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Adsorption of Staphylococcus Bacteriophage by Serum Globulins.

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It is well known that bacteriophage may be precipitated from bouillon by alcohol, acetone, and magnesium sulphate. The lytic principle is only slightly impaired by this procedure though length of time of exposure and temperature have been shown to be limiting factors. It is thought that the lytic principle is adsorbed onto protein and hence is naturally carried down with the precipitate. Hauduroy[1] has shown that gelatin, as well as other similar substances, such as agar, gum, etc., will arrest the action of the bacteriophage. Bronfenbrenner[2] states that the influence of agar or gelatin in inhibiting bacteriophagy depends on the fact that these agents prevent water from entering the bacterial cells. De Necker[3] has shown that colloidal metals, bone charcoal, and serum exercise an inhibitory effect upon the bacteriophage and this author thinks that the nature of the adsorption suggests that the lytic principle is a ferment. Angerer[4] has shown that killed Flexner *dysentery bacilli* adsorb about 70% of homologous bacteriophage within 15 minutes. Hauduroy[5] claims to have demonstrated a fixation of the lytic principle by the addition of antidysenteric serum to tubes containing *B. dysenteriae Shiga* and its homologous bacteriophage. In all of these experiments the authors have been dealing with adsorption phenomena. Recently Gay and Holden[6] have shown that a strain of herpes virus (rabbit brain emulsion) when suspended in distilled water may be precipitated with the globulins by bubbling CO_2 gas through the mixture. Resuspension of the globulins in saline when injected intracerebrally into rabbits produced encephalitis. This may also perhaps be regarded as an adsorption phenomenon.

The completeness and thoroughness of such adsorption processes is illustrated by the following experiment with a bacteriophage lytic for *Staphylococcus aureus:*

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[1] Hauduroy, Paul, *Compt. rend. Soc. de biol.*, 1924, xc, 1463.

[2] Bronfenbrenner, J., and Hetler, D., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 480.

[3] De Necker, J., *Compt. rend. Soc. de biol.*, 1922, lxxxvii, 1247.

[4] Angerer, K. v., and Rupp, H., *Arch. Hyg.*, 1928, xcix, 118.

[5] Hauduroy, Paul, *Compt. rend. Soc. de biol.*, 1922, lxxxvii, 966.

[6] Gay, Frederick P., and Holden, Margaret, *J. Infect. Dis.*, 1929, xlv, 415.

The Staphylococcus bacteriophage was titrated and found to be active in 1:10,000,000,000 dilution. One cubic centimeter of this lytic principle was added to 9 cc. of fresh rabbit serum. One cubic centimeter of the mixture was removed for titration and was found to be as active as the original lytic principle. To the remaining 9 cc. of serum-phage mixture 20 volumes of distilled water were added. CO_2 gas was then bubbled through the mixture, following which the material was centrifuged and the globulins were resuspended in 9 cc. of saline made slightly alkaline with a 1% solution of sodium carbonate. The globulin fraction was then tested for lytic activity against the sensitive *Staphylococcus aureus* and the bacteriophage was found to be present in undiminished concentration. Lytic principle, however, could not be detected in the albumin fraction. This phenomenon may be explained upon the basis of the negative charge carried by the bacteriophage and the opposite charge of serum globulin at or below its isoelectric point at pH 5.4 which is reached by treating the serum-phage mixture with CO_2 gas. The isoelectric point of serum albumin is at pH 4.7 and this degree of acidity is not reached by the method employed, the electric charge of the albumin remaining negative, and hence there is no adsorption of the negatively charged bacteriophage by this fraction of the serum.

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Further Note on the Salivary Gland Poison of Aëdes Aegypti.

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In a previous communication[1] we described the poisonous substance contained in the salivary glands of *Aëdes aegypti* and the types of reactions which are produced by introducing this substance intradermally into the human skin. This substance gives rise to a reaction which is practically identical to that following the bite of this insect. The poison is resistant to both freezing and boiling temperatures and contains no hemolytic or anticoagulant substances. It does not produce antitoxin when introduced intravenously into rabbits.

Further studies upon the properties of the salivary gland poison

[1] McKinley, E. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 806.