sharply, the increase corresponding to the decline in radioactivity in the acid-soluble fraction.

Taken in conjunction with the results described by Billing, Barbiroli & Smellie (1969), these results suggest that there are no major changes in the rate of synthesis of RNA in rat uterus until at least 6 hr. after hormone treatment and that thereafter there is a sharp increase in the rate of synthesis of RNA. These findings are consistent with the observation that the amount of RNA in rat uterus only begins to increase about 7 hr. after oestradiol treatment.

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The Effect of Tri-iodothyronine on the Biosynthesis of Deoxyribonucleic Acid in *Rana catesbeiana* Tadpoles

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Although the metabolism of RNA in the liver of the metamorphosing tadpole has been under investigation for several years (Tata, 1967; Frieden, 1967; Nakagawa & Cohen, 1967), DNA metabolism has been less intensively studied. The total amount of DNA in tadpole liver during metamorphosis has been variously reported to decrease (Finamore & Frieden, 1960), increase (Tata, 1967) and remain constant (Paik, Metzzenberg & Cohen, 1961) at different times after the onset of the process. Champy (1922) found no change in the mitotic index of tadpole liver during metamorphosis.

Recent studies in this laboratory have shown that there is an increase in the incorporation of [³H]-thymidine into the DNA of both tadpole liver nuclei and tadpole liver mitochondria after metamorphosis is induced with tri-iodothyronine. However, the incorporation into the nuclear DNA is not evident until 5 days after the induction of metamorphosis whereas that into mitochondrial DNA is evident 1 day after the induction and reaches a maximum at 2 days after tri-iodothyronine injection. The possible causes of these alterations will be discussed.

Studies on DNA polymerase *in vitro* have been carried out with thymidine [α -³²P]triphosphate as a precursor, and several of the main characteristics of this enzyme have been determined. The incorporation appears to be limited by the high rate of breakdown of the deoxyribonucleoside triphosphates under the assay conditions. This breakdown is accelerated in liver preparations from tadpoles that have been treated with tri-iodothyronine and consequently it is possible for conditions to exist

under which changes in the polymerase activity are not observable. One of the more interesting features of the polymerase activity from tadpole liver is a strong preference for native DNA over denatured DNA as template.

Under the conditions of assay determined, the DNA polymerase activity of tadpole liver rises during the early metamorphic period. Three days after metamorphosis has been induced, this change appears to be localized in the mitochondrial and postmitochondrial fractions of the cell, the greater change being in the postmitochondrial fraction. The significance of these changes will be discussed.

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Deoxyribonucleic Acid Polymerase from Landschütz Ascites-Tumour Cells

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DNA polymerase (DNA nucleotidyltransferase, EC 2.7.7.7) from Landschütz ascites-tumour cells was partially purified to fraction 3 of the method of Shepherd & Keir (1966). Further purification removing 99% of the deoxyribonuclease (EC 3.1.4.5) was achieved by fractionation on Sephadex G-150, elution being with buffer containing bovine serum albumin (1 mg./ml.), which served to minimize enzyme denaturation. Heat-inactivation, and freezing-and-thawing experiments conducted in the presence and absence of either 25% (v/v) glycerol or DNA, showed that these agents also have a protective effect on the enzyme.

Binding of DNA polymerase (fraction 3) to DNA was investigated by sedimentation analysis through 5–25% (w/v) sucrose density gradients. Binding that occurred was dependent on the buffer and ion components in the gradient. In aqueous sucrose solutions approximately half of the enzyme bound to native DNA, forming a complex with a sedimentation rate slightly greater than that of the free enzyme. When the sucrose was dissolved in a buffer solution at low ionic strength (0.2 M-tris-HCl–5 mM-2-mercaptoethanol, pH 7.5) most of the DNA and enzyme formed a complex that sedimented to the bottom of the tube. With the inclusion of 0.15 M-KCl and 4 mM-MgSO₄ little or no binding

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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*.

R. D. P. holds a Medical Research Council Studentship.

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