Pulmonary Metabolism of Mutagens and Its Relationship with Lung Cancer and Smoking Habits

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ABSTRACT

The S-12 fractions of lung peripheral parenchyma obtained from 80 male individuals, aged 17–71 years, were assayed as blind samples for the ability either to convert promutagens into bacterial mutagens or to decrease the potency of direct-acting mutagens in the Ames reversion test. In this system, lung preparations were completely ineffective in activating an N-nitroso compound (i.e., N-nitrosomorpholine) and poly-cyclic aromatic hydrocarbons [i.e., 3-methylcholanthrene and benzo(a)pyrene] or their metabolites [i.e., 3-hydroxy-benzo(a)pyrene and benzo(a)pyrene-trans-7,8-diol]. They yielded a borderline and sporadic activation of a cigarette smoke condensate, and a weak but frequent activation of an aromatic amine (i.e., 2-amino-3,4-dimethylimidazo[4,5-f]quinoline) and of a diamide (i.e., cyclophosphamide). The pulmonary metabolism was more oriented in the sense of detoxification, as shown by the consistent decrease of potency of direct-acting mutagens, including a metal (i.e., sodium dichromate), an acridine and nitrogen mustard derivative (i.e., 2-methoxy-6-chloro-9-[2-chloromethyl]amino-3,4-dimethylimidazo[4,5-f]quinoline) and an N-oxide (i.e., 4-nitroquinoline-N-oxide). As assessed by means of a numerical score quantifying the variation of mutagenicity, a marked interindividual variability (up to 20-fold) was detected in the ability of lung specimens to affect the mutagenicity of test compounds. Such variability was not significantly related to the protein concentration of S-12 fractions, nor to the age of the patients under scrutiny, who during hospitalization were on normal institutional diets and did not receive any special drug treatment. The only significant difference between 20 noncancer and 60 lung cancer patients, irrespective of the histological type, was a decreased activation of cyclophosphamide in the latter group. Probably due to the high prevalence of smokers among lung cancer patients, a significantly decreased activation of cyclophosphamide was also observed in the group of smokers. Smoking habits were associated with a stimulation of detoxifying mechanisms which, in agreement with the results of a previous study with human alveolar macrophages (F. L. Petrelli et al., J. Clin. Invest., 77: 1917–1924, 1986), was significant in the case of sodium dichromate. Such effect was further enhanced by considering only individuals smoking during the last 24 h before collecting lung specimens, and under these conditions it became significant also for ICR 191. In any case, the bulk of the interindividual variations could not be explained by the analyzed sources of variability.

INTRODUCTION

Metabolic factors are of crucial importance in affecting the first stage of the multistep carcinogenesis process, i.e., initiation, either by converting inactive chemicals into electrophilic derivatives, or by detoxifying reactive molecules into harmless derivatives. Modulation of biotransformation processes by genetic traits or by exogenous factors is likely to represent one of the major sources of interindividual variability in susceptibility to carcinogens (1), as also supported by the demonstrated possibility of preventing cancer by raising the levels of physiological protective mechanisms (2).

The metabolic fate of mutagens and carcinogens has been extensively investigated in the biochemically most active organ, i.e., the liver. Conversely, less attention has been paid to their pulmonary metabolism, although the lungs represent by far the largest surface of contact and communication between the body and the environment. Inhaled xenobiotics reaching this enormous surface area can be retained and metabolized in the variety of cells represented in the respiratory tract, prior to reacting with target molecules or to being excreted or distributed to other tissues. Even less explored has been the metabolism of mutagens and carcinogens in human lung cells, which may provide an important probe for the assessment of the reponsiveness of the respiratory target tissue to airborne chemicals, as well as a means of quantifying the susceptibility to carcinogens in humans (3).

Several years ago, we started a collaborative study involving the University of Pisa, Italy, the University of Genoa, Italy, and the International Agency for Research on Cancer, Lyon, France, aimed at assessing the pulmonary metabolism of mutagens and its interindividual variability, by using S-12 fractions of freshly collected specimens of lung obtained at surgery. So far, with few exceptions (see e.g., Refs. 4–10), the interindividual variations in susceptibility to lung cancer had been mainly evaluated by testing individual enzyme activities, such as AHH activity, and using nontarget cells, such as circulating lymphocytes (11, 12). In our study, in addition to the monitoring of various enzyme activities (which is being performed in International Agency for Research on Cancer laboratories), we are focusing on the final biological effect, i.e., the metabolic activation or detoxification of mutagens, resulting from the balance of the multiple biochemical pathways involved in their metabolism. Although S-12 fractions cannot fully reflect the metabolic competence of the intact cells (1), use of these subcellular preparations is suitable for a large-scale comparative evaluation of the phenomenon with an appreciable number of test substances and lung specimens. Three years ago we reported the progress of the study when the lung specimens from 43 individuals had been examined (13). In the present paper we communicate the cumulative results obtained with an increased number of subjects. Moreover, use of a numerical score quantifying the ability of each lung specimen either to activate several promutagens or to detoxify direct-acting mutagens has provided a tool.

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2 The abbreviations used are: AHH, aryl hydrocarbon hydroxylase; 2AA, 2-aminoanthracene; 2AF, 2-amino-fluorene; BP, benzo(a)pyrene; 2,7-diola, benzo(a)pyrene-trans-7,8-diol; CPA, cyclophosphamide; ECH, epichlorohydrin; 3-HO-BP, 3-hydroxy-benzo[a]pyrene; ICR 191, 2-methoxy-6-chloro-9-[2-chloromethyl]amino-3,4-dimethylimidazo[4,5-f]quinoline; NM, N-nitrosomorpholine; 4NQO, 4-nitroquinoline-N-oxide; PAH, polycyclic aromatic hydrocarbon; RME, relative metabolic efficiency; SDC, sodium dichromate; CSC, cigarette smoke condensate.

3 Several years ago, we started a collaborative study involving the University of Pisa, Italy, the University of Genoa, Italy, and the International Agency for Research on Cancer, Lyon, France, aimed at assessing the pulmonary metabolism of mutagens and its interindividual variability, by using S-12 fractions of freshly collected specimens of lung obtained at surgery. So far, with few exceptions (see e.g., Refs. 4–10), the interindividual variations in susceptibility to lung cancer had been mainly evaluated by testing individual enzyme activities, such as AHH activity, and using nontarget cells, such as circulating lymphocytes (11, 12). In our study, in addition to the monitoring of various enzyme activities (which is being performed in International Agency for Research on Cancer laboratories), we are focusing on the final biological effect, i.e., the metabolic activation or detoxification of mutagens, resulting from the balance of the multiple biochemical pathways involved in their metabolism. Although S-12 fractions cannot fully reflect the metabolic competence of the intact cells (1), use of these subcellular preparations is suitable for a large-scale comparative evaluation of the phenomenon with an appreciable number of test substances and lung specimens. Three years ago we reported the progress of the study when the lung specimens from 43 individuals had been examined (13). In the present paper we communicate the cumulative results obtained with an increased number of subjects. Moreover, use of a numerical score quantifying the ability of each lung specimen either to activate several promutagens or to detoxify direct-acting mutagens has provided a tool.
for analyzing some of the possible sources of interindividual variability.

PATIENTS AND METHODS

Patients. Lung specimens were collected from 80 male patients hospitalized at the Surgical Clinic of Pisa University. Twenty of them were affected by nonneoplastic, pulmonary or extrapulmonary diseases, and underwent thoracotomy and lung resection for therapeutic purposes. Sixty patients underwent unilateral pneumectomy or lobectomy due to primary bronchogenic cancer of various histology (29 squamous cell carcinoma, 18 adenocarcinoma, 9 large cell carcinoma, 2 small cell carcinoma, and 2 bronchial-alveolar carcinoma).

The age of patients ranged between 17 and 71 years, and was significantly higher (P < 0.001) in lung cancer (56.6 ± 7.2) than in noncancer patients (44.5 ± 11.1). In the days preceding the operation all the patients received normal institutional diets, and none of them were treated with anticancer drugs, nor received any special treatment. A careful record of smoking habits was obtained from all subjects. Lung specimens were used for diagnostic and research purposes with the informed consent of all patients.

Preparation of Lung S-12 Fractions. Immediately after removal, a fragment of peripheral lung parenchyma obtained from noncancer or from lung cancer patients (healthy tissue surrounding the tumor) was immersed and washed in ice-cold 0.15 M KCl. Within 3 h from surgical collection, the specimen was transferred to the laboratories of the CNR Institute of Clinical Physiology for the preparation of S-12 fractions. All the steps were performed aseptically at 0–4°C, using sterile reagents and glassware. The tissue was finely minced with scissors, wiped on gauze, weighed, immersed in three volumes (i.e., 3 ml/g of wet tissue) of 0.25 M sucrose and 50 mM Tris-HCl buffer, pH 7.4, and homogenized, first in a Polytron blender and then in a Potter-Elvehjem apparatus. The homogenate was centrifuged for 15 min at 12,000 x g and the resulting supernatant (S-12 fraction) was distributed into polyethylene tubes (3-ml aliquots) and quickly frozen at −80°C. At approximately 6-month intervals the available S-12 fractions were dispatched as coded, blind samples to the laboratories of the Institute of Hygiene of Genoa University for mutagenicity assays. In parallel, aliquots of the same specimens were dispatched to IARC for biochemical studies. The data on the protein concentration of lung S-12 fractions were kindly provided by Dr. D. S. Longfellow, National Cancer Institute, Bethesda, MD, and top agar. In each experiment, liver S-12 fractions from Aroclor-treated Sprague-Dawley rats, in amounts ranging between 5 and 50 μl/plate, were assayed in order to check the efficiency of the test system used.

The number of mutagens which could be tested with each lung specimen depended on the amount of S-12 fraction available. All the specimens and their controls were assayed in duplicate or more often in triplicate plates.

Evaluation of Metabolic Effects and Statistical Analysis. For each specimen and control, the mean number of his" revertants was calculated, along with the corresponding SD, which almost constantly fell within 10% of the mean value. Thereafter, an index expressing RME was calculated as follows:

\[ RME = \frac{A/B}{C/D} \]

where A is the mean number of revertants induced by a given mutagen in the presence of a given S-12 fraction; B is the same as A, but in the absence of S-12 fractions; C is the mean number of spontaneous revertants observed in the presence of a given S-12 fraction; D is the same as C, but in the absence of S-12 fractions. Calculation of the A/B or B/A ratio warranted a satisfactory reproducibility of results in separate experiments with different sets of specimens, as confirmed by the results obtained with controls. The correction with the C/D ratio was introduced because some lung specimens tended to raise the number of spontaneous revertants (in strain TA98 only). The RME index is scored by a scale of values, where 1 indicates no activation or deactivation. The higher the value, the higher the metabolic efficiency for both activatable and deactivatable mutagens.

The identity of tested specimens was revealed only at the end of all the experiments. The influence of possible variability factors, i.e., age of patients, protein concentration of S-12 fractions, different histological types of lung cancer, and smoking habits, on the mean RME values within each group was evaluated by analysis of variance. The relationships between the various parameters investigated was assessed by linear correlation analysis.

RESULTS

Efficiency of Lung S-12 Fractions in Metabolizing Mutagens. Table 2 summarizes the data concerning the effects of lung S-12 fractions on the mutagenicity of the 13 test compounds. Fig. 1 shows the individual RME data for the eight mutagens which

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Table 1 Experimental conditions in mutagenicity assays

<table>
<thead>
<tr>
<th>Test mutagen</th>
<th>Solvent</th>
<th>Dose (μg/plate)</th>
<th>S. typhimurium strain</th>
<th>Lung S-12 fractions (μl/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>DMSO</td>
<td>5</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>BP-7,8-diol</td>
<td>DMSO</td>
<td>10</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>3-HO-BP</td>
<td>DMSO</td>
<td>2</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>3MC</td>
<td>DMSO</td>
<td>10</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>NM</td>
<td>DMSO</td>
<td>2500</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>4NQO</td>
<td>Water</td>
<td>0.5</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>SDC</td>
<td>Water</td>
<td>30</td>
<td>TA100</td>
<td>100</td>
</tr>
<tr>
<td>CPA</td>
<td>Water</td>
<td>4000</td>
<td>TA1535</td>
<td>200</td>
</tr>
<tr>
<td>ECH</td>
<td>DMSO</td>
<td>500</td>
<td>TA1535</td>
<td>200</td>
</tr>
<tr>
<td>ICR 191</td>
<td>Water</td>
<td>2</td>
<td>TA1537</td>
<td>100</td>
</tr>
<tr>
<td>2AF</td>
<td>DMSO</td>
<td>20</td>
<td>TA98</td>
<td>200</td>
</tr>
<tr>
<td>MelQ</td>
<td>DMSO</td>
<td>0.2</td>
<td>TA98</td>
<td>200</td>
</tr>
<tr>
<td>CSC</td>
<td>DMSO</td>
<td>*</td>
<td>TA98</td>
<td>200</td>
</tr>
</tbody>
</table>

* Amount equivalent to 0.2 cigarettes per plate.
METABOLISM OF MUTAGENS IN HUMAN LUNG

Table 2. Efficiency of S-12 fractions of peripheral lung parenchyma on the mutagenicity of various compounds

<table>
<thead>
<tr>
<th>Investigated metabolic effect</th>
<th>Test mutagen</th>
<th>All patients</th>
<th>Non-cancer patients</th>
<th>Lung cancer patients</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>Recent smokers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease of mutagenicity of direct-acting mutagens</td>
<td>SDC</td>
<td>71</td>
<td>2.51 ± 0.7a</td>
<td>17</td>
<td>2.44 ± 0.6</td>
<td>54</td>
<td>2.53 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>4NQO</td>
<td>67</td>
<td>6.32 ± 2.1</td>
<td>17</td>
<td>6.78 ± 1.7</td>
<td>50</td>
<td>6.16 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>ECH</td>
<td>45</td>
<td>1.90 ± 1.7</td>
<td>12</td>
<td>1.43 ± 0.4</td>
<td>33</td>
<td>2.09 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>ICR 191</td>
<td>76</td>
<td>2.43 ± 2.0</td>
<td>18</td>
<td>2.10 ± 1.1</td>
<td>58</td>
<td>2.53 ± 2.2</td>
</tr>
<tr>
<td>Activation of promutagens to mutagenic metabolites</td>
<td>BP</td>
<td>70</td>
<td>1.01 ± 0.1</td>
<td>19</td>
<td>1.01 ± 0.1</td>
<td>51</td>
<td>1.00 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>3-HO-BP</td>
<td>5</td>
<td>0.82 ± 0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>BP-7,8-diol</td>
<td>19</td>
<td>1.10 ± 0.2</td>
<td>5</td>
<td>1.13 ± 0.2</td>
<td>14</td>
<td>1.11 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>3MC</td>
<td>5</td>
<td>1.04 ± 0.1</td>
<td>1</td>
<td>0.99</td>
<td>4</td>
<td>1.05 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>NM</td>
<td>21</td>
<td>0.98 ± 0.1</td>
<td>4</td>
<td>0.96 ± 0.1</td>
<td>17</td>
<td>0.99 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>CSC</td>
<td>66</td>
<td>1.26 ± 0.4</td>
<td>16</td>
<td>1.26 ± 0.4</td>
<td>50</td>
<td>1.25 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>2AF</td>
<td>54</td>
<td>2.02 ± 0.9</td>
<td>14</td>
<td>2.22 ± 1.2</td>
<td>40</td>
<td>1.95 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>CPA</td>
<td>39</td>
<td>1.62 ± 0.5</td>
<td>12</td>
<td>1.90 ± 0.5</td>
<td>27</td>
<td>1.49 ± 0.5c</td>
</tr>
<tr>
<td></td>
<td>MelQ</td>
<td>10</td>
<td>1.65 ± 0.4</td>
<td>3</td>
<td>1.61 ± 0.3</td>
<td>7</td>
<td>1.67 ± 0.5</td>
</tr>
</tbody>
</table>

*a Smoking during the last 24 h preceding surgical operation.
*b Significantly higher (P < 0.05) as compared to noncancer patients.
*c Significantly higher (P < 0.01) as compared to noncancer patients.
*d Significantly lower (P < 0.05) as compared to noncancer patients.
*e Significantly lower (P < 0.05) as compared to noncancer patients.

The liver S-12 fractions from Aroclor-treated rats, used as controls in each experiment, efficiently decreased the mutagenicity of direct acting compounds and activated promutagens to mutagenic metabolites (data not shown). The only exception was NM, which was never activated by the rat liver preparations (mean ± SD = 32.8 ± 12.6 mg/ml). In fact, the correlation indices calculated for the mutagens under scrutiny.

Influence of Protein Concentration and of Storage of Samples. In general, there were no relationships between the intensity of metabolic effects produced by lung S-12 fractions and their content in total protein, which ranged between 12.0 and 76.3 mg/ml (mean ± SD = 32.8 ± 12.6 mg/ml). In fact, the correlation indices relative to the above parameters were not statistically significant for SDC (r = -0.106), 4NQO (r = 0.125), ICR 191 (r = 0.171), BP (r = -0.028). There was a weak yet significant (P < 0.01) positive correlation only in the case of ECH (r = 0.378) and a negative correlation in the case of CPA (r = -0.440). Therefore, on the whole, the observed interindividual variability appears to reflect variations in specific activity rather than in protein concentration.

Moreover, there was no relationship between the storage time of S-12 fractions at -80°C (for a maximum of 6 months) and the corresponding metabolic efficiency, as shown by the very low and nonsignificant values of the correlation indices calculated for the mutagens under scrutiny.

Influence of Age and of Lung Cancer. Considering all together the individuals under scrutiny, there was no significant correlation between age and metabolism for any of the test mutagens. Moreover, by dividing the individuals into age classes (≤ 40, 41-50, 51-60, ≥ 61 years), the analysis of variance did not point out any age-related, significant difference in the metabolism of the investigated compounds.

As shown in Table 2, there was no significant difference between the lung preparations of noncancer patients and the preparations of healthy tissue surrounding the tumor in lung cancer patients. The only exception was CPA, the pulmonary activation of which was slightly inhibited (P < 0.05) in cancer patients. Within lung cancer cases, no significant variation was detected in the metabolism of test substances, as related to the histological classification (not shown).
Influence of Smoking Habits. On the whole, for any mutagen the intensity of metabolic effects was not significantly correlated with the cumulative number of cigarettes smoked lifetime by patients (pack-years) (not shown).

By dividing all the subjects into two groups, i.e., smokers and nonsmokers (including individuals who had never smoked and those who had refrained from smoking during the last 6 months), the only significant differences ($P < 0.05$) pointed out by statistical analysis were an inhibition of CPA activation and an enhancement in SDC metabolism in the group of smokers. A similar trend was also observed with other two direct-acting mutagens undergoing detoxification, i.e., ECH and ICR 191, but the recorded differences were not significant, due to an increased scattering of data among smokers (Table 2).

Smokers were further divided into subgroups, according to the time elapsed between smoking the last cigarette and surgical operation. By this way, no significant variation could be detected between nonsmokers and smokers withdrawing from cigarette smoke for one week or more (not shown). Conversely, as shown in the last column of Table 2, the inducing effects of smoke became more evident in patients smoking during the last 24 h preceding collection of lung specimens. In fact, in spite of the lower number of subjects within each group, a significant effect ($P < 0.05$) was also detected in the case of ICR 191, whereas the difference became more pronounced ($P < 0.01$) in the case of SDC. The metabolism of the latter compound was also weakly yet significantly correlated with the number of cigarettes smoked during the day preceding intervention ($r = 0.223, P < 0.05$). In contrast, inhibition of CPA activation was no longer significant in the group of recent smokers, which suggests that such a phenomenon did not depend on a metabolic effect produced by cigarette smoke but rather on the high
prevalence of smokers among lung cancer patients (who, as described in the previous section, were less active in metabolizing CPA). In fact, the number of pack-years in lung cancer patients (49.1 ± 24.6) was significantly higher \((P < 0.001)\) than in noncancer patients (19.2 ± 18.7).

**DISCUSSION**

The results herein presented confirm, on the basis of an almost doubled number of specimens, the indications that emerged from our previous study (13). Additionally, they give some insight about the identification of at least a part of the sources of the observed interindividual variability.

Once more, there was a clear evidence that human lung S-12 fractions are more efficient in decreasing the potency of certain direct-acting mutagens rather than in activating promutagens. In fact, lung preparations were completely ineffective in activating PAHs (i.e., BP and 3MC) and their proximate metabolites (i.e., BP-7,8-diol and 3-HO-BP) in sufficient amounts to give a mutagenic response. They also failed to activate an N-nitroso compound (i.e., NM) and yielded only a borderline activation of an unfractionated cigarette smoke condensate. More convincing and more frequent, albeit weak, was the activation of an aromatic amine (i.e., 2AF), of a heterocyclic amine (i.e., MelQ) and of a diamide (i.e., CPA). It is noteworthy that, for all three compounds, the first metabolic step is a hydroxylation process. Our results are in agreement with those reported by other investigators (15) who, by testing human lung S-9 fractions from three autopsy cases, detected in the Ames test some activating ability for another aromatic amine (2AA) and no activation of PAHs nor of various CSC fractions. Recently, some activation of MelQ to bacterial mutagens has been also obtained in the presence of rabbit and mouse lung microsomes or of freshly isolated rabbit lung cells, i.e., in decreasing rank of efficiency, Clara cells, type II cells, and alveolar macrophages (16).

On the other hand, the same lung preparations consistently produced a loss of potency of direct-acting mutagens, including a metal (SDC), an acridine and nitrogen mustard derivative (ICR 191), an epoxide (ECH), and, above all, an N-oxide (4NQO). Interestingly, there was a certain parallelism in the metabolism of some mutagens, which underlies either common metabolic pathways or biochemical processes governed by similar regulatory mechanisms. For instance, both 4NQO and SDC, in spite of their chemical diversity, can be metabolized by DT diaphorase, which converts 4NQO into its hydroxyamine derivative (17) and also participates in the cytosolic reduction of chromium(VI) (18).

Application of the RME index provided an easy and reliable quantification of the metabolic changes induced in the test system used by equivalent (in terms of original tissue weight) amounts of lung preparations. The variations in this index among the investigated patients were rather broad, up to approximately 10–20-fold, and reflected variations in specific activity, being mostly unrelated to the protein content of S-12 fractions. Thus, also by evaluating a composite biological endpoint, such as the metabolic variation of mutagenicity, it is possible to point out and to quantify the interindividual variability in the pulmonary metabolism of mutagens, which had so far been assessed by measuring individual enzyme activities, or the yield of metabolites by means of analytical methods, or the DNA-binding of a mutagen to cultured bronchus explants (5–10).

By ruling out known variability factors (19), such as sex, age, diet, and drugs, it was of interest to assess the influence of smoking habits and of lung cancer. In exploring the possible metabolic consequences of lung cancer, only the healthy tissue surrounding the neoplasia was examined, since it has been already demonstrated that the metabolism of the pulmonary tumoral tissue is different from that of the normal tissue, as shown by a lower activity of both AHH (9) and of epoxide hydrolase (7). Irrespective of the histological type, the only significant effect observed in cancer patients was a slight inhibition of the metabolic activation of CPA. Since this antineoplastic and immunosuppressive agent is also used in the treatment of certain malignant neoplasms of the lung (20), a decreased metabolic activation in lung cancer patients, at least in the pulmonary tissue, would imply a decreased carcinogenic risk in treated patients. However, in case the alkylating metabolite responsible for mutagenicity and carcinogenicity of CPA may be the same responsible for its antitumor activity [which has been identified as the phosphoramid mustard (21)], the observed phenomenon would also imply a loss of therapeutic activity.

The effects of smoking habits on the pulmonary metabolism of carcinogens represent an open question. Most studies facing this problem have focused on the metabolic activation of BP, with conflicting findings between animal and human data. In fact, exposure of rodents to cigarette smoke has been found to induce the pulmonary AHH activity and to enhance the binding of reactive PAH metabolites to the DNA of lung cells (22–24). In contrast, the low activity of AHH does not appear to be stimulated by cigarette smoke in human lungs (8–10, 25). Also, human bronchial cells derived from explanted tissue of smokers did not show any induction of BP metabolism (26) nor any increase in the DNA-binding of BP metabolites (5). However, use of cultured bronchial cells involves several days of *in vitro* growth, during which a reversible induction may be lost.

Our results provided evidence that, apart from an inhibition of CPA activation which was clearly amenable to the high prevalence of smokers among lung cancer patients, cigarette smoke is capable of inducing some detoxifying processes. Such effect was already significant for SDC by comparing nonsmokers with all smokers, and became more pronounced (and significant also for ICR 191) by comparing nonsmokers with individuals smoking during the 24-h preceding the collection of lung specimens. This clearly demonstrates that cigarette smoke can modify the local metabolism to such an extent to be detected in a mutagenicity test system, and that the inducing effects are reversible and relatively short-lived.

The induction of SDC reduction by cigarette smoke, as revealed by the examination of blind specimens of peripheral lung parenchyma, is in full agreement with the conclusions drawn in a previous study on chromium metabolism in pulmonary alveolar macrophages (27). In fact, at equivalent number of cells, chromium(VI) reduction, total protein, and some oxidoreductase activities had been found to be significantly increased in smokers, especially in recent smokers, without any appreciable variation between lung cancer and noncancer patients. The specific chromium reducing activity of alveolar macrophages, mostly mediated by cytosolic, enzyme-catalyzed mechanisms, was significantly higher than that of corresponding preparations of human peripheral lung parenchyma or bronchial tree, or of rat lung or liver (27). Therefore, it is likely that macrophages may have supplied an important contribution to the SDC reduction detected in the mixed-cell populations present in the lung specimens tested in this study. Together with other chromium(VI)-reducing mechanisms operating out-
side and inside cells, this detoxifying process is likely to determine thresholds in the pulmonary carcinogenicity of chromium, and it is noteworthy that the cell reaction to cigarette smoke leads to a stimulation of such protection device.

In any case, also cigarette smoke provided only a limited justification to the observed amplitude of the interindividual variability in the pulmonary metabolism of mutagens. In fact, the bulk of the phenomenon was not explained by the analyzed factors and may be thus attributable to other, hardly predictable exogenous sources or, more probably, to host factors, such as genetic traits, which are suspected to play an important role in determining the susceptibility to pulmonary carcinogens in humans (11, 28, 29). We hope that the continuation of this study (involving the examination of additional lung specimens), together with the biochemical results obtained at IARC on the same materials, and with the analysis of additional anamnestic data of patients, may provide in the near future some further element in order to elucidate this complex problem.

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