THE PHYSICAL STATE OF VIRAL NUCLEIC ACID AND THE SENSITIVITY OF VIRUSES TO ULTRAVIOLET LIGHT

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ABSTRACT Ultraviolet light action spectra in the range 2250 to 3020 A have been determined for the plaque-forming ability of the following bacteriophage and animal viruses: T-2, ϕx-174, R-17, fr, MS2, 7-S, fd, vesicular stomatitis, vaccinia, encephalomyocarditis, reovirus-3, and polyoma. Absolute quantum yields for the plaque-forming ability of MS2, fr, fd, ϕx-174, and T-2 were determined over the range 2250 to 3020 A. Relative quantum yields for plaque-forming ability indicated that viruses with single-stranded nucleic acid were on the average ten times more sensitive to UV than double-stranded viruses. In addition for ten of the twelve viruses a relation existed between the shape of their action spectra and the stranded state of their nucleic acid. The ratio of the inactivation cross-section at 2650 A to that at 2250 A for these viruses was 1.0 for single-stranded viruses and 2.0 for viruses with double-stranded nucleic acid. The above relations were dependent on the stranded state of the nucleic acid not the ribose or deoxyribose form of the sugar present.

INTRODUCTION

The relative sensitivity to ultraviolet light (UV) of a number of viruses has been examined by several workers (1-3) and attempts have been made to correlate the UV sensitivity of these viruses with the nature of their nucleic acid. Known differences between the nucleic acids of different viruses, such as whether they are single- or double-stranded, DNA or RNA, of low or high molecular weight, might be expected to affect their susceptibility to UV. Unfortunately, such attempts at correlation have been limited by the types of viruses available for quantitative study.

Since the number of viruses whose chemical composition and physical structure are fairly well known has increased in recent years, it was of interest to examine the UV sensitivity of these viruses in light of the earlier results. Also, in the earlier studies, the UV source usually used was a germicidal lamp whose major UV band is at 2537 A. Since specific events at the chemical level can be dependent on the wavelength of the UV light used, more information may be obtained if survival curves are determined for a series of wavelengths and action spectra constructed.

Such action spectra are useful in comparing the UV sensitivity of different viruses. For example, Setlow and Setlow have shown that double-stranded T-2 DNA and *Haemophilus influenzae* transforming DNA do not have the same UV action spectra as single-stranded DNA (4, 5). For these reasons, in the present investigation, the survival curves for viral plaque-forming ability were determined for each of twelve bacterial and animal viruses at 8 to 11 different wavelengths in the range 2250 to 3020 A. The relation of the results to the chemical and physical structure of the viruses will be discussed.

MATERIALS AND METHODS

Properties of the Viruses Examined. Table I gives a list of the twelve viruses studied, as well as the hosts in which they were propagated and assayed, and information about their physical and chemical properties.

Bacteriophage. The action spectrum for each bacteriophage was determined using a single lysate. Bacteria were grown in MS2 broth (16) at 37°C. Bacteriophage was prepared by infecting the appropriate host bacteria in the logarithmic phase of growth and shaking until lysis was complete. Chloroform was added to release virus from unlysed bacteria (17). Bacterial debris was then spun down by low speed centrifugation. Such crude lysates were further purified by two cycles of ammonium sulfate precipitation. The resulting stock lysates had a titer of 10^{11} to 10^{14} plaque-forming units (pfu) per ml depending on the bacteriophage. For irradiation, the virus stock was diluted in phosphate-buffered saline (PBS) (18) to give 10^{7} to 10^{8} pfu per ml. Viruses were irradiated in a quartz cuvette with a 1 cm light path at an optical density (OD) of 0.1 or less depending on the wavelength used.

Bacteriophage samples were removed after various times of irradiation, diluted in PBS, and plated on agar plates using the appropriate bacterial host, given in Table I. The plating techniques and the materials for all bacteriophages were the same as those given by Davis and Sinsheimer for MS2 (16). All handling of the bacteriophage after irradiation was carried out under yellow light to avoid any possible photoreactivation.

Animal Viruses. Polyoma virus lysates were prepared using secondary cultures of mouse embryo monolayers (19). Monolayers of cells were grown at 37°C in glass bottles containing CMRL 1066 medium (20) plus antibiotics and 5 per cent calf serum. Monolayers were infected with polyoma virus and lysis was considered complete when these monolayers detached as a sheet from the glass surface. The crude lysates were disrupted further by sonication to remove attached virus from cellular material. Cellular debris was then spun down by centrifugation at 600 $\times$ g for 15 minutes and the resulting supernatant used as the polyoma virus stock lysate. The lysates for viruses 8 to 11 of Table I were prepared by infecting suspension cultures of L cells (21). Suspension cultures were grow at 37°C in glass roller tubes in a revolving drum (22) or when large quantities of cells were required, in 1000 ml spinner flasks (Bellco Glass Co., Vineland, New Jersey). The medium in which the L cells were grown was CMRL 1066 medium (20) containing antibiotics plus 5 per cent normal horse serum. The viruses were partially purified by differential centrifugation after freezing and thawing the lysates. For irradiation, aliquots of the lysates were diluted in PBS so that their OD was less than 0.150 over all the wavelengths used for inactivation. The virus titers in the aliquots used for irradiation ranged from 10^{5} to 10^{7} pfu per ml. After irradiation, dilutions were made in PBS or in CMRL 1066 medium.

TABLE I

THE PHYSICAL AND CHEMICAL PROPERTIES OF VIRUSES

Virus	Host	Viral nucleic acid	Molecular weight of viral nucleic acid	Virus shape + size	Base content A	U or T	G	C	Molecular weight of virus	Reference
1. MS2	*E. coli* CR63	RNA	1×10^6	200–250 A spherical	23	25	27	25	3.6×10^6	(6)
2. R–17	*E. coli* CR63	RNA	1.3×10^6	200–250A spherical	23	25	26	25	4.2×10^6	(7)
3. *fr*	*E. coli* CR63	RNA	1.2×10^6	200–250 A spherical	24.3	23.7	27.1	24.9	4.2×10^6	(8)
4. 7–S	*P. aeruginosa*, H–1	RNA	1×10^6	200–250 A spherical	23.8	25.8	24.6	25.6	4×10^6	(9)
5. ϕX–174	*E. coli* C	DNA	1.7×10^6	200–250 A spherical	24.6	32.7	24.1	18.4	6.2×10^6	(10)
6. fd	*E. coli* CR63	DNA	1.3×10^6	7000 × 50 A rod-shaped	24.4	34.1	19.9	21.7	10.8×10^6	(8)
7. T–2	*E. coli* B	DNA	120×10^6	650 × 950 A head and tail	32.5	32.5	17.5	17.5	300×10^6	(11)
8. Vaccinia	Mouse L-60	DNA	150×10^6	2300 × 2500 A brick-shaped	29.5	29.9	20.6	20.0	2000×10^6	(11)
9. EMC	Mouse L–60	RNA	2.1×10^6	270 A spherical	27.3	25.3	23.5	23.5	7×10^6	(12)
10. VSV	Mouse L–60	RNA	—	1750 × 700 A bullet-shaped		–			–	(13)
11. Reovirus–3	Mouse L–60	RNA	$\geq 10 \times 10^6$	700 A spherical	29.7	30.5	19.3	20.5	$\geq 70 \times 10^6$	(14)
12. Polyoma	Mouse embryo	DNA	3.5×10^6	440 A spherical	26	26	24	24	40×10^6	(15)
Herpes simplex	HeLa	DNA	60×10^6	1000 A spherical	16	16	34	34	–	(36)
Adenovirus	KB	DNA	10×10^6	700 A spherical	22	21	27	29	200×10^6	(37)
Polio	HeLa	RNA	2×10^6	250 A spherical	30.5	24.8	25.5	19.2	7×10^6	(11)

Polyoma was titrated by plaque assay on secondary cultures of mouse embryo cells after the method of Dulbecco and Freeman (19). The other animal viruses were assayed on mouse L cells essentially as described by Dulbecco and Vogt (23). In all cases cells were first grown to monolayers on plastic Petri dishes (Falcon Plastics, Los Angeles, California). After removal of the growth medium 0.1 ml samples of various viral dilutions were plated, in triplicate, allowed to adsorb for 1 hour, and overlaid with growth medium containing 1 per cent agar. The plates were then incubated at 37°C in a humidified incubator flushed with a 5 per cent CO_2 atmosphere. Incubation times were 24 hours for vesicular stomatitis virus (VSV), 2 to 3 days for vaccinia and encephalomyocarditis virus (EMC), 6 days for reovirus-3, and 14 days for polyoma virus.

At the end of the incubation period, the dishes containing L cells and the appropriate viruses were fixed with a 3:1 ethanol–acetic acid solution, the agar removed, and the plates stained with a saturated solution of methylene blue. Plaques of polyoma virus were observed after staining directly under agar for 20 hours with a 1:20,000 solution of neutral red in 1066 medium.

Irradiations. Irradiations were carried out with a large diffraction grating monochromator (24). At the exit slit the dispersion of this instrument was 40 A per cm so that for the maximum slit width, 0.6 cm, the beam was monochromatic to about $\lambda \pm 12$ A. Scattered radiation was less than 0.01 per cent at all wavelengths. After passing through the exit slit (0.6 cm wide and 15 cm high), the beam was focussed to a spot approximately 0.5 cm wide by 1.5 cm high at the center of a Zeiss spectrophotometer cell ($1 \times 1 \times 4$ cm). A small motor-driven stainless steel propeller positioned just above the focussed beam spot stirred the virus suspension and insured that a uniform dose was delivered to it. The light passed through the cell without striking its walls.

The total number of photons incident on the virus solution, N_i, was measured by a photocell which had been calibrated with malachite green leucocyanide (MGL) (25). The average flux density of photons through the cell was determined by correcting for absorption of the virus suspension, the incident beam's pathlength through the cell, and the volume of virus suspension irradiated. The details of this calculation are also given in reference 25. The correction for absorption of the virus suspension is similar to the correction of Morowitz (26).

Despite rapid stirring it was not possible to obtain a straight line for the log of the surviving fraction *versus* dose for a survival less than 0.01 per cent. The inactivation curves tended to become concave upwards, due to the survival of a small percentage of virus adsorbed to the walls of the cell and therefore unexposed to the UV beam. If the irradiation was carried out under conditions where the irradiation cell was cylindrical and rotated on its axis so that the whole cell was exposed to the UV flux this effect disappeared. However, the fixed cell geometry was used for all irradiations, since the energy flux calibration of the monochromator was more precise, higher intensities were available, and the survival curves to 0.01 per cent were satisfactory for slope determinations.

RESULTS

Typical survival curves for an animal virus (VSV) and a bacteriophage (MS2) are shown in Fig. 1. All the viruses gave similar curves, except for T-2, which showed a slight shoulder. Some difficulty was experienced in obtaining good survival curves for vaccinia and polyoma viruses. At times an initial sharp drop in survival to the

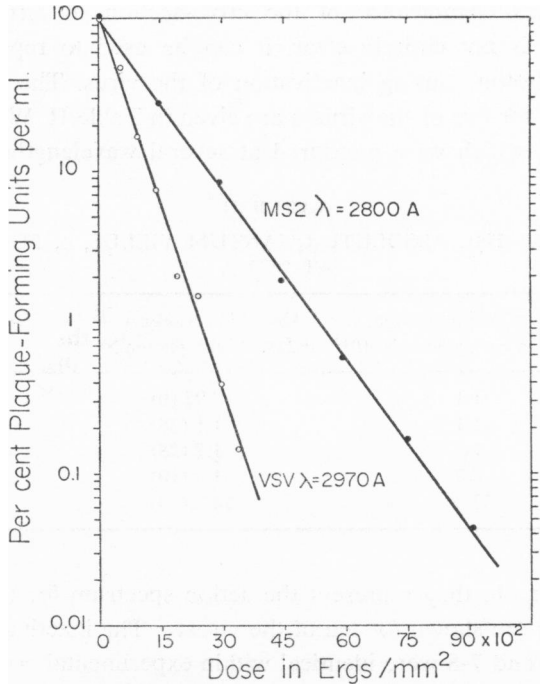

FIGURE 1 Typical survival curves of viral plaque-forming ability *versus* dose of ultraviolet light (UV) for an animal and bacterial virus. The wavelength of light used to inactivate the vesicular stomatitus virus (VSV) was 2970 A and that to inactivate MS2 bacteriophage was 2800 A.

5 to 10 per cent level was seen for vaccinia and polyoma. There followed a rapid decrease in slope until a constant value was reached which was the same as the single slope found for these two viruses when this unusual effect was not present. The reason for this occasional change in curve shape was not connected with any genetic heterogeneity of the virus (the same stock was used throughout), photoreactivation, or multiplicity effects. Only the curves which did not show this effect were used for the determination of the viral sensitivity.

The experimental curves in Fig. 1 can be represented by the relation

$$\frac{n}{n_0} = e^{-\sigma D} \tag{1}$$

where n_o is the initial virus titer and n is the titer after a dose D is delivered, σ is a constant which is proportional to the sensitivity of a particular virus at a given wavelength.

In the above equation if D is given in units of photons per cm^2, then the constant σ can be referred to as a cross-section and has dimensions of cm^2 per photon.

Though the physical significance of the cross-section in terms of the dimensions of the virus is not entirely clear, it can be used to represent the relative probability of a photon causing inactivation of the virus. The inactivation cross-sections at 2537 A for five of the viruses are given in Table II. When these inactivation cross-sections, which were measured at several wavelengths, are plotted as a

TABLE II

CALCULATION OF THE ABSOLUTE QUANTUM YIELDS, ϕ, FOR FIVE VIRUSES AT 2537 A

Virus	Inactivation cross-section, σ, at 2537 A cm^2/photon $\times 10^{16}$	Absorption cross-section S, at 2537 A cm^2/photon $\times 10^{18}$	Quantum yield $\phi = \sigma/S$ at 2537 A $\times 10^4$
MS2	1.1	0.92 (6)	12
fr	1.1	1.1 (28)	10
fd	7.8	1.2 (28)	65
ϕX-174	8.7	1.9 (10)	47
T-2	37	74 (29)	5

function of wavelength, they represent the action spectrum for the virus. In Fig. 2 such action spectra are shown for ten of the viruses. The inactivation cross-sections for MS2, R-17, fr, and 7-S were identical within experimental error.

The action spectrum for reovirus-3 is not included in Fig. 2 but is shown separately in Fig. 3. It is similar in shape to the curves shown in Fig. 2 above 2650 A, but below this wavelength the inactivation cross-section becomes relatively larger as the wavelength decreases. This is particularly apparent at 2250 A where it is four times as great as it was at 2650 A. For comparative purposes the action spectrum of herpes simplex virus as determined by Powell (27) is also shown in Fig. 3 and as can be seen it is similar in shape to the action spectrum for reovirus-3.

In Fig. 4 the action spectrum of fd is shown along with that of ϕX-174 for comparison. From Table I it can be seen that these two viruses have a similar nucleic acid content and composition. These similarities are reflected in the near equivalence of their inactivation cross-sections above 2400 A. Below 2400 A the inactivation cross-section of fd becomes increasingly smaller than that of ϕX-174, until at 2250 A it is one-half that of ϕX-174.

As can be seen from Figs. 2 to 4, the relative sensitivity of the viruses studied varies over a factor of 100 at any one wavelength.

The measure of the probability that a photon will inactivate a virus is its inactivation cross-section, σ while the measure of the probability that a photon will be absorbed in a virus is its absorption cross-section, S. If σ and S are known the quantum yield ϕ for viral inactivation can be calculated. It is defined

$$\phi = \frac{\sigma}{S} \tag{2}$$

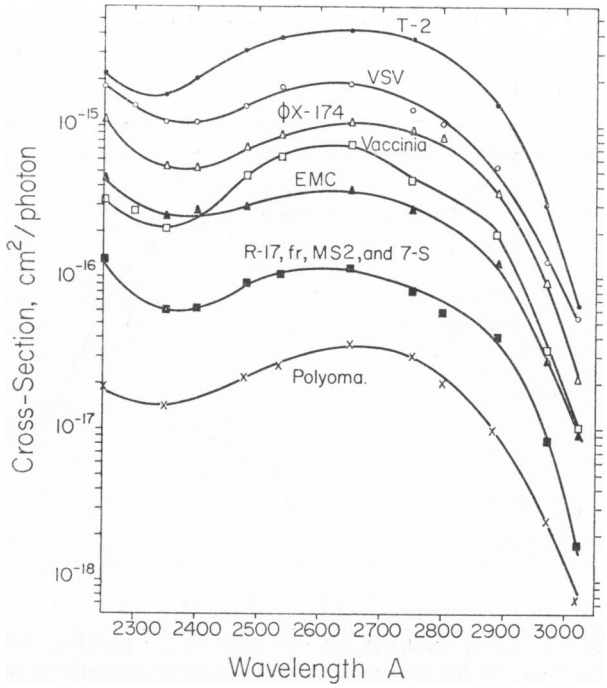

FIGURE 2 The UV action spectra for viral plaque-forming ability for ten of the viruses studied. The spectra for MS2, fd, R-17, and 7-S bacteriophages were identical within experimental error.

and ϕ is an absolute measure of viral sensitivity to ultraviolet light. The values of σ were obtained from the survival curves for each virus and are given in Table II for five of the viruses at 2537 A and Figs. 2 to 4, for all the viruses at a number of wavelengths. Unfortunately absorption cross-sections have been measured for only a few of the viruses examined here. The data for those five viruses (MS2, fr, fd, ϕX-174, and T-2) whose absorption cross-sections have been measured are shown in Table II, as well as the quantum yield at this wavelength. The variation of quantum yield with wavelength for these five viruses can also be calculated since data on both inactivation and absorption cross-sections were available at numerous wavelengths. These are shown in Fig. 5*a* for MS2 and fr, Fig. 5*b* for fd and ϕX-174, and Fig. 5*c* for T-2.

It should be pointed out that the absorption cross-sections given in the literature for MS2, fd, fr, and ϕX-174 probably include both absorption and scattering components. To make an estimate of this scattering component a sample of MS2 bacteriophage was purified by the method used by Loeb and Zinder (30) for their small RNA bacteriophage f2. The absorption spectrum of MS2 was measured and a correction made for light scattering using the same method as that used by

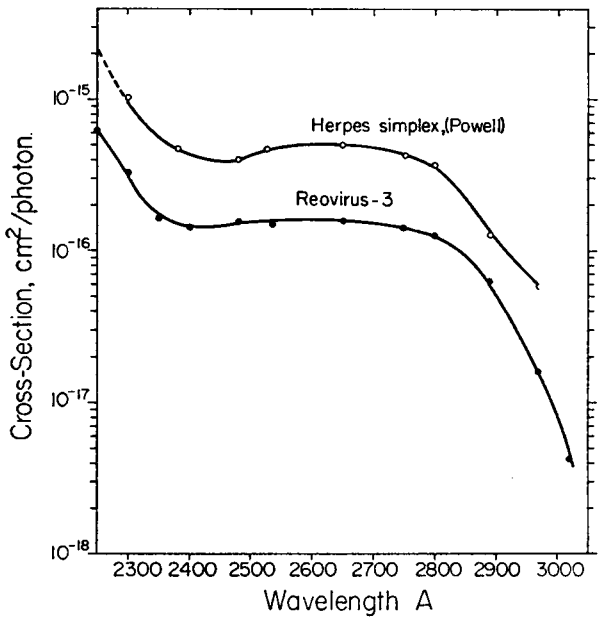

FIGURE 3 The UV action spectrum for reovirus-3 plaque-forming ability compared to the action spectrum for herpes simplex virus plaque-forming ability as measured by Powell (27).

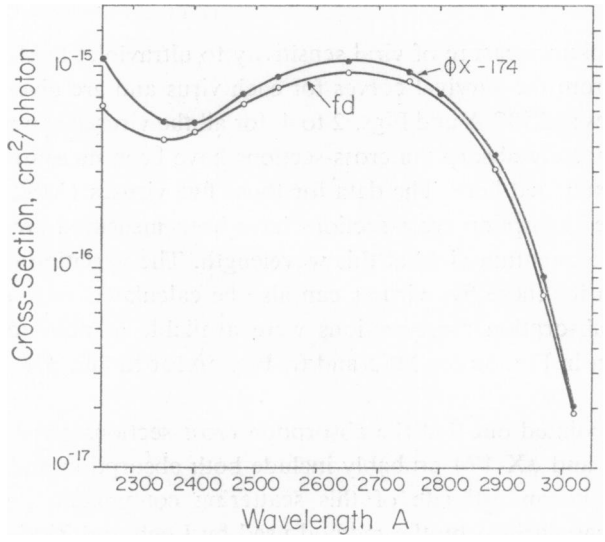

FIGURE 4 The UV action spectrum for fd bacteriophage plaque-forming ability compared to the action spectrum for φX-174 bacteriophage plaque-forming ability.

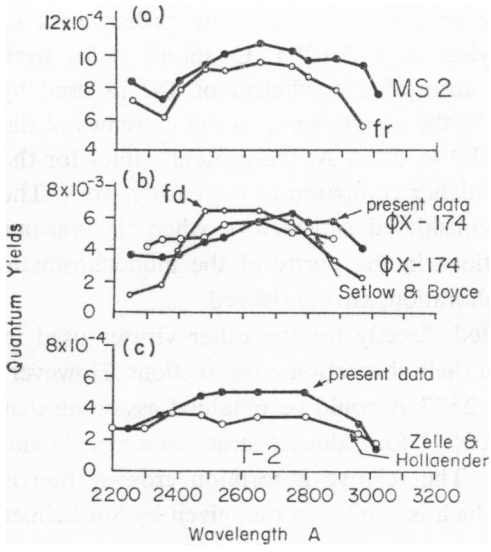

FIGURE 5 Absolute quantum yields as a function of wavelength for the UV inactivation of viral plaque-forming ability: (*a*) a comparison of two similar RNA containing bacteriophages fr and MS2; (*b*) a comparison of the present results for ϕX-174 bacteriophage with the earlier work of Setlow and Boyce (32) and with the present results for fd bacteriophage; (*c*) a comparison of the present results for T-2 bacteriophage with the earlier work of Zelle and Hollaender (29).

Dulbecco (31). At 3020 A the percentage correction to the measured absorption spectrum due to scattering was large (25 per cent) because the absorption cross-section of the virus is small at this wavelength. At 2600 A, though the amount of scattering is larger than at 3020 A, the percentage correction to the measured absorption spectrum is small (2 per cent) because the absorption cross-section is large. Therefore for the small bacteriophages such as MS2, fr, and ϕX-174 it appears that the corrections for scattering are almost negligible at lower wavelengths but become appreciable at about 2800 A and higher. The curve shown for MS2 in Fig. 5*a* was corrected for the scattering components of the measured absorption spectrum of the bacteriophage. It can be seen that the quantum yields for MS2 and fr, a bacteriophage very similar to MS2 in its physical properties, are about the same throughout the low wavelengths but diverge a little towards higher wavelengths. This divergence is probably due to the fact that the absorption data for MS2 were corrected for light scattering whereas those for fr were taken directly from Hoffman-Berling *et al.* (28) without correction.

Fig. 5*b* also includes the variation in quantum yield for ϕX-174 determined by Setlow and Boyce (32). It can be seen that the present data agree reasonably well with theirs over nearly all wavelengths and that the bacteriophages fd and ϕX-174 have similar quantum yields over nearly all wavelengths except for the very low and very high ends of the spectrum. The differences at high wavelengths are probably due to lack of correction for light scattering in the measured absorption spectra for ϕX-174 and fd and the difference at the low wavelength is probably due to the difference in physical properties of fd and ϕX-174, as will be discussed later.

A comparison was made between the present results and the earlier work of Zelle and Hollaender on the quantum yield of T-2 (29). Quantum yields were calculated using the same value for the absorption coefficient of T-2 as used by Zelle and Hollaender. As is seen in Fig. 5*c* the results agree at the extremes of the wavelength range, but in the range of 2480 to 2800 A, the present values for the quantum yield appear 30 to 60 per cent higher compared to the earlier work. The degree of agreement however can be considered satisfactory when the various sources of error possible, such as variations in the purity of the monochromatic light and differences in incident energy calibration, are considered.

Quantum yields could not be calculated directly for the other viruses used in this study since data were not available on their absorption cross-sections. However, a relative absorption cross-section, S_r, at 2537 A could be obtained assuming that the OD/mg/ml of nucleic acid at 2537 A is 17 for a double-stranded molecule and 21 for a single-stranded molecule (33). The relative absorption cross-section is then given by the following expression, which is similar to that given by Sinsheimer in reference 34.

$$S_r = (\text{OD/mg/ml} \times \text{mol. wt.}) \times 3.83 \times 10^{-21} \quad (3)$$

where mol. wt. is the molecular weight of the viral nucleic acid for which S_r is being calculated. The term in parentheses is the extinction coefficient for the viral nucleic acid and the number multiplying it is one thousand times the natural logarithm of ten divided by Avogadro's number. Equation (3) assumes that the absorption cross-section is due only to the viral nucleic acid, which at 2537 A absorbs 20 to 40 times more UV light per mg than protein (35). The validity of this assumption will be weakest of course in those viruses in which the nucleic acid forms only a small per cent of the total viral material. However, in the viruses studied here the nucleic acid constituted 10 to 50 per cent by weight of the total viral material. This can be seen by dividing the molecular weight of the nucleic acid by that of the whole virus (Table I).

The relative viral absorption cross-sections at 2537 A, S_r, are given in Table III. From these a relative quantum yield at 2537 A could be calculated and these are also in Table III. The relative quantum yields for the viruses MS2, fr, ϕX-174, fd, and T-2 are in fair agreement with the absolute quantum yields as given in Table II and Fig. 5. The differences between the two will be due to (*a*) the lack of consideration of viral protein in calculating the relative absorption cross-sections and (*b*) to the fact that the OD/mg/ml for nucleic acid used to calculate the relative absorption cross-sections S_r, may not be exactly correct due to differences in hypochromic effect and specific base composition for the various viral nucleic acids.

The relative quantum yields for herpes simplex, adenovirus, and polio virus as calculated from the data of other workers are given at the bottom of Table III for

TABLE III

RELATIVE ABSORPTION CROSS-SECTIONS, S_r, AND RELATIVE QUANTUM YIELDS, ϕ_r, AT 2537 A FOR VARIOUS VIRUSES

Virus	Nucleic acid	Stranded state	Molecular weight of viral nucleic acid $\times 10^{-6}$	Viral absorption cross-section S_r* at 2537 A cm^2/photon $\times 10^{14}$	Viral inactivation cross-section σ at 2537 A cm^2/photon $\times 10^{16}$	Relative quantum yield ϕ_r, at 2537 A $\times 10^4$
MS2 (R-17, fr, 7-S)	RNA	1	1	8.0	1.1	14
EMC	RNA	1	2	13	3.5	27
ϕX-174	DNA	1	1.7	14	8.7	62
fd	DNA	1	1.3	10	7.8	78
Vaccinia	DNA	2	150	980	6.5	0.7
T-2	DNA	2	120	780	38	4.9
Polyoma	DNA	2	3.5	23	0.26	1.1
Reovirus-3	RNA	2	10	65	1.5	2.3
Herpes simplex	DNA	2	60	380	4.6 (27)‡	1.2
Adenovirus	DNA	2	10	65	0.2 (38)	0.3
Polio	RNA	1	1.8	15	3.5 (39)	23

* Estimated by assuming: (*a*) that only the viral nucleic acid absorbs and (*b*) that the extinction coefficient for double-stranded DNA or RNA is 17 cm^2/mg and for single-stranded DNA or RNA it is 21 cm^2/mg.

‡ The numbers in parentheses refer to the references in which the viral inactivation cross-sections quoted are given.

comparative purposes. Some of the physical properties of these viruses are given at the bottom of Table I.

DISCUSSION

As can be seen from Table III the inactivation cross-sections for the viruses studied here vary over a factor of one hundred at any one wavelength. This would be expected if the absorption cross-sections also differed by the same factor and if no other phenomena other than energy absorption were involved in virus inactivation by UV light. However, if this were so, the ratio of inactivation cross-section to absorption cross-section, that is, the quantum yield, would be the same for all viruses. The data described in Tables II and III and Fig. 5 show that this is not the case and that the quantum yields can also vary over a factor of one hundred. Thus other factors must be involved in determining the sensitivity of viruses to UV light. Some of these factors will now be discussed.

Effect of the Stranded State on the Relative Quantum Yield, ϕ_r. The ϕ_r in Table III for single-stranded nucleic acids are seen to be at least ten times greater

than the ϕ_r for double-stranded nucleic acids. T-2 is an exception having an unusually large quantum yield. Thus, measurements of survival of viral plaque-forming ability indicate that nucleic acid in the single-standed state is some ten times more sensitive to UV than in the double-stranded state. A similar difference in UV sensitivity was found by Sinsheimer *et al.* (40) between single-stranded infectious ϕX-174 DNA and its replicative form which is thought to be double-stranded. Ginoza, using ionizing radiation, has demonstrated a tenfold difference in sensitivity between double- and single-stranded nucleic acids (41).

As seen in Table III, differences in sensitivity between double- and single-stranded nucleic acid were found for both RNA and DNA containing viruses. For example, reovirus-3 the one representative of a double-stranded RNA virus, has a quantum yield which is in the region of that for T-2, polyoma, vaccinia, and adenovirus, all viruses that contain double-stranded nucleic acid. It may also be noted that the single-stranded RNA viruses in Table III have relative quantum yields smaller than the single-stranded DNA viruses by a factor of 3 to 4. Since only two single-stranded DNA viruses are available for comparison, the significance of this difference is uncertain.

Effect of the Stranded State on the Shape of the Action Spectra. Single-stranded nucleic acid is not only more sensitive to UV than is double-stranded nucleic acid at 2537 A, but its action spectra differ in the wavelength region below 2480 A.

In Table IV values are shown of the ratio, *R*, of the inactivation cross-section at 2650 A to that at 2250 A for all the viruses examined. It is seen that for viruses with double-stranded nucleic acid, *R* is approximately 2.0 while for a single-stranded virus the ratio is about 1.0. Setlow and Boyce found such an effect for ϕX-174, (32) but they described it as a change in the position of the minimum of the action spectrum from 2350 A for double-stranded DNA to 2400 A for single-stranded DNA. The change in the ratio *R* observed above can be expressed as a consequence

TABLE IV

RATIO *R* OF THE INACTIVATION CROSS-SECTION AT 2650 A TO THE INACTIVATION CROSS-SECTION AT 2250 A

Single-stranded viruses		Double-stranded viruses	
Virus	*R*	*Virus*	*R*
MS2	0.9	T-2	2.0
R-17	0.9	Vaccinia	2.3
fr	0.9	Polyoma	2.0
7-S	0.9		
ϕX-174	1.0		
EMC	0.85		
VSV	1.0		
fd	1.5	Reovirus-3	0.3

of this shift. It is seen that this change in R occurs for RNA as well as for DNA single-stranded viruses. Two exceptions to this pattern are reovirus-3 and fd whose behavior will be discussed later.

Since the difference in action spectra takes place at low wavelengths where viral protein absorption is not negligible, it might be thought that viral protein sensitizes single-stranded nucleic acid to UV light. However, Setlow and Setlow (5) have shown that deproteinized *H. influenzae* transforming principle has a similar change in its action spectrum when irradiated at temperatures above its melting point. Thus, this shift also occurs in purified DNA, where protein has less of a chance to be involved.

In the light of this problem, it is interesting to consider the action spectra of bacteriophages fd and ϕX-174. These are shown in Fig. 4. Many of the properties of the two phages are the same (Table I) so it is not surprising that the action spectra parallel each other between 2400 and 3020 A, and that the absolute values of the inactivation cross-sections are almost equal. The difference between the two viruses is largest below 2400 A, however, and this is reflected in the ratio of the inactivation cross-sections of fd at 2650 to 2250 A. As seen in Table IV, this ratio is 1.5 midway between 1.0, the value characteristic for single-stranded nucleic acid, and 2.0, the value characteristic for double-stranded nucleic acid. Despite the many similarities of fd and ϕX-174 they are very different in physical shape (see Table I). ϕX-174 is a sphere, while fd is rod-shaped. Preliminary x-ray diffraction patterns from partially orientated gels of fd can be interpreted in terms of a helical structure with a 60 A pitch and six repeating units per turn (8). If this helical structure is the nucleic acid, it may be in an "organized" state even though it is single-stranded. Such "organization" may be the explanation for the high value of R observed for fd, relative to the other single-stranded viruses (see Table IV).

The Role of Protein in Viral Sensitivity to UV. In Table IV it is seen that reovirus-3 has an unusually low value for R. By comparing the action spectrum for reovirus-3 (Fig. 3) with the action spectra of the other viruses it can be seen that below 2650 A the inactivation cross-section of reovirus-3, is increasing relatively more rapidly than the inactivation cross-sections for the other viruses. The action spectrum for herpes simplex, determined by Powell (27), is included in Fig. 3 since it shows a similar behavior. The "peculiar" shape of the action spectrum of reovirus-3 cannot be a property only of double-stranded RNA containing viruses since herpes simplex is a double-stranded DNA containing virus. To explain this phenomenom for herpes simplex, Powell has invoked an effect due to the protein of the virus. It is possible that in contrast to most of the other viruses the proteins of herpes simplex and reovirus-3 are relatively sensitive to UV light. This would cause an increase in their inactivation cross-section in the low wavelength region where the viral protein absorbs a larger fraction of the incident photons.

Another instance of protein affecting the viral sensitivity to UV light is found in

the U-1 strain of TMV which is ten times more resistant to UV light at 2537 A than the infectious RNA extracted from it (42). In this case the protective effect of the protein does not appear to be due to shielding of the nucleic acid and various hypotheses have been put forward to explain it (43).

From the above discussion it is seen that effects due to protein can be invoked to explain an increase or decrease in viral sensitivity below 2480 A. A rigorous test of these hypotheses would require the determination of the sensitivity of the infectious nucleic acid of the virus separated from its protein shell as was done for the U-1 strain of TMV (42).

Host Cell Effects. The ability of the host cell to modify UV damage in the infectious virus has been demonstrated in several different cases. For example, Jansz *et al.* (44) have shown that the factor of ten difference in the UV sensitivity of infectious ϕX-174 DNA and its replicative form (40), which is double-stranded, is due in large part to an ability of the host cell to reactivate double-stranded DNA to a higher level than single-stranded DNA. Therefore the difference in UV sensitivity between single- and double-stranded nucleic acid may not be due to differences in the initial damage put into it by UV light, but due to differences in the system used to detect this damage.

From Table III it is evident that even among viruses with the same stranded state and nucleic acid type, there are still large differences in the relative quantum yields. Some of these differences may be due to host cell effects. The quantum yield for inactivation of T-2 phage can be varied over a factor of two by means of photoreactivation (31). This process presumably involves the repair of UV lesions by enzymes supplied by the host cell (45). Recent work with T-1 phage has revealed that the survival level for irradiated phage is dependent on the genetic and physiological state of its host bacteria (46, 47). It is not unreasonable to assume that for the animal viruses, there may also be host-dependent repair mechanisms. These effects may be strictly host-dependent or may require both viral and host cell information (45).

The group of small RNA bacteriophages all have the same UV sensitivity as a function of wavelength. Preliminary tests in this laboratory with an early antiserum (11 days) produced by mice against MS2 indicate that R-17 is serologically related to it while fr and 7-S show little or no cross-reaction. It may be significant that four independently isolated phages, only two of which seem related, have the same quantum yield. This is presumably due to the similarity of their physical properties (Table I) and a uniform lack or presence of host cell interactions. This similarity in quantum yield is particularly interesting since the host for one of these viruses, 7-S, is different than for the other three. Also, none of the RNA phages showed significant photoreactivation (unpublished results).

Molecular UV Lesions and Viral Sensitivity. The quantum yields for the reduction of viral plaque-forming ability are in the range of 1 to 6 $\times$ 10^{-3} for the

viruses containing single-stranded nucleic acid and typically an order of magnitude or more lower for those containing double-stranded nucleic acid. However, the quantum yields for the formation of UV photoproducts of pyrimidines (48) are in the range 1×10^{-2} to 1×10^{-3}.

If one considers ϕX-174, it has a quantum yield for loss of its plaque-forming ability of 0.6×10^{-2} at 2537 A (Table II). Since half of its bases are UV-resistant purines (Table I) its measured quantum yield appears to be half what it would be if purines were sensitive to UV light. Therefore the quantum yield of ϕX-174 and that for alteration of a single pyrimidine base are similar. In the case of the other single-stranded nucleic acid viruses and the double-stranded nucleic acid viruses the number of altered bases required to inactivate the virus appears higher. As mentioned above this difference may be due to the existence of host cell repair for some viruses and not for others. Thus, the biological activity of the viral nucleic acids may not be sensitive to each UV lesion because the virus host cell complex is able to repair, remove, or bypass (49) a part of the damage to the genome.

SUMMARY

1. *The absolute quantum yields* for the viral plaque-forming ability of MS2, fr, fd, ϕX-174, and T-2 were determined over the range 2250 to 3020 A. The results for ϕX-174 and T-2 were in reasonable agreement with previous work.

2. *Relative quantum yields* determined for twelve viruses indicated that viruses with single-standed nucleic acid are about ten times more sensitive to UV than double-stranded viruses as measured by viral plaque-forming ability. This effect was observed for viruses containing either ribose or deoxyribose nucleic acid.

3. The ratio of the inactivation cross-sections at 2650 to 2250 A for ten of the twelve viruses was near 2.0 for the double-stranded viruses, and near 1.0 for the single-stranded type. This effect was independent of the form of the sugar in the nucleic acid.

4. Two of the viruses, fd and reovirus-3, behaved differently from the other viruses in regard to statement 3. This behavior may reflect a role of protein in effecting viral sensitivity to UV light.

5. Remaining differences in viral quantum yields may be due to host cell repair mechanisms.

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