These results permit the conclusion that hemagglutination and toxicity are properties associated with separate particles. This multiplicity in the nature of crystalline toxin is in conflict with the apparent homogeneity demonstrated by diffusion, electrophoresis, and ultracentrifugation studies and by supernatant

TABLE 1

Effect of types A, B, and C botulinal antitoxins on hemagglutination and toxicity of a type A culture supernatant

ANTITOXIN TYPE	UNITS	TEST MATERIAL	LD_{50}	HEMAGGLUTINATION TITER
None	0	Culture	570,000	21.1
A	1.5	Culture		8.0
	3.0	Culture		4.2
	4.5	Culture	270,000	<2
	4.5	Sterile broth		<2
B	1.5	Culture		<2
	3.0	Culture		<2
	4.5	Culture	570,000	<2
	4.5	Sterile broth		<2
C	1.5	Culture		19.0
	3.0	Culture		17.2
	4.5	Culture		17.2
	4.5	Sterile broth		<2

Antitoxins were of equine origin and were purchased from the Jensen-Salsbery Laboratories.

Toxin titer was determined in 20-gram white mice, and hemagglutination by a modification of the Hirst and Pickels electrophotometric method.

tests for antigen and antibody in the zone of equivalence in the antigen-antibody reaction (Putnam *et al.*: J. Biol. Chem., **176,** 401, 1948). The possibility that under certain conditions the hemagglutinin and toxin form stable complexes or combinations acting as homogeneous materials is under investigation.

NUCLEIC-ACID-FREE T2 VIRUS "GHOSTS" WITH SPECIFIC BIOLOGICAL ACTION

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T. F. Anderson (J. Appl. Phys., **21,** 70, 1950) has reported that rapid dilution of a concentrated salt solution of the T2 virus of *Escherichia coli* results in a loss of

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virus infectivity. To this process he gave the name "osmotic shock." I had occasion to "shock" some T2 virus and confirmed Anderson's results. In addition, however, I found that the solution of "shocked" T2 virus prevented the host cells from multiplying either in solution or on plates and also lysed them. A number of fractionation experiments using a high-speed centrifuge accompanied by tests for lytic activity and by electron microscope examinations[2] indicated that these biological properties are associated with the "ghosts."[3]

To shock the virus, 2 volumes of 3 molar sodium sulfate solution were added to 1 volume of T2 virus having at least 1×10^{13} plaque-forming units per ml (approximately 1 mg N per ml), and after a minute or two 50 volumes of water were added rapidly. The infectivity (plaque-forming value) dropped to less than 1 per cent of a control. However, when the dilution of the concentrated salt-virus mixture was very gradual, there was no measurable loss. The infective unit of the virus before shocking is a convenient reference in establishing the lytic activity of the ghosts. Since the lytic activity of a number of shocked virus preparations was proportional to the original virus infectivity, the shocking procedure either destroys very little or a constant fraction of the lytic activity. In making quantitative determinations of lytic activity 1 ml of a saline dilution of the ghosts is added to 10 ml of 3.3×10^8 *E. coli* B per ml in the log phase of growth in nutrient broth. This suspension in a 22-by-175-mm pyrex tube is examined immediately in a Coleman junior spectrophotometer set at 650 mμ and again after 15 minutes' agitation at 37 C. The most reproducible results were obtained when the saline dilution contained between 1×10^{10} and 3×10^{10} ghosts per ml, corresponding to an average of 3 to 9 ghosts per bacterium in the test system. An average of 9 ghosts per cell reduces the turbidity to below 50 per cent of the initial value in 15 minutes. Very little is known about the reactions leading to lysis of the host, but it is assumed in the present instance that a drop in turbidity is a measure of lysis. Electron micrographs[2] and light microscope examinations of bacterial suspensions treated with ghosts or virus lend strong support to this assumption.

The ghosts have a host range specificity similar to that of the virus from which they were derived. Thus broth cultures of *E. coli* B and B/4 are lysed by T2 ghosts but B/2 organisms are not.

The addition of the equivalent of 3 ghosts per cell to a log phase culture of cells in synthetic media completely blocked the increase in absorbancy at 260 mμ for an hour. The very slow increase following this interval was also observed in controls containing the few cells calculated from Poisson's equation that should be free of ghosts in the experimental system. It seems likely therefore that there was no net increase in nucleic acid during the 2.5-hour period of observation.

Preliminary chemical studies on purified T2 ghosts show them to contain little

[2] The electron microscope studies were kindly made by Dr. James S. Murphy, Fellow in the Department of Medicine, The Johns Hopkins Medical School. A complete study will be published later.

[3] The word "ghost" has been used because of its obvious relationship to the red cell ghost produced in a somewhat similar manner. It consists of the deflated head and the tail or appendage.

or no phosphorus (P/N < 0.01), about 3.5 to 4 per cent tryptophan, and very little tyrosine. Solutions of crystalline trypsin and chymotrypsin caused a marked decrease in the lytic property of the ghosts, but crystalline desoxyribonuclease (courtesy of Dr. M. Kunitz), crude papain, and crystalline pepsin were without effect. More experiments are needed to prove that the destructive action of the first two enzymes was due to proteolysis. Light-scattering measurements indicate that the mass of a ghost is about 20 per cent of the intact virus.

The work described indicates that the nucleic acid and other possible phosphostructures of this virus are not essential for the specific adsorption, for the power to block the host's multiplication, or for lysis of the host. This limits the site of the foregoing properties of the virus to the protein portion or the small amount of lipid reported by Taylor (J. Biol. Chem., **168,** 271, 1946). This then is a beginning in the physical separation of the various biological functions of a virus and the correlation of these functions with certain morphological and chemical properties.