treated for 2–3 h with dimethyl sulphoxide solutions of the epoxides, 3-methylcholanthrene 11,12-oxide, dibenz[*a,h*]anthracene 5,6-oxide and 7-methylbenz[*a*]anthracene 5,6-oxide were found to be strongly mutagenic. Under these conditions phenanthrene 9,10-oxide gave a very slight mutagenic effect, but chrysene 5,6-oxide and benz[*a*]anthracene 5,6-oxide were inactive. Similar results were obtained when bacteriophage were treated with these epoxides for longer periods and the frequency of plaque-type mutants was determined. The active epoxides were highly effective mutagens when compared with ethyl methanesulphonate, which was used as a positive control (Loveless, 1959). It may also be significant that only those 'K-region' epoxides that are derived from potent carcinogenic hydrocarbons were strongly mutagenic to bacteriophage in these tests. Some possible mechanisms of action of polycyclic hydrocarbon epoxides as mutagens will be discussed in relation to the role that these metabolic intermediates may play in hydrocarbon carcinogenesis.

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Binding of Polylysine to Chromatin Extracted from Various Tissues

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Previous studies on the accessibility of the DNA component of rat thymus chromatin have shown that about 40% of the DNA phosphate groups are available for binding poly-L-lysine hydrobromide of various molecular weights (Itzhaki, 1970, 1971a). This work has now been extended to the DNA-protein complex extracted from a variety of sources.

Mixtures of DNA-protein and polylysine in 0.7 mM-sodium phosphate buffer, pH 6.9, were made over a range of DNA-protein concentrations such that all the DNA-protein was precipitated. Estimates were then made of the excess of unbound polylysine by titration with Methyl Orange.

It has been found that, in chromatin from the livers of normal rats and from rats treated with dimethylnitrosamine, in calf thymus nucleohistone and in avian erythrocyte chromatin, 40–44% of the DNA phosphate groups are available for binding

polylysine, and these are present in stretches sufficiently long to bind most or all of each polylysine molecule. In DNA-protamine from salmon sperm about 24% of the DNA phosphate groups are available for binding, but these free phosphate groups are present only in very small stretches.

Attempts were made to find if there was appreciable binding of polylysine to the chromatin protein carboxyl groups. Two methods were used: first, the binding to rat thymus chromatin was measured at pH 4.5 so as to decrease the dissociation of the protein acidic groups; secondly, the binding to a precipitate consisting mainly of chromatin protein was measured. Both methods showed that the binding of polylysine to the chromatin protein was negligible.

On the basis of the previous studies with polylysine and other studies on the effect of added nucleases on chromatin (Itzhaki, 1971b) it was proposed that the DNA in chromatin is extensively covered by protein but that there are many zones of free DNA beneath the protein. The present results suggest that the structure of chromatin from relatively active cells is similar to that of chromatin from the inactive avian erythrocyte. In fact, calculation of the ratio of protein basic groups to DNA phosphate groups in the extracts shows that the value is lower rather than higher in the DNA-protein complex from the avian erythrocyte and from sperm. It is concluded that any repression of gene activity by histones is unlikely to be due to a simple coverage of the DNA.

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Formation of Polydeoxyadenylylthymidylate after Deoxyribonucleic Acid Synthesis by a Deoxyribonucleic Acid Polymerase from *Mycobacterium smegmatis*

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When the DNA polymerase from *Mycobacterium smegmatis* is incubated with a small amount of primer DNA and an excess of deoxynucleoside triphosphates, synthesis of DNA stops before 50% 'replication' of the primer has taken place. However, this is followed by a phase of exponential incorporation of thymidine, and evidence has been obtained that this is due to synthesis of poly-[d(A-T)] (McNulty & Winder, 1972). The following results support this conclusion.

The polymerase was purified 500-fold from iron-limited *M. smegmatis*, as will be described elsewhere.

The reaction mixtures contained, in 0.48 ml, 1.25 μ g of native DNA, 40 nmol of each of the four deoxynucleoside triphosphates, of which either dTTP or dCTP was labelled with tritium, 2 μ mol of magnesium chloride, 0.3 μ mol of 2-mercaptoethanol, 0.3 mg of bovine serum albumin, 20 μ mol of glycine-NaOH buffer, pH 9.2, 35 μ mol of sodium chloride and 10 units of enzyme. These were incubated at 37°C. Samples were taken at intervals and acid-insoluble radioactivity was counted in a toluene-based scintillation fluid after precipitation on filter-paper discs (Bollum, 1959).

As in previous experiments there were two successive phases of thymidine incorporation into polymer. No incorporation of deoxycytidine took place during the second phase, supporting the conclusion that a polymer containing only deoxyadenosine and thymidine was formed. Incubation of the product of [³H]dTTP incorporation with formic acid-diphenylamine (Burton & Petersen, 1960) rendered the thymidine incorporated into polymer in the second phase entirely acid-soluble, indicating that it was the alternating co-polymer poly[d(A-T)].

When the products of a polymerase reaction, in which [³²P]DNA and [³H]dTTP were employed, were centrifuged in alkaline caesium chloride density gradients, the main bulk of tritium banded at the same density as poly[d(A-T)]. Neither the distribution of tritium between this band and the [³²P]DNA band, nor the distribution of ³²P in the gradient, suggested that there was any appreciable amount of DNA covalently linked to the poly-[d(A-T)], thus providing no evidence that the DNA served any primer role in the poly[d(A-T)] synthesis.

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Absence of Single-Stranded Regions in Deoxyribonucleic Acid during the Action of an Adenosine Triphosphate Dependent Deoxyribonuclease

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The ATP-dependent deoxyribonuclease from *Mycobacterium smegmatis* excises short oligonucleotides from the ends of strands of DNA, and evidence

has been obtained that it attacks both strands of the duplex more-or-less simultaneously (Winder & Lavin, 1971). It was important to confirm the latter point, since it is probable from work with other micro-organisms that this enzyme is involved in recombination (Oishi, 1969; Barbour & Clark, 1970; Vovis & Buttin, 1970) and since exonucleolytic attack on both strands of the duplex does not readily fit into most models of recombination (see Whitehouse, 1970). Adsorption on nitrocellulose is a sensitive test for single-stranded regions (Berger & Irvin, 1970), which should be formed if the enzyme preferentially digests one strand of the DNA.

The enzyme was purified about 180-fold from iron-limited *M. smegmatis* by a modification of the procedure of Winder & Lavin (1971). Incubation mixtures contained, in 1 ml, 1.68 nmol of [³²P]DNA from *Escherichia coli* (which had been passed through Millipore filters, type HAWP), 2 μ mol of ATP, 20 μ mol of magnesium chloride, 75 μ mol of tris-HCl buffer, pH 8.0, 50 μ mol of potassium chloride, 0.15 μ mol of dithiothreitol and 288 units of enzyme. After incubation at 37°C, mixtures were deproteinized with phenol-sodium dodecyl sulphate, pH 9.0, and dialysed against 0.15 M-sodium chloride-15 mM-sodium citrate, pH 7.0. Samples were removed, dried on Millipore filters and their radioactivities counted. The remainder was made 0.3 M with respect to sodium chloride and 30 mM with respect to sodium citrate, pH 7.0, and passed through Millipore filters (which had been pre-washed with the same buffer), followed by washing with buffer. The filters were dried and their radioactivities counted in a toluene-based scintillation fluid.

At zero time 6% of the [³²P]DNA recovered from the reaction mixtures was adsorbable on the filters. After 0.5, 1, 2, 3, 5 and 10 min of reaction the total amount of DNA recovered had fallen by 14, 25, 51, 73, 76 and 79% respectively, and the DNA adsorbable on the filters had fallen by 1, 15, 20, 36, 52 and 49% respectively. Thus the action of the enzyme led to a continuous decline in the amount of DNA adsorbable on the filters (even if this was slightly slower than the decline in total DNA), instead of the increase that would be expected if the enzyme produced single-stranded regions. This is further evidence that the enzyme attacks both strands more-or-less simultaneously.

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