SPECIAL ARTICLES

THE SIZE OF STREPTOCOCCUS BACTERIOPHAGES AS DETERMINED BY X-RAY INACTIVATION

INFORMATION about the particle size of bacteriophages has been obtained chiefly by ultrafiltration and centrifugation methods. Data of various authors, as collected by Elford,[1] seem to indicate that the particles of each phage are homogeneous in size; and that particle size is a characteristic property of each phage. Coli-dysentery phages have particles ranging from 8–20 mμ (phage "S13") to 50–75 mμ diameter (phage "C16"). Staphylococcus phage "K" has a diameter of about 50–70 mμ. The largest phage at present known has particles of 80–120 mμ (Subtilis phage).

While the particle size values as given above are usually accepted, it must be mentioned that diffusion studies[2] have suggested that phage particles may be much smaller and inhomogeneous in size.

A new method has recently been developed,[3] which, on the basis of radiation experiments, seems to give useful information about the particle size of bacteriophages.

When suspended in a suitable medium the sensitivity to x-rays of a phage strain, measured by the percentage of active phage which remains after a certain amount of radiation has been given, is a highly reproducible property of that strain, and appears to be a function only of the size of its particles. The irradiation experiments interpreted in terms of the "hit theory" indicate that a phage particle is inactivated by a single ionization (or excitation) process. In order to be effective this process must take place within a "sensitive volume," the size of which can be calculated from the rate of inactivation. This volume is found for each strain of phage to be of the same order of magnitude as the volume of the phage particle itself, as determined by ultrafiltration and centrifugation.

We have now used this method to obtain information about the particle sizes of three streptococcus bacteriophages, which had not previously been measured. These phages have been described by A. C. Evans.[4] They are distinguished by their serological specificity, by bacterial host specificity and by the type of plaque they produce on solid medium.

Samples of the bacteriophages suspended in peptone broth in small celluloid tubes were exposed to x-rays (475 kv equivalent constant potential, 13 ma, .25 mm Cu plus .45 mm Al filter, 960 roentgens/min. measured in air at 37 cm focal distance, absorption and scattering corrections negligible). Afterward the phage content of each sample was determined by plaque count. The results are shown in Fig. 1.

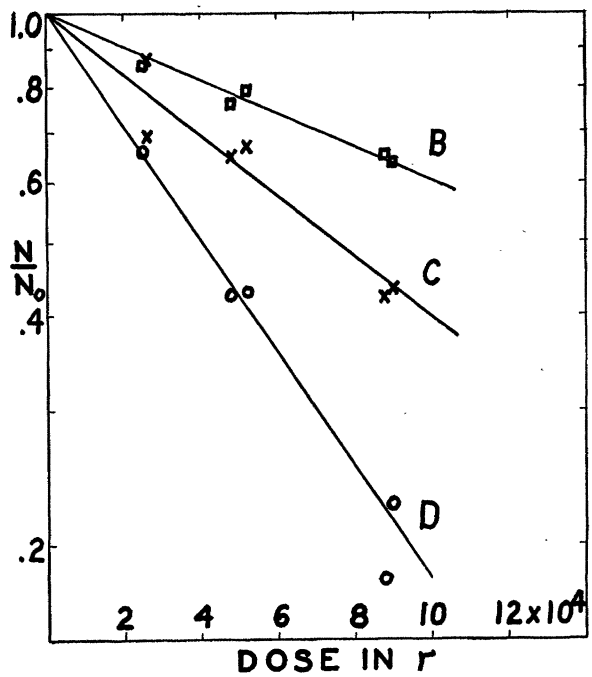

FIG. 1. Inactivation curves for three streptococcus bacteriophages. Abscissas: dose of x-rays in roentgens. Ordinates: proportion of bacteriophage particles remaining active after irradiation.

It is evident that in each case the experimental points can be fitted by an exponential curve (straight line on semi-logarithmic plot) within the limits of the experimental errors, which in plaque counts with streptococcus phages are likely to be rather large. Having established the exponential relation with great precision for other phages,[5] we shall assume it to apply here.

Table I gives the diameters of the "sensitive volumes," calculated from the curves in Fig. 1 by Lea's method.[3] Assuming that the relationship which exists for other phages between size of sensitive volume and particle size is also valid for the streptococcus phages we can tentatively consider the values of Table I as estimates of particle size. It should be mentioned that the recognized uncertainties in the absolute measurement of x-ray dosage at 500 kv are not large enough

[1] W. J. Elford, in Doerr and Hallauer, Handbuch d. Virusforschung, p. 126, Julius Springer, Wien, 1938.

[2] J. Bronfenbrenner, *Jour. Exp. Med.*, 45: 873, 1927; D. M. Hetler and J. Bronfenbrenner, *Jour. Gen. Physiol.*, 14: 547, 1931; J. H. Northrop, *Jour. Gen. Physiol.*, 21: 335, 1938.

[3] E. Wollman, F. Holweck and S. Luria, *Nature*, 145: 935, 1940; D. E. Lea, *Nature*, 146: 137, 1940.

[4] A. C. Evans, U. S. Public Health Reports 49: 1386, 1934.

[5] S. E. Luria and F. M. Exner, *Proc. Nat. Acad. Sci.*, 27: 370, 1941.

TABLE I

Bacteriophage	Streptococcus strain	Inactivation dose in r*	Sensitive volume diameter mμ
B	563	200,000	26
C	594	110,000	33
D	693	60,000	43

* Dose giving inactivation ratio $\frac{N}{N_0} = 1/e$.

to influence the present results, since particle diameters depend (through volumes) on cube roots of inactivation rates.

A comparison with previously measured phages[5] shows that phage D is a medium size phage; phage C falls among small phages (like C13 Burnet); and phage B is still smaller.

The classification of these three phages as separate entities, based up to now on their different biological properties,[4] finds further justification in their different particle sizes.

A general relationship of inverse proportionality has been shown to exist between the size of phage particles and the size of the plaques they produce on agar (Elford, Burnet). Such comparisons are definite only for plaques produced in presence of the same host strain of bacteria, which was not the case in our experiments. Nevertheless, it is probably not meaningless that the smallest phage B is a "large plaque forming" strain, whereas the larger phage D produces very small plaques.

We hope soon to check the particle size values as given here by other methods (ultra-centrifugation, electron microscope).

We are indebted to Dr. A. C. Evans for supplying the strains of bacteriophages and of host bacteria. Only three out of four strains were studied, owing to difficulty in obtaining reproducible counts of the plaques formed by phage A.

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THE DETECTION OF POLIOMYELITIS VIRUS IN FLIES[1]

THE present note describes two instances in which the virus of poliomyelitis has been detected in collections of flies made in the field during epidemics of this disease. The first positive test was obtained from a summer camp (Camp S.) in Connecticut, where at least three frank cases of poliomyelitis occurred during the latter half of July, 1941, among a varying population of about 100 children, and where during the first half of August, two other proven, convalescent (intestinal) carriers were present among the campers. A sample of roughly 1,000–1,200 flies, caught out of doors in the vicinity of the camp kitchen on August 6th and 8th, was stored in the refrigerator until ready for inoculation. The bulk of this sample was made up of several varieties, including in particular, three types of green-bottle flies or *Lucilia* (*viz.: sericata, caesar* and *sylvarum*) and one variety of blow-fly, *Phormia regina.* In lesser numbers there were also representatives of the common house fly (*Musca domestica*) and flies of the following species: *Muscina stabulans, Sarcophaga haemorrhoidalis, Ophyra leucostoma, Protocalliphora,* and some questionable examples of *Stomoxys.*[2]

Two types of inocula were prepared: (*a*) an emulsion of 100–300 flies macerated in 200 cc of sterile water; and (*b*) washings from 400–600 flies in 50 cc of water. Sample *a* was centrifuged and from the mid-layer a 30 cc portion was frozen and set aside for nasal instillation, while to another 20 cc mid-layer portion, 15 per cent. ether was added (for bactericidal purposes) and it was allowed to stand in the ice box overnight before being injected intraperitoneally. Sample *b* was filtered through gauze and used for nasal instillation. On August 12th, 10 cc of the etherized portion of sample *a* was injected intraperitoneally, and on 3 successive days 2 cc amounts of samples *a* and *b* were instilled intranasally into one cynomolgous monkey (No. 1676). This animal developed poliomyelitis after an incubation period of 15 days.

The second specimen of flies to yield the virus was obtained in the vicinity of Jasper, Alabama, where poliomyelitis was epidemic during July and August, 1941. On August 20th, a fly trap was placed near a privy used by three households where cases of poliomyelitis had recently occurred. On August 24th, a sample of flies, representing about 200 specimens (unidentified as to species, except for the presence of green-bottle flies, blow-flies and probably house flies) were removed from the trap, packed in dry ice and mailed to New Haven, where they were prepared and inoculated into one cynomolgous monkey (No. 1840) which developed poliomyelitis after an incubation period of 9 days. The methods used were essentially the same as those described in the first animal.

Criteria for the identification of the virus in these two instances have been that the monkey developed signs and symptoms of the experimental disease; that typical histological lesions were found in the cervical

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] We are indebted to Dr. R. B. Friend, of the Connecticut Agricultural Experiment Station of New Haven, for the identification of the specimens.