# POLYCYCLIC HYDROCARBON EPOXIDES: THE INVOLVEMENT OF 8,9-DIHYDRO-8,9-DIHYDROXYBENZ (a) ANTHRACENE 10,11-OXIDE IN REACTIONS WITH THE DNA OF BENZ (a) ANTHRACENE-TREATED HAMSTER EMBRYO CELLS

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## 1. Introduction

Carcinogenic polycyclic hydrocarbons are metabolized to epoxides by mammalian microsomal monooxygenases [1–4] and it has been suggested that this type of reactive intermediate is responsible for the biological effects, including carcinogenesis, attributed to the parent hydrocarbons [5]. Although synthetic K-region epoxides possess many properties relevant to the initiation of tumours [6], some previous work has indicated that K-region epoxides are not the actual epoxides involved in reactions with the nucleic acids of cells treated with the parent hydrocarbons [7]. 8,9-Dihydro-8,9-dihydroxy-benz(a)anthracene 10,11-oxide, a new type of epoxide that is formed on the isolated 10,11-double bond of the 8,9-diol of benz(a)-anthracene, has recently been detected as a metabolite of the dihydrodiol and has also been prepared by synthesis [8]. The experiments described here provide evidence that when hamster embryo cells in culture are treated with benz (a) anthracene, the DNA products formed result from the reaction of 8,9-dihydroxybenz(a)anthracene 10,11-oxide with this nucleic acid. Similar results have been obtained when rat-liver microsomal preparations are incubated with the 8,9-dihydrodiol of benz(a)anthracene and DNA. As an important corollary, these results also provide some of the strongest evidence so far obtained in support of the hypothesis that epoxides are the metabolically-activated intermediates formed from polycyclic hydrocarbons.

## 2. Materials and methods

### 2.1. *Materials*

$^{3}$H-Labelled benz(a)anthracene (specific activity 26 Ci/mmole) was obtained from the Radiochemical Centre, Amersham, Bucks and $^{3}$H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)anthracene and $^{3}$H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide (both of specific activity 0.6 Ci/mmole) prepared using the method described [8]. Dulbecco's MEM and foetal calf serum were obtained from Biocult Laboratories Ltd., Paisley, Scotland, caffeine from Hopkin and Williams Ltd., Chadwell Heath, Essex and DNA, Type III from Salmon testes, from the Sigma Chemical Co., St. Louis, Mo., USA. Glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADPH were purchased from Boehringer, Mannheim, W. Germany.

### 2.2. *Reaction of epoxides with DNA*

8,9-Dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide (2.5 mg) or benz(a)anthracene 5,6-oxide (2.5 mg) in ethanol (25 ml) was mixed with a solution of Salmon sperm DNA (50 mg) previously deproteinized by a detergent-salt procedure [9], in Tris buffer pH 7.4, 0.01 M (50 ml) and incubated at 37°C for 4 hr. After incubation, the solution was extracted with solvent and the DNA reisolated as described previously [5].

### 2.3. *DNA from hamster embryo cell cultures*

Embryo cell suspensions were prepared from 11–12 day Syrian hamster embryos [10], seeded ($1 \times 10^{8}$



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cells) into Dulbecco's MEM (250 ml) containing foetal calf serum (15% v/v) and cultured at 37°C in Thompson bottles (2l) under 10% $CO_2$ in air for 4 days. Cell monolayers were treated with $^3$H-labelled benz(a)anthracene (2 μg/ml), added to the medium as a solution in DMSO (final concentration, 0.1%), and caffeine (1 mM) and 24 hr later the medium was removed and the cells harvested using an EDTA solution (0.02%). The DNA was isolated and purified as previously described [11].

### 2.4. *Microsomal incubations with DNA*

Deproteinized Salmon sperm DNA (60 mg), dissolved in Tris buffer pH 7.4, 0.01 M (40 ml), was mixed with a washed rat-liver microsomal preparation [12] (≡ 5 g liver) resuspended in Tris buffer (40 ml) containing NADPH (24 mg), glucose 6-phosphate (120 mg) and glucose 6-phosphate dehydrogenase (14 units) to which $^3$H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)-anthracene (262 μg) was added in acetone (0.2 ml). After incubation at 37°C for 30 min, the microsomes were pelleted by centrifugation and the DNA reisolated from the supernatant and purified as described [12].

### 2.5. *Sephadex LH20 column chromatography*

DNA from reactions with epoxide, from treated hamster cell cultures or from microsomal incubations with hydrocarbon derivatives was hydrolysed enzymically to nucleosides [7,13] and chromatographed on columns (75 × 1.5 cm) of Sephadex LH20 eluted with methanol–water gradients [7,13]. Fractions (5.3 ml) were collected and examined at 260 nm for U.V. absorbing materials and the radioactivity present determined by liquid scintillation counting.

### 2.6. *Thin-layer chromatography*

Fractions eluted from chromatography columns that contained DNA-hydrolysis products were pooled and evaporated to a small volume and the solution applied to Eastman Kodak 6060 Chromogram sheets that were developed with solvent (a) butan-1-ol: propan-1-ol:aq. 2M-$NH_3$ (2:1:1, by vol) or with solvent (b) ethyl acetate:methanol:water:formic acid, 90% (100:25:20:1, by vol). Chromatograms were examined under U.V. light and U.V. absorption, at 260 nm, and radioactivity measured after elution of areas of the chromatograms with ethanol.

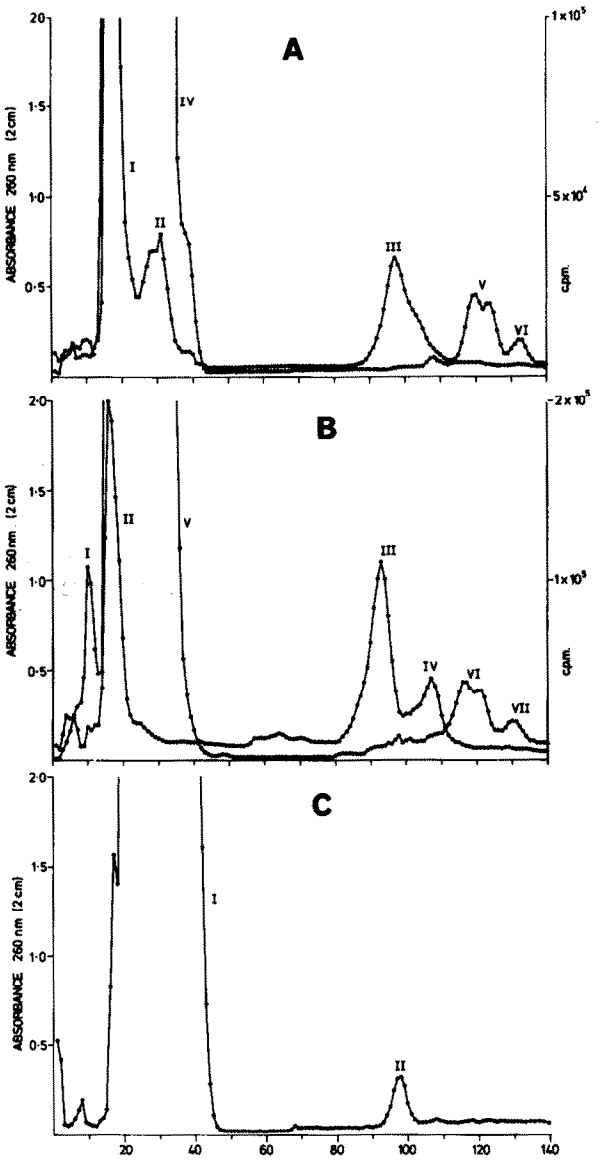


Fig. 1. Sephadex LH20 column elution profiles obtained following reactions with benz(a)anthracene derivatives. (A) Hydrolysate of DNA from hamster embryo cells treated with $^3$H-benz(a)-anthracene (○——○) co-chromatographed with that of DNA reacted with benz(a)anthracene 5,6-oxide (●——●). (B) Hydrolysate of DNA reisolated after incubation with $^3$H-8,9-dihydro-8,9-dihydroxybenz(a)anthracene and a microsomal preparation (○——○). co-chromatographed with that of DNA reacted with benz(a)anthracene 5,6-oxide (●——●). (C) Hydrolysate of DNA reacted with 8,9-dihydro-8,9-dihydroxy-benz(a)anthracene 10,11-oxide.

## 3. Results and discussion

The Sephadex LH20 column chromatographic system used here separated polycyclic hydrocarbon-deoxyribonucleoside products present in DNA hydrolysates from unreacted deoxyribonucleosides [7,13] which elute earlier (fig. 1A, I, II and IV; fig. 1B, I, II and V; fig. 1C, I; fig. 2, I, II and V; fig. 3, I, II and IV). When a mixture of hydrolysates of (a) DNA from hamster embryo cells that had been treated with $^{3}$H-labelled benz(a)anthracene and (b) DNA that had been reacted with benz(a)anthracene 5,6-oxide was examined by Sephadex LH20 column chromatography, the elution profiles of the two hydrolysates differed (fig. 1A). This confirms previous findings with 7-methylbenz(a)anthracene [7]. Hydrolysates of DNA that had been incubated with $^{3}$H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)anthracene and microsomes gave profiles that showed the presence of a radioactive product (fig. 1B, III) in a position similar to that occupied by the product (fig. 1A, III) obtained from hydrocarbon-treated cells: DNA-products were not formed in microsomal incubations that lacked the cofactors necessary for the monooxygenase. This result indicated that further metabolism of the 8,9-diol of benz(a)anthracene, one of the principle metabolites of this hydrocarbon in cells, may be involved in the reactions with DNA that occur following treatment of these cells with benz(a)anthracene. Metabolic oxidation of the isolated 10,11 double bond present in the 8,9-diol seemed likely and, when the synthetic 10,11-oxide was prepared [11] and reacted with DNA, the profile shown in fig. 1C was obtained from the hydrolysate. The peak (fig. 1C, II) that eluted in the region normally occupied by DNA-hydrocarbon products [7] was in a position similar to that of the product from the DNA of benz(a)anthracene-treated cells (fig. 1A, III).

Further comparisons confirmed this close similarity. Fig. 2 shows the elution profiles obtained when hydrolysates of (a) DNA reacted with 8,9-dihydro-8,9-dihydroxy 10,11-oxide and (b) DNA that had been incubated with $^{3}$H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)anthracene and microsomes were mixed and chromatographed on the same Sephadex column. The elution profiles obtained when hydrolysates of (a) DNA reacted with 8,9-dihydro-8,9-dihydroxybenz(a) anthracene 10, 11-oxide and (b) DNA from $^{3}$H-labelled benz(a)anthracene treated cells were mixed and chromatographed are shown in fig. 3. The products

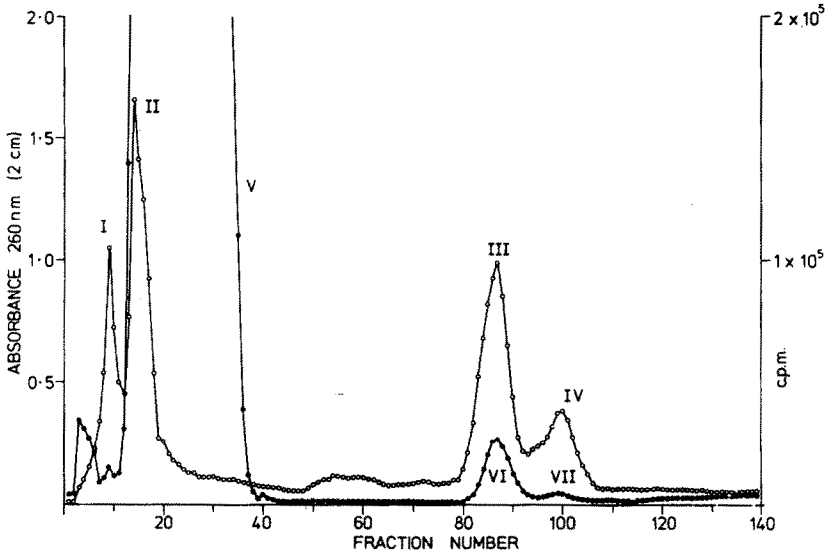


Fig. 2. Sephadex LH20 column elution profiles obtained from a hydrolysate of DNA reisolated after incubation with $^{3}$H-8,9-dihydro-8,9-dihydroxybenz(a)anthracene and a microsomal preparation (○——○) and co-chromatographed with that of DNA reacted with 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide (●——●).

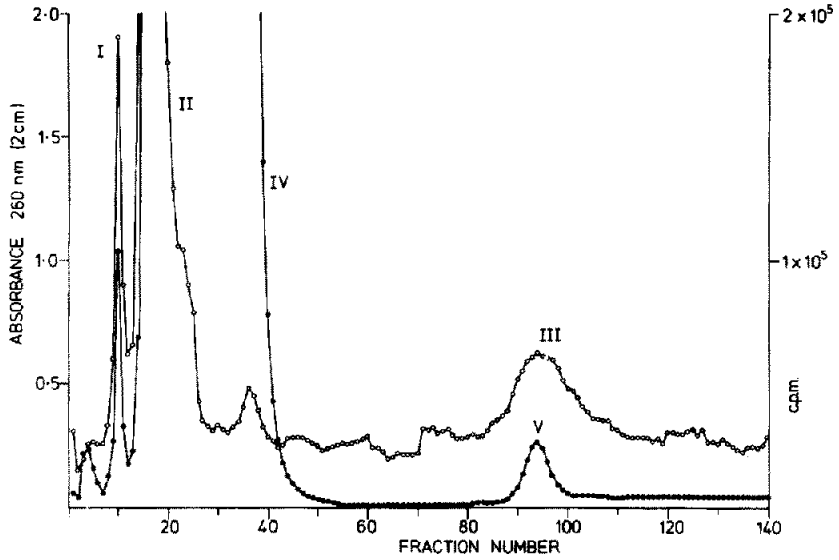


Fig. 3. Sephadex LH20 column elution profiles obtained from a hydrolysate of DNA from hamster embryo cells treated with $^{3}$H-benz(a)anthracene (○——○) co-chromatographed with that of DNA reacted with 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10, 11-oxide (●——●).

present in the coincident peaks (fig. 2, III, IV and fig. 3, III, V) were further examined by thin-layer chromatography. The results obtained showed that in reactions with DNA, 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide and the microsomal metabolite of 8,9-dihydro-8,9-dihydroxybenz(a)anthracene gave products that were not separable, in the two solvent systems used, from the DNA products that are formed in cells treated with benz(a)anthracene.

Although restricted to benz(a)anthracene, the evidence presented strongly supports the general concept that epoxides are the activated intermediates that are formed from polycyclic hydrocarbons by metabolism and that react with cellular nucleic acids. Investigations are in progress to determine whether diol-epoxides that are similar in structure to 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide are involved in the reactions of other, more carcinogenic, hydrocarbons with cellular nucleic acids. In addition, the possible biological activity of diol-epoxides is being examined and explanations for the apparent reactivity of this type of epoxide with the DNA of cells in culture are being sought.

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