Biochemical Pharmacology, Vol. 22, pp. 661–666. Pergamon Press, 1973. Printed in Great Britain.

# K-REGION EPOXIDES OF POLYCYCLIC HYDROCARBONS: REACTIONS WITH NUCLEIC ACIDS AND POLYRIBONUCLEOTIDES

PHILIP L. GROVER and PETER SIMS

The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, England

(*Received* 29 *July* 1972; *accepted* 14 *October* 1972)

**Abstract**—The extents of reaction that occurred between the K-region epoxides of $^3$H-labelled polycyclic hydrocarbons and DNA, RNA and apurinic acid have been estimated after incubation at 37° in neutral aqueous solution. Phenanthrene 9,10-oxide was less reactive than benz[a]anthracene 5,6-oxide, 7-methylbenz[a]anthracene 5,6-oxide or dibenz[a,h]anthracene 5,6-oxide, all of which reacted much more extensively with DNA and RNA than with apurinic acid. In similar experiments with polyribonucleotides, the K-region epoxides were reactive towards poly(G), reacted less with poly(A), poly(X) and poly(I) but did not react appreciably with poly(U) or poly(C). The results of these experiments suggest that the purine moieties of nucleic acids are the main sites for reaction with polycyclic hydrocarbon epoxides.

MICROSOMAL metabolism of the double bonds of carcinogenic polycyclic hydrocarbons such as benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene involves the formation of epoxides.[1–4] The suspicion that reactive intermediates of this type could be responsible for the biological effects produced by the parent hydrocarbons led to efforts to synthesize polycyclic hydrocarbon epoxides. Those prepared have been mainly K-region derivatives[5–7] but the synthesis of a range of non-K-region epoxides is now in progress in this laboratory.[8–10] The positive results obtained in biological tests with K-region epoxides gave rise to the formation of our working hypothesis which states that the mechanism of polycyclic hydrocarbon carcinogenesis involves somatic mutations caused by epoxides produced by metabolism. This hypothesis is supported by investigations that have shown that K-region epoxides are formed by metabolism[1,3,4] and; (a) are alkylating agents[8,11] that react with nucleic acids chemically and in cells in culture;[11–13] (b) are more active than their parent hydrocarbons in effecting the malignant transformation of rodent cells in culture[14–16] and; (c) are mutagenic in bacteriophage,[17] in bacteria,[18] in mammalian cells in culture[19] and in *Drosophila*.* In whole animals, however, K-region epoxides have so far proved to be less active as carcinogens than the parent hydrocarbons.[20,21] The reasons for this are not known but may be associated with the relative instability of the epoxides.

The chemical reactions that occur between $^3$H-labelled K-region epoxides and nucleic acids[11] have not previously been examined in detail and consequently the structures of the products formed are not known. This paper describes the results

* O. G. Fahmy and M. J. Fahmy, unpublished observations.

obtained in some investigations of the reactions of $^3$H-labelled K-region epoxides derived from phenanthrene, benz[a]anthracene, 7-methylbenz[a]anthracene and dibenz[a,h]anthracene with nucleic acids and with synthetic polyribonucleotides.

## EXPERIMENTAL

*Materials.* Polycyclic hydrocarbons generally labelled with tritium were obtained from the Radiochemical Centre, Amersham, Bucks., and the $^3$H-labelled K-region epoxides phenanthrene 9,10-oxide (sp. act. 39 mCi/mmole), benz[a]anthracene 5,6-oxide (sp. act. 57 mCi/mmole), 7-methylbenz[a]anthracene 5,6-oxide (sp. act. 271 mCi/mmole) and dibenz[a,h]anthracene 5,6-oxide (sp. act. 198 mCi/mmole) were prepared from the hydrocarbons by methods described previously.[5,22,23] DNA from salmon testes (Sigma Chemical Co., St. Louis) was deproteinized by a detergent–salt procedure;[24] apurinic acid was prepared from deproteinized DNA by treatment with acid.[25] Synthetic polyribonucleotides were purchased from Miles Seravac Ltd., Maidenhead, Berks and RNA (highly polymerized from yeast) from British Drug Houses Ltd., Poole.

*Reactions of epoxides with macromolecules.* Nucleic acid or polyribonucleotide ($\equiv$ 4·0 $\mu$mole-P) was dissolved in distilled water (pH 7·4, 2 ml) and mixed with an ethanol solution (1 ml) of the $^3$H-labelled hydrocarbon epoxide ($\equiv$ 0·04 $\mu$mole). After incubation for 2 hr at 37° in subdued light, the mixture was extracted with ether (3 × 3 ml) and the aqueous portion applied to the top of a G25 Sephadex column (0·9 × 15 cm) which was eluted with distilled water of pH 7·4. Fractions (0·5 ml) were collected and analysed both for u.v. absorbance at 260 nm and for radioactivity. Extents of reaction were calculated from elution profiles using $E^{0\cdot1\,cm}_{260\,nm}$ values for nucleic acids and polyribonucleotides and the specific activities of the $^3$H-labelled hydrocarbon epoxides.

## RESULTS

*Reactions with DNA, RNA and apurinic acid.* G25 Sephadex column elution profiles obtained following incubation of DNA, RNA and apurinic acid with $^3$H-labelled benz[a]anthracene 5,6-oxide are shown in Fig. 1. The amounts of reaction that were

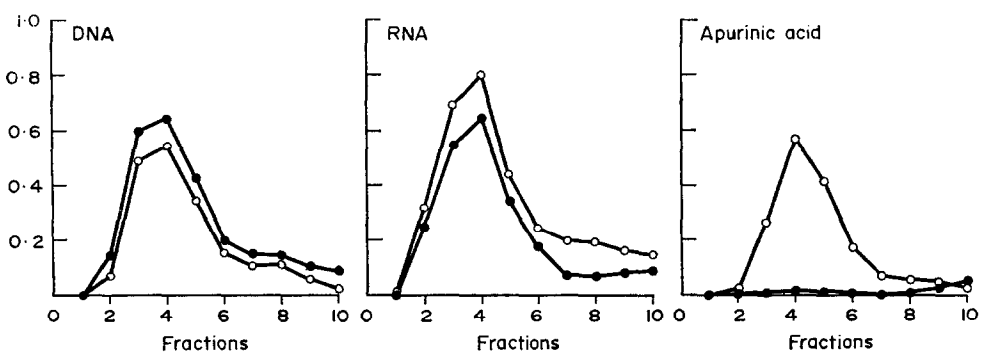


FIG. 1. G25 Sephadex column elution profiles obtained following reaction of $^3$H-labelled benz[a]anthracene 5,6-oxide with DNA, RNA and apurinic acid. ○—○ $E^{0\cdot1\,cm}_{260\,nm}$; ●——● counts/min × $10^{-5}$/0·2 ml. Reactions were carried out as described in the text.

found to occur when these nucleic acids were incubated with the K-region epoxides derived from phenanthrene, benz[a]anthracene, 7-methylbenz[a]anthracene or dibenz[a,h]anthracene are given in Table 1. These results show that, under the conditions used, phenanthrene epoxide is not as reactive towards DNA and RNA as the

TABLE 1. REACTIONS OF $^3$H-LABELLED K-REGION EPOXIDES OF POLYCYCLIC HYDROCARBONS WITH DNA, RNA AND APURINIC ACID

| K-region epoxide | μMoles/mole-P | | |
|---|---|---|---|
| | DNA | RNA | Apurinic acid |
| Phenanthrene 9,10-oxide | 15 | < 7 | < 7 |
| Benz[a]anthracene 5,6-oxide | 680 | 505 | 21 |
| 7-Methylbenz[a]anthracene 5,6-oxide | 1630 | 960 | 51 |
| Dibenz[a,h]anthracene 5,6-oxide | 327 | 417 | 20 |

Nucleic acid (4·0 μmole-P) dissolved in distilled water (pH 7·4, 2·0 ml) was mixed with a solution of the epoxide (0·04 μmole) in ethanol (1 ml) and incubated at 37° for 2 hr. Extents of reaction were calculated as described in the text following passage through a G25 Sephadex column.

other epoxides and that the K-region epoxides derived from benz[a]anthracene, 7-methylbenz[a]anthracene and dibenz[a,h]anthracene react to a much greater extent with DNA and RNA than with apurinic acid.

*Reactions with polyribonucleotides.* Figure 2 shows the Sephadex column elution profiles for u.v. absorbance and radioactivity obtained following incubation of the synthetic polyribonucleotides poly(G), poly(A), poly (X), poly(I), poly(C) and poly(U) with $^3$H-labelled benz[a]anthracene 5,6-oxide. The extent of reaction that occurred when these polyribonucleotides were incubated with each of the four K-region epoxides are given in Table 2. The most extensive alkylations occurred with poly(G) and lower levels of reaction took place with poly(A), poly(X) and poly(I). Significant reactions were not detected with the pyrimidine polymers poly(C) and poly(U).

## DISCUSSION

The investigation of epoxides as possible reactive intermediates that are involved in polycyclic hydrocarbon carcinogenesis was stimulated by the finding that microsomal metabolism of $^3$H-labelled polycyclic hydrocarbons leads to the formation of products that react with DNA.[26] K-region epoxides derived from phenanthrene and dibenz-[a,h]anthracene were then shown to be alkylating agents that reacted with 4-(*p*-nitrobenzyl)pyridine,[11] a result that led to studies of the reactivity of K-region epoxides towards biological macromolecules both in solution[11] and in cells in culture.[12,13]

Results reported here confirm the reactivity of K-region epoxides towards DNA and RNA but also indicate that compounds of this type do not react appreciably with apurinic acid (Table 1). The possibility that the epoxides react with the purine moieties of the nucleic acids is borne out by results obtained using synthetic polyribonucleotides. In these experiments appreciable reactions occurred between K-region

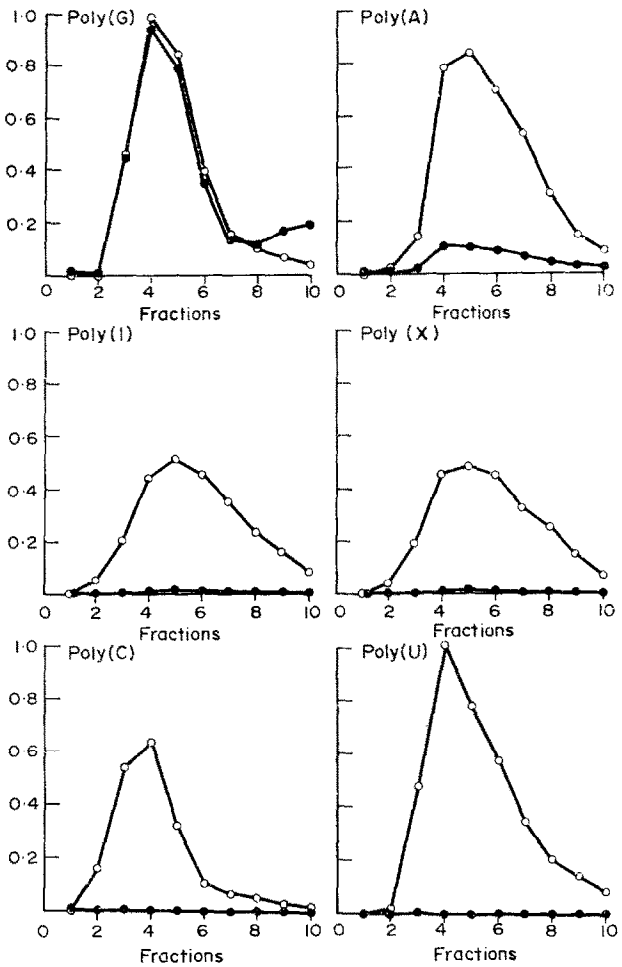


FIG. 2. G25 Sephadex column elution profiles obtained following reaction of $^3$H-labelled benz[a]anthracene 5,6-oxide with polyribonucleotides. ○——○ $E^{0\cdot1\,cm}_{260\,nm}$; ●——● counts/min × $10^{-4}$/0·2 ml
Reactions were carried out as described in the text.

epoxides and poly(G) or poly(A) that were not detected with the pyrimidine polymers poly(C) or poly(U) (Table 2). Furthermore the reactivity of the epoxides towards poly(X) or poly(I) was in general lower than the corresponding reactivity towards poly(G) or poly(A) (Table 2) suggesting that the amino groups of the purines may be involved in reactions with epoxides. Experiments with a model alkylating derivative of a polycyclic hydrocarbon, 7-bromomethylbenz[a]anthracene, have also indicated that reactions occur with the purine amino groups of nucleic acids.[27]

In the present work $^3$H-labelled K-region epoxides that have been prepared by synthesis from the parent hydrocarbons have been used for reactions with nucleic acids and polynucleotides. $^3$H-labelled K-region epoxides of pyrene, of benzo[a]pyrene and of 7,12-dimethylbenz[a]anthracene have not so far been prepared by synthesis but they have been obtained as microsomal metabolites; these epoxides have also been found to react with poly(G).[3,4]

TABLE 2. REACTIONS OF $^3$H-LABELLED K-REGION EPOXIDES OF POLYCYCLIC HYDROCARBONS WITH POLYRIBONUCLEOTIDES

| K-region epoxide | μMoles/mole-P | | | | | |
|---|---|---|---|---|---|---|
| | Poly(G) | Poly(A) | Poly(X) | Poly(I) | Poly(U) | Poly(C) |
| Phenanthrene 9,10-oxide | 280 | 22 | 31 | 42 | < 9 | < 9 |
| Benz[a]anthracene 5,6-oxide | 870 | 161 | 86 | 45 | < 7 | < 7 |
| 7-Methylbenz[a]anthracene 5,6-oxide | 1310 | 566 | 265 | 80 | 27 | 8 |
| Dibenz[a,h]anthracene 5,6-oxide | 1860 | 35 | 90 | 51 | < 5 | < 5 |

Polyribonucleotide (4·0 μmole-P) dissolved in distilled water (pH 7·4, 2·0 ml) was mixed with a solution of the epoxide (0·04 μmole) in ethanol (1 ml) and incubated at 37° for 2 hr. Extents of reaction were calculated as described in the text following passage through a G25 Sephadex column.

Attempts to detect reactions of polycyclic hydrocarbon epoxides with purines or pyrimidines, nucleosides or mononucleotides have not been successful. This is probably due partly to the lower activity of polycyclic hydrocarbon epoxides as alkylating agents in comparison with, for example, aliphatic mustards and partly to the much lower reactivity of, say, guanine towards alkylation when present in guanosine or guanylic acid than when present in a polymer.[28,29] In addition, the activity of polycyclic hydrocarbon epoxides as frameshift mutagens in a bacterial system[18] may mean that intercalation of the hydrocarbon epoxide between adjacent bases of a nucleic acid precedes covalent reaction. Stacking interactions of this type seem less likely to occur in dilute solutions of monomeric nucleotides.

The significance of variations in the reactivity of different K-region epoxides towards nucleic acids (Table 1) and towards polynucleotides (Table 2) is not yet clear but the lower reactivity of phenanthrene epoxide merits comment. Unlike the K-region epoxides of benz[a]anthracene, 7-methylbenz[a]anthracene and dibenz[a,h]anthracene, phenanthrene 5,6-oxide has not been found to be carcinogenic,[20] mutagenic[17,18] or active in the malignant transformation of $C_3H$ mouse prostate cells[15] although positive results were obtained with hamster embryo cells.[16]

The biological effectiveness of polycyclic hydrocarbon epoxides may well be linked to their reactivity towards nucleic acids. In the wider context of hydrocarbon carcinogenesis, however, an understanding of other factors including the sites of enzymic oxidation and the relative rates of epoxide formation and further metabolism may be required.

*Acknowledgements*—The authors wish to thank Mr. A. Hewer for excellent technical assistance. This investigation was supported by grants to the Chester Beatty Research Institute from the Medical Research Council and the Cancer Research Campaign.

## REFERENCES

1. P. L. GROVER, A. HEWER and P. SIMS, *FEBS Letts*, **18**, 76 (1971).
2. J. K. SELKIRK, E. HUBERMAN and C. HEIDELBERGER, *Biochem. biophys. Res. Commun.* **43**, 1010 (1971).
3. P. L. GROVER, A. HEWER and P. SIMS, *Biochem. Pharmac.* **21**, 3316 (1972).
4. G. R. KEYSELL, J. BOOTH, P. SIMS, P. L. GROVER and A. HEWER, *Biochem. J.*, **129**, 41 P (1972).
5. M. S. NEWMAN and S. BLUM, *J. Am. Chem. Soc.* **86**, 5598 (1964).
6. E. BOYLAND and P. SIMS, *Biochem. J.* **97**, 7 (1965).
7. P. SIMS, *Biochem. J.* **98**, 215 (1966).

8. P. SIMS, *Biochem. J.* **125,** 159 (1971).
9. J. F. WATERFALL and P. SIMS, *Biochem. J.* **128,** 265 (1972).
10. P. SIMS, *Biochem. J.*, **130,** 27 (1972).
11. P. L. GROVER and P. SIMS, *Biochem. Pharmac.* **19,** 2251 (1970).
12. P. L. GROVER, J. A. FORRESTER and P. SIMS, *Biochem. Pharmac.* **20,** 1297 (1971).
13. T. KUROKI, E. HUBERMAN, H. MARQUARDT, J. K. SELKIRK, C. HEIDELBERGER, P. L. GROVER and P. SIMS, *Chem.Biol. Int.* **4,** 389 (1972).
14. P. L. GROVER, P. SIMS, E. HUBERMAN, H. MARQUARDT, T. KUROKI and C. HEIDELBERGER, *Proc. natn. Acad. Sci. U.S.A.* **68,** 1098 (1971).
15. H. MARQUARDT, T. KUROKI, E. HUBERMAN, J. K. SELKIRK, C. HEIDELBERGER, P. L. GROVER and P. SIMS, *Cancer Res.* **32,** 716 (1972).
16. E. HUBERMAN, T. KUROKI, H. MARQUARDT, J. K. SELKIRK, C. HEIDELBERGER, P. L. GROVER and P. SIMS, *Cancer Res.*, **32,** 1391 (1972).
17. M. J. COOKSON, P. SIMS and P. L. GROVER, *Nature New Biol.* **234,** 186 (1971).
18. B. N. AMES, P. SIMS and P. L. GROVER, *Science*, **176,** 47 (1972).
19. E. HUBERMAN, L. ASPIRAS, C. HEIDELBERGER, P. L. GROVER and P. SIMS, *Proc. natn. Acad. Sci. U.S.A.* **68,** 3195 (1971).
20. E. BOYLAND and P. SIMS, *Int. J. Cancer*, **2,** 500 (1967).
21. E. C. MILLER and J. A. MILLER, *Proc. Soc. exp. Biol. N.Y.* **124,** 915 (1967).
22. E. BOYLAND and P. SIMS, *Biochem. J.* **95,** 788 (1965).
23. P. SIMS, *Biochem. J.* **105,** 591 (1967).
24. E. R. M. KAY, N. S. SIMMONS and A. L. J. DOUNCE, *J. Am. Chem. Soc.* **74,** 1724 (1952).
25. E. CHARGAFF, in *The Nucleic Acids* (Eds. E. CHARGAFF and J. N. DAVIDSON) Vol. 1, p. 341. Academic Press, New York (1955).
26. P. L. GROVER and P. SIMS, *Biochem. J.* **110,** 159 (1968).
27. A. DIPPLE, P. BROOKES, D. S. MACKINTOSH and M. P. RAYMAN, *Biochemistry*, **10,** 4323 (1971).
28. P. D. LAWLEY, *Ann. Rep. Br. Emp. Cancer Camp.* **36,** 16 (1958).
29. W. J. C. ROSS, *Biological Alkylating Agents*, p. 45. Butterworths, London (1962).