A MODIFICATION OF THE METHOD USING PRECIPITATION IN AGAR TO COMPARE TWO ANTIGEN-ANTISERUM SYSTEMS

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The method using precipitation in agar became particularly effective after Ouchterlony modified it to compare complex antigens with each other [4]. The fact that this modification permits one to establish the immunological affinity of the components of complex systems, as well as to determine the number of antigens in a system, makes it particularly important.

Different variations of Ouchterlony's method are in use, most of which are essentially an elementary system consisting of two comparable antigens diffusing towards a serum or, conversely, to antisera diffusing towards one antigen [3]. Whith this arrangement, an antigen found only in one system forms a "spur" with the homologous serum, which does not run together with the precipitation spectrum of the heterologous system.

In many cases, however, depending chiefly on the strength of the antigen and serum as well as on the complexity of the spectrum of the preparations being compared, it is difficult to determine the true "spur" and to distinguish it from a false one caused by a difference in the concentrations of the antigens being compared. This difficulty is aggravated by the fact that the "spur" forms in the zone farthest from the diffusion centers, so that these most important parts of the spectrum are often not clearly apparent.

We often encountered these difficulties in work with tissue antigens and therefore developed a simple modification of Ouchterlony's method in order to more clearly demonstrate specific antigens. With the use of this modification, the precipitation zones are situated so that the whole length of the line of the antigen specific to one system only differs in direction from the spectrum of the antigens common to the preparations being compared and crosses this spectrum in the optimal reaction zone. In addition, this modification permits the comparison of two complete antigen-antiserum systems at once.

Two systems of antigen and its corresponding serum (AC-ac and BC-bc, where A,B,C are the antigenically unrelated components and a,b,c are their corresponding antibodies) are arranged crosswise at opposite corners of a square (Fig. 1, I).

The presence of other, unrelated antigens, antibodies or the precipitates formed by them in the agar does not affect the reaction of a specific antigen-antibody pair. Therefore, in the interest of clarity, the reaction of each antigen-antibody pair can be considered separately as if it had occurred in an isolated system.

Figure 1, II shows the reaction between antigen A and its antibody a. The precipitate Aa forms where the diffusion zones of A and a (depressions 1 and 3) meet; because depressions 2 and 4 do not contain related components, the preparations in them do not affect the position of Aa. With optimal A and a correlations, the precipitate line passes through the center, and its ends rest on the depressions of the heterologous system. If, however, there is a large surplus of one of the components, the line passes below or above depressions 2 and 4, and the ends of the line Aa become somewhat distorted.

The phenomenon is demonstrated in Fig. 1, III. The antibody, when there is a greater amount than the antigen A, diffuses and enters the fluid-filled depressions 2 and 4, mixing into them. Owing to this, part a, diffusing through the depressions, overlaps the common diffusion front in the agar, and the portions of zone A adjoining depressions 2 and 4 bend under symmetrically, as shown in Fig. 1, III.

The above is also true of system B-b, the precipitate of which is located between depressions 2 and 4, at right angles to Aa (Fig. 1, IV). In the presence of an antigen common to the preparations being compared (C) and antibodies to it (c), the line of precipitation runs in a different direction (Fig. 1, V). This is because C, diffusing simultaneously from two depressions (1 and 2) forms a common antigen front, moving from left to right to meet the common front of the antibodies diffusing from the serum depressions 3 and 4. The precipitate which forms at the meeting place of the fronts Cc, therefore, is located in the zone between the antigen and serum depressions.

Therefore, if two systems which have a common antigen, as well as one specific to each system, are compared, the lines of the precipitates for the specific antigens form a cross, while the precipitate line formed for the common

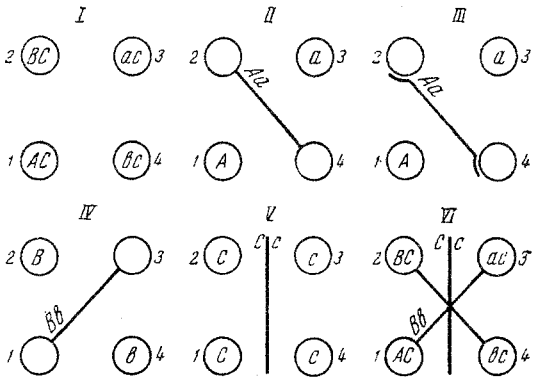

Fig. 1. Schematic representation of the modified precipitation-in-agar reaction (see text for explanation).

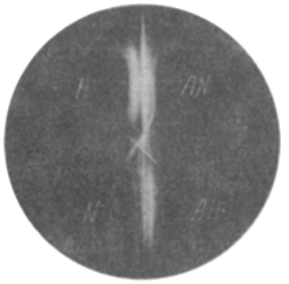

Fig. 2. Comparison of two systems with common antigens and antigens specific to each system. N) Antigen from mouse liver; H) antigen from mouse hepatoma; AN) antiliver serum; AH) antihepatoma serum.

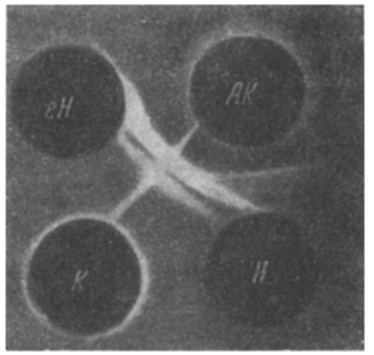

Fig. 3. Nonidentity reaction of specific hepatoma antigen with kidney antigens. H) Antigen from mouse hepatoma; K) antigen from mouse kidney; eH) antibodies to specific hepatoma antigen; AK) antikidney serum.

antigen is vertical (Fig. 1, VI). It is obvious that the same figure is also formed in more complex systems.

The proposed experimental arrangement has certain advantages over the usual arrangement. In the first place, it facilitates the demonstration of qualitatively different antigens, because the whole length of the lines formed by such antigens differ in direction from the spectrum of common antigens, which these lines cross in the optimal reaction zone, i.e. at the shortest distance from the centers of diffusion. In the second place, the proposed arrangement permits one to compare two complete antigen-antiserum systems and to obtain of full serological characterization of them simultaneously. Moreover, the lines of a specific precipitate form only if analogous antigen and the antibodies to it are not present in the heterologous system, which creates more rigid and objective criteria of the specificity of the given component.

We have used this modification extensively in the study of organospecific and specific tumor antigens [1,2].

Figures 2 and 3 show examples of the modified precipitation-in-agar reaction, using different antigens and corresponding antisera.

SUMMARY

Antigens and antisera are placed in depressions inside the angles of a square. Each antigen is located diagonally opposite the homologous serum. The antigens specific for each system give precipitation lines crossing at the center of the square at right angles to each other, their ends resting against the depressions of the heterologous system. The antigens common for the systems under comparison produce lines passing between the reservoirs of both antigens and sera. This method facilitates the detection of qualitatively different antigens in the preparations under comparison and permits the analysis of two complete antigen-antiserum systems.

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