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METABOLISM OF [^{3}H]BENZO(a)PYRENE BY DIFFERENT PORTIONS OF THE RESPIRATORY TRACT

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Abstract—The metabolism of benzo(a)pyrene by isolated perfused lungs and tracheal, bronchial and lung cultures has been studied in both rats and hamsters. The ethyl acetate-soluble metabolites from the medium are qualitatively similar in the tracheal and bronchial cultures, whereas, in the lung cultures large amounts of a new metabolite (X) derived from 3-hydroxybenzo(a)pyrene are formed. An unidentified metabolite (Y) migrating between 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene and 3-hydroxybenzo(a)-pyrene was observed in rat and hamster lung cultures and isolated rat lung perfusions. Dihydrodiols, in particular 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, are the major metabolites formed in the tracheal and bronchial cultures. In contrast to this 3-hydroxybenzo(a)pyrene and a metabolite derived from it (X) are the major metabolites formed by the isolated perfused lungs and lung cultures respectively. In the lung cultures much higher concentrations of water-soluble metabolites are produced than in the tracheal and bronchial cultures. The formation, accumulation and relative resistance to metabolism to water-soluble metabolites of dihydrodiols in the trachea and bronchi may partially explain the higher susceptibility of these areas to hydrocarbon carcinogenesis compared to the lungs.

Lung cancer is a major cause of death in many countries. Based on clinical, experimental, epidemiological and autopsy data, cigarette smoking has been identified as a major risk factor in the development of lung cancer [1]. The majority of these lung cancers in man are bronchogenic carcinomas which arise from a metaplastic squamous differentiation of the large bronchi. Thus this high and increasing incidence of lung tumours involves only a very small portion of respiratory epithelium and suggests a remarkable susceptibility of this tissue to carcinogenesis. Polycyclic aromatic hydrocarbons, such as benzo(a)pyrene, found in cigarette smoke and as environmental pollutants, have been associated with lung cancer in man [2]. As most chemical carcinogens have to be metabolically activated in the body before exerting their carcinogenicity [3], it is important to study the metabolic fate of benzo(a)pyrene in the different areas of the respiratory tract.

Polycyclic aromatic hydrocarbons are initially metabolised by the microsomal mixed function oxidase system to epoxides, which can then either be converted (i) enzymically by the microsomal epoxide hydrase system to the corresponding dihydrodiols or (ii) to glutathione conjugates by the soluble enzyme glutathione *S*-epoxide transferase or (iii) by spontaneous rearrangement to the corresponding phenols [4]. Whilst the above reactions are thought to be detoxication reactions, the epoxides, in what is believed to be their toxic reactions can also react with nucleic acids and proteins. Sims and co-workers have also shown that dihydrodiols may be further metabolised by the microsomal mixed function oxidase system to diol-epoxides which can then bind to DNA, for example, the conversion of 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene to 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide and its subsequent reaction with DNA [5].

Whilst the liver has been shown to be the major organ for the metabolism of anutrients, benzo(a)pyrene metabolism has been shown to occur in a wide number of extrahepatic tissues, including kidney, skin, intestine, placenta and lung [6]. Benz(a)anthracene, 7-methylbenz(a)anthracene and benzo(a)pyrene are metabolised by both rat lung homogenate and microsomal preparations [7] and by cultures of human bronchi and trachea of rat and hamster [8]. In both of these studies benzo(a)pyrene was converted to a number of ethyl acetate-extractable metabolites including 3-hydroxybenzo(a)pyrene, 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene [7, 8].

The rat and hamster were chosen for this study because they have been shown to be suitable models for the study of respiratory carcinogenesis [9, 10] and histologically the rodent trachea is similar to the human bronchus since it contains cartilage, compound mucous glands and goblet cells. In one of the major animal models for studying bronchogenic carcinoma repeated intratracheal instillations of a mixture of benzo(a)pyrene and ferric oxide to Syrian golden hamsters result in a high incidence of tumours in both the trachea and the bronchi but only a low occurence of peripheral lung tumours [9], which is a similar tumour distribution to that found in man.

In this study we report the metabolism of benzo(a)pyrene by both isolated perfused rat and hamster lungs and compare the metabolite patterns obtained in these experiments with those from short term organ culture of rat and hamster trachea, bronchi and lungs. The structural integrity of the lungs was maintained in the isolated lung perfusion experiments, thus more closely approximating the *in vivo* situation than in previous studies utilising lung microsomal preparations [7]. However, as no anastamoses have been

described between the pulmonary and bronchial circulations of the rat, perfusion of the isolated lungs via the pulmonary artery would not bring benzo(a)-pyrene into contact with either the trachea or bronchi, i.e. those areas which are most susceptible to respiratory carcinogenesis in both man and the animal model described above. Thus in these studies, we are able to compare the metabolic activation of benzo(a)-pyrene in different areas of the respiratory tract. The higher amounts of the dihydrodiols detected in the tracheal and bronchial cultures relative to those of the lung may partially explain the sensitivity of these areas to tumour formation.

MATERIALS AND METHODS

Materials. [^{3}H]benzo(a)pyrene (Sp. act., 25 Ci/m-mole) was obtained from the Radiochemical Centre, Amersham, Bucks. It was further purified according to the method of De Pierre *et al.* [11]. Benzo(a)pyrene, was obtained from Koch–Light Laboratories Ltd., Colne, Bucks. Leibovitz L-15 medium and foetal calf serum were obtained from Biocult Laboratories Ltd., Paisley, Scotland, bovine serum albumin (Fraction V—Lot 45C-0420) and DNA (Calf thymus Type I) from Sigma Chemical Co., St. Louis, Mo. U.S.A. and pentobarbitone Na from Abbot Laboratories Ltd., Kent. Pre-coated t.l.c. aluminium sheets coated with Silica gel 60 (0.25 mm thickness) were obtained from Merck, Darmstadt, Germany.

Animals. Male Syrian golden hamsters DSN (100–140 g) were obtained from D. Roberts, Basingstoke, England. They were maintained on Sterolit bedding (Engelhard Corp., Edison, N.J., U.S.A.). Male Wistar albino rats (200–300 g) bred at the University of Surrey were maintained on wood shavings. All animals were allowed food and water *ad lib.* and were kept under conditions of constant temperature (22°), relative humidity (50%) and lighting (12-hr alternating light–dark cycle).

Tissue preparations

(a) *Isolated rat and hamster lung preparations.* The isolated lungs were perfused by the method of Junod [12] as follows. The animals were anaesthetized by an intraperitoneal (i.p.) injection of pentobarbitone Na (60–90 mg/kg) and a tracheotomy was performed. After having opened the diaphragm, the animals were ventilated by positive pressure ventilation, using an electronically controlled animal ventilator (C. F. Palmer (London) Ltd.), with a tracheal air pressure of 15 cm H_2O, respiration frequency 45 cycles/min, inspiration time 30%, expiration time 50%, and pause 20%. The lungs were perfused via the pulmonary artery and the aorta was tied off. The perfusion medium was 5% bovine serum albumin in Krebs–Ringer bicarbonate and the perfusate was collected via a cannula placed in the left ventricle. After an initial perfusion period of 5 min to allow both removal of all traces of blood and temperature equilibrium of the lungs to 36–37°, the lungs were perfused with the above medium containing [^{3}H]benzo(a)pyrene (2 μM) at a flow rate of 5–6 ml/min using a Watson–Marlow roller pump. The perfusate was recirculated and samples of perfusion medium were removed from the reservoir at various times for analysis of metabolites as described below.

(b) *Tracheal, bronchial and lung cultures.* The cultures were carried out according to the method of Kaufman *et al.* [13] with minor modifications as follows. The animals were anaesthetised as described above and exsanguinated. The entire trachea and main bronchi were exposed and removed. The trachea was slit down the membranous posterior wall and separated from the bronchi at the tracheal bifurcation. The trachea, bronchi and lungs were then washed gently in Dulbecco's phosphate-buffered saline [14], and placed in separate flasks containing 10 ml of Leibovitz L 15 medium with 2 mM L-glutamine, 10% foetal calf serum, Penicillin 100 U/ml, Streptomycin 100 μg/ml and [^{3}H]benzo(a)pyrene (2 μM). The flasks were then incubated for either 3 or 24 hr. For the 24-hr cultures, attempts were made to maintain sterile conditions and either the trachea or bronchi or lungs of a single animal were used. In the 3-hr experiments, pooled trachea or bronchi from five animals or lungs from two animals were used. At the end of the timed incubations the trachea, bronchi and lungs were removed either for fixation in formal–saline and subsequent examination by light microscopy or for determination of DNA [15] content. After 24 hr culture, the trachea and bronchi appeared normal but the lungs, whilst remaining structurally intact, had darker staining nuclei in haematoxylin and eosin stained sections. The culture medium was examined for benzo(a)pyrene metabolites as described below.

Metabolism of benzo(a)pyrene by tissue preparations

Aliquots of the perfusion and culture media were extracted with ethyl acetate (1 vol × 2), and portions of the extracts examined by t.l.c. using the method of Sims [16]. In some experiments the ethyl acetate extracts were dried with anhydrous sodium sulphate but this step did not appear to affect the results obtained. Unlabelled reference compounds 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene and 3-hydroxybenzo(a)pyrene were prepared and added as cold carriers [16]. Thin-layer chromatograms were developed either in solvent system (a) benzene–ethanol (9:1, v/v) or (b) benzene–ethanol (19:1, v/v) and the products located by inspection of the wet plates in u.v. light. The metabolites of [^{3}H]benzo(a)pyrene were quantified by cutting the chromatograms into equal segments carrying the individual metabolites and the intermediate areas. The percentage radioactivity from different segments of the t.l.c. plate of appropriate controls were subtracted from the corresponding values obtained in the experimental determinations. The radioactivity was determined by liquid scintillation counting using a Packard Tri-Carb scintillation spectrometer, (Model 3320) using a toluene solution containing 5 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene per litre. The ^{3}H-labelled metabolites were identified according to their chromatographic properties and it was assumed that the metabolites had the same specific activity as the original [^{3}H]benzo(a)pyrene.

RESULTS

(a) *Metabolite formation by isolated perfused rat and hamster lung preparations.* Figure 1 shows results obtained from analysis of the perfusate of an isolated rat lung preparation after 120 min of perfusion. The major ethyl acetate-soluble metabolites formed during the perfusion were 3-hydroxybenzo(a)pyrene, 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene and a quinone. Lower amounts of the dihydrodiols 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene were also formed. The presence of benzo(a)pyrene 3,6-quinone was suggested by both its red colour and the $R_f = 0.85$ (relative to benzo(a)-pyrene) [17]. Whether this quinone arises by air oxidation of 3-hydroxybenzo(a)pyrene or by enzymic means is not known. An unknown metabolite (Y) with an $R_f = 0.58$ (relative to benzo(a)pyrene) and which fluoresced silver grey in u.v. light was formed in some perfusions (Fig. 1). The formation of the various metabolites during the perfusion is shown in Fig. 2. Production of all the metabolites appeared to follow similar patterns during the perfusion and 3-hydroxybenzo(a)pyrene appeared to be the major metabolite. In some experiments a decline in the amount of 3-hydroxybenzo(a)pyrene was observed after 90 min of perfusion (Fig. 2). This decline is probably due either to enzymic or chemical conversion of 3-hydroxybenzo(a)pyrene to further metabolites. The decline was generally accompanied by a concomittant decrease in the percentage of ethyl acetate-extractable metabolites and an increase in the water-soluble metabolites supporting the conversion of 3-hydroxybenzo(a)pyrene to a water-soluble product.

In contrast to the results seen with the isolated perfused rat lungs much smaller amounts of ethyl acetate-extractable metabolites were detected after isolated perfused hamster lung experiments, where the major metabolite was 3-hydroxybenzo(a)pyrene.

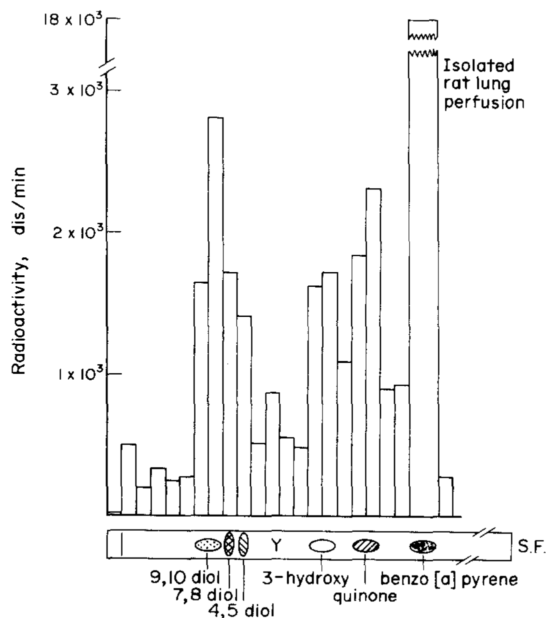

Fig. 1. Ethyl acetate-extractable metabolites from the perfusate after 120 min perfusion of isolated rat lung with [^{3}H]benzo(a)pyrene. The radioactive peaks co-chromatograph with the reference unlabelled metabolites as shown. The products were separated by t.l.c. in a mixture of benzene and ethanol (9:1 v/v). S.F. indicates solvent front.

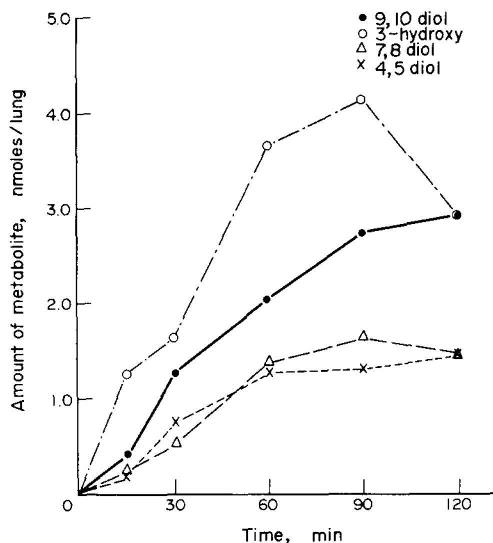

Fig. 2. Cumulative production of ethyl acetate-extractable metabolites in the perfusate during an isolated rat lung perfusion with [^{3}H]benzo(a)pyrene. 3-hydroxybenzo(a)-pyrene. ○——○; 9,10-dihydro-9,10-dihydroxybenzo(a)-pyrene ●——●; 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, △---△; 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene ×---×.

(b) *Cultures of hamster trachea, bronchi and lungs.* The metabolite patterns obtained by analysis of the media of hamster tracheal and bronchial cultures were similar (Fig. 3). Radioactive peaks which co-chromatographed with 3-hydroxybenzo(a)pyrene, 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene in both solvent systems (a) and (b) were obtained. The high concentration of dihydrodiols, particularly 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, relative to 3-hydroxybenzo(a)pyrene was very striking (Fig. 3). A similar pattern was seen after 3 hr culture, but smaller amounts of metabolites were formed. A small radioactive peak which moved just beyond the origin in solvent systems (a) or (b) was detected after analysis of the media of some cultures particularly those of the trachea. The amount of radioactivity connected with this peak increased with increasing duration of the cultures. This peak as well as the other metabolites were not obtained when either the trachea or bronchi was cultured at 4° for 24 hr. In the majority of the culture experiments, insufficient amounts of 3-hydroxybenzo(a)-pyrene and 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene were formed for accurate quantitation. Thus in Table 1 only the amounts of 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene and 7,8-dihydro-7,8-dihydroxybenzo-(a)pyrene formed using the different culture systems are shown.

In cultured hamster lungs benzo(a)pyrene was converted to 9,10-dihydro-9,10-dihydroxybenzo(a)-pyrene, and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene whereas little or no 4,5-dihydro-4,5-dihydroxybenzo-(a)pyrene or 3-hydroxybenzo(a)pyrene was detected

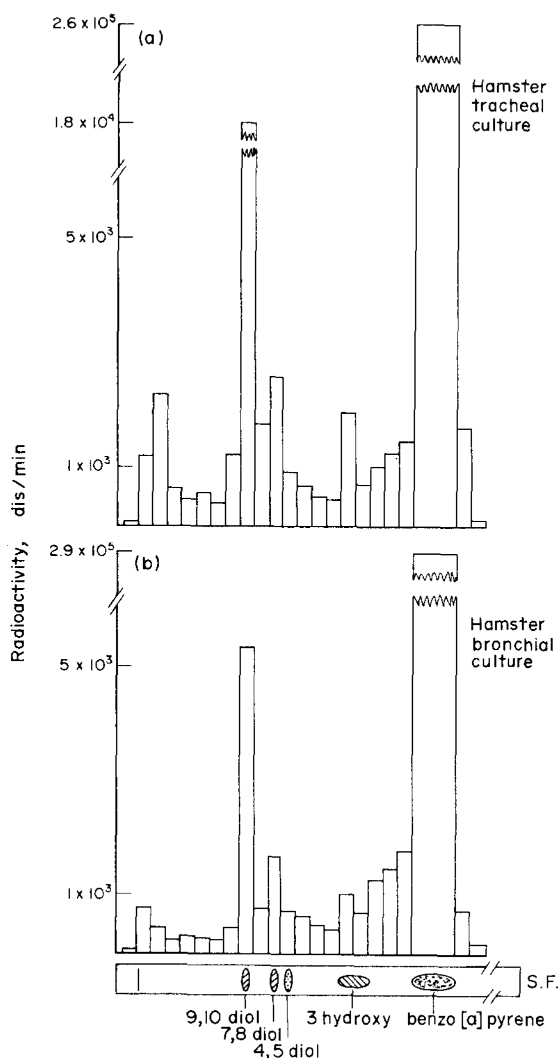

Fig. 3. Ethyl acetate-extractable metabolites from the medium after 24 hr culture with [^{3}H]benzo(a)pyrene of (a) hamster trachea, and (b) hamster bronchi. The radioactive products were separated by t.l.c. in a mixture of benzene and ethanol (9:1, v/v). S.F. indicates solvent front.

(Fig. 4b). The major ethyl acetate-extractable metabolite (X) migrated just beyond the origin in solvent systems (a) and (b). A radioactive peak, with similar chromatographic properties to Y (Fig. 1), migrating between 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene and 3-hydroxybenzo(a)pyrene was also observed (Fig. 4b).

(c) *Cultures of rat trachea, bronchi and lungs.* The ethyl acetate-extractable metabolites formed by rat tracheal and bronchial cultures were similar to those formed by the hamster cultures and co-chromatographed in solvent systems (a) and (b) with the unlabelled reference compounds of 3-hydroxybenzo(a)-pyrene, 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene, 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene (Fig. 5). A small radioactive peak which moved just beyond the origin in both solvent systems (a) and (b) was also seen in the rat tracheal cultures. The amounts of 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene formed in rat tracheal and bronchial cultures are shown in Table 1.

In rat lung cultures the major ethyl acetate-extractable metabolite (X) remained near the origin in a similar manner to that seen with hamster lung culture (Fig. 4). The majority of the radioactivity associated with this spot, which remained near the origin in solvent systems (a) and (b) moved away from the origin in a mixture of benzene and ethanol (4:1, v/v) with an $R_f = 0.15$ (relative to benzo(a)pyrene). This spot appeared blue in u.v. light.

In preliminary experiments to determine the nature of this metabolite(X), [^{3}H]7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, [^{3}H]9,10-dihydro-9,10-dihydroxybenzo(a)pyrene, [^{3}H]4,5-dihydro-4,5-dihydroxybenzo(a)-pyrene and [^{3}H]3-hydroxybenzo(a)pyrene were synthesized and incubated with rat lung. Only [^{3}H]3-hydroxybenzo(a)pyrene gave a product with similar chromatographic and fluorescent properties to X. Thus this major metabolite (X) in both the rat and hamster lung cultures is a further metabolite of 3-hydroxybenzo(a)pyrene.

An unidentified radioactive peak, migrating between 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene and 3-hydroxybenzo(a)pyrene was also observed after the rat lung culture (Fig. 4a). This unknown metabolite had similar chromatographic properties to the product (Y) formed after hamster lung culture (Fig. 4b) and isolated lung perfusion (Fig. 1).

Percentage of ethyl acetate-extractable radioactivity. The percentage of ethyl acetate-extractable radioactivity from the medium after lung culture was significantly lower than the corresponding values obtained from the media after tracheal or bronchial cultures. This difference was most marked after 24 hr when the percentages of ethyl acetate-extractable radioactivity from the media after rat and hamster lung cultures were 17.5 ± 2.9 and 12.0 ± 0.2 respectively, whereas those for the media after rat and hamster tracheal cultures were 54.3 ± 8.0, and 48.5 ± 0.8, respectively, and those obtained for rat and hamster bronchial cultures were 67.5 ± 3.8 and 67.1 ± 7.3, respectively. (All values represent mean $\pm$S.E.M. of at least 3 determinations). These differences are even more marked when percentages are corrected for the percentage ethyl acetate-extraction of 70.5 ± 1.6 of [^{3}H]benzo-(a)pyrene (2 μM) after 24 hr incubation with the culture media at 37°, e.g. the percentages for hamster bronchial and lung cultures become 95.2 and 17.0 respectively. Thus even considering the differences in tissues weights, the lungs of both rat and hamsters appear to convert the benzo(a)pyrene more readily to water-soluble metabolites than either the trachea or bronchi.

DISCUSSION

These studies have shown that benzo(a)pyrene is metabolised (Figs. 3 and 5) qualitatively to similar metabolites by cultured trachea and bronchi of rat and hamster, whereas isolated perfused lungs of rat and cultured lungs of the rat and hamster produce an unidentified metabolite (Y) (Figs. 1 and 4), and the cultured lungs also produced a new metabolite

Table 1. Amount of ethyl acetate-soluble metabolites formed*

Tissue	Incubation time (hr)	pmoles/g/min		pmoles/mg DNA/min†	
		9,10-diol	7,8-diol	9,10-diol	7,8-diol
Hamster Trachea	3	1.85 ± 0.45	0.34 ± 0.06	1.08 ± 0.32	0.19 ± 0.05
Hamster Trachea	24	8.27 ± 0.39	1.49 ± 0.14	—	—
Hamster Bronchi	3	1.05 ± 0.25	0.40 ± 0.04	0.60 ± 0.12	0.25 ± 0.05
Hamster Bronchi	24	8.45 ± 2.91	2.11 ± 0.83	—	—
Hamster Lungs	3	0.070 ± 0.007	0.029 ± 0.001	0.0096 ± 0.0007	0.0040 ± 0.0001
Hamster Lungs	24	0.15 ± 0.03	0.050 ± 0.008	—	—
Rat Trachea	3	1.14 ± 0.30	1.48 ± 0.33	0.55 ± 0.01	0.74 ± 0.07
Rat Trachea	24	1.59 ± 0.40	0.37 ± 0.11	—	—
Rat Bronchi	3	2.15 ± 0.55	2.37 ± 0.23	0.79 ± 0.13	0.91 ± 0.07
Rat Bronchi	24	2.29 ± 0.91	0.58 ± 0.22	—	—
Rat Lungs	3	0.71 ± 0.05	0.26 ± 0.02	0.11 ± 0.01	0.04 ± 0.004
Rat Lungs	24	0.061 ± 0.026	0.077 ± 0.012	—	—

All values represent mean ± S.E.M. of 3 determinations.

* The amounts of metabolites were calculated after having cut the t.l.c. plates into segments and determined the radioactivity, and then subtracted the percentage activity from corresponding plates from a control flask incubated with [^{3}H]benzo(a)pyrene at 37° for an appropriate time.

† Results expressed on a DNA basis are shown only for 3-hr pooled samples where sufficient amounts of tissue were present for accurate estimations. 9,10-Diol is 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene and 7,8-diol is 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene.

(X) (Fig. 4). Quantitatively the major ethyl acetate-extractable metabolites identified in the medium, after having cultured with trachea and bronchi, were the dihydrodiols particularly 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene (Table 1 and Figs. 3 and 5), whereas relatively small amounts of 3-hydroxybenzo(a)pyrene were formed. This was further emphasised by the observation that no major radioactive peak attributable to a further metabolite of 3-hydroxybenzo(a)pyrene was detected near the origin in the tracheal and bronchial cultures. In contrast to this, the major ethyl acetate-extractable metabolite in the perfusate from isolated perfused lungs was 3-hydroxybenzo(a)pyrene (Fig. 2), and in the cultured lungs it was a further metabolite of 3-hydroxybenzo(a)pyrene (X) (Fig. 4). Thus the dihydrodiols appeared to be the major ethyl acetate-extractable metabolites formed in the trachea and bronchi, whereas 3-hydroxybenzo(a)pyrene was formed predominantly in the lungs.

In the lung cultures 3-hydroxybenzo(a)pyrene is further metabolised to an unknown metabolite (X). Experiments are in progress to determine the nature and reactivity of X. Capdevila *et al.* [18] have shown a cytochrome P-450-linked activation of 3-hydroxybenzo(a)pyrene by rat lung microsomes to a metabolite which binds covalently to DNA. The further metabolism of 3-hydroxybenzo(a)pyrene observed in this and other studies [18, 19] further complicates the interpretation of the many studies measuring benzo(a)pyrene 3-monooxygenase (EC 1.14.14.2) by the fluorescence of 3-hydroxybenzo(a)-pyrene.

7,8-Dihydro-7,8-dihydroxybenzo(a)pyrene in the presence of microsomes will bind to a purified DNA, prepared from cultured hamster embryo cells, approximately 15-fold greater than benzo(a)pyrene itself [17], and Sims *et al.* have shown that 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene is further metabolised to 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene-9,10-oxide and that it is this type of diol-epoxide which reacts with DNA in primary cultures of Syrian hamster embryo cells *in vitro* [5]. Their results also suggested that the 9,10-dihydrodiol was more resistant to further activation than the 7,8-dihydrodiol [5]. Thus the high concentrations of dihydrodiols formed in the trachea and bronchi in these experiments may be further metabolised to diol-epoxides which initiate the tumourigenic response. The higher amounts of 9,10-dihydrodiol to 7,8-dihydrodiol formed in our experiments (Table 1) are consistent with a higher rate of formation and/or a decreased susceptibility to further metabolic activation of the 9,10-dihydrodiol. The higher amounts of dihydrodiols formed in the trachea or bronchi relative to the lungs is very striking, when the results are expressed either on a wet weight of tissue basis or on DNA content (Table 1), e.g. the amounts of 7,8-dihydrodiol formed in the hamster trachea and bronchi at 24 hr relative to the lung at the same time period are approx 30- and 42-fold respectively when results are expressed on a wet weight of tissue basis. These differences were more marked when results were expressed on a DNA basis because of the higher content of DNA in the lung and the weight of cartilage included in the wet weight of trachea.

The significantly lower values obtained for the ethyl acetate-extractable radioactivity from the medium after lung cultures compared to the other cultures suggest that the lung cultures convert the benzo(a)-pyrene to water-soluble products more rapidly than either the trachea or bronchi. This further metabolism of the metabolites in the lung by preventing an accumulation of carcinogenic metabolites, may contribute to the lower sensitivity of the lung to hydrocarbon carcinogenesis. However, this may mean that more dihydrodiols are produced in the lung than indicated in Table 1, but once formed are readily converted to water-soluble, probably less toxic, metabolites. This suggestion is supported by the much more rapid formation of metabolites by isolated perfused rat lung compared to the rat lung culture (Fig. 2 and Table 1). In the perfused lung experiments when metabolite

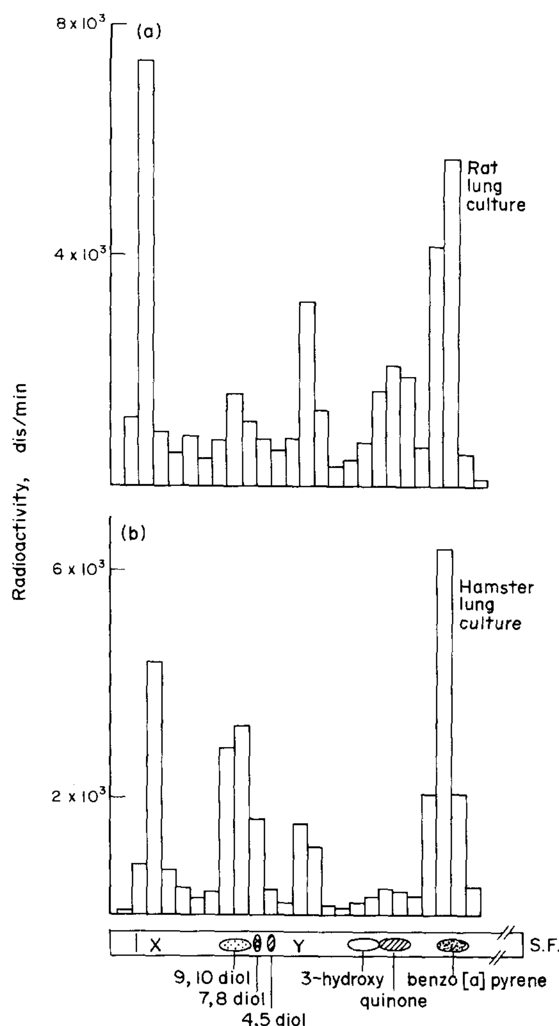

Fig. 4. Ethyl acetate-extractable metabolites from the medium after 24 hr culture with [^{3}H]benzo(a)pyrene of (a) rat lung and (b) hamster lung. The radioactive products were separated by t.l.c. in a mixture of benzene and ethanol (9:1, v/v). The major metabolite migrated just beyond the origin. S.F. indicates solvent front.

formation was still almost linear at 90 min the rates of formation of 3-hydroxybenzo(a)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene were approximately 44 and 31 pmoles/g/min, respectively (Fig. 2). This relatively high rate of formation of metabolites could be due to the better perfusion of the lung in the perfusion experiments compared to the cultures.

Many chemical carcinogens such as the polycyclic aromatic hydrocarbons require metabolic activation to exert their carcinogenic action. These results, in agreement with Pal *et al.* [8], show a metabolic activation of benzo(a)pyrene in those areas of the respiratory tract most susceptible to carcinogenesis. This is supported by the observations that benzo(a)pyrene will bind to DNA in cultured human bronchial epithelium [20] and in hamster tracheal epithelium [21].

Thus the metabolism of benzo(a)pyrene in trachea and bronchi, the high amounts of the dihydrodiols and their retention as dihydrodiols in these areas relative to the lungs may partially explain the sensitivity of the trachea and bronchi to hydrocarbon carcinogenesis. The results and thereby the conclusions in this study on the amounts and types of metabolites obtained under the different experimental conditions may be affected by many factors, in particular further metabolism of the initial metabolic products and binding of activated metabolites to tissue macromolecules. Both of these factors would lead to an underestimation of the production of any particular metabolite. However considering these limitations, the higher amounts of the dihydrodiols formed in the trachea and bronchi and their retention as dihydrodiols in susceptible tissues as determined from the lower amounts of water soluble metabolites found after tracheal and bronchial cultures possibly contribute to the different susceptibility of areas of the respiratory tract to carcinogenesis. The susceptibility of a particular tissue will also be dependent on many other factors such as the absorption, distribution, dose and accumulation of the carcinogen within the tissue, the stage of the cell in the cell cycle, the ability of the cell to repair damage to DNA, the presence of tumour

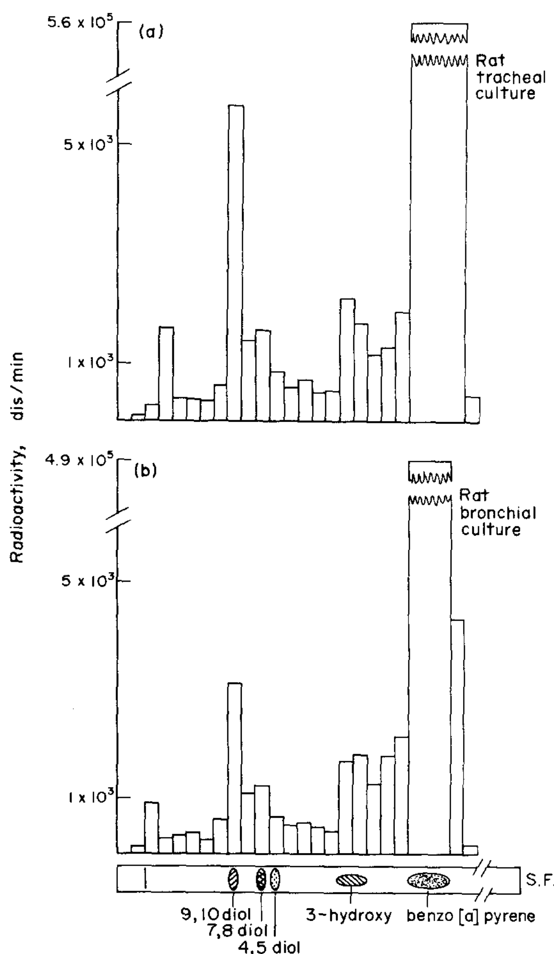

Fig. 5. Ethyl acetate-extractable metabolites from the medium after 24 hr culture with [^{3}H]benzo(a)pyrene of (a) rat trachea, and (b) rat bronchus. The radioactive products were separated by t.l.c. in a mixture of benzene and ethanol (9:1, v/v). S.F. indicates solvent front.

promoters as well as co-factor and enzymatic activity of a particular cell which will determine the further metabolite fate of a carcinogen such as benzo(a)-pyrene.

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