A Comparison of Ultraviolet Action Spectra for Vaccinia Virus and T2 Bacteriophage

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The effect of monochromatic u.v. light on vaccinia virus was first studied by Rivers & Gates (1928) using irradiations at several wavelengths between 230 and 312 nm. Although their titration methods were understandably limited they found greatest activities at the wavelengths between 250 and 280 nm with a peak at 267 nm. A similar result was obtained by Rauth (1965), who included vaccinia virus in a comparison of action spectra of several animal and bacterial viruses. Numerous other reports of action spectra of viruses have been published, e.g. tobacco mosaic virus (Kleczkowski, 1963), bacteriophages T1 and T2 (Zelle & Hollaender, 1954) and influenza virus (Tamm & Fluke, 1950) and, in general, they too show peaks in the region of maximum nucleic acid absorption (260 to 265 nm).

However, a number of studies made with pox viruses irradiated with u.v. light from germicidal lamps have revealed a curious difference in the sensitivity of different early virus functions. Thus, while some early functions appear highly resistant, others seem almost as sensitive as infectivity itself (McAuslan, 1963; Jungwirth & Joklik, 1965; Woodson, 1967). These differences are difficult to reconcile with random damage in nucleic acid and have raised the possibility that u.v. light might act principally by inactivating a protein constituent of the particle (Joklik, 1966). Although the basis of the argument has been altered by the discovery of the particle-associated RNA polymerase (Kates & McAuslan, 1967), the difference in sensitivity of early functions remains unexplained and has prompted us to re-examine the action spectrum for the inactivation of vaccinia virus and to compare it with that of a T-even phage which has been widely accepted as typical of the 'nucleic acid type'.

A clone of the LISTER INSTITUTE strain of vaccinia virus was derived by three serial pock pickings on the chorioallantoic membrane. Stocks were grown on the chorioallantois, purified by the method of Joklik (1962) and stored frozen in replicate amounts at −70 °C. A typical preparation, that was used in all the determinations included in this report, had a titre of $1.2 \times 10^8$ p.f.u./ml, a particle count of $1.4 \times 10^{10}$/ml and a protein concentration of $0.125$ mg/ml.

The virus was dispersed by ultrasonic vibration before and after diluting 1/100 in 4 mM-phosphate buffer, pH 7.2, and 1 ml amounts irradiated in 6 cm diameter glass Petri dishes, with an average depth of fluid less than 0.5 mm.

The u.v. source was a high intensity xenon arc fitted with a diffraction grating monochromator (Bausch and Lomb catalogue Nos. 33-86-20-01 and 33-86-01, respectively). Exit and entrance slits were arranged to allow the wavelength under study to pass at full intensity with a band width of 9·6 nm. The light was reflected through 90° by a polished aluminium mirror to reach the sample, which was placed on a reciprocating platform at a total distance of 21·25 cm from the exit slit of the monochromator.

The monochromator was calibrated using a vacuum thermopile and given absolute values by repeated estimations at 250 nm employing potassium ferrioxalate actinometry (Hatchard & Parker, 1956). Incident energies ranged from 0.07 ergs mm$^{-2}$ s$^{-1}$ at 220 nm to 1.86 ergs mm$^{-2}$ s$^{-1}$ at 300 nm.

Inactivation curves were established for wavelengths at 5 nm intervals from 220 to 300 nm.
Each determination was based on five to seven experimental points, including that of the un-irradiated control, selected to cover the range of survival down to 10%. Multicomponent survival curves have been recorded using more prolonged irradiation from a germicidal lamp (Abel, 1962) and we have obtained a similar result with monochromatic light at 260 nm.

Virus infectivity was assayed on HeLa cell monolayers grown in 6 cm diameter glass Petri dishes. Incubation was at 35 °C, with a liquid medium overlay, and plaques were counted after 3 days.

Wild-type T2 bacteriophage was propagated and assayed on Escherichia coli strain B/s. Phage lysates were partly purified by successive centrifuging at low and high speed, the final pellet being suspended in 4 mM-phosphate buffer at a titre of 3.5 × 10⁹ p.f.u./ml (protein concentration 0.14 mg/ml). The phage was diluted 1/100 in buffer for irradiation and inactivation curves were established as for vaccinia virus.

At the relatively low doses examined here, the logarithm of the survival of both viruses was proportional to the dose, suggesting that inactivation resulted from a single hit at all the wavelengths studied. Best-fitting straight lines were calculated by the method of least squares and the doses required to inactivate to 37% survival (e⁻¹) derived from them. The action spectra in Fig. 1 are the reciprocals of these doses plotted against wavelength. Most points represent the means of two determinations, but four or more values were obtained at a number of wavelengths in the regions of peak inactivation for which the standard errors are shown in the Figs.

The action spectrum for vaccinia virus (Fig. 1A) has a broad peak from 255 to 275 nm. This may possibly be split into two separate peaks at 255 to 260 nm and at 270 nm, but the differences in this region are small and of doubtful significance. There is a much reduced sensitivity to wavelengths longer than 275 nm and a sharply increasing sensitivity to wavelengths shorter than 235 nm. The action spectrum for T2 bacteriophage (Fig. 1B) has two peaks, a major one at 260 to 270 nm and a secondary one at 280 nm, and a gradual rise at wavelengths below 230 nm. The two viruses are of approximately equal sensitivity to u.v. light in the region of 260 nm. Their action spectra both diverge in shape from a typical nucleic acid absorption spectrum, the discrepancy being particularly marked for vaccinia virus at 270 nm and for T2 at 280 nm. In the latter instance the sharp difference in values between the trough at 275 nm and the secondary peak at 280 nm may well have been underestimated because of the overlap inherent in irradiations at 5 nm intervals with a band width of 9-6 nm.

These action spectra may be compared with those previously published for these and similar viruses. The data for bacteriophages, converted where necessary to logarithmic plots of the inactivation cross section, are summarized in Fig. 2 and a corresponding treatment of the data for vaccinia virus in Fig. 3. Absolute inactivation doses cannot be obtained for the data of Rivers & Gates (1928) and their curve in Fig. 3 has been ‘normalized’ to our own action spectrum on the basis of the value at 280 nm. Our values for the absolute sensitivity of T2 bacteriophage and vaccinia virus clearly differ from those obtained by others. These differences may not be very meaningful because the values obtained depend upon many factors concerning the u.v. light source, the virus strain and preparation and the conditions of irradiation and assay. There is also a difference in the relative sensitivity of T2 bacteriophage and vaccinia virus in the two studies which have included these viruses. Whereas Rauth (1965) found T2 to be five times more sensitive than vaccinia virus, our values are intermediate and suggest a roughly equal sensitivity. We have also found this relationship in experiments with u.v. light from a germicidal lamp, the D₃₇ (e⁻¹) of T2 being 30 ergs mm⁻² and that for vaccinia virus 26 ergs mm⁻² (unpublished). The relative sensitivity of these
viruses cannot readily be predicted from the properties of their particles. Although the DNA of vaccinia virus is larger with a mol. wt. of $160 \times 10^6$, it lies within a substantial protein coat and constitutes only 5% of the particle. The smaller DNA of T2 has a mol. wt. of $120 \times 10^6$, but accounts for 54% of the particle. The DNA's are of similar base composition except for the substitution of 5-hydroxymethyl cytosine in T2. Differences in the u.v. light sources and their calibration cannot contribute to the difference in relative sensitivity found by Rauth (1965) and ourselves. The difference is therefore an indication of the wide variation which can result from the biological factors involved.

Of greater importance, then, is a comparison based upon the shapes of the different action spectra. Taking first those for bacteriophages (Fig. 2), the data of Zelle & Hollaender (1954) for T2 bacteriophage bear a remarkably constant relationship to our own and illustrate the importance of extra values between 260 and 280 nm in calling attention to the secondary peak at 280 nm.

The action spectra of Winkler, Johns & Kellenberger (1962) and Rauth (1965), although mutually consistent, are of a rather different shape with relatively higher values at 275 nm. In neither is there a suggestion of a second peak at 280 nm, but both lack data for the critical points at 280 and 285 nm. However, Franklin, Friedman & Setlow (1953) obtained an action spectrum for *Bacillus megatherium* phage M5 which had a secondary peak at 280 nm and a very similar shape to our spectrum for T2. It is of interest that an animal DNA virus,
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Fig. 2. A comparison of our action spectrum for T2 bacteriophage ($\Delta-\Delta$) with those published for T2 by Zelle & Hollaender (1954) ($\square-\square$), Rauth (1965) ($\bullet-\bullet$), and Winkler et al. (1962) ($\triangle-\triangle$), and for M5 by Franklin et al. (1953) ($\circ-\circ$).

Fig. 3. A comparison of our action spectrum for vaccinia virus ($\Delta-\Delta$) with those published by Rivers & Gates (1928) ($\square-\square$) and Rauth (1965) ($\circ-\circ$).
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Table 1. 260/280 ratios for extinction and inactivation

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>Extinction</th>
<th>Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single stranded DNA</td>
<td>Yarus &amp; Sinsheimer (1967)</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>(φX174)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein aldolase</td>
<td>Setlow &amp; Doyle (1957)</td>
<td>0.42</td>
<td>0.4*</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>Rivers &amp; Gates (1928)</td>
<td>--</td>
<td>1.6*</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>This paper</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>Rauth (1965)</td>
<td>--</td>
<td>2.38*</td>
</tr>
<tr>
<td>T2 bacteriophage</td>
<td>This paper</td>
<td>1.52</td>
<td>1.16</td>
</tr>
<tr>
<td>T2 bacteriophage</td>
<td>Zelle &amp; Hollaender (1954)</td>
<td>--</td>
<td>1.97*</td>
</tr>
<tr>
<td>T2 bacteriophage</td>
<td>Winkler et al. (1962)</td>
<td>1.50</td>
<td>1.67*</td>
</tr>
<tr>
<td>T2 bacteriophage</td>
<td>Rauth (1965)</td>
<td>--</td>
<td>1.8*</td>
</tr>
<tr>
<td>M5 bacteriophage</td>
<td>Franklin et al. (1953)</td>
<td>--</td>
<td>1.17*</td>
</tr>
</tbody>
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* Values obtained by interpolation where necessary.

herpes simplex virus, has recently also been shown to have an action spectrum with a secondary peak at 280 nm (Cameron, 1973). However, this clearly is not the case with vaccinia virus (Fig. 3). Although both of the previous action spectra for vaccinia have rather sharper peaks at 265 nm than we have found, all three agree in suggesting much reduced activity at 280 nm. The action spectrum of Rauth (1965) differs from ours in the greater reduction in inactivation at wavelengths above 256 nm and in the absence of any sharp rise at wavelengths below 235 nm. It is possible that these effects and perhaps also the lower sensitivity which he found for vaccinia virus might be explained by the shielding effects of protein, since it would appear that his virus preparations were derived from less purified stocks than our own.

Another way of looking at the data, which may be useful in distinguishing between effects in protein and nucleic acid, is to examine the ratios between inactivations at 260 and 280 nm and to compare these with extinction data (Table 1). Light scattering creates problems with these large viruses and our own extinction ratios, derived from measurements on purified virus preparations, owe much to correction which has been made both by the extrapolation method and the Rayleigh formula but they are similar to those of others (see Table 1 and Joklik, 1962) and appropriate to the known DNA and protein content of the virus particles. Because of the magnitude of the correction, particularly with vaccinia virus, we have not thought it justifiable to derive quantum yields. The 260/280 inactivation ratios for the different sets of phage data are of the order 1.1 except for the higher values of Winkler et al. (1962) and Rauth (1965), which both depend heavily on interpolated values at 280 nm. The low value of 1.1 is a reflection of the secondary peak at 280 nm and both features strongly implicate the involvement of absorption by protein as well as nucleic acid in the inactivation of T2 bacteriophage. This finding is important as it has been suggested (for discussion see Jagger, 1967) that T2 be used as a standard for the DNA type of inactivation with which other spectra should be compared when deciding if protein is involved. The situation with vaccinia virus in which the 260/280 inactivation ratio is greater than the 260/280 extinction ratio is in marked contrast to that of phage. Although the action spectrum of vaccinia virus does not exactly follow the curve of absorption in nucleic acid, the much higher value of 1.7 for the 260/280 inactivation ratio would appear to emphasize the importance of nucleic acid as the prime target for inactivation in this virus. Moreover, the detail of our action spectrum leaves little scope for the emergence of any major hidden peaks in further studies of this kind. Our findings do not help to explain the curious difference in sensitivity of early virus functions mentioned above and we are currently examining the effect of monochromatic u.v. light on
the individual functions themselves in an effort to resolve this phenomenon. The sharp rise that we have found in the action spectrum of vaccinia virus at wavelengths below 235 nm would suggest that breakage of peptide bonds is important in this region although this feature was not evident in the action spectrum of Rauth (1965).

A general point which may be made about the action spectra presented in this and an accompanying paper (Cameron, 1973) is that viruses may show secondary peaks more commonly than has been recognised and that absorption in protein may thus be more important in the inactivation of viruses by u.v. light than has been realized in the past. Hitherto, the example of B. megatherium phage M5 (Franklin et al. 1953) has been regarded as rather exceptional. Our experience suggests that secondary peaks may be easily overlooked if observations are not made at closely spaced wavelengths.

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REFERENCES


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