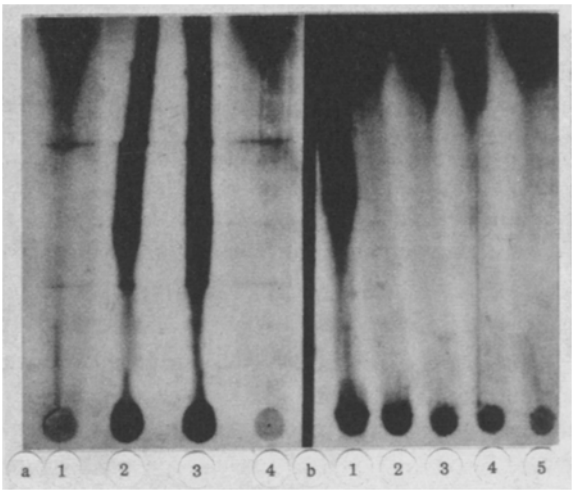

Fig. 2. Formation of a precipitate during the reaction of antigen with serum preliminarily dried on paper. a) Serum (0.0151 ml), lipoid antigen: 1) serum (latent syphilis) + 96° ethyl alcohol (0.011 ml); 2) the same + antigen (0.0051 ml); 3) the same + antigen (0.011 ml); 4) donor's serum + antigen (0.0051 ml). b) Precipitating serum of a rabbit (0.0151 ml), antigen — horse serum (0.0141 ml); 1) immune serum + antigen (112.5 μg of protein); 2) the same + antigen (56.25 μg); 3) the same + antigen (22.5 μg); 4) the same + antigen (11.25 μg); 5) the same + human serum (110.5 μg of protein).

of the sera of some other patients (with leprosy, cancer, tuberculosis, etc.) by this method gave negative results. It should be pointed out that in some cases of latent syphilis, when the Wassermann reaction and the precipitation tests (Kahn, Sachs-Witebsky)* were strongly positive, it was not possible to demonstrate any increase in the protein at the site of application of the serum and antigen after chromatography. Of the 23 Wassermann-positive sera from patients with different forms of syphilis which we examined, in only 6 cases did we observe an obviously positive paper precipitation reaction.

Variant 2. Inactivated experimental and control sera were applied to strips of paper in volumes of 0.0151 ml. After completely drying in the air, to the site of application of the sera was carefully applied undiluted lipoid antigen in a volume of 0.0051 ml. Such accuracy in the measurement of the volume of liquid was achieved by means of an automatic micropipette [2]. After 2-3 minutes the strips of paper were placed in a chamber for descending chromatography. Chromatography was continued for 3 hours. The treatment of the chromatograms and the reading of the reactions were done as was described above.

The results obtained in these experimental conditions showed that the majority of syphilitic sera possess a well-marked property of forming a precipitate with entigen on paper. At the site of formation of the precipitate, the nonspecific proteins were evidently also partially held up, but in the process of chromatography these were gradually washed out, leaving traces on the paper in the direction of flow of the buffer (Fig. 2, a). It must be pointed out that a similar hold-up of part of the protein at the site of formation of the precipitate (this happens most often when the optimum amount of antigen is added) is observed during chromatography of precipitating sera of rabbits immunized with serum proteins (see Fig. 2, b).

* The serological examinations were carried out by E. V. Kantimulina and E. B. Panova in the Regional Venereological Dispensary.

There are indications in the literature that Wassermann-positive sera (++++), may react directly with a protein antigen fixed on paper by means of a haloid alkylate [4]. It was shown in our experiments that the interaction of immune proteins of specific sera with antigen on paper may be obtained without preliminary fixation of the lipoid antigen.

It is still too early to speak of the diagnostic value of the reaction described, for this would demand a comparative evaluation of the test on extensive clinical material. Nevertheless the positive results obtained may be of definite interest for research in this direction.

SUMMARY

Some Wassermann-positive serums give precipitation reaction on paper with diluted lipoid antigen. Preliminary drying of serum on paper and employment of nondiluted antigen in certain ratios considerably increased the sensitivity of this method. It is presumed that Wassermann reagents are precipitated on paper with lipoid antigen.

LITERATURE CITED

[1] V. S. Gostev, N. A. Shagunova, Byull. Éksptl. Biol. i Med., 44, No. 10, 121-125(1957).*

[2] A. E. Gurvich, Laboratornoe Delo, No. 3, 3-9 (1955).

[3] A. E. Gurvich, Biokhimiya, 5, 550-553 (1955).

[4] A. E. Gurvich, Laboratornoe Delo, 6, 1028-1034 (1957).

[5] A. E. Gurvich, R. B. Kapner, Laboratornoe Delo, No. 2, 23-26 (1958).

[6] E. Kabat and M. Heidelberger, J. Exper. Med., 1937, v. 66, p. 229-250.

*Original Russian pagination. See C. B. Translation.