Estimation of scrapie nucleic acid MW from standard curves for virus sensitivity to ionizing radiation

IN an unusually rancorous communication to this forum[1], Alper protests Fig. 4 of my letter[2] in which I constructed standard curves for the inactivation of single-stranded (ss) or double-stranded (ds) viruses by ionizing radiation versus their nucleic acid molecular weights (MWs). Extrapolation of either line to the inactivation rate constant (D_{37}) of the scrapie virus gave virus-like values for the scrapie genome. This is in sharp contrast to values of 150,000 daltons or less calculated from the 'target theory' by Alper[3] and others[2].

In defending her target calculation[1], Alper denounces my use of previous reviews (as a source of data for constructing these curves, incorrectly), asserting that "some of the references are wrong" and that few of the references were checked. She gives the following four examples:

Yellow fever virus (YFV). Alper: "Molecular weight too low, about one-tenth of what we took from our independent source. Rohwer's source makes no mention of the MW of this virus." The YFV RNA has been sequenced and has a MW of 3.7×10^6 daltons[4], in good agreement with the value plotted in Fig. 4, 3.5×10^6 daltons (as given by) my source[5] (on page 18). The "independent source[6]" preferred by Alper is an ultrastructural study of intact YFV virions. Her value was apparently estimated from the virion core diameter.

Vaccinia. Alper: "D_{37} incorrectly quoted by Rohwer from K & M [Kaplan and Moses] who cited McCrea incorrectly." Kaplan and Moses[7] give a range of values for vaccinia obtained from multiple determinations by McCrea[8] and Wilson[9], both of whom they cite. I plotted the mean of the range. Alper ignores Wilson[9], selects the single value from McCrea[8] most favourable to her argument, which is also the only determination that appears to have been made under vacuum, and then objects to the use of vacuum.

Shope papilloma. Alper: "D_{37} plotted as too high to fit the Lea theory. K & M's source is Syverton *et al.*, who gave the dose for 'total inactivation'—an unknown multiple of the inactivation dose...". Indeed Syverton *et al.*[10] summarize their data in terms of total inactivation rather than inactivation rate constant. However, the rate constant is easily calculated from the data in experiments II–IV and this is the value cited by K & M and plotted in Fig. 4. The identical value was obtained by Lea *et al.*[11] in their analysis of the completely independent measurements of Friedewald and Anderson[12].

Newcastle[12] disease virus (NDV). Alper: "Inactivation dose too low... Rubin and Temin... irradiated the virus in suspension." NDV has also been irradiated dry[13], giving a rate constant even smaller than that plotted in Fig. 4. Most investigators, aware that viruses tolerate drying poorly, suspend viruses in broth, serum or tissue homogenates, either wet or frozen, to protect against indirect effects, and for this reason most of the available data have been obtained in suspension. While Alper insists that viruses be irradiated dry, she invokes this argument to discard only the particularly inconvenient NDV point from Fig. 4. If we apply her criteria consistently and consider only the five viruses irradiated dry in Fig. 4 (three from her own laboratory), both the line for the ss viruses and that for the ds viruses closely parallel the original regression lines in Fig. 4 predicting ds scrapie genome of MW 2.0×10^6 daltons or a ss genome of 0.86×10^6 daltons. These results are consistent with a larger study to be published separately (manuscript in preparation), in which each state, liquid, frozen and dry, was considered separately.

Using these four highly contrived 'examples', Alper alleges that the integrity of Fig. 4 is compromised by the use of previous reviews in documenting the data. *Nature*'s succinct format necessitated the use of reviews where possible and did not permit elaboration on individual data points in the two paragraphs of text devoted to Fig. 4.

Alper intimates that low-temperature inactivations may be in error. While it is true that some viruses[14], but not others[15], show enhanced protection at liquid nitrogen temperatures (−196 °C), the only frozen specimens in Fig. 4 were at dry ice temperatures (−78 °C), where the available evidence[15,16], including that for scrapie[17,18], suggests virus inactivation kinetics similar to those obtained dry.

Alper's most surprising suggestion is that ss and ds viruses (and even proteins) be regressed as a single group. This is in spite of overwhelming evidence that ss viruses show a 20–40-fold greater sensitivity to inactivation than do ds viruses of similar genome size[7,19–24]. This difference derives in part from the ease with which many otherwise lethal single-strand lesions can be repaired in ds DNA.

As noted by Alper, crude tissue suspensions of the scrapie agent show greater sensitivities to inactivation at ultraviolet wavelengths below the 254 nm maximum typical of some viruses[25]. However, a similar inactivation spectrum is observed for tobacco mosaic virus (TMV)[26] and, to a lesser extent, potato X virus[27]. The absence of other examples may only reflect the limited number of viruses for which an inactivation spectrum has been determined rather than the actual prevalence of this presumed anomaly in nature. It may be noteworthy that TMV and potato X share a filamentous morphology with the scraple-associated fibril[28], a candidate structure for the scrapie virus[29].

Standard curves, such as those in Fig. 4, are the preferred method of measurement in biophysical procedures such as sedimentation and electrophoresis where adequate standards exist. Now that D_{37} values can be calibrated against exact, sequence-based MWs for dozens of viruses[30], it no longer makes sense to attempt the estimation of nucleic acid MWs from first principles using the target theory. Moreover, past attempts to do so have seriously misled scrapie investigation.

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