**Virus Development in Enucleate Cells: Echovirus, Poliovirus, Pseudorabies Virus, Reovirus, Respiratory Syncytial Virus and Semliki Forest Virus**

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**SUMMARY**

A group of RNA viruses, echovirus, poliovirus, reovirus, respiratory syncytial virus and Semliki Forest virus have been examined for ability to grow in enucleate African green monkey kidney (BSC1) cells. Semliki Forest virus produced an almost normal yield of virus but poliovirus, echovirus, reovirus and respiratory syncytial virus, although showing clear evidence of virus replication when compared with a nuclear DNA virus (pseudorabies virus) gave much lower yields than those from nucleate cells. Analysis of enucleate cells infected with echovirus and reovirus showed no evidence of a specific block in the synthesis of any virus-specified polypeptide. Infection with vesicular stomatitis virus at intervals after enucleation demonstrated a diminishing ability to support virus growth with increasing time. It is suggested that the yield of virus obtained from an enucleate cell is related to the length of the growth cycle of the virus, the reduced yield obtained with some viruses reflecting the declining ability of the enucleate cell to support virus growth.

**INTRODUCTION**

Until recently our knowledge of the role of the nucleus in the replication cycle of certain RNA viruses has been obtained from experiments involving inhibitors of known nuclear functions such as DNA transcription and replication. With some viruses, e.g. vesicular stomatitis virus (VSV) and Semliki Forest virus (SFV), virus growth can proceed normally in high concentrations of inhibitors of DNA transcription and replication (Taylor, 1965; Black & Brown, 1968). With other viruses such as reovirus there is clear evidence that high concentrations of an inhibitor such as actinomycin D can affect virus growth as well as blocking nuclear function (Shatkin, 1965). In such systems it is thus impossible to block completely a nuclear function without affecting virus growth; information on the role of the nucleus is usually inferred from the results obtained with sub-optimal concentrations of inhibitor where virus growth is unaffected but the nuclear function remains operative, albeit at a low level.

Recently, an alternative approach to the problem of examining the role of the nucleus in certain virus infections has been made possible by the development of techniques to enucleate cells using the drug cytochalasin B (Carter, 1967; Prescott, Myerson & Wallace, 1972). In such cells it is possible to test whether the growth of a particular virus is dependent on the physical presence of a nucleus.
In previous studies it has been shown that the growth of VSV is completely independent of the presence of a nucleus, that influenza viruses will not replicate in an enucleate cell and that they require a nucleate cell before the synthesis of any virus-specific products can be initiated, and that vaccinia virus will produce all normal virus-specific products in enucleate cells but in reduced yield (Follett et al. 1974; Kelly, Avery & Dimmock, 1974; Pennington & Follett, 1974). It was therefore of considerable interest to examine the growth potential of enucleate cells for other RNA viruses.

In this paper we report our findings for representative viruses of the main categories of RNA viruses: the picornaviruses – echovirus type 1 and poliovirus type 3; the group A arbovirus – Semliki Forest virus; the diplomavirus – reovirus type 3; and respiratory syncytial virus, an enveloped RNA virus, provisionally classified as a metamyxovirus. We have also examined two additional rhabdoviruses; Mokola virus representing the rabies virus subgroup and Klamath virus, a rodent virus unrelated to VSV or rabies virus. The nuclear DNA virus, pseudorabies virus, has been used to determine the residual amount of virus synthesis due to the small proportion of nucleate cells surviving the enucleation procedure.

METHODS

**Materials.** A continuous line of African green monkey kidney cells (BSC1) was used in all enucleation experiments. Cells were plated out at a density of 2 to 3 × 10^5/3 cm dish in Eagle's medium supplemented with 10 % foetal bovine serum (EF 10) and incubated overnight at 37 °C. The virus strains studied and their origins were as follows. All virus stocks were passed at least once in BSCI cells at 31 or 37 °C to obtain some degree of adaptation. The stocks of VSV (Indiana, Cocal and New Jersey serotypes), reovirus type 3 (Dearing strain), respiratory syncytial virus (RSN-2 strain) and pseudorabies virus (Kaplan strain) used in these experiments were derived from rigorously cloned wild type stocks maintained in the Institute of Virology by one of us (C.R.P.). The origin of the other viruses was as follows: echovirus type 1 (Farouk strain), Dr M. C. Timbury, Institute of Virology, Glasgow; Semliki Forest virus, Dr J. S. Porterfield, N.I.M.R., Mill Hill, London; poliovirus, type 3 (Sabin strain), Dr Y. G. Cossart, Public Health Laboratories, Colindale, London; Klamath and Mokola viruses, Dr F. A. Murphy, Communicable Disease Center, Atlanta, Georgia, U.S.A., and measles virus (Beckenham 31 strain), Dr D. McCahon, Wellcome Research Laboratories, Beckenham, Kent.

**Cell enucleation.** Enucleation was carried out using the system described previously (Follett, 1974). Cells were grown on 3 cm Petri dishes (Sterilin or Nunc) and the Petri dish bases were supported in an inverted position in polycarbonate centrifuge tubes (MSE 10 × 100 rotor) in medium containing 5 µg/ml cytochalasin B. The tubes were centrifuged for 20 min at 9000 rev/min in the MSE 65 ultracentrifuge. Temperatures were maintained as near 37 or 31 °C as possible depending on the requirements of the subsequent experiment. Batches of either ten or twenty dishes were enucleated per run. After centrifuging, the cells were incubated for 1 h in normal medium at 37 °C or 2 h at 31 °C before use in any experiment. One dish from each batch of ten was stained with Giemsa. Enucleation efficiency was determined by counting at least 500 cells from six separate areas of each dish. In some experiments cells were enucleated using the Beckman type 21 rotor and the Beckman L3-50 ultracentrifuge. To allow for differences in the geometry between the Beckman and MSE rotors an increased speed of 10000 rev/min was necessary.

**Virus growth.** The ability of the various viruses to grow in enucleate cells (E) was compared with their ability to grow in nucleate cells treated with cytochalasin B (5 µg/ml) for
the same time as the enucleation procedure, but not centrifuged (T). After centrifuging or
cytochalasin treatment, the cells were allowed 1 h for recovery in normal medium at 37 °C
or 2 h at 31 °C before infection. The viruses were absorbed for 30 min at 37 °C, the infected
monolayers washed twice with EF10, 2 ml EF10 added to each dish and the cells incubated
at 37 °C. The input multiplicity was approx. 1 p.f.u./cell. The total virus yield at intervals
thereafter was determined by freezing and thawing the cells plus medium from a single dish
(T or E) and titrating a sample in the appropriate cells, either BHK 21/C13 or BSC1 cells.
Each point on the resulting growth curve thus represented the yield from a complete Petri
dish of cells.

Enucleate cell ‘competence’. One of the difficulties of experimenting with enucleate cells
is that there is no way of determining the ‘viability’ of the cells and thus comparing and
controlling the condition of the cells from experiment to experiment. In order to have some
control in the system an infection with VSV was always included in experiments to measure
virus growth in enucleate cells. If the final titre of VSV after 12 h incubation was > 70 %
of that in nucleate cells then the enucleate cells were considered ‘competent’ to support virus
growth, and any differences in the test virus titre likely to be significant. A yield of < 70 %
denoted, if not ‘incompetence’ then ‘less competence’ and the results were discarded.

Polypeptide analysis. To investigate the production of virus-specified proteins in enucleate
cells, cells were infected and described above and individual dishes (T, E or N, normal cells)
pulse-labelled with [35S]-methionine for 15 min at intervals thereafter. The cells were washed
twice with methionine-free medium and [35S]-methionine (10 μCi/dish) added in 0·2 ml
methionine-free medium. After 15 min the label plus medium was removed and the cells
scraped into 0·4 ml 0·001 M-tris buffer, pH 9, and stored at −70 °C until required for
analysis. Samples were adjusted to a final concentration of 2 % SDS, 10 % mercaptoethanol,
boiled for 2 min and electrophoresed in 9 %, acrylamide gel using the buffer systems
described by Laemmli (1970). Gels were stained in Coomassie brilliant blue, destained in
acetic acid (3·5 %), methanol (50 %) and allowed to swell in 7·5 % acetic acid. Individual
gels were sliced longitudinally and dried using the method of Fairbanks, Levinthal & Reeder
(1965). Autoradiographs were prepared using Kodak Blue Brand or Kodirex KD54T
film. Densitometer tracings were prepared using a Joyce–Loebl densitometer.

RNA synthesis. The ability of enucleate cells to synthesize virus RNA was examined by
following the incorporation of [3H]-uridine into acid-precipitable material at intervals after
infection. After enucleation, cells were infected as described above and at intervals labelled
with [3H]-uridine (10 μCi/dish) for 1 h. After labelling, the cell monolayer was dissolved in
0·4 ml NETS buffer (0·1 M-NaCl, 0·01 M-EDTA, 0·01 M-tris, 1 % SDS). Samples of this
solution were spotted on to filter paper discs, washed seven times with 5 % TCA containing
5 % sodium pyrophosphate, dried in alcohol and diethyl ether and counted on an Inter-
technique liquid scintillation counter.

RESULTS

Enucleation

Satisfactory levels of enucleation (>97 % of cells in Giemsa-stained dishes) were con-
sistently achieved over a period of many months. Lowering the temperature to 31 °C during
the enucleation procedure and subsequent incubation had no significant effect on the
efficiency of enucleation although 2 h as against 1 h at 37 °C was now required before the
cells completely regained their original morphology. Cell loss occurring during the enuclea-
tion procedure was consistently < 10 % and only preparations with an efficiency of enuclea-
tion >97 % and a cell loss of <10 % were used in virus studies.
VSV was used as a control in all growth experiments in order to determine if the enucleate cells were 'viable' and contributing to the final yield of VSV and thus potentially capable of contributing to the final yield of the virus under test. To determine the contribution to the final yield of the residual nucleate cells in enucleate cell preparations, cells were infected with pseudorabies virus, a herpes virus which multiplies in the nucleus, and yields determined up to 48 h (Fig. 1). Some multiplication in the enucleate preparations was apparent as indicated by the increase in the titre at 48 h post-infection compared to that at 1 h. However, this was very slight and the difference of 3 log units between the final yield in treated and enucleate cells indicated that of the cells productively infected in the nucleate preparation, on average, only one cell in a thousand in the enucleate preparation was producing infectious virus. As this preparation contained between ten and twenty residual nucleate cells per thousand, these results suggest that all cells scored as nucleate by Giemsa staining of an enucleate preparation may not be capable of supporting virus growth.

Reovirus

Measurements of the yields of infectious virus produced in nucleate cells at intervals up to 24 h after infection demonstrated that reovirus could multiply in the enucleate cell preparations, i.e. that there was a significant rise in titre compared to that immediately after the absorption period (Fig. 2). The final yield of virus in the enucleate preparations never
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reached that obtained in nucleate treated cells and was normally of the order of 10% after incubation at 37 °C. This yield was significantly more than could be attributed to the remaining nucleate cells in the cultures (< 3%) and it could thus be concluded from these results that reovirus multiplied in enucleate cells, but much less efficiently than in nucleate cells.

Enucleate cells have a relatively short lifetime. By 12 to 15 h post-enucleation some cells have detached from the substrate and others show morphological changes especially at the cell periphery. Attempts to extend the lifetime by changes in serum concentration, medium composition and by incubation in conditioned medium produced no significant increase. It was only when the temperature of enucleation and subsequent incubation was lowered to 31 °C that a significant extension of the morphological lifetime was apparent. Under these conditions many more enucleate cells remained morphologically intact at 24 and 48 h post-enucleation than after incubation at 37 °C. Reovirus can replicate at 31 °C and an examination of its growth in enucleate cells showed that an improved yield of virus was obtained compared to that in treated cells (~ 30%) (Fig. 2), although the growth cycle was longer and the final yield in the nucleate, treated cells less than at 37 °C.

To investigate further this reduction in yield in enucleate cells, the production of virus-specified polypeptides in enucleate and nucleate cells was examined by gel electrophoresis at intervals after infection at 37 °C (Fig. 3). All virus-specified polypeptides were produced in enucleate cells. No difference could be detected either in their time of appearance or their rate of migration when compared to those in nucleate, treated cells. The most significant difference was in the quantity produced in enucleate cells. From their earliest time of

Fig. 2. Growth of reovirus in enucleate (○—○) and cytochalasin-treated (●—●) BSC1 cells at 37 and 31 °C.
Fig. 3. (a) Autoradiogram of polyacrylamide gels of BSC1 cells infected with reovirus and labelled for 15 min with [35S]-methionine at intervals after infection. Enucleate, E; cytochalasin-treated, T. New virus-specified polypeptide bands are indicated (▲). (b) Densitometer tracings of autoradiographs of gels from (a) labelled 9 h post infection. Note reduced synthesis in enucleate cells.
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Fig. 4. Uptake of [3H]-uridine into acid-precipitable material following infection of BSC1 cells with reovirus. (a) Both enucleate and treated cells incubated with 0.3 μg/ml actinomycin D added immediately after virus absorption. Pulse time was 1 h. Enucleate (○—○); cytochalasin-treated (●—●). (b) Actinomycin D concentration in treated cells 2.5 μg/ml, in enucleate 0 μg/ml. Enucleate (○—○); cytochalasin-treated (●—●).

appearance a reduction in the amount synthesized in enucleate cells was evident and at no time was the amount made in enucleate cells more than 60% of that in nucleate cells. There was no evidence from such gels of any specific defects in the synthesis of any polypeptides sufficient to account for the reduction in yield of infectious virus noted above at 37 °C. These results suggested that enucleate cells were capable of producing sufficient virus-specified proteins to permit a yield of infectious virus in excess of the 10% obtained.

Enucleate cell preparations provide an ideal system for examining virus RNA synthesis as the background host cell RNA synthesis is extremely low (Follett, 1974). After infection of enucleate cells with reovirus, a peak of RNA synthesis was observed at 9 h post-infection (Fig. 4a). To obtain a quantitative comparison between enucleate and nucleate cells it was necessary to incubate the nucleate cells with actinomycin D which at the concentration used (0.3 μg/ml) produced some inhibition of host but not virus RNA synthesis. It was found that the amount of new RNA synthesized in enucleate cells was extremely small, ranging between 6 and 9% of that in nucleate, treated cells. Further increasing the concentration of actinomycin D improved the extent of host inhibition and demonstrated that the time course of the new RNA synthesis in the reovirus-infected nucleate and enucleate cells was identical (Fig. 4b). At this concentration (2.5 μg/ml) however, the amount of new virus RNA synthesis in nucleate cells is also drastically inhibited (Fig. 4b) (Shatkin, 1965). From these results it would appear that the limiting factor in the production of infectious reovirus in enucleate cells is the synthesis of new virus RNA.
Fig. 5. Growth of poliovirus in enucleate (○—○) and cytochalasin-treated (●—●) BSC1 cells at 37 °C.

Fig. 6. Growth of echovirus in enucleate (○—○) and cytochalasin-treated (●—●) BSC1 cells at 37 °C. Enucleate and treated cells from the same preparation were infected either after 1 h incubation in normal medium or 17 h later, i.e. after 18 h incubation at 37 °C and the yields determined at times shown. The yield at 24 h is omitted to avoid confusion; the titres were similar to those at 12 h. Yields from VSV-infected enucleate (△—△) and treated cells (▲—▲) infected at identical times are also shown.
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Echovirus and poliovirus

In an earlier study using cells enucleated on glass coverslips Pollack & Goldman (1973) showed that poliovirus could multiply in enucleate cells but that the yield obtained was reduced to 10% of that in nucleate cells. Experiments with our system have confirmed their findings (Fig. 5) and show a higher yield of virus from the enucleate cells. The reduction in yield is less than that which occurs with reovirus, the final yield being 30 to 40% of that in the nucleate cells.

Another picornavirus, echovirus, was also examined for growth in enucleate cells. This virus multiplied very poorly; there was a considerable time lag between the appearance of new virus in the enucleate and nucleate cells and the final yield was less than 10% of that in nucleate cells (Fig. 6). To confirm that enucleate as well as residual nucleate cells were contributing to the rise in titre, enucleate cell preparations were infected 18 h post-enucleation. At this time the majority of the enucleate cells are in the process of detaching from the surface and can be shown to be unable to support the growth of VSV (see below). Any increase in titre can therefore be attributed to the nucleate cells remaining. A comparison of the growth curves (Fig. 6) showed that significantly more virus was made in the enucleate cells infected immediately post-enucleation than 18 h later, indicating that some multiplication of echovirus was taking place in the enucleate cells. The control titrations of VSV are also included in Fig. 6 to illustrate the different capabilities of the enucleate preparations to support virus growth.

Further evidence that virus-specified products were being made in enucleate cells was obtained from analysis of polypeptides present at intervals after infection (Fig. 7). New bands appeared in gels from enucleate and nucleate cells at 4 h post-infection, reached a maximum intensity at 8 h and had declined by 12 h. All virus-specified polypeptides could be distinguished in enucleate cells although the amount present was reduced to 20 to 30% of that in nucleate cells. The observation of these bands in enucleate preparations also indicates that post-translational cleavage of the large precursor polypeptide can occur in the absence of a nucleus.

Respiratory syncytial virus

Respiratory syncytial virus (RS virus) grows extremely slowly in BSC1 cells and can take up to 48 h to reach maximum yield. The majority of enucleate cells become detached by this time if maintained at 37 °C and consequently the growth of RS virus was examined both at 37 and 31 °C (Fig. 8). The yield from enucleate cells infected and incubated at 37 °C was less than 10% of that from nucleate cells but at 31 °C the yield had increased to 30 to 40%, indicating a significant level of multiplication in the enucleate cells.

Semliki Forest virus

Semliki Forest virus grows rapidly in BSC1 cells, new virus becoming detectable at 5 h post-infection. In enucleate cells the growth is equally rapid and the final titre achieved is greater than 70% of that in nucleate cells (Fig. 9). The multiplication of this virus, like that of VSV, is therefore completely independent of the presence of a nucleus.

Enucleate cell competence

In all the growth experiments the ability of VSV to replicate efficiently in enucleate cells was used as a measure of the competence of the cells to support virus growth. From the echovirus studies it was apparent that this competence was much reduced 18 h post-enucleation and to examine this further, cells were enucleated and infected with VSV at intervals...
post-enucleation both at 31 and 37 °C. The virus yields were measured 12 h later (Fig. 10). It is clear that at 37 °C the best yield is obtained immediately post-enucleation and that thereafter the cells become gradually less competent to support virus growth. After 12 h the yield of virus can all be attributed to the remaining nucleate cells. At 31 °C a consistently higher yield was obtained at each time apart from the initial infection. The poor growth at this time probably indicates that the time given for the cells to recover from the enucleation treatment (1 h) was insufficient at 31 °C. Experiments have shown that cell protein synthesis does not reach its optimum level until 2 h after enucleation at 31 °C whereas 1 h is sufficient at 37 °C. Although an improved yield of virus was obtained from enucleate cells at 31 °C as compared to 37 °C, consistently less virus was obtained from the nucleate cells at 31 °C than at 37 °C (Fig. 10). Enucleate cells thus support a higher proportion of the nucleate cell’s potential virus yield at 31 than at 37 °C and can thus be considered more competent at 31 °C. The findings with reovirus already described also illustrate this conclusion. At 31 °C the yield of reovirus in treated nucleate cells is less than at 37 °C but the yield in enucleate cells is not less at 31 °C than at 37 °C, reflecting the greater competence of the enucleate cell at 31 °C.

Vesicular stomatitis virus has a short growth cycle in BSC1 cells. In enucleate BSC1 cells the growth rate of all strains of VSV (Indiana, New Jersey and Cocal) was also rapid and the
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Fig. 8. Growth of respiratory syncytial virus (RS virus) in enucleate (○—○) and cytochalasin-treated (●—●) BSC1 cells at 37 and 31 °C.

Fig. 9. Growth of Semliki Forest virus (SFV) in enucleate (○—○) and cytochalasin-treated (●—●) BSC1 cells at 37 °C.
Fig. 10. Effect on virus yield of infecting enucleate BSC1 cells at increasing times post-enucleation. Enucleate cells and cytochalasin-treated cells were allowed 1 h for recovery in normal medium at either 37 or 31 °C, infected with VSV at 0, 3, 6, 9, 12, 15 and 18 h thereafter and the virus yield determined 12 h later. O—O, Cytochalasin-treated, 37 °C; □—□, cytochalasin-treated, 31 °C; □—□, enucleate, 31 °C; ○—○, enucleate, 37 °C. The points in the bottom left-hand corner represent the titres in the various cells at the end of the absorption period.

The final yield obtained normally > 70 % of that in cytochalasin-treated cells (Follett et al. 1974). In order to determine if increasing the length of the growth cycle had any effect on the yield obtained from enucleate cells the growth of two other rhabdoviruses, Klamath and Mokola, which have prolonged growth cycles in BSC1 cells, was compared to that of the VSV Cocal strain used as a standard in all other experiments (Fig. 11). The results clearly indicated that the longer the virus took to achieve its maximum titre then the greater was the reduction in yield in the enucleate cells. The yield of Klamath virus from enucleate cells was reduced to 10 % of that in treated cells while the yield of Mokola virus which has the longest growth cycle was only 2 to 3 % of normal. It should be noted that the growth curve of Mokola virus may not represent a single cycle yield because of the necessarily low multiplicity of infection.

These results also demonstrate that enucleate cells can support the growth of viruses which mature at the plasma membrane (VSV) or at intracytoplasmic sites (Klamath and Mokola).

**DISCUSSION**

The results obtained with enucleate cells confirm in a direct manner that the conclusions previously drawn from studies using metabolic inhibitors are correct. The growth of Semliki Forest virus is completely independent of the presence of a nucleus; poliovirus, echovirus and reovirus can grow in enucleate cell preparations but less efficiently than either VSV or Semliki Forest virus. There is, however, sufficient evidence of growth to conclude that these viruses also do not require the presence of a nucleus for replication. RS virus has not yet
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been fully characterized and has been tentatively classified in a new group, the metamyxo-
viruses. Our results indicate that the growth of this virus is independent of the presence of a
nucleus. We have also been examining the growth of another myxovirus, measles, in enu-
clideate cells. From growth curves alone it would appear that measles virus grows poorly if at
all in such cells. However, such is the time course of measles development that it could be
argued that by the time the measles virus should appear the enucleate cells are no longer
capable of supporting virus growth. Experiments designed either to prolong the competent
life of enucleate cells or to detect early virus-specified antigen are being pursued.

It is apparent from previous studies on vaccinia virus replication in enucleate cells (Pen-
nington & Follett, 1974) and the results reported here on reovirus and echovirus, that poor
growth of these viruses in enucleate cells is not the result of a specific block on any one virus-
specified protein. All proteins normally specified in nucleate cells are synthesized in enucleate
cells but in reduced amounts. Moreover, there is usually more protein made than is required
for the yield of infectious virus obtained and the low level of reovirus RNA synthesis found
in enucleate cells would suggest that RNA synthesis and not protein synthesis is the factor
limiting the yields of infectious virus in enucleate cells.

Now that several viruses have been examined for growth in enucleate cells it is apparent
that viruses with a short, rapid growth cycle such as VSV and Semliki Forest virus grow
almost as well in enucleate as nucleate cells but that viruses with longer growth cycles tend
to have a significantly reduced yield in enucleate cells. This is not too surprising in view of
our present knowledge of the lifetime of enucleate cells. By 15 h post-enucleation cells can
be seen to be rounded up and detached from the substrate in uninfected cultures. Protein
synthesis is reduced on enucleation and declines gradually thereafter (Follett, 1974). The
enucleation procedure results in some cytoplasm being removed with the nucleus and
measurements of the ribosomal RNA content of enucleate cells indicate an immediate reduction to 25 to 30% on enucleation (E. A. C. Follett & W. H. Wunner, personal communication). Clearly, lack of a nucleus and/or the process of enucleation produces a population of cells of diminishing metabolic activity which would not be expected to support virus growth as effectively as a population of healthy growing cells. Reducing the temperature of incubation to 31 °C prolongs the lifetime of enucleate cells as judged by their appearance and by the number remaining attached to the substrate 24 h post-enucleation. The improved relative yields obtained by incubation of reovirus and respiratory syncytial virus at 31 °C suggest that the ability of the enucleate cell to support virus growth can also be prolonged by lowering the temperature. Yield is also dependent on the time after enucleation when infection occurs as evidenced by the results obtained from infecting with VSV at varying times after enucleation (Fig. 10). The decreased yields obtained in that experiment may, again, merely reflect the general decline in the metabolic activity of the enucleate cells as a whole or they may be indicative of a more specific effect such as a surface alteration leading to less efficient absorption of the virus.

The single most important factor governing the ultimate yield obtained from an enucleate cell would, however, appear to be the length of the growth cycle. The experiments using different rhabdoviruses suggest that there is a direct correlation between the length of the growth cycle and the difference in yield between treated and enucleate cells (Fig. 11). Lack of apparent production of infectious virus from an enucleate cell cannot therefore be taken as conclusive evidence for the requirement of a nucleus for virus multiplication. The enucleation technique in its present form thus results in a cell preparation for virus experimentation which can provide only an unequivocal negative answer to the question of whether a virus requires the presence of a nucleus for multiplication. Positive answers must be treated with extreme caution.

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