An Improved Technique for Obtaining Enhanced Infectivity with Herpes Simplex Virus Type 1 DNA

By N. D. STOW AND N. M. WILKIE

M.R.C. Virology Unit, Institute of Virology, Church Street, Glasgow, G11 5JR, Scotland

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SUMMARY

Cells infected with herpes simplex virus type 1 (HSV-1) DNA by the calcium phosphate precipitation technique produce virus which leads to the formation of plaques (Graham, Veldhuisen & Wilkie, 1973). In the study reported here we show that treatment of cell monolayers with dimethyl sulphoxide (DMSO) solutions after infection with DNA-calcium phosphate complexes leads to a considerable increase in the number of plaques obtained. The conditions for this enhancement of infectivity have been optimized for baby hamster kidney (BHK) cells, and increases in plaque numbers of over 100-fold have been obtained. The treatment appears to increase the proportion of cells which respond to DNA infection by initiating plaque formation, and results in a large increase in the measured specific infectivity of HSV-1 DNA. DMSO causes similar (but quantitatively different) responses in various other cell lines infected with HSV-1 DNA. BHK cells infected with either virus particles, or virus DNA by the DEAE-dextran technique (Laithier & Sheldrick, 1975), do not exhibit this massive enhancement following exposure to DMSO.

INTRODUCTION

The genomes of several different groups of RNA and DNA viruses have been shown to be infective when susceptible cells are exposed to the extracted nucleic acid (Vaheri & Pagano, 1965; Tovell & Colter, 1967; McCutchan & Pagano, 1968; Warden & Thorne, 1968; Nicolson & McAllister, 1972; Graham & van der Eb, 1973). The infective potential of DNA isolated from herpes simplex virus type 1 (HSV-1) was first suggested in 1969 by Lando & Ryhiner, who observed a typical c.p.e. in rabbit kidney and fibroblast cells, but did not carry out progeny tests to determine whether any infectious particles had been produced. Lando & Ryhiner used DEAE-dextran in an attempt to maximize the infectious property of the HSV DNA. Subsequently Graham & van der Eb (1973) published a new method of infecting cells in which DNA was co-precipitated with calcium phosphate. Cells readily took up the DNA, and plaques were produced in monolayers infected with both SV40 and adenovirus 5 DNA. In the same year two reports showed that plaques, and progeny virus, were produced after infection with HSV-1 DNA; Sheldrick et al. (1973) used the DEAE-dextran method, while Graham et al. (1973) employed the new calcium phosphate technique.

Using this latter technique to infect BHK cells, specific infectivities of about 10^{11} p.f.u./μmol have been obtained for HSV-1, HSV-2 and pseudorabies virus DNAs (Wilkie et al. 1974). This corresponds to several thousand p.f.u./μg of DNA.

Recently we have observed that the specific infectivities of HSV-1 DNA preparations have
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decreased considerably, concomitant with a decrease in the yield of virus per infected cell. On several occasions titres of less than 10 p.f.u./μg have been obtained.

In this report we describe a modification of the calcium phosphate technique which results in a 100-fold increase of the infectivity of HSV-1 DNA.

METHODS

Cells. Baby hamster kidney (BHK 21 C13) cells were grown in Eagle's medium supplemented with 10% tryptose phosphate and 10% calf serum (ETC10) as previously described (Macpherson & Stoker, 1962). Subconfluent cell monolayers (2 × 10⁶ cells per dish) in 50 mm diam. plastic (Flow) Petri dishes were used throughout for DNA infection experiments.

Virus growth and virus DNA preparation. Cells grown in slowly rotating 80 oz bottles were infected at low input multiplicities (1/100 to 1/300) with herpes simplex virus type 1 (HSV-1 Glasgow strain 17) and incubated at 37 °C for 2 days. Cell-released and cell-associated virus was prepared from infected cells as described by Wilkie (1973). HSV DNA was obtained from the virions by phenol extractions in the presence of 0.01 M-EDTA, pH 7.4, and 2% SDS, and the final aqueous phase was dialysed extensively against 0.1 x SSC. In some cases the DNA was further purified by isopycnic banding on cesium chloride gradients (Wilkie, 1973). HSV DNA concentrations were estimated by extinction at 260 nm, or by buoyant density centrifugation in the analytical ultracentrifuge.

Infection of monolayers with DNA. The method used was essentially that of Graham et al. (1973). HSV DNA was diluted to the required concentration in Hepes buffered saline (HeBS; Graham et al. 1973) containing 30 μg/ml carrier DNA (calf thymus or uninfected BHK cell DNA). CaCl₂ (2m) was added to a final concentration of 130 mM, and 0.4 ml samples of the fine suspension which formed were added to BHK cell monolayers from which the growth medium had been removed. Four ml fresh ETC5 (5% calf serum) was added to the plates after 40 min incubation at 36 °C. The monolayers were washed with ETC5 at 4 h post-infection (p.i.), and incubation was continued in 4 ml ETC5 or ETHu5 (5% human serum, which contains neutralizing antibodies to HSV).

Treatment of monolayers with DMSO solutions. At various times following infection with DNA the monolayers were drained and washed once with ETC5. 1 ml of a solution of DMSO was added for a short time, as described in Results and then poured off. The plates were washed again with ETC5 and incubation was continued for about 40 h at 36 °C in 4 ml ETHu5. Plates were fixed and stained in Giemsa, and the plaques were scored. All points shown represent the average of four replicate plates.

RESULTS

Effect of DEAE-dextran on HSV-1 DNA infectivity

In early experiments in this study it was found that monolayers of BHK cells infected with HSV-1 DNA by the calcium phosphate technique (Graham et al. 1973) supported the appearance of more plaques when briefly exposed to a solution of 150 μg/ml DEAE-dextran in 10% DMSO. Cells exposed to this solution for 10 min at 4 h p.i. titrated HSV-1 DNA at 700 p.f.u./μg as compared with the figure of about 50 p.f.u./μg obtained with cells infected using the calcium phosphate precipitation technique alone.

DEAE-dextran has been used to increase the specific infectivity of a number of virus RNA and DNA genomes (Vaheri & Pagano, 1965; McCutchan & Pagano, 1968; Sheldrick et al. 1973). Accordingly, the effect of varying the DEAE-dextran concentration on HSV-1 DNA
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Fig. 1. The effect of varying the concentration of DEAE-dextran (DEAE-D) on the infectivity of HSV-1 DNA. Cells were infected with 0.2 µg of HSV-1 DNA per plate as described. Four h p.i. monolayers were exposed for 10 min to varying concentrations of DEAE-D dissolved in HEB (○—○) or HEB containing 10% DMSO (●—●), and the number of plaques per dish counted at 40 h p.i.

Fig. 2. The effect of DMSO concentration on the enhancement of HSV-1 DNA infectivity. As for Fig. 1 but cells were infected with 0.04 µg of virus DNA per plate and exposed for 4 min to varying concentrations of DMSO dissolved in HEB (●—●) or Eagle’s medium (■—■).
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infectivity was measured. Cell monolayers infected with 0.2 μg of HSV-1 DNA were treated for 10 min at 4 h p.i. with various concentrations of DEAE-dextran dissolved in either HeBS or HeBS containing 10% DMSO. Fig. 1 shows that maximum infectivity was obtained with 10% DMSO in the complete absence of DEAE-dextran, the number of plaques obtained being increased 60-fold over the control plates. Since DEAE-dextran appeared to have an inhibitory effect in the presence of DMSO, and no stimulatory effect when used alone, it was omitted in all subsequent experiments.

Effect of DMSO on HSV-1 DNA infectivity

Fig. 2 shows the effect of varying the DMSO concentration on the number of plaques appearing on BHK cell monolayers infected with 0.04 μg HSV-1 DNA. The DMSO was dissolved in either Eagle’s medium or HeBS, and cells were treated for 4 min at 4 h p.i. Increasing numbers of plaques were obtained with increasing concentrations of DMSO up to 25% using either medium. Above this figure areas of the cell sheets were frequently destroyed, even after only brief exposure (less than 1 min) to DMSO. Slightly fewer plaques were obtained with Eagle’s medium than with HeBS, and plaque counts were less reproducible. In all subsequent work HeBS was used.

The effect of varying the length of time for which monolayers were exposed to 25% DMSO at 4 h p.i. is shown in Fig. 3. Short exposure (less than 1 min) is clearly sufficient to cause a large enhancement in plaque numbers. Some cell destruction was observed with prolonged exposure times which probably accounts for the fall off in plaque numbers at later times. Between 4 and 15 min appeared to be an optimal time for BHK cells.

Fig. 4a shows the effect of varying the time at which DMSO exposure takes place. When cells were exposed to DMSO prior to infection, no enhancement was observed, but the
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Fig. 4. (a) Time course for treatment with DMSO. Monolayers were infected with 0.028 μg (○), or 0.014 μg (■) of HSV DNA and exposed to 25% DMSO in HeBS for 4 min at the various times post-infection. Plaques were counted 36 h p.i. (b) Time course for resistance to DNase. Monolayers were infected with 0.028 μg HSV DNA and 12.5 μg DNase I (Sigma) added at the times shown. The monolayers were treated with DMSO for 4 min at 4 h p.i., and DNase (3 μg/ml) added to the final incubation medium. Plaques were counted 36 h p.i.

results clearly indicate that cells should be left in contact with the CaPO₄-DNA precipitate for at least 3 h for maximum enhancement to be realized. The plaque-forming ability of DNA in the calcium phosphate precipitate becomes resistant to DNase I one to two h after contact with susceptible cells (Fig. 4b). This is much earlier than the time at which the DMSO boost is maximally effective. In experiments not shown, it was found that the plaque-forming ability of HSV-1 DNA became resistant to sonication immediately the CaPO₄-DNA precipitate had formed. Indeed, precipitates which had lost almost all their infectivity through prolonged standing could be fully restored by brief periods of sonication.

Fig. 5 shows that DMSO treatment had relatively little effect on the plaque counts obtained following infection of BHK monolayers with herpes simplex virus particles. Treatment with DMSO at various times post- and pre-infection led to no more than a 30% increase in plaque numbers at the time of maximum effect (2 to 8 h p.i.). This increase is clearly insufficient to account for the massive enhancement of DNA infectivity which results from DMSO treatment.

The effect of DMSO on the infectivity of DNA as assayed by the DEAE-dextran technique was also investigated. BHK monolayers which had been pre-exposed to DEAE-dextran as described by Laithier & Sheldrick (1975), were infected with 0.2 μg of HSV-1 DNA. Four ml of ETHu5 was added to each plate after 1 h. At 4 h p.i., the medium was decanted from one group of plates and 1 ml of 25% DMSO in HeBS added for 4 min. These monolayers were then washed and incubation continued in ETHu5. The ETHu5 was left on the remaining group of plates for the whole duration of the experiment. It can be seen from Table 1 that although DMSO enhanced the infectivity of the calcium phosphate-precipitated DNA
Fig. 5. Effect of DMSO treatment on the number of plaques obtained following infection with wild type HSV-1 virus. 0.4 ml of virus suspended in PBS was added to each plate ('zero time') and allowed to adsorb for 40 min before the addition of growth medium. Monolayers were exposed to 25% DMSO for 4 min at the times indicated either before or after infection. Final incubation was for 48 h in ETC5. The arrow indicates the number of plaques found on control plates not treated with DMSO.

Table 1. Comparison of the effects of DMSO treatment on the infectivity of HSV-1 DNA assayed by the calcium phosphate and DEAE-dextran techniques*

<table>
<thead>
<tr>
<th>Technique</th>
<th>HSV-DNA µg/plate</th>
<th>Carrier DNA µg/plate</th>
<th>Plaques without DMSO treatment</th>
<th>Plaques with DMSO treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEAE-dextran</td>
<td>0.2</td>
<td>0</td>
<td>45.0</td>
<td>39.3</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>0.04</td>
<td>4</td>
<td>0.0</td>
<td>91.3</td>
</tr>
</tbody>
</table>

* Cell monolayers were infected and treated as described in the text. Plaque numbers were counted 48 h p.i.

controls, it had no effect on the number of plaques appearing on monolayers infected with DNA using the DEAE-dextran method.

Optimum conditions for enhancement of infectivity

In order to maximize the specific infectivity of HSV DNA the following procedure was adopted. Four hours after infection with the CaPO₄-DNA precipitate, the monolayers of cells were washed once with ETC5. They are then exposed to 1 ml of 25% DMSO in HeBS for 4 min, washed once with ETC5 and finally incubated in ETC5 or ETHu5. Fig. 6 shows that omitting the wash prior to DMSO exposure reduces the effectiveness of the assay, as does the addition of pure DMSO directly to 25% in the medium containing the CaPO₄-DNA complex. The wash following exposure to DMSO is necessary to prevent cell killing by residual DMSO.
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Fig. 6. Effect of different washing procedures prior to DMSO treatment. At various times after infection with 0.017 µg of virus DNA per plate, BHK monolayers were treated with DMSO as follows: O—O, the medium was poured off and the cells washed once prior to exposure to 25% DMSO in HeBS; ●●●, the medium was poured off but 25% DMSO in HeBS was added without washing the cells; ■■■, the medium was left on and made 25% with respect to DMSO by adding the pure compound. The monolayers were washed once following a 4 min exposure to DMSO and then incubated in ETHu5. Plaques were counted 40 h p.i.

Dose-response curve for HSV-1 DNA

The number of plaques obtained per dish with increasing amounts of HSV-1 DNA using the technique described above is shown in Fig. 7. The presence of a plateau (at 600 plaques per plate) suggests that at any one time only a limited number of cells in the population can respond to virus DNA infection by producing progeny virus and eventual plaque formation.

Previously published dose-response curves (Graham et al. 1973) were of a similar shape but with between only 30 and 60 plaques per plate at the plateau level (Fig. 7). It is clear that the higher level of infectivity obtained using the DMSO boost is not merely due to an extension of the first order response portion of the curve to higher virus DNA concentrations, but results from an enhancement in the number of plaques obtained throughout the range of DNA concentrations. However the number of cells capable of producing virus remains limiting. The specific infectivity calculated from the linear portion of the curve shown in Fig. 7 is $8 \times 10^9$ p.f.u./µg HSV-1 DNA. Assuming a mol. wt. of $100 \times 10^6$ for HSV-1 DNA (Kieff, Bachenheimer & Roizman, 1971; Wilkie, 1973) this corresponds to one infectious molecule in every $8 \times 10^6$. The specific infectivity calculated from control plates, which received the same amounts of virus DNA, but were not exposed to DMSO, was only about 10 p.f.u./µg (Fig. 7).

In the case of both adenovirus 5 and HSV-1 DNA it has been observed that when the calcium phosphate technique is used a decrease in plaque number with high concentrations of virus DNA frequently results (Graham & van der Eb, 1973; Graham et al. 1973). Although not seen in the experiment shown in Fig. 7 this phenomenon has also been observed when the DMSO boost is used. It is particularly striking when DNA preparations with low
Fig. 7. Dose-response curve for HSV-1 DNA using the infectivity boost technique. Using the optimized DMSO enhancement method described in the text, the number of plaques per dish obtained following infection with varying amounts of virus DNA was measured 36 h p.i. (●—●, with DMSO boost; ○—○, control plates without DMSO boost). The dotted line is taken from the data of Graham et al. (1973).

overall infectivities are titrated, and remains, as yet, unexplained. The DMSO treatment has no effect on the optimum concentration of carrier DNA required which was found to be 10 μg/ml as previously observed (Graham & van der Eb, 1973).

**HSV-1 DNA infectivity in other cell lines**

To test the effect of DMSO treatment on the infectivity of HSV-1 DNA in various cell lines, subconfluent monolayers of BHK C13, HEp2, BSC-1, Vero and HeLa cells were infected with 0.2 μg of HSV DNA. After 4 h the cells were treated with 10% or 20% DMSO in HepBS for 4 min as before. DNA-infected control plates received no DMSO but were washed with ETC5 prior to continuation of incubation. Half the plates were then incubated in ETC5 and the remainder in ETHu5. The incubation was terminated at 72 h p.i. and the ETHu5 plates were fixed, stained and the number of plaques counted. The ETC5 plates were harvested, sonicated, and the virus yield assayed by titration on BHK cell monolayers in the presence of human serum. Table 2 gives the results of this experiment. Plaques could be observed on the stained monolayers of each cell line following DNA infection, but of course differed considerably in their morphology. With HeLa, HEp2 and BSC-1, although the plaques consisted of only a small number of rounded up cells, they could nevertheless be readily distinguished and scored. It can be seen from Table 2 that DMSO treatment led to considerable increases in the plaque numbers on monolayers of BHK, Vero and HeLa cells. A less pronounced increase was observed in HEp2 cells and no detectable effect could be seen with BSC-1 cells.

In all the cell lines the virus yields were clearly enhanced by DMSO treatment. However, the increase in virus yield with increasing concentrations of DMSO was usually less than the increase in the number of plaques. This may be due to the late time of harvesting and the use of calf serum in the virus yield experiment. Under these conditions progeny arising from
Table 2. Effect of DMSO treatment on the infectivity of HSV-1 DNA in various cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Plaques/plate (0 % DMSO)</th>
<th>Plaques/plate (10 % DMSO)</th>
<th>Plaques/plate (20 % DMSO)</th>
<th>Virus yields p.f.u./plate (0 % DMSO)</th>
<th>Virus yields p.f.u./plate (10 % DMSO)</th>
<th>Virus yields p.f.u./plate (20 % DMSO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHK (C13)</td>
<td>5.5†</td>
<td>140.5†</td>
<td>557.5†</td>
<td>7 × 10⁷</td>
<td>2.6 × 10⁸</td>
<td>5.6 × 10⁸</td>
</tr>
<tr>
<td>HEP2</td>
<td>1.0</td>
<td>2.0</td>
<td>28.5</td>
<td>7 × 10⁷</td>
<td>4.4 × 10⁸</td>
<td>1.7 × 10⁸</td>
</tr>
<tr>
<td>BSC-1</td>
<td>0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.1 × 10⁴</td>
<td>9.2 × 10⁸</td>
<td>9.8 × 10⁸</td>
</tr>
<tr>
<td>Vero</td>
<td>24.0</td>
<td>83.5</td>
<td>495.5</td>
<td>7 × 10⁴</td>
<td>9 × 10⁷</td>
<td>5.6 × 10⁷</td>
</tr>
<tr>
<td>HeLa</td>
<td>7.5</td>
<td>132.5</td>
<td>243.0</td>
<td>6.6 × 10⁴</td>
<td>8.8 × 10⁶</td>
<td>3.5 × 10⁶</td>
</tr>
</tbody>
</table>

* Cell monolayers were infected with 0.2 μg HSV DNA and exposed to DMSO solutions at 4 h p.i. as described in the text. Plaque numbers were counted and virus yields titrated at 72 h p.i.
† Plaque numbers counted 48 h p.i.

initial DNA infections might re-infect cells in many parts of the plate, causing more virus to be produced than would be expected from plaque counts.

As expected, it was found that the different cell lines differed in their tolerance to exposure to DMSO. BSC-1 and Vero cells were the least resistant to high DMSO concentrations.

DISCUSSION

In this paper we have presented clear evidence that exposure to DMSO of cell monolayers, infected with HSV-1 DNA by the calcium phosphate technique, increases the infectivity of the DNA preparations by as much as two log units. The conditions for maximal increase have been investigated.

Several of our observations are pertinent to the poorly understood mechanisms by which virus nucleic acids are taken up and expressed by susceptible cells. DEAE-dextran appears to increase infectivity by interacting with both the virus nucleic acid and the cell membrane (Pagano, 1970), and is most effective when added to monolayers either immediately before infection (Al-Moslih & Dubes, 1973; Laithier & Sheldrick, 1975), or mixed with the infecting nucleic acid (Vaheri & Pagano, 1965; Sheldrick et al. 1973). There is no requirement for the addition of carrier DNA to the virus nucleic acid. Exposure of cells to virus nucleic acids in the presence of DMSO has also been shown to cause an increase in their infectivity (Amstey & Parkman, 1966; Tovell & Colter, 1967). The calcium phosphate method has previously been shown to differ from the DEAE-dextran technique in requiring the presence of excess carrier DNA during the formation of the calcium phosphate-DNA complex (Graham et al. 1973). In addition, no additivity between the methods is observed when cells infected by the calcium phosphate technique are treated with DEAE-dextran, either before (Graham & van der Eb, 1973) or 4 h after (Fig. 1) infection. A further important difference between the two techniques is revealed in Table 1 which shows that DMSO treatment has no effect on the number of plaques appearing on monolayers infected with DNA using the DEAE-dextran method. These observations provide evidence that the initial stages of infection with DNA differ considerably according to whether the calcium phosphate or the DEAE-dextran technique is used.

DMSO has been shown to cause an increase in the number and size of plaques obtained after SV40 infection of sensitive cells (Cleaver, 1974). It seemed possible, therefore, that in enhancing HSV-1 DNA infectivity, DMSO was affecting processes which followed the onset of virus replication rather than acting directly on the initiation of an infectious cycle by the
DNA. If this were the case one might expect the infectivity of both intact virus and DNA to be enhanced to a similar degree. However, the data presented in Fig. 5 shows that this is not the case and DMSO had relatively little effect on plaque numbers obtained after infection with virus particles.

When HSV DNA is co-precipitated with calcium phosphate it rapidly becomes resistant to damage by sonication. However, the infectivity of the DNA in the complex remains sensitive to DNase for up to 4 h, even when a fairly thick precipitate of calcium phosphate has formed (results not shown). In contrast, when the calcium phosphate-DNA complex is allowed to interact with cells, the DNA becomes resistant to DNase within 1 h (Fig. 4b). This suggests that some stable interaction with the cells, probably at the surface membrane, has occurred. If the DMSO boost is applied at this time the full infectivity of the DNA is not expressed (Fig. 4a), and the maximum infectivity is not realized until a further 2 to 3 h have elapsed (Fig. 4a). This must reflect some further change in the association of the DNA with the cell which must be completed before the DMSO can have its full effect in boosting the infectivity. It is not yet known whether this change is a second stage in uptake, such as the penetration of the DNA into the interior of the cell, or some other event in the infectious process, such as the transport or expression of the DNA.

Previous reports on the calcium phosphate co-precipitation technique have shown that after infection with virus DNA, only a small proportion of cells can produce virus which leads to plaque formation, although the entire population is fully competent for infection with virus (Graham & van der Eb, 1973; Graham et al. 1973). A similar situation was found for HSV DNA infection using the DEAE-dextran technique (Sheldrick et al. 1973; Laithier & Sheldrick, 1975). Although we have succeeded in increasing this fraction with the use of DMSO in conjunction with the calcium phosphate technique, the value remains very small. It is not clear whether the cells which do respond are in some particular stage of the cell cycle, or are in other ways phenotypically different from the majority of the cells in culture. Studies are currently in progress to examine some of these possibilities.

Over a period of some 2 years we have noted that the specific infectivity of HSV-1 DNA preparations has decreased to levels far below those previously obtained by Graham et al. (1973) and Wilkie et al. (1974) using the same strain of virus, the same cell line and the same method of infection (the calcium phosphate technique). The reasons for this change are not known, but over the same period it has been noted that virus yield per cell has frequently been reduced, and that DNA produced from such infections has an increased incidence of alkali-labile single-strand interruptions (Frenkel & Roizman, 1972; Wilkie, 1973). These events may reflect a change in the physiological state of the cells in culture in this laboratory rather than a change in the genetic make-up of the virus. The efficiency of the infectivity assay was also found to be decreased when original early passage BHK cells were revived and used, and when HSV-1 DNA prepared in other laboratories was assayed on our cells by the calcium phosphate technique. It should, however, be noted that DMSO treatment has been shown to boost the infectivity of HSV DNA in another laboratory using a different line of BHK cells, and preliminary results indicate that it has a similar effect upon the infectivity of other virus nucleic acids (our unpublished observations).

When the DEAE-dextran method was used in the current series of experiments the level of infectivity obtained was much greater than with the unmodified calcium phosphate technique, but substantially less than when the DMSO boost was used in conjunction with the latter method (e.g. Table 1). Since our experience with the DEAE-dextran technique is limited, we are not able to say whether its efficiency has also changed over the last two years. We have given and discussed evidence which suggests that the two techniques apparently enhance
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Infectivity by different mechanisms. Hence, it is possible that changes in the cells in culture could effect one method more than the other. Despite this, the modification of the calcium phosphate method described in this paper routinely gave HSV-1 DNA infectivities substantially higher than those previously published for either technique (Sheldrick et al. 1973; Graham et al. 1973; Wilkie et al. 1974).

The DMSO boost technique was developed using BHK cells but was also found to be effective to varying extents in other cell lines (Table 2). Both the number of plaques (and the specific infectivity) and the yield of virus following DNA infection varies for the different cell lines. This may be due to differences in the inherent susceptibility of the different lines to infection with HSV-1 DNA or to the fact that the conditions of infection were not optimized separately for each cell line tested. We are currently investigating the effect of DMSO treatment on the infectivity of other virus nucleic acids.

Although the processes involved in nucleic acid uptake and expression and the DMSO boost are still not understood, the method offers considerable practical advantages for experiments involving the use of infectious herpesvirus DNA, such as studies of marker rescue for genetic mapping, or on transformation with DNA and DNA fragments (Wilkie et al. 1974; N. D. Stow, J. H. Subak-Sharpe & N. H. Wilkie, unpublished data).

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