[CANCER RESEARCH 45, 0000-0000, November 1985]

Enhanced Formation of Benzo(*a*)pyrene:DNA Adducts in Monocytes of Patients with a Presumed Predisposition to Lung Cancer

Hugo W. Rüdiger,[1] Dennis Nowak,[2] Klaus Hartmann, and Peter Cerutti

Unit of Hereditary and Constitutional Diseases, Department of Internal Medicine, University of Hamburg, Germany [H. W. R., D. N., K. H.], and Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, Epalinges/Lausanne, Switzerland [P. C.]

ABSTRACT

Blood monocytes from 45 selected patients with lung cancer and 30 healthy controls were incubated with [G-^{3}H]-benzo(*a*)pyrene for 30 h, and the formation of covalently bound DNA adducts was determined. The lung cancer patients were either relatively young (below 46 yr), nonsmokers, or had at least one first degree relative with lung cancer. Therefore, they might be considered cancer prone (2.07 fmol of benzo(*a*)pyrene per μg of DNA). The DNA adducts were significantly elevated in 22 patients with early age cancer (4.34 fmol/μg of DNA; $P < 0.04$). In 12 familial cases, the slight elevation (2.77 fmol/μg of DNA) was not statistically significant in comparison to healthy controls. Benzo(*a*)pyrene:DNA adduct levels did not differ significantly between smokers and nonsmokers. Eight of 9 lung cancer patients with DNA adducts equal or above 4.5 fmol/μg of DNA but only 16 of 36 with adducts below this value had either oat cell or squamous cell cancer ($P < 0.05$). The observed enhanced formation of covalent DNA adducts in blood monocytes exposed to a carcinogenic polycyclic hydrocarbon may be genetically determined and could play a role in the development of lung cancer at an early age.

INTRODUCTION

A 4-fold increased risk for lung cancer has been observed in individuals with at least one affected first degree relative (1). This familial incidence is independent of smoking habits and is probably based on genetic predisposition. On the other hand, lung cancer is considered to be caused by environmental factors. This is convincingly illustrated by the more than 100-fold increased incidence of this tumor during the last century (2). Hence, it seems reasonable to speculate that genetic factors modulate the individual susceptibility to certain carcinogens (3–6). Much attention has been focused on polycyclic aromatic hydrocarbons which are activated in exposed cells to their ultimate carcinogenic forms by genetically controlled enzyme systems (7, 8). Although the contribution of this class of carcinogens to the development of lung cancer in humans remains unclear, several investigators have attempted to relate carcinogenic risk to individual activating or inactivating enzymes, using benzo(*a*)pyrene as a substrate. Promising results were obtained in animals where large differences of B(a)P[3] carcinogenicity could be related to different metabolic capacities (9, 10). However, the validity of this approach for a risk assessment in humans remains controversial (11–19). According to some reports, lung cancer patients produced enhanced levels of activated benzo(*a*)pyrene metabolites with the capacity to bind to DNA (20–22). If genetic predisposition plays a role, this correlation should become more pronounced for patients with cancer at an early age, patients with multiple primaries, and patients with a family history of lung cancer.

Here we report our studies on the formation of B(a)P:DNA adducts in blood monocytes from three groups of lung cancer patients: (*a*) patients having one or more first degree relative with lung cancer; (*b*) life-long nonsmokers; and (*c*) patients developing cancer below 46 yr of age. Healthy smokers and nonsmokers without a family history of lung cancer served as controls.

MATERIALS AND METHODS

Patients and Controls. Blood from patients with lung cancer was referred to us by seven hospitals between June 1982 and November 1983. The patients were either nonsmokers, smokers and nonsmokers below 46 yr of age, or had at least one first degree relative with lung cancer. Manifestation of lung cancer below 46 yr is being considered an unusually early onset of the disease, because an evaluation of the case records of our department of internal medicine between 1976 and 1981 revealed that only 10% of lung cancer patients were less than 46 yr old. The diagnosis was confirmed histologically or cytologically, and the tumors were classified according to WHO recommendations (23) as squamous cell, oat cell, large cell, or adenocarcinomas. Mixed tumors were evaluated separately. A detailed family and case history was obtained for each individual, in particular with respect to preexistent lung diseases and smoking habits. Blood was drawn prior to the onset of therapy, with informed consent of the patients. A total of 45 patients were studied of which 22 were below the age of 46 yr, and 12 had at least one first degree relative with lung cancer. Among the latter group were five patients with additional cases of cancer in the family, and three patients were below 46 yr of age. Among 11 nonsmokers with lung cancer, there were 8 females and 2 males below 46 yr of age. Thirty healthy volunteers served as controls, 22 smokers and 8 nonsmokers, 16 individuals being below 46 yr of age. Seven groups of patients and controls were defined for a separate statistical evaluation as indicated in Table 1.

Preparation of Monocytes. Fifty ml of venous blood were drawn into two heparinized plastic syringes from patients early in the morning prior to breakfast, smoking, or intake of drugs. Isolation of cells was started within 3 h after the blood was taken. Mononucleated cells were isolated after centrifugation on Ficoll:Paque according to Bøyum (24) and washed 2 times with culture medium. We used Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco), penicillin (50 mg/liter), neomycin (100 mg/liter), and streptomycin (100 mg/liter) buffered with 200 mM 2,4-(2-hydroxyethyl)piperazinyl(*a*)ethanesulfonate (Serva, Heidelberg, German Federal Republic) together with $NaHCO_3$ (1 g/liter). The pH was adjusted to 7.5 prior to sterile filtration.

The cells were resuspended in 10 ml of culture medium, and an aliquot was measured in a Coulter Counter connected with a Channelyzer C 1000, which allowed the determination of the monocyte content. Usually

[1] To whom requests for reprints should be addressed.
[2] This study is part of a thesis for a medical doctorate.
[3] The abbreviations used are: B(a)P, benzo(*a*)pyrene; PAH, polycyclic aromatic hydrocarbons.

Received 11/19/84, revised 7/24/85, accepted 7/25/85

Table 1
Groups of patients and controls

1	All patients (n = 45)	*vs*	All controls (n = 30)
2	All patients below 46 yr of age (n = 22)	*vs*	All controls below 46 yr of age (n = 16)
3	All patients above 46 yr of age (n = 23)	*vs*	All controls above 46 yr of age (n = 14)
4	Patients with family history of lung cancer (n = 12)	*vs*	All controls (n = 30)
5	All patients who smoke (n = 34)	*vs*	All healthy smokers (n = 22)
6	Patients who smoke, below 46 yr of age (n = 20)	*vs*	Healthy smokers below 46 yr of age (n = 14)
7	All nonsmoking patients (n = 11)	*vs*	All healthy nonsmokers (n = 8)

between 2 and 4 × 10^7 cells were obtained from 50 ml of blood. The cell suspensions were inoculated in two 175-cm^2 cell culture bottles (Nunc, Proskilde, Denmark), each containing 20 ml of culture medium, and the monocytes were allowed to adhere to the bottom during 18 h of incubation at 37°C in a moist incubator equilibrated with a 4% CO_2 atmosphere. Thereafter the cell layer was carefully rinsed with warm Dulbecco's phosphate-buffered saline (25) to remove loose cells, *i.e.*, mainly lymphocytes and residual erythrocytes. Each bottle was processed separately in order to obtain duplicate determinations from each donor.

Determination of Benzo(*a*)pyrene:DNA Adducts. The culture bottles were subsequently incubated for 30 h under the conditions described, together with 20 ml of fresh culture medium containing 300 nM [G-^{3}H]-benzo(*a*)pyrene. The labeled benzo(*a*)pyrene was purchased from Amersham Buchler (Braunschweig, German Federal Republic) with a special radioactivity between 40 and 50 Ci/mmol and was purified prior to use by thin layer chromatography as described (21). Less than 15% of the radioactive B(a)P was converted to H_2O-soluble material during the incubation period as determined by extraction of the culture medium with organic solvent (26). Pilot experiments with repetitive cell counts indicated that the loss of monocytes was negligible during incubation. After incubation, the medium was decanted, the cell layer was rinsed 3 times with a 0.9% NaCl solution, and the cells were removed by the addition of 5 ml of 0.05% trypsin (Gibco) in a 0.9% NaCl solution. The cellular DNA was isolated according to a method described previously (27) Aliquots of about 2 × 10^6 cells were transferred each to a 25-mm polycarbonate filter (Nucleopore Corporation, Pleasonton, CA) with a 2-μm pore diameter, and the cells were lysed on the filter by incubation for 15 min with 0.05 M phosphate buffer, pH 6.7, containing 2% sodium dodecyl sulfate (Serva) and proteinase K (0.5 mg/ml) (Merck, Darmstadt). The DNA remaining attached to the filter was washed twice carefully with 5 ml of 0.02 M disodium EDTA, pH 6.7, and subsequently exposed for 15 min to 5 ml of EDTA buffer containing RNase A (150 μg/ml; Boehringer, Mannheim, German Federal Republic). The RNase had been pretreated for 2 h at 56°C to destroy contaminating DNase activity. After additional rinsing with 0.0005 M phosphate buffer, pH 7.0, a solution containing DNase (0.01 mg/ml) in 0.001 M $MgCl_2$, pH 7.0, was added, and after incubation for 30 min at 37°C, the fragmentated DNA was eluted from the filter. One ml of bidistilled water was used to remove residual DNA fragments from the filter. Subsequently DNA concentrations were determined by a fluorimetric assay (28). On average we obtained 8.7 μg of DNA per 10^6 cells, which represents a recovery of 60 to 70%. In order to minimize unspecific radioactivity associated with DNA, the DNA samples were digested enzymatically with DNase I, phosphodiesterase, and alkaline phosphatase (Boehringer) according to Baird and Brookes (1) and subsequently adsorbed to Sep-Pak C 18 cartridges (Waters, Milford, MA). After washing with 30% methanol:H_2O to remove unspecific hydrophilic components, the B(a)P nucleoside adducts were eluted with by 5 ml of 80% methanol The total radioactivity contained in the B(a)P nucleoside adducts was determined by scintillation counter. We obtained between 200 and 8.000 dpm per sample

Analysis by High-Performance Liquid Chromatography. High-performance liquid chromatographic analysis was performed in selected experiments. The 80% methanol eluates from Sep-Pak C_{18} were concentrated in a nitrogen stream to a volume of 200 to 400 μl One hundred-μl samples were analyzed on two 25-cm C_{18}-Bondapak columns connected in tandem and eluted isocratically with 50% methanol at a flow rate of 1 ml/min. We used a Hewlett Packard Model 1084 A chromatograph. Peaks were identified using authentic standards and by comparison to data in the literature (Refs 29 and 30, Chart 1)

RESULTS

The concentrations of covalent DNA adducts in human blood monocytes from lung cancer patients and healthy individuals were determined following incubation for 30 h with B(a)P. We were particularly interested in young patients and patients with a family history of lung cancer. The fact that they developed the disease may reflect a predisposition. Adduct concentrations in these "predisposed" patients were compared to corresponding healthy control groups. In addition, the smoking history of patients and healthy donors was taken into account. The definitions of the donor groups are given in Table 1. In Table 2, the B(a)P:DNA adduct concentrations are listed for the patients groups and the corresponding healthy control groups. Among groups of patients, the highest values were found for patients below 46 yr (Group 2: all patients, 4.34 ± 2.8; Group 6: smokers, 4.52 ± 2.89). In all groups, the adduct concentrations were higher in patients than in healthy controls. Highly significant differences were observed for Group 1 (all patients relative to all controls), Group 2 (all patients below 46 yr relative to all healthy donors below 46 yr), and Group 6 (patients below 46 yr who smoke relative to healthy smokers below 46 yr), No significant

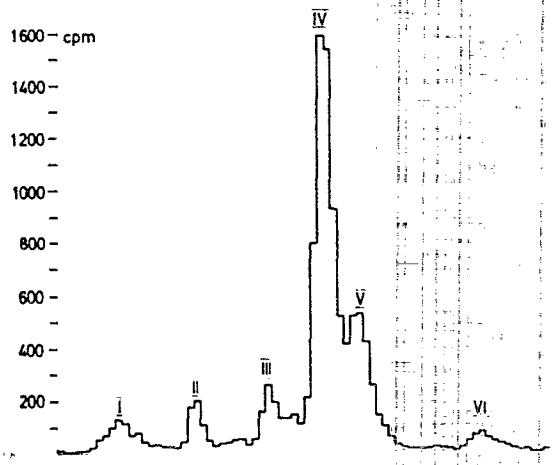

Chart 1. Typical elution profile of DNA adducts from two 25-cm C_{18}-Bondapak columns connected in series. The assignment of peaks was as follows *Peak I* is unspecific radioactive material, *Peaks II* and *VI* are tentatively assigned to adducts to cytosine and adenine. *Peak III* is (7*S*)-N^2-(7*d*,8,9,-trihydroxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene-1-yl)deoxyguanosine. *Peak IV* is (7*R*)-N^2-(7*d*,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene-10-yl)deoxyguanosine. *Peak V* is (7*R* or 7*S*)-N^2-(7*d*,8,9*d*-trihydroxy-7,8,9-tetrahydroxybenzo(*a*)pyrene-10-yl)deoxyguanosine

Table 2

Statistical evaluation (one-sided Wilcoxon test) of DNA adducts in patients versus controls

1 All patients $n = 45$ 3 44 ± 2 30[a] fmol/µg DNA	vs	All controls $n = 30$ 2 07 ± 0 81	$P < 0\ 000001$
2 All patients below 46 yr of age $n = 22$ 4.34 ± 2 8	vs	All controls below 46 yr of age $n = 16$ 1.92 ± 0.90	$P < 0\ 0025$
3 All patients above 46 yr of age $n = 23$ 2 58 ± 1 20	vs	All controls above 46 yr of age $n = 14$ 2.24 ± 0 70	$0\ 3 < P < 0\ 4$ (NS)[b]
4 Patients with family history of lung cancer $n = 12$ 2.77 ± 1 79	vs.	All controls $n = 30$ 2.07 ± 0 81	$0\ 2 < P < 0\ 3$ (NS)
5 All patients who smoke $n = 34$ 3 67 ± 2.56	vs	All healthy smokers $n = 22$ 2.19 ± 0.87	$P < 0\ 007$
6. Patients who smoke, below 46 yr of age $n = 20$ 4 52 ± 2 89	vs	Healthy smokers below 46 yr of age $n = 14$ 1.96 ± 0.95	$P < 0\ 004$
7. All nonsmoking patients $n = 11$ 2 74 ± 1 06	vs	All healthy nonsmokers $n = 8$ 1.72 ± 0.38	$P < 0\ 04$

[a] Mean ± SE
[b] NS, not significant

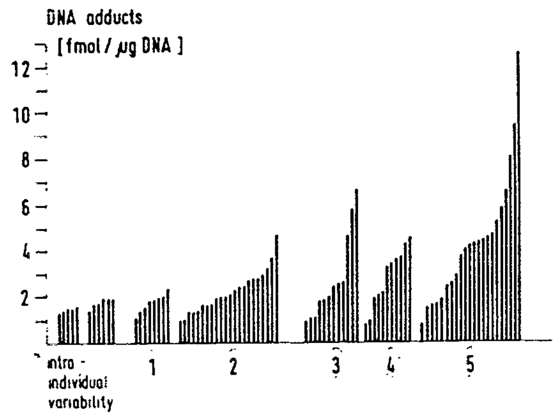

Chart 2. B(a)P DNA adducts from all patients and healthy controls The groups of data in this chart are not identically numbered as in Tables 1 and 2. Individual variability represents multiple determinations in weekly intervals in monocytes from 2 healthy controls. 1, healthy nonsmokers ($n = 8$). 2, healthy smokers ($n = 22$). 3, lung cancer patients with at least one affected first degree relative ($n = 12$); 4, nonsmoking lung cancer patients ($n = 11$). 5, lung cancer patients below 46 yr of age ($n = 22$)

differences were observed between patients with a family history of lung cancer and healthy controls, and the smoking habits of the donors had only a minor effect. For example, the adduct concentrations were only slightly higher for healthy cigarette smokers (Group 5: 2.19 ± 0.89) than healthy nonsmokers (Group 7: 1.72 ± 0.38). The number of cigarettes smoked the day before the blood was taken had no measurable effect. Chart 2 contains a visual presentation of adduct data for patient and control groups which allow an evaluation of interindividual variations. It also shows that the variability for multiple blood samples taken

Table 3

Distribution of tumor cell type

Group of patients	No of patients with: Type I oat, squamous cell	Type II adeno-, large cell	Statistical significance (χ^2 test)
All patients ($n = 45$)[a]	24	22	$P < 0.05$[b]
<46 yr of age ($n = 22$)	13	9	NS[b, c]
Positive family history ($n = 12$)[a]	11	2	NS[b]
Nonsmokers ($n = 11$)	3	8	$P < 0\ 001$[b]
Patients with DNA adducts <4.5 fmol/µg DNA ($n = 36$)[a]	16	21	$P < 0\ 05$[d]
Patients with DNA adducts ≥4.5 fmol/µg DNA ($n = 9$)	8	1	

[a] One patient had two independent tumors (adeno- and squamous cell carcinoma)
[b] Compared with the distribution of tumors of types I and II in the general population being 619 *versus* 268 according to Kreyberg (31).
[c] NS, not significant.
[d] Types I and II distribution compared in patients with DNA adducts below or above 4.5 fmol/µg of DNA (this value is the mean ± 3 σ of the distribution of all healthy controls).

after an interval of several days from the same healthy donor was small (about 7%).

Table 3 shows that, according to the distinction of Kreyberg (31), approximately equal numbers of our patients had type I tumors (oat cell plus squamous cell carcinoma) and type II tumors (adeno- and large cell carcinomas). The relatively high numbers of type II tumors in young patients (below 46 yr) and nonsmoking patients account for this result. It should be noted that, in the general population, type I tumors predominate (31). It is interesting that 8 of 9 patients with very high adduct concentrations (*i.e.*, >4.5 fmol/µg of DNA) had type I tumors.

DISCUSSION

Peripheral blood monocytes were used for our study because they are readily obtained from patients and healthy donors and

because they are closely related and possibly identical to the alveolar phagocytes of the lung. Monocytes constitutively metabolize PAH and may release metabolites into the neighboring lung tissue. Many enzymes participate in PAH metabolism, and for B(a)P, more than 40 metabolites have been identified. Some of these enzymes detoxify PAH; others activate them to metabolites which cause macromolecular damage (32). DNA is believed to be an important cellular target for carcinogen action (33). Therefore, we measured the capacity of monocytes from different groups of patients and healthy donors to form covalent B(a)P:DNA adducts without attempting to assess differences in the activities of individual metabolic enzymes (34). Smoking habits did not exert a measurable effect on adduct formation, suggesting that the overall activation to DNA binding metabolites was a constitutive process. Our results are in agreement with experiments using dogs on smoke inhalation machines in which the concentrations of B(a)P:DNA adducts were determined by a radioimmunological method (35). In contrast, the activity of aryl hydrocarbon hydroxylase alone, a key enzyme in B(a)P metabolism, is induced by cigarette smoke (36). The concentration of covalent B(a)P:DNA adducts following 30-h incubation with B(a)P is the result of adduct formation and removal by repair processes. At least for mice, genetic differences in adduct formation (37), rather than repair (38), appear to determine the susceptibility to the induction of cancer by B(a)P. No decrease in repair capacity was detected in mice with increased sensitivity to B(a)P. For human monocytes, the efficiency of DNA repair is unknown.

In earlier work we found that monocytes from lung cancer patients contained significantly higher B(a)P than did control monocytes (22). In contrast to the previous study, we now selected patients who might genetically be predisposed to the disease. In predisposed patients, carcinogen exposure might be lower, *e.g.*, in nonsmokers or young individuals with lung cancer, but their sensitivity to the carcinogen might be higher due to endogenous factors. An increased efficiency of DNA adduct formation could play an etiological role in these individuals. Increased adduct levels were indeed observed in young patients (below 46 yr) in the present work. However, it is conceivable that the increased adduct concentrations were a consequence rather than a cause of the disease. Our findings argue against this possibility. Only a slight elevation of adduct levels was found in patients with a family history of lung cancer. A substantial increase would have been expected also in this group if the disease were the cause of the observed change. It should be realized that the group of patients with family history of lung cancer is relatively ill defined. The probability that a second case of lung cancer occurs in a particular family depends on the family size. This factor could not be taken into account in the present study. In addition, the diagnosis for relatives reported to be affected by lung cancer could often not be ascertained.

Epidemiological studies on lung cancer patients in Denmark (31) have revealed that predominantly oat cell and squamous cell cancers of the lung (type I cancers) are related to an increased carcinogeic exposure by occupational or environmental air pollutants and, in particular, to cigarette smoke. If these carcinogens induce predominantly type I tumors of the lung, and if the individual sensitivity to carcinogens is mediated by metabolic activation of procarcinogens as proposed, then a preponderance of type I cancer can be expected in patients with high efficiency of formation of B(a)P:DNA adducts. Indeed 8 of 9 patients at the upper end of the distribution of adduct values in all lung cancer patients had type I cancer (Table 3). However, this argument is weakened by the considerable, albeit not statistically significant, number of type I cancers among low adduct patients. Type II tumors (normally adeno- and large cell carcinomas) predominated for patients who did not smoke.

Studies with monozygotic and dizygotic twins suggested that genetic, rather than environmental, factors are mainly responsible for interindividual variations in the inducibility of aryl hydrocarbon hydroxylase in humans (39, 40). These and our present findings support the concept of ecogenetics (41, 42) in the etiology of at least some types of lung cancer.

ACKNOWLEDGMENTS

We are deeply indebted to the consultants of the collaborating hospitals who referred patients to us: H. Finck, E. Hain, G. Jungst, E. Kehler, D. Sommerwerck, E. Wandelt-Freerksen, and K. von Windheim.

REFERENCES

1. Tokuhata, G. K., and Lilienfeld, A. M. Familial aggregation of lung cancer in humans. J. Natl. Cancer Inst., *30*: 289–312, 1963.
2. Spencer, H. (ed.). Pathology of the Lung. Ed. 3, Vol. 2, pp. 773–000. Oxford: Pergamon Press, 1977.
3. Lynch, H. T., Fain, P. R., Albano, W. A., Ruma, T., Black, L., Lynch, J., and Shonka, M. Genetic/epidemiological findings in a study of smoking-associated tumors. Cancer Genet. Cytogenet., *6*: 163–169, 1982.
4. Lynch, H. T., Lynch, P. M., and Guirgis, H. A. Host-environmental interaction and carcinogenesis in man. *In*: R. E. Koun (ed.), Genetic Differences in Clinical Carcinogenesis, Chap. 7, pp. 185–209. Boca Raton, FL: CRC Press, 1980.
5. Mulvihill, J. J. Host factors. *In*: C. C. Harris (ed.), Pathogenesis and Therapy of Lung Cancer, pp. 53–71. New York: Marcel Dekker, 1978.
6. Tokuhata, G. K. Cancer of the lung: host and environmental interaction. *In*: H. T. Lynch (ed.), Cancer Genetics, Chap. 13, pp. 213–232. Springfield, IL: Charles C. Thomas Publisher, 1976.
7. Brookes, P., and Lawley, P. D. Evidence for the binding of polynuclear aromatic hydrocarbons to the nucleic acids of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to deoxynbonucleic acid. Nature (Lond.), *202*: 781–784, 1964.
8. Duncan, M., Brookes, P., and Dipple, A. Metabolism and binding to cellular macromolecules of a series of hydrocarbons by mouse embryo cells in culture. Int. J. Cancer, *4*: 813–819, 1969.
9. Nebert, D. W., Benedict, W. F., and Koun, R. E. Aromatic hydrocarbon-produced tumorigenesis and the genetic differences in arylhydrocarbon hydroxylase. *In*: P. O. P. Tso and J. A. Di Paolo (eds.), Chemical Carcinogenesis, Chap. 12, pp. 271–288. New York: Marcel Dekker, 1974.
10. Nebert, D. W., Boobis, A. R., Yagi, H., Jenna, D. M., and Koun, R. E. Genetic differences in benzo(a)pyrene carcinogenic index *in vivo* and in mouse cytochrome P_{448}-mediated benzo(a)pyrene metabolite binding to DNA *in vitro*. *In*: D. J. Jallow, J. J. Kocsis, R. Snyder, and H. Vainio (eds.), Biological Reactive Intermediates, pp. 125–145. New York: Plenum Publishing Corp., 1977.
11. Cohen, G. M., Mehta, R., and Meredith-Brown, M. Large interindividual variations in metabolism of benzo(a)pyrene by peripheral lung tissue from lung cancer patients. Int. J. Cancer, *24*: 129–134, 1979.
12. Guirgis, H. A., Lynch, H. T., Mate, T., Harris, R. E., Wells, I., Caha, L., Anderson, J., Maloney, K., and Rankin, L. Aryl hydrocarbon hydroxylase activity in lymphocytes from lung cancer patients and normal controls. Oncology (Basel), *33*: 105–109, 1976.
13. Kellermann, G., Shaw, C. R., and Lyten-Kellermann, M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. N. Engl. J. Med., *289*: 934–937, 1973.
14. Koun, R. E., McKinney, C. E., Slomiany, D. J., Snodgrass, D. R., Wray, N. P., and McLemore, R. L. Positive correlation between high AHH activity and primary lung cancer as analyzed in cryopreserved lymphocytes. Cancer Res., *42*: 5030–5037, 1982.
15. McLemore, T. L., Martin, R. R., Springer, R. R., Wray, N., Cantrell, E. T., and Busbee, D. L. Aryl hydrocarbon hydroxylase activity in pulmonary alveolar macrophages and lymphocytes from lung cancer and noncancer patients: a correlation with family histones of cancer. Biochem. Genet., *17*: 795–806, 1979.
16. McLemore, T. L., Martin, R. R., Busbee, D. L., Richie, R. C., Springer, R. R., Toppell, K. L., and Cantrell, E. T. Aryl hydrocarbon hydroxylase activity in pulmonary macrophages and lymphocytes from lung cancer and noncancer patients. Cancer Res., *37*: 1175–1178, 1977.
17. Oesch, F., Schassmann, H., Ohnhaus, E., Altham, U., and Lorenz, J. Monoox-

ygenase, epoxide hydrolase, and glutathione-S-transferase activities in human lung. Variations between groups of bronchogenic carcinoma and non-cancer patients and interindividual differences. Carcinogenesis (Lond.), *1*: 827–835, 1980.

18. Paigen, B., Gurtoo, H. L., Minowada, J., Houten, L., Vincent, R., Paigen, K., Bejba Parker, N., Ward, E., and Thompson Hayner, N. Questionable relation of aryl hydrocarbon hydroxylase to lung cancer risk. N. Engl. J. Med., *297*: 346–350, 1977.
19. Ward, E., Paigen, B., Steenland, K., Vincent, R., Minowada, J., Gurtoo, H. L., Sarton, P., and Havens, M. B. Aryl hydrocarbon hydroxylase in persons with lung or laryngeal cancer. Int. J. Cancer, *22*: 384–389, 1978.
20. Harris, C. C., Autrup, H., Connor, R., Barrett, L. A., McDowell, E. M., and Trump, B. F. Interindividual variation in binding of benzo(*a*)pyrene to DNA in cultured human bronchi. Science (Wash. DC), *194*: 1067–1069, 1976.
21. Rudiger, H. W., Marxen, J., Kohl, F.-V., Meldens, H., and von Wichert, P. Metabolism and formation of DNA adducts of benzo(*a*)pyrene in human diploid fibroblasts. Cancer Res., *39*: 1083–1088, 1979.
22. Rudiger, H. W., Heisig, V., and Hain, E. Enhanced benzo(*a*)pyrene metabolism and formation of DNA adducts in monocytes of patients with lung cancer. J. Cancer Res. Clin. Oncol., *96*: 295–302, 1980.
23. Sobing, L. H. The WHO histologic classification of lung tumors. *In*: Muggia and M. Rosenzweig (eds.), Lung Cancer, Progress in Therapeutic Research, pp. 83–90. New York: Raven Press, 1979.
24. Bøyum, A. Separation of lymphocytes, lymphocyte subgroups, and monocytes: A review. Lymphology, *10*: 71–76, 1977.
25. Dulbecco, R., and Vogt, M. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med., *99*: 167–182, 1954.
26. Huberman, E., and Sachs, L. Metabolism of the carcinogenic hydrocarbon benzo(*a*)pyrene in human fibroblasts and epithelial cells. Int. J. Cancer, *11*: 412–418, 1973.
27. Leadon, S. A., and Cerutti, P. A. A rapid and mild procedure for the isolation of DNA from mammalian cells. Anal. Biochem., *120*: 282–288, 1982.
28. Fiszer-Szafarz, B., Szafarz, D., and de Murillo, A. G. A general, fast, and sensitive micromethod for DNA determination: application to rat and mouse liver, rat hepatoma, human leukocytes, chicken fibroblasts, and yeast cells. Anal. Biochem., *110*: 165–170, 1981.
29. Brown, H. S., Jeffrey, A. M., and Weinstein, I. B. Formation of DNA adducts in 10T ½ mouse embryo fibroblasts incubated with benzo(*a*)pyrene or dihydrodiol oxide derivatives. Cancer Res., *39*: 1673–1677, 1979.
30. Meehan, T., and Straub, K. Double-stranded DNA stereoselectively binds benzo(*a*)pyrene diol epoxides. Nature (Lond.), *277*: 410–412, 1979.
31. Kreyberg, L. Aetiology of Lung Cancer. A Morphological, Epidemiological, and Experimental Analysis. Oslo: Universitetsforlaget, 1969.
32. Gelboin, H. V. Benzo(*a*)pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed function oxidases and related enzymes. Physiol. Rev., *60*: 1107–1166, 1980.
33. Baird, W. M., and Brookes, P. Isolation of the hydrocarbon-deoxyribonucleoside products from the DNA of mouse embryo cells treated in culture with 7-methylbenzo(*a*)anthracene-^{3}H. Cancer Res., *33*: 2378–2385, 1973.
34. Perera, F. P., and Weinstein, I. B. Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. J. Chron. Dis., *35*: 581–600, 1982.
35. Perera, F. P., Poirier, M. C., Yuspa, S. H., Nakayama, J., Jeretzki, A., Curnen, M. M., Knowles, D. M., and Weinstein, I. B. A pilot project in molecular cancer epidemiology: determination of benzo(*a*)pyrene-DNA adducts in animals and human tissues by immunoassays. Carcinogenesis (Lond.), *3*: 1405–1410, 1982.
36. Nebert, D. W., Winker, J., and Gelboin, H. V. Aryl hydrocarbon hydroxylase activity in human placenta from cigarette smoking and smoking women. Cancer Res., *29*: 1763–1769, 1969, *39*: 1083–1088, 1979.
37. Nebert, D. W., Levitt, R. C., and Pelkonen, O. Genetic variation in metabolism of chemical carcinogens associated with susceptibility to tumorigenesis. *In*: A. C. Griffin and C. R. Shaw (eds.), Carcinogens: Identification and Mechanisms of Action. New York: Raven Press, 1979.
38. Kulkarni, M. S., and Anderson, M. W. Persistence of benzo(*a*)pyrene metabolite:DNA adducts in lung and liver of mice. Cancer Res., *44*: 97–101, 1984.
39. Okuda, T., Vesell, E. S., Plokin, E., Tarona, R., Bast, R. C., and Gelboin, H. V. Interindividual and intraindividual variations in aryl hydrocarbon hydroxylase in monocytes from monozygotic and dizygotic twins. Cancer Res., *37*: 3904–3911, 1977.
40. Nebert, D. W. Genetic control of carcinogen metabolism leading to individual differences in cancer risk. Biochimie, *60*: 1019–1029, 1978.
41. Omenn, G. S. Predictive identification of hypersusceptible individuals. J. Occupational Med., *24*: 369–374, 1982.
42. Strong, L. C. Genetic and environmental interactions. Cancer (Phila.), *40*: 1861–1866, 1977.