

Target Molecular Weight of Foot-and-Mouth Disease Virus and Poliovirus

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The use of ionizing radiation has given much information on the size and structure of virus particles (Ginoza, 1963; Contreras & Ohlbaum, 1968). The relation between virus particle size and sensitivity to inactivation by ionizing radiation was postulated by Lea (1940); later, the target theory was developed (Lea, 1946; Timofeeff-Ressovsky & Zimmer, 1947). The amount of genetic material in a virus can be determined by calculating the target molecular weight (Ginoza, 1967). Even though it is generally accepted that the RNA of picornaviruses has a molecular weight of about 2×10^6 (Allison & Burke, 1962), there is very little information about the molecular weight of the genome of many of them. The present study was undertaken to determine the size of the genome of foot-and-mouth disease virus by means of γ -irradiation. For the purpose of comparison, we irradiated simultaneously another enterovirus, poliovirus, under identical experimental conditions.

Foot-and-mouth disease virus, type O, strain 3, propagated in BHK cells was kindly given by the Bacteriological Institute of Chile. In our laboratory it was propagated for at least eight passages in primary cell monolayers, prepared from foetal pig kidney. The virus stock was kept at -20° and contained 10^7 p.f.u./ml. Foot-and-mouth disease virus was assayed in foetal pig kidney cells (Sellers, 1955). Poliovirus, type 1, BRUNHILDE strain, was propagated in HEp 2 cell monolayers where it reached a concentration of 10^7 to 10^8 p.f.u./ml. It was partially purified by ECTEOLA column chromatography (Levintow & Darnell, 1960), suspended in 0.02 M-phosphate buffer pH 7.4 and kept at -20° , at concentration of 10^9 p.f.u./ml. Poliovirus was assayed in HEp 2 cell monolayers following Hsiung's procedure (Grado, Fischer & Contreras, 1965).

The ^{137}Cs source of the Faculty of Physics and Mathematics of the University of Chile was used for the irradiation. The dose rate was 10^4 rad/hr, repeatedly checked by means of ferrous sulphate dosimetry (Weiss, Allen & Schwarz, 1956). The viruses used for irradiation were suspended in 10% horse serum in water, at a concentration of 10^6 to 10^7 p.f.u./ml. Adequate controls were kept at the same temperature of irradiation ($16^\circ \pm 1^\circ$) throughout the observation period.

Suspensions of foot-and-mouth disease virus were exposed to several doses of irradiation from 10^4 to 10^6 rads. The irradiation experiments were done with five samples of foot-and-mouth disease virus, resulting in 17 experimental values. There was a direct relation between irradiation dose, measured in rads, and the percentage of survival of foot-and-mouth virus infection (Table 1).

Poliovirus was irradiated similarly (Table 2). Eight virus suspensions were used; of these, three were purified samples, while the remainder were obtained from poliovirus-infected cells without further purification. Crude and purified virus preparations showed no differences in their response to irradiation. A total of 39 experimental points were obtained under these conditions.

Both viruses followed a single exponential curve of inactivation rate (Fig. 1). Foot-and-mouth disease virus had a D_{37} of 16×10^4 rads, while poliovirus had a D_{37} of 28.8×10^4 rads.

The target molecular weight of the nucleic acid was calculated according to the formula

of Ginoza (1967), on the assumption that none of the inactivation was due to the effects of radiolysis products from the suspending medium.

$$\text{Target molecular weight} = \frac{6.03 \times 10^{23}}{D_{37} \text{ rad} \times 10^{11} \times 9}$$

Applying this formula we found a target molecular weight of 4.18×10^6 daltons for the RNA of foot-and-mouth disease virus, and 2.23×10^6 daltons for poliovirus RNA.

We believe that the inactivation of virus activity observed in our experiments was due exclusively to the direct effect of the ionizing radiation, because the indirect effect was suppressed by the addition of 10% serum to the irradiation media. In previous work

Table 1. *Effect of γ -irradiation on the biological activity of foot-and-mouth disease virus*

Dose (rad $\times 10^{-4}$)	Residual infectivity (p.f.u./ml.)	Percentage survival
0	3.0×10^6	100.0
19.8	1.3×10^6	33.3
38.6	1.8×10^5	6.0
106.5	0.8×10^4	0.3

Table 2. *Effect of γ -irradiation on the biological activity of poliovirus*

Dose (rad $\times 10^{-4}$)	Residual infectivity (p.f.u./ml.)	Percentage survival
0	5.5×10^8	100
8	5.0×10^8	90
16	3.5×10^8	63
33	1.0×10^8	18
64	0.3×10^8	6

(Contreras & Ohlbaum, 1968), we showed that the addition of protein (5 mg./ml.) provided complete protection against the indirect effects of ionizing radiation, since the slope of the inactivation curve resulting from γ -irradiation of vaccinia virus was the same when the virus was irradiated in either the solid or the liquid state. Watson (1952) showed that 10% serum was equivalent to protein (5 mg./ml.) in affording complete protection against the indirect effect of irradiation of bacteriophages. It is also known that when the dose rate of irradiation is changed, the survival curve has the same slope, provided that the indirect effect is excluded. We obtained the same D_{37} when polio-virus was irradiated with higher doses, using a ^{67}Co source, suggesting again that the survival curve observed in our experiments was indeed due to the direct effect of γ -irradiation.

We have found no data in the literature on the direct effect of ionizing radiation on poliovirus. Our experiments yielded a D_{37} of 28.8×10^4 rads for poliovirus, equivalent to a target molecular weight of 2.23×10^6 daltons. This agrees well with the 2×10^6 daltons measured by electron microscopy (McGregor & Major, 1968).

Foot-and-mouth disease virus is a picornavirus presenting characteristics of both enteroviruses and rhinoviruses (Plummer, 1965). Although it shows many similarities with other enteroviruses it differs from them in some respects. It is remarkable that foot-and-mouth disease virus has a buoyant density of 1.43 g./cm.^3 in CsCl (Trautman & Breese, 1962); this value is similar to that of rhinovirus (Chapple & Harris, 1966; McGregor, Phillips & Major, 1966), in contrast to other acid-insensitive picornavirus which band in the range of 1.32 to 1.34 g./cm.^3 (Schaffer & Frommhagen, 1965). We found a D_{37} of 16×10^4 rads

for foot-and-mouth disease virus, which is less than the one reported by Polatnick & Bachrach (1968). With our irradiation data, we estimated that the RNA of foot-and-mouth disease virus had a molecular weight of about 4×10^6 . Brown, Newman & Steward (1963) found that the infective RNA of foot-and-mouth disease virus had a molecular weight of 3.1×10^6 . A similar molecular weight was found by Strohmaier & Mussgay (1959). The

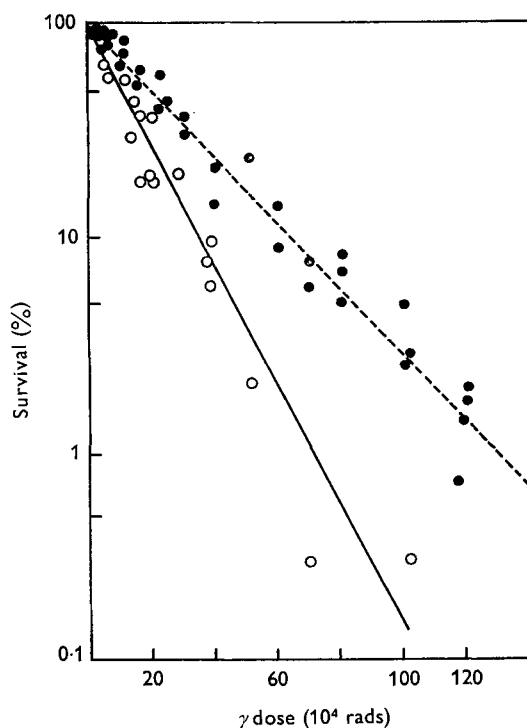


Fig. 1. Mean survival curves of foot-and-mouth disease virus and poliovirus after γ -irradiation. The curves were obtained from five experiments with foot-and-mouth disease virus (○—○), and eight with poliovirus (●—●).

demonstration that the RNA of foot-and-mouth disease virus is apparently larger than the RNA of other enteroviruses may explain some of its peculiarities, especially those related to its thermal and acid instabilities (Bachrach *et al.* 1957).

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