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QUALITY CHANGES IN VACCINIA VIRUS DURING ADAPTATION TO GROWTH IN CULTURES OF EARLE'S L CELLS

D. G. SHARP, P. SADHUKHAN,[1] AND G. J. GALASSO

Biophysics Laboratory, Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina

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ABSTRACT

SHARP, D. G. (University of North Carolina School of Medicine, Chapel Hill), P. SADHUKHAN, AND G. J. GALASSO. Quality changes in vaccinia virus during adaptation to growth in cultures of Earle's L cells. J. Bacteriol. 88:309-312. 1964.—Although particle production improves rapidly with passage of vaccinia (mouse neurotropic strain) virus in L cells, plaque production improves much more slowly. Beyond the passage when maximal particle yield was reached (18th), further improvement in plaquing quality of 1,000-fold was observed. Particle count and plaque titrations showed that plaquing efficiency improves at comparable rates in either plaque-to-plaque passage on plates or in tube cultures inoculated at high multiplicity. The host cell is probably exerting some influence in addition to that of the selection of mutants from the initial population.

The adaptive process of a virus in a new host is often characterized by an initial period of poor growth followed by an increase in virus production which tends to become stable with continued passage. Frequently, such an adapted virus is found to differ qualitatively from the starting material. A measurement of this change can be obtained by making titrations and virus particle counts by electron microscopy, at several stages in the adaptive process. The ratio of these two numbers gives the specific infectivity or quality of the virus at each step. Some initial data of this kind for vaccinia virus in animals was published by Overman and Sharp (1959).

The present work reveals changes in both quantity and quality of vaccinia virus during its adaptation to growth in cultures of L cells. Our purpose was to investigate, in a single series of experiments, some scattered observations made during a long series of passages (330) of WR (mouse neurotropic strain) virus in L cells. Eighth-passage virus was found to yield about 1,000 new virus particles per infected L cell (Smith and Sharp, 1960), but it produced no plaques upon monolayer cultures of L cells prepared for titration. Later (18th) passage virus produced 3,000 to 9,000 virus particles per infected cell (Smith and Sharp, 1961). The 18th passage was the first from which the virus was capable of producing reliable plaque titrations and, thus, the first point in the passage series at which quality measurements could be made. This 18th-passage virus was used as the starting point for this work.

[1] National Institute of Health Postdoctorate trainee whose experimental results are the basis of this paper, on leave of absence from the Biophysics Division, Saha Institute of Nuclear Physics, Calcutta, India.

MATERIALS AND METHODS

Virus. The vaccinia virus, WR (mouse neurotropic) strain, was obtained from the American Type Culture Collection. It has been passed 330 times in L cells with samples of various passages preserved at −62 C.

L cells. The L cell culture was obtained through the kindness of Wilton Earle of the National Cancer Institute. These cells have been kept in continuous passage in a nutrient medium consisting of Hanks balanced salt solution with 16.6% horse serum and 0.15% Yeastolate (Difco). The medium contained 400 units of penicillin and 40 μg of streptomycin per ml. Cells were grown in screw-capped tubes (16 by 127 mm), incubated slantwise, and in 2-oz stoppered prescription bottles. Cell suspensions were obtained by scraping the cells from the glass surface and agitating them by rapid pipetting. Counts were made in a hemocytometer.

Virus particle counting. The agar sedimentation and electron microscopic method of counting

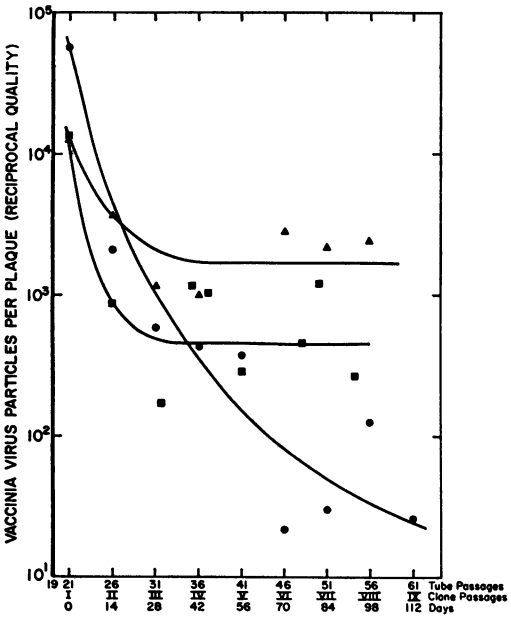

FIG. 1. *Vaccinia virus quality, shown in reciprocal form as the logarithm of the number of particles per plaque, plotted as a function of passage number. Two plaques were selected from the 18th-passage starting virus. Virus from plaque A was passed TT (■) and PTP (▲). Virus from plaque B was passed PTP (●).*

virus particles (Sharp, 1960) was employed to determine the number of virus particles per ml of the original suspension and to measure the virus yield in all samples afterward. Each count datum quoted was calculated from the average of at least five electron micrographs which usually contained 100 to 300 virus particles each. Such counts have been shown to be repeatable with a standard deviation of 15% of the mean or better.

Plaque titration. The procedure employed here for plaque titration of the virus on cell monolayers was described previously (Galasso and Sharp, 1962). One point only, in the plaque technique, was different from that previously used; plaques were prepared in 50-mm Pyrex petri dishes rather than in 1-oz prescription bottles. Briefly, monolayers containing about 5 million cells were prepared in petri dishes, inoculated with 0.2 ml of growth medium containing the virus, and incubated 3 hr at 37 C, with periodic tilting, in a humidified incubator with a 5% CO_2 atmosphere. The first overlay was then applied. After 3 days, a second overlay containing neutral red was added. Plaques were counted on the fifth day of incubation throughout.

Passage procedure. Frozen 18th-passage virus was thawed, dispersed with 9-kc sonic waves, counted, and used to inoculate test-tube cultures. These were prepared in advance by placing 5×10^5 L cells in 1 ml of growth medium in screw-capped test tubes (16 by 127 mm) and incubating slantwise for 2 to 3 days at 37 C. The cultures then contained about 10^6 cells, and virus inocula containing 5×10^7 to 10×10^7 particles were used. [This is very much less than one plaque-forming unit (PFU) per cell.]

After incubation for 48 hr, the infected cells were scraped from the glass and treated with sonic waves to release and disperse the virus particles. This 19th-tube passage virus was spread thinly upon L-cell monolayers for isolated plaque development. Two well-separated plaques (A and B) were punched out of the plates with thin-walled stainless-steel tubing (0.32 cm in diameter). These small agar plugs were transferred to 1 ml each of fresh growth medium and treated with sonic waves to dislodge infected cells and to disperse the virus. Two more tube passages were made with both A and B virus. This passage-21 virus was counted and titrated to give the first two data which appear on Fig. 1. Beyond this point, two different passage procedures were followed. In the first, the virus was passed from tube to tube (TT) at 2- to 3-day intervals, with particle counts and plaque titrations every fifth passage. The second procedure consisted of a series of four tube passages followed by a plaque titration. Virus from one of these isolated plaques was then put through four more tube passages, and the process was repeated eight times. This will be called plaque-tube-plaque (PTP) passage.

RESULTS

The virus from plaque A was passed 35 times TT, with plaque titrations and particle counts yielding the ratios shown by the squares on Fig. 1. These data show a 40-fold improvement in plaquing efficiency or quality of the virus in the first 10 passages but essentially no further change in the next 25 passages. The virus seemed to have reached a steady level of 400 particles per plaque.

Passage of the same (plaque A) virus PTP indicated a tenfold improvement in plaquing quality during the first 10 passages, but the average or level of the plateau established by the

data from the last 26 passages was 2,000 particles per plaque (triangles on Fig. 1). Although there is substantial scatter among the points, the fivefold difference between the final average level for PTP and TT passage virus is clearly significant.

Passage of the virus from plaque B began at a quality of one plaque per 50,000 particles. After a total of 40 passages PTP, including 8 plaque passages, the quality was improved to about 1 plaque per 20 particles. The curve of these data (circles, Fig. 1) suggests that further improvement, perhaps reaching the value of 1:10 previously reached in a much longer set of TT passages (Galasso and Sharp, 1963), might be attained.

Passage of plaque A virus was also made directly from plaque to plaque (PP) without intermediate tube passage. Under these conditions, there was not enough virus available, at each passage, for count by electron microscopy. After five such passages, the virus was put in tube culture for increase to counting level. Titration and particle count data from three such experiments showed the final virus quality to be 1:280, 1:140, and 1:190. The average, about one plaque for 200 particles compared with 1:17,000 for the starting virus, represents an 85-fold improvement. These data indicate an improvement in plaquing quality somewhat greater than that produced in five TT passages in the first experiment above.

Virus taken from the plaque passage III of the PTP series on virus from plaque A produced about 1 plaque per 1,000 particles (Fig. 1). This was passed PP in three sets of five plates and then grown out in tubes for particle count and titration. None of these produced any further improvement in virus quality.

DISCUSSION

Passage of the mouse neurotropic strain of vaccinia virus in tube cultures of Earle's L cells produced a population of particles that grows to high yield in these cells. At 18th passage, the earliest at which plaque formation was sufficient for titration purposes, the yield of virus per cell of infected cultures was already maximal. No significant improvement in yield was observed through 300 additional passages, but the plaquing efficiency of the virus improved over 1,000-fold.

The experimental evidence presented here shows something of the progress of these changes in virus quality. Possibly the initial difference in quality of virus from plaques A and B was the result of chance selection from a genetically inhomogeneous population (19th tube passage) of virus. This may account also for the observation that the two virus populations showed different capabilities for subsequent improvement with continued passage. Improvement was observed, however, with either tube or plaque passage of virus from either starting sample. These are not special cases, but demonstrations of the change that we observed many times in less complete experiments with this virus in L cells.

According to Fenner and Cairns (1959), the adaptation of a virus to a new situation must be pictured as the product of a single change or succession of changes augmented by intense selection pressure. Presumably, the first 18 tube passages in L cells exerted sufficient pressure to effect the selection, from the whole population, of high-yielding virus in tube cultures inoculated at a particle-to-cell multiplicity of 10:20. It may be argued also that, among these, the potentiality for plaque development is a rather broadly peaked distribution which yields more slowly to the selection process and thus continues to improve, with TT passage, after quantity of particle production has reached a maximum. Some features of the results, however, are difficult to explain on a basis of selection alone. Although several PP passages, beginning at passage III or plaque A virus, made no further change and seemed to indicate uniformity (as confirmed by the horizontal lines of Fig. 1 for continued tube passage), there is other evidence that virus selected repeatedly by plaque propagation is still capable of further quality improvement by further tube passage. Thus, the virus from plaque B, although repeatedly selected from well-separated plaques, shows continued improvement through several such steps. Also, three sets of five PP passages of plaque A virus beginning at quality 1:17,000 showed quality improvement comparable with that obtained with five TT passages of the same virus. We are aware of the possibility that PP passage of low-quality virus may carry a few infectious but nonplaque-forming particles with the population; however, we would have expected the preponderance of progeny from plaque-forming particles at each step to quickly eliminate the possibility of further improvement in quality by the presumably less selective TT passage, unless quality improvement is wrought within the L

cell by means other than selection of existing mutants alone.

ACKNOWLEDGMENT

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