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A Method for the Purification of the Bacteriophage.

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Numerous observers have reported the adsorption of bacteriophage corpuscles by both electro-positive and electro-negative colloids. D'Herelle[1] in a review of the literature concludes that in an alkaline medium the bacteriophage is adsorbed only by electro-positive colloids and therefore carries a negative charge, a conclusion in agreement with cataphoresis studies.

De Necker[2] reported the partial flocculation of the bacteriophage by aluminum hydroxide, an electro-positive sol. When the alumina mass is dissolved by the careful addition of acetic acid the bacteriophage reappears in the liquid. Sommer, Sommer, and Meyer[3] reported the isolation of botulinus toxin by selective adsorption on colloidal aluminum hydroxide, elution with secondary ammonium phosphate, removal of salts by dialysis, and evaporation to dryness at 40° C. Their method was used in the following attempt to purify the bacteriophage.

When a suspension of the staphylococcus bacteriophage in Martin's broth (pH 7.8) is stirred with an equal volume of a 3.5% suspension of aluminum hydroxide for one hour the bacteriophage is completely removed from the mother liquor. After washing the precipitate 4 to 6 times with distilled water no bacteriophage corpuscles can be detected in the final wash water. When the precipitate is triturated with a 0.5% solution of secondary ammonium phosphate (pH 8.0) the bacteriophage is liberated and can be separated from the alumina by centrifugation or filtration.

The final procedure adopted is as follows: 50 cc. of a suspension of the staphylococcus bacteriophage in Martin's broth is stirred for one hour with an equal volume of the aluminum hydroxide suspension. This is collected on a Buchner funnel and washed 5 times with distilled water. The washed alumina mass is stirred for ½ hour with 50 cc. of the secondary ammonium phosphate solution and then placed in the 32° C. incubator over night. The alumina is fil-

[1] D'Herelle, "The Bacteriophage and its Behavior," Williams and Wilkins, Baltimore, 1926.

[2] De Necker, *Compt. rend. Soc. de Biol.*, 1922, **87**, 1247.

[3] Sommer, Sommer, and Meyer, *J. Infect. Dis.*, 1926, **39**, 345.

tered off by suction and the bacteriophage is recovered in the filtrate.

The original bacteriophage suspension was active in a dilution of 10^{-9}. No activity could be detected in the original filtrate or in the final wash-water but after elution the purified phage was active in a dilution of 10^{-7}. The ordinary color tests for proteins were negative. No precipitate was formed by ½ or complete saturation with ammonium sulfate.

Further purification studies by means of selective adsorption are now in progress and also attempts to remove salts by dialysis and to concentrate the suspension *in vacuo*.

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Capillary Circulation in Skeletal Muscle in Rest and in Exercise.

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Method—Gracilis muscles in anesthetized dogs were injected with India ink introduced under pressure centrally into the femoral artery below the origin of the branch artery to the muscle without interrupting the circulation. The injection was continued until the venous blood showed complete blackening. The muscle was quickly clamped off, excised, and dropped at once into Bouin's fluid for fixation. Paraffin sections about 10 to 15μ thick were made for counting. To study the effect of exercise, a muscle was stimulated directly through the obturator nerve by means of brief tetani repeated about once a second for about 3 to 5 minutes. India ink was injected while the muscle was being stimulated.

Results on muscles of amytalized dogs. In resting gracilis about 925 capillaries were injected per sq. mm. of tissue. These are figures for injected bundles. There were also many bundles in which larger vessels contained ink but capillaries none. In exercised gracilis about 1900 capillaries were injected per sq. mm. tissue. An occasional uninjected bundle was seen in these muscles, but far fewer than in resting muscles.

$$\text{Ratio } \frac{1900}{925} = 2.05$$

The maximum number of capillaries per sq. mm. (about 2200) was seen in muscles injected ½ hour after death. No uninjected bundles were seen.