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Ultrafiltration of Type I Antipneumococcal Sera.

KENNETH GOODNER, FRANK L. HORSFALL, JR., AND JOHANNES H. BAUER.

From the Hospital of the Rockefeller Institute for Medical Research and the Laboratories of the International Health Division of the Rockefeller Foundation, New York.

Elford, Grabar and Fischer[1] have reported that the "antibody activity" of antipneumococcal horse-serum is associated with the "larger complex protein in the serum." Thus it was estimated by the method of optimal proportions that 75% of the antibody passed a 140mμ membrane, rather less than one percent passed an 80mμ membrane, while the filtrate from a 54mμ membrane showed no trace of antibody-activity.

The membranes used in the present experiments were prepared by the method of Elford[2] with certain modifications described by Bauer and Hughes.[3] For filtration, Type I antipneumococcal horse- and rabbit-sera were diluted 1:5 in broth. Concentrated antipneumococcal horse-serum was diluted 1:10 in the same reagent. For evaluating the amount of antibody in the filtrate the quantitative precipitation method of Heidelberger, Sia, and Kendall[4] was used. Because of the difficulties in securing large amounts of filtrates complete analyses over a wide range of amounts of polysaccharide were impossible. Therefore an arbitrary amount (0.2 mg. per cc. of original serum) of the Type I acetyl polysaccharide* was employed for each filtrate. Determinations of nitrogen in the washed precipitates were carried out by the gasometric micro-Kjeldahl method of Van Slyke.[5]

The end-points in filtration of the specific antibodies of Type I antipneumococcal horse- and rabbit-sera are shown in Fig. 1. The results are plotted in terms of the percentage of total specifically precipitable nitrogen recovered in the various filtrates against average pore-diameters. With antipneumococcal rabbit-serum no

1 Elford, W. J., Grabar, P., and Fischer, W., *Biochem. J.*, 1936, **30**, 92.

2 Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

3 Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

4 Heidelberger, M., Sia, R. H. P., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

* This preparation of the viscous form of the capsular polysaccharide was kindly supplied by Dr. M. Heidelberger.

5 Van Slyke, D. D., *J. Biol. Chem.*, 1926, **71**, 235.

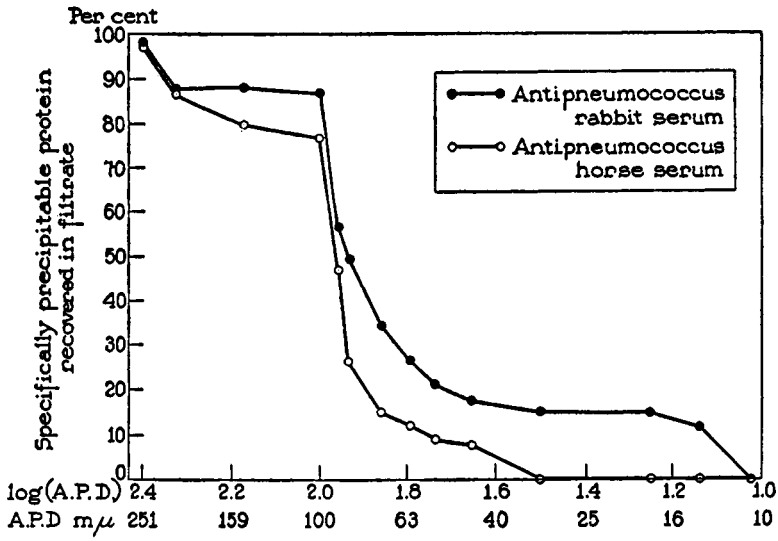

FIG. 1.

specifically precipitable protein passed through a membrane with average pore-diameter of 10.6mμ. A 13.8mμ filtrate contained 11.9% of the total specifically precipitable protein of the serum. Slightly greater amounts were then recovered as the pore-sizes were increased up to 73.0mμ. At this point the curve rose sharply until at 102.5mμ the filtrate contained 86.6% of the total amount of antibody.

With antipneumococcal horse-serum, on the other hand, the smallest pore permitting the passage of antibody was 45.2mμ. Again, between 73.0mμ and 102.5mμ, the curve rose steeply until at the latter porosity 76.7% of the antibody was recovered.

With a concentrated antipneumococcal horse-serum the end-point was relatively sharp, no antibody being recovered at 150.4mμ, while 100% was found in the filtrate at 188mμ.

These findings have been checked qualitatively on several lots of immune sera. In all experiments, both quantitative and qualitative, the rates of filtration agree well with the findings. An obvious difficulty, and one which cannot be eliminated from this technic, is that large particles tend to be fixed in the membrane and thus decrease the actual porosity of the filter.

In generalizing it may be assumed that the smallest specific antibody of antipneumococcal rabbit-serum corresponds to a pore-size of 11mμ, the smallest in horse-serum to a size of 44mμ, while both horse- and rabbit-serum have large specific aggregates correspond-

ing roughly to a pore-size of 88mμ. Furthermore, the antibody of concentrated horse-serum requires a pore-size of approximately 176mμ. It will be noted that these figures are multiples of 11. Elford and Ferry[6] have shown that the approximate pore-size required for normal horse-pseudoglobulin in the isolated form is 11 to 12 mμ.

The sizes of these aggregates are apparently not fixed properties, for it was possible to obtain marked dispersion of equine antibodies by dilution in broth with a final NaCl concentration of 5%. The large aggregate of the concentrated serum is easily dispersed by dilution in normal horse- or rabbit-serum and even more dispersed by injection into a normal rabbit. The state of aggregation is therefore probably a function of some unknown factors of the environment. Slight variations in salinity, or variations in pH from 6.2 to 8.6, have no apparent effect.

The full import of these results, however, cannot be gained from the consideration of the amounts of specifically precipitable protein. Assuming that these particles of antibody are spheroidal and that the pore-size is a function of the diameter, it is obvious that a comparison of the volumes of the antibody-aggregates is most striking. It seems, furthermore, that the amount of nitrogen in each unit of antibody would be a function of this volume. Therefore it becomes apparent that although a very large percent of the total precipitable nitrogen is found in the larger aggregates, this may not be a true indication of the actual number of antibodies, assuming that each aggregate functions as a single antibody. On this basis of reasoning, not only is the mass of the smallest rabbit-antibody 64 times less than the smallest horse-antibody, but the ratio of small to large antibodies is very much greater in rabbit- than in horse-serum.

These observations not only offer an additional differentiation between the properties of the immune sera of these 2 animal species, but may serve to explain certain of the heretofore poorly understood properties.

[6] Elford, W. F., and Ferry, J. D., *Biochem. J.*, 1934, **28**, 650.