MULTIPICITY REACTIVATION OF NEWCASTLE DISEASE VIRUS

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ABSTRACT

Drake, John W. (University of Illinois, Urbana). Multiplicity reactivation of Newcastle disease virus. J. Bacteriol. 84:352-356. 1962.—A very weak multiplicity reactivation occurs among Newcastle disease virus particles inactivated by ultraviolet irradiation. The preliminary steps in the infection of embryonic chicken fibroblasts are complex, and their relation to multiplicity reactivation is discussed.

Multiplicity reactivation (MR) has been demonstrated to occur to a very limited extent among ultraviolet (UV) irradiated polioviruses (Drake, 1958), and has been invoked to explain the apparent reactivation of irradiated influenza viruses (Henle and Liu, 1951; Kilbourne, 1957; Barry, 1961). The demonstration of MR in influenza viruses has never satisfied the strict test of measuring an excess of virus-yielding cells over the input multiplicity of UV survivors, but its postulated existence is supported by cross-reactivation studies (Appleby, 1952; Baron and Jensen, 1955; Gotleib and Hirst, 1956; Kilbourne and Murphy, 1960; Simpson and Hirst, 1961). MR has been claimed (Barry, 1962) not to occur with Newcastle disease virus (NDV), and in initial experiments we also found it very difficult to demonstrate. However, NDV exhibits anomalies of adsorption and penetration which may interfere with the measurement of viral interactions. Under certain conditions, a small amount of MR can be demonstrated.

MATERIALS AND METHODS

Virus and cell strains and virus assay. The L-Kansas 1948 strain of NDV was used unless otherwise indicated. The growth of cells and the virus assay have been described (Drake and Lay, 1962). Virus was assayed by plaque formation on embryonic-chicken monolayers, and fractions of infected cells were assayed by plating optically counted cells for plaque formation. As with the poliovirus system (Drake, 1958), the free virus titer was one-half to one-third that obtained when washed suspended cells were infected, adsorption was measured by plating free virus before and after adsorption, and the cells were assayed for plaque-forming ability. This latter titer was always used in calculations of the multiplicity. The fraction of infected cells was normalized to the fraction of cells (60 to 90%) capable of forming plaques after infection at large multiplicities. Control experiments demonstrated that adsorbed virus did not elute from cells to a significant extent.

UV irradiation. Irradiation was performed with a Champion 15-w germicidal bulb. Although diluents absorbing in the 260-m region were used, the dose rate was held uniform by irradiating layers less than 2 mm deep and by agitation. The virus was inactivated exponentially to survivals of at least 10-6, so long as multiple infection did not occur on the assay plates. Upwards deviations occurred at large UV doses, possibly because of MR on the plate (see also Gotleib and Hirst, 1956). Surviving fractions were measured by assay of free virus, since preliminary measurements produced identical survival ratios by assaying either free virus or cells infected at low multiplicities.

RESULTS

Infection of cells by unirradiated virus. At low multiplicities, the normalized fraction of infected cells was strictly equal to the adsorbed NDV multiplicity (Fig. 1). At multiplicities approaching and exceeding one, however, two anomalies tended to arise. First, the fraction of virus adsorbing to the cells decreased as the multiplicity increased (Fig. 2). Second, the fractions of infected cells deviated from those expected from the adsorbed multiplicities. With random adsorption to a uniform population of cells, the fraction of infected cells I is related to the multiplicity m by log (I - I) = -0.4 m. When the cells are heterogeneous in their ability
to adsorb virus particles, an upwards deviation occurs on a plot of log \((1 - I)\) vs. \(m\) (Drake, 1958). When chicken fibroblasts adsorbed NDV with a short average time interval between successive virus particles, the anticipated upwards deviation was seen (Fig. 3, top line). However, when more time elapsed on the average between successive “adsorptions” to a given cell, a downwards deviation occurred (Fig. 3, circles).

**Fig. 1.** NDV infection of chicken fibroblasts at low multiplicities. Circles and squares: two different experiments.

**Multiplicity reactivation.** When UV-irradiated NDV was adsorbed slowly to cells, multiplicities above one could be achieved, but the fraction of plaque-forming cells was generally equal to or less than the adsorbed multiplicity of UV survivors. Occasionally it was higher. When adsorption was made more rapid by using a high cell density, an excess of yielders over the input of UV survivors usually resulted (Fig. 4). This excess was not observed below multiplicities of...
FIG. 2. Relation between input multiplicity and adsorption. Circles and squares as in Fig. 3.

FIG. 3. NDV infection of chicken fibroblasts at multiplicities near one. Solid line: expected fraction of uninfected cells with random adsorption. Dashed line (squares): rapid adsorption, cell density $2 \times 10^6$/ml in adsorption tube. Circles: slow adsorption, cell density $5 \times 10^6$/ml in adsorption tube.
approximately 0.2, and it also tended to disappear at multiplicities above approximately 4. The efficiency of MR (the excess of yielders at a given multiplicity) was equal for virus irradiated to survivals between $10^{-4}$ and $10^{-3}$.

Virus purified by adsorption and elution on chicken red blood cells and by differential centrifugation was as susceptible to MR as was a fresh allantoic-fluid stock. Attempts to further increase the level of MR by adsorbing in media of high or low tonicity, treating infected cells with antiserum to remove slowly penetrating virus, or allowing adsorption to proceed at 4°C and inducing synchronous penetration at 37°C (Marcus, 1959), proved ineffective. The Hik strain and a heat-resistant Beaudette strain (Granoff, 1959a) were no more efficient in MR than the L-Kansas strain. The L-Kansas strain can be separated by adsorption and elution on chicken red blood cells into fast-adsorbing and slow-adsorbing fractions (unpublished data). The fast-adsorbing fraction exhibited no more MR than did the parental virus.

**DISCUSSION**

NDV has to date displayed a paucity of genetic interactions. Attempts to demonstrate genetic recombination (Granoff, 1959a) and cross-reactivation (with influenza virus: Simpson and Hirst, 1961) have been unsuccessful. Barry (1962) reported the absence of MR, but his measurements would not have clearly revealed the small amounts reported here. (His Table 1 in fact suggests an excess of yielders amounting to 40 to 60%.)

This situation may be understood, at least in part, in terms of the early steps of infection by NDV. A very rapid homologous interference operates by blocking superinfection (Baluda, 1959). This presumably accounts for the observation that adsorption is multiplicity-limited (Fig. 2), and for the excess of yielders with unirradiated virus compared to the expectations from random adsorption (Fig. 3). Except under conditions of rapid adsorption, multiple infection will not easily occur, and MR will be suppressed. At higher multiplicities, even the input of UV survivors will tend to be excluded. An antagonism between homologous interference and MR has also been reported with influenza virus (Kibbourn, 1957).

Even when multiple infection has occurred, only a fraction of the adsorbed virus may proceed to successive steps of infection. Thus, although adsorbed unirradiated NDV becomes resistant to antiserum in only a few minutes, interference by irradiated virus is reversible for up to 30 min (Baluda, 1959). HeLa cells multiply infected by unirradiated NDV may even survive as colony-formers (Marcus, 1959). If only a fraction of the adsorbed virus particles can interact, it is possible that NDV is much more efficient in MR than the present data indicate.

Certain kinds of interactions do occur readily in cells infected with NDV. Phenotypic mixing and heterozygote formation have been demonstrated
(Granoff, 1959b) under conditions where adsorption was apparently not multiplicity-limited (Granoff, 1959a). Attempts to measure MR under Granoff's conditions were limited by technical difficulties, however.

As with MR in polioviruses (Drake, 1958), no choice is yet possible between a genetic and a nongenetic mechanism. Although MR in bacteriophages is closely associated with recombination (Epstein, 1958), a possibly nongenetic reactivation occurs in heated poxviruses (Joklik, Holmes, and Briggs, 1960), and normally nonplaque-forming NDV particles may be reactivated without apparent recombination under conditions of multiple infection (Granoff, 1961).

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LITERATURE CITED


