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The Association Between Severe Nephropathy and Pheochromocytoma in the Male F344 Rat—The National Toxicology Program Experience*

ABRAHAM NYSKA,[1] JOSEPH K. HASEMAN,[1] JAMES R. HAILEY,[1] SAMUEL SMETANA,[2] AND ROBERT R. MARONPOT[1]

[1]*National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA, and* [2]*Nephrology Institute, Wolfson Medical Center, Holon 58100, Israel*

ABSTRACT

The possible correlation between the severity of chronic progressive glomerulonephropathy (CPN) and the incidence of adrenal pheochromocytoma was examined in selected studies of male Fischer 344 (F344) rats at the National Toxicology Program (NTP). The NTP historical control database was first examined in order to determine whether there was association between the severity of CPN and the occurrence of adrenal pheochromocytoma in unexposed animals. Following this analysis, the 125 most recent NTP studies conducted in F344 rats were examined in order to determine how frequently chemicals that cause increased severity of CPN showed an increased incidence of pheochromocytoma. Finally, we examined the association between the incidence of pheochromocytoma and the severity of CPN in those NTP studies with chemically related increased rates of pheochromocytoma. In control male F344 rats surviving beyond 21 mo, the incidence of adrenal pheochromocytoma was consistently higher in animals with more severe CPN. This association was significant ($p < 0.05$) both for 900 NTP inhalation study controls and 900 NTP feeding study controls. An association was not consistently observed when dosed groups were considered. Although 22% (28/125) of NTP studies reported a chemically related increased severity of CPN, only 3 of these reported a corresponding significant increase in the incidence of pheochromocytoma. Of 6 NTP studies that reported increased incidence of pheochromocytoma, animals with pheochromocytoma from 5 of those studies had some degree of increased severity of CPN. However, the estimated strength of the correlation with the severity of CPN varied from study to study and was often quite different from that indicated by an analysis of the more extensive NTP control databases. The possible correlation between the severity of CPN and the incidence of pheochromocytoma may influence interpretation of carcinogenic effects observed at this site.

Keywords. Rat adrenal medullary tumors; glomerulonephropathy; calcium; nongenotoxic; carcinogenicity

INTRODUCTION

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 (23). The same adrenal lesions are rarely observed in humans and other animal species (24). In most instances, the exogenous agents that induce adrenal medullary neoplasia lack the ability to cause DNA damage, and so it has been suggested that many of these agents influence the carcinogenic response of the adrenal medulla through an indirect mechanism (24).

Tischler et al (25) showed that marked stimulation of chromaffin cell proliferation occurs following oral administration of vitamin D to the rat *in vivo*. Vitamin D is known to stimulate Ca^{2+} absorption. In contrast, use of vitamin D, its active metabolite calcitriol, lactose, or xylitol in adrenal medullary cell culture did not result in a mitogenic effect. It was, therefore, hypothesized that the mitogenic effects of altered Ca^{2+} homeostasis may be related to presynaptic changes in the nature or intensity of neurally derived signals that stimulate chromaffin cell proliferation. A direct effect of Ca^{2+} on the nicotinic or muscarinic acetylcholine receptors of the chromaffin cell may also be involved.

Chronic progressive glomerulonephropathy (CPN) is a commonly occurring spontaneous disease in aging F344/N rats. The severity of CPN is greater in male than in female rats. Chronic renal failure is known to be associated with the inability to secrete phosphate, which results in hyperphosphatemia. Also associated with this disease are reduced production of the active metabolite of vitamin D (because of the decreased number of nephrons) and hypocalcemia (secondary to decreased calcium intestinal absorption) (19, 22, 26). The low serum calcium levels stimulate parathyroid hormone secretion. In severe cases of CPN in rats, which are associated with disturbed calcium/phosphorous homeostasis, there might be chronic

* Address correspondence to: Dr. Abraham Nyska, National Institute of Environmental Health Sciences (NIEHS), P.O. Box 12233, Research Triangle Park, North Carolina 27709; e-mail: Nyska@niehs.nih.gov.

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stimulation of the chromaffin cells toward proliferation, which may eventually lead to hyperplasia and neoplasia. Thus, the possible association between the severity of CPN, associated changes related to secondary hyperparathyroidism, and adrenal pheochromocytoma were examined in selected studies carried out at the National Toxicology Program (NTP). Since higher spontaneous incidence of both severe CPN and pheochromocytoma occurs in males, only data derived from males was used in the current investigation.

Materials and Methods

The NTP historical control database was examined for correlation between the severity of CPN and the occurrence of adrenal pheochromocytoma in unexposed animals. The data were derived from 2-yr carcinogenicity studies, in which the rats were approximately 6 wk of age at study start. Approximately 900 male F344 chamber control rats from 18 recent NTP inhalation studies and another 900 untreated controls from 18 recent NTP feeding studies were included in this evaluation.

Following this analysis, data from the 125 most recent NTP studies in F344 rats were reviewed, and all studies with an increased severity of CPN in treated male F344 rats as compared with controls were examined in order to determine whether or not there was evidence of an increased incidence of pheochromocytoma. Studies showing an increased incidence of pheochromocytoma were then examined for a significant association between CPN severity and the incidence of adrenal pheochromocytoma. The strength of this association in these studies was compared with that observed in the control database in order to determine whether the relationship might provide a possible mechanism for the increased incidence of adrenal pheochromocytomas.

Logistic regression techniques were used to model adrenal pheochromocytoma incidence as a function of survival, CPN severity, and (when applicable) chemical dose (20). The general logistic regression model for predicting the incidence of pheochromocytoma in control animals may be written as follows: Tumor rate = $1/(1 + \exp(T))$. The specific model that maximizes the likelihood of the observed data was found to be $T = 23.4942 - 0.0614S + 0.0000399S^2 + 0.5838N - 0.1610N^2$ for inhalation study controls and $T = 6.6415 - 0.00795S - 0.1483N$ for feeding study controls, where S indicates survival (days) and where N indicates severity of CPN. In addition to CPN, related nonneoplastic lesions, which are known to occur in severe cases of CPN (6) (including metastatic mineralization of the kidney, heart, lung, and glandular stomach, parathyroid hyperplasia, and bone osteodystrophy) were also included in this evaluation. These changes are commonly associated with secondary hyperparathyroidism resulting from a calcium/phosphorous imbalance created by compromised functional capacity of the kidney.

Severity grades for CPN were based upon the percentage of the renal parenchyma that exhibited the tissue changes. Alterations were graded with a numerical system of 0–4 (0: no involvement; 1: minimal, up to 25%; 2: mild, 26–50%; 3: moderate, 51–75%; or 4: marked, >76% of involvement). There was a high degree of consistency of severity grading within a study; however, because these studies were evaluated by different pathologists, severity grading between studies was not always exactly identical.

Results

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 did smaller neoplasms. Malignant pheochromocytomas were identified when there was invasion into or beyond the adrenal capsule.

Typical morphological changes associated with CPN in the kidney included the following: varying degrees of tubular dilation and distortion with cyst formation; proteinaceous tubular casts; regeneration and hypertrophy of the renal tubular epithelium; thickening of the renal tubule and glomerular basement membranes; interstitial fibrosis; scattered foci of suppurative inflammation, primarily within degenerating renal tubules; and varying numbers and aggregates of mononuclear inflammatory cells within the interstitium.

For control animals that survived more than 21 mo, the incidence of adrenal pheochromocytoma was consistently higher in animals with the more severe CPN (Table I). For the inhalation study controls, only animals with grade 4 CPN appeared to be at increased risk of pheochromocytoma, whereas with feeding study controls, animals with grade 3 or 4 CPN were at increased risk. Logistic regression analysis (adjusting for survival) indicates that this association is statistically significant for both the inhalation study controls ($p < 0.01$) and for the feeding study controls ($p < 0.05$).

Inhalation study controls had a much higher incidence of grade 4 CPN (237/900) than did feed study controls (73/894). For animals with grade 4 CPN that survived until the final sacrifice at 24 mo, the incidence of adrenal pheochromocytoma was higher in inhalation study controls (61%) than in feeding study controls (38%). The reasons for these differences are unknown.

Since there appeared to be an association between CPN severity and the incidence of pheochromocytoma in control male F344 rats, the NTP studies with chemical-related increases in CPN severity were investigated in order to determine how frequently there was an associated increase in pheochromocytoma. Of the 125 NTP studies evaluated, 22% (28/125) reported an increase of more than 0.5 in mean CPN severity in dosed male F344 rats relative to controls; these 28 studies are summarized in Table II. Only 3 of these studies showed a significant increase in adrenal pheochromocytoma incidence, and 3 actually showed significantly decreased incidences of pheochromocytoma relative to controls. It is clear that, despite the correlation between CPN severity and pheochromocytoma incidence observed in the control populations, increased CPN severity in chemically exposed

TABLE I.—Incidence of adrenal pheochromocytoma (%) vs chronic progressive nephropathy severity at different survival times in control male F344/N rats from National Toxicology Program 2-yr studies.

Nephropathy severity	Survival time (mo)				Total
	0–15	15–21	21–24	24	
Inhalation study controls					
0–2	0/39 (0)	34/212 (16)	30/84 (36)	20/53 (38)	84/388 (22)
3	0/1 (0)	12/81 (15)	36/93 (39)	37/100 (37)	85/275 (31)
4	0/0	4/33 (12)	59/103 (57)	62/101 (61)	125/237 (53)
Total	0/40 (0)	50/326 (15)	125/280 (45)	119/254 (47)	294/900 (33)
Feeding study controls					
0–2	2/38 (5)	20/185 (11)	48/146 (33)	60/209 (29)	130/578 (22)
3	0/1 (0)	3/28 (11)	32/73 (44)	59/141 (42)	94/243 (39)
4	0/1 (0)	1/7 (14)	11/25 (44)	15/40 (38)	27/73 (37)
Total	2/40 (5)	24/220 (11)	91/244 (37)	134/390 (34)	251/894 (28)

male F344 rats is generally not associated with a corresponding increase in pheochromocytoma incidence.

NTP studies that showed an increased incidence of pheochromocytoma were examined in order to determine to what extent, if any, these incidences may be related to CPN severity. In order to minimize the effect of potentially confounding variables, we excluded from consideration chemicals that increased the incidence of adrenal pheochromocytoma when administered by inhalation [which is often hypothesized to increase adrenal pheochromocytoma by stress-related mechanisms (16)] or chemicals that were administered at doses that resulted in markedly increased mortality.

Among the 125 chemicals evaluated (subject to the conditions noted above), 6 were identified that the NTP regarded as causative of increased incidences of pheochromocytoma in male F344 rats. These chemicals are C.I. pigment red 3 (14); phenolphthalein (17); oxymetholone (18); hexachloroethane (12); para-chloroaniline hydrochloride (HCl) (13); and polysorbate 80 (15). The incidences of adrenal pheochromocytoma in these 6 NTP studies are presented in Table III, along with corresponding mean CPN severities.

The increases in the incidence of adrenal pheochromocytoma observed in these 6 studies fall into 3 broad categories: chemicals showing a significantly increased tumor incidence only at the top dose (oxymetholone, polysorbate 80, *p*-chloroaniline HCl); chemicals showing an increased tumor incidence of approximately the same magnitude at all 3 doses (C.I. pigment red 3, phenolphthalein); and a chemical with significantly increased tumor incidence only at the low dose (hexachloroethane).

In the phenolphthalein, C.I. pigment red 3, and oxymetholone studies, the severity of CPN was increased in treated groups that displayed increased incidences of pheochromocytoma (Table III). Such an association was less clear in the polysorbate 80 and hexachloroethane studies. The difference in the severity of nephropathy between control and treated groups from these 2 studies was not as pronounced as it was in the other 3 studies. Furthermore, although the severity of nephropathy was slightly higher in the treated groups with the increased incidence of pheochromocytomas, other treatment groups within these studies had a similar severity of nephropathy with no concomitant increase in pheochromocytomas. In the *p*-chloroaniline HCl study, the severity of nephropathy in the treatment group with the increased incidence of pheochromocytoma was actually decreased.

Logistic regression analysis can be used to better assess whether an association exists within these studies. This analysis uses individual animal data to model the incidence of adrenal pheochromocytoma as a function of survival, CPN severity, and possible differences among dosed groups. In each of the 6 studies (Table IV), there was a positive correlation between the severity of CPN and the incidence of adrenal pheochromocytoma that was either statistically significant (4 studies) or suggestive of an effect (2 studies). In every study except one (*p*-chloroaniline HCl), adjusting for this association reduced the statistical significance of the chemically related change in incidence of pheochromocytoma; for C.I. pigment red, oxymetholone, and polysorbate 80, the increased incidence of pheochromocytoma was no longer statistically significant ($p < 0.05$) when adjustment is made for nephropathy severity (Table IV).

The incidences of parathyroid hyperplasia, mineralization of the glandular stomach, and osteodystrophy, which are often associated with severe CPN (6), were low or variable among the studies (data not shown). These nonneoplastic lesions were not significantly correlated with adrenal pheochromocytoma.

DISCUSSION

The aim of the present retrospective analysis was to examine the possible correlation between CPN, lesions secondary to severe CPN, and pheochromocytoma. The development of spontaneous CPN in the rat is influenced by many factors, such as the amount and quality of dietary protein and carbohydrates, levels of dietary intake, caloric intake, sodium, and other components (2). CPN progresses more rapidly and with greater severity in male than in female rats (2, 21). The higher prevalence of CPN among aged male rats is attributable to an age-related physiological change in the kidney that results in progressive deterioration in terms of that organ's capacity to handle proteins, and male rats are spontaneously more proteinuric than are females rats of the same strain (21). At 2 yr of age, the mean severity score of CPN among male rats of both the Sprague-Dawley (SD) and F344/N strains is at least double that present in females of the

TABLE II.—Incidence of adrenal pheochromocytoma in male F344/N rats seen in National Toxicology Program 2-yr carcinogenicity studies in which an increased severity of chronic progressive nephropathy was observed.

Dose group	Pheochromocytoma incidence				Mean CPN severity			
	Control	Low	Mid	High	Control	Low	Mid	High
Significant ($p < 0.05$) increase in pheochromocytoma								
Phenolphthalein[a]	18/50	35/50*	35/50*	35/50*	1.7	2.8	3.1	3.1
C.I. pigment red 3[a]	24/50	32/50	37/50*	36/50*	2.4	3.1	3.6	3.8
Oxymetholone[a]	19/51	25/50	21/50	29/49*	1.7	2.4	2.7	2.6
No significant change in pheochromocytoma								
o-Nitroanisole[a]	12/49	10/50	10/50	10/49	2.2	2.4	2.6	3.2
Quercetin	13/50	IH	IH	12/49	2.7	2.7	3.0	3.2
Ethyl benzene[a]	13/50	13/50	9/49	14/48	2.3	2.4	2.3	3.5
Furan[a]	9/50	4/47	11/48	10/49	1.6	2.4	3.2	3.2
Furfuryl alcohol[a]	19/50	27/50	24/50	26/50	2.9	2.9	3.1	3.7
Acetaminophen	17/44	22/49	19/49	21/46	2.3	2.6	2.6	2.8
Primidone[a]	23/50	28/50	24/50	12/49[b]	2.2	2.9	3.4	3.8
Chloroprene[a]	19/50	21/50	21/49	18/50	2.8	3.0	3.1	3.5
D & C yellow no. 11[a]	10/50	9/50	14/51	8/54	2.3	2.8	3.2	3.0
4,4'-Thiobis (6-*t*-butyl-*m*-cresol)	14/50	14/50	10/50	9/49	1.4	1.4	1.6	2.3
o-Benzyl-*p*-chlorophenol[a]	14/50	7/50	10/49	15/50	2.3	2.8	2.9	3.3
Coumarin	9/49	5/50[b]	5/50[b]	0/50[b]	2.0	2.9	3.6	3.6
3,4-Dihydrocoumarin	18/50	11/49[b]	12/49[b]	8/50[b]	2.2	2.9	3.2	3.2
Mercuric chloride[a]	24/48	18/50	—	23/49	2.7	3.1	—	3.3
2,4-Diaminophenol dihydrochloride	15/49	8/50	—	9/50	2.8	2.7	—	3.3
1,2,3-Trichloropropane	10/50	7/50	13/48	0/51[b]	2.0	2.0	2.6	2.4
Benzofuran[a]	14/50	6/50[b]	—	10/47[b]	1.6	3.3	—	2.9
alpha-Methylbenzyl alcohol[a]	16/50	20/50	—	4/49[b]	2.0	3.5	—	3.5
Phenylbutazone	19/50	20/49	—	21/49	2.1	2.6	—	3.0
Hydroquinone	14/55	19/48	—	21/55	2.8	2.7	—	3.4
8-Methoxypsoralen[a]	14/50	14/50	—	10/49	1.9	2.5	—	2.7
Hydrochlorothiazide[a]	18/50	22/49	17/50	22/50	NR	NR	NR	NR
Significant ($p < 0.05$) decrease in pheochromocytoma								
Oxazepam[a]	14/50	9/50	6/50*	3/50*	1.9	2.3	2.7	3.2
Salicylazosulfapyridine	16/50	7/49*	7/50*	8/49	1.6	1.9	2.0	2.5
Tetrafluoroethylene[a]	20/50	12/50	9/50*	10/50	2.3	1.9	2.7	3.5

Abbreviations: CPN = chronic progressive glomerulonephropathy; IH = incomplete histopathology for low mid-dosed groups; NR = not reported.
[a] These chemicals also had increased incidences of bone fibrous osteodystrophy.
[b] Apparent decrease due to significantly ($p < 0.01$) reduced survival.
* $p < 0.05$ vs controls.

same age and strain (11, 21). In F344/N rats, the rate of pheochromocytoma is 6 times higher among males than females (mean rate 31.9% in males vs 5.1% in females) (5). Similarly, the incidence of pheochromocytomas among male SD rats is at least 3 times more frequent than among females (mean rate 20.9% in males vs 6.2% in the females) (11).

In the present investigation, severe CPN was often associated with parathyroid hyperplasia and fibrous osteodystrophy. Secondary hyperparathyroidism associated with chronic renal disease has been described in humans, rats, and dogs. The mechanism responsible for the renal secondary hyperparathyroidism is reduced filtration of the glomeruli, which leads to the retention of phosphorus, thus resulting in progressive hyperphosphatemia, decrease of blood calcium, and decreased levels of calcitriol (8). The reduction in the synthesis of active vitamin D by the kidneys leads to reduction in calcium absorption from the intestine (19). Fibrous osteodystrophy is known to result from increased secretion of parathyroid hormone as a manifestation of primary or secondary hyperparathyroidism (26). It is considered to be the most significant

TABLE III.—Incidences of adrenal pheochromocytoma and mean chronic progressive nephropathy severities in F344 male rats in 6 selected National Toxicology Program studies.

Chemical	Incidence of pheochromocytoma					Mean CPN severity			
	Controls	Low	Mid	High	Study result	Controls	Low	Mid	High
Phenolphthalein[a]	18/50	35/50	35/50	35/50	CE	1.7	2.8	3.1	3.1
C.I. pigment red 3	24/50	32/50	37/50	36/50	SE	2.4	3.1	3.6	3.8
Oxymetholone	19/51	25/50	21/50	29/49	EE	1.7	2.4	2.7	2.6
Polysorbate 80	21/50	19/50	—	29/50	EE	2.7	3.0	—	3.0
p-Chloroaniline HCl	13/49	14/49	15/48	26/48	EE	2.6	2.9	2.8	2.1
Hexachloroethane	15/50	28/45	—	21/49	EE	2.3	2.6	—	2.7

Abbreviations: CPN, chronic progressive glomerulonephropathy; CE = clear evidence of carcinogenic activity; SE = some evidence of carcinogenic activity; EE = equivocal evidence of carcinogenic activity.
[a] For phenolphthalein, the "clear evidence" conclusion was based not only on adrenal pheochromocytomas, but also on increased incidences of kidney renal tubule tumors (1/50, 10/50, 16/50, and 16/50).

TABLE IV.—Significance of the association between adrenal pheochromocytoma incidence and chronic progressive nephropathy severity in the 6 National Toxicology Program studies as determined by logistic regression.

Chemical	Significance
Phenolphthalein	$p = 0.15$
C.I. pigment red 3[a]	$p < 0.001$
Oxymetholone[a]	$p = 0.048$
Polysorbate 80[a]	$p = 0.012$
p-Chloroaniline HCl	$p = 0.084$
Hexachloroethane	$p = 0.004$

[a] For these 3 chemicals, adjusting for chronic progressive glomerulonephropathy severity eliminates the statistical significance ($p < 0.05$ of the increased pheochromocytoma incidence.

consequence of increased parathyroid hormone levels because of chronic renal disease and associated uremia (19, 22).

Advanced renal failure may lead to hypercalcemia (3). Hypercalcemia in the rat (secondary to hyperparathyroidism) is well documented in the literature (10). Hypercalcemia leads to calcification of normal tissues (3). This metastatic calcification of soft tissues occurs in rats as a relatively late manifestation of CPN (2). Metastatic calcification occurs in epithelial cells and basement membrane of the gastric glands; connective tissue of the lamina propria and muscularis mucosae in the stomach; muscular layers in the stomach and intestine; epithelial cells and the basement membranes of the renal tubules and Bowman's capsule in the kidney; alveolar walls in the lung; tunica media in the large arteries; and tunica media of the moderate-sized arteries in the kidney, testis, pancreas, stomach, heart, tongue, lung, spleen, salivary gland, and skeletal muscle (6).

In human patients suffering from chronic renal disease associated with hyperparathyroidism, the serum levels of calcium, alkaline phosphatase, and parathyroid hormone and associated soft-tissue calcification were reduced after subtotal parathyroidectomy (7). Although serum Ca^{2+} analysis was not performed in the studies included in our investigation, the pathologic evidence of metastatic mineralization (i.e., gastric glandular mineralization and bone fibrous osteodystrophy) is evidence for a state of hypercalcemia. An association between hypercalcemia and increased incidence of pheochromocytomas has been previously reported in rats (9). In that study, retinol acetate (RAC) was administered to F344/DuCrj rats in drinking water for 2 yr and was found to be associated with higher incidences of benign and malignant pheochromocytomas and hyperplasias of the adrenal medulla of the RAC-treated groups. Hypercalcemia was also reported in the treated rats, but without severe bone lesions. It has been suggested that the RAC enhanced the absorption of calcium from the gut, which promoted the development of the adrenal medullary tumors.

Some slowly digested nonmutagenic sugar alcohols, such as sorbitol, xylitol, lactitol, and lactose, induce proliferation of adrenal medullary cells and pheochromocytomas in rats, presumably by means of a nongenotoxic secondary mechanism that involves perturbations in calcium homeostasis (1, 4, 27). These alterations in calcium homeostasis and the secondary changes in adrenal medullary cells are analogous to what we hypothesize may explain the association between CPN and pheochromocytomas observed in the present study. In addition to alterations in calcium homeostasis, it is apparent that genetic susceptibility also plays a role in adrenal medullary cell response to these various polyols, since these agents do not measurably affect calcium homeostasis or the occurrence of pheochromocytomas in humans (27).

In our investigation, some studies (e.g., phenolphthalein) showed only a marginal correlation between the incidence of pheochromocytoma and the severity of CPN, and adjusting for CPN severity did not totally account for the observed increase in pheochromocytoma.

In the NTP C.I. pigment red 3, oxymetholone, and polysorbate 80 studies, CPN severity was significantly correlated with the incidence of adrenal pheochromocytoma, and this association could account (statistically) for the increased incidences of adrenal pheochromocytoma observed in the dosed groups, especially for C.I. pigment red 3. However, the strength of the association in these studies appears to be quite different from that observed in the control groups. For example, neither of the 2 logistic regression models derived from the control databases predict accurately the incidence of pheochromocytoma observed in the dosed groups in the C.I. pigment red 3 study. In particular, the apparent impact of the severity of CPN on the incidence of adrenal pheochromocytoma is much greater (and the impact of survival less) in the C.I. pigment red 3 study than in the inhalation or feeding study control groups. The reasons for these differences are unclear. As a result, the models derived from the 2 control databases substantially underpredict the incidence of adrenal pheochromocytoma observed in the C.I. pigment red 3 groups. Similar results were found for oxymetholone and polysorbate 80.

Furthermore, most NTP studies with marked treatment-related increases in the severity of CPN did not show a corresponding increase in adrenal pheochromocytoma incidence. The reason for this is unknown, but one possible explanation is that although the severity of CPN may be increased, the resulting enhanced CPN in certain studies may not be severe enough to cause marked alteration in the calcium homeostasis. Interestingly, each of the 3 studies with the strongest increase in pheochromocytoma incidence (phenolphthalein, C.I. pigment red 3, and oxymetholone) showed significant increases both in bone fibrous osteodystrophy and severity of CPN (data of fibrous osteodystrophy not shown). In contrast, many other studies with less striking increases in CPN severity and/or no corresponding increase in fibrous osteodystrophy showed no increase in the incidence of pheochromocytoma (Table II). Other alternatives that might explain the increased incidence of pheochromocytomas in male rats were discussed in relation to similar results noted with inhalation exposure to talc (16). With talc, these speculations included a nonspecific effect of stress (i.e., increased catecholamine synthesis) as a result of the treatment-related chronic pulmonary inflammation and effects of cytokines (growth factors) released from macrophages

and other inflammatory cells that were infiltrating the affected lungs.

The existence of an association between CPN severity and pheochromocytoma incidence does not definitively establish a cause and effect relationship between the two. Moreover, our findings indicate that more work is needed to better understand this correlation and what effect, if any, it may have on the interpretation of experimental results. Specific issues that require further work include the following: the reason why, despite the correlation between the severity of CPN and the incidence of pheochromocytoma observed in the control populations, most NTP studies with increased severity of CPN in male F344 rats do not have a corresponding increase in the incidence of pheochromocytoma. In addition, for those chemicals that do show an increase in pheochromocytoma, the estimated strength of the correlation with the severity of CPN varies from study to study and is often quite different from that indicated by an analysis of the more extensive NTP control database. Moreover, this association cannot always totally account for the increased incidence of adrenal pheochromocytoma observed in dosed animals.

Investigators evaluating a chemical's potential carcinogenic effect on the adrenal medulla in male F344 rats should be aware of the possible correlation between CPN severity and pheochromocytoma incidence and the impact that this association may have, in some instances, on the interpretation of carcinogenic effects observed at this site.

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For more information, contact:

Ms. Ruth Murphy
Department of Medical Education
Armed Forces Institute of Pathology
Washington, DC 20306-6000
(202) 782-2637 or (800) 577-3749
came@afip.osd.mil
World Wide Web site: http://www.afip.org