QUANTITATIVE DETERMINATION OF ANTIBODIES BY THE GEL DIFFUSION REACTION

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In the gel diffusion reaction the antibody concentration is directly proportional to the antigen concentration at which the precipitation band disappears (in the zone of antigen excess). The concentration of antibodies in immune sera can thus be determined.

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The gel diffusion reaction is now widely used for the identification of antigens and its sensitivity is much higher than that of the precipitation test in tubes. Work has therefore been done recently on the use of this reaction for the quantitative determination of antigen [3, 4, 6, 7].

In the investigations cited, the antigen concentration was determined by comparison with the results of parallel titration of a standard solution of the same antigen.

It is known from the literature that the precipitate dissolves readily in the zone of excess of antigen. There was reason to suppose that for each dose of antibodies there must be a certain concentration of antigen at which the precipitation band formed in the agar in Ouchterlony's test must disappear, and that this could be used for quantitative determination of the antibodies.

A commercial preparation of bovine serum albumin was used in the work. Antisera against it were obtained by immunization of rabbits. The antibody concentration in the antisera was determined by Heidelberger's method [5].

The gel diffusion reaction was carried out in Gusev and Tsvetkov's micromodification [2] of Ouchterlony's method.

Melted agar was poured on to a glass slab divided into three equal parts, so that 5 ml was applied to each part (strip). After the agar had solidified, the slab was transferred to a humid chamber. Slabs were used the same or next day. Before use, wells 6 mm in diameter were cut out of the agar by means of a punch. Antisera were poured into the central wells in a given dilution, while antigen in different concentrations was poured into the peripheral wells. The slabs were then allowed to stand at room temperature in the humid chamber for 20 h, after which the results of the reaction were read. The concentration of antigen at which the precipitation band disappeared was thus determined.

The experiment was carried out in two stages. In the first stage each dilution of serum was titrated with different dilutions of antigen, the differences between the concentrations in two successive dilutions amounting to 5-10 mg protein/ml. The concentration at which the precipitation band was no longer recordable was determined. An example of such a titration is illustrated in Fig. 1a.

In the second stage the same dilution of serum was titrated with dilutions of antigen from a point without precipitate to a point where a precipitation band was present, at intervals of 1 mg. A specimen of such a titration is illustrated in Fig. 1b. In this way the boundary of the antigen concentration at which the precipitation band disappeared could be determined precisely. Four antisera against bovine serum albumin were tested by this method.

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TABLE 1. Relationship between Antibody Concentration in Antiserum against Bovine Serum Albumin and Antigen Concentration at Which Precipitation Band Disappears

Antigen protein (in mg)	2,8	4,0	4,0	5,0	6,0	8,0	8,0	9,0
Antibody nitrogen (in μg)	24,37	32,5	36,2	43,5	48,7	54,5	65,0	70,5

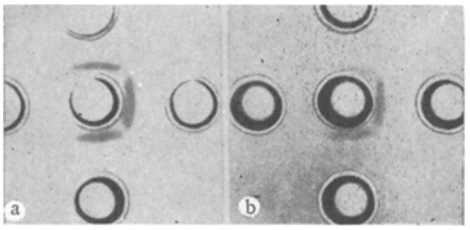

Fig. 1

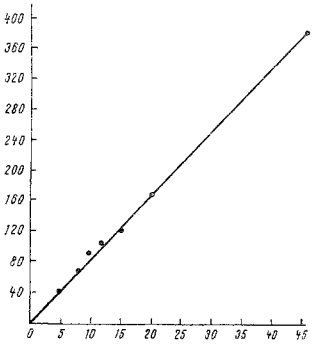

Fig. 2

Fig. 1. Titration of serum with different doses of antigen: a) antiserum diluted 1:4 in center, antigen in doses of 5, 10, 15, and 20 mg protein/ml (wells contain 1.25, 2.5, 3.75, and 5 mg protein); b) serum diluted 1:4 in center, antigen in doses of 16, 17, 18, and 19 mg protein/ml (wells contain 4.0, 4.25, 4.5, and 4.75 mg protein).

Fig. 2. Relationship between antibody concentration and antigen concentration at which precipitation band disappears. Abscissa, content of antigen protein (in mg); ordinate, content of antibodies (in μg nitrogen).

EXPERIMENTAL RESULTS

The results of several experiments are given in Table 1. Their comparison shows that the following relationship exists between the antibody concentration in the antiserum and the antigen concentration at which the precipitation band disappears (in the zone of excess antigen): with an increase in antibody concentration the dose of antigen causing disappearance of the precipitation band also increases.

This relationship can be expressed by a straight line (Fig. 2) passing through the zero point and described by the equation of direct proportionality $y = kx$. In this equation y represents the concentration of antibodies in the test antiserum and x the concentration of antigen in the zone of antigen excess at which the precipitation band is absent; the coefficient of proportionality k can be calculated as the mean for the points shown on the graph. In the experiments with bovine serum alubmin it was 9.1 ± 0.33. By means of this equation the antibody concentration in an unknown serum can be determined with accuracy.

The results of several such determinations are given in Table 2, where they are compared with data obtained by testing the same antisera by Heidelberger's method.

It will be clear from Table 2 that the concentration of antibodies determined by the two methods did not differ significantly. The differences lay within limits of 0-12%.

TABLE 2. Results of Determination of Antibody Concentration by Heidelberger's Method and by Gel Diffusion Reaction

Concentration of antibodies (in μg N_2/ml)		Difference (in %)
by Heidelberger's method	by gel diffusion reaction	
24,4	25,5	4,5
36,2	36,5	0
48,7	54,7	12,3
65,0	72,9	12,1
97,5	91,2	6,4
108,0	109,4	1,3
390,0	410,4	5,2

The gel diffusion reaction can thus be used to determine the concentration of antibodies in unknown antisera provided that the tests are carried out in the zone of excess of antigen. It must also be pointed out that if an antiserum with a known concentration of antibodies, determined by the methods of Heidelberger [5] or Gurvich [1], is available the opposite problem can be solved: the unknown concentration of antigen can be determined by using the equation given above.

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