*MULTIPLICITY ACTIVATION OF VACCINIA VIRUS IN L CELLS**

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Demonstration of multiplicity reactivation (MR) of irradiated or otherwise damaged vaccinia virus has been made by two methods. The first and oldest of these was devised by Luria,[1] who worked with the coliphages. It consists in showing that as input multiplicity is increased and made to approach one vaccinia virus particle (VP) per cell (and substantial numbers of cells receive two or more) a sharp increase in infectivity is observed.[2, 3] A second method makes use of aggregated virus particles at very low *average* input multiplicity. Multiplicity reactivation occurs when the host cells receive clumps of virus particles.[4] This MR tends to disappear when the virus particles are dispersed. Analysis of clump size and frequency by electron microscopy has supplied a means for quantitation of this MR as a function of the physical state of the VP.[5–10] This has provided a highly sensitive means of detecting the superiority of clumps over individual irradiated virus particles as plaque-forming units (PFU). Evidence has now appeared that MR-like behavior is not limited to irradiated or otherwise partially inactivated virus. This note will describe a multiplicity activation (MA) of some fresh preparations of early-passage vaccinia virus.

Vaccinia virus WR (mouse neurotropic) strain obtained from the American Type Culture Collection has been maintained in this laboratory beyond 300 passages in Earle's L cells. Culture methods, media, and plaque assay procedure have been previously described.[6] Although the virus grows poorly in the first few passages, the yield usually exceeds 1000 VP per cell at 16th passage and 3000 VP per cell at 24th passage. Plaquing efficiency or quality, however, improved much more slowly, eventually reaching a maximum of about 1 plaque-forming unit (PFU) for each 10 VP at about the 50th passage and continuing at this level thereafter.

Experiments and Results.—If the course of this adaptive process is broken, as it was in these experiments, by a single passage of the virus in rabbit kidney (RK) cells, a dramatic change is observed in the ability of the progeny virus to produce plaques on L cells. Growth of the 15th-passage L cell virus on its first passage in RK cells was excellent, yielding 3000 to 5000 VP (average) per cell of the inoculated monolayer culture. Plaque production, however, was about 50 PFU per 100,000 VP, whereas that of the 15th L cell passage virus that was used for inoculum was ten times greater. This initial observation was followed by a series of repeated titrations of the progeny virus in various states of aggregation. A critical dependence of plaque titer on aggregation was observed (Fig. 1). This was apparent (Table 1) not only as a decrease in PFU when aggregated preparations were dispersed but also as an increase when reaggregation was induced by pelleting the virus in a centrifuge and gently resuspending it. These reversible changes in PFU/ml (Table 1, column 2) are the small observable result of two strong but opposing influences. The first of these is the superior plaque-forming potency of aggregates and the second is the reduced number of suspended or active units of virus that

remain to form plaques. This number of potentially active units (AU) is just the total of single particles, pairs, triplets, etc. that is observed in electron micrographs prepared by sedimenting a dilute suspension of the virus upon agar and analyzing pictures of pseudoreplicas from the agar surface.[6] When the efficiency of plaque production (PFU/AU) is observed (Fig. 1 and Table 1, column 3), the changes wrought by aggregation emerge more clearly and they are seen to be about 20-fold.

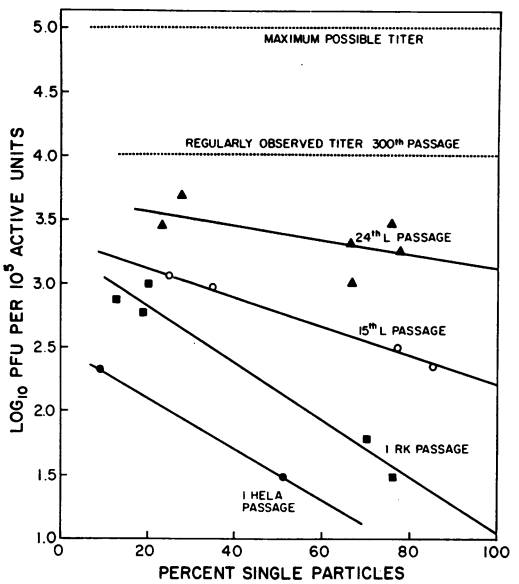

FIG. 1.—The plaquing efficiency on L cells of early passage (15th on L cell) vaccinia virus is dependent on aggregation of the particles whereas that of well-adapted virus is not. Intermediate (24th L) passage virus is less dependent but 15th L passed once in either RK or HeLa cells shows maximum sensitivity to aggregation or capacity for multiplicity activation (MA).

TABLE 1

Physical state	Per cent single particles	PFU per ml	PFU per AU
Aggregated	13	11,000	733
Dispersed	76	2,700	31
Reaggregated	19	12,000	600

Virus particle aggregation increases the plaque titer of vaccinia virus that has been passed once in RK cells. This increase is reversible and the plaquing efficiency (PFU per suspended or active unit, AU) may be altered more than 20-fold by changing the degree of aggregation of this virus.

The nature of the aggregation among virus particles recovered from infected RK cells was found (Fig. 2) to be similar in both type and magnitude to that previously shown for virus from L cells.[6] When the frequency (N) of the groups of size i is plotted log-log fashion against i, the groups fall quite well upon a straight line.

Repeated demonstrations have shown that well-established (200th-passage) L cell virus yields a constant value of PFU/AU over a wide range of aggregation (Fig. 1). Previous work has shown only irradiated virus departing from this behavior, pre-

sumably because of MR. We have therefore investigated early-passage L cell virus and found to our surprise (Fig. 1) that the plaquing quality of 15th-passage virus, just prior to its passage in RK cells, was somewhat dependent upon aggregation and that even 24th-passage virus showed a trace of this dependence, which apparently decreases with continued passage.

Passage of 15th-passage L cell virus once in monolayer cultures of HeLa cells produced virus particles in numbers just as great as regularly observed from L or in RK cells, as just described, but the plaque efficiency of their progeny on L cells was even lower than those from RK cells and a similar aggregation-dependence was observed although precise determination of the slope (Fig. 1) is doubtful because of the extremely low efficiency (3 PFU per 100,000 VP) indicated for single particles.

Passage in RK cells of established (200th-passage) L cell virus yielded normal numbers of virus particles (3000 per cell) and these progeny were just as efficient in plaque production on L cell monolayers as the starting virus.

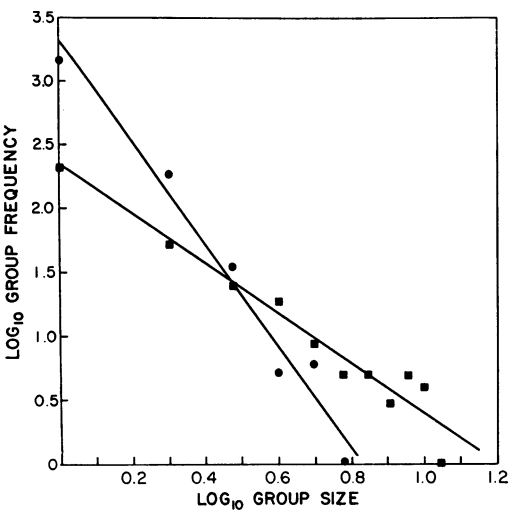

FIG. 2.—The nature of the virus particle aggregation in the experiments of Fig. 1 and Table 1 is shown here as frequency of the various group sizes seen in electron micrographs of particles sedimented, from dilute suspension, upon an agar surface. Squares and circles represent preparations containing 28 and 84% single particles, respectively.

Discussion.—These experiments show that the plaquing efficiency of early L cell passage vaccinia virus is not only low but sensitive to particle aggregation, in the same manner as virus whose infectivity has been reduced by irradiation[6, 8, 9] or by treatment with nitrogen mustard[7] or formaldehyde.[10] The observed MA gives evidence that some, perhaps all, of the particles can cooperate in the act of plaque formation, presumably in the act of infecting an L cell. These particles, or at least these populations, show a deficiency not observed in well-adapted virus, a deficiency that is gradually reduced by continued passage in L cells but which is sharply increased by one passage in RK or HeLa cells. The nature of the deficiency is not

known except that it resembles that of irradiated virus which exhibits MR for which the recombination concept of Luria[1] and the uncoating protein concept of Joklik[2] may prove to be the explanation.

The deficiency revealed here is probably not what limits plaque production to 1 in 10 VP of well-adapted fresh preparations on L cell monolayers. It is as though every one of the well-adapted VP is capable and infection is limited only by the cell culture which denies entry to 9 out of 10 of the VP on some nondiscriminatory basis. Perhaps VP can be uncoated only in certain ports of entry constituting only 1 out of 10 of those available. Both early-passage virus and irradiated virus are probably subject to this same kind of limitation but in addition they suffer from imperfection, part of which may be compensated for by MR or MA. Thus their low quality is the product of two factors, only one of which is a virus property and thus subject to change through the selective pressure of further cell passage.

The practical importance of MA is evident to anyone making plaque titrations in order to determine virus quantity or passing virus in different cells. Not only is the plaque titer subject to wide variations depending on the physical state of the virus, but passage of aggregated virus, even at great dilution, will produce progeny largely through multiply infected cells. At present, only particle count and aggregation analysis by electron microscopy can provide data on the apparently critical property of a virus particle suspension—its physical state at the time of encounter with host cells.

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