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Quantitative Method for Determination of Precipitin in Small Volumes of Rabbit Anti-Crystalline-Egg-Albumin-Serum.

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The usual dilution methods for determining the precipitin titer of antiserum only roughly approximate the relative antibody strength of a given serum and give no information with regard to the absolute quantity of antibody. Analysis of precipitates from the precipitinogen-precipitin reaction for their nitrogen content by the micro-Kjeldahl method has been used by Wu and his collaborators[1] and Heidelberger and Kendall.[2] Precise measurements of the antibody per unit volume of antiserum, and exact studies on the ratio of antigen to antibody in the precipitate have thus been made possible. This method requires at least 0.5 cc. of serum for each test and cannot be used when only small volumes of antiserum are available.

We have found that the following procedure gives satisfactory results for determining the quantity of precipitin in a small volume of rabbit anti-crystalline-egg-albumin serum. The method was suggested by figures presented by Heidelberger and Kendall. It depends on the determination of the amount of antigen necessary to precipitate the precipitins completely from a given volume of an antiserum. This determination of the mg. of antigen necessary completely to precipitate the antibodies from a sample of such serum is easily accomplished without analytical methods because the point of maximum precipitation proves to be that point where antigen as well as antibody is completely precipitated and neither antigen nor antibody remains in the supernatant fluid. We have called this optimum ratio of antigen to antibody the neutralization point. Furthermore, at this optimum point antigen and antibody combine in a ratio of about 1:13. The description of the method will clarify these points.

Method. The stock solution of crystalline egg albumin was diluted with saline to provide known concentrations of the antigen expressed in mg. of nitrogen per cc. One-tenth cc. of each antigen dilution was mixed with an equal volume of the antiserum tested,

[1] Wu, H., Chang, L. H., and Li, C. P., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 853. Wu, H., Sah, P. P. T., and Li, C. P., *Ibid.*, 1929, **26**, 737.

[2] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1929, **50**, 809, and *Science*, 1930, **72**, 252.

and incubated 2 hours at 37°, then put in the icebox overnight. The next day, after centrifugation, the supernatant fluids over the precipitates were tested for antigen and for antibody. When antigen in excess had been added to the anti-egg-albumin-serum, antigen only was found in the supernatant fluid, while antibody only could be demonstrated in the supernatant fluids of the tubes in which the quantity of antigen added was insufficient to precipitate all the antibody. If the dilutions of antigen were properly spaced, the supernatant fluid of one tube would be found to contain neither antigen nor antibody within the limits of delicacy of the test. This represented our neutralization point.

The importance of the neutralization point depended on the facts that: (1) the amount of precipitate obtained at the neutralization point proved to be the maximum amount of precipitate obtainable from the antiserum and (2) the ratio of antigen to antibody in this precipitate was a constant ratio of about 1:13. That the quantity of antigen necessary to establish the neutralization point represented the amount necessary to precipitate the maximum amount of antibody was proved by carrying out micro-Kjeldahl determinations for the nitrogen content of the precipitates obtained by incubating 1 cc. portions of antiserum with equal quantities of the same antigen dilutions used in the small test tube experiments described above. That the ratio of antigen to antibody at the neutralization point is relatively constant was proved by determining the mg. of antibody precipitated by antigen at the neutralization point in a large number of sera from different animals. The results indicated: (1) the identity of the point of neutralization and the point of maximum precipitation, and (2) a constant ratio of about 1:13 between antigen and antibody in the precipitate obtained at the neutralization point. Mg. of antigen at neutralization point X 13 = Mg. antibody in serum.

TABLE I.
Identity of points of neutralization and maximum precipitation.

Serum cc.	Antigen Mg. N	Precipitate Mg. N	Substance in excess
1	.031	.358	Antibody
1	.037	.456	Antibody
1	.043	.556	Neither
1	.050	.491	Antigen
1	.056	.319	Antigen

TABLE II.
Ratio of antigen to antibody at neutralization point.

Serum No.	Antigen Mg. N	Antibody Mg. N	Ratio: Aby/Agn.
1	.008	.104	13.0
2	.008	.121	15.1
8	.025	.329	13.1
19	.040	.441	11.0
20	.040	.534	13.3
25	.048	.577	12.0
31	.060	.770	12.8
32	.060	.819	13.6