Lung Cancer 1

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edited by

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Cancer Treatment and Research

Foreword

Where do you begin to look for a recent, authoritative article on the diagnosis or management of a particular malignancy? The few general oncology textbooks are generally out of date. Single papers in specialized journals are informative but seldom comprehensive; these are more often preliminary reports on a very limited number of patients. Certain general journals frequently publish good in-depth reviews of cancer topics, and published symposium lectures are often the best overviews available. Unfortunately, these reviews and supplements appear sporadically, and the reader can never be sure when a topic of special interest will be covered.

Cancer Treatment and Research is a series of authoritative volumes which aim to meet this need. It is an attempt to establish a critical mass of oncology literature covering virtually all oncology topics, revised frequently to keep the coverage up to date, easily available on a single library shelf or by a single personal subscription.

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Where can you go to find quickly a recent authoritative article on any major oncology problem? We hope that Cancer Treatment and Research provides an answer.

WILLIAM L. MCGUIRE, M.D.
Series Editor

Preface

This volume reviews advances in several areas related to lung cancer: radiation therapy, especially for non-small cell tumors; small cell carcinoma (pathology, staging and treatment); attempts at immunotherapy, often in the 'adjuvant' setting after surgical resection; and carcinogenesis, with special attention to the predictive value of induced aryl hydrocarbon hydroxylase levels. All of these are reviewed by experts, all are controversial, and the reader should not be surprised to discover that there is not unanimity of opinion. What is missing in this volume is material related to chemotherapy for advanced, non-small cell cancers, or to surgery for resectable disease. The reasons are distressingly simple: the former has yet to be shown of proven benefit, and the latter has long since plateaued as a curative modality.

The largest part of this book is devoted to radiation therapy, because it is the editor's opinion (as a medical oncologist) that this is the modality where the application of presently available technology will make the greatest impact on the lung cancer problem in the next few years.

Doctors White and Boles emphasize the value of radiation therapy early rather than late in the treatment of regional non-small cell disease. They cite the data for split and continuous dose-fractionation schemes, and come forth with a recommendation for the continuous approach if radiation is to be used alone. Trials of combined modality approaches with chemotherapy added to radiation are reviewed, and are shown to be of no demonstrated value to date. Of particular interest is their analysis of sites of relapse, and of data suggesting the value of 'prophylactic' cranial irradiation in preventing relapse at this site in *non*-small cell patients.

Doctors Salazar and Zagars have covered new approaches to radiation therapy in a thorough fashion, yet one which is eminently comprehensible to the non-radiation oncologist. That experts do not always agree is implied by their advocacy of split-course over continuous fractionation schemes. Considerable space is devoted to unorthodox, high or varied dose regimens which have been piloted in Europe and Japan, but inadequately studied here. Basic review of such concepts as radiobiologic effectiveness and the oxygen enhancement ratio allows for a clear understanding of the potential advantages to be derived from hypoxic cell radiosensitizers and high-LET radiation, such as fast neutrons. Perhaps the most fascinating section involves Salazar's own extension of the work with hemibody irradiation, originally pioneered in

Toronto. It now appears that this technique is not only usefull for palliation of pain, but may, in conjunction with subsequent local irradiation of the primary tumor and/or chemotherapy, play an important role in reduction of systemic tumor burden.

As Dr. Green points out in his chapter on post-operative irradiation, retrospective data from his own series and that of Kirsch both strongly suggest benefit to patients with clearly resectable disease who have positive hilar or mediastinal lymph nodes. Based on their observations, it has become standard practice in many institutions to perform post-operative irradiation routinely in this setting. Green emphasizes that the appropriate studies to clarify the value of post-operative radiation therapy, namely, prospective, controlled trials with careful staging, have *not* been done. Sherman and Weichselbaum's chapter reviews the data for pre-operative irradiation. They cite previous, large-scale, controlled trials which failed to show any benefit versus surgery alone, and point out that these trials failed to use modern staging, employed lower-dose, protracted radiation therapy, and involved a long period between administration of radiation therapy and the anticipated surgery. Their own pilot trial of short-course, pre-operative radiation therapy, followed in two weeks by surgery, produced encouraging results in a group of patients considered 'marginally resectable' by pre-operative surgical evaluation. Unfortunately, what constitutes 'marginal' resectability varies from one institution to another, and even what makes a tumor 'T3' and therefore 'Stage 3' may vary: the usual criteria cited involve invasion of the mediastinum, diaphragm or chest wall and/or too close a margin to the main carina, but in Sherman's series, involvement of the main pulmonary artery was also considered a criterion for classification as 'T3'. Such patients did particularly well in their combined modality pilot.

A second major topic in this volume is small cell lung cancer, an area in which tremendous changes have taken place. Mary Matthews describes the role of pathologic subclassification among the small cell tumors and makes two extremely important points: 1) among tumors in which small cells are the 'only' tumor cells present, subclassification as to 'lymphocyte-like' versus 'spindle,' 'fusiform,' etc. is of no prognostic value; 2) small cell tumors in which there is a significant admixture of large cells (more generous cytoplasm, clearing of nuclear chromatin, prominent nucleoli) should be *treated* like other small cells, but analyzed separately. The reason for this is that many more will respond to chemotherapy than would be expected for large cell tumors in general, and some will even have long-term complete remissions. but median survival and overall long-term survival are compromised, relative to 'pure' small cell.

Hansen and Dombernowsky review the staging of small cell lung cancer. Especially noteworthy is their observation that blastic new bone formation

often accompanies marrow involvement in this disease, unlike the other types of lung cancer, and that it may persist in the absence of demonstrable tumor after therapy. This could lead to a problem with the interpretation of sequential bone scans and/or radiologic bone surveys, especially in patients who present with marrow metastasis. Bunn and Ihde exhaustively review the therapeutic results in treatment of small cell lung cancer, and add some suggestions for future directions if we are to progress beyond the present plateau. It is unquestionably clear that chemotherapy has improved the results of treatment for the average patient with this disease, and it is probable that any of several combinations are superior to single-agent cyclophosphamide. Far from resolved are the questions of how (or if) radiation therapy should be integrated with chemotherapy in management of these patients.

An area of major interest in recent years has been the immunotherapy of cancer, now more fashionably referred to as 'biological response modification,' to include the concept that some of the effects mediated by agents such as BCG, Corynebacterium parvum, thymosin and transfer factor may be by mechanisms other than immunologic. McGuire reviews adjuvant immunotherapy for resected lung cancer patients, as well as the trials of immunotherapy which have been carried out in small cell lung cancer. Hints remain of some advantage for intrapleural BCG after surgery in adequately staged Stage I patients: the rest is negative or inevaluable at present. However, several controlled trials in the United States, Canada and Europe should provide definitive answers in the next two to three years.

From a practical point of view, it may be considered as fact that cigaret smoking is causally related to lung cancer development in the vast majority. Yet only 1 in 20 heavy smokers develops lung cancer. Aside from the other health problems associated with smoking, it would be of great potential value to be able to prospectively identify individuals at special risk *in advance* and try to alter their behaviors and/or exposure to a cancer-causing environment. McLemore and Martin provide in-depth consideration of one very promising avenue: the measurement of aryl hydrocarbon hydroxylase (AHH) levels. As with treatment of this disease, controversy abounds as well in carcinogenesis research. In particular, use of the lymphocyte as a tool with which to measure AHH activity has been criticized, since in patients *with* lung cancer other tissues have more frequently shown elevated levels of AHH. As the authors point out, however, the readily available peripheral blood lymphocyte may still be useful for measuring the inducibility of AHH in those individuals who are free of detectable cancer. Certainly, the time has come to put their hypothesis to the test: prospective studies should be done in high risk individuals (e.g., cigaret smokers), classifying them according to AHH inducibility, then measuring the frequency of lung cancer development and corre-

lating this with AHH activity. It might then be possible to focus intensive preventive efforts on the 5 to 10% of smokers who are really 'high risk.'

Dr. Holmes' chapter on immunotherapy of lung cancer indicates a somewhat more optimistic point of view. While one may debate with some of the conclusions regarding the outcome of adjuvant trials with systemic immunotherapy, his observations of the value of intralesional BCG, injected either via the bronchoscope or directly into the tumor through the chest wall, are extremely provocative. They should stimulate confirmatory efforts in other centers.

We are far from preventing lung cancer, although perhaps the tools are at hand to do so. We are far from curing most cases, and probably lack the tools at present to do more than improve modestly on today's results. Even modest improvement, however, would translate into thousands of lives saved each year. What *has* happened in the last decade is a major ferment of interest, and disappearance of the apathy about the problem which once prevailed in the medical scientific community. These are essential first steps to real progress.

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Contents

1. Pulmonary Carcinogenesis: Aryl Hydrocarbon Hydroxylase

THEODORE L. McLEMORE and R. RUSSELL MARTIN

1. INTRODUCTION

Especially during the past two decades, there has been a growing awareness that exposure of individuals to exogenous environmental agents is responsible for various kinds of cancer. Chemical carcinogenesis in man was first documented in 1775 by the British physician Percival Pott, who attributed the high incidence of scrotal cancer in London chimney sweeps to their chronic exposure to soot and coal tars [1]. In the two hundred years following that initial observation, at least 1000 chemicals have been shown to induce cancer in a wide variety of tissues [2]. As our civilization has become industralized, our food, air, and water have undergone increased contamination with a number of cancer producing chemicals. It is currently estimated that approximately 75 to 85 percent of human cancer may be directly associated with exposure to these environmental carcinogens [3].

Probably the best established and the most extensively studied example of human environmental carcinogenesis is the relationship between cigarette smoking and the development of lung cancer. This area has aroused both medical and public concern and is a major interest for investigators involved in the study of chemical carcinogenesis. Prior to 1920, lung cancer was a rare cancer type seen only infrequently by physicians. However, as the quantity of tobacco products (especially cigarettes) consumed in the United States rose, there was a concomitant increase in the incidence of lung cancer. By 1950, lung cancer had become the number one cause of cancer deaths in adult males in the United States. Since that time with a further increase in cigarette smoking by females, lung cancer is now steadily rising in this subpopulation

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as well. In fact, it is estimated that by 1985, lung cancer will be the number one cause of cancer-related deaths in the total United States adult population [4].

Numerous reports have implicated tobacco (specifically components of cigarette smoke condensate) to be carcinogenic in laboratory animals [5–20], and epidemiological studies in man have directly associated heavy consumption of cigarettes with increased risk of lung cancer [21–27]. However, the precise mechanisms responsible for the initiation of pulmonary neoplasia have remained undefined. There is evidence that genetic factors may play a role in human susceptibility to lung cancer. Tokuhata [28] found that the incidence of lung cancer was greater when a family history of cancer was present, either for nonsmokers or smokers. Furthermore, there was an exponential increase in lung cancer risk among cigarette smokers with a family history of lung cancer. The combination of these two factors, smoking and family history, increased the risk of lung cancer several fold. Tokuhata [29] further suggested that the organ site at which a tumor is likely to develop is largely under genetic control. He further indicated that synergism between genetics and environmental factors is of great importance.

The major carcinogenic agents in cigarette smoke condensate are the polycyclic aromatic hydrocarbons (PAHs). These compounds, which include benzo(a)pyrene (BP) and benzanthracene (BA), are products of incomplete combustion and are among the active components of cigarette smoke condensate [21, 23, 30, 31], air pollution [32, 33], coal tar [34–36], and smoked foods [37]. It is now known that many of these PAHs are converted to active forms after their entry into the body tissues [2, 3, 38–42]. PAHs are only weakly carcinogenic before being converted to electrophilic forms which readily favor covalent binding to protein and nucleic acids within the cell [43–45]. These reactive compounds have been implicated in the process of chemical carcinogenesis in man [46–57]. One enzyme system which is responsible for the conversion of these compounds to their active metabolic forms is aryl hydrocarbon hydroxylase (AHH). This membrane-bound, oxygen-dependent, enzyme system is found in many tissues in the human body and is capable of converting components of cigarette smoke to potent intermediates with enhanced mutogenic [43, 45, 58–60] and carcinogenic potential [61–63]. Specific details of the structure and mechanisms for activation of carcinogens by AHH will be discussed later in this chapter.

In order to understand the intricate interactions between PAHs and human lung tissues (particularly the bronchus, which is the target tissue for lung cancer production), we must understand how formation of these potent intermediate compounds could theoretically fit into the overall scheme of pulmonary carcinogenesis in man. As noted in Figure 1, different stages of the carcinogenic process are interrelated. The initial step in this pathway is the

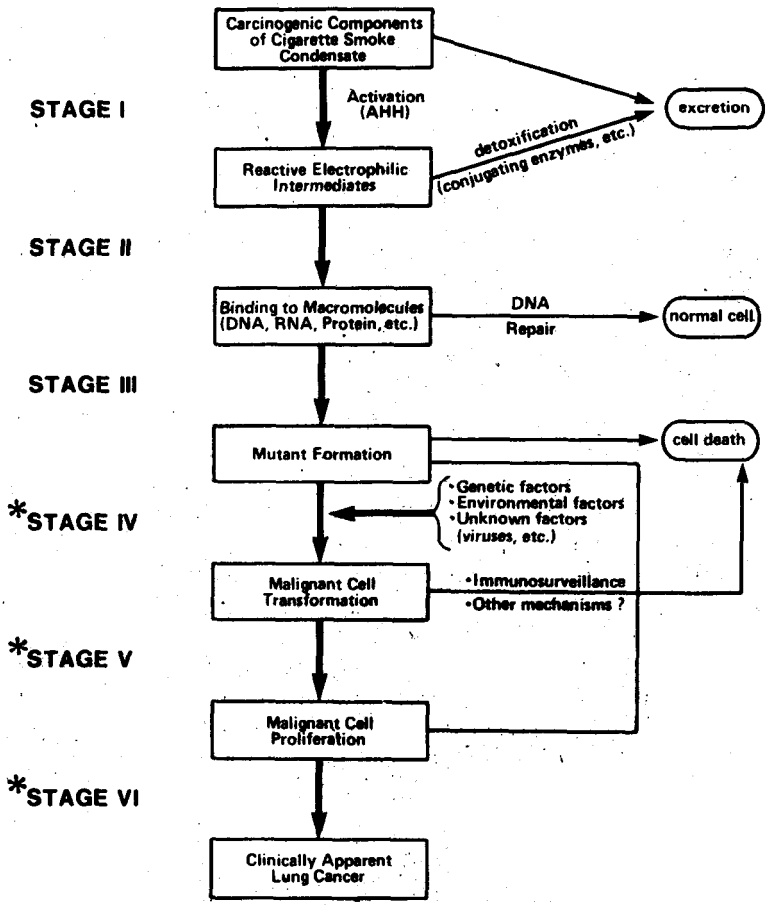


Fig. 1. Theoretical pathways for human pulmonary carcinogenesis.
* Represents those stages requiring long latency periods.

activation of PAH components of cigarette smoke by the AHH enzyme system. The reactive intermediate metabolites which are produced may undergo detoxification (via conjugation and other enzymatic reactions) and be excreted from the body. Metabolites which are not excreted may undergo binding with macromolecules within the cell (stage II), initiating mutagenesis (stage III). These alterations may be rectified by DNA repair mechanisms, in which case normal cellular function would resume. Therefore, in an individual cigarette smoker, many DNA mutations could be formed for a given unit of time, but the efficiency of the individual's DNA repair mechanisms might

allow for reversal of these harmful effects. It is only when the DNA repair mechanisms falter that mutations would persist and ultimately lead to malignant transformation (stage IV) in lung tissues. Environmental factors, such as viruses, co-carcinogens (such as asbestos), promoters (such as phorbol esters, which are constituents of cigarette smoke), and various undefined factors may also be involved in promotion of malignant transformation. At some point, the transformed malignant cell may be destroyed by immunosurveillance or other internal protective mechanisms. Alternatively, malignant cell proliferation (stage V) may occur, eventually leading to the appearance of clinically apparent lung cancer (stage VI). Even after proliferation of the malignant cells (before they are clinically apparent) immunosurveillance or other protective body mechanisms may contain the spread or eradicate the malignant tissues. A latency period (as long as 20 years) occurs in progression from stage IV to stage VI, and undoubtedly many abortive progressions through the various stages could occur before the defense mechanisms are overwhelmed and clinically apparent cancer results.

It is apparent that the process of pulmonary carcinogenesis in man is multifactorial and highly complicated in nature. In this chapter, we will discuss in detail only one aspect of this hypothesized scheme for pulmonary carcinogenesis. Biochemical pathways for metabolism of PAHs by human tissues will be reviewed and current concepts defining the relatonship between levels of AHH and human lung cancer susceptibility will be discussed, with emphasis on recent reserach developments. This portion of the hypothetical scheme for pulmonary carcinogenesis could be the most relevant since it represents the rate limiting step without which progression to pulmonary neoplasia could not occur (Fig. 1).

2. STRUCTURE AND FUNCTION OF THE AHH ENZYME COMPLEX

AHH represents a labile, multicomponent enzyme system which is tightly bound to membranes and requires NADPH and/or NADH and molecular oxygen for enzymatic activity [64, 65]. Studies by Lu *et al.* [66] and other investigators [67–71] have indicated that the microsomal enzyme is composed of at least three components: 1) a phospholipid portion (intimately associated with the membrane), 2) a reductase (presumably either cytochrome c or cytochrome b5 reductase), and 3) a cytochrome(s) P-450 fraction. It has been proposed that the cytochrome P-450 and the reductase portions of the enzyme are in a halo of phospholipid matrix which is bound to the cell membrane and which allows lateral mobility of these enzymes in the matrix [71]. The substrate specificity of the AHH system is dependent upon the cytochrome P-450 fraction [72–86]. Reducing equivalents for the reactions

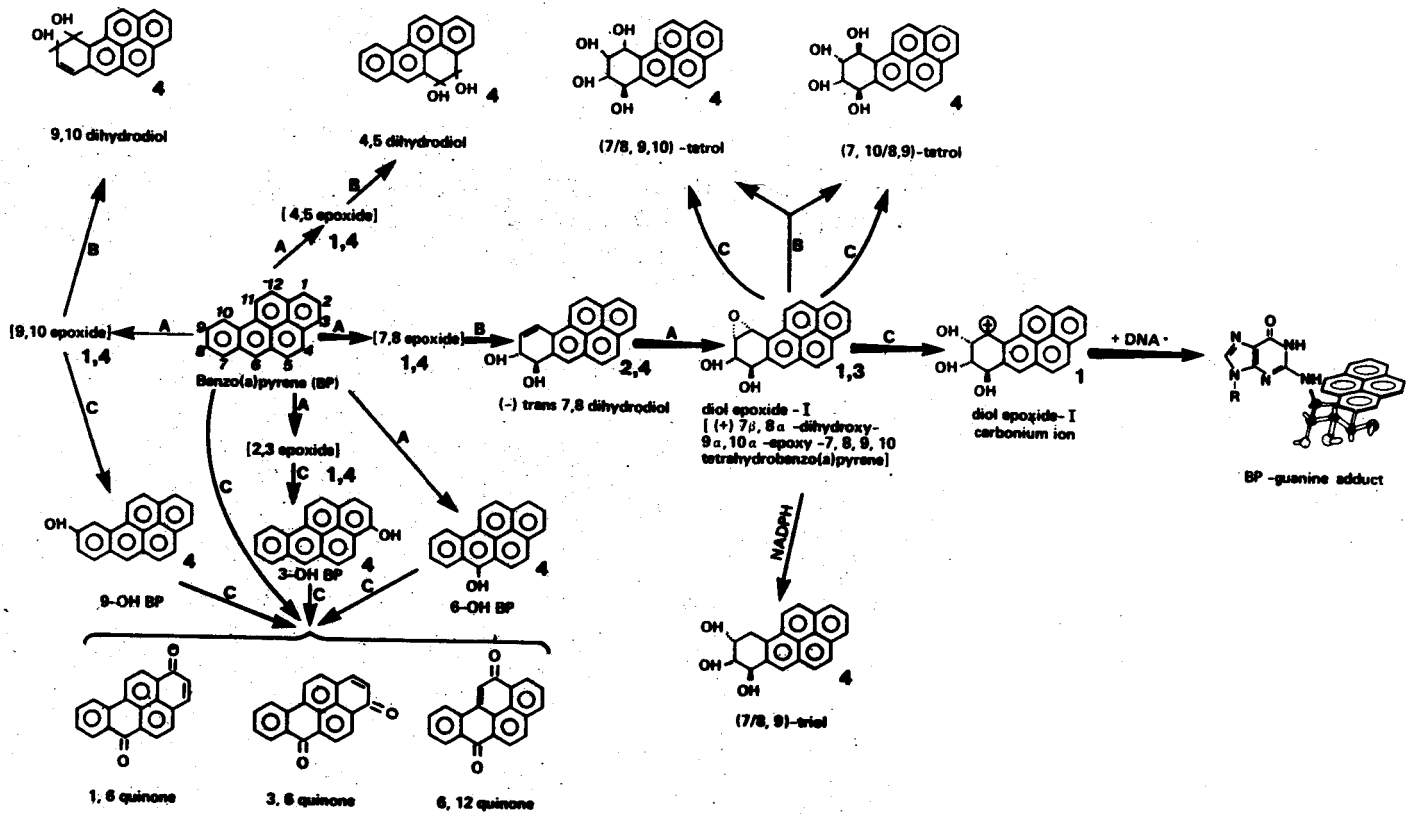
may be supplied by NADPH and/or NADH, and ultimately reach hydrophobic substrates through cytochrome P-450 or cynanide-sensitive factor [66, 67, 69, 71, 87].

Numerous xenobiotics, including PAH components of cigarette smoke, are highly lipophilic and would remain in the body tissues indefinitely if it were not for enzymes such as the AHH enzyme complex. During metabolism by AHH, one or more polar groups (hydroxyls) are introduced into nonpolar molecules, thereby making them more hydrophilic. These more polar substrates can then be readily conjugated, excreted from cells and eventually cleared from the body [88].

AHH converts PAHs to numerous metabolites by hydroxylation pathways (Fig. 2). BP is the prototype PAH whose metabolism has been extensively studied. The acceptance of one atom of molecular O_2 into an unsaturated BP molecule results in formation of reactive arene oxide (epoxide) intermediates with increased capacity for macromolecular binding. These can undergo nonenzymatic spontaneous rearrangement to form phenols (which may in turn undergo conjugation reactions); be further metabolized by epoxide hydratase to nontoxic dihydrodiols (these may also be conjugated); interact with cellular macromolecules including DNA and RNA; or undergo conjugation reactions (for review see references [89–91]).

Although all epoxide derivatives of BP are electrophilic species capable of binding macromolecules such as DNA, the most reactive of these known to date is the diol epoxide I. As demonstrated (Fig. 2), the proposed pathway for production of the proximate and ultimate carcinogenic BP derivatives involves both primary and secondary metabolism of BP. The 7,8-dihydrodiol (the proximate carcinogenic metabolite of BP) is produced by initial metabolism of the BP molecule by AHH and subsequent conversion of the 7,8-epoxide to the 7,8-dihydrodiol by epoxide hydratase. Further metabolism of this derivative by AHH results in formation of the ultimate carcinogen of BP, the (+)7β,8α-dihydrodiol-9α-10α-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene (diol epoxide I). This molecule subsequently undergoes spontaneous rearrangement to a carbonium ion at the 10 position, which is postulated to then react with cellular macromolecules. Specifically, when this reactive species interacts with DNA guanine base pairs, they form BP-guanine adducts (Fig. 2). Note that at various points along the activation pathways, detoxification pathways can intervene, producing tetrols, triols, and conjugation products which are readily execreted from the cell (Figure 2) (for review see references [89–91]).

AHH is known to metabolize a number of endogenous substrates such as steroids, cholesterol, fatty acids, bilirubin, biogenic amines, indoles, and ethanol. AHH is also essential in the metabolism of a number of xenobiotics. These hydrophobic exogenous substrates include PAHs such as BP, BA, and


9,10 dihydrodiol
4,5 dihydrodiol
(7/8, 9,10) -tetrol
(7, 10/8,9)-tetrol
[4,5 epoxide]
1,4
[9,10 epoxide]
1,4
Benzo(a)pyrene (BP)
[7,8 epoxide]
1,4
(-) trans 7,8 dihydrodiol
2,4
diol epoxide - I
[(+) 7β, 8α -dihydroxy-
9α,10α -epoxy -7, 8, 9, 10
tetrahydrobenzo(a)pyrene]
1,3
diol epoxide- I
carbonium ion
+ DNA
BP -guanine adduct
[2,3 epoxide]
1,4
9-OH BP
3-OH BP
6-OH BP
NADPH
(7/8, 9)-triol
1, 6 quinone
3, 6 quinone
6, 12 quinone

3-methylcholanthrene (MC); halogenated hydrocarbons such as polychlorinated and polybrominated biphenyls, insecticides and ingredients in soaps and deodorants; strong mutagens such as N-methyl, N-nitro nitrosoguanidine and nitrosamines; aminoazo dyes and diazo compounds; N-acetylarylamines and nitrofurans; numerous aromatic amines, such as those found in hair dyes, nitro aromatics and heterocyclics; wood terpenes; epoxides; carbamates; alkyl halides; safrole derivatives; certain fungal toxins and antibiotics; many of the chemotherapeutic agents used to treat human cancers; and most commonly used drugs [20, 31–37, 41, 87, 88, 92].

An important characteristic of AHH is its inducibility. The levels of the enzyme fluctuate depending upon exposure to specific inducers which include the exogenous as well as the endogenous chemicals previously described. The precise mechanisms whereby AHH inducers increase enzyme action have not been completely defined, but synthesis of more heme protein is involved [74, 93, 94]. Exposure to inducers produces accelerated *de novo* protein synthesis and associated increases in the cytochrome P-450 fractions [94]. Poland *et al.* have isolated a cytosol receptor protein molecule which appears to bind AHH inducers and possibly is associated with initiation of gene recognition, transcription, and translation of the cytochrome heme proteins [95].

Figure 3 shows a hypothetical scheme associated with the induction of different P-450s of the AHH system. Inducers, such as the PAHs might bind the cytosol receptor proteins in the cell. These receptors are themselves under genetic regulation and can vary with each individual [82, 95, 96]. The binding of the receptor to inducer molecules activates the genomes thus stimulating the process of transcription, translation, and ultimately synthesis of the cytochrome(s) P-450 which are associated with increased monooxygenease activity [82].

Multiple forms of the cytochrome P-450 heme protein have been identified in microsomes [94, 97, 103], and various AHH inducers have been associated with different forms of cytochrome P-450 species. These inducers have been classified as Type I or Type II depending on the predominant species of the cytochrome P-450 present [67, 104–107]. Pretreatment of experimental animals with a Type I inducer (e.g. phenobarbital) results in a nonspecific

←

Fig. 2. Pathways for activation and detoxification of benzo (a) pyrene (BP) by the aryl hydrocarbon hydroxylase (AHH) enzyme system. 1 – epoxide intermediates with enhanced macromolecular binding capabilities. 2 – the proximate carcinogen of BP metabolism. 3 – the ultimate carcinogen of BP metabolism. 4 – BP derivatives which may be substrates for conjugation (detoxification) pathways. A, B, and C represent reactions mediated by AHH, epoxide hydratase, and nonenzymatic rearrangement, respectively. Dark arrows represent the major pathways for activation of BP.

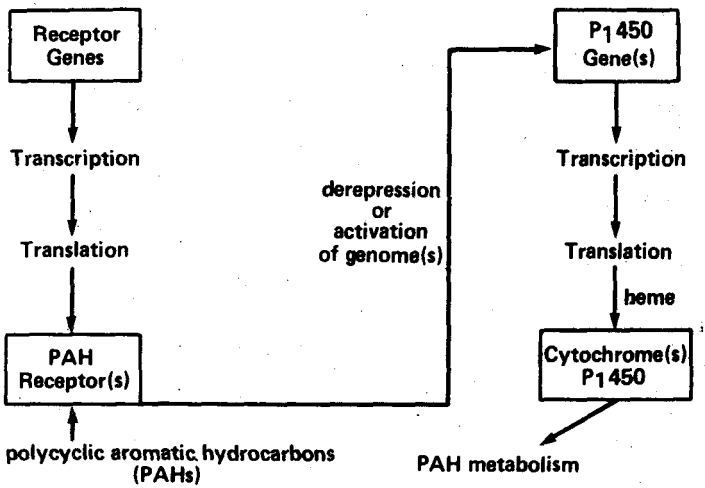


Fig. 3. Hypothetical scheme for polycyclic aromatic hydrocarbon (PAH) induction of different cytochrome P-450s of the AHH enzyme system.

increase in the metabolism of PAH as well as other drugs by the AHH system [72, 73, 75, 77, 78]. Studies utilizing AHH inhibitors [75] or observing metabolite profiles [107] indicate that Type I induced hepatic activity may be only quantitatively different from the constitutive enzyme activity. The liver appears to be the only tissue in which AHH activity is increased by Type I substrates [67, 104–107]. Type II inducers include PAHs and other xenobiotics. Pretreatment of experimental animals with PAHs results in specific increased metabolism of these compounds in hepatic as well as extrahepatic tissues, but AHH activity toward most other substrates remains unchanged [67, 104–107]. Type I induction is associated with an absorbance of the reduced CO-bound cytochrome complex at 450 nm (or cytochrome P-450) [78, 81, 108, 109]. However, Type II enzyme induction has been characterized by an increased absorbance maximum of the reduced, CO-bound complex from 450 to 448 nm (cytochrome P-448) for liver microsomes [109] and from 450 to 454–446 nm (or cytochrome P_1-450) for nonhepatic tissues [83, 110]. The presence of different heme proteins can also be supported by their electron paramagnetic resonance spectroscopy [78], and by a preferential inhibition of BP hydroxylation *in vitro* [73]. The specific type of cytochrome P-450 induced may influence the distribution of metabolites produced [107–111], as well as the ratios of intermediate products formed [20, 112].

Induction of one or more forms of cytochrome P-450 is associated with induction of numerous monooxygenase activities which are related to PAH