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Association of Adrenal Pheochromocytoma and Lung Pathology in Inhalation Studies with Particulate Compounds in the Male F344 Rat—The National Toxicology Program Experience

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ABSTRACT

Systemic hypoxemia, occurring in space-occupying lung pathologies such as inflammation and neoplasms, reduces the gas exchange area and stimulates catecholamine secretion from the adrenal medulla where chronic endocrine hyperactivity may lead to hyperplasia and neoplasia. We investigated the possible correlation between nonneoplastic chronic pulmonary lesions and adrenal pheochromocytoma in 9 recent, NTP, 2-year particulate inhalation studies in male F344 rats. Re-evaluation for chronic active inflammation, interstitial fibrosis, alveolar epithelial hyperplasia, squamous metaplasia, proteinosis, and histiocytosis revealed significant associations of pheochromocytoma only with the severity of inflammation and fibrosis. Nickel oxide, cobalt sulfate, indium phosphide, talc, and nickel subsulfide studies showed chemical-related incidences of adrenal pheochromocytoma and significant ($p < 0.01$) associations with inflammation and fibrosis. Gallium arsenide, vanadium pentoxide, molybdenum trioxide, and nickel sulfate hexahydrate studies revealed an increased incidence and/or severity of nonneoplastic lung lesions, but no increased incidence of pheochromocytoma. Although gallium arsenide and molybdenum trioxide showed no dose-related increase in pheochromocytoma, a significant ($p < 0.01$) correlation of the latter with the severity of fibrosis and inflammation occurred. In the vanadium pentoxide and nickel sulfate hexahydrate studies, no relationship between nonneoplastic lung lesions and pheochromocytoma was manifested. Our investigation assessed the strength of these various associations and supports the possible roles of 2 chronic pulmonary lesions—fibrosis and inflammation—and hypoxemia in the induction of pheochromocytoma in the F344 male rat.

Keywords. Pheochromocytoma; adrenal medulla; lung; fibrosis; inflammation; F344 rat; inhalation; particulate.

INTRODUCTION

In recent years, the NTP (National Toxicology Program) has performed several 2-year inhalation studies in rats, testing particulate compounds such as nickel subsulfide, nickel oxide, talc, indium phosphide, cobalt sulfate, vanadium pentoxide, molybdenum trioxide, nickel sulfate, and gallium arsenide. Results of these studies indicated the occurrence of variably extensive pulmonary inflammatory lesions, alveolar-bronchiolar tumors and squamous cell carcinomas, and significantly increased incidences of adrenal medullary hyperplasias and pheochromocytomas in males and females with the first 5 compounds. Carcinogenic effects of each chemical on the lung or adrenal medulla have been summarized in Table 1 from the NTP technical reports (28–35).

Hyperplasia and neoplasia arise frequently in the rat adrenal medulla, either spontaneously in the course of aging or in response to a wide variety of xenobiotic agents, but proliferative lesions of the adrenal are rarely observed in humans and other animal species (37). In F344/N rats, the rate of spontaneous pheochromocytoma is 6 times higher among males than females (mean rate 31.9% in males vs 5.1% in females) (13). Similarly, the incidence of pheochromocytoma among male SD rats is at least 3 times more frequent than among females (mean rate 20.9% in males vs 6.2% in females) (22). Multiple factors that affect the pathogenesis of pheochromocytoma in rats include genetic background, chronic high levels of growth hormone or prolactin associated with pituitary tumors, dietary factors, and stimulation of the autonomic nervous system (38). In most instances, the exogenous agents that induce adrenal medullary neoplasia do not cause DNA damage; thus, many of these agents may influence the carcinogenic response of the adrenal medulla through an indirect mechanism (41).

In response to various stimuli that increase the secretion of catecholamines (CA) from the adrenal medulla into the plasma, the rate of biosynthesis of CA in the adrenal gland rises to meet the increased demand (8, 19). Cells in mammalian tissues are primarily aerobic and highly dependent upon a continuous supply of oxygen. Inadequate oxygen delivery to tissues—hypoxemia—is followed by hyperventilation, which is mediated by the O_2-sensitive type I cells in the carotid body (36). This chemoreceptor releases dopamine in response to a reduction in arterial O_2-tension. The activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of CA such as dopamine, is enhanced in the carotid body (10) and adrenal gland (14) during hypoxemia. Czyzyk-Krzeska et al (7) reported that hypoxia enhances tyrosine hydroxylase gene expression in the rat carotid body and stimulates both the rate of tyrosine hydroxylase gene transcription and mRNA stability in pheochromocytoma PC12 cells.

Tyrosine hydroxylase catalyzes the formation of 3, 4-dihydroxyphenylalanine (DOPA) from L-tyrosine and oxygen (27). Systemic hypoxemia is one of the stimuli that

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TABLE 1.—Summary of incidence of nonneoplastic pulmonary lesions by re-evaluation of 9 inhalation studies of particulate compounds in male F344 rats; incidence of neoplastic lung lesions, pheochromocytoma, and hyperplasia in adrenal medulla as reported by the NTP.

Study/findings	Control	Low	Mid	High
Nickel subsulfide	0 mg/m^3	0.15 mg/m^3	—	1.0 mg/m^3
Lung				
Proteinosis	3/58 (1.7)**	49/58 (1.6)	—	57/58 (3.0)
Fibrosis, interstitium	1/58 (1.0)	56/58 (2.3)	—	52/58 (2.3)
Inflammation, chronic active	20/58 (1.3)	58/58 (2.6)	—	58/58 (2.6)
Alveolar epithelium hyperplasia	5/58 (1.6)	58/58 (2.4)	—	56/58 (2.4)
Metaplasia, squamous epithelium	0/58	58/58 (2.5)	—	56/58 (2.3)
Histiocytosis	7/58 (1.1)	58/58 (2.1)	—	58/58 (2.6)
Alveolar/bronchiolar adenoma	0/58	3/58	—	6/58
Alveolar/bronchiolar carcinoma	0/58	3/58	—	7/58
Adrenal medulla				
Pheochromocytoma*	14/58	30/57	—	42/58
Hyperplasia	26/58 (2.2)	22/57 (1.9)	—	11/58 (2.3)
Nickel oxide	0 mg/m^3	0.62 mg/m^3	1.25 mg/m^3	2.5 mg/m^3
Lung				
Proteinosis	0/59	1/58 (1.0)	1/58 (1.0)	53/57 (2.6)
Fibrosis, interstitium	9/59 (1.6)	46/58 (1.6)	52/58 (2.3)	56/57 (2.0)
Inflammation, chronic active	23/59 (1.5)	52/58 (1.8)	58/58 (2.5)	57/57 (2.5)
Alveolar epithelium hyperplasia	14/59 (1.5)	34/58 (1.5)	57/58 (1.7)	56/57 (1.9)
Metaplasia, squamous epithelium	4/59 (1.5)	46/58 (1.5)	58/58 (2.0)	56/57 (2.1)
Histiocytosis	6/59 (1.0)	53/58 (1.7)	54/58 (1.6)	57/57 (2.6)
Alveolar/bronchiolar adenoma	0/59	1/58	3/58	2/57
Alveolar/bronchiolar carcinoma	0/59	0/58	3/58	2/57
Squamous cell carcinoma	1/59	0/58	0/58	0/57
Adrenal medulla				
Pheochromocytoma*	27/59	24/57	29/58	35/57
Hyperplasia	25/59 (2.2)	27/57 (2.1)	27/58 (2.6)	25/57 (2.5)
Talc	0 mg/m^3	6 mg/m^3		18 mg/m^3
Lung				
Proteinosis	2/49 (3.0)	15/50 (1.0)	—	44/50 (2.3)
Fibrosis, interstitium	8/49 (1.0)	34/50 (1.2)	—	42/50 (2.1)
Inflammation, chronic active	16/49 (1.5)	50/50 (2.4)	—	50/50 (3.1)
Alveolar epithelium hyperplasia	7/49 (1.7)	35/50 (1.5)	—	44/50 (1.7)
Metaplasia, squamous epithelium	2/49 (1.5)	44/50 (1.7)	—	49/50 (2.3)
Histiocytosis	5/49 (1.4)	50/50 (2.5)	—	50/50 (2.8)
Alveolar/bronchiolar adenoma	0/49	1/50	—	1/50
Alveolar/bronchiolar carcinoma	0/49	0/50	—	1/50
Adrenal medulla				
Pheochromocytoma*	26/49	32/48	—	37/47
Hyperplasia	20/49 (2.7)	8/48 (2.3)	—	9/47 (3.3)
Cobalt sulfate	0 mg/m^3	0.3 mg/m^3	1.0 mg/m^3	3.0 mg/m^3
Lung				
Proteinosis	0/50	1/50 (1.0)	36/50 (2.1)	49/50 (3.4)
Fibrosis, interstitium	1/50 (1.0)	48/50 (1.6)	48/50 (2.6)	49/50 (2.4)
Inflammation, chronic active	2/50 (1.0)	49/50 (1.7)	49/50 (2.7)	49/50 (2.5)
Alveolar epithelium hyperplasia	12/50 (1.2)	42/50 (1.6)	48/50 (2.5)	48/50 (2.4)
Metaplasia, squamous epithelium	0/50	49/50 (1.5)	47/50 (2.6)	48/50 (2.4)
Histiocytosis	5/50 (1.0)	33/50 (1.3)	47/50 (1.7)	50/50 (1.9)
Alveolar/bronchiolar adenoma	1/50	4/50	1/50	6/50
Alveolar/bronchiolar carcinoma	0/50	0/50	3/50	1/50
Adrenal medulla				
Pheochromocytoma*	15/50	19/50	25/49	20/50
Hyperplasia	34/50 (2.0)	23/50 (2.5)	29/49 (2.1)	30/50 (2.1)
Indium phosphide	0 mg/m^3	0.03 mg/m^3	0.1 mg/m^3	0.3 mg/m^3
Lung				
Proteinosis	0/50	50/50 (2.4)	45/50 (1.8)	50/50 (3.3)
Fibrosis, interstitium	0/50	50/50 (2.6)	49/50 (2.7)	50/50 (3.0)
Inflammation, chronic active	11/50 (1.4)	50/50 (3.0)	50/50 (3.0)	50/50 (3.3)
Alveolar epithelium hyperplasia	14/50 (1.3)	49/50 (2.8)	50/50 (2.8)	50/50 (2.9)
Metaplasia, squamous epithelium	1/50 (1.0)	49/50 (2.8)	50/50 (3.3)	50/50 (2.9)
Histiocytosis	6/50 (1.0)	50/50 (2.8)	47/50 (1.9)	50/50 (2.7)
Alveolar/bronchiolar adenoma	6/50	13/50	27/50	30/50
Alveolar/bronchiolar carcinoma	1/50	10/50	8/50	16/50
Adrenal medulla				
Pheochromocytoma*	10/50	26/52	18/49	24/50
Hyperplasia	26/50 (2.2)	26/50 (2.4)	24/49 (2.4)	32/50 (2.3)
Vanadium pentoxide	0 mg/m^3	0.5 mg/m^3	1.0 mg/m^3	2.0 mg/m^3
Lung				
Proteinosis	0/50	0/50	0/50	0/50
Fibrosis, interstitium	1/50 (1.0)	3/50 (2.3)	3/50 (1.0)	30/50 (1.5)
Inflammation, chronic active	2/50 (1.0)	17/50 (1.4)	15/50 (1.1)	42/50 (1.5)
Alveolar epithelium hyperplasia	9/50 (1.6)	27/50 (1.5)	38/50 (1.4)	43/50 (2.8)
Metaplasia, squamous epithelium	0/50	1/50 (3.0)	0/50	1/50 (1.0)
Histiocytosis	2/50 (1.5)	28/50 (1.4)	45/50 (1.5)	46/50 (2.7)
Alveolar/bronchiolar adenoma	4/50	8/49	5/48	6/50
Alveolar/bronchiolar carcinoma	0/50	3/49	1/48	3/50

(Continued)

TABLE 1.—Summary of incidence of nonneoplastic pulmonary lesions by re-evaluation of 9 inhalation studies of particulate compounds in male F344 rats; incidence of neoplastic lung lesions, pheochromocytoma , and hyperplasia in adrenal medulla as reported by the NTP. *(Continued)*

Study/findings	Control	Low	Mid	High
Adrenal medulla				
Pheochromocytoma*	5/50	13/50	8/49	6/50
Hyperplasia	16/50 (2.1)	8/50 (2.4)	12/49 (3.0)	10/50 (2.7)
Nickel sulfate hexahydrate	0 mg/m^3	0.12 mg/m^3	0.25 mg/m^3	0.5 mg/m^3
Lung				
Proteinosis	0/54	0/53	16/53 (1.2)	39/53 (1.7)
Fibrosis, interstitium	3/54 (1.0)	5/53 (1.4)	35/53 (1.3)	44/53 (1.6)
Inflammation, chronic active	17/54 (1.2)	18/53 (1.3)	46/53 (1.6)	52/53 (2.1)
Alveolar epithelium hyperplasia	6/54 (1.5)	7/53 (1.4)	7/53 (1.0)	8/53 (1.3)
Metaplasia, squamous epithelium	0/54	1/53 (2.0)	14/53 (1.1)	22/53 (1.2)
Histiocytosis	15/54 (1.1)	16/53 (1.3)	52/53 (1.4)	53/53 (1.9)
Alveolar/bronchiolar adenoma	0/54	0/53	0/53	2/53
Alveolar/bronchiolar carcinoma	1/54	0/53	1/53	1/53
Squamous cell carcinoma	1/54	0/53	0/53	0/53
Adrenal medulla				
Pheochromocytoma*	16/54	19/53	13/53	12/53
Hyperplasia	28/54 (2.4)	20/53 (2.3)	18/53 (2.3)	26/53 (2.2)
Molybdenum trioxide	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Lung				
Proteinosis	0/50	0/50	0/50	0/50
Fibrosis, interstitium	5/50 (2.0)	4/50 (1.0)	7/50 (1.0)	45/50 (1.8)
Inflammation, chronic active	10/50 (1.8)	7/50 (1.6)	37/50 (1.2)	48/50 (2.1)
Alveolar epithelium hyperplasia	12/50 (1.8)	14/50 (1.6)	16/50 (1.2)	7/50 (1.3)
Metaplasia, squamous epithelium	2/50 (1.0)	0/50	2/50 (1.0)	27/50 (1.1)
Histiocytosis	11/50 (1.9)	13/50 (1.3)	35/50 (1.3)	46/50 (1.7)
Alveolar/bronchiolar adenoma	0/50	0/50	0/50	3/50
Alveolar/bronchiolar carcinoma	0/50	1/50	1/50	1/50
Adrenal medulla				
Pheochromocytoma*	15/50	13/50	18/50	18/50
Hyperplasia	32/50 (2.1)	27/50 (2.1)	28/50 (2.1)	29/50 (2.5)
Gallium arsenide	0 mg/m^3	0.01 mg/m^3	0.1 mg/m^3	1.0 mg/m^3
Lung				
Proteinosis	0/50	21/50 (1.0)	50/50 (2.4)	50/50 (3.5)
Fibrosis, interstitium	5/50 (1.0)	30/50 (1.4)	50/50 (2.6)	50/50 (2.8)
Inflammation, chronic active	16/50 (1.6)	46/50 (1.6)	50/50 (2.7)	50/50 (3.7)
Alveolar epithelium hyperplasia	9/50 (1.6)	11/50 (1.4)	35/50 (1.3)	48/50 (1.4)
Metaplasia, squamous epithelium	0/50	6/50 (1.0)	50/50 (1.1)	48/50 (1.5)
Histiocytosis	11/50 (1.7)	41/50 (1.6)	50/50 (2.0)	50/50 (2.1)
Alveolar/bronchiolar adenoma	1/50	0/50	3/50	2/50
Alveolar/bronchiolar carcinoma	2/50	0/50	2/50	1/50
Squamous cell carcinoma	1/50	0/50	0/50	0/50
Adrenal medulla				
Pheochromocytoma*	16/50	12/49	23/49	14/50
Hyperplasia	22/50 (2.5)	26/49 (2.5)	23/49 (2.6)	33/50 (3.0)

Numbers in parentheses indicate the mean grade of severity.

*Benign and malignant pheochromocytom a combined.

**Alterations were graded with a numerical system of 0–5 (0 - no involvement; 1 - minimal, up to 5 small lesions, up to 10% of the area; 2 - mild, 10–25%; 3 - moderate, 26–50%; 4 - marked, 51–75%; 5 - severe, more than 75% involvement).

augments the secretion of CA from the adrenal gland into the plasma (1, 21, 26, 40). Thus, a proposed mechanism for the direct effect of hypoxemia implicates increased CA secretion (24). Prolonged functional stimulation of endocrine cells may lead to their hypertrophy, followed by hyperplasia, and finally adenoma (4).

Our working hypothesis was that the percentage of the area of the lungs involved by the pathological process correlated positively with the degree of hypoxemia. We could not use the clinical data for the assessment of the grade of hypoxemia, as this functional parameter was not scored objectively throughout the studies. In contrast, the lung pathology data, which were re-evaluated in the present project by one pathologist, used a consistent criterion for all 9 studies.

Consequently, the possible association between the chronic pulmonary lesion, which might induce hypoxemia followed by hyperventilation, and adrenal pheochromocytoma or hyperplasia was examined in the 9 selected studies conducted at the NTP. Although chemically related increased pheochromocytoma was noted in both males and females, only the male rats were chosen for statistical evaluations, because the pulmonary damage appeared to be of essentially identical severity in both sexes (28–30, 33, 35).

MATERIALS AND METHODS

Histopathology

Pathology diagnosis in NTP studies is subjected to a rigorous peer review to assure accuracy and consistency of both neoplastic and nonneoplastic diagnoses within a study. Similarly, there is an effort to assure consistency in the diagnostic approach for neoplastic lesions across NTP studies; however, uniformity in diagnosing nonneoplastic effects from study to study is not always achieved. For the purpose of this retrospective evaluation, conducting a microscopic re-evaluation of the lungs to assess accurately nonneoplastic effects across the studies was therefore necessary. The lungs of males from 9 of the NTP particulate inhalation studies conducted in F344

rats for 2 years were re-evaluated histopathologically relative to the following chronic pulmonary lesions: proteinosis, interstitial fibrosis, chronic active inflammation, alveolar hyperplasia, alveolar epithelial squamous metaplasia, and histiocytosis. The severity grades for pulmonary change were based upon the proposed quantitative assessment of lung pathology (6), which is the percentage of the lung area that exhibited the tissue change. Alterations were graded with a numerical system of 0–5 (0- no involvement; 1- minimal, up to 5 small lesions and 10% of the area; 2- mild, 10–25%; 3- moderate, 26–50%; 4- marked, 51–75%; 5- severe, more than 75% involvement). Pulmonary lesions were re-evaluated by the same pathologist in all 9 studies; thus, the criteria of severity grading were identical.

Statistical Analyses

Logistic regression procedures (9) were used to determine whether the severity of each of the 6 lung lesions was predictive of pheochromocytoma incidence, after adjusting for possible confounding factors, such as survival (days of study). Statistical analyses were conducted on an individual-animal basis. Because there was a strong correlation between the severity of many of the 6 nonneoplastic lung lesions, the single lung lesion showing the strongest association with pheochromocytoma occurrence was first identified by logistic regression. The remaining lung lesions were then added to the model to determine whether they added significantly to the prediction of pheochromocytoma occurrence.

Each of the 9 chemicals was evaluated individually by logistic regression with adjustments for survival and chemical dose. In addition, data from the 9 control groups were assessed in a single logistic regression analysis, a model which adjusted for survival and study-to-study variability, to determine if there were any naturally occurring associations between the severity of nonneoplastic lung lesions and the occurrence of pheochromocytoma. Finally, all of the data from all chemicals and dosed groups were included in a single logistic regression model, which evaluated associations after adjusting for survival, dose, and study-to-study variability.

RESULTS

Histopathology

Results of the re-evaluation of chronic pulmonary nonneoplastic lesions as well as the incidence of neoplastic pulmonary lesions, pheochromocytoma, and hyperplasia in the adrenal medulla, as reported by the NTP for the 9 studies, are included in Table 1. Histopathological characteristics of chronic nonneoplastic lesions in the lung have been categorized according to definitive criteria. Proteinosis was typified by aggregates of homogeneous to granular eosinophilic material within alveolar lumina. Fibrosis within the interstitia consisted of varying amounts of dense fibrous tissue within alveolar septa and lumina; fibrosis was considered secondary to the inflammatory processes. Inflammation, characterized as chronic active, consisted of histiocytes, mononuclear cells, and pleomorphonuclear (PMNL) inflammatory cells within the alveoli and interstitia. Alveolar epithelial hyperplasia was manifested by increased numbers of uniformly cuboidal, type II epithelial cells lining septa with maintenance of normal architecture. An atypical hyperplastic lesion, characterized by proliferation of somewhat pleomorphic alveolar epithelial cells along septa that were distorted and often thickened by interstitial fibrosis, was also present and included under the diagnosis of alveolar epithelial hyperplasia. Squamous metaplasia of the alveolar epithelium was usually associated with inflammation and characterized by the replacement of alveolar type I and type II pneumocytes by well-differentiated squamous cells. Histiocytosis was manifested by the accumulation of alveolar macrophages with foamy cytoplasm, occasional multinucleated giant cells and cholesterol clefts, cell debris, and very few neutrophils.

Adrenal medullary hyperplasia consisted of irregular, small foci of small-to-normal-sized medullary cells arranged in packets or solid clusters slightly larger than normal; compression of surrounding parenchyma was minimal or absent. Benign pheochromocytomas arising in the adrenal medulla were well-delineated masses that often exhibited altered architecture and variable compression of the surrounding parenchyma. Neoplastic cells were arranged in variably sized aggregates, clusters, and trabecular cords of varying thickness. Larger neoplasms usually exhibited greater cellular pleomorphism and atypia than smaller ones. Malignant pheochromocytomas were identified when there was invasion into or beyond the adrenal capsule. Four compounds—nickel subsulfide, nickel oxide, talc, and indium phosphide—of 9 tested by the NTP in long-term inhalation studies showed significant dose-related increased incidences of adrenal pheochromocytoma. For the 5th chemical—cobalt sulfate—the increased incidence of pheochromocytoma was limited primarily to the mid-dose group; the NTP considered this an equivocal finding. The remaining 4 studies—vanadium pentoxide, nickel sulfate hexahydrate, molybdenum trioxide, and gallium arsenide—showed an increase in nonneoplastic lung lesions but no corresponding increase in adrenal pheochromocytoma incidence.

Statistical Analyses

Evaluation of the control data, assessed in a single logistic regression analysis, was somewhat limited by the relatively low incidence of nonneoplastic lung lesions; however, even given this limitation, both fibrosis and inflammation, considered individually, were associated significantly ($p < 0.01$) with the occurrence of pheochromocytoma. The strongest correlation was seen for inflammation, as illustrated in Table 2. Although fibrosis was significant individually, it only added marginally ($p = 0.054$) to the predictability obtained by using inflammation and correcting for survival and study-to-study variability. None of the other 4 nonneoplastic lung lesions was significantly correlated with the incidence of adrenal pheochromocytoma. Table 2 illustrates that, even in

TABLE 2.—Association between the incidence of pheochromocytom a and the severity of lung inflammation in control male F344 rats from 9 NTP inhalation studies.

	Severity of lung inflammation			
Days on study	0	1	2	3
<460	4% (1/23)	0% (0/3)	0% (0/1)	—
460–625	15% (17/112)	22% (4/18)	60% (3/5)	—
625–725	28% (27/97)	55% (11/20)	62% (8/13)	71% (5/7)
>725	31% (38/121)	51% (19/37)	89% (8/9)	75% (3/4)
Total	24% (83/353)	42% (34/78)	68% (19/28)	73% (8/11)

control male F344 rats, the occurrence of pheochromocytoma increased as a function of the severity of lung inflammation.

The first time interval used in Table 2 and subsequent tables reflects essentially those animals surviving until the 15-month interim sacrifice that was conducted in certain studies. The final interval included those animals that survived until the final sacrifice at 2 years, although the talc study extended somewhat longer, and the other 2 time intervals were chosen to divide the remaining animals into approximately equal-sized groups. These 4 groupings are primarily for descriptive purposes, because the actual statistical analysis used the number of days of study as a predictor variable. Table 2 clearly shows that the incidence of pheochromocytoma increases as the severity of lung inflammation increases.

The incidence of adrenal medullary hyperplasia varied from study to study, ranging from only 16–32% in the vanadium pentoxide study to 54–64% in the molybdenum trioxide study. With 1 possible exception, however, that of gallium arsenide, there was no apparent chemical-related effect on adrenal medullary hyperplasia. Gallium arsenide showed no significant effect on pheochromocytoma incidence but a significant ($p < 0.05$) increase in the incidence of adrenal medullary hyperplasia in the high-dose group (66%) relative to controls (44%). Severity of hyperplasia was also increased in the high-dose group in that study (see Table 1).

Next, each of the 9 chemicals was evaluated individually. Although the strength of the association varied from study to study, there was a significant ($p < 0.01$) association between the occurrence of pheochromocytoma and the severity of inflammation and fibrosis in each of the 5 studies showing chemical-related increased incidences of pheochromocytoma, as presented in Table 1. Also, however, gallium arsenide and molybdenum trioxide, which showed no dose-related increase in pheochromocytoma incidence, revealed a highly significant ($p < 0.01$) correlation between adrenal pheochromocytoma incidence and the severities of fibrosis and inflammation. In 2 studies, those of vanadium pentoxide and nickel sulfate hexahydrate, there was no significant association between nonneoplastic lung lesions and pheochromocytoma incidence.

For 3 chemicals—cobalt sulfate, indium phosphide, and nickel subsulfide—the strongest correlation occurred with fibrosis, yet for 4 chemicals—talc, nickel oxide, molybdenum trioxide, and gallium arsenide—the strongest correlation was with inflammation.

Whenall 9 studies were considered, both inflammation and fibrosis showed highly significant ($p < 0.001$) associations with pheochromocytoma incidence, with each lesion adding significantly to predictability when the other was included in the model. Although the other 4 lung lesions demonstrated significant ($p < 0.05$) associations with pheochromocytoma occurrence when considered individually, these associations were much weaker than those seen for fibrosis and inflammation, and none added significantly to a model that incorporated both of these lesions. Table 3 shows this striking association for fibrosis and inflammation.

Association between lung pathology and adrenal medullary hyperplasia was also examined (data not presented), but no trend for increase was noted. According to the NTP method of data recording, when pheochromocytoma and hyperplasia are present in the same animal, only the more severe lesion (ie, the neoplasm) is included. Thus, we might

TABLE 3.—Association between the incidence of pheochromocytoma and the severity of lung inflammation and fibrosis in dosed male F344 rats from 9 NTP inhalation studies.

A. Incidence of pheochromocytoma ; low- and mid-dosed groups

	Severity of lung inflammation				
Days on study	0	1	2	3	4
<460	0% (0/18)	0% (0/9)	14% (2/14)	22% (2/9)	100% (1/1)
460–625	4% (2/51)	14% (8/57)	32% (16/50)	41% (13/32)	100% (3/3)
625–725	32% (16/50)	41% (21/51)	36% (23/64)	60% (48/80)	100% (4/4)
>725	23% (13/57)	35% (18/52)	49% (35/72)	66% (95/145)	100% (2/2)
Total	18% (31/176)	28% (47/169)	38% (76/200)	59% (158/266)	100% (10/10)
			Severity of lung fibrosis		
< 460	3% (1/29)	8% (1/12)	20% (1/5)	40% (2/5)	—
460–625	10% (9/86)	29% (14/48)	26% (10/38)	43% (9/21)	—
625–725	31% (27/86)	38% (20/52)	42% (18/43)	69% (46/67)	100% (1/1)
>725	33% (35/106)	53% (28/53)	43% (30/70)	71% (70/99)	—
Total	23% (72/307)	38% (63/165)	38% (59/156)	66% (127/192)	100% (1/1)

B. Incidence of pheochromocytoma ; high-dosed groups

	Severity of lung inflammation				
Days on study	0	1	2	3	4–5
<460	0% (0/4)	0% (0/10)	0% (0/11)	0% (0/2)	50% (1/2)
460–625	0% (0/3)	0% (0/12)	12% (5/41)	53% (23/43)	42% (8/19)
625–725	0% (0/1)	0% (0/12)	35% (14/40)	70% (43/61)	52% (12/23)
>725	0% (0/3)	19% (3/16)	43% (20/47)	75% (56/75)	58% (23/40)
Total	0% (0/11)	6% (3/50)	28% (37/139)	67% (122/181)	52% (44/84)
			Severity of lung fibrosis		
<460	0% (0/14)	0% (0/10)	0% (0/4)	—	100% (1/1)
460–625	0% (0/12)	13% (4/30)	38% (17/45)	48% (15/31)	—
625–725	23% (3/13)	25% (6/24)	56% (25/45)	62% (32/52)	100% (3/3)
>725	30% (3/10)	45% (13/29)	49% (27/55)	66% (55/83)	100% (4/4)
Total	12% (6/49)	25% (23/93)	46% (69/149)	61% (102/166)	100% (8/8)

not expect medullary hyperplasia per se to be correlated with lung-lesion severity.

DISCUSSION

The objective of this histopathological re-examination of the lung was to analyze the possible correlation between the nonneoplastic, chronic pulmonary lesion, and pheochromocytoma. As shown in the present studies, particulate compounds caused several chronic pulmonary lesions in the F344 rats. Mossman (25) reported that proliferative and mutational events observed in inhalation studies of low-solubility particulates might be mediated by oxidants, chemokines, and cytokines elaborated during the development of inflammation rather than by direct interactions of particulates with cells. Chronic pulmonary lesions, especially fibrosis and inflammation, reduce the gas exchange area leading to hypoxemia followed by hyperventilation. Gosney (11) reported that continuous exposure of adult male Wistar rats to a hypoxemic condition for 28 days, with barometric pressure of 380 mm Hg, equivalent to an altitude of 5,500 m, caused a marked increase in adrenal gland weight due to hyperplasia of both adrenal cortex and medulla. Although initial exposure to hypoxemia results in an increase in activity of the adrenal medulla (15, 18), no study has been performed in rat to show the effect upon the adrenal medulla after prolonged severe hypoxemia. Examples from other animals, like the beluga whales, in which medullary proliferation was associated with hypoxemia indicated that pulmonary disease might have been the cause (20).

Most agents that induce proliferative lesions in the rat adrenal medulla are nongenotoxic and structurally unrelated (44). That they induce lesions indirectly by stimulating chromaffin cell proliferation thereby providing a setting in which DNA damage may occur or where pre-existing initiated or responsive cells may be promoted, has been hypothesized (44, 44). Tischler divided the etiological agents inducing pheochromocytoma in rats into 4 categories based on the involved mechanism of action (42): those that affect the hypothalamic-endocrine axis, such as growth hormones and neuroleptic agents; those impacting the autonomic nervous system, such as nicotine and reserpinine; dietary factors including excess food and calcium intake; and miscellaneous agents like radiation. Various studies by Tischler et al (43–46) have demonstrated that several nongenotoxic agents, for example, vitamin D, that affect chromaffin cell function also stimulate proliferation of rat chromaffin cells, and that prolonged stimulation by these agents may lead to hyperplastic nodules and pheochromocytoma. In aged F344 rats from 125 of the most recent NTP studies, a greater incidence of pheochromocytoma was observed in animals with more severe chronic progressive nephropathy, which suggested that disturbed calcium homeostasis stimulated the proliferation of chromaffin cells (37).

We endorse the addition of hypoxemia to the list of factors stimulating chromaffin cell proliferation leading to pheochromocytoma in rats. Functional activities of both normal and neoplastic chromaffin cells are affected by hypoxia, and a hypoxia-responsive element may be present in the regulatory region of some genes involved in chromaffin cell function. We suggest that a direct effect of hypoxemia on chromaffin cells could be involved in the development of proliferative lesions in the adrenal medulla. Alternatively, hypoxemia might elicit a reflex increase in trans-synaptic stimulation of chromaffin cells.

The role of hypoxia-inducible factor 1α (HIF-1α) is under increasing scrutiny by cancer researchers (47). HIF-1α binds a specific sequence in the hypoxia-response element of various target genes (39), and the level of HIF-1α protein is inversely related to the oxygen tension in vivo (16) as well as in vitro (17). O_2-sensitive pheochromocytoma cells (PC12) in culture, increase in the HIF-1α gene expression occurs following exposure to hypoxia (23). Hypoxia reduces the proliferation of tumor cells (5) and increases the rate of apoptotic cell death (12) and metastasis (2, 3).

Statistical analyses of the relationship between chronic lung lesions and adrenal pheochromocytoma or hyperplasia provided evidence that the incidence of pheochromocytoma was associated with the severity of lung fibrosis and inflammation in certain NTP studies. This association was especially evident in dosed animals but also significant in chamber controls. These associations were apparent, with varying degrees of statistical significance, in 7 of the 9 individual studies. The 2 exceptions, which, nevertheless, were included in our overall analysis, were vanadium pentoxide and nickel sulfate hexahydrate. Although the other 4 lung lesions—proteinosis, alveolar epithelial hyperplasia, squamous epithelial metaplasia, and histiocytosis—showed dose-related increases in severity in some of the studies, these increases were generally not strongly associated with increased incidences of pheochromocytoma.

For example, in the nickel subsulfide study, a significant increase occurred in pheochromocytoma incidence (72% vs 24%) and in the incidence and mean severity of both proteinosis and fibrosis in the high-dose male rat group relative to controls (see Table 1). The incidences of pheochromocytoma, however, were essentially the same—71% (10/14), 74% (20/27), and 71% (12/17) for animals having proteinosis severities of 0–2, 3, and 4–5, respectively (data not shown). In contrast, in this same high-dose group, the incidence of pheochromocytoma was 98% (41/42) in male rats having a fibrosis severity of 2–4 but only 6% (1/16) for those animals having a severity of 0–1 (data not shown). Thus, although the incidence and severity of both proteinosis and fibrosis were increased by nickel subsulfide, the lesion whose incidence and severity correlated with the increased occurrence of pheochromocytoma was fibrosis, not proteinosis. Similar results were observed for other lung lesions in other dosed groups for other chemicals.

Table 4 shows the corresponding correlation in the molybdenum trioxide study. Note that the combined incidence of pheochromocytoma in animals with a grade 2 inflammation in the control, low-, and mid-dose groups was 66.7% (8/12)—consistent with the 68% (19/28) rate seen in control animals with grade 2 inflammation. The pheochromocytoma rate, however, in the high-dose animals with grade 2 inflammation (10/31 or 32%) was similar to the background rate of this tumor and much lower than expected based on the control data.

As a final illustration, the control data suggested that animals with a grade 3 lung inflammation should have approximately a 73% incidence of pheochromocytoma (see Table 2); however, in the high-dose nickel sulfate hexahydrate group, only 25% (3/12) of the animals with grade 3

TABLE 4.—Correlation between inflammation and pheochromocytoma occurrence in the molybdenum trioxide study.

	Control	Low dose	Mid dose	High dose
Mean survival (days)	645	617	646	654
Pheochromocytoma rate				
Overall	30.0% (15/50)	26% (13/50)	36% (18/50)	36% (18/50)
Inflammation = 0	22.5% (9/40)	16.3% (7/43)	15.4% (2/13)	0% (0/2)
Inflammation = 1	50.0% (2/4)	100% (3/3)	37.5% (12/32)	0% (0/5)
Inflammation = 2	50.0% (2/4)	75.0% (3/4)	75.0% (3/4)	32.2% (10/31)
Inflammation = 3	100% (2/2)	—	100% (1/1)	66.7% (8/12)

lung inflammation exhibited pheochromocytomas (data not shown). This 25% incidence was essentially equivalent to the background rate.

The lack of any observed association between the severity of the lung lesions and pheochromocytoma in 2 studies is not understood. Also unclear is the reason that the incidence of pheochromocytoma was not increased in 4 studies in which there was a treatment-related increase in the incidence and severity of inflammation and fibrosis. Based on the control data in Table 2, one would expect animals with grade 3 lung inflammation to show adrenal pheochromocytoma incidences >70%, yet the high-dose gallium arsenide group, which showed an average lung inflammation severity of 3.74, displayed only a 28% incidence of pheochromocytoma. Even so, there was a highly significant ($p < 0.01$) correlation between pheochromocytoma occurrence and the severity of lung inflammation in this study. We conclude that there is an overall association between chronic pulmonary fibrosis and inflammation and the elevated incidence of adrenal pheochromocytoma in the male F344 rat in the NTP inhalation studies, although for reasons that are unclear this association is not consistently seen in all studies. The hypoxemic condition, which may follow chronic pulmonary inflammation and fibrosis, may be implicated in the induction of pheochromocytoma in these studies. Our investigation may provide evidence supporting the role of chronic pulmonary fibrosis and inflammation as novel risk factors in the development of pheochromocytoma in rats. In those studies in which no association between the severity of lung pathology and pheochromocytoma was noted, the grade of hypoxemia may not have been sufficiently severe and/or prolonged to promote the proliferation of the adrenal medullary cells. Four of the lung nonneoplastic lesions—proteinosis, alveolar epithelial hyperplasia, squamous epithelial metaplasia and histiocytosis—did not correlate with the incidence of pheochromocytoma, These lesions may have contributed relatively less to the alveolar space reduction than the other 2 lesions—fibrosis and inflammation. Our findings might suggest the existence of a hypoxemic threshold, which is the factor of severity of alveolar space occupation × the duration of reduced normal oxygenation. Additional investigations are needed to test our hypothesis.

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