8770 P

Comparison of Precipitating Action of Basic Dyes on Bacteriophage and Bacterial Proteolytic Enzymes.

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Safranin is able to remove a number of biological substances from solution. Robertson[1] observed that safranin when added to a solution of trypsin would cause a precipitate. Holzberd[2] showed that this precipitate was proteolytic and later Marston[3] proved that not only safranin but all the azin dyes removed trypsin and pepsin from solution. The azin dyes also quantitatively precipitate bacterial proteolytic enzymes.[4] Vinson[5] reported that the virus of the mosaic disease of tobacco was completely removed from the infected plant juice by safranin. Krueger and Baldwin,[6] in their studies on the inactivation of bacteriophage, used safranin as one of the inactivating agents, and observed a precipitate which partly but not completely removed the phage from solution.

We examined the action of the azin and other basic dyes on bacteriophage and compared the results with those obtained by the use of the same dyes with bacterial proteolytic enzymes.

The following chemical types of basic dyes were used: azin, thiazin, oxazin, xanthane and phenylmethane. The bacteriophage employed was a *coli*-phage made in synthetic medium and free from all protein except bacterial. The titer of the phage was $1x10^{-9}$. One cc. of the phage and 7 cc. of physiologic salt-solution were placed in a 15 cc. tube and 2 cc. of M/70 solution of the dye in water added. The mixture was allowed to stand for 18 hours at room temperature, and then centrifuged. The supernatant fluid was poured off and filtered through a Berkefeld N filter. This was found to be necessary as the unfiltered supernatant fluid of both the phage and the enzyme showed the presence of small amounts of lytic and proteolytic substance. The precipitate was taken up in 10 cc. of a pH 6.8 phosphate buffer solution.

Both the precipitate and filtrate were diluted in plain broth and

1 Robertson, T. B., *J. Biol. Chem.*, 1907, **2**, 343.

2 Holzberd, H. L., *J. Biol. Chem.*, 1913, **14**, 335.

3 Marston, H. R., *Biochem. J.*, 1923, **17**, 651.

4 Walker, A. W., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 839.

5 Vinson, C. G., *Science*, 1927, **44**, 357.

6 Krueger, A. P., and Baldwin, D. M., *J. Infect. Dis.*, 1935, **57**, 207.

TABLE I.

	Azin			Thiazin			Oxazin			Xanthane	Phenyl Methane
	Safranin	Neutral red	Janus green	Thionin	Methylene green	Toluidin blue	Brilliant cresyl blue	Cresyl violet	Gallocyanin	Pyronin	Malachite green
Bacteriophage											
Precipitate	+	+	+	+	+	+	+	+	+	+	+
Filtrate	—	—	—	—	—	—	+	+	+	+	+
Proteolytic enzyme											
Precipitate	+	+	+	+	+	+	+	+	+	+	+
Filtrate	—	—	—	—	—	—	+	+	+	+	+

Presence of phage or enzyme = +
Absence of phage or enzyme = —

tested for phage in the usual manner. For the bacterial proteolytic enzymes, a Berkefeld filtrate of a 4-day culture of *B. pyocyaneus* in synthetic medium was used in the same manner as the bacteriophage. The test for the presence of the proteolytic enzyme was by inoculating the filtrate and suspended precipitate into carbol-gelatin, incubating at 37°C. for 48 hours, placing in the icebox for 1 hour, and then observing for liquefaction.

Table I shows that the bacteriophage and the proteolytic enzyme are completely precipitated by these azin and thiazin dyes, and that the other basic dyes used do not completely remove the phage or the enzyme from solution. With the exception of pyronin the dyes had little if any inhibiting action on either the phage or the enzyme. In the case of the pyronin the inhibiting action was considerable for both.

This inhibitory action has not been investigated. It may be due to the photodynamic action of the pyronin. But some of the other dyes are photodynamically active and showed no such effects; further, *coli*-phage, unlike the staphylococcal phage, is not sensitive to oxidation.

The nature of the union of phage with the dye is unknown. If it is an adsorptive phenomenon one could expect that all the dyes of the same charge and relative molecular size would act in the same manner.

Marston believes the union of trypsin and pepsin with the azin dyes to be a chemical reaction.

The chemical union of bacteriophage and dye, if proven, would be another indication of the chemical or nonliving nature of the bacteriophage.

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Preganglionic Connections of the Intramural Ganglia of the Urinary Bladder.

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The pelvic autonomic ganglia including the intramural ganglia of the urinary bladder, are connected with the lower thoracic and upper lumbar segments of the spinal cord through the splanchnic and hypogastric nerves and with the sacral segments through the pelvic nerves. The efferent fibers which enter the pelvic plexus on