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MAJOR DIFFERENCES IN THE EXTENT OF CONJUGATION WITH GLUCURONIC ACID AND SULPHATE IN HUMAN PERIPHERAL LUNG

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Abstract—Short-term organ cultures of human peripheral lung metabolise benzo(*a*)pyrene to water-soluble metabolites. Enzymic hydrolysis of these water-soluble metabolites with arylsulphatase (with saccharic acid 1,4-lactone) but not with β-glucuronidase released significant amounts of ethyl acetate-soluble radioactivity, which co-chromatographed with metabolites of benzo(*a*)pyrene. Similar studies of human peripheral lung showed that 1-naphthol, a model phenolic substrate, was metabolised extensively to its sulphate conjugate with little or no glucuronic acid conjugate being formed. In marked contrast to this, with short-term organ culture of rat lung, 1-naphthol is mainly conjugated with UDP-glucuronic acid. Thus, in human but not in rat lung, phenolic substrates such as monohydroxybenzo(*a*)pyrenes or 1-naphthol are metabolised predominantly to their respective sulphate conjugates with little or no glucuronide conjugates being formed.

Epidemiological data implicate polycyclic aromatic hydrocarbons, which are abundant in polluted atmosphere and cigarette smoke condensate, as a major factor in the aetiology of lung cancer in humans [1, 2]. It is well established that metabolism is a necessary pre-requisite for the conversion of these polycyclic aromatic hydrocarbons to ultimate carcinogenic forms [3–6].

Benzo(*a*)pyrene (BP), one of the most extensively studied member of this group of compounds, is initially metabolised to organic solvent-soluble metabolites such as epoxides, dihydrodiols, diol-epoxides, tetrahydrotetrols, a catechol, quinones and phenols [4, 5, 7, 8]. However, studies of BP metabolism *in vivo* and in intact cells and cultures have recognised the formation of more polar mainly water-soluble metabolites identified as conjugates of glutathione, glucuronic acid and sulphate [9–16]. More recently, the enzymatic formation of these conjugates from various BP derivatives has been demonstrated [17–22]. Previous studies from this laboratory have reported that BP and monohydroxybenzo(*a*)pyrenes are metabolised both by short-term organ cultures of rodent and human lung and by isolated rat hepatocytes to ethyl acetate-soluble sulphate conjugates of monohydroxybenzo(*a*)pyrenes [14, 23, 24]. The presence of water-soluble glucuronide conjugates of 4,5-dihydrodiol, 7,8-dihydrodiol, 9,10-dihydroxybenzo(*a*)pyrene or 1,(3)9,10-trihydroxy-9,10-dihydrobenzo(*a*)pyrene and monohydroxybenzo(*a*)pyrenes in short-term organ cultures of hamster lung and rodent trachea has also been described [15, 25, 26].

The conjugation step may be important in removal of carcinogenic, mutagenic and toxic intermediates, and therefore, it is necessary to determine the balance between the formation of primary metabolites and their subsequent metabolism by either oxidative or conjugation mechanisms in tissues in which the carcinogen induces biological effects. Furthermore, the nature of the conjugation pathways utilized by a particular species and tissue may give information as regards to its susceptibility to toxic or carcinogenic action since certain conjugation mechanisms, for example, conjugation with sulphate, are known to be readily saturable [27–29].

In this study, we have examined the nature of conjugated metabolites produced from both BP and 1-naphthol by short-term organ culture of human peripheral lung. Sulphate conjugates are the major metabolites formed with little or no glucuronic acid conjugates detected. In striking contrast to this, short-term organ culture of rat lung metabolised 1-naphthol predominantly to its glucuronic acid conjugate.

MATERIALS AND METHODS

Organ cultures. Male Wistar albino rats (150–180 g) bred at the University of Surrey, were maintained on wood shavings and allowed food and water *ad lib.* Peripheral lung specimens from lung cancer patients were obtained at the time of surgery. These specimens were transported to the laboratory in phosphate buffered saline (lacking calcium and magnesium salts) at 4° and were cultured within 3 hr of removal at surgery.

Samples of human lung (100 ± 10 mg), that appeared macroscopically normal, and rat lung samples (100 ± 10 mg) were cultured for 18 hr at 37° in 2.5 ml of Leibovitz L-15 medium with 2 mM L-glutamine (Biocult Laboratories Ltd., Paisley, Scotland) containing 10% foetal calf serum (Flow Laboratories, Irvine, Scotland), penicillin (100 units/ml), streptomycin (100 μg/ml) and insulin (1 mU/ml; bovine, crystalline from Sigma Chemical Company, Norbiton, Surrey, England).

Incorporation of L-[U-^{14}C]leucine (sp. act., 354 mCi/mmole; Radiochemical Centre, Amersham, England) into protein and [6-^{3}H]thymidine (sp. act., 21.5 Ci/mmole; Radiochemical Centre, Amersham) into DNA of organ cultures of human peripheral lung, cultured for periods of up to 48 hr, could be demonstrated. The amounts of precursors incorporated into macromolecules of cultured tissues were found to be

similar to pieces of fresh tissue incubated with [^{14}C]leucine and [^{3}H]thymidine. Protein isolation for the measurement of [^{14}C]leucine incorporation was carried out by the method of Massaro [30], and DNA, for the determination of [^{3}H]thymidine incorporation, was isolated by the method of Webb and Lindstrom [31].

BP metabolism. [^{3}H]-BP (sp. act., 24 Ci/mmole; Radiochemical Centre, Amersham) and unlabelled BP (Koch-Light Laboratories Limited, Colnbrook, Bucks.) were dissolved in *N,N*-dimethylformamide. After culture for 18 hr the lung samples were transferred to media (volume, 2.5 ml) containing [^{3}H]-BP (10 μCi/ml) and unlabelled BP at a final concentration of 2 μM, and incubated in a shaking water-bath at 37° for 24 hr. Tissue samples were separated from the media at the end of the incubation period.

Analysis of water-soluble metabolites. Organic solvent-soluble metabolites and unmetabolised BP from the medium were extracted by shaking the medium for 30 sec with 6 × 1 vol. of ethyl acetate and separating the aqueous and ethyl acetate phases. The medium remaining after ethyl acetate extraction was then divided into equal aliquots (0.5–0.8 ml.) and incubated with either Ketodase (5000 units/ml; pure ox liver β-glucuronidase from Warner-Chilcott Laboratories, Morris Plains, N.J.) in 0.1 M-acetate buffer, pH 5.0, or with arylsulphatase (700 units/ml; type H-1 extracted from *Helix pomatia*; Sigma Chemical Company, Norbiton, Surrey, England) and D-saccharic acid 1,4-lactone (40 mM; Sigma Chemical Company) in 0.1 M-acetate buffer, pH 5.0, or with 0.1 M-acetate buffer, pH 5.0, alone. Saccharic acid 1,4-lactone was included with arysulphatase to inhibit β-glucuronidase, a known contaminant of this form of enzyme. The hydrolyses were carried out for 20–22 hr in a shaking water-bath at 37° in sealed tubes.

The enzyme hydrolysates were then extracted with 3 × 1 vol. of ethyl acetate. The pooled ethyl acetate extracts were dried with anhydrous sodium sulphate and concentrated to dryness on a rotary evaporator. The metabolites were redissolved in ethyl acetate and separated on a Laboratory Data Control (Laboratory Data Control, Stoke, Staffordshire, England) high pressure liquid chromatograph (h.p.l.c.) fitted with Constametric I, II and II G solvent delivery systems, a Gradient Master (Model 1601), a 254 nm U.V. III monitor (Model 1203), and a Spherisorb ODS column (4.6 mm × 25 cm; 5 μM particles; Phase Separations Limited, Deeside, Clwyd, U.K.). The metabolites were eluted at room temperature with a 50 min. linear gradient from 1:1 to 4:1 methanol: water at a flow rate of 1 ml/min. The radioactive metabolites were identified by co-chromatography with authentic BP metabolites which were kindly provided by NCI Carcinogenesis Research Program, Bethesda, MD, U.S.A. Fractions of 0.5 ml each were collected and the radioactivity was measured by liquid scintillation counting, in a scintillant containing 0.5% w/v PPO (2,5-diphenyloxazole), 0.02% w/v dimethyl POPOP (1,4 bis [2-(4-methyl-5-phenyloxazolyl)] benzene), 33% v/v Metapol (Durham Chemicals Distributors Limited, Birtley, Tyne and Wear, U.K.) in toluene, using a Packard Tri-Carb Scintillation spectrometer (Model 3320).

1-Naphthol conjugation. After culture for 18 hr, the rat and human lung samples were transferred to media containing various amounts of [1-^{14}C]-1-naphthol (specific activity, 19.2 Ci/mole; Radiochemical Centre, Amersham, England) and cold 1-naphthol (10 mM; Hopkin and Williams, Chadwell Heath, Essex, England) to give final substrate concentrations of 20, 100, 1000 μM, and incubated at 37° for 90 min. Control incubations, containing tissue and similar levels of substrate as the test incubations were incubated for the same time period at 4°. At the end of the incubation period, tissue samples were separated from the media and the conjugates in the media were analysed by thin layer chromatography (t.l.c.) essentially as described previously [32].

Aliquots (100 μl) of the media were applied to silica gel t.l.c. plates (200 × 40 mm; Kieselgel 60 F_{254}, 0.2 mm thickness, aluminium-backed plates from E. Merck, Darmstadt, W. Germany). Chromatograms were developed at room temperature with a solvent system of benzene–acetic acid–acetone (2:1:2) and the metabolites associated with radioactivity were detected by radiochromatogram scanning (Varian-Berthold Radiochromatogram Scanner). These metabolites were identified by comparison with authentic 1-naphthyl-β-D-glucuronide (Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K.), 1-naphthyl sulphate (potassium salt from Sigma Chemical Co.) and 1-naphthol. The identity of the glucuronide and sulphate conjugates was further confirmed by hydrolysis with β-glucuronidase (Ketodase) and arylsulphatase with saccharic acid 1,4-lactone as described for the water-soluble metabolites of BP. The quantitation of the conjugates present in the media was carried out by cutting the chromatogram into 1 cm segments, followed by liquid scintillation counting as above. The amounts of radioactivity (1.3–2.3% of total ^{14}C on the plate) detected at similar R_f's as the conjugates in the appropriate controls were subtracted from the corresponding values obtained in the experimental determinations.

RESULTS

Short-term organ cultures of peripheral lung from four patients converted approximately 7–42 per cent of BP to water-soluble metabolites (Table 1). To determine the nature of the water-soluble metabolites remaining in the medium after exhaustive extraction with ethyl acetate, the medium was subjected to enzymic hydrolysis with either β-glucuronidase or arylsulphatase with saccharic acid 1,4-lactone to hydrolyse glucuronide and sulphate conjugates respectively. These hydrolytic procedures on the aqueous phase released varying amounts of radioactivity to ethyl acetate-soluble products (Table 1). In comparison with the amounts of ethyl acetate-soluble radioactivity released after treatment with either buffer alone or β-glucuronidase, the highest amounts of ethyl acetate-soluble radioactivity were released after treatment with arylsulphatase in all patients except patient 1, where only 350 units of arylsulphatase were employed for the hydrolysis. The ethyl acetate-soluble metabolites after enzymic hydrolysis were concentrated and analysed by h.p.l.c.

The metabolite profiles obtained following hydrolysis with β-glucuronidase were similar to those following treatment with buffer alone and consisted of a single major peak, eluting very early in fractions 3–10 and representing 74–92 per cent of the total metabolites

Table 1. BP metabolism in human peripheral lung: percentage radioactivity in water-soluble fraction, and that which is ethyl acetate extractable after various hydrolytic procedures

Patient	Per cent of initial radioactivity in water-soluble fractions*	Per cent of radioactivity from the aqueous phase released into ethyl acetate after treatment with†: 0.2 M-Acetate buffer, pH 5.0	β-Glucuronidase	Sulphatase +D-saccharic acid 1,4-lactone
I	38.0	16.3	21.3	20.4‡
II	42.4	12.6	23.2	46.3
III	6.7	23.6	27.2	32.8
IV	9.8	26.6	26.0	38.2

* [^{3}H]Benzo(*a*)pyrene (final concentration, 2 μM) was incubated for 24 hr with short-term organ cultures of human peripheral lung. The medium was extracted with 6 × 1 vol. of ethyl acetate, and the amount of ^{3}H remaining in the water-soluble fraction was determined.

† The water-soluble fraction was divided into equal aliquots and subjected to treatments with either 0.2 M acetate buffer, pH 5.0, β-glucuronidase, or sulphatase with saccharic acid 1,4-lactone as described in Materials and Methods. The hydrolysates were extracted with 3 × 1 vol. of ethyl acetate and the pooled ethyl acetate extracts were sampled for radioactivity.

‡ Only 350 units of arylsulphatase with 20 mM-saccharic acid 1,4-lactone were used to hydrolyse the aqueous medium from Patient I.

(Fig. 1; Table 2). However, in marked contrast to this, after treatment with sulphatase, the metabolite profiles consisted of at least four new peaks, and the metabolite(s) eluting in the early fraction (3–10), representing 16–58 per cent of total metabolites, was markedly reduced (Fig. 1; Table 2). The metabolites eluting in fractions 3–10 and 10–20 have not as yet been identified, but the latter probably is derived from sulphate conjugates of polyhydroxylated metabolites such as 7,8,9,10-tetrahydrotetrol, which after hydrolysis would elute in this region. Thus, treatment with arylsulphatase cleaved 3–43 per cent of the total metabolites as a probable tetrol (fractions 10–20), 3–18 per cent which co-chromatographed with 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene (9,10-diol; Fig. 1), or (7/8,9)-trihydroxy-7,8,9,10,10-pentahydrobenzo(*a*)pyrene (7/8,9-triol) [3], 10–19 per cent as 9-hydroxybenzo(*a*)pyrene (9-OH-BP) and 10–30 per cent as 3-hydroxybenzo(*a*)pyrene (3-OH-BP; Table 2). These results suggested that sulphation rather than glucuronidation was the major route of conjugation of oxidative metabolites of BP. In order to confirm and extend these observations similar studies with a model phenolic substrate, 1-naphthol, were also carried out.

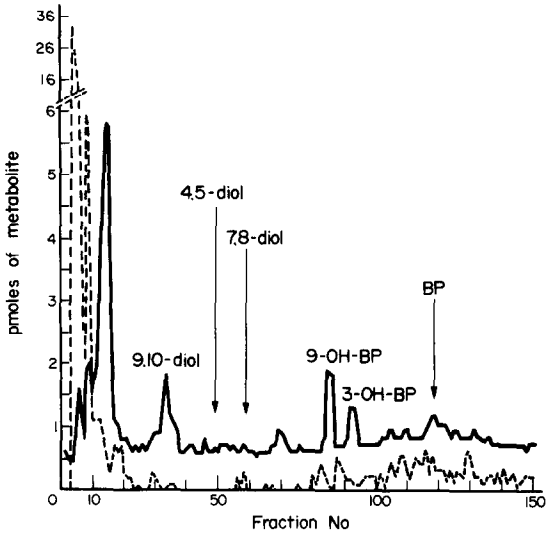

Fig. 1. H.p.l.c. of ethyl acetate soluble [^{3}H]-BP metabolites released after treatment of the water-soluble fraction with β-glucuronidase (- - -) and arylsulphatase with saccharic acid 1,4-lactone (——). The water-soluble fraction of the medium obtained from Patient II was subjected to hydrolysis with either β-glucuronidase (5000 units/ml) or arylsulphatase (700 units/ml) with saccharic acid 1,4-lactone (40 mM) as described in Materials and Methods. The hydrolysates were extracted with 3 × 1 vol. of ethyl acetate, and the pooled ethyl acetate extracts, after concentration, were chromatographed on an ODS column with a methanol–water gradient as described in Materials and Methods. The identity of the radioactive metabolites was established by co-chromatography with authentic BP metabolites. The elution position of these metabolites are marked.

When short-term organ cultures of peripheral lung from patients III and IV were incubated for 90 min with 1-naphthol (20 μM) the amounts of radioactivity, expressed as a fraction of initial radioactivity, remaining in the media after incubation were 71.8 and 69 per cent respectively. Similarly, in short-term organ culture of rat lung, after incubation with the same concentration of 1-naphthol for 90 min, 76.9 ± 1.1 per cent (Mean ± S.E., $n = 4$) of the initial radioactivity was retained in the medium.

Chromatographic analysis of the media indicated that the major metabolite produced by human lung was the sulphate conjugate of 1-naphthol (Fig. 2(a)), only minor amounts if any (0.1%) of the glucuronide conjugate being present. Similar results were obtained when fresh human lung was incubated with 1-naphthol under the same experimental conditions. In contrast, rat lung, at the same substrate concentration (20 μM), conjugated 1-naphthol mainly to its glucuronic acid conjugate [26.5 ± 4.9 per cent (Mean ± S.E., $n = 4$)], only 1.5 ± 0.6 per cent being conjugated with sulphate (Fig. 2(b)).

As the apparent lack of glucuronide conjugation in human lung may have been due to a low affinity of UDP-glucuronyltransferase for 1-naphthol preliminary

Table 2. BP metabolites in water-soluble fraction of human lung: per cent of total metabolites released into ethyl acetate after various treatments*

Fractions	Metabolite / Treatment	I†			II			III			IV		
		Buffer	Ketodase	Sulphatase	Buffer	Ketodase	Sulphatase	Buffer	Ketodase	Sulphatase	Buffer	Ketodase	Sulphatase
3–10	Solvent peak	85.6	89.3	58.2	78.3	92.2	15.8	74.2	75.5	44.1	82.0	88.8	54.6
10–20	Tetrols	2.8	1.2	4.6	10.4	3.5	43.0	0	0	3.3	2.3	2.6	4.1
27–37	9,10-Diol and/or 7/8, 9-triol	2.9	1.6	15.0	3.1	0.9	18.2	0	0	4.0	3.3	0	2.6
82–90	9-OH BP	6.2	4.7	10.0	3.6	1.6	13.0	10.9	9.8	18.9	6.4	6.5	15.3
90–95	3-OH BP	2.5	3.2	12.2	4.6	1.8	10.1	14.9	14.7	29.8	6.0	2.2	23.4

* Ethyl acetate extracts obtained after the hydrolytic procedures were analysed by h.p.l.c. as described in Materials and Methods and in legend to Fig. 1. The amounts of metabolites are expressed as a percentage of the total BP metabolites detected after h.p.l.c.
† Per cent of total metabolites after each treatment in patient.

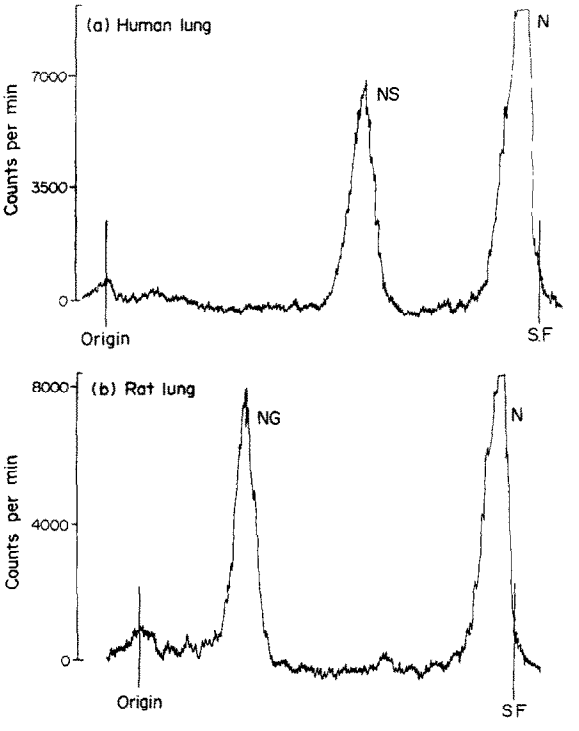

Fig. 2. T.l.c. separation of 1-naphthol and its conjugates in the media after incubation of short-term organ cultures of (a) human peripheral lung and (b) rat lung with [1-^{14}C]-1-naphthol. [1-^{14}C]-1-Naphthol was incubated with short-term organ cultures of the rat and human lungs for 90 min, and aliquots of the media were analysed by t.l.c. as follows. Chromatography was carried out on Kieselgel 60 F_{254} (0.2 mm thickness) aluminium-backed plates (E. Merck, Darmstadt, W. Germany). Chromatograms were developed at room temperature with benzene:acetic acid:acetone (2:1:2) and the identities of the metabolites, detected by radiochromatogram scanning, were established by comparison with authentic compounds. NG = 1-naphthyl glucuronide; NS = 1-naphthyl sulphate; N = 1-naphthol; S.F. = solvent front.

studies have also been carried out at higher concentrations (100 and 1000 μM). Similar results were found at these higher concentrations i.e. naphthyl sulphate was the major metabolite and little or no naphthyl glucuronide was detected.

DISCUSSION

A significant proportion (7–42 per cent) of BP metabolites produced by short-term organ culture of human peripheral lung were water-soluble. Such large variation in the presence of water-soluble metabolites is most likely due to inter-individual differences in overall rates of BP metabolism; thus, at the end of a 24 hr incubation period, the amounts of unmetabolised BP in the medium, as analysed by h.p.l.c. of the initial ethyl acetate extracts, were 19.7, 3.2, 73.8 and 78.9 per cent for patients, I, II, III and IV, respectively. (Cohen *et al.*, manuscript submitted for publication).

The present report demonstrates that detectable levels of glucuronic acid conjugates were not produced by short-term organ cultures of human peripheral lung

when these were incubated with [^{3}H]-BP. In contrast to the absence of glucuronic acid conjugates, small but significant amounts of sulphate conjugates of BP metabolites were formed. Following enzymic hydrolysis with arylsulphatase, ethyl acetate-soluble metabolites which co-chromatographed with a suspected tetrol (fraction 10–20), 9,10-dihydrodiol or 7/8, 9-triol (fractions 23–37), 9-OH-BP (fractions 82–90) and 3-OH-BP (fractions 90–95) were observed (Fig. 1). The reason only small but significant amounts of these sulphate conjugates, particularly of monohydroxybenzo(*a*)pyrenes, were found in the water-soluble fractions was most likely due to the fact that a large fraction of these sulphate conjugates were already extracted into ethyl acetate, as sulphate ester conjugates of monohydroxybenzo(*a*)pyrenes are partially organic solvent soluble [14, 23]. Stoner *et al.* [33] have also demonstrated the presence of conjugates of 9,10-diol, 7,8-diol and 3-OH-BP as water-soluble metabolites formed by cultured human peripheral lung with BP. However, as these workers used a combination of both β-glucuronidase and arylsulphatase to hydrolyse the conjugated metabolites, one cannot discern the relative importance of these two different conjugative pathways in their study.

Our findings with BP suggest that sulphate conjugation, compared with glucuronidation, is a major conjugation mechanism in human peripheral lung. This hypothesis was supported by the results with 1-naphthol, where the sulphate conjugate of the phenol was very clearly the predominant metabolite (Fig. 2(a)). These observations are further substantiated by the findings of Chin *et al.* [34] with the insecticide carbaryl (1-naphthyl-*N*-methyl-carbamate). Carbaryl readily undergoes hydrolysis in man yielding 1-naphthol as a metabolite. In explant culture of carbaryl with human liver and kidney, 1-naphthol was conjugated mainly with glucuronic acid, whereas in the lung it was conjugated almost entirely with sulphate.

Several studies have indicated that phenols can be conjugated with both glucuronic acid and sulphate, the relative extents to which this will occur depending upon the species, tissue, concentration and structure of the substrate [32, 35, 36]. Sulphation, mainly due to a limited availability of the sulphur-containing amino acids required for the synthesis of the sulphate donor, 3′-phosphoadenosine 5′-phosphosulphate, is a readily saturable process. Therefore, when the level of substrate is increased, there is a decrease in the amount of the substrate being conjugated with sulphate, and a complementary increase in that conjugated with glucuronic acid [35]. The latter mechanism is a first-order process since the conjugating agent UDP-glucuronic acid, with carbohydrate as its source, can be made readily available. However, in the present study preliminary investigations showed that with human lung even at higher concentrations, 1-naphthol did not form significant amounts of glucuronide conjugate.

Thus these results clearly indicate that sulphate rather than glucuronide conjugates are the major conjugated metabolites formed by human peripheral lung. There are several possibilities in lung to explain this including: (i) a higher proportion of the sulphating enzyme, phenolsulphotransferase (EC2.8.2.1) than the glucuronidating enzyme, UDP-glucuronyltransferase (EC2.4.1.17); (ii) the affinity of UDP-glucuronyltransferase for phenolic substrates such as 1-naphthol may be far less than for phenolsulphotransferase and (iii) the presence of a highly active β-glucuronidase in human lung which may be released into the medium on incubation. The findings of β- glucuronidase in human alveolar Type II cells and in human alveolar macrophages gives some support for the third possibility [37] although little or no β-glucuronidase is apparently released into the medium from rat lung under similar incubation conditions. If there is an active β-glucuronidase enzyme present, then this may be of some toxicological significance as it has been reported that hydrolysis of the glucuronide conjugate of 3-OH-BP with β-glucuronidase releases a product which binds to DNA [38].

In striking contrast to the results with human lung, short-term cultures of the rat lung have shown that 1-naphthol is predominantly conjugated with glucuronic acid (Fig. 2(b)). This is further supported by our earlier results with rodent lung when the major water-soluble metabolites formed from BP were glucuronide conjugates of monohydroxybenzo(*a*)pyrenes [15, 25]. Thus, in rat lung, a complete reversal of the situation encountered in human peripheral lung exists, demonstrating a major difference in the abilities of the lungs from the two species to conjugate phenolic substrates. This observation obviously needs to be further investigated; however, it may have important implications when considering the balance between activation and detoxification routes of metabolism of carcinogens such as BP, particularly in tissues like the human lung which may be constantly exposed to environmental pollutants and cigarette smoke. Although the rates of conjugation of BP derivatives with sulphate may be very high, the sulphation mechanism, being dependent on the pools of cysteine and methionine in the cell, can eventually become saturated. In situations where the glucuronidation mechanism is not effective enough to compensate for this loss, either an alternative pathway of metabolism, if available, may come into play, and/or the toxic process may ensue. For example, in the cat, which has a low capacity of glucuronidation for a number of phenols the LD_{50}'s of these phenols are very much lower in the cat compared with other laboratory animals [39].

In the case of BP in human lung, where there appears to be little or no glucuronidation, exhaustion of the sulphation mechanism may lead to an accumulation of various hydroxylated derivatives of BP such as phenols and dihydrodiols which may then undergo further oxidative metabolism to highly reactive metabolites such as phenol-oxides or diol-epoxides. The latter have been shown to be mutagenic, carcinogenic and bind covalently to nucleic acids and proteins [7, 40–43].

Of the water-soluble metabolites formed from BP by human lung, hydrolysis with arylsulphatase accounted for 32–46 per cent (excluding Patient 1, Table 1). In the present study, the remaining water-soluble radioactivity was not accounted for but may have been due in part to (i) radioactive metabolites covalently bound to proteins or other macromolecules in the medium; (ii) ^3H_2O in the medium formed by exchange; or (iii) glutathione or other yet unidentified conjugates. In similar experiments with [^{3}H]-BP and hamster embryo cells, (i) and (ii) did not account for greater than 10–20 per cent of the water-soluble radioactivity (Cohen, MacLeod and Selkirk, unpublished observations). Using a soluble-supernatant fraction of human lung, the

presence of a glutathione *S*-epoxide transferase has been demonstrated [18]. The role of this enzyme in the intact lung merits investigation as it may be a key factor in conjugating diol-epoxides and thus indirectly controlling how much metabolite is available for interaction with critical cellular macromolecules. It is also of interest that the synthesis of glutathione and 3′-phosphoadenosine 5′-phosphosulphate may both be partially dependent on the same cellular pool of cysteine.

In summary, BP and 1-naphthol are both metabolised by short-term organ cultures of peripheral human lung, primarily to sulphate conjugates with little or no glucuronic acid conjugates. In contrast to this, rat lung forms predominantly glucuronide conjugates with small but detectable amounts of sulphate conjugates.

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