9756

Mechanism of Precipitin Reaction. II. Fractionation of Immunologically Pure Precipitin.

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We have reported previously[1] that the antibody of Type I Pneumococcus in horse or rabbit serum is separable into fractions with different immunological and physical properties and that the separation can be achieved by immunological or chemical methods. We have now found that the immunologically pure precipitin of Type I Pneumococcus is itself fractionable.

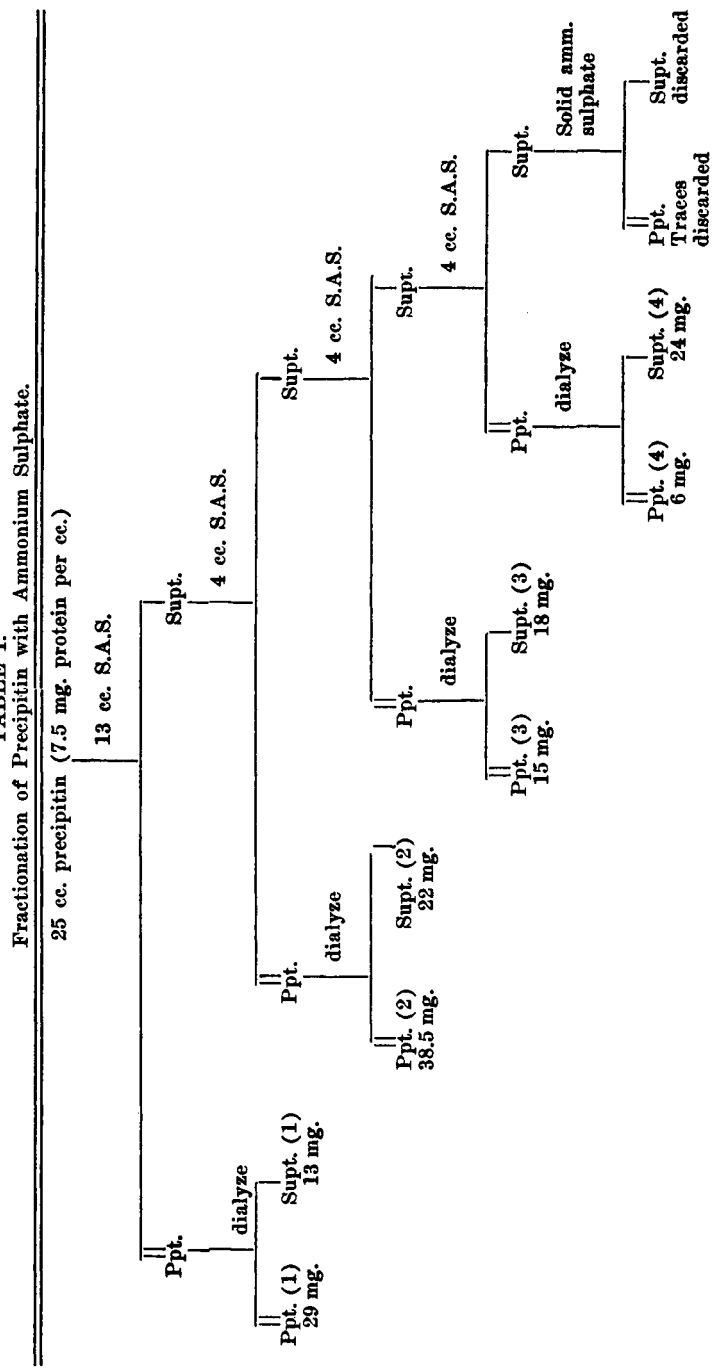
Twenty-five cc. of the antipolysaccharide precipitin solution from Type I anti-pneumococcus horse serum prepared according to our method[2] was fractionated with saturated ammonium sulphate. The scheme of fractionation and the amounts of protein obtained in each fraction are given in the diagram (Table I).

These fractions of protein were separately dissolved in water and dialyzed against pH = 5.5 acetate buffer (0.005 m) at ice-water temperature until free from sulphate. The precipitates were centrifuged and redissolved in 0.85% NaCl solution and found to be completely precipitable by the homologous polysaccharide. They represent, therefore, a fraction of the antibody with isoelectric point at pH = 5.5. The protein in the supernatant fluids was also completely precipitable by the homologous polysaccharide. The isoelectric points of these protein fractions were estimated by determination of the pH at which maximum precipitation occurred. When N/1000 HCl was added drop by drop to the supernatant fluids, precipitation of the precipitin occurred in 1 and 2 but not in 3 or 4. Therefore, the isoelectric points of the antibody in supernatant fluids 1 and 2 must be less than 5.5. When N/1000 NaOH was added, precipitation occurred in supernatant fluids 3 and 4 but not in 1 or 2. Furthermore, maximum precipitation was reached in supernatant fluid 3, before the pH of the solution was high enough to turn the color of phenol red pink, but in supernatant fluid 4 precipitation occurred at a pH red to phenol red, and definitely pink to phenolphthalein. Therefore, the isoelectric point of the precipitin in supernatant fluid 3 must lie between 5.5 and 7.6, while

[1] Chow, B. F., and Wu, H., *Proc. of the Chinese Physiol. Soc.*, 1937, 18; Chow, B. F., and Wu, H., PROC. SOC. EXP. BIOL. AND MED., 1937, in press.

[2] Chow, B. F., and Wu, H., *Chin. J. Physiol.*, 1937, **11**, 139.

TABLE I.
Fractionation of Precipitin with Ammonium Sulphate.



S.A.S. = Saturated ammonium sulphate solution.

that in supernatant fluid 4 must be above 7.6. The original precipitin gave a maximum precipitation at pH 7.6 as previously reported.

These results show that ammonium sulphate can separate the precipitin into fractions with different isoelectric points. It will be recalled that the isoelectric point of the precipitin of Type I Pneumococcus prepared by Heidelberger and Kendall's[3] method of dissociation with a concentrated NaCl solution is 4.8, whereas that prepared by alkali treatment is 7.6. This difference may indicate that different fractions of the antibody are isolated by these two different methods.

9757

Hyperlipemia in Experimental Trypanosomiasis of Rabbits.

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Although changes in the lipoid content of the blood have been described in a variety of physiological and pathological conditions, they have not been sufficiently studied in animals with protozoal infections. Nakanishi[1] observed some increase in the serum cholesterol, fatty acids and phosphatides in the early stage of syphilis in rabbits but all these elements decreased when generalized infection was established. Santos[2] and Borel, *et al.*,[3] found that in patients suffering from malaria the serum cholesterol was subnormal. On the other hand, a slight increase in the blood cholesterol in filariasis was described by Boyd.[4] Linton[5] demonstrated some increase of plasma lipoid phosphorus in rats acutely infected with *Trypanosoma equiperdum.* In a study of anemia produced by the same organism Dubin[6] found in one dog a slight increase in total lipoid of whole blood and plasma accompanied by a decrease of cholesterol. Lannoy

3 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1936, **64**, 161; Heidelberger, M., Pedersen, K. O., and Tiselius, A., *Nature*, 1936, **165**, 138.

1 Nakanishi, M., *Jap. J. Derm. and Urol.*, 1936, **40**, 215.

2 Santos, De Paula, *Trop. Dis. Bull.*, 1927, **9**, 377.

3 Borel, Pons, Advier, and Guillerm, *Ann. Inst. Pasteur*, 1926, **40**, 152.

4 Boyd, T. C., *Indian J. Med. Research*, 1930, **17**, 949.

5 Linton, R. W., *J. Exp. Med.*, 1930, **52**, 103.

6 Dubin, H., *J. Biol. Chem.*, 1913, **33**, 377.