large phosphatidylethanolamine particles are hydrolysed without the necessity for the addition of diethylether. This latter solvent probably activates the hydrolysis of lecithin particles by causing a wider spacing of the phospholipid molecules orientated at the lipid-water interface as well as preventing the accumulation of the liberated fatty acids at this interface. The lack of hydrolysis of high-pressure phosphatidylinositol films is not consistent with this idea, but here other factors may be operative. Thus such films have a much higher affinity for ^{45}Ca than either lecithin or phosphatidylethanolamine (Dawson, 1965, and unpublished work), and this may have the effect of masking the stimulating effect of the interfacial anionic groupings.

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Heat-Sensitive Deoxyribonuclease Activity in Cells Infected with Herpes Simplex Virus

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The activity of deoxyribonuclease (EC 3.1.4.5) from mammalian cells (BHK 21-C13) grown in tissue culture increases markedly after infection of the cells with herpes simplex virus (Keir & Gold, 1963). The increment could be ascribed to a stimulation or to a raised level of synthesis of the host-cell enzyme, induced by virus infection. It is also possible that the increment could arise from synthesis of new deoxyribonuclease, directed by the invading viral DNA, and this possibility is consistent with the observation that herpes-virus-specific RNA is transcribed from the herpes DNA after infection of the cells (Hay, Köteles, Keir & Subak-Sharpe, 1966). We now report briefly some observations which indicate that the increase of deoxyribonuclease activity found after infection is attributable to an enzyme(s) that differs in several respects from the corresponding enzyme(s) in the uninfected control cells.

BHK 21-C13 cells (derived from baby hamster kidney; MacPherson & Stoker, 1962) were grown as described by Russell *et al.* (1964) in 80 oz. bottles. The cell monolayers were infected with herpes

simplex virus (strain α) at a multiplicity of exposure 10. At 6-8 hr. later, the cell sheets were washed with phosphate-buffered saline (7.7 mM- Na_2HPO_4 -1.5 mM- KH_2PO_4 -0.14 M- NaCl) and removed from the glass with 0.5 mM-EDTA in phosphate-buffered saline. The cells were then washed twice with phosphate-buffered saline by centrifugation at 0° , and were stored at -75° until required. After the cells had been thawed, an initial enzyme extraction was carried out essentially as described by Keir & Shepherd (1965), to give a 105 000g supernatant fraction (S1) in 0.05 M-tris-HCl buffer (pH 8.0)-0.15 M-KCl-1 mM-EDTA-5 mM-2-mercaptoethanol. The 105 000g sediments were suspended in buffer of the same composition, homogenized vigorously for 1 min. in a Potter-Elvehjem homogenizer, and centrifuged at 105 000g to give a second soluble enzyme extract (S2). Further re-extraction of the 105 000g sediments did not yield significant amounts of enzyme activity.

Deoxyribonuclease was assayed by a standard procedure (Keir, 1962) that measured the release of acid-soluble DNA fragments from native or

Fig. 1. Heat-stability curves for deoxyribonuclease preparations from uninfected cells (BHK 21-C13) and cells infected for 8 hr. with herpes simplex virus. The enzyme fractions were submitted to a prior incubation for the indicated times at 45° and then cooled to 0°. DNA was then added and the mixtures were incubated for 60 min. at 37° for assay of surviving deoxyribonuclease activity. Each assay mixture (1.25 ml.) contained 25 μ moles of tris-HCl buffer, pH 8.0, 75 μ moles of KCl, 5 μ moles of MgSO₄, 0.5 μ mole of EDTA, 5 μ moles of 2-mercaptoethanol, 150 μ g. of Landschutz ascites-tumour cell DNA (heat-denatured) and 200 μ g. of protein from the uninfected-cell enzyme fractions (180 μ g. of protein from the infected-cell fractions). Control samples were run in parallel with the tests (a) without added DNA and (b) without enzyme. Production of acid-soluble DNA fragments is corrected for these controls and is expressed as E_{260} /mg. of protein. ○, Uninfected-cell S1 preparation; △, uninfected-cell S2 preparation; ●, infected-cell S1 preparation; ▲, infected-cell S2 preparation.

heat-denatured DNA during incubation at 37°. The reaction was linear with respect to release of acid-soluble ultraviolet-absorbing material for at least 90 min. When it was necessary to determine the ionic requirements of the reaction, the enzyme preparations were dialysed against 0.05 M-tris-HCl buffer (pH 8.0)–1 mM-EDTA–5 mM-2-mercaptoethanol.

Heat-inactivation experiments. The fractions were

submitted to a prior incubation at 45° and pH 8.0 for up to 32 min., and were then cooled to 0°. DNA was added, and surviving deoxyribonuclease activity was measured after incubation at 37°. The results (Fig. 1) showed that (a) there was substantially more of the nuclease activity in the infected than in the control preparations, (b) up to 50% of the nuclease activity in the infected preparations was destroyed by heating for 10 min. at 45°, (c) the control preparations were stable to the prior heating, and (d) though the control S1 and S2 preparations had closely similar activities, the infected S1 preparation was markedly more active than the infected S2 preparation. Since the same amounts of control and infected cells were used, and since in each case the amount of soluble protein in the S2 fraction was about 25% of that in the S1 fraction, it can be concluded that under the extraction conditions used the bulk of the nuclease increment in the infected cells was very readily soluble and extractable. It is perhaps significant that not all of the activity of the infected samples was heat-sensitive; this suggests that the infected cells contain not only the heat-sensitive deoxyribonuclease but also the heat-resistant complement of deoxyribonuclease found in the control cells. When the prior incubation was performed at 50°, the nuclease activity of the infected S1 fraction fell to that of the control S1 fraction within 2 min.; thereafter there was a slow decline of activity (25% in 30 min.) that closely followed the slow loss of activity in the control preparations.

These heat-inactivation results for control and infected preparations have been observed also with Hep-2 cells (human epitheloid carcinoma). This is of some importance since the genetic origins of BHK 21-C13 and Hep-2 cells are quite different, and the same virus stock was used in all experiments.

Physical condition of substrate. Though both the control and the infected preparations degrade heat-denatured DNA at a faster rate than native DNA, the increase of deoxyribonuclease activity that follows infection is much greater when related to native DNA as substrate than when related to denatured DNA. However, these relationships may be altered by varying the concentration of Na⁺ or K⁺ in the assay mixture.

Response to Na⁺ and K⁺. The effects of these cations were tested over the range 0–150 mM in the presence of Mg²⁺ at 2 mM and tris-HCl buffer (pH 8.9) at 50 mM: (a) the control activity towards native DNA was little affected, (b) the control activity towards denatured DNA and the infected-preparation activity towards native DNA were strongly inhibited by increasing the concentration of either Na⁺ or K⁺, and (c) the infected-preparation activity towards denatured DNA showed an optimum at 60 mM-Na⁺ or -K⁺ (representing a 30%

stimulation over the activity in the absence of Na^+ or K^+).

Requirement for a bivalent cation. All control and infected preparations displayed an absolute requirement for a bivalent cation. Mg^{2+} promoted optimum activity at 2mM, inhibited only moderately at 20mM and showed essentially the same pattern for control and infected cell fractions. Mn^{2+} promoted optimum activity at 0.5mM and inhibited nuclease activity sharply above that concentration, especially with denatured DNA as substrate. Though the activities of the control preparations were the same at 2mM- Mg^{2+} or at 0.5mM- Mn^{2+} , the activities of the infected preparations were only half as great with Mn^{2+} as with Mg^{2+} .

Optimum pH. The effect of pH over the range 6.2–10.5 was studied. With denatured DNA as substrate, the control preparations were more active at pH 6–7 than the infected preparations, although both showed a sharp optimum at pH 9.1. With native DNA as substrate, the infected preparations showed an extended optimum at pH 9–10.

General considerations. From these observations it seems reasonable to conclude that the infected cells contain deoxyribonuclease activity that is not present in the control uninfected cells. There is no clear indication whether production of the enzyme(s) is programmed by the viral or by the host-cell DNA. It would seem that this point could be conclusively proved only by syntheses *in vitro*

of enzyme directed by the respective DNA. Similar considerations apply also to DNA nucleotidyl-transferase (EC 2.7.7.7), some of the properties of which are altered after infection of BHK 21–C 13 cells of HEP–2 cells with herpes simplex virus (Keir, Hay, Morrison & Subak-Sharpe, 1966).

Satisfactory characterization of the deoxyribonuclease activities described here clearly depends on their purification. Preliminary experiments on purification have indicated that the control-cell and infected-cell nuclease activities behave differently during chromatography on DEAE-cellulose.

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