A New DNA-exonuclease in Cells Infected with Herpes Virus: Partial Purification and Properties of the Enzyme

By J. M. MORRISON AND H. M. KEIR
Institute of Biochemistry, University of Glasgow, Glasgow, W. 2, Scotland

(Accepted 2 May 1968)

SUMMARY

Infection of BHK 21 (C 13) or HEp-2 cells with herpes virus was followed by a marked increase in the activity of alkaline DNase which was prevented by actinomycin D and puromycin. Activation of a latent enzyme was not responsible for the increase. The properties of the DNase appearing after virus infection in both cell types were the same, but they differed from those of the enzyme from uninfected cells in specificity towards the secondary structure of the DNA substrate, heat-stability, requirement for thiol groups, inhibition by K⁺ and Na⁺ ions and response to various concentrations of Mg²⁺ and Mn²⁺. The activities of acid DNase and alkaline phosphomonoesterase were not significantly altered after herpes infection. Further, the activity of alkaline RNase was not altered by infection, and this implies that the induced DNase was specific for DNA. The new DNase could be separated from the DNase present in uninfected cells and from alkaline phosphomonoesterase by chromatography on columns of DEAE-cellulose; its enzymic properties were the same as those observed with soluble extracts of cells infected with herpes virus.

INTRODUCTION

Infection by DNA viruses commonly leads to elevation of the level of deoxyribonuclease (DNase) in both bacterial and animal cells (Lehman, 1967). Following from the work of Keir & Gold (1963), who showed that infection by herpes virus caused increased levels of DNase in baby hamster kidney (BHK) cells in cell culture, we have undertaken a study of this virus-induced enzyme in order to determine whether its synthesis is directed by the viral genome and if so its role in the infective process. Throughout this paper the term 'induced' is used in its broadest sense, to describe a change occurring after virus-infection without necessarily attributing this function directly to the viral genome. Preliminary accounts of some of this work have been published (Morrison & Keir, 1966, 1967).

METHODS

Reagents. Standard saline-citrate (SSC) was 0.15 M-NaCl + 0.015 M-trisodium citrate. ³²P-labelled inorganic orthophosphate was purchased from the Radiochemical Centre, Amersham, Buckinghamshire; puromycin hydrochloride from the Sigma (London) Chemical Co., Ltd., London; pancreatic DNase and RNase from the Worthington Biochemical Corp., Freehold, N.J.; pronase from Calbiochem, Ltd.,
Preparation of DNA. DNA was prepared from calf thymus or Landschütz ascites-tumour cells by the method of Kay, Simmons & Dounce (1952). [³²P]DNA was prepared by growing Escherichia coli in the presence of [³²P]orthophosphate (Lehman, 1960). The cells were washed twice, resuspended in SSC (100 ml./g. wet weight of cells), and lysed by the addition of sodium dodecyl sulphate to 1 %, followed by incubation at 37°. Pronase was then added to a final concentration of 1 mg./ml. and incubation continued overnight at 37°. The solution was gently shaken with an equal volume of 90 % (w/v) phenol in SSC, and the phases were separated by centrifugation for 15 min. at 1500 g in the general purpose swing-out rotor of the MSE Major refrigerated centrifuge. After addition of two volumes of ethanol to the aqueous phase, the DNA was 'spooled' from solution. It was then redissolved in SSC diluted 1/10, incubated for 30 min. at 37° with pancreatic RNase (which was first heated for 10 min. at 80° to inactivate any possible contamination by DNase) at a final concentration of 20 μg./ml., and the solution extracted with phenol as before. The DNA was precipitated from the aqueous phase with ethanol, redissolved in 1/10 SSC, dialysed overnight at 4° against 0.02 M-KCl and stored at 4° over chloroform. More than 95 % of the final product was rendered acid-soluble by incubation with pancreatic DNase; only about 2 % of the radioactivity in the DNA preparation was rendered acid-soluble when incubated without the pancreatic DNase.

Denaturation of DNA was carried out by heating the DNA solution (200 μg./ml. or less) for 10 min. at 100° at an ionic strength of 0.02 or less, followed by rapid cooling to 0°.

Preparation of RNA. RNA was prepared from a cytoplasmic fraction of Landschütz ascites-tumour cells by phenol extraction and two ethanol precipitations, based on the method of Kirby (1956).

Assay of enzymes. DNase was assayed by following release of acid-soluble products from DNA by measurement either of extinction at 260 nm. or of radioactivity. [³²P]DNA was measured in an automatic Nuclear Chicago gas-flow counter. The standard alkaline DNase assay (0.2 ml.) contained DNA 300 μg./ml. (non-radioactive) or 50 μg./ml. ([³²P]-labelled); Mg²⁺, 2 mM; tris+HCl buffer, pH 9, 50 mM; 2-mercaptoethanol, 10 mM, and a suitable amount of enzyme. After incubation for 30 min. at 37°, the tubes were cooled on ice. Bovine serum albumin (400 μg. in 0.2 ml.) or denatured DNA (200 μg. in 0.2 ml.) was added as co-precipitant, and finally 0.6 ml. of N-perchloric acid containing 12 mg. of Hyflo-Super-Cel. After 10 min. at 0°, the suspension was centrifuged (10 min. at 800 g in the MSE Major refrigerated centrifuge) and the extinction at 260 μm. or the radioactivity of the supernatant fluid measured. The reaction rate was linear for at least 60 min. and 50 % hydrolysis of the substrate DNA, and all experiments were conducted under these standard conditions.

A unit of DNase is that amount of enzyme which renders 10 mmoles of DNA-nucleotide acid-soluble in 30 min. at 37° under the standard conditions. The molar extinction coefficient of a solution of mixed nucleotides is taken as 10,000/cm. light path.

Acid DNase activity was measured by release of acid-soluble fragments of DNA at
New DNA-exonuclease in herpes-infected cells

pH 4.5 in 0.1 M-sodium acetate in the absence of Mg\(^{2+}\) ions. RNase was measured in a similar assay at pH 8 in 50 mM-tris+HCl buffer, 2 mM-Mg\(^{2+}\), by release of acid-soluble ultraviolet-absorbing material from ascites-tumour cell RNA, and *alkaline phosphomonoesterase* by hydrolysis of p-nitrophenyl phosphate at pH 9 as described by Curtis, Burdon & Smellie (1966). DNA polymerase was measured under optimal conditions for assay of the enzyme activities from uninfected and virus-infected cells (Keir *et al.* 1966). The assay measures incorporation of \([\alpha-32\text{P}]\)deoxythymidine 5'-triphosphate into acid-insoluble polydeoxyribonucleotide in the presence of the 5'-triphosphates of deoxyadenosine, deoxycytidylne and deoxyguanosine, and DNA template.

Protein was measured by the method of Lowry *et al.* (1951). Precipitation of protein with 5% trichloroacetic acid preceded the assay when necessary; for example, when high levels of KCl or 2-mercaptoethanol (both of which interfere with the estimation) were present.

*Heating of enzymes before assay.* Enzyme fractions were incubated at the elevated temperature for various times, cooled on ice and assayed under standard conditions. The amount of enzyme present before incubation was within the limits of linearity of the assay.

*Growth of cells and infection with virus.* BHK 21 (C 13) (Macpherson & Stoker, 1962) and HEp-2 cells were used. They were grown in monolayer cultures in Petri dishes, or, for large-scale work, in rotating 80 oz bottles (House & Wildy, 1965) in a modified Eagle's medium (Vantsis & Wildy, 1962) containing 10% tryptose phosphate broth and 10% calf serum. Infection with herpes simplex virus (strain HFEM2) was performed at an input multiplicity of 10 to 15 p.f.u./cell with an adsorption period of 30 min. Over 90% of cells were infected under these conditions.

*Preparation of extracts.* Cells were harvested and extracts prepared at 0° to 4° as described by Morrison & Keir (1966), or by subjecting the cells to ultrasonic vibrations (MSE Ultrasonic Disintegrator) in 0.1 M-tris+HCl buffer pH 8, 2 mM-EDTA, 10 mM-2-mercaptoethanol, followed by centrifugation for 30 min. at 30,000 g in rotor no. 69181 of the MSE ‘Hi-Speed 18’ centrifuge.

Nuclei were prepared from BHK cells by homogenization in 0.25 M-sucrose, 3 mM-CaCl\(_2\), 20 mM-tris+HCl buffer pH 8, 5 mM-2-mercaptoethanol after scraping the cells from the glass in the same medium. The nuclei were sedimented (10 min. at 800 g in the MSE Major refrigerated centrifuge), resuspended in the above medium by homogenization and centrifuged again. The two supernatant fractions were pooled, dialysed overnight against two changes of 0.15 M-KCl, 20 mM-tris+HCl buffer pH 8, 1 mM-EDTA, 5 mM-2-mercaptoethanol and centrifuged for 30 min. at 30,000 g; the supernatant fluid was termed the *cytoplasmic* fraction. The washed nuclei were resuspended in the above dialysis buffer, disrupted by ultrasonic vibration, dialysed as above, and centrifuged for 30 min. at 30,000 g. The supernatant fluid was termed the *nuclear* fraction.

Before disruption, the nuclei were examined microscopically after staining with aceto-orcein-fast-green (Kurnick & Ris, 1948). Contamination with cytoplasmic material was minimal; further treatments did not improve the quality of the nuclei even when the non-ionic detergent Triton X-100 (1%) was used.

Extracts were stored at 0° in the presence of 5 to 10 mM-2-mercaptoethanol. DNase activity was stable for at least 1 month under these conditions. Storage as a suspension
in an 80% (w/v) saturated solution of ammonium sulphate gave stability over longer periods.

RESULTS AND DISCUSSION

Levels of DNase in cells infected with herpes virus

When BHK or HEp-2 cells are infected with herpes virus, marked increases in the levels of DNA polymerase (DNA nucleotidyltransferase EC 2.7.7.7) and DNase are observed (Keir & Gold, 1963). We confirmed and extended these observations (Fig. 1). DNase activity on denatured DNA substrate also rose over the same period, but less markedly, because of the greater activity of the host cell DNase on this substrate (see below). DNA polymerase rose sevenfold over the period 5 to 12 hr and infective virus first appeared between 8 and 12 hr after infection. From other experiments conducted under the same growth conditions (J. Hay, J. M. Morrison & H. M. Keir, unpublished results) the most active period of viral DNA synthesis was 6 to 12 hr after infection.

![Graph showing DNase activity following infection of BHK cells with herpes virus.](image)

**Fig. 1.** Induction of DNase activity following infection of BHK cells with herpes virus. ○, Uninfected cells; ●, cells infected at an input multiplicity of 15 p.f.u. per cell; ▲, cells infected at an input multiplicity of 15 p.f.u. per cell, in the presence of puromycin hydrochloride (50 μg./ml. throughout the experiment). Native DNA was used as substrate.

Other enzymes

The levels of several other enzymes before and after infection of BHK cells were also examined (Table 1).

Herpes-infected HEp-2 cells also showed no increased in acid DNase activity. These results are in agreement with those of McAuslan et al. (1965) and Flanagan (1966), but not with those obtained by Newton (1964). Thus, herpes virus differs from vaccinia virus which does induce an acid DNase (McAuslan & Kates, 1967).

Effect of inhibitors

Puromycin added at the time of infection inhibited the increase in DNase caused by herpes infection (Fig. 1). It also prevented the increase in DNA polymerase. This
New DNA-exonuclease in herpes-infected cells

implies that synthesis of new protein is required for induction of both enzymes. Actinomycin C completely abolished the increased enzyme levels due to herpes infection when added to cultures 2 hr after infection or earlier, but the effect became progressively less the later the drug was added (Fig. 2), suggesting that m-RNA for the induced enzymes is made between 2 and 3 hr after infection onwards, by transcription from DNA.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Units/mg. of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid DNase*</td>
<td>Control</td>
</tr>
<tr>
<td>Native DNA</td>
<td>38</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>15</td>
</tr>
<tr>
<td>Denatured DNA</td>
<td>20</td>
</tr>
<tr>
<td>Nucleus</td>
<td>8</td>
</tr>
<tr>
<td>Alkaline RNase*</td>
<td>9.1</td>
</tr>
<tr>
<td>Alkaline phosphomonoesterase†</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* One unit = that amount of enzyme which renders acid-soluble 10 μmoles of nucleic acid nucleotide in 30 min. at 37°C.
† One unit = that amount of enzyme which liberates 10 μmoles of p-nitrophenol from p-nitrophenylphosphate in 60 min. at 37°C.

Fig. 2. Effect of actinomycin D on enzyme induction by herpes virus. ○, DNA polymerase activity assayed under conditions optimal for the induced enzyme (Keir et al. 1966); ●, DNase activity on native DNA substrate. The final concentration of actinomycin D was 0.5 μg./ml.

Intracellular location of induced DNase

Nuclear and cytoplasmic fractions were prepared from control and herpes-infected BHK cells, 6 hr after infection, when the bulk of the DNase increment was located in the cytoplasm, in contrast to the increment of DNA polymerase which had mainly a nuclear location. Subcellular fractionation of infected cells harvested at various times after infection showed that a greater proportion of the induced DNase was found in the nuclear fraction at later times (Table 2).
Mixing of control and herpes-infected cell extracts

When extracts of control and infected cells were assayed for DNase separately and after mixing in various proportions, the activities of the mixtures were approximately the same as the sum of the activities of the component extracts, provided that the total did not exceed the limits of the assay; that is, 50% hydrolysis of substrate. This result suggests that there is neither excess of an inhibitor of the virus-induced DNase in uninfected cells nor of an activator in herpes-infected cells.

Partial purification of the herpes-induced DNase

Purification of the herpes-induced DNase was undertaken with the specific aims of separating the induced enzyme activity from the DNase activity of uninfected cells, and removing factors (for example, phosphomonoesterase) which would interfere with further studies on the enzyme.

Table 2. Intracellular location of herpes-induced enzymes*

<table>
<thead>
<tr>
<th></th>
<th>Cytoplasm</th>
<th>Nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNase†</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>183 (86%)</td>
<td>37 (14%)</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA polymerase†</td>
<td>945</td>
<td>297</td>
</tr>
<tr>
<td>Control</td>
<td>1166 (18%)</td>
<td>1302 (82%)</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Enzymes prepared from non-infected BHK cells and from cells infected with virus for 6 hr.
† Results expressed as total units of enzyme per subcellular fraction (see Methods).
Figures in parentheses represent the proportions of the enzyme increments found after infecting in each fraction.

Salt fractionation. Fractional precipitation with ammonium sulphate at pH 8 and 0º gave the DNase activity from both control and herpes-infected cells in the 25 to 50% saturated fraction. No separation of activities was obtained, but this procedure was a useful concentration step before column chromatography; moreover, a two- to threefold purification was generally achieved. In addition, a greater measure of stability was conferred upon the enzyme by making a suspension of the fraction in 80% saturated ammonium sulphate for long-term storage.

Gel filtration. Passage of extracts of infected cells through columns of Sephadex G-200 yielded two peaks of DNase activity, one eluting with the void volume and the other at an elution volume close to that of haemoglobin. Extracts of uninfected cells contained only the latter enzyme peak, and this is probably similar to that purified by Curtis et al. (1966) from rat liver. Phosphomonoesterase activity was also divided between two peaks, one of which coincided with the void volume. Thus, although gel filtration separated the DNases of control and herpes-infected cells, the presence of the phosphomonoesterase, combined with a poor recovery of enzyme activity (less than 20%), precluded its use as a routine purification step. The observed behaviour of the DNase was not due to aggregation or association with high molecular weight material (for example, nucleic acids) since treatment with the non-ionic detergent Triton X-100 or prior passage through DEAE-cellulose did not alter the elution volume of the enzyme. These experiments indicated therefore a high molecular weight for the herpes-induced DNase.
New DNA-exonuclease in herpes-infected cells

Ion-exchange chromatography. Following the observation of Curtis et al. (1966) that a rat liver DNase was not adsorbed to DEAE-cellulose under conditions where the bulk of the other protein was retained, extracts of herpes-infected cells were passed through DEAE-cellulose in 0.05 M tris-HCl, pH 8. It was found that the unadsorbed DNase was similar in properties and quantity to that found in uninfected cells.

![Graph showing chromatography of DNases of herpes-infected BHK cells on DEAE-cellulose.](image)

Fig. 3. Chromatography of the DNases of herpes-infected BHK cells on DEAE-cellulose. The enzyme preparation was applied to a 10 x 1 cm. column in 0.05 M-tris-HCl, pH 8, 1 mM-EDTA, 5 mM-2-mercaptoethanol, and the column flushed with the same buffer. The linear elution gradient was started at the point indicated by the arrow, with 100 ml. of the above buffer in the mixing chamber and 100 ml. of buffer containing 0.5 M-KCl in the reservoir. Fractions of 5 ml. vol. were collected. Δ --- Δ, E_{ase}; • --- •, DNase activity on native DNA substrate; ○ --- ○, DNase activity on denatured DNA substrate; --- --- ---, linear KCl concentration gradient.

DEAE-cellulose chromatography of an ammonium sulphate fraction (25 to 50% saturation) of herpes-infected BHK cells was performed with elution by a linear KCl gradient. Two distinctly resolved peaks of DNase activity were obtained (Fig. 3). Peak I, which was not retained by DEAE-cellulose, was most active with denatured DNA as substrate, while peak II, which was adsorbed and subsequently eluted between 0.1 M and 0.15 M-KCl, preferentially hydrolysed native DNA and had properties similar to those of the DNase increment induced by herpes infection. A more rapid separation of the two DNases could be achieved by stepwise elution with 0.1 M and 0.2 M-KCl, the bulk of the induced DNase appearing in the 0.2 M eluate. Nuclear and cytoplasmic fractions of herpes-infected cells both showed the same qualitative elution pattern, while extracts of uninfected cells gave only peak I, clearly indicating that peak II represented the DNase activity appearing after herpes infection. Phosphomonoesterase was eluted by the gradient before peak II.

Use of DEAE-cellulose separated, in one step, the two most serious contaminants...
from the herpes-induced DNase. Recovery of enzyme was satisfactory (Table 3) and at least tenfold purification of the herpes-induced DNase was achieved (an underestimate because of removal of the host-cell DNase). Increased lability of the enzyme after passage through the ion-exchanger (see below) made further purification difficult. Work is in hand, however, to resolve the herpes-induced DNase and DNA polymerase, which elute together from DEAE-cellulose, in order that these two activities may be studied separately.

Table 3. Purification of the herpes virus-induced DNase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml.)</th>
<th>Total protein (mg.)</th>
<th>Activity of DNase* (Total units)</th>
<th>(Units/mg. of protein)</th>
<th>Recovery (%)</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic extract</td>
<td>50</td>
<td>125</td>
<td>495</td>
<td>14</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>(NH4)2SO4 precipitate</td>
<td>6</td>
<td>50</td>
<td>366</td>
<td>25</td>
<td>74</td>
<td>2</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-retained KCl eluate</td>
<td>25</td>
<td>6-2</td>
<td>310</td>
<td>155</td>
<td>72</td>
<td>11</td>
</tr>
<tr>
<td>KCl eluate peak tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* One unit = that amount of enzyme which renders acid-soluble 10 μmole of DNA-nucleotide in 30 min. at 37°.

Properties of the DNases of herpes-infected cells

When the properties of the DNase activity induced by herpes infection were examined and compared with those of the DNase of uninfected cells (both BHK and HEp-2), the following outstanding points of difference emerged.

Specificity towards secondary structure of the substrate. The DNase of BHK or HEp-2 cells, active in the alkaline range of pH, preferentially hydrolysed denatured DNA, and showed little, if any, activity towards native DNA. After infection with herpes virus, however, the activity of DNase attacking native DNA increased markedly (Fig. 1); and extracts of herpes-infected cells generally hydrolysed native DNA more rapidly than denatured DNA. The partially purified enzyme displayed the same specificity (Fig. 3).

Heat stability. The DNase activity of extracts of herpes-infected cells was more heat-labile at 50° than the DNase of control cells (Fig. 4). These heat-stability data confirm the intracellular location of the induced enzyme. The bimodal nature of the inactivation curve of the DNase of the infected cytoplasmic fraction corresponds to a rapid inactivation of the herpes-induced DNase followed by a slower destruction of the pre-existing host cell enzyme. The heat-inactivation curve of the DNase activity of unfractonated extracts of infected cells was also bimodal at 46° in 25 % (v/v) glycerol. After purification on DEAE-cellulose, the herpes-induced DNase was even more sensitive to heating (Fig. 5) and the inactivation curve at 46° was unimodal, suggesting that only one enzyme activity was present in this fraction. In contrast, the unadsorbed DEAE-cellulose fraction, from either infected or uninfected cells, was stable at 46° like the DNase of unfraktionated host cell extracts (Fig. 5).

Stabilization by 2-mercaptoethanol. In extracts of herpes-infected cells prepared in
the absence of 2-mercaptoethanol, less than 20% of the normal DNase increment was observed and the activity was only partly restored by adding the reducing agent before assaying. The DNase of the host cell was not dependent on the presence of mercaptoethanol in the extracting medium.

Fig. 4. Heat inactivation of the DNase activities from nuclear and cytoplasmic fractions of control and herpes-infected BHK cells. Denatured DNA (200 μmoles of DNA-nucleotide in each assay) was the substrate. O, Control cytoplasmic; □, control nuclear; ●, cytoplasmic 6 hr after infection; ■, infected nuclear.

Effect of univalent cations. The DNase of uninfected cells (after passage through DEAE-cellulose, see Fig. 3) was 55% inhibited at 50 mM-NaCl or KCl and 80% at 100 mM. On the other hand, the partially purified, herpes-induced DNase was 20% and 40% inhibited at the same respective concentrations of NaCl and KCl. These results differed somewhat from those obtained using the initial soluble extracts of cells (Morrison & Keir, 1966) in that the stimulation of the induced DNase at 60 mM-KCl was not observed; however, the herpes-induced DNase was always clearly less sensitive to inhibition by NaCl or KCl than the host enzyme, both before and after purification.

Effect of bivalent cations. The response of the herpes-induced DNase to varying concentrations to bivalent cations differed somewhat from that of the host-cell DNase. Both enzymes were absolutely dependent upon the presence of a bivalent cation for activity, both Mg²⁺ and Mn²⁺ being capable of fulfilling this function (Morrison & Keir, 1966). Using the partially purified DNases (after chromatography on DEAE-
cellulose, see Fig. 3) the following results were obtained. The optimal Mg$^{2+}$ ion concentration for the host-cell DNase was 0·5 to 1·0 mM; the activities at 4 mM, 8 mM and 16 mM were respectively 85%, 70% and 53% of that at the optimum. On the other hand, the herpes-induced DNase displayed a Mg$^{2+}$ optimum at about 8 mM; the activities at 1 mM, 2 mM, 4 mM and 16 mM were respectively 56%, 68%, 80% and 94% of that at 8 mM.

When the partially purified enzymes were assayed with Mn$^{2+}$ ions, further differences were observed. The host-cell DNase was optimally active at 0·25 mM Mn$^{2+}$; the activities at 1 mM, 2 mM, 4 mM and 8 mM were respectively 70%, 62%, 37% and 1% of that at 0·25 mM. In contrast, the virus-induced DNase was optimally active at 1 mM Mn$^{2+}$; at 0·25 mM-, 0·5 mM-, 2 mM-, 4 mM- and 8 mM-Mn$^{2+}$, the activities were 47%, 74%, 96%, 72% and 30% of that at 1 mM Mn$^{2+}$. The ratio of DNase activity at the Mn$^{2+}$ optimum to that at the Mg$^{2+}$ optimum was 2·15 for the host-cell enzyme and 0·4 for the herpes-induced enzyme.

**Substrate specificity**

The absence of stimulation of RNase activity after herpes-infection suggests that the herpes-induced nuclease is specific for DNA. In addition, analysis of the products of digestion of RNA by extracts of control and herpes-infected cells revealed that they
New DNA-exonuclease in herpes-infected cells

were qualitatively and quantitatively similar, only oligonucleotides being produced. In view of the exonucleolytic action of the herpes-induced DNase (Morrison & Keir, 1967), the absence of mononucleotides suggests that RNA is not hydrolysed by the induced DNase.

We thank Professor J. N. Davidson, F.R.S., and Professor R. M. S. Smellie for their interest and support, and Dr H. Subak-Sharpe for kindly co-operating in the growth of cells and virus. We also acknowledge with thanks the skilled technical assistance given by Miss Helen Moss. The investigation was aided by a grant from the British Empire Cancer Campaign.

REFERENCES


(Received 6 February 1968)