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Influence of Diethylaminoethyl-Dextran on Uptake and Degradation of Polyoma Virus Deoxyribonucleic Acid by Mouse Embryo Cells

D. WARDEN[1] AND H. V. THORNE[2]
Institute of Virology, University of Glasgow, Glasgow, Scotland

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The uptake of ^{32}P-labeled polyoma virus deoxyribonucleic acid (DNA) (I and II + III) by mouse embryo cells was increased from two- to fivefold in the presence of 500 μg of diethylaminoethyl-dextran (DEAE-D) per ml. This concentration of DEAE-D gives maximal enhancement of infectivity; however, the increase is many thousand-fold. As the DEAE-D concentration was increased from 0 μg/ml, uptake and infectivity increased to flat maxima and then decreased in a similar manner, except that at low DEAE-D concentrations uptake was relatively much greater than infectivity. Several other polycations also increased DNA uptake but did not enhance infectivity, and uptake of viral DNA was unaffected by the presence of mouse DNA, although infectivity was reduced. Thus, increased uptake is not the sole basis for the enhancement of infectivity produced by DEAE-D. The possibilities that DNA complexed with DEAE-D penetrates more rapidly or is stabilized against degradation do not completely account for enhancement since complexes formed in mixtures of DNA and DEAE-D, which sedimented heterogeneously from 40 to 60*S*, were infectious only for monolayers treated with DEAE-D. A more likely factor in enhancement is inhibition of the cellular nuclease activity detected, since virus DNA exposed to cells was much more degraded in the absence than in the presence of DEAE-D. The nuclease activity produced single-strand breaks in double-stranded DNA. Treatment of monolayers with deoxyribonuclease after adsorption of DNA in the presence of DEAE-D reduced cell-associated radioactivity by about 70%, although the number of plaques formed was not affected. In the absence of DEAE-D, 90 to 100% was removed by deoxyribonuclease. Thus, in both cases most of the DNA was adsorbed extracellularly. The greater deoxyribonuclease-resistant fraction in the presence of DEAE-D would be consistent with another possibility: that enhancement results from an increase in DNA penetration rate due to some action of DEAE-D on the cell.

We recently reported use of diethylaminoethyl-dextran (DEAE-D) to increase the infectivity of polyoma virus deoxyribonucleic acid (DNA) for mouse embryo cells and to assay relative infectivities of different molecular fractions (13, 15). This system has advantages, also, for determining the general properties and optimal conditions of interaction of DNA with mammalian cells, since the virus DNA is well defined physically and the plaque-forming response provides a measure of the amount of DNA which penetrates into the cell. Therefore, in continuation, we have examined the effect of DEAE-D on the quantitative uptake of DNA under various conditions and on the degradation of virus nucleic acid by cells. This should help to determine the mechanism of action of DEAE-D and to specify conditions permitting higher efficiencies of infection.

[1] Present address: Glaxo Laboratories, Greenford, Middlesex, England.

[2] Member of the Medical Research Council Experimental Virus Research Unit. Present address: Département de Microbiologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, P.Q. Canada.

MATERIALS AND METHODS

Cell culture, plaque assay, growth, and purification of virus. Methods for cell culture, plaque assay, growth, and purification of radioactive virus, extraction of DNA, and fractionation of DNA by zonal sedimentation into components I and II + III were those previously described by Warden and Thorne (15).

Sedimentation velocity analysis. DNA was analyzed by centrifugation on sucrose gradients containing

(*p*H 8.5) 0.02 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, 0.1 M NaCl and 0.01 M ethylenediaminetetraacetate (EDTA) (NaCl-EDTA), or 0.3 N KOH, 0.1 M NaCl, and 0.01 M EDTA in Spinco SW 39 or SW 65 rotors. Fractions were collected dropwise on glass-fiber paper, dried, and assayed for radioactivity by liquid-scintillation procedures.

Agar electrophoresis. DNA was analyzed by the method described by Thorne (12).

Measurement of ^{32}P-labeled DNA uptake. Confluent mouse embryo monolayers in 60-mm petri plates were washed once with 3 ml of phosphate-buffered saline [PBS (4)] before addition of 0.1 ml of a solution of DEAE-D (1,000 μg/ml, unless stated otherwise) in PBS, followed by 0.1 ml of ^{32}P-labeled DNA solution in PBS and incubation for 1 hr at 37 C (unless stated otherwise). Monolayers were then washed three times with PBS to remove unadsorbed radioactivity and were suspended in Eagle's medium plus 10% calf serum either with a silicone rubber-tipped scraper or by treatment with 0.05% trypsin in 0.0006 M EDTA for 15 min. After centrifugation, supernatant fluids were discarded and cell pellets were dried in an oven at 80 C. Each dried cell pellet was moistened with 10 μliters of distilled water, suspended in 1 ml of Hyamine, and incubated at 60 C for 4 hr before being mixed with 5 ml of toluene scintillator fluid [containing 0.05 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene and 4 g of 2,5-diphenyloxazole per liter] for radioactive assay. DNA uptake was defined as the amount of radioactivity remaining in the cell pellet after the above procedures.

Reagents. DEAE-D of mean molecular weight 2×10^6 was obtained from Pharmacia Inc., New Market, N.J.; poly-L-ornithine (mol wt 200,000), poly-L-lysine hydrobromide (mol wt 3,000), dextran sulfate (mol wt range 5 to 40×10^6), and crystalline pancreatic deoxyribonuclease were obtained from Sigma Chemical Co., St. Louis, Mo. Protamine sulfate (mol wt 6,000) was obtained from Calbiochem, Los Angeles, Calif.

RESULTS

Effect of DNA concentration and presence of DEAE-D on uptake of ^{32}P-labeled DNA. The uptake of DNA by mouse embryo monolayers at 37 C varied with DNA concentration (Fig. 1). In PBS alone, uptake increased approximately linearly for inputs below 2 to 5 μg/monolayer, but leveled off between 10 and 20 μg. In the presence of DEAE-D at 500 μg/ml, the concentration used for infectivity assay (15), uptake increased approximately linearly for DNA inputs up to at least 20 μg/monolayer.

At 37 C, with either PBS or DEAE-D, uptake increased linearly with time up to at least 60 min; the rate of uptake in DEAE-D was two- to threefold greater than in PBS.

The amount of uptake varied considerably between experiments with different cell culture batches; in PBS, with near-saturation inputs, uptake varied between 3 and 40% of the input.

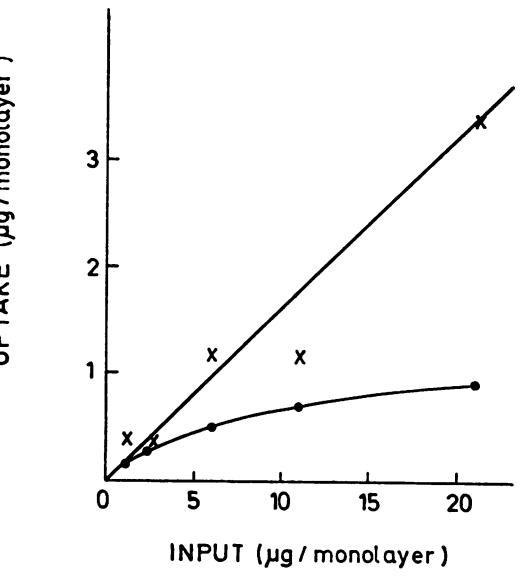

FIG. 1. *Dependence of uptake of component I on concentration in PBS and in the presence of DEAE-D. (Symbols: ●, PBS; ×, PBS + 500 μg of DEAE-D per ml.*

Although the cause of this variation remains unknown, uptake in DEAE-D was always greater than in PBS, usually by a factor of two- to five-fold; occasionally higher values (up to 14-fold) were obtained.

The uptake of components I and II + III followed closely the behavior found for unfractionated DNA.

In subsequent experiments, DNA concentrations falling within the linear ranges for uptake were used.

Dependence of uptake and infectivity of ^{32}P-labeled DNA on DEAE-D concentration. The concentration of DEAE-D present during adsorption influenced the amount of radioactive DNA taken up. In Fig. 2, uptake is compared with infectivity at different DEAE-D concentrations. (It was necessary to use much higher doses of DNA for measurement of radioactive uptake than for infectivity.) The responses to DEAE-D concentration were similar with broad maxima at 200 to 500 μg/ml, but uptake was relatively much greater than infectivity below 50 μg of DEAE-D per ml.

Effect of different adsorption conditions on ^{32}P-labeled DNA uptake. The magnitude of uptake was similar when DNA was added immediately after DEAE-D, when DEAE-D was added 15 min before DNA, or when DNA and DEAE-D were premixed for 20 min at 25 C before addition to the cells. These different

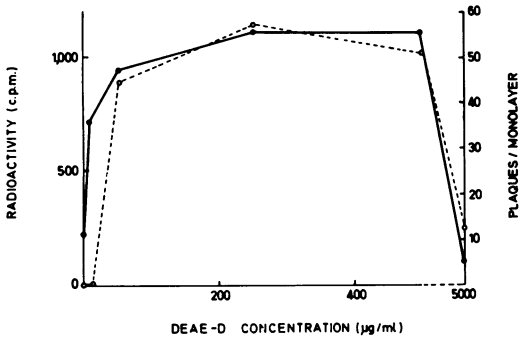

FIG. 2. *Dependence of uptake and infectivity of component I on DEAE-D concentration. Symbols:* ●, *radioactive uptake, input 2.5 μg/monolayer;* ○, *infectivity, input 2.5* × 10^{-2} *μg/monolayer.*

procedures applied in the infectivity assay gave the same number of plaques.

After treatment of the cells with DEAE-D, uptake was not changed significantly by washing the cells three times in PBS either before or after addition of labeled DNA. The same procedures applied in the infectivity assay resulted in a threefold or greater reduction in plaque count.

These results imply that for maximal infectivity the optimal concentration of DEAE-D must be maintained for a function other than that of increasing uptake.

Demonstration of DNA-DEAE-D complex formation. Mixtures of component I and component II + III with DEAE-D analyzed by sedimentation velocity gave asymmetric peaks sedimenting at about 40 to 60*S* (Fig. 3). Other analyses showed that 100-fold dilution of the mixtures in NaCl-EDTA did not dissociate the complexes. Thus, the complexes which result upon mixing DNA and DEAE-D are stable.

Fractions from the peak regions did not produce plaques in mouse embryo monolayers unless DEAE-D was added at the time of infection, as in the normal procedure.

Although it appears probable from preceding experiments that DNA complexed with DEAE-D has a stronger affinity for cells than does free DNA, possibly because of reduced negative charge, formation of complexes alone is insufficient to enhance infectivity.

Effects of other polyionic substances on DNA uptake and infectivity. The effects on uptake of other polycations known to enhance viral RNA infectivity (6, 11) were investigated. Since the average molecular weights and concentrations of the polyions varied, relative molecular concentrations are given in Table 1.

All polycationic substances stimulated uptake at least threefold compared to uptake in the presence of PBS only (Table 1). On a molecular basis, DEAE-D and polyornithine, which were higher in molecular weight, were much more

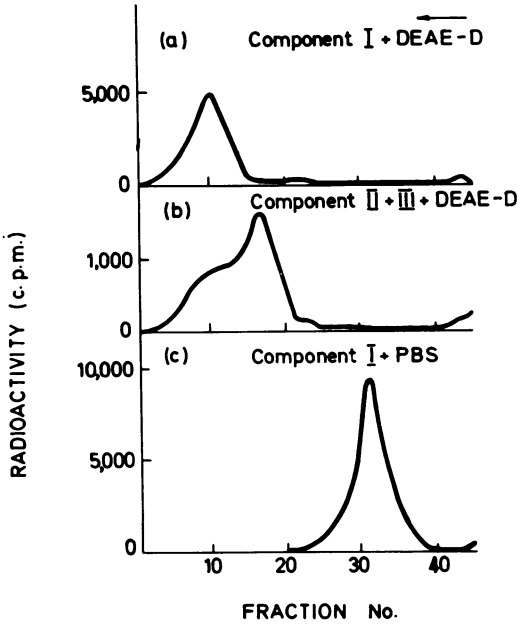

FIG. 3. *Sedimentation velocity analysis of complexes formed between polyoma virus DNA and DEAE-D. (a) Component I + DEAE-D (500 μg/ml). (b) Component II + III + DEAE-D (500 μg/ml). (c) Component I + PBS. Mixtures were incubated for 30 min at 37 C and then centrifuged for 3 hr at 39,000 rev/min on 10 to 30% sucrose gradients at pH 8.5.*

TABLE 1. *Uptake of* ^{32}*P-labeled polyoma virus DNA by mouse embryo cells in the presence of different polyions*[a]

Substance	Approx mol wt	Amt present/ monolayer (μg)	Ratio of no. of molecules of polyion to DEAE-D	Ratio of no. of molecules of polyion to DNA	Uptake (counts/min)
PBS					273
DEAE-D	2×10^6	100	1	300	2,346
Polyornithine	2×10^5	10	1	300	1,561
Protamine sulfate	6×10^3	10	33	10,000	852
Polylysine	3×10^3	10	67	20,000	970
Dextran sulfate	2×10^7	100	0.1	30	18

[a] Approximately 0.5 μg of unfractionated DNA was used per monolayer. Input radioactivity was 4,630 counts/min.

effective than protamine or polylysine. A polyanion, dextran sulfate, included for comparison, almost totally inhibited uptake.

Of these polyions, only polyornithine promoted formation of plaques, but at much lower efficiency than DEAE-D (Table 2). Polyornithine was grossly cytotoxic at concentrations greater than 10 μg/ml. DEAE-D added to cell monolayers already treated with low concentrations of polyornithine and DNA increased the plating efficiency to near that obtained with DEAE-D alone; the plating efficiency decreased at higher polyornithine concentrations (Table 2).

Complex formation between polyornithine and DNA, similar to that obtained with DEAE-D, was demonstrated by sedimentation analysis.

It is possible, since enchancement of SV40 DNA infectivity increases with increasing molecular weight of DEAE-D (7), that higher molecular weight preparations of the other polycations would be more effective.

Effect of mouse DNA on virus DNA uptake. The presence of mouse embryo cell DNA during adsorption, at concentrations greater than 0.1 μg/monolayer, markedly reduced the number of plaques formed by virus DNA in DEAE-D-treated monolayers (15). In our present work, the effect of mouse DNA on uptake of radioactive virus DNA was determined.

In the absence of DEAE-D, cell-associated radioactivity decreased steadily with increasing mouse DNA concentration; in the presence of DEAE-D it remained roughly constant up to 10 μg of mouse DNA per monolayer (Table 3). Total DNA uptake in PBS or DEAE-D, calculated from radioactive uptake and assuming that virus DNA and mouse DNA interact similarly, increased in a manner similar to that for virus DNA (Fig. 1). Treatment of monolayers with mouse DNA followed by washing in PBS inhibited radioactive virus DNA uptake 10-fold in the absence of DEAE-D, but had no effect when DEAE-D was added with virus DNA.

These results suggest that, although uptake capacity is high in the presence of DEAE-D, competition between virus and mouse DNA for a limited number of sites at a stage(s) after attachment reduces the number of plaques formed. Since mouse DNA at similar concentrations, in the presence of 500 μg of DEAE-D per ml, was without effect on the plating efficiency of intact virus (Table 4), interference at an intracellular site does not occur between mouse DNA and virus DNA released from virus particles. This suggests that mouse DNA inhibits the DNA infectivity response by competing for a limited number of sites available for transport of DNA into the cell.

Inhibition of cellular nuclease activity by DEAE-D. To study possible action of cellular nucleases we examined the sedimentation characteristics of DNA remaining unadsorbed after infection of washed monolayers with component I. Inocula were removed after an adsorption time of 1 hr and stored at 4 C in the presence of 0.01 M EDTA. Before analysis by sucrose gradient centrifugation or agar gel electrophoresis, sodium dodecyl sulfate (SDS) was added to a concentration of 0.5% to inhibit further nuclease degradation and to attempt to dissociate DNA-DEAE-D complexes. For reasons not yet determined, dissociation of DEAE-D complexes by SDS was not always

TABLE 2. *Plaque formation in the presence of polyornithine*

Sample	No. of plaques per plate at polyornithine concn (μg/ml)[a]					
	0	0.01	0.1	1	10	100
Without DEAE-D....	0	0	0	0.5	3	Lysis
With DEAE-D.	94	66	60	18	9	Lysis

[a] Values are averages of two plates.

TABLE 3. *Effect of mouse DNA on the uptake of ^{32}P-labeled component I*[a]

Dose of mouse DNA (μg)	Uptake[b]	
	Without DEAE-D	With DEAE-D
μg	*counts/min*	*counts/min*
0	227 (0.03)	842 (0.11)
0.1	211 (0.04)	1,066 (0.16)
1	137 (0.06)	836 (0.38)
10	57 (0.21)	682 (2.3)

[a] Dose of component I was 0.4 μg/monolayer. Input radioactivity was 3,150 counts/min per monolayer.

[b] Figures in parentheses represent estimated total DNA uptake, expressed in micrograms.

TABLE 4. *Plaque response to polyoma virus adsorbed in the presence of mouse DNA and DEAE-D*

DNA dose (μg)	No. of plaques/plate[a]
0	37
1	28
5	41
25	42

[a] Average plaque count in the absence of DNA and DEAE-D = 47. Values are averages of four plates.

complete, and a significant fraction of radioactivity sometimes remained in complex.

In the absence of DEAE-D, inocula stored for 24 hr at 4 C after removal from monolayers frequently showed complete degradation of component I to components of 0 to 10*S* (Fig. 4a); in other cases, all component I was eliminated, but the major fraction of the radioactivity sedimented in the II and III component regions.

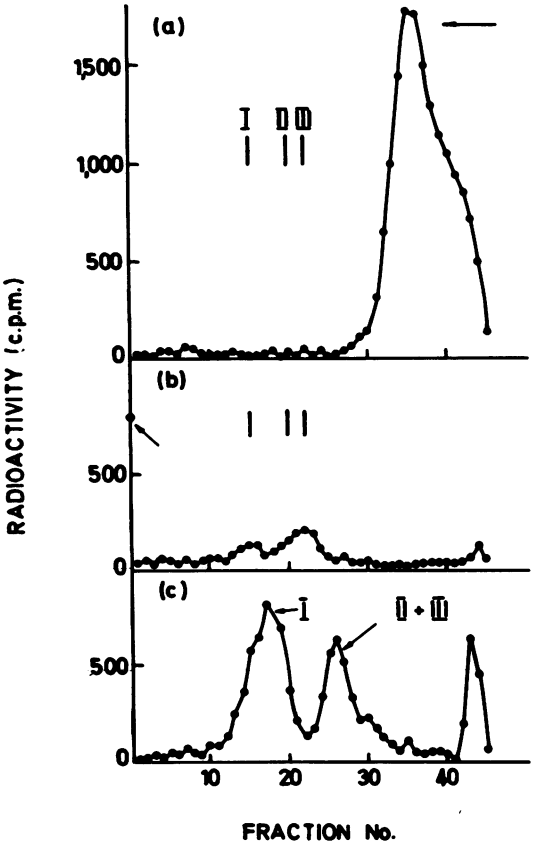

FIG. 4. *Sedimentation and electrophoretic analyses of radioactivity remaining unadsorbed after infection of mouse embryo monolayers with component I. (a) Adsorption in PBS. (b) Adsorption in PBS + 500 μg of DEAE-D per ml. (c) Adsorption in PBS + 500 μg of DEAE-D per ml. Analysis was by electrophoresis. Monolayers washed once with PBS were treated with 0.1 ml of DEAE-D (1,500 μg/ml) or with PBS in controls for 15 min at 37 C before the addition of 0.2 ml of component I containing about 100,000 counts/min. After 60 min at 37 C, the inoculum volume was removed and stored at 4 C in the presence of 0.01 M EDTA. SDS was added at 0.5% before analysis of 50 μliters by centrifugation for 4 hr at 48,000 rev/min on 5 to 25% sucrose gradients at pH 8.5 or by agar gel electrophoresis for 17 hr at 110 v. Vertical lines indicate positions of components in cosedimented, ^{3}H-labeled, unfractionated polyoma virus DNA in this and subsequent figures.*

In contrast, when inocula contained DEAE-D, at least 60% of the radioactivity recovered from the gradient sedimented as I or II + III with about 30% sedimenting to the bottom of the tube in an unresolved complex (Fig. 4b). Since about 70% of the radioactivity was lost on the tube walls during centrifugation at *p*H 8.5 in the presence of DEAE-D, alkaline gradient centrifugation and agar gel electrophoresis, which gave high recoveries, were preferable methods of analysis. Agar electrophoresis which gave nearly 100% recovery showed (Fig. 4c) that at least 50% of the radioactivity in inocula containing DEAE-D remained in the component I form; a minor proportion remained in complex at the origin. Similarly, when monolayers were infected with component II + III, extensive degradation occurred in PBS but not in the presence of DEAE-D.

Similar results were obtained with monolayers washed three times with PBS before infection and with monolayers grown at low serum concentration, which yield extracts of relatively low nuclease activity (5).

When SDS was added to inocula immediately after removal from monolayers, degradation in PBS did not proceed beyond the conversion of I to II + III components. In alkaline gradient analyses of similarly treated inocula containing DEAE-D, proportions of 50 and 40% sedimented in 53*S* and 16 to 18*S* regions, corresponding to component I and single-stranded DNA of substantially unaltered molecular weight, respectively; about 10% sedimented slower than 16*S*. Therefore, when SDS was added at the end of the adsorption period, degradation was still substantially greater in PBS than in the presence of DEAE-D.

Characteristics of cell-associated radioactivity. Attempts were also made to determine the sedimentation characteristics of cell-associated radioactivity. In one procedure, monolayers were washed with PBS at the end of the adsorption period, solubilized with 0.5% SDS, and extracted with phenol; samples of the aqueous layers were analyzed on sucrose gradients (*p*H 8.5). After infection in PBS, cell-associated virus DNA was much less degraded than that remaining in the inoculum, and a significant proportion of component I remained (Fig. 5a); this greater stability has yet to be explained. In the presence of DEAE-D, the proportion of component I was considerably higher (Fig. 5b), but only 30 to 40% of the total radioactivity was recovered in the aqueous phase; the remainder was in the interface and phenol layer, presumably as unresolved complex.

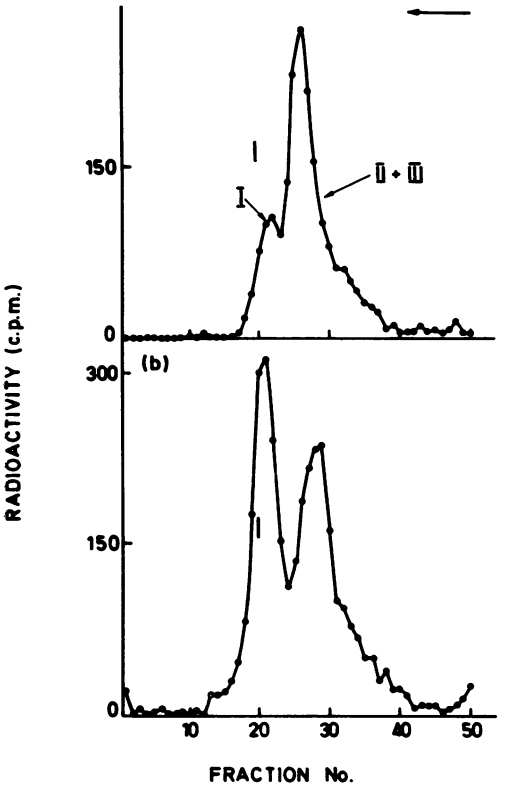

FIG. 5. *Sedimentation analysis of phenol-extracted cell-associated radioactivity after infection of mouse embryo monolayers with component I. (a) Adsorption in PBS. (b) Adsorption in PBS + 500 μg of DEAE-D per ml. Monolayers were treated and infected as described in Fig. 4. After removal of inocula monolayers were washed four times with PBS (minus Ca^{++} and Mg^{++}), drained, and then solubilized by the addition of 0.4 ml of 0.5% SDS containing 0.14 M NaCl and 0.1 M EDTA. After a single phenol extraction, 0.2 ml of the aqueous phases was centrifuged for 4 hr at 48,000 rev/min on 10 to 30% sucrose gradients at pH 8.5.*

In the second procedure, applied in a different experiment, SDS-solubilized cells were analyzed, without phenol extraction, on alkaline sucrose gradients. The proportion of freely sedimenting radioactivity recovered as component I was about 2.5 times greater in DEAE-D than in PBS (Fig. 6). In this procedure, about 50% of the radioactivity in monolayers infected in DEAE-D remained complexed and sedimented to the bottom of the tube (Fig. 6b).

Unless the unresolved complexes contain mainly 16 to 18S and slower-sedimenting components, it can probably be concluded that DEAE-D protects cell-associated as well as unadsorbed virus DNA against nuclease action.

Susceptibility of adsorbed DNA to the action of pancreatic deoxyribonuclease. The preceding experiments showed that a high proportion of the adsorbed DNA remained as component I. Most of this DNA is unavailable for infection since infection by DNA, even aided by DEAE-D, is very inefficient. To explore its location in the cell, the effect of pancreatic deoxyribonuclease on cell-associated virus DNA was examined.

After adsorption of DNA for 1 hr at 37 C, monolayers were washed twice with PBS, incubated with 100 μg of deoxyribonuclease per ml for 15 min at 25 or 37 C, washed to remove deoxyribonuclease and degraded DNA, and solubilized with SDS for measurement of radioactivity.

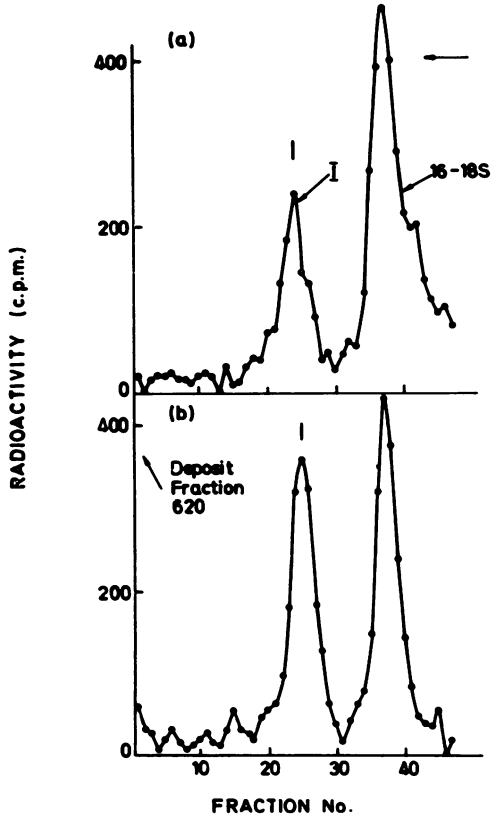

FIG. 6. *Sedimentation analysis on alkaline gradients of SDS-extracted cell-associated radioactivity after infection of mouse embryo monolayers with component I. (a) Adsorption in PBS. (b) Adsorption in PBS + 500 μg of DEAE-D per ml. Monolayers were infected as described in Fig. 4, but with about 40,000 counts/min per monolayer, and were solubilized with SDS as described in Fig. 5. To 0.1 ml of the SDS extracts, 0.1 ml of 5% SDS was added, followed by 20 μliters of 1·2 N KOH, before centrifugation on 10 to 30% alkaline sucrose gradients for 1.5 hr at 48,000 rev/min.*

The cell-associated radioactivity of monolayers infected in the absence of DEAE-D was reduced to less than 10% of that in untreated cells, and all sedimented between 0 and 10S. In the presence of DEAE-D, it was reduced to 20 to 30% of that in untreated monolayers, about half sedimenting as component I and half as a group of components between 0 and 16S.

Since it was possible that deoxyribonuclease could enter the cells and degrade intracellular DNA, these results alone possibly were insignificant. However, it was found that deoxyribonuclease applied in a similar manner at concentrations up to 400 μg/ml had little effect on the plating efficiency of DNA in the infectivity assay (Table 5). This showed that deoxyribonuclease did not degrade intracellular DNA, and it supports the conclusion that at least 70 to 80% of the DNA attaching to cells in the presence of DEAE-D (and presumably nearly 100% in its absence) was located at the surface and accessible to deoxyribonuclease. The amount of the deoxyribonuclease-resistant remainder which was intracellular is uncertain since deoxyribonuclease action probably is partially inhibited by residual DEAE-D. However, under similar conditions in the absence of cells, 80% of component I was degraded to components of 0S, even in the presence of 500 μg of DEAE-D per ml.

Whether intracellular or not, the increased proportion of deoxyribonuclease-resistant DNA could be a factor determining infectivity enhancement.

DISCUSSION

Although sedimentation analyses show that stable complexes, probably electrostatic in nature (1, 10, 14), form between polyoma virus DNA and DEAE-D, it is doubtful, for the following reasons, that this alone leads to increased infectivity. First, preformed complexes isolated by centrifugation were noninfectious unless monolayers were treated as usual with DEAE-D. Second, the plaque response was considerably reduced if monolayers were washed after adsorption, although this did not reduce bound radioactivity or, probably, dissociate complexes.

Also, although DNA-DEAE-D interaction may cause increased uptake, it is unlikely that increased infectivity results only from this, since infectivity is increased by a much bigger factor than uptake at the optimal DEAE-D concentrations; also, uptake is increased at DEAE-D concentrations below 50 μg/ml which only slightly increase plating efficiency. In addition, other polycations which increased uptake had little effect on infectivity.

It seems probable, therefore, that increased plating efficiency depends on more than one action of DEAE-D. The present evidence indicates that intra- and extracellular inhibition of the strong nuclease activity associated with these cells is an important factor. The discrepancy between the magnitudes of increase of infectivity and radioactivity uptake would be explained if high DEAE-D concentrations were necessary for effective nuclease inhibition; lower concentrations, although sufficient to increase uptake, might inhibit nuclease insufficiently. The failure of other polycationic substances tested could also result from insufficient nuclease inhibition. It has often been suggested that hypertonic methods for increasing infectivity may depend on inhibition of nucleases, since many nucleases are inhibited by high salt concentrations. Possibly, DEAE-D inhibits more efficiently than high salt concentrations. The existence of a cell-associated nuclease which degrades viral ribonucleic acid (RNA) has been established by Bases and Huppert (2), and it has been reported that DEAE-D partially protects viral RNA against digestion with pancreatic ribonuclease (1, 8, 9).

The possibility that inhibition of a nuclease which destroys messenger RNA coded by viral DNA increases infectivity is unlikely because DEAE-D does not significantly influence the infectivity of intact polyoma virus (Table 4; *unpublished data*).

The sedimentation properties of the degradation products of component I indicate that endonuclease activity producing single-strand breaks predominated. This activity, like that of deoxyribonuclease II, may be activated by EDTA (3); this could contribute to the increased degradation when addition of SDS was delayed. Examination of enzymological properties and of possible inhibitors of greater activity than DEAE-D might

TABLE 5. *Effect of postadsorption treatment with pancreatic deoxyribonuclease on plating efficiency of component I*[a]

Adsorption time (min)	No. of plaques per plate at deoxyribonuclease (μg/ml)[b]			
	0	10	100	400
5	11	5	5	6
30	39	34	29	20
60	96	91	75	67

[a] Monolayers previously treated with DEAE-D (0.1 ml, 1,000 μg/ml) were incubated at 37 C with DNA dilutions (0.1 ml), washed twice with PBS, and then incubated with deoxyribonuclease in 0.25 ml of PBS containing 0.01 M $MgCl_2$ for 15 min at 25 C before addition of overlay medium.

[b] Values are averages of four plates.

lead to greater plating efficiencies for infectious DNA. Since strong nuclease activity, inhibited by DEAE-D, was also found to be associated with BHK21 cells (*unpublished data*), improved transformation efficiencies for DNA, using these cells, could also be expected.

The effect of deoxyribonuclease on plaque formation in monolayers infected in the presence of DEAE-D demonstrates that DNA initiating infection becomes immune from the action of the enzyme. This may be because deoxyribonuclease does not enter the cell, because DNA forms an enzyme-resistant association with a cellular component, or because DEAE-D continues to protect the DNA. The location of the remainder of the deoxyribonuclease-resistant radioactivity, which rough estimates indicate still represents at least 1,000 times the amount of DNA needed to initiate infection on the basis of one molecule per cell, is not clear. It may be mainly intracellular, which might indicate that DEAE-D increases the rate of penetration of DNA by some action on the cell, apart from increasing the amount of undegraded DNA available for penetration; however, the reduction in infectivity after washing with PBS is against this possibility. Alternatively, the radioactivity may be mainly superficially located but protected from deoxyribonuclease by residual DEAE-D.

Our results demonstrate the considerable reduction in the background of nonparticipating DNA which can be achieved by the use of deoxyribonuclease and they suggest that further analysis of the location and macromolecular state of the deoxyribonuclease-resistant fraction should help to resolve these questions.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Bachrach, H. L. 1966. Ribonucleic acid of foot-and-mouth disease virus: an ultrasensitive plaque assay. Proc. Soc. Exp. Biol. Med., 123:939–945.
2. Bases, R. E., and J. Huppert. 1966. Impairment of EMC viral infectivity by cell-associated ribonuclease. Virology 29:596–604.
3. Bernardi, G. 1966. Spleen acid deoxyribonuclease, p. 102–121. *In* G. L. Cantoni and David R. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York and London.
4. Dulbecco, R., and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis virus. J. Exp. Med. 99:167–182.
5. Fried, M., and J. D. Pitts. 1968. Replication of polyoma virus DNA: a resting cell system for biochemical studies on polyoma virus. Virology 34:761–770.
6. Koch, G., N. Quintrell, and J. M. Bishop. 1966. An agar-cell-suspension plaque assay for isolated viral RNA. Biochem. Biophys. Res. Commun. 24:304–309.
7. McCutchan, J. H., and J. S. Pagano. 1968. Enhancement of the infectivity of Simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. J. Nat. Cancer Inst. 41:351–356.
8. Maes, R., W. Sedwick, and A. Vaheri. 1967. Interaction between DEAE-dextran and nucleic acids. Biochim. Biophys. Acta 134:269–276.
9. Pagano, J. S., J. H. McCutchan, and A. Vaheri. 1967. Factors influencing the enhancement of the infectivity of polio virus ribonucleic acid by diethylaminoethyl-dextran. J. Virol. 1:891–897.
10. Pagano, J. S., and A. Vaheri. 1965. Enhancement of infectivity of poliovirus RNA with diethylaminoethyl-dextran (DEAE-D). Arch. Gesamte Virusforsch. 17:456–464.
11. Smull, C. E., and E. H. Ludwig. 1962. Enhancement of the plaque-forming capacity of poliovirus ribonucleic acid with basic proteins. J. Bacteriol. 84:1035–1040.
12. Thorne, H. V. 1967. Electrophoretic characterization and fractionation of polyoma virus DNA. J. Mol. Biol. 24:203–211.
13. Thorne, H. V., J. Evans, and D. Warden. 1968. Detection of biologically defective molecules in component I of polyoma virus DNA. Nature 219:728–730.
14. Tovell, D. R., and J. S. Colter. 1967. Observations on the assay of infectious viral ribonucleic acid: effects of DMSO and DEAE-dextran. Virology 32:84–92.
15. Warden, D., and H. V. Thorne. 1968. The infectivity of polyoma virus DNA for mouse embryo cells in the presence of diethylaminoethyl-dextran. J. Gen. Virol. 3:371–377.