the anaphylactic reaction in the dog might contribute some information to these various and possibly related problems.

Chondroitin was administered to 13 horse-serum sensitized dogs in doses of 10 gm. per day. In 8 animals it was given during the last 10 days of the incubation period, and in 5 it was given for 10 days prior to the sensitizing injection and throughout the incubation period. The animals were anesthetized so that the degree of shock resulting from the assaulting dose of serum could be recorded by the blood pressure tracing. Definite shock occurred in all animals, 3 reactions being fatal, the remainder moderate to severe. The distribution of the various grades of severity of shock was identical with that in a large number of controls.[3] Consequently it is concluded that the administration of chondroitin does not influence in any way the anaphylactic reaction in the dog.

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Purified Bacteriophage from Lysogenic Cultures.

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The desirability of freeing bacteriophage from extraneous materials, such as media constituents, bacterial proteins and products of bacterial growth, has long been recognized and needs no emphasis. Efforts to obtain phage in a pure state have been numerous, and reports concerning the properties of phages of relative purity indicate that they differ from broth phage. The purpose of this paper is to describe a simple method for obtaining phage of low nitrogen content without the use of special media or equipment.

Bordet and Ciuca[1] noted that the late bacterial growth occurring after the period of maximum phage action continued to carry the phage through a series of transfers on artificial media. They called these lysogenic* cultures. During the course of a study of cultures

[3] Mills, M. A., and Dragstedt, C. A., *J. Immunol.*, 1936, **31**, 1.

[1] Bordet, J., and Ciuca, M., *Compt. rend. de Soc. de Biol.*, 1920, **83**, 1293.

* The term 'lysogenic' was later applied to and is now commonly used for cultures of the type first described by Lisbonne and Carrère;[2] *i. e.*, recently isolated cultures (usually *B. coli*) whose filtrates contained phage active on heterologous strains (usually dysentery bacilli of the Shiga or Flexner type).

[2] Lisbonne, M., and Carrère, L., *Compt. rend. de Soc. de Biol.*, 1922, **86**, 569.

having this property, it was observed that the growth from agar slants could be washed repeatedly with physiological salt solution or distilled water, and that even the twenty-seventh washings still showed the presence of phage in easily detectable amounts. It seemed reasonable to suppose that the earlier washings would remove the greater part of other proteins and that later washings might contain the phage in purer form; it was found, in fact, that repeated washings of lysogenic cultures could be used as a means of purifying phage for *B. coli, Staphylococcus aureus,* and *B. graveolens.* The phage for *B. coli* was chosen for further investigative work because of its relative stability.

Bacteriophage active in the 10^{-8} dilution was prepared from sewage for a non-sucrose fermenting strain of *B. coli* recovered from human feces. A 1:10 dilution in broth was incubated with the susceptible bacteria for 3 to 5 days. Lysis occurred, followed by an overgrowth from which a lysogenic culture was prepared by transferring 2 loopfuls to a beef extract agar slant. Scattered colonies appearing in 24 hours were streaked over the surface of the slant and the growth resulting in 3 to 5 days was suspended in 2 or 3 cc. of sterile distilled water. Six-inch petri dishes with a thin layer of 2% agar were inoculated with 2 drops of this suspension, which were then streaked over the surface of the plates with sterile bent glass rods. After 48 hours' incubation, the growth was washed from the surface of the plates into physiological salt solution or distilled water, using 3 cc. per plate. The suspension was centrifuged three times in 50 cc. round-bottomed centrifuge tubes. The supernatant fluid was carefully decanted each time, the sediment drained and resuspended in approximately the same amount of fluid. The first, second and third washings were filtered through Berkefeld N or W filters, new filters being used for the third supernatant fluid to prevent the addition of extraneous nitrogen by the procedure. Titrations for phage content were made by the serial dilution method in broth, separate pipettes being used for each dilution; also 3 plaque counts were made on different dilutions of each phage sample.

Although the titer sometimes dropped slightly in successive washings, the titer of the third supernatant fluid was consistently high (10^{-7}) (see Table I). The total nitrogen content of the third washings, determined by the microkjeldahl method of Koch and McMeekin,[3] ranged from 0.66 to 0.88 mg. per 100 cc. in different experiments, varying slightly with the unavoidable slight variation in the number of bacteria washed and the amount of suspending

[3] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

TABLE I.
Phage titer and total nitrogen content of representative samples of phage washed from lysogenic cultures.

Experiment	Supernatant fluid	Total nitrogen mg./100 cc.	Phage titer
Growth washed from agar plates into physiological salt solution.			
A	1	74.92	10^{-8}
	2	3.34	10^{-7}
	3	0.66	10^{-7}
B	3	0.88	10^{-7}
C	3	0.84	10^{-7}
Growth scraped from agar plates into distilled water.			
D	1	6.06	10^{-7}
	2	0.62	10^{-7}
	3	0.31	10^{-7}
E	1	5.82	10^{-7}
	2	1.01	10^{-7}
	3	0.27	10^{-7}
Crude broth phage		180.0	10^{-8}

fluid used each time. Rabbits immunized with the third supernatant fluid, containing 0.36 to 0.49 mg. of nitrogen in the amount of phage injected, produced no agglutinins for the homologous organism detectable in a 1:10 dilution of serum and only faintly demonstrable precipitins with antigen undiluted or diluted 1:2 (none with antigen diluted 1:4). Antibodies for bacteriophage were present in dilutions from 1:320 to 1:640.

Because of the faint precipitin reaction, indicating the presence of some bacterial protein, the technique of making the phage was modified slightly. The 48-hour growth of lysogenic cultures on agar plates was scraped with a rubber kitchen plate scraper instead of being washed from the surface of the medium. The suspension was then washed in sterile distilled water as previously described. The reduction in total nitrogen content occurring as a result of this modification (from between 0.66 and 0.88 mg. to between 0.27 and 0.31 mg. per 100 cc.) indicated that a large part of the nitrogen in previous samples had come from medium proteins. The possible sources of the remaining nitrogen are the filters, the glassware, the water (distilled only once), the bacteria and the phage.

Phage purified in the manner described containing 0.27 to 0.31 mg. of nitrogen per 100 cc. was concentrated from 25 cc. to approximately 0.5 cc. by filtering through collodion membranes having an average pore diameter of 34 millimicrons prepared by the method of Bauer and Hughes.[4] The titer was increased 5 to 7 times by this

[4] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

procedure. It is believed that this may also serve as a means of further purification.

Complete inactivation of the purified phage occurred in 30 minutes by exposure to 65°C., whereas homologous broth phage of similar titer (10^{-7}) and pH (6.1 to 6.2 by the indicator method) required a temperature of 75°. The purified phage was completely inactivated by 50% C. P. acetone in 24 hours at room temperature, though broth phage of similar titer and pH still had a titer of 10^{-5} after the same period of time under the same conditions. After evaporation to dryness in a Freas vacuum oven at 22°C. and resuspension in one-fourth the original volume of water, the titer of the purified phage was 10^{-2}; that of the crude broth phage, 10^{-6}. These results appear to support the findings of other workers that purified phage is more susceptible to inactivation by physical and chemical agents than is crude broth phage.

Summary. Serological tests and nitrogen determinations indicate that bacteriophage prepared by repeated washing of lysogenic cultures of *B. coli* contains little bacterial or other protein. Methods of preparation and concentration of such purified phage are described and some of its properties compared with those of homologous crude broth phage of similar titer and pH.†

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Comparative Study of Water Metabolism in Amphibians Injected with Pituitrin.

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Since the work of Brunn[1] it has been shown by several other investigators that frogs *(Rana pipiens)* when injected with pituitrin, increase markedly in weight due to the absorption of water. This increase, occurring in the case of frogs, gave rise to the problem of comparing the amount of water uptake in different animals in the class Amphibia when similarly treated.

The animals selected for this comparative study were: the toad *(Bufo americanus)*, mud puppy *(Necturus maculatus)*, and two

† The collodion membranes used in this work were prepared by Dr. Evelyn B. Tilden of the Department of Research Bacteriology.

[1] Brunn, F., *Z. ges. exp. Med.*, 1921, **25**, 170.