

THE NUMBER OF PARTICLES OF *SERICESTHIS* IRIDESCENT VIRUS REQUIRED TO PRODUCE INFECTIONS OF *GALLERIA* LARVAE*

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For the majority of viruses, the ratio of total countable particles to infective units is more than 10 : 1. Reasons underlying these ratios are reviewed by Sharp (1965) and by Wildy and Watson (1962). Cheng (1961), Kaplan and Valentine (1959), and Overman and Tamm (1956) report infection ratios closer to unity, all using *in vitro* assays on cell suspensions or on chorioallantoic membrane. In this paper we shall show that *Sericesthis* iridescent virus (SIV) has high infectivity when assayed in the intact host.

A number of possible reasons have been advanced for the apparent requirement for a number of virus particles to produce infection:

- (1) Many viruses produce non-infectious particles, containing either no nucleic acid or an amount less than that present in the infectious particles.
- (2) Particles of some viruses tend to clump, and a group of particles may then give a single infection.
- (3) The assay system may be inefficient for any of a number of reasons.
- (4) Immunity mechanisms of the host may destroy or inhibit some otherwise infectious particles.

Day and Mercer (1964) reported that the total particle/infectious particle ratio of SIV was not greater than 10 : 1, but the experiments were based on tenfold dilutions, and the question has therefore been examined more closely.

Virus was prepared from infected *Galleria mellonella* larvae by centrifugation in buffer and in a sucrose density gradient (Day and Mercer 1964). SIV is unusually easy to purify, and no special precautions to overcome problems of contamination are necessary. Three different stocks of virus were prepared, and assayed for infectivity. The infectivity of a given stock of virus decreases with time (Table 1). The results in Table 1 refer to virus kept as a dilute preparation in a refrigerator, but the undiluted stock of virus also lost infectivity.

The concentration of each virus stock is found by mixing a sample (suitably diluted) with a solution of polystyrene latex particles of known concentration (Williams and Backus 1949). The ratio of the two kinds of particle is counted in electron micrographs of drops of the mixture sprayed onto an electron microscope grid. Assuming that the two kinds of particle are equally easy to count, it may be shown that the accuracy (for a given labour input) is greatest when the ratio is 1 : 1. The virus and latex samples were diluted accordingly. The latex particles (but not the

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virus) tended to stick together on the electron microscope grid, but statistical tests showed no abnormal variation in the virus : latex ratio from drop to drop, and so it appears that the virus and latex were well mixed when sprayed onto the grid. Light microscopy confirmed that the latex particles were not aggregated in solution. It proved helpful to add bovine serum albumin to the mixture to improve the spreading of the droplets. Enough particles were counted (about 3000 in each experiment) for the percentage standard error of the estimated viral concentration to be small compared with the error of the assay itself. The diameter of the latex particles (Dow Chemical Co. Lot LS-061A) was 3650 Å, and the electron micrographs were taken at a magnification of 2000 diameters (enlarged to 8000) in a Siemens Elmiskop I microscope. No metal shadowing or negative staining was necessary to enhance countability.

TABLE 1
DECREASE IN INFECTIVITY OF SIV DURING STORAGE
Infection of *Galleria* larvae by standard small dose of one stock
of SIV at successive dates

Date of Assay	No. of Larvae Inoculated	No. of Larvae Infected (%)
February 4	16	94
March 2	31	65
March 23	109	39
April 28	114	14

In assays of infectivity, 8.3 µl of suitably diluted virus stock was injected into each *Galleria* larva by micropipette. Larvae were then incubated for 3 weeks at 22°C and scored for infection. It may be shown that infectivity is most accurately estimated when each dose contains approximately 1.6 infective particles on average (probability of infection = 0.8), but that doses containing between 0.8 and 2.0 infections are nearly as informative. The assay dilutions were adjusted to cover this range of dose. About 10% of larvae died from causes other than virus infection. They would not greatly alter the results even if all were counted either as "infected" or as "not infected".

The average numbers of virus particles from three different stocks of virus required to infect one *Galleria* larva were 1.7 (± 0.21), 6.9 (± 0.90), and 3.9 (± 0.52). These values were estimated by "maximum likelihood", and standard errors of each determination are given in brackets. It is not entirely satisfactory to quote standard errors, since the distribution of errors is asymmetric. But a variance ratio test, performed on the logarithmic scale, shows that the three stocks of virus had different infectivities; so the standard errors merely indicate the accuracy of each individual assay. The reasons for the differences in infectivity between stocks, and for the loss of infectivity on storage, are unknown.

The results show that SIV is more infective in whole animals than any other virus assayed previously. Although capable of attacking species of Coleoptera, Diptera, and Lepidoptera (Day and Mercer 1964), the virus has been found wild only in larvae of one beetle species. It thus seems likely that *Galleria mellonella* has no immunity mechanism against SIV. SIV has two other characteristics which may contribute to its infectiousness. In the electron micrographs, the virus does not aggregate, and particles lacking a central core are rare. In assays of infectivity of SIV in insect tissue culture cells (Bellett 1965) the virus showed lower specific activity than that described above.

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