occurred. There was no evidence of specificity of binding of the tumour-cell enzyme to homologous DNA since similar results were obtained with bovine DNA.

Sedimentation coefficients of DNA polymerase and deoxyribonuclease were 7.1s and 4.3s respectively, as determined by the method of Martin & Ames (1961) with a haemoglobin marker.

The product of the DNA polymerase reaction after 5 hr. incubation was subjected to zonal CsCl density-gradient ultracentrifugation; less than half of the product sedimented with the denatured DNA marker, suggesting an association with the DNA template.

The remainder sedimented very slowly, suggesting a product of low molecular weight. The presence of a low- and a high-molecular-weight product was confirmed by chromatography on DEAE-cellulose paper (Furlong, 1967). The low-molecular-weight product was not apparent after shorter periods of incubation, suggesting endonuclease action. Fractionation of the product in a polyethylene glycol-dextran two-phase system (Alberts, 1967) showed it to behave as double-stranded DNA after only 10% replication of the template.

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Possible Association of Virus-Induced Deoxyribonucleic Acid Polymerase and Deoxyribonucleic Acid Exonuclease in Herpes-Virus-Infected Cells

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Since the discovery (Keir & Gold, 1963) that infection of mammalian cells with herpes virus is followed by increases in DNA polymerase and deoxyribonuclease, much evidence has accumulated to support the hypothesis that these increases represent new proteins distinct from the corresponding activities of the uninfected host and that the synthesis of these proteins is very probably virus-directed (Keir, 1968). Purification of the DNA polymerase induced by herpes virus has been undertaken in order that its properties and mode of replicating DNA may be adequately characterized. Such information should make it possible to corre-

late enzymic function *in vitro* and DNA replication in the herpes-infected cell.

Chromatography on DEAE-cellulose has proved to be a useful technique for separating herpes-induced DNA exonuclease from host-cell deoxyribonucleases (Morrison & Keir, 1968). In the course of this fractionation, however, herpes-induced DNA polymerase was eluted along with the herpes exonuclease. In view of the known association of polymerase and exonuclease in bacterial systems (Lehman & Richardson, 1964; Goulian, Lucas & Kornberg, 1968), this observation suggested that a similar situation might obtain in the herpes-infected cell.

When extracts of herpes-infected cells were chromatographed on hydroxyapatite, two peaks of exonuclease were observed, one that was not retained by the hydroxyapatite and that had no polymerase activity and a second that was adsorbed and subsequently eluted at exactly the same phosphate concentration as the herpes DNA polymerase. Similar results were obtained when polymerase-exonuclease preparations that had been purified on DEAE-cellulose were fractionated on hydroxyapatite. The two exonuclease peaks have different heat-stabilities, but are probably both virus-induced, since exonuclease activity in uninfected cells is very low.

Further evidence in support of polymerase-exonuclease association comes from isokinetic sucrose-gradient-sedimentation analysis. By using this technique, DNA polymerase and exonuclease were found to sediment together with an apparent $S_{20,w}$ value in the range 9.3–9.8s (corresponding to a molecular weight of $190\,000 \pm 20\,000$). Earlier work (Morrison & Keir, 1968) had shown that the two activities were eluted together during gel filtration on Sephadex G-200. Thus the failure to separate polymerase and exonuclease by these four techniques suggests that these two activities may well be associated, either in a single polypeptide chain, as in the *Escherichia coli* enzyme, or in a multichain protein complex. The relationship of hydrolytic to synthetic functions in DNA polymerases is clearly crucial to an understanding of the operation of the enzyme *in vivo*.

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