K-REGION EPOXIDES OF POLYCYCLIC HYDROCARBONS: FORMATION AND FURTHER METABOLISM OF BENZ[a] ANTHRACENE 5, 6-OXIDE BY HUMAN LUNG PREPARATIONS

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1. Introduction

In animal tissues, carcinogenic polycyclic hydrocarbons are known to be metabolised to epoxides [1—4]. These epoxides, which are formed by the action of the NADPH-dependent microsomal mixed-function oxidase on the double bonds of the hydrocarbons [5], are biologically active [6—8] and are suspected of being responsible for the effects produced by the hydrocarbons. Aromatic hydrocarbons, which are present in tobacco smoke and in the urban atmosphere [9], are carcinogenic to rat-lung [10] and are also metabolized to K-region epoxides by preparations of this tissue [P.L. Grover, unpublished results]. Since polycyclic hydrocarbons are almost certainly carcinogenic in man, it seemed relevant to examine their metabolism by human tissue.

In the experiments described here, human lung preparations have been used for the first time to demonstrate: a) that an epoxide, which has been identified as the K-region derivative, benz[a] anthracene 5, 6-oxide, is formed as a microsomal metabolite of benz[a] anthracene and b) that this epoxide can be further metabolised to the corresponding dihydrohiol, *trans*-5, 6-dihydro-5, 6-dihydroxybenz[a] anthracene, and to the glutathione conjugate, S-(5, 6-dihydro-6-hydroxybenz[a] anthracene-5-yl) glutathione by enzymes present in these preparations.

2. Materials and methods

2.1. *Materials*

^{3}H-Labelled benz[a] anthracene (specific activity 510 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, Bucks, and [^{3}H] benz[a] anthracene 5, 6-oxide (specific activity 7.9 mCi/mmole) was prepared from the hydrocarbon [11]. Glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADPH were purchased from Boehringer, Mannheim, W. Germany and cyclohexene oxide from R.N. Emmanuel, Wembley, Middlesex. S-(5, 6-Dihydro-6-hydroxybenz[a] anthracene-5-yl) glutathione and *trans*-5, 6-dihydro-5, 6-dihydroxybenz[a] anthracene were prepared as described [12, 13].

2.2. *Lung preparations*

Specimens of human lung tissue were placed on ice immediately following removal at thoracotomy. Within an hour of removal, lung tissue, which was macroscopically free of tumour, was homogenised in phosphate buffer (0.1 M, pH 7.4, 4 vol) for 1.5 min in an Atomix blender and the mixture strained through cotton gauze to remove fragments of fibrous connective tissue. Washed microsomal and soluble supernatant fractions were then prepared as described [14].

2.3. *Microsomal metabolism of benz[a] anthracene*

Microsomal incubations were carried out using a human lung microsomal fraction (ca. 40 g lung), ^{3}H-labelled benz[a] anthracene (400 μg) and the co-factors for the NADPH dependent microsomal mixed function oxidase in the amounts previously described for rat liver [1, 3, 4]. The 3, 4-dihydronaphthalene 1, 2-oxide previously used as an 'epoxide hydrase' inhibitor was replaced by cyclohexane oxide (30 μmoles). After being incubated at 25°C for 10 min, the mix-

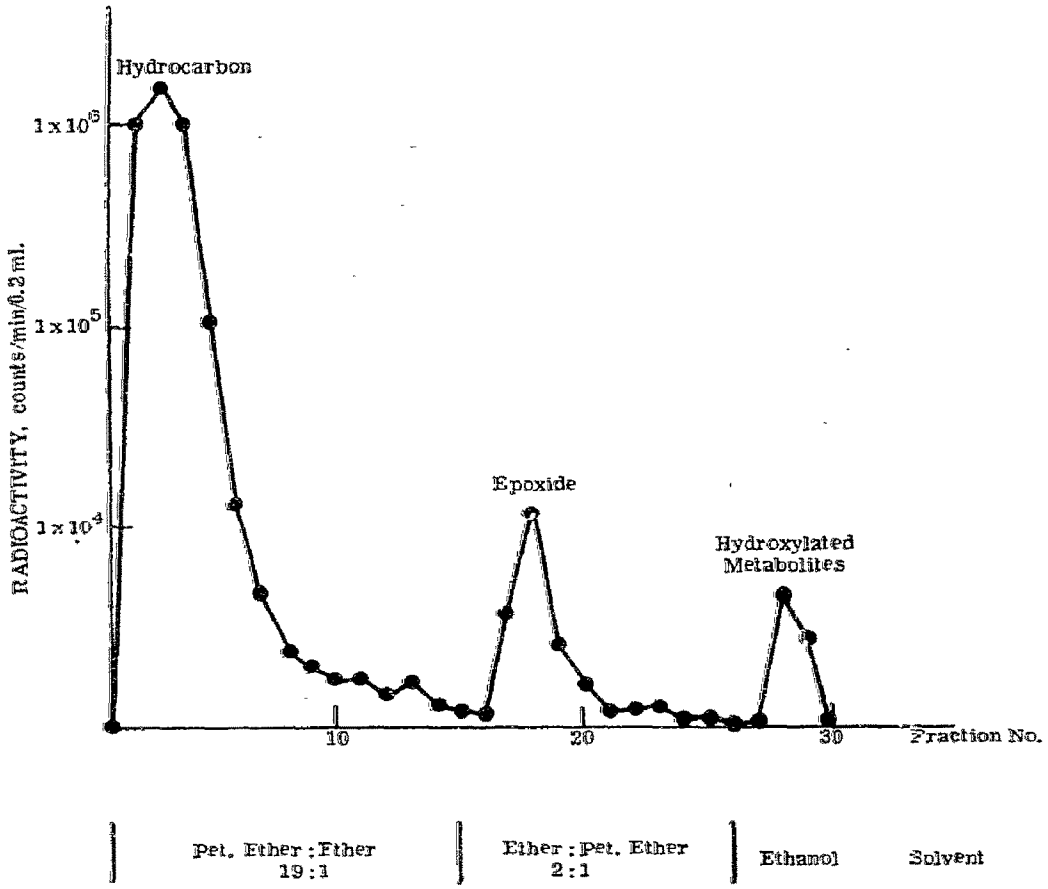

Fig. 1. Column chromatography of [^{3}H] benz[a] anthracene metabolites. The concentrated ether extract from a human lung microsomal incubation (see text) was applied to a column (1.3 × 4 cm) of basic alumina (M. Woelm Eschwege, Germany; deactivated by the addition of water, 6% w/w, to Wolem grade III) and eluted with solvent. 100 Drop fractions were collected and radioactivity measured.

ture was extracted with ether and the ether extract dried, concentrated and chromatographed on an alumina column as described in the legend to fig. 1. Fractions containing radioactive epoxide metabolites were pooled, the materials present subjected to procedures known to convert K-region epoxides into dihydrodiols, into phenols or into glutathione conjugates [1, 3, 4] and the products examined by chromatography using the appropriate unlabelled reference compounds derived from benz[a] anthracene.

2.4. *Detection of microsomal 'epoxide hydrase' activity*

Mixtures that contained human lung microsomal fraction (ca. 1g lung) resuspended in phosphate buffer (0.1 M, pH 7.4, 5 ml) and [^{3}H] benz[a] anthracene 5, 6-oxide (1 μmole), added in acetone (0.2 ml), were incubated at 37°C for 20 min and extracted with ethyl acetate (2 ml). Portions (0.1 ml) of the extract were co-chromatographed with unlabelled *trans*-5, 6-dihydro-5, 6-dihydroxybenz[a] anthracene as a marker as described in the legend to fig. 3 and the radioactive dihydrodiol formed detected by liquid scintillation counting.

2.5. *Detection of 'glutathione S-epoxide transferase' activity*

[^{3}H] benz[a] anthracene 5, 6-oxide (0.5 μmole) was added in ethanol (0.1 ml) to a mixture consisting of soluble supernatant fraction prepared from human lung (1 ml. ca. 0.2 g tissue) and phosphate buffer (0.1 M, pH 7.4, 4 ml) containing GSH (14.5 mg).

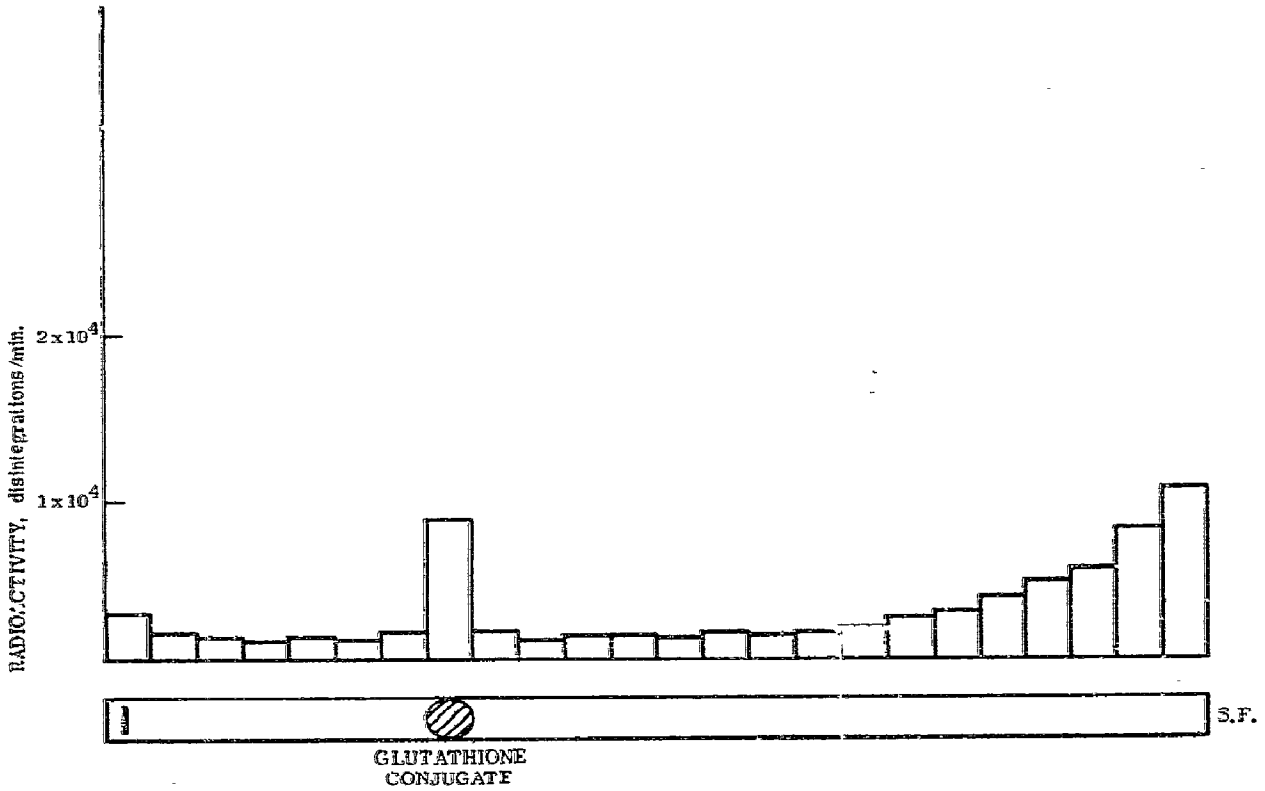

Fig. 2. Formation of a glutathione conjugate from a radioactive benz[a]anthracene epoxide metabolite. Fractions containing the radioactive metabolite, obtained by column chromatography of an ether extract of a [^{3}H]benz[a]anthracene incubation with human lung microsomal fraction were pooled, evaporated to dryness and reacted with GSH [1]. The products were co-chromatographed with unlabelled S-(5, 6-dihydro-6-hydroxybenz[a]anthracen-5-yl) glutathione on paper chromatograms (Whatman 3MM) developed with butan-1-ol: propan-1-ol: aq. 2 M NH_3 (2 : 1 : 1, by vol) which were then treated with ninhydrin, cut into strips and the radioactivity present determined.

After incubation at 37°C for 1 hr acetone (5 ml) was added, the mixture centrifuged and portions (0.2 ml) of the clear supernatant co-chromatographed with unlabelled S-(5, 6-dihydro-6-hydroxy-benz[a]anthracen-5-yl) glutathione as described in the legend to fig. 4. The radioactive glutathione conjugate was detected by liquid scintillation counting.

3. Results and discussion

When the ether-soluble products obtained from the metabolism of [^{3}H]benz[a]anthracene by the NADPH-dependent microsomal mixed function oxidase of human lung were examined by chromatography on alumina columns, profiles of radioactivity like that shown in fig. 1 were obtained. The radioactive peak that elutes after that containing the unchanged hydrocarbon but prior to that containing the hydroxylated products is in an identical position to the peak that has been previously shown to contain benz[a]anthracene 5, 6-oxide [1]. The epoxide formed as a metabolite of benz[a]anthracene by human lung preparations in the present experiments was identified as the K-region derivative, benz[a]anthracene 5, 6-oxide since it could be converted a) by treatment with acid, into a product indistinguishable on chromatograms from 5-hydroxybenz[a]anthracene and b) by treatment with rat-liver 'epoxide hydrase' into a product indistinguishable from *trans*-5, 6-dihydro-5, 6-dihydroxybenz[a]anthracene. The epoxide metabolite could also be converted by reaction with GSH into a radioactive product with the chromatographic characteristics of S-(5, 6-dihydro-6-hydroxybenz[a]anthracene-5-yl) glutathione as shown in fig. 2.

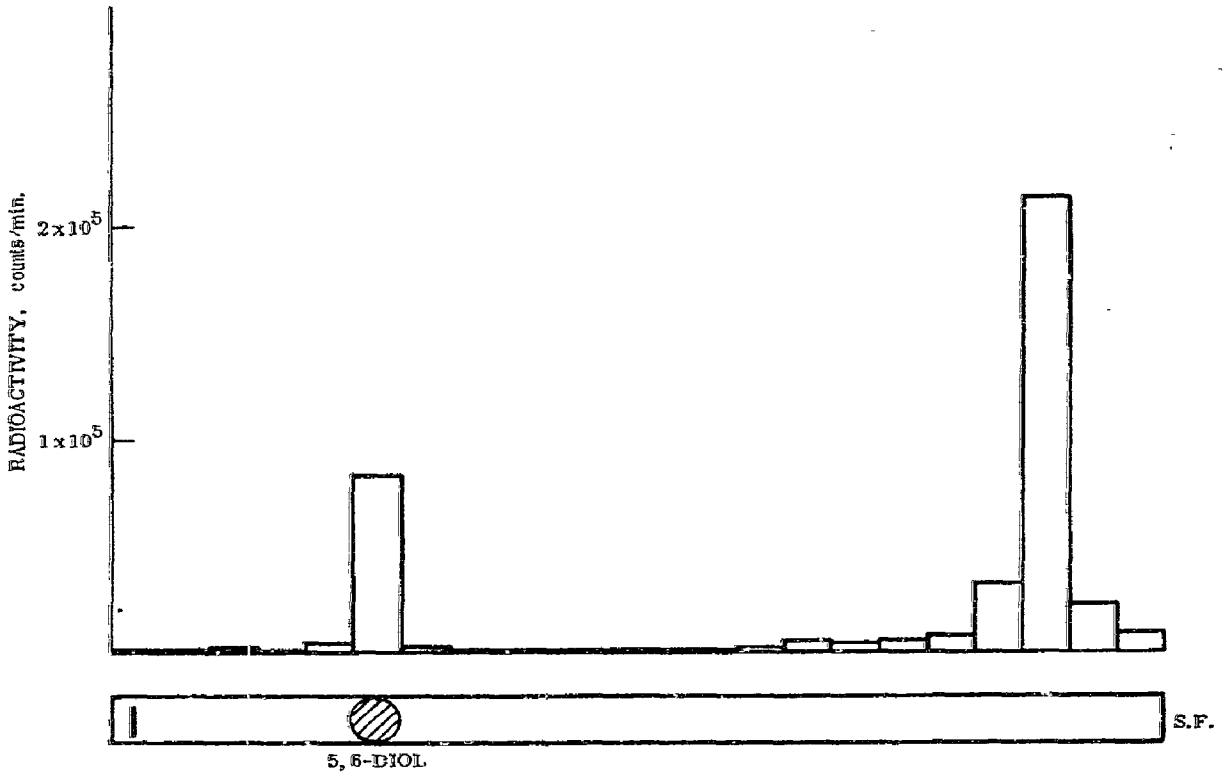

Fig. 3. Detection of human lung 'epoxide hydrase' activity. [^{3}H] benz[a] anthracene 5, 6-oxide was incubated with a microsomal preparation of human lung (see text) and the products co-chromatographed with unlabelled *trans*-5, 6-dihydro-5, 6-dihydroxybenz[a] anthracene on thin-layer chromatograms of silica gel G developed in benzene:ethanol, 10 : 1 v/v. Bands of silica gel were marked off and the radioactivity present determined.

When synthetic [^{3}H] benz[a] anthracene 5, 6-oxide was incubated with a human lung microsomal preparation in the absence of cofactors, a product indistinguishable from *trans*-5, 6-dihydro-5, 6-dihydroxybenz[a] anthracene was formed (fig. 3). The enzymic formation of this product, which could not be detected if microsomal suspensions that had been heated at 100°C for 5 min were used, demonstrates the presence of a microsomal 'epoxide hydrase' [15] in human lung that can catalyse the conversion of a benz[a] anthracene epoxide into the corresponding dihydrodiol.

A radioactive product, identical in its chromatographic characteristics with S-(5, 6-dihydro-6-hydroxybenz[a] anthracen-5-yl) glutathione, was formed when synthetic [^{3}H] benz[a] anthracene 5, 6-oxide was incubated with GSH and a soluble-supernatant fraction prepared from human lung (fig. 4). The conjugate was not detected when soluble supernatant fraction that had been heated to 100°C for 5 min was used. The formation of this GSH conjugate shows that a 'glutathione S-epoxide transferase' like that described in rat tissues [16] is also present in human lung.

Collectively the results presented here show that all three enzymes known to be involved in the formation and further metabolism of polycyclic hydrocarbon epoxides are present in human lung. The mixed function oxidase, which catalyses the initial oxidation of the aromatic double bonds to epoxide intermediates, may well be involved in the metabolic activation of the carcinogenic polycyclic hydrocarbons. The activity of this enzyme cannot be considered in isolation however, since the epoxides formed can be inactivated by further metabolism to dihydrodiols and to glutathione conjugates by enzymes present in human lung. It is hoped to carry out other more detailed studies on the metabolic activation of polycyclic hydrocarbons in human lung since these compounds are strongly suspected of contributing to the incidence of cancer of the pulmonary tract in man.

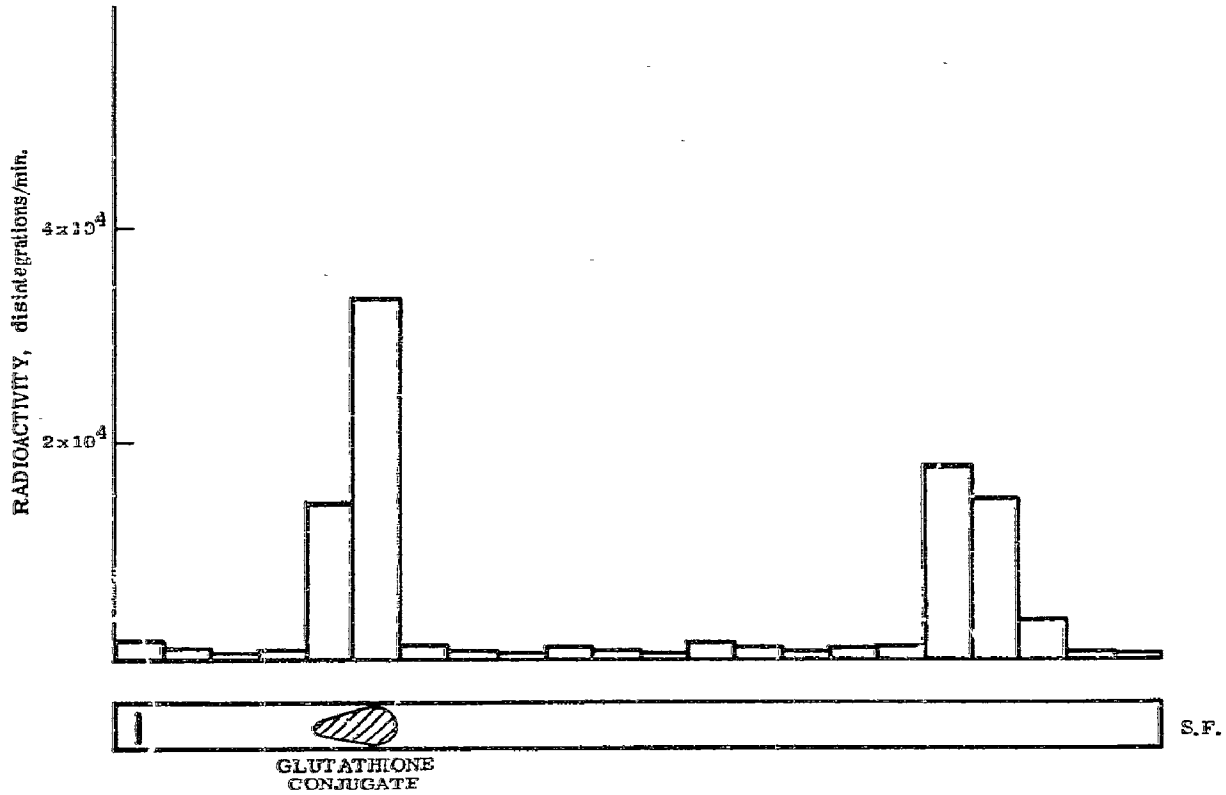

Fig. 4. Detection of human lung 'glutathione S-epoxide transferase' activity. [^{3}H] benz[a]anthracene 5, 6-oxide was incubated with GSH and soluble supernatant fraction prepared from human lung (see text) and the products co-chromatographed with unlabelled S-(5, 6-dihydro-6-hydroxybenz[a]anthracen-5-yl) glutathione on thin-layer chromatograms of silica gel S developed with butan-1-ol: propan-1-ol aq. 2M-NH_3 (2 : 1 : 1, by vol). The dried chromatograms were treated with ninhydrin, bands of silica gel removed and the radioactivity present determined.

Acknowledgements

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