Nuclear Deoxyribonucleic Acid Polymerases from Landschütz Ascites-Tumour Cells

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Highly purified nuclei were prepared from cells of the Landschütz ascites tumour by the method of Lazarus & Sporn (1967).

The nuclei were extracted with 20mM-tris-HCl buffer (pH 7.8)-1mM-EDTA-1.0M-KCl and dialysed against 20mM-tris-HCl buffer (pH 7.8)-1mM-EDTA-20mM-KCl. The extract was applied to a column of DEAE-cellulose previously equilibrated against the latter buffer.

During the ensuing chromatography at least three DNA polymerase activities were detected. The first (DNA polymerase I) was eluted with the peak of protein that did not bind to the column, was most active with 'activated' DNA as template (Aposhian & Kornberg, 1962) and had a marked preference for native DNA compared with denatured DNA as template. No deoxyribonuclease could be detected in the peak, as assessed by ability to render [^3H]DNA acid-soluble. The Mg^{2+} and Mn^{2+} optima for DNA polymerase I were 2.5mM and 10mM respectively.

DNA polymerase II was eluted from the DEAE-cellulose in a KCl gradient at 75-100mM-KCl, had highest activity with 'activated' DNA as template and marginally preferred denatured to native DNA. Its Mg^{2+} and Mn^{2+} optima were 2.4mM and 4mM respectively. No deoxyribonuclease activity was detected.

DNA polymerase III was eluted from the DEAE-cellulose in a KCl gradient at 250-300mM-KCl, displayed the same template preference as DNA polymerase II, but although its Mg^{2+} optimum was the same as those for DNA polymerases I and II its Mn^{2+} optimum was above 20mM. A small peak of deoxyribonuclease activity was eluted with the leading edge of DNA polymerase III.

Purified nuclei were swollen in 10mM-tris-HCl buffer (pH 7.8)-50mM-thioglycerol, frozen and thawed thrice, homogenized and centrifuged at 100000g for 60min. The sediment was re-extracted and centrifuged, and the two supernatant fractions were pooled (nucleoplasmic fraction). The second sediment was extracted with 20mM-tris-HCl buffer (pH 7.8)-1mM-EDTA-1.0M-KCl, centrifuged and the supernatant fluid (solubilized fraction) dialysed as above. The DNA polymerase activities in the nucleoplasmic and solubilized fractions each showed an elution pattern from DEAE-cellulose similar to that obtained by the total extraction of whole purified nuclei.

Chromatography of whole nuclear extracts on

columns of phospho-cellulose likewise revealed at least three polymerase peaks.

These results are compatible with, although distinct from, observations by others on mammalian tumour cells (Iwamura *et al.*, 1968; Ballal *et al.*, 1970; Furlong & Gresham, 1971; Harm & Hilz, 1971), on cells grown in culture (Lindsay *et al.*, 1970; Weissbach *et al.*, 1971) and on mammalian tissues (Chang & Bollum, 1971; Haines *et al.*, 1971).

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- Aposhian, H. V. & Kornberg, A. (1962) *J. Biol. Chem.* **237**, 519
 Ballal, N. R., Collins, M. S., Halpern, R. M. & Smith, R. A. (1970) *Biochem. Biophys. Res. Commun.* **40**, 120
 Chang, L. M. S. & Bollum, R. J. (1971) *J. Biol. Chem.* **246**, 5835
 Furlong, N. B. & Gresham, C. (1971) *Tex. Rep. Biol. Med.* **29**, 75
 Haines, M. E., Holmes, A. M. & Johnston, I. R. (1971) *FEBS Lett.* **17**, 63
 Harm, K. & Hilz, H. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1469
 Iwamura, Y., Ono, T. & Morris, H. P. (1968) *Cancer Res.* **28**, 2466
 Lazarus, H. M. & Sporn, M. S. (1967) *Proc. Nat. Acad. Sci. U.S.A.* **57**, 1386
 Lindsay, J. G., Berryman, S. & Adams, R. L. P. (1970) *Biochem. J.* **119**, 849
 Weissbach, A., Schlabach, A., Fridlender, B. & Bolden, A. (1971) *Nature (London)* **231**, 167

Applications of a Simple Staining Technique for Steroids on Thin-Layer Chromatograms

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Advances in techniques for steroids have frequently involved increasingly complicated experimental procedures and ever-increasingly expensive materials and apparatus. Contrary to this trend, the additions now described to the usual techniques of t.l.c. have the advantages of slight cost and extreme simplicity.

The chromatogram is run on silica gel, is dried to remove solvents and is exposed for a few minutes to the vapour from a little conc. HCl. Various steroids give coloured and/or fluorescent products, and by choosing suitable conditions certain important types of steroid can be distinguished. Saturated steroids and simple $\alpha\beta$ -unsaturated ketones generally do not react. The common 3β -hydroxy-5-enes react at room temperature or, better, a little above (e.g. 40°C) to give coloured (usually pink) spots, though a 7-oxo group can prevent this reaction. At lower temperatures (e.g. 0°C) the 3β -hydroxy-5-enes scarcely react, but the 3β -hydroxy-4-enes still react even at -10°C much as they do at 40°C. 3,7-Dihydroxy-5-enes react