

*Conclusions*—1. Coproporphyrin I is excreted at a constant rate in normal dogs, proving the existence of the dualism of the porphyrins in that animal. 2. Experimental evidence is given that coproporphyrin I excretion seems to be directly proportional to and a measure of hematopoietic activity. 3. Coproporphyrin I is not a product of or related to blood destruction.

I wish to express my gratitude to Dr. W. B. Hawkins for allowing me to make use of his dogs and to include his blood and bile pigment values in this study.

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### Inactivation of Bacteriophage by Ethyl Alcohol.

CHARLOTTE A. COLWELL. (Introduced by Arthur I. Kendall.)

*From the Department of Research Bacteriology, Northwestern University Medical School, Chicago.*

General agreement has prevailed among investigators that phage freed from a major portion of extraneous materials is more susceptible to inactivation by physical and chemical agents than is crude broth phage. One exception to this statement has been noted. Bronfenbrenner<sup>1</sup> reported that phage free from nitrogen as determined by Nessler's reagent or ninhydrin was not weakened in lytic activity when mixed with 10 volumes of alcohol and left for 8 days at 22 to 25°C., although broth phage was inactivated under similar conditions in a very short time. Since Kligler and Olitzki<sup>2</sup> have reported that the presence of protein delays the inactivation of phage by alcohol and Callow<sup>3</sup> found that both purified and broth phages were inactivated by alcohol after 3 days in the refrigerator, corroboration of Bronfenbrenner's findings appears to be worth recording.

Bacteriophage for a strain of *B. coli* was purified from lysogenic cultures by a method recently described.<sup>4</sup> Purified phages used in these experiments contained between 0.3 and 0.5 mg. of nitrogen per 100 cc. as determined by the microkjeldahl method of Koch and McMeekin.<sup>5</sup> Homologous crude broth phage was adjusted to approximately the same titer and pH (6.3). Each kind of phage was

<sup>1</sup> Bronfenbrenner, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **24**, 372.

<sup>2</sup> Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 393.

<sup>3</sup> Callow, B. R., *J. Infect. Dis.*, 1927, **41**, 124.

<sup>4</sup> Colwell, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 100.

<sup>5</sup> Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

mixed with an equal amount of 95% ethyl alcohol and allowed to stand in corked test tubes at room temperature. Titrations of both samples for phage content were made by the serial dilution method in broth every hour for 5 hours and after 16, 18, 24, 48, 72, 96 hours and 5 days.

It was found repeatedly that although the titer of broth phage fell to  $10^{-1}$  or  $10^{-2}$  in one hour and completely disappeared in 2 to 4 hours, the purified phage still had a titer of  $10^{-6}$  after 6 hours,  $10^{-5}$  after 24 to 48 hours and  $10^{-3}$  after 72 hours. After 4 days, 2 cc. of the phage-alcohol mixture contained no detectable phage, although the titer of an aliquot of the same phage mixed with sterile distilled water instead of alcohol maintained under the same conditions was still  $10^{-7}$ . Inactivation of the purified phage therefore occurred earlier in this work than in that of Bronfenbrenner previously quoted, owing perhaps to slightly different conditions; but the 2 experiments illustrate the same principle, namely, that purified phage, contrary to general opinion, is more resistant to inactivation by ethyl alcohol than is crude broth phage.

Bronfenbrenner believes the more rapid inactivation of broth phage by alcohol to be due to the precipitation of carrier particles of the phage. When the carrier particles are removed by purification, the phage itself is not so readily inactivated. No alternative explanation of the greater resistance of purified phage to alcohol presents itself at the present time.

TABLE I.  
Inactivation of Bacteriophage by Alcohol.

	Titer before alcohol	After 1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Purified phage	$10^{-7}$	$10^{-6}$ to $10^{-7}$	$10^{-6}$ to $10^{-7}$	$10^{-6}$	$10^{-6}$	$10^{-6}$	$10^{-5}$	$10^{-5}$	$10^{-3}$	0
Crude broth phage	$10^{-7}$	$10^{-1}$ to $10^{-2}$	0 to $10^{-2}$	0 to $10^{-2}$	0	0	0	0		