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HOST AND ENVIRONMENTAL FACTORS ENHANCING CARCINOGENESIS IN THE RESPIRATORY TRACT[1]

P. Nettesheim

National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

D. C. Topping

Eastman Kodak Company, B-320 Toxicology Section, Health, Safety and Human Factors Laboratory, Rochester, New York 14650

R. Jamasbi

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

INTRODUCTION

Bronchogenic carcinoma is a neoplasm with complex etiology. Its induction is usually linked to repeated exposures to complex chemical mixtures. The prime example is, of course, cigarette smoke, which has been firmly established as the major etiological factor for lung cancer induction through numerous epidemiological studies (e.g. 1, 2). However, there is ample evidence suggesting that various occupational exposures are also important either as causative agents or as cofactors in the pathogenesis of respiratory tract neoplasms [for recent review see (3)]. This has been most convincingly demonstrated in the case of uranium miners (4), asbestos workers (5, 6), and coke oven workers (7). The role of "general" air pollution in the etiology

of lung cancer remains uncertain (8). Particularly in dealing with occupational lung cancer, it is often difficult to identify the major causative agent(s) or cofactor(s) in the complex work environment. The problem is further compounded by various factors that may predispose individuals or groups of individuals to the development of cancer. Among the factors that have to be considered are the genetic control of drug metabolism (e.g. 9, 10), and a number of genetic aberrations (11). Similarly, various acquired diseases and physiological or nutritional states have been implicated in certain types of cancer, though precise interrelationships have not been defined (12).

Clearly, much work remains to be done in the identification of modifiers of the carcinogenic process by both endogenous and exogenous factors. In relation to bronchogenic carcinoma, it seems to be particularly urgent that we learn to identify individuals who are exceptionally vulnerable to carcinogenic insults, conditions predisposing to cancer, and agents promoting or accelerating the development of neoplastic disease.

Over the past 10 years, our laboratory has been engaged in studies concerned with the pathogenesis of lung cancer, with particular attention given to the possible interaction of multiple endogenous and exogenous factors. We review those studies and major contributions made by other investigators which might have a bearing on the problem of the multifactor etiology of lung cancer. We discuss first experiments relating to modification of host susceptibility, and second studies concerned with the interaction of environmental or occupational agents.

STUDIES ON AGE-SPECIFIC SUSCEPTIBILITY OF RESPIRATORY TRACT EPITHELIUM TO CARCINOGENS

Because cancer is in general a disease of the more advanced age groups, it has been suggested that the aging process might increase the susceptibility to carcinogenic agents (see 13). However, the increased incidence of many cancers with advancing age might simply be due to the time required to accumulate an effective carcinogen dose, as suggested by the experiments of Peto et al (14), or to the long latency period typical of many neoplasms. On the other hand, the limited data presently available do not permit us to rule out the possibility that the susceptibility of tissues to carcinogens changes with age or that the defense mechanisms, declining with age, render the host more susceptible to carcinogenic agents. It has been shown, for example, that in humans, aryl hydrocarbon hydroxylase inducibility changes with age (10). Some experimental studies using a rodent model of skin carcinogenesis have suggested that senescent skin grafted to young hosts is more susceptible to the tumorigenic effects of chemical carcinogens

than skin from younger animals (15). However, VanDuuren et al (16), using the two-stage carcinogenesis model, showed that the skin of old mice was more refractory to tumor induction following application of promoting agents than the skin of younger animals. These results are not necessarily contradictory, but might instead indicate that the aging process differentially modifies the efficacy with which complete carcinogens, initiators, and promoting agents exert their tumorigenic potential. There are also many biochemical studies that have demonstrated age-dependent variation of drug metabolism, including carcinogen metabolism [for discussion see (13)].

In our own studies, we chose as the experimental model the tracheal transplant system which was developed several years ago for laboratory investigations of respiratory tract carcinogenesis (17). Since several of the studies to be discussed here utilize this model, its principles are briefly described. Whole excised tracheas are transplanted to the subcutis of syngeneic hosts in the retroscapular region. Histologically, such tracheal transplants are virtually indistinguishable from host tracheas within several weeks after grafting, and it was shown that they produce and secrete mucus for many months. To produce tumors in these tracheal grafts, one inserts pellets containing carcinogen into the tracheal lumen. Squamous cell carcinomas, adenocarcinomas, and mixed adenosquamous carcinomas develop within 10–24 months in a carcinogen dose-dependent fashion (18–20).

Utilizing this experimental model, we asked: Are there age-specific differences in the susceptibility to carcinogens, inherent in the target tissues themselves, independent of other age-related changes in the host (e.g. hormonal or immunological changes)? Tracheas were transplanted from 10-week-old and from 2-year-old Fischer 344 rats into young adult recipients of the same strain, thus eliminating systemic host effects as variables. In this way both sets of tracheas were also ensured an equally long "survival time" and thus an equal chance to develop tumors. Because of the long tumor induction times, this is not the case in many other studies concerned with aging and carcinogenesis; the older animals commonly die from various age-related diseases before the tumor response under study has time to develop fully. The results from this study are summarized in Table 1. As can be seen, no increase in the susceptibility of senescent tracheal epithelium to the effects of the carcinogenic polycyclic aromatic hydrocarbon (PAH) was observed. On the contrary, at the lower carcinogen dose, the senescent epithelium appeared somewhat more refractory to the carcinogenic effects (D. C. Topping and P. Nettesheim, unpublished observations). Thus, while there may be host-related factors increasing the risk to aged individuals of developing lung cancer, this experiment suggests that the respiratory tract epithelium itself does not become more susceptible to the carcinogenic PAH

Table 1 Tumor response in tracheal grafts from 10-week-old posed to dimethylbenz(*a*) anthracene (DMBA)[a]

Carcinogen dose (μg)	Age of donor animal	Number of tracheas[b]	Number of palpable carcinomas	Number of carcinomas (all types)[c]
100	10 wk	40	17	17
100	2 yr	43	4	7
150	10 wk	41	16	21
150	2 yr	41	14	23
0	2 yr	29	0	0

[a] Established tracheal grafts were implanted with beeswax pellets containing DMBA. Grafts remained on the host animal until palpable tumors developed or until the host animal died.
[b] Only those tracheas grafted to animals that survived for 20 or more weeks after implantation of the beeswax-carcinogen pellets are included.
[c] Includes microinvasive and noninvasive carcinomas.

with increasing age. There is evidence from carcinogen-DNA binding studies (see Table 2) suggesting that conducting airways in animals younger than those tested in our aging study may actually be at a greater risk of developing cancer than those of adults (21). The great sensitivity of embryonic and neonatal lung for a variety of carcinogens has been noted for many years (22–24).

THE EFFECT OF IMMUNE SUPPRESSION ON THE DEVELOPMENT OF RESPIRATORY TRACT TUMORS

The immune system has been shown to decrease in competence with age (e.g. 25), and this coincides with the increasing appearance of various types of cancer. It is well established that the impairment of immune functions is associated with an increased cancer incidence in both man and experi-

Table 2 Specific activities of binding of [^{3}H] benzo(*a*)pyrene [B(a)P] to hamster tracheal DNA: Effect of age of hamsters[a]

In vitro incubation conditions	Specific binding[b] for hamsters of ages: 4 wk	8 wk	12 wk
[^{3}H] B(a)P, 37°C	41.7	27.3	11.2
[^{3}H] B(a)P, 7,8-benzoflavone, 37°C	2.9		

[a] In all cases, hamsters received an intratracheal administration of 5 mg B(a)P plus 5 mg Fe_2O_3 48 hr before they were killed [from (21)].
[b] All results are expressed as dpm [^{3}H] B(a)P/μg DNA.

mental animals (e.g. 26). The only type of respiratory tract tumor that has been studied in this regard is the mouse lung adenoma (24)—a tumor different in its origin, histological appearance, and biology from bronchogenic carcinoma in man. We conducted carcinogenesis studies with rat trachea as a model for airway mucosa (see above). The purpose of these investigations was to determine whether a temporary state of immune suppression existing only for the first few months after carcinogen exposure would significantly affect the incidence of squamous carcinomas induced by a chemical carcinogen. The tracheal graft model was again used for these studies since it is possible in this experimental system to immunosuppress the host with chemical immunosuppressants or X radiation without exposing the tissue that is the target for the carcinogen, in this case the tracheal epithelium. Because the state of immune impairment occurred only during the early phase of tumor induction, we hoped that this experiment would give us some clue regarding the time of appearance of antigenically distinct cells during neoplastic development. We knew from previous studies that fully developed respiratory tract carcinomas have tumor-associated transplantation antigens (27, 28). Prospective recipient animals were immunosuppressed by means of thymectomy and whole-body X irradiation. Subsequently, these recipients were grafted with tracheas from isogenic donors. One month later, tracheas were exposed to carcinogen as described above. Assessment of the immunocompetence (skin homograft rejection and anti-sheep red blood cell antibody response) showed that the animals were severely immunosuppressed shortly after X irradiation and thymectomy, but that the immune responsiveness had fully recovered 4 months after the start of carcinogen exposure. The results of this study (Figure 1) clearly showed an enhanced tumor response in tracheas carried by immunosuppressed recipients, even though the immunosuppression lasted at most for 4 months (R. Jamasbi and P. Nettesheim, unpublished). In the group exposed to 100 μg DMBA, no tumors developed in the first 6 months, and the mean tumor induction time was more than 1 year. The same enhancement of the tumor response was seen in a second group of animals in which a dose of 200 μg of DMBA was used. These results suggest that early events in the carcinogenesis of respiratory tract mucosa may be controlled to some extent by immune defense mechanisms. This supports the concept that suppressed immunosurveillance permits the emergence and proliferation of initiated and transformed cells, some of which might otherwise be destroyed by a functional immune system. Furthermore, our findings suggest that neoantigens might develop early in carcinogenesis during the preneoplastic phase, probably before the "carcinogen-altered" or initiated cells are fully transformed and before they can be recognized as neoplastic growths. We fully realize that the specifics of these findings are likely to be a product of the experimental approach used (i.e. transplanted tracheas rather than in

situ tracheas); however, we have no reason to believe that in principle these results are not applicable to cancer development in the intact respiratory tract. Antigens in the preneoplastic phase have been reported also in other tumor induction systems such as liver carcinogenesis (29).

THE ROLE OF DIETARY VITAMIN A IN THE SUSCEPTIBILITY TO LUNG CANCER INDUCTION

A number of nutritional factors are thought to be important as modifiers of carcinogenesis (30–32). One of these factors is vitamin A. It is known that this vitamin is involved in the differentiation and maintenance of normal mucosa, and severe vitamin A deficiency leads to abnormal cellular differentiation and proliferation in several types of epithelia (33, 34). Whether and in what way these tissue alterations are related to the development of preneoplastic and neoplastic lesions is difficult to ascertain. However, there is at least one epidemiological study which suggests that inadequate vitamin A intake might be associated with an increased lung cancer risk (35). We have conducted a number of studies in which the role of dietary vitamin A on the development of respiratory tract tumors was examined in rats following intratracheal injection of the carcinogen 3-methylcholanthrene (MCA) (36–38). One major goal of these studies was to determine whether subnormal vitamin A intake would predispose the animals to develop lung cancer. Groups of rats were maintained on less than adequate, adequate, and exces-

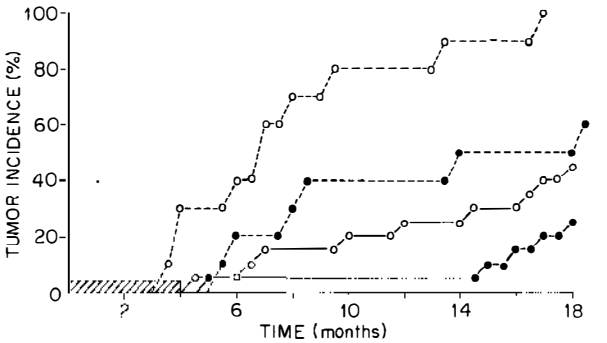

Figure 1 Effect of temporary immune suppression on tracheal carcinoma incidence. Normal tracheas were implanted into thymectomized, X-irradiated (600 R whole-body irradiation) syngeneic rats (○) or into sham-operated, nonirradiated rats (●). One month later, the tracheas were exposed to pellets containing either 100 (——) or 200 μg (- - -) DMBA. The capacity to produce antibodies to sheep red blood cells or to reject skin homografts was examined in a separate group of animals. The duration of immune suppression is indicated by the hatched horizontal bar.

sive levels of vitamin A in the form of retinyl acetate (RA), starting at 3–4 weeks of age. Five weeks after their vitamin A intake was adjusted to the respective levels, the animals were given intratracheal injections of MCA at one of four dose levels. Results of this study, summarized in Table 3, indicate that rats maintained on a low level of vitamin A (approximately 100 μg RA/week is considered to be adequate for rats) are more susceptible to lung cancer induction by the carcinogenic PAH MCA than rats maintained on an adequate or high vitamin A level. It is important to note that this increased susceptibility exists in rats that are not totally deprived of vitamin A. Rather, the low RA level is sufficient to allow rats not exposed to carcinogen to grow at a normal rate and to live a normal life span. The greater lung cancer susceptibility of the deficient rats is manifested in a higher lung cancer incidence and a shortened survival due to death from lung cancer. The other important finding is that RA levels 10 times in excess of "adequate" levels do not confer any added protection against the carcinogenic effects of MCA in the lung. We also showed recently (39) that pharmacological doses of 13-*cis*-retinoic acid and of a synthetic retinoid (R011-1430) failed to inhibit the development of tracheal carcinomas in hamsters. The absence of reproducible antineoplastic effects of at least three retinoids (RA, 13-*cis*-retinoic acid, R011-1430) in various experimental models of respiratory tract carcinogenesis is noteworthy since these and other vitamin A–related compounds have been shown to effectively inhibit tumor development in other organ systems including the skin, the bladder, and the mammary gland [for review of literature and discussion see (40)]. One possible explanation is that retinoids are most effective as chemopreventive agents in those tumor systems in which internal or external promoters are essential in tumor induction. In the respiratory tract carcinogenesis models presently in use, promotion may not be an important factor. If this interpretation is correct, then this may point to a serious shortcoming of the

Table 3 Effect of RA on the incidence of squamous cell carcinomas in rats[a]

	Effect at carcinogen dose of								
	10 mg		5 mg		2.5 mg		1.3 mg		Combined
RA dose (μg/wk)	Percentage of TBA[b]	MST[c] (wk)	Percentage of TBA	MST (wk)	Percentage of TBA	MST (wk)	Percentage of TBA	MST (wk)	percentage of TBA[d]
1744.0	66	77	20	104	9	110	10	111	24
174.0	40	85	21	100	10	105	0	112	16
17.4	93	70	65	82	27	101	23	103	48

[a]Each of the 12 subgroups consist of 15–22 "effective" animals (233 rats total). MCA was administered intratracheally; the RA by gavage (2 doses/wk) [from (38)].

[b]TBA = tumor-bearing animals. All tumors were invasive squamous carcinomas.

[c]MST = mean survival time.

[d]For each RA level, the % TBAs of all carcinogen dose groups were combined; there were 77–79 rats per RA level.

existing lung cancer models, since promotion factors are almost certain to play a decisive role in the pathogenesis of lung cancer in humans.

A question still to be answered is, What is the mechanism by which vitamin A deficiency increases the risk of developing cancer in respiratory tract epithelium? Studies of Genta et al (41) indicate that covalent binding of the PAH B(a)P to DNA of tracheal epithelium is increased in vitamin A deficiency. Conversely, Hill & Shih (42) have suggested that retinol and some of its derivatives inhibit the formation of epoxides during the metabolism of B(a)P. Thus one possible mechanism by which vitamin A deficiency might enhance carcinogenesis is by way of increased metabolic activation and/or DNA binding of carcinogen. However, as we have previously shown (36, 37), vitamin A deficiency also appears to enhance the carcinogenic processes during the "postinitiation phase," i.e. after the crucial interactions of the ultimate carcinogen with the molecular target have occurred.

Other observations made in the respiratory tract of vitamin A–deficient animals may also be relevant to this discussion. It has been known for many years that squamous metaplasias develop in the respiratory tract of vitamin A–deficient animals (33, 43, 44). More recently, it was shown that changes in cell type distribution and cell proliferation occur in respiratory tract epithelium of deficient animals. In the tracheal epithelium of such animals, the relative number of basal cells is increased and the relative number of ciliated cells is decreased (45). Labeling index and mitotic index are significantly increased above normal levels (46). Such changes in epithelial proliferation and differentiation may indeed be important factors in the induction and development of neoplastic cell populations in vitamin A–deficient mucociliary epithelium.

THE ROLE OF RESPIRATORY TRACT INFECTIONS IN THE PATHOGENESIS OF LUNG CANCER

For decades clinical investigators and laboratory researchers have considered the possibility that certain respiratory infections might predispose in some ways to the development of lung cancer. However, both the clinical data and the experimental findings are for the most part rather ambiguous. Infections and/or inflammatory conditions that have been studied in this connection are pneumonitis (particularly influenza), tuberculosis, and chronic bronchitis [for discussion see (47, 48)]. Because of the conflicting results of numerous clinical and pathological case studies, it is difficult to come to any firm conclusion regarding the role of inflammatory processes in the development of bronchogenic carcinoma and the so-called scar-cancer. This uncertainty is reflected in the ambiguity with which the subject is discussed in several reviews on lung cancer etiology. Two fairly recent epidemiological studies deny completely the existence of any compelling

evidence for or against an association of lung cancer with pulmonary infection, or for that matter with any other "precursor disease." It appears to us that one of the reasons for this confusion is that tobacco smoke, the major etiological factor in pulmonary carcinogenesis, also interferes with the pulmonary defense systems against infectious agents, making it difficult to single out the effect of respiratory tract infection in the pathogenesis of human bronchogenic carcinoma. [For discussion and review of the relevant literature see (49).] Several years ago, a series of investigations was conducted to examine various aspects of this complex problem (49). In one of these studies a nitrosamine, which upon ingestion causes squamous carcinomas in the lungs of rats, was administered to germfree rats, specific-pathogen-free (SPF) rats, and rats suffering from confirmed chronic murine pneumonia (50). The tumor incidence data showed that the infected rats had the highest lung cancer incidence (Table 4). The mechanism of this enhancement of the lung tumor response in the animals with respiratory tract infection is not clear. Subsequent studies with different carcinogens and different infectious agents suggested several possible alternative mechanisms such as alteration of local immune competence (51) and of pulmonary carcinogen metabolism (52). Other mechanisms that might possibly result in an enhanced lung tumor response if the carcinogen is deposited in the lungs by inhalation exposure are disturbances of pulmonary clearance. This possibility was explored in studies in which the clearance of radioactive particles was examined during and after pulmonary infection with influenza viruses (53–55). As is evident from the data summarized in Figures 2 and 3, infection of the lower respiratory tract can cause drastic and longlasting defects in the ability of the respiratory tract system to clear itself of particles. In the case of repeated exposures to carcinogenic agents, this is likely to result in increased residence time as well as accumulation of carcinogenic particulates in the lungs. Morphological studies showed that the labeled

Table 4 Tumor response to *N*-nitrosoheptamethyleneimine in germfree and SPF rats and rats with chronic murine pneumonia[a]

Group	Effective number of rats[b]	Number of rats with lung tumors[c]	Number of rats with esophageal papillomas[c]
Carcinogen-treated males			
Germfree	12 (12)	2 (17)	12 (100)
SPF	19 (21)	7 (37)	14 (74)
Chronic murine pneumonia	29 (30)	24 (83)	22 (76)

[a] Modified from (50).

[b] Only animals surviving the entire carcinogen treatment course were included in this study; numbers in parentheses indicate the number of rats at start of experiment.

[c] Numbers in parentheses indicate the percentage of tumor-bearing rats per group.

particles were concentrated in areas of scarring remaining from the infection. This increased local concentration of retained material might be an additional factor in the induction of cancers in the affected areas

However, not all experimental data support the concept that respiratory tract infections necessarily promote the development of respiratory tract cancers. Considering the complexity of the organ system involved, this is not really surprising. The route of entry and type of carcinogen, the site of tumor development, and the characteristics of the infection all are likely to play an important role in this interaction of infection and carcinogenesis. We have shown, for example, that the pulmonary adenoma response in mice can be suppressed by infection with either Sendai virus or PR8-influenza virus (see Table 5). This is observed following exposure to various types of respiratory tract carcinogens and in several strains of mice [for discussion and literature see (49, 56)]. The mechanism of this inhibitory effect on the pulmonary adenoma development is probably complex. One factor may be the loss of target cells due to the viral infection; another may be changes in carcinogen metabolism (depending on the time relationship between carcinogen exposure and induction of the infection). New information concerning drug-metabolizing activity of the nonciliated cells of the terminal airways (57) and the origin of a significant proportion of chemically induced pulmonary adenomas from these cells (58) removes such explanations from the purely speculative, offering possibilities to reconcile some of the seemingly contradictory findings.

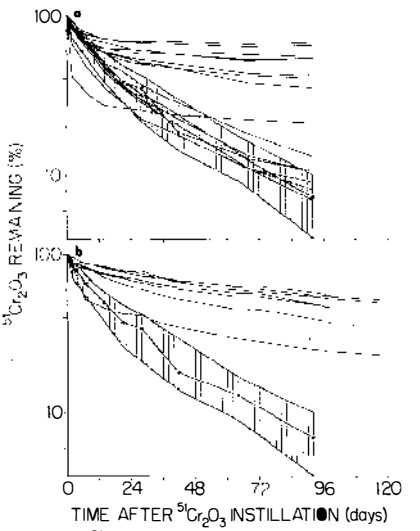

Figure 2 Pulmonary clearance of $^{51}Cr_2O_3$ by influenza virus–infected mice (solid lines). Data expressed as percentage of initially deposited $^{51}Cr_2O_3$. $^{51}Cr_2O_3$ administered (*a*) within 0.5 hr and (*b*) 7 days after virus inoculation. Uninfected controls (●) with high–low ranges (hatched). [From (54).]

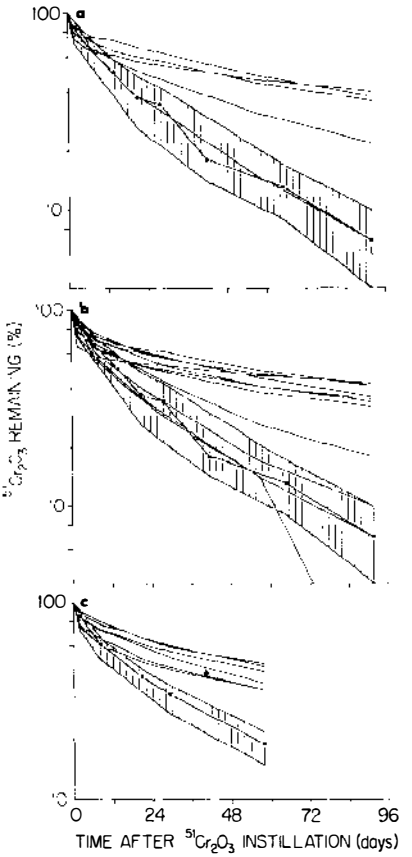

Figure 3 Pulmonary clearance of $^{51}Cr_2O_3$ by influenza virus–infected mice after acute stage of virus infection (solid lines). Data expressed as percentage of initially deposited $^{51}Cr_2O_3$ and corrected for physical decay. $^{51}Cr_2O_3$ administered at (*a*) 3 weeks, (*b*) 9 weeks, and (*c*) 56 weeks after virus inoculation. Uninfected controls (●) with high–low ranges (hatched). [From (54).]

TWO-STAGE CARCINOGENESIS IN RESPIRATORY TRACT EPITHELIUM

A great number of urban and industrial air pollutants have toxic effects on respiratory tract epithelium. Among these are particulates such as soot and coal particles, silica, asbestos, and sulfates—to name but a few—and reactive gases such as oxides of nitrogen, ozone, and aldehydes. At the concentrations encountered they may cause either short-lived irritation of airway mucosa or chronic inflammation, which, as in the case of occupational exposure to asbestos, may result in the development of pulmonary fibrosis. A question that remains largely unanswered is whether such air contami-

Table 5 Effect of viral infection on the urethan-induced pulmonary adenoma response in BALB/c mice[a]

Time of viral inoculation (days)	Type of virus	Mean number of tumors per mouse			
		Urethan and virus	Urethan only	Virus only	Vehicle only
−60	Sendai	11.1 ± 0.7	12.2 ± 0.7	0.2	0
	PR8	10.2 ± 0.8	11.7 ± 0.9	0	0
−21	Sendai	10.3 ± 1.0	11.1 ± 0.5	0.1	—
	PR8	11.9 ± 0.9	13.6 ± 1.2	0.1	—
−9	Sendai	6.9 ± 0.9	12.3 ± 1.3	—	—
	PR8	4.0 ± 0.9	11.2 ± 1.0	—	—
−3	Sendai	all died	—	—	—
	PR8 (5 mice)	4.2 ± 1.0	—	—	—
+1	Sendai	all died	—	—	—
	PR8 (4 mice)	3.5 ± 0.9	—	—	—
+30	Sendai	14.6 ± 0.9	13.2 ± 1.2	0	—
	PR8	13.1 ± 1.0	12.0 ± 1.7	0	—
+90	Sendai	11.2 ± 0.9	11.5 ± 0.8	0.1	0.1
	PR8	12.7 ± 1.3	12.4 ± 1.9	0.1	0

[a] Urethan was injected at 0 time; viral inoculation was performed before (−) or after (+) urethan injection. Data are based on 15 mice per group except on days −3 and +1. Mice were killed 4 months after urethan injection [from (56)].

nants are important factors in the pathogenesis of lung cancer. In the case of asbestos this question has clearly been answered in the affirmative (see below). Various hypothetical mechanisms have been invoked by which some of the gaseous and particulate air contaminants might act as "cocarcinogens" or "promoters." The term cocarcinogenesis is used here to denote a more than additive tumor response resulting from any combination of insults. Tumor promotion is a special case of cocarcinogenesis in which exposures to two agents (an initiator and a promoter, see below) occur sequentially. For a general discussion of the concepts of cocarcinogenesis and tumor promotion, the reader is referred to a recent review by Berenblum (59), and for discussion of pathogenetic mechanisms in respiratory tract carcinogenesis to a review by Nettesheim & Schreiber (60). The phenomena of cocarcinogenesis and tumor promotion have been most clearly established and most thoroughly studied in mouse skin. In classical two-stage carcinogenesis studies, a subtumorigenic dose of a carcinogen such as B(a)P is applied to the skin of mice. Subsequently, the "initiated" skin is exposed repeatedly and for many months to a promoting agent like croton oil or TPA (12-0-tetradecanoylphorbol-13-acetate) which by itself is non-tumorigenic. As a result of the initiation and promotion, multiple skin

tumors develop. Exposure to either agent alone induces no or only a few tumors. This two-stage model of carcinogenesis [for reviews see (61, 62)] has in recent years been extended to tumor induction systems in other organs and to other initiating and promoting agents (63).

In terms of the pathogenesis of bronchogenic carcinoma, two questions must be asked: Is there any evidence that two-stage carcinogenesis, in principle, occurs in respiratory tract mucosa? And what agents are present in our environment that might act as tumor promoters (or cocarcinogens)? To answer the first question, we conducted classical initiation-promotion experiments with either MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) or DMBA as initiator and TPA as promoting agent; instead of mouse skin the target tissue was rat tracheal mucosa. Our studies were carried out in two experimental systems: tracheal organ cultures (64, 65, 65a) and heterotopic tracheal transplants. The mucociliary epithelium of the trachea was first exposed to the initiating agent and subsequently to the promotor. The in vitro studies showed that repeated TPA exposures markedly increased the frequency of transformation of neoplastic epithelial cell lines and shortened the time required for neoplastic transformation to occur (Table 6). In the tracheal carcinogenesis studies carried out in vivo, the incidence of tracheal carcinomas induced by a brief exposure to DMBA was tripled following chronic exposure to TPA, and the tumor latency (Figure 4) was significantly shortened (65b). Thus, using classical initiating and promoting agents, we concluded that two-stage carcinogenesis can indeed occur in airway mucosa. Promotion of pulmonary adenomas induced in mice by urethan has previously been shown to occur following systemic administration of phorbol (66) and butylated hydroxytoluene (67). These studies have demonstrated that various epithelial tissues of the respiratory tract, once initiated, are susceptible to tumor promotion. Thus, the possibility has to be considered that promotion might indeed be an important pathogenetic mechanism in the induction of bronchogenic carcinoma. This has been

Table 6 Promotion of cultured tracheal epithelium by TPA[a]

MNNG (μg/ml)	TPA (μg/ml)	Time of appearance of morphologically altered cells (days)	Time to first malignant cell line (days)	Frequency of tumorigenic cell lines (%)
0	1.0	190	0	—
0.1	0.0	209	365	30
0.1	1.0	132	150	73

[a] Tracheal organ cultures were first exposed to MNNG and subsequently to TPA. Epithelial outgrowths developed from the exposed organ cultures; from these outgrowths, primary cultures and, where possible, cell lines were established. The tumorigenicity of cell lines was tested in isogenic, immunosuppressed hosts [modified from (65a)].

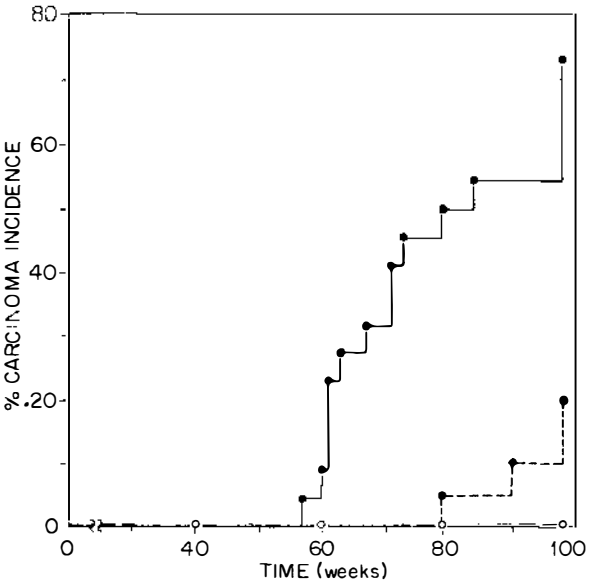

Figure 4 Tumor response in tracheal transplants exposed to DMBA and subsequently to TPA. ●——● , 4-week exposure to 188 μg DMBA followed by exposure to 100 μg TPA (22 tracheas); ●- - -● , 4-week exposure to 188 μg DMBA followed by exposure to blank beeswax pellets (20 tracheas); ○- - -○ , 4-wk exposure to beeswax followed by exposure to 100 μg TPA (20 tracheas). (From Topping, D. C., Nettesheim, P. Promotion-like enhancement of tracheal carcinogenesis in rats by TPA. To be submitted for publication.)

previously suggested because of the presence of promoters of mouse skin carcinogenesis in tobacco smoke condensate (e.g. 68, 69). However, direct evidence for tumor promotion in the respiratory tract was missing until recently. A most important question that now has to be answered is: Are there chemicals among the various air pollutants (urban or occupational) that have tumor-promoting activity for respiratory tract epithelium? Obviously, TPA is not a candidate except in the environment of the laboratory. The studies on tobacco smoke cocarcinogens (see above) can provide important leads in this regard. Also to be considered are "systemic" promoters such as butylated hydroxytoluence (see above).

COCARCINOGENIC EFFECTS OF CHRYSOTILE ASBESTOS

It is well established that inhaled asbestos fibers can induce two fatal diseases of the respiratory tract: asbestosis and pleural mesothelioma. Epidemiological data also suggest that asbestos inhalation markedly increases the incidence of bronchogenic carcinoma in smokers (5, 70).

Whether asbestos itself is an effective carcinogen for the mucosa of the conducting airways is not clear, however. In inhalation studies with animals exposed to various types of asbestos, mostly peripheral lung tumors seem to have been induced (71–73). Studies carried out in our laboratory addressed two main problems: (*a*) Is chrysotile asbestos a carcinogen for the mucosa of conducting airways? (*b*) Does asbestos promote the tumor formation in respiratory tract mucosa previously exposed to a carcinogen (initiation-promotion)? The tracheal transplant system was used for these studies (74) since it is possible in this system to keep the asbestos fibers in prolonged contact with the tracheal mucosa (see Figure 5). First, the carcinogenicity of asbestos was established in a tumorigenesis study in vivo. We found a low incidence of squamous carcinomas (5%) induced by 2 mg of chrysotile asbestos. Because carcinomas have never been observed in untreated or vehicle-treated transplants, we concluded that asbestos must be considered a weak but definite carcinogen for the epithelium of the conducting airways (75). In a subsequent study, tracheal mucosa was first exposed to various doses of carcinogenic PAH and later to asbestos. The results

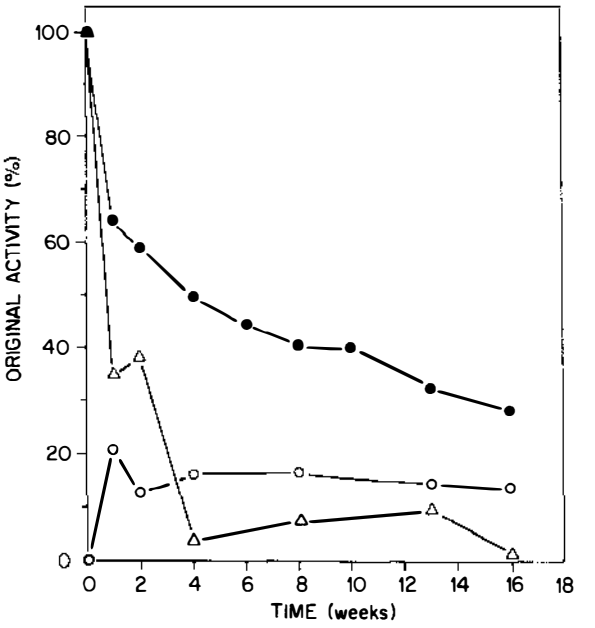

Figure 5 Location of radioactivity following implantation of 280 μg of neutron-activated chrysotile A into established tracheal grafts. Amounts expressed as percentage of original radioactivity corrected for decay. ● , whole body; ○ , tracheal wall; △ , tracheal lumen. This approach underestimates the amounts of asbestos persisting because of leaching of the metal ions from the fibers. [From (75)].

(Table 7) showed that asbestos markedly increases the tumor incidence in tracheas previously exposed to low doses of another carcinogen (74). This effect is reminiscent of tumor promotion. It was not seen at a DMBA dose which is by itself carcinogenic (100 μg DMBA only, inducing 18% tracheal carcinomas). But it did occur at the next two lower DMBA dose levels (50 and 25 μg DMBA), which are subcarcinogenic but apparently doses at which initiation takes place. The subsequent exposure to chrysotile forced the expression of the initiated cells and their progression to neoplasia. The lowest DMBA dose (12.5 μg) obviously did not initiate enough cells. The asbestos dose used in this two-stage experiment was one tenth the dose necessary to induce a low tumor incidence by itself. These data suggest that chrysotile asbestos may have a promotion-like effect. This may be related to the hyperplastic and metaplastic effects of asbestos for tracheal epithelium noted in previous studies (75, 76). That asbestos acts as a cocarcinogen when administered simultaneously with carcinogenic PAHs has been described previously (77, 78). This has been attributed to the increased retention and penetration of the PAH adsorbed to the asbestos fibers (see also 79). The two-stage carcinogenesis studies, however, indicate that the cocarcinogenic activity of asbestos has yet another component, namely a promotion-like effect.

THE ROLE OF SO-CALLED INERT PARTICLES IN RESPIRATORY TRACT CARCINOGENESIS

The role of a variety of "carrier" particles as cofactors in respiratory tract carcinogenesis has been the subject of many studies (80–83). This topic was

Table 7 Effect of asbestos on carcinoma incidence in tracheas preexposed to DMBA[a]

Exposure (μg)[b]		Number of carcinomas	Percentage of tracheas with metaplastic-dysplastic lesions[d]
DMBA	Asbestos	No. of tracheas (% in parentheses)[c]	
100	200	9/40 (23)	28
100	—	7/38 (18)	16
50	200	6/40 (15)	13
50	—	0/34 (0)	12
25	200	9/40 (23)	10
25	—	0/38 (0)	10
12.5	200	1/40 (3)	8
12.5	—	1/36 (3)	0
—	200	0/40 (0)	0

[a] From (74).
[b] The DMBA dose was delivered within 4 wk; the tracheas were then exposed to asbestos.
[c] Includes invasive carcinomas and carcinomas in situ.
[d] Includes metaplasias with different degrees of atypia (exclusive of all carcinomas).

extensively discussed during the Symposium on Experimental Lung Cancer held in Seattle, Washington, in June 1974 (84). The underlying hypothesis is that carcinogens generated during various types of combustion processes will adsorb to airborne particles, and that this might markedly affect their deposition, retention, translocation, and clearance pattern once they enter the respiratory tract and thus decisively influence their carcinogenic efficacy. In many of these studies metal oxide particles, particularly ferric oxide particles, were used. A carcinogen such as B(a)P was either added to the particle suspension or adsorbed onto the particle surface. When the particle-carcinogen mixtures were intratracheally injected into animals, generally two observations were made: (*a*) B(a)P adsorbed to particles persisted in the lungs longer than B(a)P administered alone (85, 86); (*b*) the tumor response resulting from intratracheal administration of B(a)P-particle suspensions was much higher than that following the intratracheal injection of B(a)P alone (80, 85). Similar findings were reported with a variety of carcinogen-particle combinations (87). This suggested that one major mechanism of the enhancement of respiratory tract carcinogenesis by noncarcinogenic particles might be the increased retention of the carcinogen. However, data were reported from several laboratories (87, 88) (Table 8) indicating that this interaction was perhaps more complex than initially anticipated: particle-carcinogen preparations with the slowest carcinogen clearance rates (carcinogen clearance was inversely related to carrier particle size) were not always the most carcinogenic.

Because of the possible importance of the particle-carcinogen interaction in the pathogenesis of lung cancer, we studied this problem further, with the aim of measuring not only the retardation of carcinogen clearance by carrier particles, but in addition the rate of dissociation in the lungs of the carcinogen from the particles (89). We found that the rate of elution of B(a)P from the carbon particles used in this study depends on the size of

Table 8 Effect of particle size on clearance of B(a)P-coated carbon particles and tumor incidence[a]

Particle size (μ)	Time required for removal of 50% inoculated B(a)P	Time required for removal of 95% inoculated B(a)P	Percentage of tumor-bearing animals
15–30	60 hr	503 days	36
5–10	51 hr	496 days	54
2–5	24 hr	44 days	37
0.5–1	9.6 hr	24 days	70

[a] For clearance studies, animals were injected intratracheally with 0.2 ml of gel-saline containing 0.2% B(a)P-carbon particles. For tumorigenesis study, animals were injected intratracheally with the same mixture twice weekly for 25 wk [modified from (87, 88)].

the particles (Figure 6). Both types of particles markedly increased retention of B(a)P, but little if any B(a)P dissociated from the large particles while they were retained in the lungs. It was this latter type of particle which, with B(a)P adsorbed to its surface, had been shown to have relatively low carcinogenic activity (87). This finding points to what appears to be an important principle in the particle-carcinogen interaction: The enhancing effect of carrier particles seems to be closely tied not only to the clearance rate (of particles and carcinogen) but also to the elution rate of the carcinogen from the particle. If the elution rate is too slow, then substantial amounts of carcinogen are not released from the particles before they are cleared; the release of the carcinogen from the particle does seem to be essential for the carcinogen to become effective. Recent attempts to study the particle-mediated carcinogen transport at the subcellular level suggest that carrier particles might facilitate the intracellular uptake of B(a)P and the transfer of the carcinogen to membranes (79, 90). It should not be forgotten, however, that "inert" particles may also be cocarcinogenic via a completely different mechanism. We showed that inhaled iron oxide particles (not carrying any carcinogen) enhance the lung tumor response in-

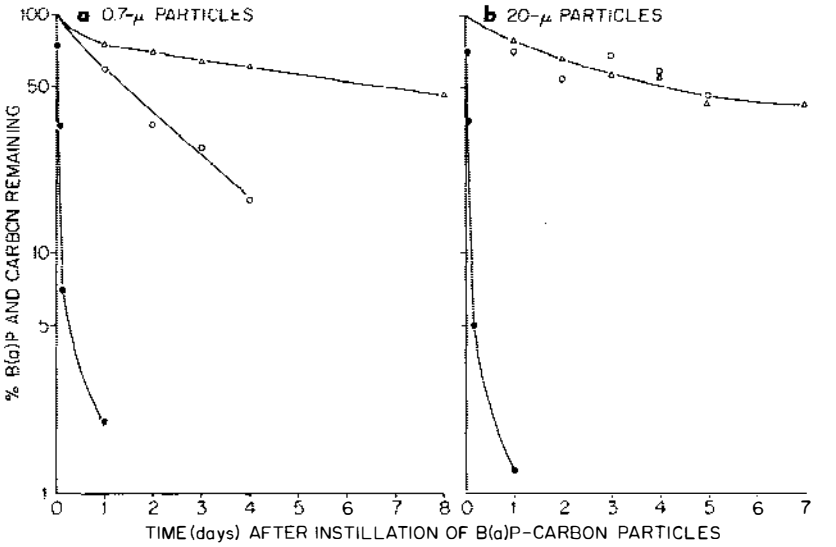

Figure 6 Elimination of B(a)P from the respiratory tracts of mice. The mice were injected intratracheally with B(a)P crystals alone or with B(a)P crystals adsorbed to (*a*) 0.7- or (b) 20-μ carbon particles. Data are expressed as percentage of the initial lung burden. ●, B(a)P crystals alone; ○, B(a)P eluted from carbon particles; △, carbon particles. Carbon particles were tagged with ^{103}Ru and mixed with B(a)P-coated carbon particles. Analysis of B(a)P content of the lungs was by chemical extraction and spectofluorimetric analysis. [From (89).]

duced by a systemically administered nitrosamine (91, 92). This cocarcinogenic effect is suspected to be mediated by the irritant effects of iron oxide (promotion?). Such a conclusion is supported by a number of respiratory tract tumor induction studies (93) and by various in vitro studies which have shown the irritant effects of particles such as carbon, iron oxide, and asbestos for mucociliary epithelium (76, 78, 94). However, some of the controversies surrounding this topic remain unsettled [for discussion and literature see (95)].

NICKEL SUBSULFIDE AS A POSSIBLE COCARCINOGEN IN OCCUPATIONAL LUNG CANCER

Epidemiological studies clearly indicate that nickel workers are at an increased risk of developing respiratory tract cancer (96, 97). However, what is not known is whether in these occupational groups nickel is the primary etiological agent, or whether it acts perhaps synergistically with other air contaminants. Studies in experimental animals have established that a variety of nickel compounds are carcinogenic, but with a few exceptions (98, 99) most do not involve respiratory tract tissue. A problem encountered in the determinaton of the carcinogenic potency of nickel toward the respiratory tracts of experimental animals is that the concentration which can be achieved in the lungs by inhalation exposure is considerably less than that achieved locally when nickel is injected intramuscularly or intrarenally. To overcome this problem, we implanted nickel subsulfide (Ni_3S_2) into tracheal transplants (100). In this way, we were able to keep a moderate amount of nickel in intimate contact with the respiratory mucosa for extended periods of time. Analysis of transplants for residual nickel indicated that we were able to achieve a continuous exposure for 7–9 months. We found that high doses of Ni_3S_2 (3 mg/tracheal transplant) caused considerable erosion and submucosal inflammation and a high percentage of fibrosarcomas and myosarcomas, but only 1 squamous carcinoma occurred in 64 transplants (Table 9). However, when 1 mg of Ni_3S_2 was administered, fewer sarcomas

Table 9 Tumor induction study with Ni_3S_2[a]

Amount of Ni_3S_2 per pellet (mg)	Number of effective tracheas	Number of tracheas with	
		Sarcoma	Carcinoma
1	60	6	6
3	64	44	1

[a] At 20 months all surviving animals were killed [modified from (100)].

developed, but the carcinoma incidence was 10%. These findings indicate that Ni_3S_2 is indeed a carcinogen for the mucosa of the conducting airways, but that the airway epithelium is relatively resistant. Subsequently, we carried out further experiments similar to those described for asbestos except that the Ni_3S_2 in this case was incorporated into beeswax pellets, which allow only minute amounts of nickel to be released over many months (unpublished observations). The tracheas were first exposed to the carcinogenic PAH and subsequently to Ni_3S_2 pellets. As is shown in Figure 7, the tracheal carcinoma incidence was more than doubled in the group sequentially exposed to DMBA and Ni_3S_2 to cause neoplasias in tracheas not previously exposed to the PAH. The tracheal tumor response summarized in Table 9 was induced by free nickel crystals released from gelatin

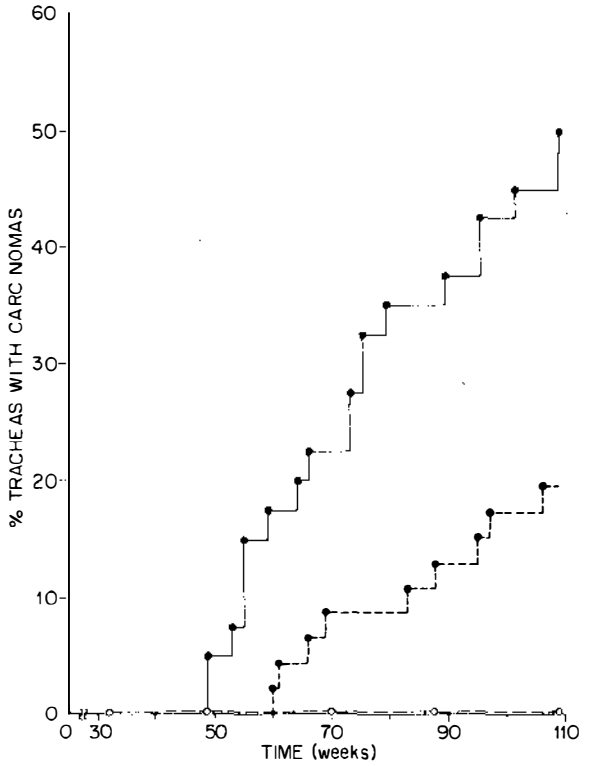

Figure 7 Tumor response in tracheal transplants preexposed to DMBA and subsequently exposed to Ni_3S_2. Tracheas were first exposed to DMBA; 165 μg of the carcinogen was delivered during the period of 4 weeks from intraluminal beeswax pellets. Thereafter the DMBA pellets were replaced by pellets containing 1 mg of Ni_3S_2, which remained in the grafts for the rest of the study. ●——● , 165 μg DMBA followed by 1 mg Ni_3S_2; ● - - - ● , 165 μg DMBA followed by beeswax pellets; O- - - O , 1 mg Ni_3S_2. 40–45 tracheas per group.

pellets which dissolve within minutes after insertion into the tracheal lumina. Our findings, therefore, lend support to the notion that, besides nickel, other carcinogens may also be involved in the causation of respiratory tract cancers in nickel workers (101). Also in support of this theory is the finding by other investigators that simultaneous administration of hydrocarbons and nickel compounds results in an increase of the tumor response (102, 103). The importance of Ni_3S_2 in the pathogenesis of lung cancer may lie mostly in its action as a syncarcinogen or cocarcinogen.

STUDIES ON BENZO(e)PYRENE [B(e)P] AS A COCARCINOGEN

A great variety of PAHs are produced during incomplete combustion of organic matter (104). Among these are classic carcinogens such as B(a)P and a number of compounds regarded as only weakly carcinogenic or noncarcinogenic. B(e)P belongs to this latter group (105). While much has been learned about the carcinogenicity of individual PAHs, little is known about their combined effects. The carcinogenic potency of some combustion products, e.g. tobacco smoke (condensate), has led to the speculation that it may stem from the synergistic interaction of individual components. This view is supported by recent evidence indicating that B(e)P acts as a cocarinogen when applied together with its carcinogenic analogue B(a)P to the skin of mice (Table 10) (106). We recently conducted a survey of the toxicity of various PAHs for tracheal mucosa using morphological end points: B(e)P caused the least amount of toxic alteration (107). Stimulated by these findings and the observations made by Van Duuren (105, 106), we

Table 10 Cocarcinogenicity of B(e)P on mouse skin[a]

Coupound and dose	Number of mice	Mice with papillomas: Total papillomas	Mice with carcinomas
1 mg B(e)P[b]	20	0/0	0
15 μg B(e)P 3 × weekly[c]	50	0/0	0
5 μg B(a)P 3 × weekly[c]	50	16/26	10
5 μg B(a)P + 5 μg B(e)P 3 × weekly[c]	50	24/33	9
5 μg B(a)P + 15 μg B(e)P 3 × weekly[c]	50	33/79	27

[a] Modified from (105, 106).
[b] Applied once only in 0.1 ml acetone.
[c] Applied in 0.1 ml acetone each time.

decided to determine whether the simultaneous exposure of tracheal mucosa to B(a)P and B(e)P would produce effects similar to those described for mouse skin. Tracheal transplants were exposed to pellets containing both B(a)P and B(e)P at two different ratios (107a). As can be seen in Figure 8, the two hydrocarbons are continuously released for 6–8 months. The tumor responses are summarized in Figure 9. There was no indication of a cocarcinogenic effect of B(e)P. Actually, when tracheas were exposed to equal amounts of the two PAHs, the tumor response to B(a)P was suppressed. In addition to the carcinomas, peritracheal sarcomas were observed; these developed around the polyethylene tubing which is transplanted together with the tracheal grafts (to prevent the grafts from curling up in the graft site). The background incidence of these sarcomas in controls has been 10–15%. The incidences revealed in the present study were 10, 10, and 15% in the groups receiving 0.5 mg B(a)P, 1.0 mg B(a)P, and 1.0 mg B(e)P, respectively. However, in the two groups receiving B(a)P plus B(e)P the sarcoma incidences were 27 and 28% respectively, which is significantly greater ($P \leq 0.02$) than that in control grafts or in grafts receiving B(e)P or B(a)P alone. That inhibition of metabolism (108) and of carcinogenesis (109) of one PAH by another can occur was recently shown in mouse epidermal cell cultures and in a mouse skin carcinogenesis assay, respectively. What our studies show in addition is that inhibition can occur in one target tissue (tracheal epithelium) and enhancement in another, topographically in close proximity (peritracheal connective tissue). This illustrates the complexities involved in the search for substances with cocarcinogenic activity and suggests that generalizations concerning presence or

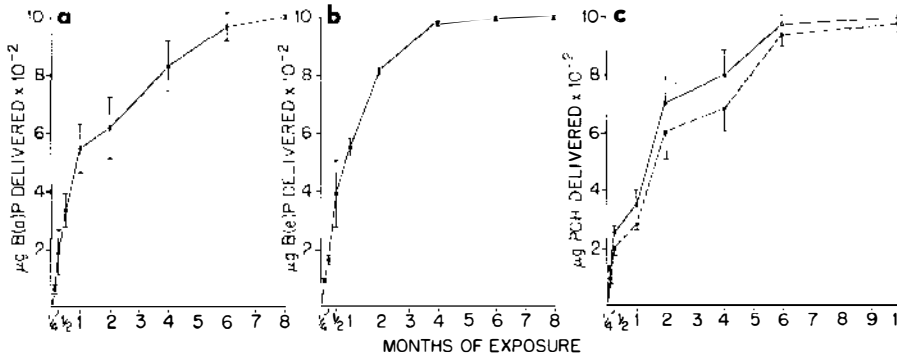

Figure 8 Delivery of B(a)P and B(e)P to tracheal grafts from intraluminal beeswax pellets. Points represent the amount of PAH released to the grafts. Each point represents the mean ± SD of 6 pellets. The original amount of each chemical was 1000 μg. (*a*) Release of B(a)P to grafts; (*b*) release of B(e)P to grafts; (*c*) simultaneous release of B(a)P and B(e)P (1000 μg each) to tracheal grafts. ●——● , B(a)P; ● - - - ● , B(e)P. [From (107a).]

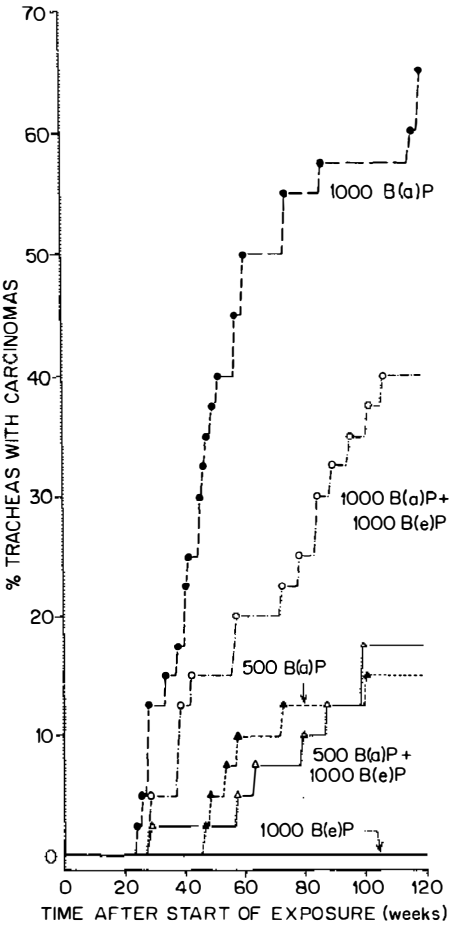

Figure 9 Carcinoma incidence in tracheal grafts implanted with beeswax pellets containing B(a)P and/or B(e)P. ● , 1000 μg B(a)P; ○ , 1000 μg B(a)P + 1000 μg B(e)P; ▲ , 500 μg B(a)P; △ , 500 μg B(a)P + 1000 μg B(e)P; — , 1000 μg B(e)P. Numbers refer to amounts of hydrocarbon initially in the pellets. 40 tracheas per group. [From (107a).]

absence of cocarcinogenic activities of any given chemical may not be warranted.

THE ROLE OF IRRITANT GASES AND VAPORS AS COCARCINOGENS IN THE RESPIRATORY TRACT

During incomplete combustion of organic materials, a variety of reactive gases and vapors are generated that have irritant and cytotoxic effects on

the airways. The concern is that such air contaminants, which are common in highly industrialized and densely populated areas (auto exhaust), might not only act as inflammatory agents on the conducting airways and the pulmonary parenchyma, but might also act as cocarcinogens or promoting agents. The irritation of airway epithelium with the resulting increase in cellular proliferation might render the epithelium more susceptible to carcinogenic air contaminants or accelerate or increase the expression of malignant transformations. Several gaseous contaminants of ambient air have been studied in this regard such as sulfur dioxide, nitrogen dioxide, and the aldehydes formaldehyde, acrolein, acetaldehyde, and furfural. Most of these studies have failed so far to provide any definitive evidence for cocarcinogenic effects [for discussion and literature see also (60)]. The most notable exception, however, is the study of Laskin, Kuschner & Drew (110), in which rats were exposed to B(a)P aerosols and 10 ppm of sulfur dioxide. The animals exposed to B(a)P alone developed a 3% lung tumor incidence, while animals exposed to both agents developed a 20% tumor incidence. A large series of cocarcinogenesis studies was carried out several years ago with over 1000 hamsters (P. Nettesheim, unpublished) to determine whether nitrogen dioxide and the aldehydes formaldehyde and acrolein were cocarcinogenic. The hamsters were exposed to B(a)P by repeated intratracheal instillations either before, during, or after the chronic inhalation exposure to the reactive gases (10 ppm NO_2; 10, 20, or 50 ppm formaldehyde; 10 or 15 ppm acrolein; 1 or 5 days/week for 4 months or for life). These studies produced no evidence for either the carcinogenicity or the cocarcinogenicity of these three reactive gases in hamsters. In similar studies with 4 ppm acrolein (111) and 1500 ppm acetaldehyde (112) in which hamsters were the test animals, no cocarcinogenic activity was detected [inhalation exposure: 7 hr/day, 7 days/week, 52 weeks; carcinogen: B(a)P, intratracheally injected, and diethyl nitrosamine, administered subcutaneously; diethyl nitrosamine induces tumors in various parts of the respiratory tract upon subcutaneous injection]. In earlier studies in which B(a)P and furfural were simultaneously administered by intratracheal instillation, an increased incidence of peritracheal sarcomas was observed in the combined exposure group (33% vs 3%), but the incidence in respiratory tract carcinomas was not significantly increased by addition of furfural to the B(a)P (113). The biological significance of the increase in sarcomas due to the addition of furfural is somewhat uncertain. It might be an indication of the toxicity of the mixture to the tracheobronchial epithelium, allowing more of the PAH to reach the submucosa. Since it was recently found that inhalation of formaldehyde at concentrations of 15 ppm causes nasal carcinomas in rats (113a), it is important to stress that there is presently no

evidence in any of the studies discussed above that formaldehyde or related aldehydes are carcinogenic in hamsters. That rats are particularly susceptible to the toxic effects of aldehydes was noticed earlier by Feron et al (114) in subchronic inhalation studies in which hamsters, rats, and rabbits were exposed to acetaldehyde at concentrations ranging from 0.4–4.9 ppm. In all we have to conclude that, with the exception of SO_2, there is at present little experimental evidence to indicate that gaseous air pollutants increase the risk of developing lung cancer. But it is quite obvious that considerably more experimental work is needed in this area.

SUMMARY AND CONCLUSION

In the prevention of lung cancer, obviously the first and foremost objective is to identify the source of the carcinogenic contaminant and to reduce or prevent exposure. However, to develop sound and effective environmental health measures or industrial hygiene programs, one must be aware of and understand the different risk factors involved. The purpose of our presentation has been to exemplify, with some selected laboratory studies, what has been learned during the last decade about the multicomponent etiology of lung cancer. It seems that it is rarely possible to link the induction of this disease, or that of most other neoplastic diseases, to exposure to a single chemical agent. To determine the relative importance of the main etiological or pathogenetic factors is, therefore, essential to an understanding of the principles involved in induction of bronchogenic carcinoma.

The major interaction of agents or factors may lie *within* a given environmental or occupational setting. For example, it might be the combination of exposures to agents such as carcinogenic PAHs, arsenic, and particulates which is responsible for the high lung cancer risk in certain smelter workers. The danger of such combined exposures might be drastically reduced if one could eliminate just one of the components involved through modification of the industrial process or through industrial hygiene measures. In other instances it appears to be the interaction of an occupational with a nonoccupational exposure that results in a high lung cancer risk. The most convincing example of this kind appears to be the high incidence of bronchogenic carcinoma in smoking asbestos workers. Finally, certain host factors (e.g. age, immune status), nutritional factors, or preexisting diseases may predispose individuals and render them highly susceptible to carcinogenic agents in the environment. Still very little information exists to date about the genetic basis of most cancers, including lung cancer. The laboratory data clearly show a marked strain dependency (genetic basis) of susceptibility to tumor induction (e.g. 9, 115, 116) and begin to provide some insight into

the biochemical basis of this long-known fact. It remains to be established whether the large differences between individuals in carcinogen metabolism and DNA binding of bronchial mucosa (Figure 10), which were recently demonstrated (117), are linked to an increased lung cancer risk and whether they have a genetic basis.

Laboratory studies such as the ones discussed here can rarely, if ever, prove the importance of specific factors in the causation of lung cancer in specific urban or occupational environments. However, by uncovering important principles of interactions in the pathogenesis of lung cancer, they can alert us to situations and conditions that might be particularly hazardous. Together with epidemiological investigations, such mechanistic studies contribute important information needed to reduce the lung cancer risks in our motorized, industrialized, and tobacco-habituated society.

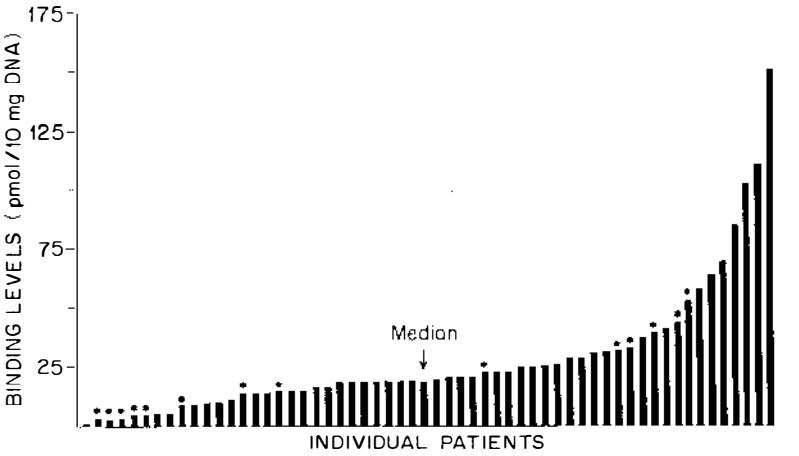

Figure 10 Binding of B(a)P to DNA varies markedly among individuals. The asterisks denote patients without lung cancer. [From (117).]

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