SENSITIVITIES OF DIFFERENT BACTERIOPHAGE SPECIES TO IONIZING RADIATIONS¹

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Under appropriate conditions ("direct effect" of Luria and Exner, 1941; Watson, 1950; and others) bacteriophages are known to be inactivated by ionizing radiations according to a simple exponential function of the dose D of radiation received by the phage

$$S = e^{-RD} \quad (1)$$

where S is the survival ratio (number of plaque forming phage particles after exposure to a dose D of radiation)/(number of plaque-forming phage particles before exposure), and R is the radiosensitivity of the phage (for a recent discussion of this subject, see Pollard, 1954). The radiosensitivities of a number of bacteriophage species have been measured, and are presented here and compared with data available in the literature, as a contribution to phage taxonomy and as a possible help in the choice of material for future investigations.

MATERIAL AND METHODS

Information on the phages and host bacteria² used is given, directly or through references, in table 1.

The solid media most commonly used for phage titration (by the semisolid agar technique; see Adams, 1959, Appendix) were: nutrient agar (formula of Adams, 1959, Appendix), and LB agar (Bertani, 1951). Other media are mentioned with the appropriate reference in table 1.

For irradiation, the phage lysates were diluted into a sterile solution of yeast extract (Difco)

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(10 per cent) and gelatin (5 per cent) in distilled water, at 37 C, distributed in 5-ml amounts into paper cups (4.5 cm in diameter), and cooled to obtain gellification of the suspension. The samples were irradiated at room temperature, in the cups, without cover. Control samples were identically treated, but were not irradiated. After irradiation the samples were stored in the refrigerator, and liquefied at 37 C when needed for phage titration. Often several different phage lysates were exposed simultaneously, mixed together in the same gelatin cake, titrations being performed on differential indicators.

For all irradiations the Co⁶⁰ source of the Los Angeles Tumor Institute (Neil *et al.*, 1953) was employed,³ the samples being exposed at approximately the same distance from the source as used by Weigle and Bertani (1956). Their measurement of the radiosensitivity of phage T2 (their table 1, lowest multiplicities of infection) agrees within 10 per cent with the estimate obtained here (table 1), when correction is made for the decay of the radioactive source (half life = 5.3 years).

RESULTS

The estimates of radiosensitivities obtained are listed in table 1. The radiosensitivity is expressed as the number of "hits" received by the phage during 1-hr exposure to the Co⁶⁰ source, a "hit" being the effective damage produced by a dose of radiation for which the survival ratio S is equal to e^{-1} or 0.368. The irradiations were performed over a period of more than a year: the radiosensitivities were, however, corrected for the spontaneous decay of the radioactive source. The estimates given in the table are thus

³ The cooperation of Dr. John Anson of the Los Angeles Tumor Institute in supervising the irradiations is gratefully acknowledged.

TABLE 1
Radiosensitivities of different phage species

Phage Species	Host Bacterium*	Solid Medium Used for Phage Titration	Radiosensitivity† (as Defined in Text) in Hfts/Hr (November 1957)	Relative Radio-sensitivity	Irradiation Time (min)
T-even (T2 from S. E. Luria; T4 from A. H. Doermann). Adams (1952)	<i>Escherichia coli</i> Bc. Cohen (1959)	Nutrient agar	7.16 (7.23†, 7.11†, 7.15)	1.0	60, 60, 30
<i>Bacillus mycoides</i> phage (from A. P. Krueger). Baer and Krueger (1952) The lysate used was made from a clear plaque (virulent mutant?). P2 <i>vir</i> ³ (a virulent mutant of phage P2). Bertani (1951)	<i>B. mycoides</i> N	Nutrient agar	7.72 (7.70†, 7.75)	1.1	60, 60
φX-174 (from M. Delbrück). Sinsheimer (1959a)	<i>Shigella dysenteriae</i> Sh/s	LB agar	2.76†	0.38	60
P22 (from N. D. Zinder). Garen and Zinder (1955)	<i>E. coli</i> C (no. 122, National Collection of Type Cultures, London)	Nutrient agar	1.26, (1.16†, 1.36†, 12.5)	0.18	60, 120, 180
N1 (from H. B. Naylor). Naylor and Burgi (1956)	<i>Salmonella typhimurium</i> LT2	LB agar	1.78 (1.88†, 1.68)	0.25	60, 120
Xanthomonas phage (from C. Quadling). Katznelson <i>et al.</i> (1954)	<i>Micrococcus lysodeikticus</i> ML1	LB agar	2.66, (2.49†, 2.84)	0.37	60, 120
Pseudomonas phage 13 (from T. H. Mead) (1956)	<i>Xanthomonas phaseoli</i> XP8-1	Nutrient agar without added NaCl	4.41 (4.54†, 4.28)	0.62	60, 120
Rhizobium phage 317 (from J. Kleczkowska). Kleczkowski and Kleczkowski (1952)	<i>Pseudomonas aeruginosa</i> LIII-1b	LB agar	2.10†	0.29	60
Phage "O-1" of Felix and Callow (from Communicable Disease Center, Atlanta). Cherry <i>et al.</i> (1954)	<i>Rhizobium trifolii</i> 317	Mannitol agar (Kleczkowska, 1945)	3.31†	0.46	60
Phage 3B (serological group A) (no. 8410, National Collection of Type Cultures, London) (from M. P. Jevons). Rippon (1956); Rountree (1955)	<i>Salmonella paratyphi</i> B, phage-type 1 (76)	LB agar	4.08†	0.57	30
D32 (from R. Russell). Froman <i>et al.</i> (1954)	Staphylococcus 3B (no. 8321, National Collection of Type Cultures, London)	LB agar	1.72	0.24	30
P1 k c. (Lennox, 1955)	Mycobacterium F119	See reference	14.09 (14.3†, 15.4†, 12.5†)	1.97	15, 25, 35
	<i>S. dysenteriae</i> Sh/s	LB agar	6.09 (5.90†, 6.27†) (see text)	0.85	25, 35

* Where no reference is given, see the reference attached to the corresponding phage strain.
† Average, when more than one independent estimate is available. The estimates obtained from independent irradiations are given in parentheses, the corresponding irradiation times being given in the last column.
‡ Estimate based on the average of two titrations performed on different days of the same irradiated and control samples, rather than on one titration. Most titrations are based on sets of three or more agar plates, with total plaque counts of at least 200.

comparable, as though all irradiations had been performed at the same time (November 1957).

For several phages more than one irradiation was performed, in order either to examine the reproducibility of the measurements or to test whether the logarithm of the survival ratio was really a linear function of the dose. The latter was done for phages ϕ X-174, P22, N1, Xanthomonas phage, D32, and P1. Of these only P1 gave a survival curve inconsistent with equation (1): the logarithms of the survival ratios were indeed a linear function of the dose of radiation (three doses tested), but extrapolated to a value larger than 0 for dose 0. The accuracy of this observation is not to be doubted, particularly since phage D32 was irradiated together with P1, and did not give an aberrant survival curve. The experiment, however, was not repeated. The radiosensitivity estimates for P1 given in the table refer only to the straight part of the survival curve.

The fifth column of table 1 gives the radiosensitivities of the various phages relative to that of the T-even species.

The same irradiated preparation of P2 *vir*³ mentioned in table 1, when plated on an indicator singly lysogenic for phage P2 (strain Sh-17 of Bertani and Six, 1958), gave a radiosensitivity value of 2.40; when plated on an indicator doubly lysogenic for P2 (strain Sh-55 of Bertani and Six, 1958), it gave a value of 1.86. A similar result was obtained with P2 *vir*³ irradiated with soft X-rays (50 kv, same machine and experimental arrangement used by Harm, 1958). These observations indicate the existence of a repair mechanism involving an interaction between the damaged phage and a related prophage. A similar phenomenon occurs with phage irradiated with ultraviolet light (Jacob and Wollman, 1953).

DISCUSSION

A survey of the information on the radiosensitivities of various phages available in the literature is given in table 2. Only data are considered for which indirect effects are reasonably excluded. In most cases it was fortunately possible to calculate the relative radiosensitivity from measurements obtained by the same author with the same X-ray source for some phage of the T-even species (Adams, 1952). A greater uncertainty is attached to the relative radiosensi-

tivities calculated via physical measurements of the X-ray dose.

The data of table 2 were obtained with a variety of X-rays. Pooling of the data, and comparison with those of table 1 are permissible, however, if one assumes that, even though the absolute radiosensitivities might depend to some extent on the radiation wave length within the range 30 kv through Co⁶⁰ γ -rays, the relative radiosensitivities are independent of the wave length. This assumption is supported by the agreement between the relative radiosensitivities of phage T1, as measured by Latarjet and Fredericq (1955) with soft X-rays, and by Watson (1950) and Luria and Exner (1941) with hard X-rays; of phages S13 and ϕ X-174, as measured by Latarjet and Fredericq (1955) and by Tessman *et al.* (1957) with soft X-rays, and by us with γ -rays; of phage P2, as measured by us with γ -rays (table 1) and with soft X-rays (50 kv, same X-ray machine and experimental arrangement as used by Harm, 1958; relative radiosensitivity of P2 = 0.40).

All measurements reported in table 2 were obtained with phage irradiated in aqueous suspension, at temperatures between 0 C and room temperatures: within this range the effect of temperature on the radiosensitivity is negligible (Adams and Pollard, 1952).

The amount of information available on the biological properties of the various phages listed in tables 1 and 2 is very diverse. As a consequence, for only few of them may the criteria proposed and discussed by Adams (1953) for the recognition of different phage species be applied. In other cases, sufficiently great taxonomic differences between the host-bacteria are guarantee of a sufficiently great separation between the corresponding phages. It might be that at most one or two of the phages considered here, although grown on taxonomically different bacterial hosts, should turn out to be closely related to some other phage of those considered, upon more adequate study.⁴ It is assumed that different members of the same phage species possess identical or very similar radiosensitivities. This is consistent with the admittedly

⁴ Possibilities: *Bacillus mycoides* phage and *Bacillus megaterium* phage M25, both temperate; *Pseudomonas* phage 13 and *Salmonella* phage P22, both temperate and showing very similar plaque morphology.

TABLE 2
Radiosensitivities of different phage species. Data obtained from literature

Phage Species	Host Bacterium	Relative Radiosensitivity*	Method of Calculation†	Type of Radiation	Author
T1 (P28) (Adams and Wade, 1955)	<i>Escherichia coli</i>	0.48 0.47 0.44 0.49 0.38 0.48 0.47 0.35 1.14 0.38 0.39 (wild type); 0.33 (virulent mutant and wild type) 0.33 (virulent mutant and wild type)	A, with T-even A, with T-even A, with T-even (C16) C C A, with T-even A, with T-even A, with T-even (C16) C A, with T-even A, with T2 C	k_v 37 180-200 200-1000 250 180 37 180-200 200-1000 140 37 200	Latarjet and Fredericq (1955) Watson (1950) Luria and Exner (1941) Whitmore and Pollard (1958) Hill (1958) Latarjet and Fredericq (1955) Watson (1950) Luria and Exner (1941) Buzzell and Lauffer (1952) Latarjet and Fredericq (1955) Epstein and Englander (1954)
T3, T7, C13 (Adams and Wade, 1954; Burnet, 1933)	<i>E. coli</i>				Marcovich (1956)
T5 (Adams, 1952)	<i>E. coli</i>				Latarjet and Fredericq (1955)
λ	<i>E. coli</i>				Alper (1954)
S 13 (same species as ϕ X-174; Zahler, 1958; Tessman, 1959)	<i>E. coli</i> ; <i>Shigella</i>				Tessman <i>et al.</i> (1957)
P8	<i>Pseudomonas</i>				Tobin (1953)
3K	<i>Staphylococcus</i>				Luria and Exner (1941)
B (Evans, 1934)	<i>Streptococcus</i>				Exner and Luria (1941)
C (Evans, 1934)	<i>Streptococcus</i>				Exner and Luria (1941)
D (Evans, 1934)	<i>Streptococcus</i>				Exner and Luria (1941)
Lactobacillus phage	<i>Lactobacillus casei</i>				Walter (1958)
M1 (Friedman and Cowles, 1953)	<i>Bacillus megaterium</i>				Woese (1959)
M5 (Friedman and Cowles, 1953)	<i>B. megaterium</i>				Friedman (1954)
M25	<i>B. megaterium</i>				Woese (1959)
P22 (virulent mutant)	<i>Salmonella typhimurium</i>				Garen and Zinder (1955)

* Radiosensitivity of T-even phages set to be equal to 1.

† A = direct comparison by the author cited with the radiosensitivity of a T-even phage or other phage for which a direct comparison with T-even phages exists; B = direct comparison by the author cited with the radiosensitivity of another phage for which no direct comparison with T-even phages exists; C = calculated from X-ray dose measurements, assuming an "inactivation dose" (the dose for which the survival ratio S is equal to e^{-1}) for T-even phages of 40,000 r.

Figure 1. Frequency distribution of relative radiosensitivities of different phage species. The radiosensitivity of phages of the T-even species is set to be equal to 1. The figure is based on the data of tables 1 and 2: where more than one estimate was available for a given phage species the arithmetic mean was calculated and used.

limited information available on this point, the only exception being that of phage λ as compared with one of its virulent mutants (Epstein and Englander, 1954), an observation which Marcovich (1956) was unable to confirm, although it is not certain that identical phage strains were used in the two cases.

The data of tables 1 and 2 are pooled and plotted in figure 1. The most common relative radiosensitivity value for bacteriophages appears to be in the range 0.3 to 0.6. The radiosensitivity values cover more than a 10-fold range. The frequency distribution is highly asymmetric, favoring the larger values. One might be led to suspect the existence of more than one class of bacteriophages, different in their average radiosensitivities.

If one excludes from the tally the case of the only Mycobacterium phage examined, there does not seem to be a significant difference in radiosensitivity between the phages of gram-negative and those of gram-positive bacteria, nor a correlation between radiosensitivity and temperate (as opposed to virulent) character of the phage.

It would be very desirable to see whether the high radiosensitivity of the Mycobacterium phage is shared by other phages attacking Mycobacterium, Nocardia, Actinomyces, or Streptomyces.

One might speculate on the apparent coincidence in value of the relative size of the "big piece" of deoxyribonucleic acid (DNA) in phage T2 (Levinthal, 1955; Thomas, 1959) with the average relative radiosensitivity (0.42; range: 0.20 to 0.67) for the 18 most resistant phages (ϕ X-174 excluded because of the unusual prop-

erties of its DNA; see Sinsheimer, 1959b, and Tessman, 1959) of figure 1. If one accepts the notion of a rough correspondence between radiosensitivity and DNA content (Epstein, 1953), the possibility should be considered that the "big piece" represents an evolutionarily older, structural "packing unit" of DNA, the only DNA possession of some phages, larger and more evolved phages having acquired other DNA elements in addition to it.

SUMMARY

The sensitivities ("direct effect") to ionizing radiations (γ -rays from Co^{60}) of 13 different phage species have been measured, and compared with the information available in the literature for still other species of phage (to a total of 26 species). The most sensitive (a Mycobacterium phage) is about 13 times more sensitive than the most resistant (coli-dysentery phage ϕ X-174, S13). The most common radiosensitivity value corresponds to about $\frac{1}{10}$ of the radiosensitivity of the T-even phages.

Lysogenic hosts may repair a fraction of the damage produced in phage by ionizing radiations.

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