THE PRECIPITIN REACTION BETWEEN TYPE III PNEUMOCOCCUS POLYSACCHARIDE AND HOMOLOGOUS ANTIBODY

II. Conditions for Quantitative Precipitation of Antibody in Horse Sera*

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Experiments were reported several years ago by the writers (1) leading to an absolute method for the determination of precipitins (2–4). It was originally thought that by following the usual immunological technique, namely incubation of precipitin reactions at 37°C. for 2 hours and letting stand in the ice box overnight, conditions had been established for the maximum precipitation of antibody. While this has been found to be the case for rabbit antisera, experiments such as those given below have shown that the usual immunological practice does not result in maximum precipitation, or in the attainment of true equilibria, in the case of pneumococcus antisera produced in the horse.

It appears, moreover, that objections are still being made to the absolute chemical method for the estimation of precipitins on the ground that non-specific protein nitrogen might be included in the values actually found. Since non-specific protein was shown to be without influence by Marrack and Smith (5) the writers have hitherto refrained from publishing their own data on this point, but are now including such material.

EXPERIMENTAL

1. Incompleteness of Precipitation When Carried Out Both at 37° and 0°.— According to the usual immunological technique, precipitin tests are incubated for

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2 hours at 37°C. and allowed to stand overnight in the ice box. The writers have shown that in rabbit antisera the same amount of specifically precipitable protein is thrown down whether the above conditions are employed, or whether the entire reaction is carried out at 0° (4[1]). This is, however, not the case with antipneumococcus horse sera nor with antibody solutions prepared from horse sera.

Duplicate tubes were set up containing 1.0 cc. of Type III antipneumococcus horse antibody Solution B 61[2] and 0.05 mg. of S III in a total volume of 4.0 cc. In the first pair of tubes the reagents were chilled to 0°C. before mixing, after which the tubes were kept in the ice box overnight and whirled in the refrigerating centrifuge.[3] A second pair of tubes was kept at 37° for 2 hours, allowed to stand overnight in the ice box, and was then centrifuged in the cold. A third pair of tubes was incubated for 2 hours at 37° and was then stoppered and shaken in an ice bath for 3 hours in order to favor the establishment of equilibrium. A fourth pair of tubes was placed in an incubator at 37°C. for 2 hours and whirled in a small Swedish angle centrifuge[4] in the same incubator. The precipitates were washed twice with chilled saline (3) and analyzed for nitrogen by a modification of the micro Kjeldahl method. The results were as follows:

Tubes mixed at, °C. Tubes centrifuged at, °C.	0 0–10	37 0–10	37 Shaken at 0	37 37
Antibody N pptd. by 0.05 mg. S III	1.38 1.38	1.13 1.12	1.15 1.15	1.00 0.97
Maximum specifically pptble. N in solution (0.15 mg. S III)	2.11 2.15			1.85 1.85

It will be seen that the largest quantity of antibody nitrogen is precipitated by a given amount of S III when the solutions are kept at low temperatures throughout. Under these conditions the precipitate forms more slowly and is less gelatinous than at 37°. Allowing the tubes to stand at 0° for an additional 24 hours does not appreciably increase the amount of antibody nitrogen precipitated. When the reaction is started at 37° and completed at 0° far less antibody nitrogen is precipitated, although the amount is greater than if the tubes are centrifuged at 37°. This appears to be due to a partial attainment of the final equilibrium arrived at when the reaction is carried out entirely at 0°, since shaking the tubes for several hours at 0° brings the amount of antibody nitrogen somewhat closer to the 0° value. Similiar results were obtained with another antibody solution, B 60, and with Type III antipneumococcus horse serum. Experiments

[1] Heidelberger, Kendall, and Soo Hoo, page 142.

[2] Prepared according to Felton (6).

[3] Manufactured by the International Equipment Co., Boston.

[4] Supplied by Eimer and Amend, New York.

at 37° for 4 and 24 hours showed that no more antibody was precipitated than in 2 hours.

2. Effect of Non-Specific Protein.—From Table I in the following paper (III) it is clear that at 0° or at 37° the ratios of nitrogen to S III in the precipitate are of the same order whether the analysis is carried out in whole serum (607), in which the antibody constitutes about 15 per cent of the total protein, or in the antibody solution (B 62) prepared from it, in which antibody is 50 to 60 per cent of the total protein. In comparing actual ratios it should be noted (see also Table VI, Paper III) that the antibody solution contains less precipitable nitrogen per cubic centimeter than the serum. This would tend to make the ratio lower at any given S level.

That the above findings are due to the actual antibody content in each instance and not to the presence of other serum components is shown in the following experiment, in which parallel determinations were made with antibody solution alone, and with antibody solution to which an equal volume of normal horse serum had been added. Reiner and Reiner (7) have shown that normal horse serum contains globulin very similar to the antibody fraction in antipneumococcus serum, so that it would be expected that a portion at least of this globulin would be carried down by S if non-specific protein were reactive.

Duplicate tubes containing 1.0 cc. of a Type I pneumococcus antibody Solution B 72 and 0.05 mg. of S I[5] were set up at 0°, at 37°, and at 37° and 0° as described above, and the nitrogen precipitated was compared with a similar series to which 1 cc. of normal horse serum had been added. The results are given in the following tabulation and confirm those of Marrack and Smith (5) on other immune systems.

Effect of Non-Specific Protein

Antibody Nitrogen Precipitated by 0.05 Mg. S I

Temperature, °C. / Centrifuged at, °C.	0 / 0	37 / 0	37 / 37
1.0 cc. B 72	0.92	0.57	0.51
1.0 cc. B 72 + 1 cc. normal horse serum	0.92	0.58	0.52

The above experiments show that addition of non-specific protein has no effect upon the amount of antibody nitrogen precipitated under any of the conditions of temperature investigated.

DISCUSSION AND SUMMARY

The experiments recorded above show that in the case of antipneumococcus horse serum or purified antibody the arbitrary im-

[5] Experiments have shown that the reaction between Type I pneumococcus polysaccharide and its homologous antibody closely parallels that of S III.

munological procedure (37° for 2 hours, overnight in the ice box) does not permit either the establishment of a true equilibrium or the precipitation of the maximum amount of antibody nitrogen. Analyses of such horse sera for antibody content should therefore be carried out at 0° and the determinations should be allowed to stand in the cold for at least 24 hours in order to insure the completion of the reaction.

It is believed that the similarity of the nitrogen : S III ratios in the specific precipitate, whether obtained from whole serum or from purified antibody, and the failure of added serum to influence the amount of nitrogen precipitated show that the absolute chemical method for the estimation of antibody actually measures antibody and not antibody plus a more or less indefinite amount of non-specific protein. An objection to the use of the method is thus shown to be unfounded.

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