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A Study of Antigens and Antibodies by the Monolayer Film Technique of Langmuir.

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Methods have been developed by Langmuir and his coworkers¹⁻³ for the adsorption of dissolved substances onto the surface of suitable plates covered with multiple monomolecular layers of stearate, and for the study of various properties of these adsorbed films. Thus, through the use of simple optical techniques, the thickness of monolayers of a number of substances of biological interest (*e.g.*, egg albumin, crystalline tobacco mosaic virus protein, diphtheria toxin, chlorophyll, digitonin, sterols) has been determined.⁴⁻⁶ These results check well with molecular dimensions found by other workers using different methods, such as ultra-centrifugation, filtration through collodion membranes of graded porosity, osmotic pressure measurement, etc.

With the aid of the Langmuir technic† we have made observations on the behavior of several antigen-antibody systems. The method employed consisted essentially in the preparation of Y-films of stearate made up of the desired number of layers deposited on polished chromium surfaces. A small amount of the solution under investigation was applied to the stearate surface and rubbed over it to assure adequate exposure, by passing a glass rod back and forth several hundred times in contact with the upper surface of the drop. The film was then washed, dried, and the change in thickness due to the adsorbed layer measured by comparing the interference colors

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¹ Langmuir, I., *J. Franklin Inst.*, 1934, **218**, 143.

² Blodgett, K. B., *J. Am. Chem. Soc.*, 1934, **56**, 495; *Ibid.*, 1935, **57**, 1007.

³ Langmuir, I., Schaefer, V. J., and Wrinch, D. M., *Science*, 1937, **85**, 76.

⁴ Langmuir, I., and Schaefer, V. J., *J. Am. Chem. Soc.*, 1937, **59**, 1406; *Ibid.*, 1937, **59**, 2075.

⁵ Blodgett, K. B., and Langmuir, I., *Physical Rev.*, 1937, **51**, 964.

⁶ Langmuir, I., Schaefer, V. J., and Sobotka, H., *J. Am. Chem. Soc.*, 1937, **59**, 1751.

† One of us (M.F.S.) has had the privilege of studying these methods in the laboratory of Dr. I. Langmuir, General Electric Company, Schenectady, N. Y., through his kindness and with the encouragement of Dr. James B. Conant and Dr. Hans Zinsser.

of the experimental film with those of a standard Y-film stearate reference gauge, using daylight as a source of illumination.

A 2% aqueous solution of crystalline egg albumin applied to the surface of stearate wetted it readily and the area of albumin-treated stearate showed an increase in thickness of approximately 40 Å. Anti-ovalbumin rabbit serum applied to the albuminized area further increased the thickness by 100 Å. The specificity of the reactions, when immune serums were applied to antigen-treated areas in this and the other experiments, was shown by the fact that the antisera did not adhere when applied directly to a stearate surface although they wetted it immediately. Furthermore normal rabbit or horse serums or heterologous immune serums of horse or rabbit failed to adhere to a stearate surface on which a monolayer of antigen had been deposited, although they readily wetted it.

On attempting to deposit a second layer of egg albumin on the upper surface of the anti-ovalbumin antibody already adherent to a base of albumin, the albumin solution was found to remove the antibody, leaving attached to the stearate surface only the original albumin layer. This experiment not only shows an analogy to the effect sometimes observed when an excess of antigen is added to an already existing immune serum-antigen complex, but also may indicate the relative forces existing between antigen and antibody as compared with those which bind albumin to stearate.

A 1% solution of pneumococcus type III carbohydrate (prepared either according to the methods of Heidelberger, Kendall, and Scherp⁷ or of Hornus and Enders⁸) gradually wetted the stearate surface and yielded a film of 50 Å maximum thickness. Antipneumococcus type III rabbit serum applied to such an area increased the thickness by 100 Å. In contrast, the application of antipneumococcus type III horse serum to such an area increased the thickness 240 Å. Four samples of antipneumococcus type III horse serum, each of which was obtained from a different source and differed from the others in its antibody concentration, gave identical results.

Different findings were obtained, however, when similar experiments were carried out with pneumococcus types V and VIII carbohydrates and homologous antisera. Pneumococcus type V car-

⁷ Heidelberger, M., Kendall, F. G., and Scherp, H. W., *J. Exp. Med.*, 1936, **64**, 559.

⁸ Hornus, G. J. P., and Enders, J. F., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 188.

bohydrate in 1% concentration gave films of 50 Å thickness. Application of the homologous immune serums of both horse and rabbit to such areas gave films of 100 Å. Pneumococcus type VIII carbohydrate in a concentration of 0.4% gave films of 25 Å. Here again, application of homologous immune serums from either horse or rabbit to the treated areas further increased the thickness 100 Å.

Our results with antipneumococcus type III horse and rabbit serums are in general agreement with those of Heidelberger and Pedersen⁹ and Kabat and Pedersen.¹⁰ The findings with types V and VIII antipneumococcus serums, on the other hand, strongly suggest that in the horse at least the size of the antibody protein molecule or aggregate varies according to the type of pneumococcus employed in its production. The reactions between other stearate-adsorbed antigens and their antibodies are now being investigated. A more extensive discussion of the material presented above, together with additional data, will be given in a subsequent communication.

Summary. From the above results it may be concluded that the monolayer film method provides a valuable means for the study of antigen-antibody systems as regards molecular dimensions, combining properties, and other aspects of their behavior.

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Effect of Cyclopropane on Intestinal Activity *in Vivo*.

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In a communication reported by Peoples and Phatak¹ it was shown that in the isolated rabbit's intestine cyclopropane up to 25% in oxygen when added to the solution in which the strip of intestine is suspended causes an increase in intestinal tonus. It seemed desirable to repeat this work in the intact animal. Accordingly, experiments were conducted on normal dogs prepared with Thiry-Vella fistulas² of the jejunum or of the ileum.

⁹ Heidelberger, M., and Pedersen, K. O., *J. Exp. Med.*, 1937, **65**, 393.

¹⁰ Kabat, E. A., and Pedersen, K. O., *Science*, 1938, **87**, 372.

¹ Peoples, S. A., and Phatak, N. M., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 287.

² Gruber, C. M., and Brundage, J. T., *J. Pharm. and Exp. Therap.*, 1935, **53**, 120.