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Rhythmic Precipitation of Pneumococcal Soluble Specific Substances and Antipneumococcal Sera.

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In order to extend the observations on pneumococcal antigen-antibody reactions *in vitro*, precipitations were allowed to proceed slowly in a solid medium. The resulting rhythmic phenomenon resembles in many respects that noted by Liesegang when crystalloids react in a gel.¹ Other workers (Bechhold,² Petrie,³ Kirkbride and Cohen,⁴ and Reiner and Kopp⁵) have noted this phenomenon also in serological reactions.

For the present experiments, antipneumococcal sera were mixed with 3% gelatin in physiological salt solution in tubes (11- by 75-mm), and the solution of antigen layered over the solidified gelatin. The tubes were corked and the reactions allowed to proceed for several weeks at refrigerator temperature. Eighteen types of unconcentrated and 4 types of concentrated antipneumococcal horse sera, as well as types 1 to 32 rabbit sera were used. Highly purified soluble specific substances,⁶ pneumococcal "C" substance, and pneumococcal nucleoprotein were taken as antigens. Results were duplicated readily when conditions were carefully controlled. The concentration of both reagents and the age and concentration of the gel were important. Failure in any instance to obtain rhythmic precipitation may be due to improper experimental conditions.

There was great variation in the character of the results obtained when the specific carbohydrate solution was layered over the homologous serum-gelatin mixture. (Plate I) Series of closely packed, very fine and sharply defined rings, as well as series of broad and more or less indistinct bands, and intermediate types of reactions were observed. The pattern obtained varied with the type of pneu-

¹ Liesegang, R. E., *Naturwiss. Wchnschr.*, 1896, **11**, 353 (Cited in Freundlich, Herbert, *Colloid and Capillary Chemistry*, 3d ed., New York, Dutton & Co., 1922, p. 732.)

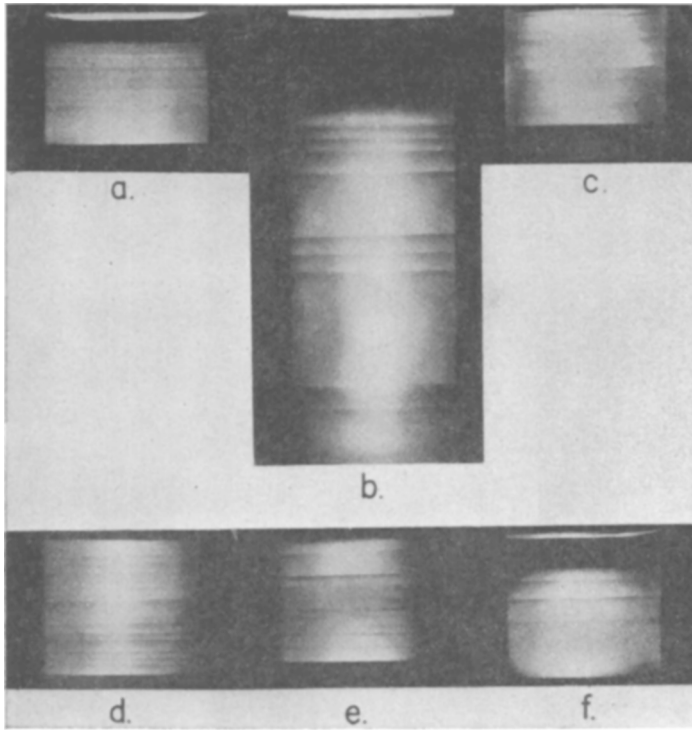
² Bechhold, H., *Z. f. physik. Chem.*, 1905, **52**, 185.

³ Petrie, G. F., *Brit. J. Exp. Path.*, 1932, **13**, 380.

⁴ Kirkbride, M. B., and Cohen, S. M., *Am. J. Hyg.*, 1934, **20**, 444.

⁵ Reiner, L., and Kopp, H., *Kolloid-Z.*, 1927, **42**, 335 (Cited in *Chem. Abs.*, 1927, **21**, 3949.)

⁶ Brown, Rachel, *J. Immunol.*, 1939, **37**, 445.



Rhythmic precipitation exhibited by homologous soluble specific substance and antipneumococcal serum. *a.* Type 3 rabbit serum; *b.* Type 5 rabbit serum; *c.* Type 12 horse serum; *d.* Type 14 horse serum; *e.* Type 19 horse serum; *f.* Type 25 rabbit serum.

mococcus. Also, the character of the reaction was dependent on the individual serum or animal and on the preparation of soluble specific substance. In general, the reaction was more extensive in the tubes of horse serum than in those of rabbit serum. Particularly characteristic of the latter was a clear interval just below the contact surface and above a zone of precipitation. The extent of the reaction was not related to the viscosity of the antigen solution.

Certain immune horse sera exhibited a more complex pattern than others of the same type. This was especially true of all bleedings examined from a particular horse immunized against type-12 pneumococcus. (Plate I) Sera from this horse also reacted with pneumococcal "C" substance and with nucleoprotein. Sera of very low potency gave a continuous hazy reaction rather than the rhythmic reaction of more potent sera. By adjustment of the quan-

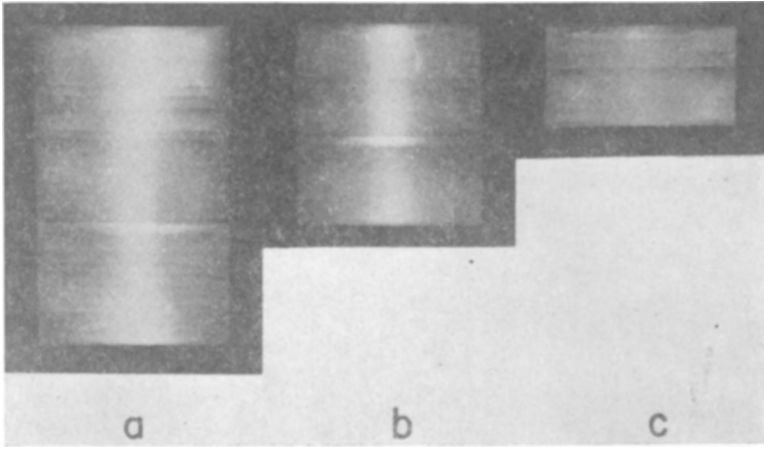


PLATE II.

Rhythmic precipitation exhibited by decreasing amounts of type 3 soluble specific substance and a constant amount of type 3 antipneumococcal horse serum. *a.* 1:400 dilution; *b.* 1:2000 dilution; *c.* 1:10,000 dilution.

tities of both reagents, a definite zone was determined in which maximal reaction occurred.

In one experiment, 3 type-2 horse antisera of different potencies according to the mouse-protection test, showed very similar results when the same number of units of each was used with the same optimal amount of soluble specific substance; but when equal volumes of these 3 sera were taken, marked variations were noted.

In another experiment with decreasing amounts of type-3 soluble specific substance and a constant amount of homologous horse antiserum, the pattern was similar but progressively less extensive. (Plate II)

Conclusion. These observations on the rhythmic precipitation of pneumococcal antigen-antibody reactions suggest that the phenomenon is comparable to that occurring with crystalloids in a gel.