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Metabolism-related Tissue-binding of Halogenated Hydrocarbons

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INTRODUCTION

The halogenated hydrocarbons comprise a huge group of chemicals with different persistence and toxicity. Although certain types of compounds exert a high toxicity per se, it appears to be common that toxic effects are caused by metabolites formed during biotransformation in different tissues. Studying halogenated hydrocarbons, autoradiography (12) has proved to be a useful tool to uncover tissue-selective interactions in experimental animals. Below, examples will be given to demonstrate metabolism-related binding of polychlorinated biphenyls (PCBs) and 1,2-dibromoethane (DBE), mainly in the surface epithelia of the respiratory and alimentary tracts.

Binding of PCBs in lung tissue is due to reversible high-affinity interactions of PCB methyl sulphone metabolites ($MeSO_2$-PCBs) with a secretory protein (5,10), whereas the epithelial binding of DBE is mediated by a high local bioactivation to reactive metabolites (7). While DBE is a documented carcinogen in the respiratory and upper alimentary tracts of experimental animals (see refs. in 7), chronic PCB intoxication has been associated with respiratory distress in humans (11).

Formation and tissue-binding of PCB methyl sulphone metabolites. $MeSO_2$-PCBs are lipophilic and persistent metabolites formed during biotransformation of PCBs in the mercapturic acid pathway (1-3). Depending on structure, $MeSO_2$-PCBs have been shown by autoradiography to be selectively accumulated in one or more of the following locations in mice, rats and/or quail: non-ciliated bronchiolar (Clara) pulmonary cells (Fig. 1), proximal tubular kidney cells, intrauterine luminal fluid, prostate (Fig. 2), large intestinal epithelium (Fig. 2) and brain (5, and references therein; unpublished data). In one case, a $MeSO_2$-PCB metabolite was accumulated in fetal (but not in maternal) soft tissue (6). Using 4,4'-bis(^{3}H-$MeSO_2$)-2,2',5,5-tetrachlorobiphenyl as a model ligand, the uptake in the pulmonary Clara cells has been shown to be due to a specific $MeSO_2$-PCB-binding protein (10). The protein-$MeSO_2$-PCB complex is se-

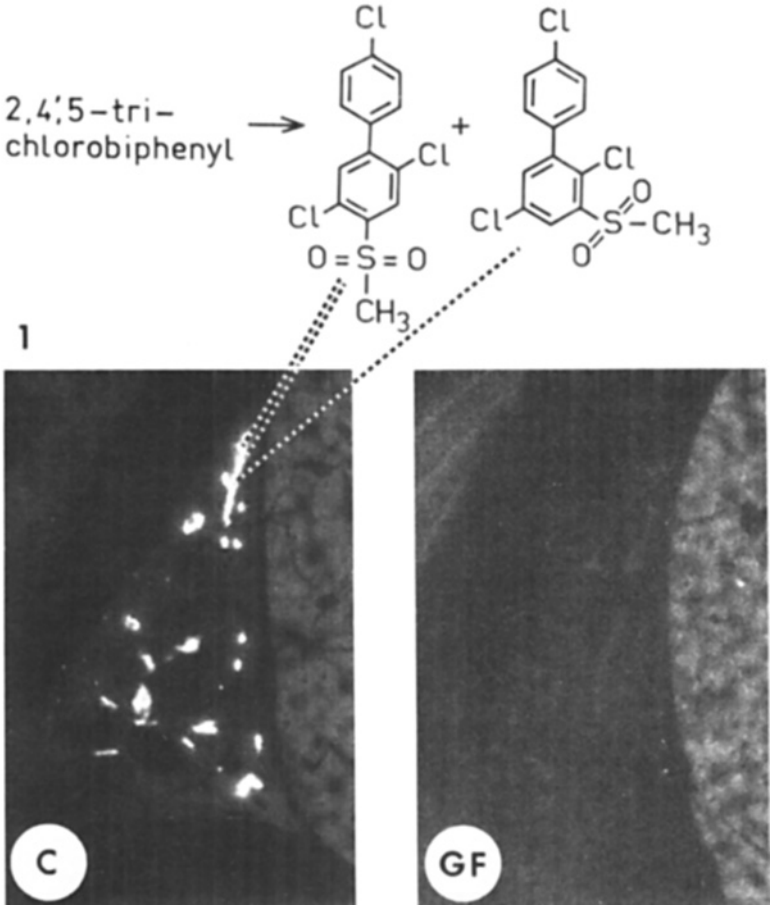
creted into the bronchiolar lumen and spread over the surface lining (5). While accumulation in the bronchiolar epithelium has been observed for at least 7 different $MeSO_2$-PCBs, localization of $MeSO_2$-PCBs in the other sites mentioned is less frequent. Hence it seems as binding of $MeSO_2$-PCBs in the latter structures occurs by a different mechanism than that in lung tissue. The existence of other sulphone-binding proteins could possibly explain these binding phenomena.

Given the specific tissue-localizations of $MeSO_2$-PCBs, whole-body autoradiography was combined with analytical methods (GC, GC-MS) to study the route of formation of $MeSO_2$-PCB. Initially, the parent PCB is epoxidized in the liver and conjugated with glutathione (GSH). The GSH conjugate is degraded to the corresponding cysteine conjugate and eliminated via biliary secretion. In the large intestine, the conjugate is further metabolized by a microbial C-S lyase, the PCB-thiol formed is methylated, reabsorbed from the intestine and sulphoxidized (presumably in the liver) to the corresponding $MeSO_2$-PCB, which is finally distributed to the target tissues (see refs. in 5). The involvement of the intestinal microflora in this metabolic sequence was demonstrated by experiments with germ-free mice and bile-duct cannulated rats (1,4). As can be seen in Fig. 1, there was no binding of $MeSO_2$-PCB in the bronchial epithelia of germ-free mice given the parent PCB, while conventional mice accumulated $MeSO_2$-PCB in the bronchial epithelium.

To summarize, the $MeSO_2$-PCBs are formed in a metabolic sequence involving enterohepatic circulation and metabolism by the intestinal microflora. Despite the reversible binding to a secretory protein, the $MeSO_2$-PCBs are retained in lung tissue for long periods of time (several months). Possibly, the presence of a pulmonary-entero-hepatic circulation could contribute to the persistence of $MeSO_2$-PCB in lung tissue; the secreted protein-$MeSO_2$-PCB complex could be transported by the ciliary escalator, swallowed, and the $MeSO_2$-PCB absorbed from the gut and redistributed to the lung.

Figure 1. Details of autoradiograms showing the lung region of a conventional (C) and a germ-free (GF) mouse 24 h after injection of 2,4',5-trichloro[^{14}C]-biphenyl. In the conventional mouse, a C-S lyase in the intestinal microflora contributes to the formation of the methyl sulphone metabolites, which are subsequently accumulated in the bronchial epithelium. In the germ-free mouse, lacking intestinal microflora, a limited formation of methyl sulphone metabolites is contributed by C-S lyase present in the tissues. Hence, the bronchial accumulation is insignificant in the germ-free mouse.

Figure 2. Detail of an autoradiogram obtained from a rat 6 days after injection of the PCB metabolite 4,4'-bis(^{3}H-methylsulphonyl)-2,2',5,5'-tetrachlorobiphenyl. There is a marked accumulation of the metabolite in the ventral prostate and the large intestinal epithelium. Note also labelling of intestinal contents.


2,4',5-tri-
chlorobiphenyl
Cl
Cl
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O=S=O
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+
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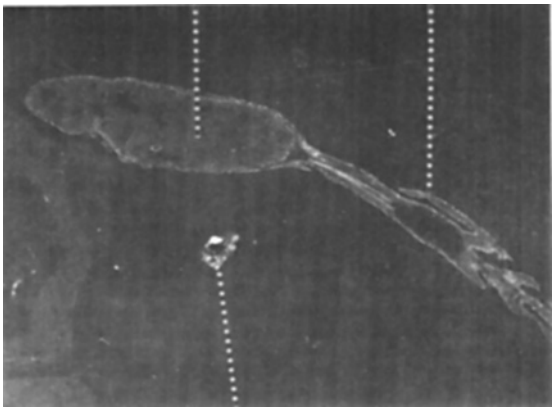
S-CH3
O
1
C
GF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Bioactivation of 1,2-dibromoethane in surface epithelia. The pesticide and lead scavanger DBE is a strong carcinogen, inducing tumour in the forestomach, nasal mucosa, lung and liver of mice and rats. The metabolic activation of DBE involves both an oxidative and a reductive cytochrome P-450 dependent pathway, and a glutathione-S-transferase catalysed reaction yielding a reactive GSH-conjugate (refs. in 7). Whole-body autoradiography showed that DBE is taken up and bound in the surface epithelia of the entire respiratory and the upper alimentary tracts of mice and rats. Using a combination of *in vivo* and *in vitro* experiments, autoradigraphy of solvent-extracted tissue showed a relationship between the sites of covalent binding of DBE in the surface epithelia and the sites of tumours observed after DBE administration (7).

Experiments with pregnant mice demonstrated that metabolic activation and epithelial binding of DBE is pronounced also in fetal tissues. Unlike adult animals, which showed the highest binding in the respiratory system, the fetal DBE-binding was most marked in the upper alimentary tract; according to computer-assisted image analysis of extracted sections, the binding in the fetal oral epithelium was about 3 times higher than that in the maternal liver, while binding in the fetal forestomach and nasal cavity equalled that in the maternal liver. These results suggest that DBE may also be a transplacental carcinogen in rodents (8).

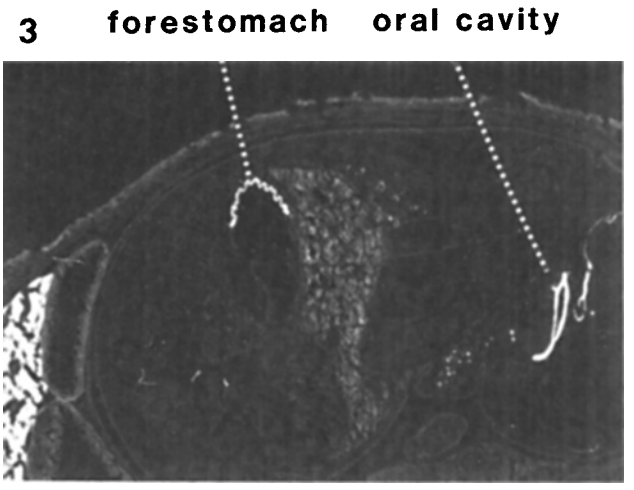

Figure 3. Detail of an autoradiogram showing a late gestational fetus of a mouse 90 min. after injection of the potent carcinogen 1,2-dibromo[^{14}C]ethane. Note the high and selective binding in the epithelia of the fetal forestomach and oral cavity.

CONCLUSIONS

Examples have been given, where biotransformation of halogenated hydrocarbons results in tissue-selective formation and binding of metabolites. In the case of the PCBs, reversible binding to a secretory protein in lung tissue is mediated by $MeSO_2$-PCBs, formed in an entero-hepatic cycle involving both hepatic and intestinal microbial metabolic transformations. In contrast, the epithelial binding of DBE is apparently due to a high metabolic activation *in situ*, resulting in enrichment of covalently bound adducts in the surface epithelia. While the latter results hopefully will help to understand the previously known organo-specific induction of tumours caused by DBE, the binding of $MeSO_2$-PCBs to a secretory protein in the Clara cells represents a hitherto fairly unexplored mechanism of accumulation. The significance of such binding of $MeSO_2$-PCBs for the development of respiratory distress in PCB-exposed humans remains to be investigated. Recently, a $MeSO_2$-PCB was found to decrease cytochrome P-450-dependent drug metabolism in the mouse lung (9). Also this finding stresses the need to clarify the physiological function of the $MeSO_2$-PCB-binding protein.

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