The Metabolism of Carcinogenic Polycyclic Hydrocarbons by Tissues of the Respiratory Tract

KALYANI PAL, PHILIP L. GROVER and PETER SIMS

The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London SW3 6JB, U.K.

Carcinogenic polycyclic hydrocarbons undoubtedly require metabolic activation, a process currently thought to involve epoxide formation. Previous studies have shown that the NADPH-dependent microsomal mono-oxygenase that catalyses the formation of epoxides is present in preparations of human and rat lung tissue (Grover et al., 1973, 1974). These tissues are also capable of the further metabolism of hydrocarbon epoxides since, with both species, the conversion of benz[a]anthracene 5,6-oxide into the corresponding dihydrodiol, catalysed by microsomal epoxide hydrase, and into the corresponding glutathione conjugate, catalysed by glutathione S-epoxide transferase, was detected (Grover et al., 1973; Grover, 1974).

The polycyclic hydrocarbons that are present in tobacco smoke (Wynder & Hoffman, 1959) are suspected of contributing to the increased incidence of respiratory cancer in man (Doll & Hill, 1950). The human respiratory tumours (Kreyberg type I), whose occurrence appears to be most closely associated with tobacco smoking (Kreyberg, 1962), arise in the bronchial epithelium. In view of this, efforts are being made to investigate the metabolic activation of polycyclic hydrocarbons in bronchial mucosa, which is probably more relevant to the etiology of human lung cancer than the mainly alveolar tissue preparations that were used in the earlier work.

In the present studies, the metabolism of the 3H-labelled polycyclic hydrocarbons benz[a]anthracene (sp. radioactivity 610 mCi/mmol), 7-methylbenz[a]anthracene (sp. radioactivity 457 mCi/mmol) and benzo[a]pyrene (sp. radioactivity 475 mCi/mmol) by Wistar rat and Syrian hamster trachea and by human bronchial tissue maintained in short-term culture was examined. The freshly removed animal trachea were sliced into rings and portions weighing approx. 200 mg were placed in plastic tissue-culture flasks (30 ml) (Falcon Plastics, Oxnard, Calif., U.S.A.) that contained Dulbecco's MEM (10 ml) (Biocult Ltd., Paisley, U.K.) supplemented with foetal calf serum (10%, w/v) and incubated at 30°C under 10% CO₂ in air. Segments of human bronchus that were macroscopically free of tumour were placed in culture in a similar way within 1 h of removal at thoracotomy. After 24 h in culture, the medium surrounding the respiratory tissue was replaced by medium (10 ml) to which a 3H-labelled hydrocarbon (4 μg) (The Radiochemical Centre, Amersham, Bucks., U.K.) had been added as a solution in ethanol (2 μl). After incubation for a further 24 h, the media were removed, extracted with ethyl acetate (2 × 1 vol.) and the pooled extracts were evaporated to dryness. The residue was redissolved in ether, small amounts of appropriate authentic reference compounds were added and the mixtures were examined on t.l.c. developed with benzene-ethanol (9:1, v/v) as described previously (Grover et al., 1974). Bands that contained the reference compounds were marked off under U.V. light and these and intermediate bands were removed, transferred to glass vials, and the radioactivity present was determined by liquid-scintillation counting. Protein present in homogenates of tracheal and bronchial tissues was determined (Fincham, 1954) by using casein as a standard protein. Other specimens were examined histologically.

With benz[a]anthracene, radioactive products were formed that were inseparable on t.l.c. from 5,6-dihydro-5,6-dihydroxybenz[a]anthracene, 8,9-dihydro-8,9-dihydroxybenz[a]anthracene and 9-hydroxybenz[a]anthracene. With 7-methylbenz[a]anthracene radioactive products with the chromatographic characteristics of the related 5,6- and 8,9-dihydrodiols were formed together with 7-hydroxymethylbenz[a]anthracene. The metabolism of [3H]benzo[a]pyrene by respiratory tissues yielded products with the chromatographic characteristics of 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; 3-hydroxybenzo[a]pyrene was also detected. The results obtained show that rat
and hamster tracheal rings and human bronchial segments each appear to metabolize the three polycyclic hydrocarbons studied in a qualitatively similar manner. The data obtained so far also suggest that, in quantitative terms, the amounts of each of the metabolites formed from the hydrocarbons by the respiratory tissues examined are of the same order.

The microsomal mono-oxygenase that catalyses the initial oxidation of aromatic double bonds to epoxides is an enzyme whose activity is inducible in liver and in other tissues (Conney, 1967), but this aspect has not been investigated so far with trachea since it may be difficult to determine the extent of mono-oxygenase induction present in human bronchial segments. The present studies show that rat and hamster trachea and human bronchus can metabolize polycyclic hydrocarbons; the site of this metabolism within these tissues was not determined. The detection of dihydrodiols, which are formed as metabolites from polycyclic hydrocarbons by these respiratory tissues, may well be relevant to hydrocarbon carcinogenesis for two reasons. First, the trachea of the rat and the hamster are known to be susceptible to tumour induction by polycyclic hydrocarbons (Kuschner et al., 1957; Saffiotti et al., 1968) and trachea from these species appear to metabolize hydrocarbons to the same products formed by human bronchial tissue. Secondly, the detection of dihydrodiols as metabolites indicates that these respiratory tissues possess both the mono-oxygenase that catalyses epoxide formation and the epoxide hydrase that is involved in their hydration to dihydrodiols. The metabolic activation of polycyclic hydrocarbons may also involve dihydrodiols since recent work has shown (a) that a new type of epoxide is formed by the further metabolism of a dihydrodiol (Booth & Sims, 1974) and (b) suggests that these diol-epoxides are the active intermediates involved in reactions with cellular nucleic acids (Swaisland et al., 1974; Sims et al., 1974).

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Anaerobic Dechlorination of Trichlorofluoromethane by Liver Microsomal Preparations in vitro

C. ROLAND WOLF, LAURENCE J. KING and DENNIS V. PARKE

Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

The toxicity of the aerosol propellant gas, trichlorofluoromethane, has aroused some interest since the reports that its inhalation can induce cardiac arrhythmia (Taylor & Harris, 1970). Routine toxicity studies indicated that it was non-toxic and biologically