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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Grover, P. L., Forrester, J. A. & Sims, P. (1971). *Biochem. Pharmac.* **20**, 1297.

Grover, P. L. & Sims, P. (1970). *Biochem. Pharmac.* **19**, 2251.

Loveless, A. (1959). *Proc. R. Soc. B*, **150**, 497.

Sims, P., Hewer, A. & Grover, P. L. (1971). *Biochem. J.* **125**, 28P.

Binding of Polylysine to Chromatin Extracted from Various Tissues

By R. F. ITZHAKI and H. K. COOPER. (*Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, U.K.*)

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.

Itzhaki, R. F. (1970). *Biochem. biophys. Res. Commun.* **41**, 25.

Itzhaki, R. F. (1971a). *Biochem. J.* **122**, 583.

Itzhaki, R. F. (1971b). *Biochem. J.* **125**, 221.

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

By F. G. WINDER, M. S. McNULTY and D. S. BARBER. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

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.