

# Monooxygenase, epoxide hydrolase, and glutathione-S-transferase activities in human lung. Variation between groups of bronchogenic carcinoma and non-cancer patients and interindividual differences.

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## Abstract

Activities of microsomal monooxygenases (MO) and epoxide hydrolase (EH) and cytoplasmic glutathione-S-transferases (GST) will contribute to controlling the pool of reactive intermediates, enzymatically derived from polynuclear aromatic hydrocarbons (PAH) within the cells of target organs such as the human lung. Therefore, we studied what interindividual differences exist in these enzyme activities and whether there is a correlation between the activities of these epoxide forming and metabolizing enzymes in preparations from peripheral lung samples and the occurrence of bronchogenic carcinomas in smokers and non-smokers. 57 samples obtained from surgery were studied. Among them were 12 samples from non-smoking patients without cancer as a control group. It is not known whether this control group behaves, with respect to the investigated parameters, identically to fully healthy people, since in all cases indications existed which justified the removal of lung biopsies. Using very sensitive standard assays with benzo[a]pyrene, biphenyl, 7-ethoxyresorufin and 7-ethoxycoumarin as substrates, MO activity could only be determined as O-deethylation of 7-ethoxycoumarin and only after modification of the assay method. Evidence was obtained for the presence of a diffusible, but not dialysible, MO inhibitor in human lung microsomes. The MO activity (substrate: 7-ethoxycoumarin) in this fraction was extremely low in human (100-fold lower than in rat lung preparations), whereas EH (substrate: benzo[a]pyrene 4,5-oxide) was slightly (about 2-fold) higher in human and GST (substrate: 2,4-dinitrochlorobenzene) had similar activities in both species. Interindividual variations of enzyme activities in human lung were considerable: MO, 40-fold; EH, 5-fold; GST 10-fold. Compared to the

control group (non-smokers without cancer) MO activities were slightly but significantly higher in lungs from bronchogenic carcinoma patients whether they were smokers (170% of controls,  $p < 0.0005$ ) or non-smokers (320% of controls  $p < 0.025$ ). MO activities of smokers without cancer were only very slightly elevated (140%) of controls,  $p < 0.05$ ). Specific EH activities compared to the control group were slightly but significantly increased in smokers without cancer (160% of controls,  $p < 0.0125$ ) and in bronchogenic carcinoma patients whether they used tobacco products (130% of controls,  $p < 0.005$ ) or not (140% of controls,  $p < 0.05$ ). Specific GST activities showed no significant differences ( $p > 0.1$ ) between the various groups studied. The substrate specificity of human lung EH, which was studied using five K-region epoxides of various PAH as substrates, corresponded to that in human and rat liver and in human, mouse and rat skin and to the pure enzyme isolated from rat liver. In contrast to rat liver hepatoma preparations, where EH had been shown to be increased in the tumor tissue and had been identified as a preneoplastic antigen, EH activity in lung microsomal preparations from samples of peripheral squamous cell carcinomas of two subjects had in the tumor tissue only one third of the activity of non-diseased areas of the same lung.

## Introduction

Polynuclear aromatic hydrocarbons (PAH)\* are widespread airborne pollutants. They are constituents of cigarette smoke, the inhalation of which is associated with an increased risk of bronchogenic carcinoma (1), one of the most frequent cancers in man.

However, PAH exert their adverse effects only after metabolic activation (2-10). Subcellular fractions of mammalian organs catalyse the metabolism of a great number of endogenous and exogenous compounds (11-13), and are responsible for the formation of electrophilically reactive intermediates of PAH leading to their covalent binding to tissues constituents including proteins and nucleic acids and to cytotoxic, mutagenic and carcinogenic effects (2-10). PAH are transformed by MO to electrophilically reactive epoxides (monofunctional arene oxides) which may bind to tissue constituents (14) but are frequently so rapidly inactivated by EH and/or GSH (15,16) that their contribution to toxic effects is minor in many situations. In angular PAH those pro-

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\*Abbreviations: PAH, polynuclear aromatic hydrocarbons; MO, monooxygenases; EH, epoxide hydrolase; GST, glutathione S-transferases; AHH, aryl hydrocarbon hydroxylase.

ducts of the EH-catalyzed reaction which have their hydroxyl groups at the two positions of the benzoring opposite to the bay region can be transformed by a second MO-catalyzed step to vicinal dihydrodiol-bay region epoxides (17,18). Results from the past few years suggest that these (e.g. benzo[a]pyrene 7,8-dihydrodiol 9,10-oxide) might be the most potent mutagens and carcinogens formed from several angular PAH, especially benzo[a]pyrene (19-24) one of the most widely distributed and most intensively studied PAH. Thus monooxygenases (MO), epoxide hydrolase (EH) and glutathione-S-transferases (GST) are critical for the control of reactive metabolites derived from PAH.

Most of these studies were performed with liver tissue from experimental animals. However, some pertinent information on human lung is also available: PAH are metabolized by human lung subcellular fractions and cultured bronchial cells to epoxides, dihydrodiols and diol epoxides (25-29). Harris *et al.*, determined the specific activity of benzo[a]pyrene MO and benzo[a]pyrene 4,5-epoxide hydrolase in human bronchial mucosa explants (30) and Cantrell *et al.*, measured benzo[a]pyrene hydroxylase in human pulmonary macrophages (31,32). McLemore *et al.* (32), reported on a connection between the occurrence of lung tumors and the level of aryl hydrocarbon hydroxylase AHH (reflecting those MO forms which transform benzo[a]pyrene to fluorescent phenols) in alveolar macrophages. Referring to the studies of Kellermann *et al.* (33), they suggested a correlation between the AHH activity in pulmonary macrophages and AHH induction in lymphocytes in the same subject for non-smoking cancer patients (32). However, extensive studies by other investigators have failed to confirm a connection between AHH inducibility in lymphocytes and the risk of bronchogenic carcinoma development while some studies could reproduce these results to various extent (34-39). One of the reasons for these confusing and contradictory results may be the fact that the attempted correlation was restricted to AHH whereas other MO forms and further enzymes such as EH and GST also contribute to the control of reactive metabolites of PAH.

Therefore in this study, three specific enzyme activities which play an important role in controlling the pool of electrophilically reactive PAH intermediates were measured simultaneously in 57 human lung samples. MO and GST were determined by broad-spectrum substrates which are converted by many of their multiple forms. Benzo[a]pyrene 4,5-oxide was used as a substrate for EH, where the situation with respect to possible broad-spectrum substrates for multiple forms is as yet not resolved.

MO is localized predominantly in the microsomal fraction whilst several other membrane fractions possess much lower and the cytoplasmic fraction no measurable activity (13). GST activity is very high in the cytoplasmic fraction (40), whilst membrane fractions possess much lower albeit significant activities (41-43). These enzymes were investigated in the present study in those subcellular fractions where they are predominantly located, MO in the microsomal and GST in the cytoplasmic fraction. EH is localized both in membrane (4) and cytoplasmic (44) fractions. The activity of the cytoplasmic EH is quite high for some substrates (44) but very low for all epoxides derived from PAH tested to date (45), whereas the microsomal EH is very active towards the latter (16). Amongst the various membrane fractions the microsomal had by far the highest activity (13). EH activities were therefore investigated in microsomal fractions in this study. Some of these individual lung samples had already been measured earlier and the results have been communicated in preliminary form in a meetings proceedings (46). Since the population in that preliminary study was so small, which was especially true for the control group, medical indication for thoracotomy in non-cancer patients being infrequent ( $n = 7$ , but now 12), none of the differences were statistically significant. The present study shows significant differences between the control and each of the three other groups in MO and EH but not GST activities. Moreover in the preliminary meeting report (46) patients with primary cancers outside the lung having lung metastasis were included (this group was called "lung-cancer patients"), whilst the greater number of samples from patients with primary bronchogenic carcinoma now available has allowed the formation of an exclusive group now called "bronchogenic carcinoma".

## Materials and Methods

### Chemicals

[<sup>3</sup>H]Benzo[a]pyrene (generally labelled, specific activity 21 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, UK and diluted to a specific activity of 1.25 mCi/mmol. [<sup>3</sup>H]Benzo[a]pyrene 4,5-oxide was synthesized according to the method of Dansette and Jerina (47) under conditions as described (48) and had a specific activity of 1.2 mCi/mmol. The other tritium-labelled epoxides were a generous gift from Dr P.Sims, Institute of Cancer Research, Royal Cancer Hospital, London, UK. They were generally labelled. The specific radioactivities used were (mCi/mmol): phenanthrene 9,10-oxide, 3.8; 7-methylbenz[a]anthracene 5,6-oxide, 10.0; benz[a]anthracene 5,6-oxide, 2.2; 3-methylcholanthrene 11,12-oxide, 3.0.

7-Ethoxycoumarin was prepared by standard procedure as described (49). Ethoxyresorufin was a generous gift from Dr J.Dent, DIIT, Research Triangle Park, NC, USA.

Other chemicals were of the purest grade commercially available.

#### Animals

Adult male Sprague-Dawley rats (200-220 g) were obtained from Versuchstierzuchtanstalt WIGA, Sulzfeld, GFR.

#### Patient population and statistical analysis

Lung samples from 57 patients suffering from bronchogenic carcinoma or non-tumorous diseases were obtained from surgery, frozen immediately in liquid nitrogen and kept at  $-70^{\circ}\text{C}$  until preparation. No measurable loss in enzyme activities was observed after a storage under these conditions for at least one year. Except where specified all samples were from non-tumorous peripheral tissue. None of the patients investigated was on long-term treatment with drugs known to be inducers of the microsomal enzyme system in man. In addition, none of the subjects had received x-ray treatment or chemotherapy prior to surgery.

Diagnoses were confirmed histologically. Only those patients were classified as non-smokers who had not used tobacco products for at least 6 months. Observations by Cantrell *et al.* had shown that induced monooxygenase (substrate: benzo[a]pyrene) activity levels in pulmonary macrophages of smokers revert to levels of non-smokers within 3 months after cessation of smoking (31). Most of the non-smokers had not smoked for a much longer time (means of 11 years for cancer patients and 26 years for non-cancer patients). All smokers smoked at least until the third last day before the biopsy was taken. A survey of the subjects is shown in Table I.

For statistical analysis of the specific enzyme activities of the different groups of patients a mean and its standard deviation were calculated. The variances of all groups were tested by an F-test for homogeneous distribution (50). If the variances of two samples were found to be significantly different, a Wilcoxon-Rank test was performed, while in the case of a homogeneous distribution a Student T-test for unconnected random samples was performed (50). Differences with  $p < 0.05$  were considered as statistically significant.

#### Preparation of subcellular fractions

Rats were killed by cervical dislocation. The lungs from groups of three animals were excised and pool-

Table I

Details of patients included in this study

	Noncancer	Bronchogenic carcinoma <sup>a</sup>
Total	19	38
Cigarette smokers <sup>b</sup>	7	31
Nonsmokers <sup>b,c</sup>	12	7
Male	14	34
Female	5	4
Age	8-73	39-80
Mean age	53	58

a) Patients with primary tumors outside the lung having lung metastasis were not included.

b) The distribution of sex and age was similar for smokers and non-smokers.

c) Patients who had never smoked or who had not smoked for at least 6 months prior to surgery.

ed in ice-cold 1.15% KCl containing 10 mM K-phosphate buffer, pH 7.4. The organs were freed from adhering tissues. The frozen samples from human lung (see above) were rapidly warmed up to  $0^{\circ}\text{C}$  and transferred into the same medium. Tissues were minced and homogenized in about 3 volumes of 1.15% KCl containing 10 mM K-phosphate buffer pH 7.4 in an Ultra Turrax homogenizer. The homogenates were centrifuged at 10,000 g for 15 min and the resulting supernatant fractions were centrifuged at 100,000 g for 1 h. The 100,000 g supernatant fraction was isolated while the microsomal pellet was washed by resuspending in buffered KCl and recentrifuging at 100,000 g for 30 min. Washed microsomes were resuspended again in the same medium, which resulted in final concentrations of 1-8 mg protein/ml for material from both rat and human lung. Protein recovery was low in human lung microsomes ( $1.61 \pm 0.42$  mg/g wet tissue weight) compared to rat lung microsomes ( $4.22 \pm 0.41$  mg/g wet tissue weight). Protein concentrations were determined by the method of Lowry *et al.* (51).

Activities of the three enzymes measured were found to be differently stable in ice cold suspensions of subcellular fractions. Whereas ethoxycoumarin-O-deethylase showed a measurable loss in activity after only 4 h, benzo[a]pyrene 4,5-oxide hydrolase remained stable for at least 24 h, and GST had constant activities during 3 days. Therefore assays were performed promptly after preparation in the sequence MO, EH, GST.

#### Assays

MO activities were determined with 4 different sub-

strates. The assay of 7-ethoxycoumarin O-dealkylation was carried out according to the method of Ullrich and Weber (52) with one modification. Cofactor concentrations in the assay mixture were altered, because both NADP and umbelliferone fluoresce at 460 nm when excited at 385 nm. We reduced the NADPH amount within the range of saturating concentrations in order to diminish the resulting fluorescence quenching, which was important when tissues with extremely low enzyme activities such as the human lung were studied. The optimal conditions adopted for this study were:  $4 \times 10^{-5}$  M NADPH,  $2 \times 10^{-5}$  M NADH,  $3 \times 10^{-3}$  M glucose 6-phosphate, 0.56 U glucose 6-phosphate dehydrogenase in the assay mixture. Benzo[a]pyrene hydroxylase was determined by the methods of Nebert and Gelboin (53) and DePierre *et al.* (54). Biphenyl hydroxylase (2-hydroxylation and 4-hydroxylation) was measured according to a method of Creaven *et al.* (55). Ethoxyresorufin deethylation was measured as described by Burke and Mayer (56). EH activities were determined using the radiometric extraction assay with [ $^3$ H]benzo[a]pyrene 4,5-oxide as substrate (48) and for the other substrates as described (16). GST activities were measured as described by Habig *et al.* (57), using 2,4-dinitrochlorobenzene as substrate.

All results reported were obtained under conditions where linearity with time and protein was ascertained. All samples were assayed in duplicates. Two samples were divided each into two and these prepared and measured separately. Intra- and inter-experimental variation proved to be low (< 5%).

## Results and Discussion

Human lung microsomes have a very low MO activity compared to rat lung fractions. This was not due to freezing or storing of the tissue. Four human lung samples from smoking cancer patients and one from a non-smoking patient without cancer had similar specific MO activities when they were measured immediately after surgery or after storage at  $-70^\circ\text{C}$ . The same was true for EH and GST. In order to find optimal assay conditions for human lung MO activity, several substrates and assay methods were tested using the most sensitive assays. O-Deethylation of ethoxycoumarin was the only assay which gave measurable activities after the modification described in **Materials and Methods**. Specific activities in rat lung microsomal fractions were determined in parallel as a positive control (Table II). The use of 7-ethoxycoumarin has the advantage that, on one hand it is a broad-spectrum substrate converted by several MO forms which are inducible by different groups of chemicals (58). On the other hand, there

**Table II**

Substrate specificities of rat and human lung monooxygenases

Substrates	Specific activity (pmol product per mg per min)	
	Rat lung <sup>a</sup>	Human lung <sup>b</sup>
Benzo[a]pyrene hydroxylase <sup>c</sup>	4.2 ± 0.3	n.d.
Benzo[a]pyrene hydroxylase <sup>d</sup>	2.1 ± 0.2	n.d.
Ethoxycoumarin O-deethylase	68.4 ± 2.9	1.2
Ethoxyresorufin O-deethylase	detectable <sup>e</sup>	n.d.
Biphenyl 2-hydroxylase	42 ± 6	n.d.
Biphenyl 4-hydroxylase	65 ± 5	n.d.

Assays were carried out with microsomal fractions from rat and human lung. Microsomes from human lung were samples of smokers with bronchogenic carcinomas which were prepared immediately after surgery.

a) Values represent means ± S.D. The organs of three animals were pooled. Assays were performed using three aliquots of the microsomal pool each of which was measured in duplicate at two protein concentrations.

b) Results of determination in three human lungs. Determinations were performed in samples of individual lungs, each of which was measured in duplicate at two protein concentrations, n.d. = not detectable.

c) Assay method after Nebert and Gelboin (53).

d) Assay method after DePierre *et al.* (54).

e) Specific enzyme activity cannot be given quantitatively for lack of a product standard.

exist data which indicate that this substrate is especially well transformed by that group of cytochrome P-450's (P-448, P<sub>1</sub>-450) which is also especially active in the biotransformation of PAH. These data include concerted inductions and common genetic control of inducibility (59).

One of the reasons for the extremely low specific enzyme activity in human lung preparations could be inhibitors present in the tissue. We therefore incubated rat lung and human lung microsomes together. It could be demonstrated that 7-ethoxycoumarin O-deethylase activity of the rat lung microsomes was inhibited by the presence of human lung microsomes (Table III). This finding suggested that diffusible inhibitors may be present in human lung microsomal fractions. Although higher proportions of human to rat lung microsomes generally led to higher inhibition (Table III), this dependence was not linear, possibly due to limited accuracy of the assay where the remaining activity was only slightly over blank, or else due to limited diffusibility of the inhibiting factor. Indeed, dialysis with 10 mM EDTA could not remove this inhibitor. The somewhat higher MO activities with the use of EDTA and

Table III

Inhibition of rat lung 7-ethoxycoumarin O-deethylase by human lung microsomes<sup>a</sup>

Ratio rat/human <sup>b</sup>	Specific activity in rat lung microsomes <sup>c</sup>	Theoretical specific activity in rat lung microsomes <sup>d</sup>	% Inhibition of rat lung monoxygenase <sup>e</sup>
10/0	63.0	63.0	0
9/1	31.8	56.7	44
3/1	20.0	47.3	58
1/1	15.8	31.5	50
1/3	3.0	25.8	81
1/9	0 (<0.1)	6.3	total inhibition

a) The microsomal fractions of rat lung and human lung (from a smoking cancer patient), both with equal protein concentrations, were mixed in different ratios, but with a constant total protein concentration in the assay mixture (0.42 mg). The assay was carried out after a preincubation time of 15 min at 37°C.

b) Ratio of rat to human lung microsomes (w/w).

c) Specific enzyme activities given as pmol product per mg protein per min.

d) Supposed enzyme activities in rat lung microsomes, given as pmol product per mg protein per min without human lung microsomes.

e) The inhibition was calculated assuming zero contribution of human lung MO activity to the measured total MO activity. Although in the sample containing the highest amount of human preparation no MO activity was measurable, the assumption may not be fully correct in the samples where the remaining activity was very little above blank (samples having more human than rat lung microsomes).

glycerol in microsomal suspension from human lung reported by Prough *et al.* and Šipal *et al.* (26,28,29) may be due to partial removal of inhibitor.

Specific MO activities in human lung microsomes from the control group were about two orders of magnitude lower than in rat lung preparations with the only substrate which allowed quantitation, 7-ethoxycoumarin (Table II). This is, at least in part, due to the presence of an MO inhibitor in these human lung preparations (Table III). On the other hand, specific EH activities in human microsomes of the control group were slightly higher than in rat lung microsomes (about 2-fold) and GST activities were very similar to those of the rat (Table IV).

Considerable interindividual variations were observed in enzyme activities of human lung samples. Figure 1 shows a 40-fold variation from the lowest to the highest extremes for MO, a 5-fold variation for EH and a 10-fold variation for GST. This coincides with findings of other investigators (26,28,29,60) concerning specific activities of MO, glucuronyl- and sulfotransferase in human lung preparations, with results on the variation in metabolism of

benzo[a]pyrene by human lung tissue (61) and with a report of Harris *et al.* (62) on a 75-fold inter-individual variation for the binding of benzo[a]pyrene to DNA in cultured human bronchus explants. The wide range in specific enzyme activities between individuals may in part reflect genetic differences but is also influenced by, *e.g.*, environmental factors or variation in the composition of cells between the samples.

Significant differences in some of these enzyme activities were observable between the different groups of patients (Table IV). Compared to non-smoking patients without cancer (control group), non-smoking cancer patients had 3-fold higher specific MO activities ( $p < 0.025$ ) and 1.4-fold higher EH activities ( $p < 0.05$ ), whereas no significant differences in specific GST activities were observable between the two groups ( $p > 0.1$ ). A similar relation existed when the control group was compared to smoking cancer patients. The latter group had 1.7-fold higher specific MO activities ( $p < 0.0005$ ), 1.3-fold higher specific EH activities ( $p < 0.005$ ), but again not significantly different specific GST activities ( $p > 0.1$ ) in comparison with the control patients. Patients who were smokers, but not suffering from bronchogenic carcinoma, had slightly but significantly higher MO (1.4-fold,  $p < 0.05$ ) and EH (1.6-fold,  $p < 0.0125$ ). Again, specific GST activities did not differ between these two groups ( $p > 0.1$ ).

On the other hand, no significant differences in any of the three enzyme activities were noticed between smokers without cancer and those with bronchogenic carcinoma nor between cancer patients who smoked and those who did not. Differences in specific activities between non-smoking cancer patients and smokers without cancer were not statistically significant for EH and GST ( $p > 0.1$ ), whilst the difference in MO just reached statistical significance ( $p < 0.05$ ).

Thus the connection between smoking or the development of malignant tumors dependent on the use of tobacco products and the level of specific activities of enzymes which are important for the metabolism of PAH in human lung appears to be complex. Furthermore it should be emphasised that for surgical reasons the lung tissues were from peripheral parts of the organ which contained mostly alveolar tissue and small bronchioles where only a minority of human cancers originate, while the majority of lung cancers develop from bronchial epithelium. However, the following trends may be deduced.

The specific activity of GST was very similar in all groups, whilst MO and EH were higher in all cancer and smoker groups compared to controls. These dif-

Table IV

Activities of epoxide-forming and -metabolizing enzymes in human lung subcellular fractions of various groups of samples and species differences between human and rat lung.

Source of lung preparations	Specific activities* (% of controls)		
	Monoxygenase <sup>b</sup>	Epoxide hydrolase <sup>c</sup>	Glutathione S-transferase <sup>d</sup>
Patient groups			
A Control <sup>e</sup> (no cancer, Non-smokers)	0.62 ± 0.26 (100)	739 ± 198 (100)	78,400 ± 47,000 (100)
B No cancer <sup>f</sup> Smokers	0.88 ± 0.37 (142)	1,219 ± 428 (165)	68,000 ± 36,300 (87)
C Bronchogenic <sup>g</sup> carcinoma Non-smokers	1.96 ± 1.40 (316)	1,048 ± 513 (142)	80,900 ± 44,500 (103)
D Bronchogenic carcinoma Smokers	1.05 ± 0.20 (169)	954 ± 233 (129)	64,000 ± 32,200 (82)
Rat <sup>h</sup>	68.4 ± 2.9	384 ± 21	76,000 ± 3,200

a) Specific activities are given as pmol product per mg protein per min. Numbers in brackets represent % of controls. Values are means ± S.D. Samples were measured individually in duplicates at two protein concentrations.

b) Assays were carried out with microsomal fractions using 7-ethoxycoumarin as substrate.

c) Assays were carried out with microsomal fractions using benzo[a]pyrene 4,5-oxide as substrate.

d) Assays were carried out with 100,000 g supernatant fractions using 2,4-dinitrochlorobenzene as substrate.

e) Compared to group B: p < 0.05 (MO), p < 0.0125 (EH), p > 0.1 (GST).

Compared to group C: p < 0.025 (MO), p < 0.05 (EH), p > 0.1 (GST).

Compared to group D: p < 0.005 (MO), p < 0.05 (EH), p > 0.1 (GST).

f) Compared to group C: p < 0.05 (MO), p > 0.1 (EH), p > 0.1 (GST).

Compared to group D: p > 0.1 (MO), p > 0.05 (EH), p > 0.1 (GST).

g) Compared to group D: p < 0.05 (MO), p > 0.1 (EH), p > 0.1 (GST).

h) Values represent means ± S.D. The organs of three animals were pooled. Assays were performed using three aliquots, each of which was measured in duplicate at two protein concentrations.

ferences between the mean MO and EH activities of the various groups compared with the controls were rather modest (maximally 3.2-fold) but all statistically significant. Cantrell *et al.* (31) found a 12-fold AHH induction in human alveolar macrophages from healthy smokers compared to non-smokers. Since in our study MO levels of smokers did not differ from controls as distinctly as in the above study, it may appear that macrophages are among the cell types of higher inducibility within the cell population present in the samples we investigated.

The specific initial rates for the hydration of five K-region epoxides by human lung microsomal EH are shown in Figure 2. The rate of hydration of all substrates was easily measurable and the order of rates was phenanthrene 9,10-oxide > 7-methylbenzo[a]anthracene 5,6-oxide ≅ benzo[a]pyrene 4,5-oxide ≅ benz[a]anthracene 5,6-oxide > 3-methylcholanthrene 11,12-oxide (Figure 2). The relationship between the substrates is very similar to that found in microsomal preparations from human and rat liver, from human, rat and mouse skin and with pure EH

isolated from rat liver (16,63-65). This suggests the presence of either one enzyme or different enzymes under the same control in these species and tissues.

Specific EH activity was determined in two samples of bronchogenic carcinoma tissue. Microsomes were prepared from two tumors histologically specified as squamous cell bronchogenic carcinomas, situated at peripheric areas of the lung. Enzyme activities in a non-diseased, also peripherically situated lung area of the same two subjects, were also determined. In both cases specific activity of microsomal EH was three fold lower in the preparation from the tumors (Table V). This is in marked contrast to the situation in rat liver where, as Levin *et al.* (66) have reported, a precarcinogenic antigen in preneoplastic nodules, which have developed following administration of 2-acetylaminofluorene, was identified as microsomal EH. They found a 5- to 7-fold higher EH activity in hyperplastic nodules and in chemically induced hepatomas compared to livers of control rats. The results with the two squamous cell bronchogenic carcinomas obtained in this study indicate that an in-

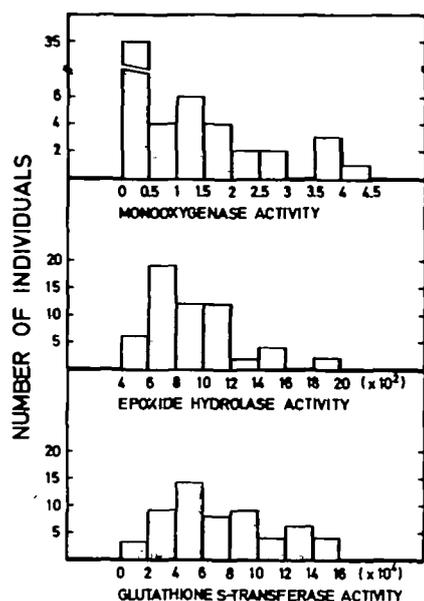


Fig. 1. Interindividual differences of specific enzyme activities (pmol product per mg protein per min) of microsomal MO, microsomal EH and cytoplasmic GST in human lung samples. The lowest (highest) specific enzyme activity measured was 0.10 (4.15) pmol product per min per mg protein for MO (substrate: 7-ethoxycoumarin), 406 (1890) pmol product per min per mg protein for EH (substrate: benzo[a]pyrene 4,5-oxide) and 15.4 (156) nmol product per min per mg protein for GST (substrate: 2,4-dinitrochlorobenzene).

Table V

Epoxide hydrolase activity in microsomes from tumor tissue and non-diseased lung areas of two bronchogenic carcinoma patients.

Source of lung sample	Specific activity* (pmol product per mg protein per min)	Ratio A/B
Patient I		
A) Tumor	460	0.32
B) Non-diseased tissue	1,440	
Patient II		
A) Tumor	380	0.34
B) Non-diseased tissue	1,106	

Samples of histologically verified squamous cell carcinomas and of microscopically non-diseased areas of the lungs were taken out during thoracotomy. All samples were taken from peripheric parts of the lungs. Preparation of subcellular fractions and assays were performed immediately after surgery.

a) Assays were carried out with microsomal fractions using benzo[a]pyrene 4,5-oxide as substrate.

crease of EH in tumor tissue does not represent a general phenomenon.

In this study conditions were established where specific MO activities (as ethoxycoumarin

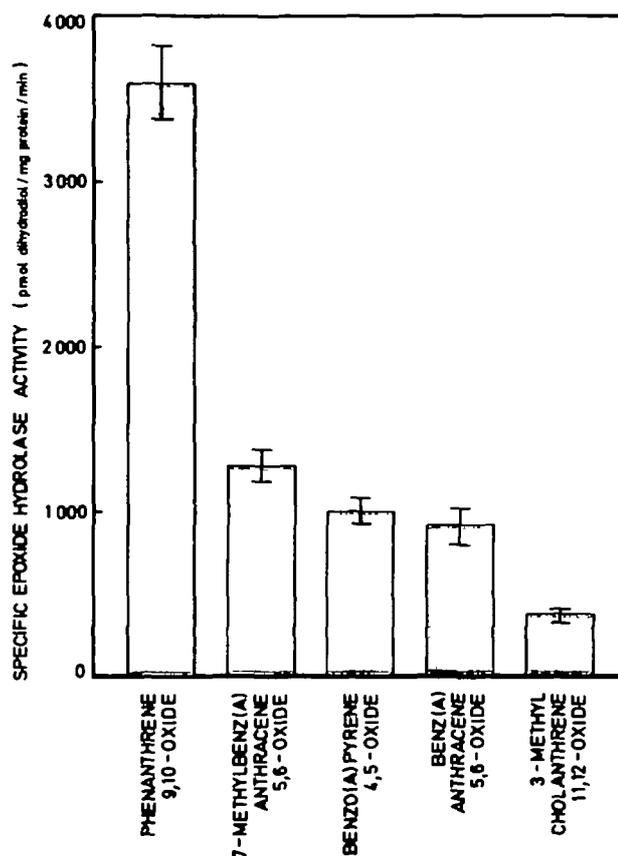


Fig. 2. Substrate specificity of human lung microsomal EH towards five K-region epoxides derived from PAH. The values represent the average  $\pm$  S.D. of three measurements of lung microsomes from a smoking cancer patient. Microsomes were incubated with the tritium-labelled epoxides for 20 min at 37°C. Assays were carried out as described (16).

O-dealkylation) became clearly measurable in human lung microsomal suspensions. These specific activities were determined simultaneously with those of EH and GST in lung samples of different groups of patients.

After a long term use of tobacco products the pattern of activities of the three enzymes differed from that of the control patients. This change of the pattern resembled that of the cancer patients, whether they were smokers or not. Further investigations on these enzymes in cell types and lung regions known to be affected most often by cancer development will be necessary to deepen our understanding of relations between carcinogen metabolizing enzymes and bronchogenic carcinomas.

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