NCI-Black-Reiter (NBR) Male Rats Fail to Develop Renal Disease following Exposure to Agents That Induce α -2u-Globulin (α_{2u}) Nephropathy¹

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NCI-Black-Reiter (NBR) Male Rats Fail to Develop Renal Disease following Exposure to Agents That Induce α_{2u} -Globulin (α_{2u}) Nephropathy. DIETRICH, D. R., AND SWENBERG, J. A. (1991). *Fundam. Appl. Toxicol.* 16, 749-762. The NCI-Black-Reiter (NBR) rat is the only strain of male rat known not to synthesize the hepatic form of the low molecular weight protein, α_{2u} -globulin. In previous studies, NBR rats were shown not to develop renal disease when exposed to decalin, a compound known to induce α_{2u} -globulin nephropathy in other rat strains. The objective of this study was to show that the presence of α_{2u} -globulin (α_{2u}) is essential for the development of this syndrome in rats exposed to 2,2,4-trimethylpentane (TMP), 1,4-dichlorobenzene (DCB), isophorone (IP), PS-6 unleaded gasoline (UG), and *d*-limonene (*d*-L). The induction of α_{2u} -nephropathy in F344 male rats with lindane was used as a positive control and this response was contrasted to male NBR and female F344 rats treated with lindane. Five to seven 11-week-old male NBR rats were exposed to TMP (500 mg/kg/day), DCB (500 mg/kg/day), IP (1000 mg/kg/day), UG (500 mg/kg/day), *d*-L (1650 mg/kg/day), or lindane (10 mg/kg/day) and five 11-week-old male and female F344 rats were exposed to lindane (10 mg/kg/day) by oral gavage on 4 consecutive days. NBR male and F344 male and female rats gavaged with corn oil were incorporated in the study as vehicle controls. The presence of hyaline droplets was assessed in perfusion-fixed kidneys by staining paraffin sections with Mallory-Heidenhein stain and in GMA sections with Lee's methylene basic blue fuchsin stain. Paraffin sections were also analyzed immunohistochemically for the presence of α_{2u} . Under exposure conditions that clearly induce α_{2u} -nephropathy in male F344 rats, no lesions, hyaline droplets, or α_{2u} were detectable in treated or control male NBR and female F344 rats. It is thus concluded that the presence of α_{2u} is causal to the development of renal disease in rats exposed to TMP, DCB, IP, UG, *d*-L, and lindane. © 1991 Society of Toxicology.

2,2,4-Trimethylpentane (TMP), 1,4-dichlorobenzene (DCB), isophorone (IP), unleaded gasoline (UG), and *d*-limonene (*d*-L) have all been shown to cause renal disease in male rats (Short *et al.*, 1986; NTP, 1987; NTP, 1986; Strasser *et al.*, 1988; Charbonneau *et al.*, 1987;

Short *et al.*, 1987, 1989a,b, Olson *et al.*, 1987; NTP, 1988; Kanerva and Alden, 1987; Webb *et al.*, 1989). This renal disease, known as α_{2u} -globulin nephropathy, occurs specifically in male rats. Female rats or either sex of mice, guinea pigs, dogs, or monkeys are refractory to this disease (MacEwen and Vernot, 1978; Gaworski *et al.*, 1980, 1981; Alden, 1986; Swenberg *et al.*, 1989). The acute stage of α_{2u} -globulin nephropathy is characterized by an accumulation of protein droplets in lysosomes of the proximal convoluted tubules (Alden *et al.*, 1984; Busey and Cockrell, 1984; Halder

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et al., 1985; Short *et al.*, 1986; Stone *et al.*, 1987; Johnson, 1987), subsequent degeneration and necrosis of individual epithelial cells lining the protein absorbing segment (P_2) of the proximal tubule, and regeneration of proximal tubule epithelium. These protein droplets have been shown to contain accumulations of α_{2u} -globulin (α_{2u}) using immunohistochemistry (Olson *et al.*, 1987; Burnett *et al.*, 1989). α_{2u} , a low molecular weight protein of 18,700 Da, is synthesized under androgenic control by the hepatic parenchymal cells in mature male rats (Roy *et al.*, 1966; Roy and Neuhaus, 1966; Ekstrom and Hoekstra, 1984). Important industrial and environmental chemicals and/or their metabolites including TMP, DCB, IP, and *d*-L were shown to be bound reversibly to α_{2u} after *in vivo* or *in vitro* exposure (Lock *et al.*, 1987; Charbonneau *et al.*, 1989; Strasser *et al.*, 1988; Lehman-McKeeman *et al.*, 1989), suggesting that the formation of the chemical- α_{2u} complex may be responsible for the accumulation of α_{2u} in the proximal tubule cells of exposed male rats, thus inducing the cascade of lesions previously described as being characteristic of α_{2u} -nephropathy.

In contrast to the commonly used F344 male rat, the inbred NCI-Black-Reiter (NBR) male rat does not synthesize the androgen-dependent α_{2u} (Chatterjee *et al.*, 1989). These authors proposed that the lack of androgenic induction of α_{2u} in the liver of NBR rats is the result of a tissue- and gene-specific regulatory defect, since no endocrinological or reproductive abnormalities are apparent in these animals and the mRNA for the androgen-repressible hepatic protein SMP-2 is normally regulated. Ridder *et al.* (1990) reported that NBR rats do not develop renal disease following exposure to decalin, a known α_{2u} -nephropathy-inducing agent.

Lindane, the γ -isomer of 1,2,3,4,5,6-hexachlorocyclohexane, is a widely used organochlorine pesticide. The application of lindane in soil, foliar, and seed treatment for a large variety of fruit and vegetable crops; its application on livestock, pets, and on agricultural

premises; in pharmaceutical preparations; and in public health pest control could lead to the exposure of humans to low concentrations of this compound. Various experiments were carried out in order to assess the toxicity and carcinogenicity of lindane (Reuber, 1979; Xu *et al.*, 1984). In some of these experiments, male rat specific renal toxicity was observed. This toxicity was characterized by an exacerbated formation of hyaline droplets, enlargement of lysosomes in the proximal tubules, and necrosis of proximal tubule epithelial cells (Yixang and Yunzhen, 1986; Sulik and Derogowski, 1987). These lesions appeared very similar to the lesions characteristic of α_{2u} -nephropathy.

Based on the assumption that lindane induces α_{2u} -nephropathy, the objectives of this study were to compare the response of male and female F344 and male NBR rats treated with lindane as well as to characterize the response of male NBR rats to several industrial and environmental chemicals, including TMP, DCB, IP, UG, and *d*-L, known to induce α_{2u} -nephropathy in other strains. These experiments test the hypothesis that the presence of α_{2u} is required for the development of renal disease in rats exposed to TMP, DCB, IP, UG, *d*-L, or lindane.

MATERIALS AND METHODS

Animals and housing. All male and female F344 rats (Charles River Laboratories Inc., Raleigh, NC) and male NBR rats (National Cancer Institute) were purchased at 9 weeks of age and quarantined for 2 weeks prior to the onset of the experiments. The National Research Council's guidelines for the care and use of laboratory animals were followed. The animals were housed in pairs or singly in polycarbonate shoebox cages on stainless-steel racks fitted with an inline automated water system. The cages were partially filled with Alpha-Dry paper bedding (Henly Paper Co., Raleigh, NC) which was exchanged twice weekly. Housing conditions were: humidity $65 \pm 5\%$, temperature $24 \pm 1^\circ\text{C}$, and a 12-hr light/dark cycle. Feed (Purina brand No. 5001 rat chow) and water (Durham city tap water filtered with a $5\text{-}\mu\text{m}$ filter) were given *ad libitum* during the quarantine period as well as throughout the experiments.

Experimental procedure. For every experiment, the rats were divided randomly into two groups of five to seven

animals per group, one group serving as the control, the other as the exposure group. All rats were gavaged daily for 4 consecutive days and killed 24 hr after the last dose. Controls were gavaged with corn oil (3 ml/kg, Mazola, Best Foods, CPC International, Inc., Englewood Cliffs, NJ), while exposure groups were gavaged with the respective dose of the compound dissolved in corn oil (Table 1). Test chemicals included 2,2,4-trimethylpentane (Aldrich Chemical Co., 99.8% pure, Lot No. 03215JV), 1,4-dichlorobenzene (Aldrich Chemical Co., +99% pure, Lot No. 04719KV), isophorone (Aldrich Chemical Co., 97% pure, Lot No. 01314HM), PS-6 unleaded gasoline (American Petroleum Institute), *d*-limonene (Sigma Chemical Co., +99% pure, Lot No. 115F-0094), and lindane (γ -hexachlorocyclohexane, Aldrich Chemical Co., 97% pure, Lot No. 02916HV). With the exception of lindane, all of the chemicals and doses had been shown to induce α_{2u} -nephropathy in male F344 rats in previous studies (Short *et al.*, 1986; NTP, 1987, 1986; Olson *et al.*, 1987; Short *et al.*, 1987; NTP, 1988; Kanerva and Alden, 1987).

Perfusion procedure. Twenty-four hours after the last dose, the animals were anesthetized with Metofane (Pitman-Moore Inc., Washington Crossing, NJ) and the kidneys flushed *in situ* via retrograde perfusion of the abdominal aorta with 185 mOSM (pH 7.4) sodium phosphate buffer via a rotary infusion pump (Masterflex, Cole-Parmer, Chicago, IL) until both kidneys were well drained of all blood, and then perfused with 50–100 ml of a 864 mOSM fixative solution comprised of a 2% paraformaldehyde–1% glutaraldehyde (Ted Pella Inc., Redding, CA) solution in sodium phosphate buffer (pH 7.4). Upon fixation, the kidneys were weighed, sliced in half, the halves reimmersed in fixative solution for a maximum of 24 hr, then washed with 185 mOSM sodium phosphate buffer, reimmersed in the buffer, and stored at 4°C until being processed for histology.

Histopathology. The halves of the kidneys used for light microscopic examination were embedded in glycol-methacrylate (GMA) and paraffin and were sectioned at 2 and 4–5 μ m, respectively. GMA sections were stained with Lee's methylene basic blue fuchsin (LMBBF), whereas the paraffin sections were stained with H&E and the Mallory-Heidenhein (MH) protein stain. An α_{2u} -specific immunostain was used to identify and locate α_{2u} in GMA and paraffin sections.

Immunohistochemical localization of α_{2u} -globulin. The staining procedure used was a modification of the plastic-embedded tissue method published by Burnett *et al.* (1989). This method was adapted to paraffin-embedded tissue sections. In brief the procedure entailed the following steps: The sections were deparaffinized in xylene and the xylene was removed by dipping the sections in ethanol of decreasing concentrations: 100, 95, and 70% EtOH. The sections were dried and a circle was etched around each section with a PAP-pen (Newcomer Supply, Oak Park, IL). The endogenous tissue peroxidase was blocked (1 ml 30% H₂O₂ in 15 ml methanol) by immersing the sections in H₂O₂–

TABLE 1

DESIGN OF EXPERIMENTS PERFORMED WITH 11-WEEK-OLD MALE NBR AND MALE AND FEMALE F344 RATS

Compound/control	Dose (mg/kg/day)	Strain/sex
2,2,4-TMP	500	NBR/male
Control	—	NBR/male
1,4-DCB	500	NBR/male
Control	—	NBR/male
Isophorone	1000	NBR/male
Control	—	NBR/male
Unleaded gasoline	500	NBR/male
Control	—	NBR/male
<i>d</i> -Limonene	1650	NBR/male
Control	—	NBR/male
Lindane	10	NBR/male
Control	—	NBR/male
Lindane	10	F344/male
Control	—	F344/male
Lindane	10	F344/female
Control	—	F344/female

methanol for 8 min. The sections were then washed with 95% ethanol and rehydrated in 10% Biomed Automation buffer for 90 sec (Biomed No. M30, Biomed Corp., Foster City, CA). Three drops of trypsin were put onto each section (0.1% trypsin [Sigma Type III Bovine Pancreas No. T-8253] in 10% Biomed buffer with 0.1% CaCl₂) and allowed to stand for 20 min, and then the slides were washed in buffer three times. Subsequent instructions followed those outlined in Biomed Antibody No. BM 06-601B (Autoprobe 150 kit). Biomed primer was placed on the sections for 20 min to reduce unspecific staining (primer: 5 drops of tissue conditioner Biomed in 5 ml of 10% Biomed buffer) and then blotted off. The slides were not rinsed! The sections were incubated with the primary antibody (1:100 dilution) for 1 hr, rinsed with buffer, incubated with Biomed peroxidase for 30 min (which includes horseradish peroxidase conjugated to antibodies which will detect primary rabbit, mouse, rat, and guinea pig antibodies), and again washed in buffer. Chromogen Biomed-Mix was applied to the sections, two times for 15 min each. The slides were rinsed in double-distilled (dd) H₂O and the sections counterstained with Biomed hematoxylin for 3 min. The slides were rinsed in dd H₂O four to five times and air-dried. As soon as the sections were dry, aqueous mounting medium (Biomed No. M02) was put onto the sections and let dry in the oven at 60°C for 20–40 min. The slides were then dipped in xylene to get rid of excess mounting medium and coverslipped using Permount (Fisher Scientific No. SP15-100).

The H&E stained section from each animal was examined for histopathological changes. Each section stained

immunohistochemically with the α_{2u} immunostain was assigned a score based on the intensity and area of positive staining, the scores being: -, negative; +, present; ++, mild; +++, moderate; +++++, severe; ++++++, markedly severe. The sections stained with LMBBF or M-H were assigned a protein droplet score using the same grading scale as for the α_{2u} immunostains. This grading scale was based on the number and size of protein droplets as well as on the number of tubules containing protein droplets.

Binding of lindane and/or lindane metabolites to low molecular weight proteins of the kidney: Preliminary experiments. In an effort to elucidate the metabolite(s) of lindane that bind to α_{2u} , the left kidneys of animals treated with lindane were excised during perfusion with the buffer solution, 33% kidney homogenates (67 mM KPO_4 buffer, pH 7.2) were prepared, and the low molecular weight proteins including α_{2u} isolated using the procedure described by Lock *et al.* (1987). In order to achieve measurable concentrations of lindane and its metabolites, the kidney homogenates of five animals per sex, strain, and treatment or control group were combined. Lindane and its metabolites were extracted from the low molecular weight protein fractions into CH_2Cl_2 , then analyzed and identified via gas chromatography coupled with a VG 70-250 SEQ hybrid mass spectrometer (GC-MS) using electron impact.

RESULTS

α_{2u} was readily detected in the kidney sections of all male F344 rats (Figs. 1A and 3A). The male F344 rats treated with lindane exhibited a marked increase in α_{2u} -staining area and intensity in comparison to the control male F344 and the treated and control male NBR rats (Table 2; Figs. 1A, 2A, 3A, and 4A). The pathological changes observed in the H&E stains of the lindane treated male F344 rats were similar to those seen in the LMBBF stained slides and occurred in the same regions of the kidney cortex which stained positive for α_{2u} . These changes were observed exclusively in the proximal tubule epithelial cells and were characterized by an exacerbated accumulation of protein droplets, various stages of hypertrophy and necrosis, pyknotic and karyolytic nuclei, exfoliation of epithelial cells, and the presence of regenerative epithelium (Fig. 3B). The protein droplets were large, round or polyangular, had a crystalline appearance, and sometimes filled the entire cytoplasm of the epithelial cells. Significantly more protein had accumulated in the renal cortex of the lindane-

treated male F344 rats than in the control male F344 rats (Table 2). The protein droplets observed in the control animals were very small and round, rarely with a crystalline appearance, and were not associated with pathological changes (Fig. 1B).

In contrast, the renal sections from all of the NBR rats, whether treated with TMP, DCB, IP, *d*-L, lindane, or controls, were negative in the α_{2u} immunostain (Figs. 2A, 4A, and 5A; Table 2). No lesions were detected in any of the NBR kidney sections stained with H&E or LMBBF. While very small protein droplets were observed in the LMBBF stained sections of all NBR rats, irrespective of whether they were control or treated animals (Figs. 2B, 4B, and 5B), only slight diffuse staining was observed in the sections stained with M-H.

In contrast to reports from other laboratories, results from this study consistently demonstrate that the LMBBF stain was superior to the M-H stain. This discrepancy may be due to interlaboratory variability in M-H staining. However, while crisp protein droplets could be observed even in the NBR rats using the LMBBF stain (Figs. 2B, 4B, and 5B), the M-H stain obtained by this laboratory reacted only with a very diffuse light staining, despite the increased clarity offered by the perfused kidney sections. Even in the lindane treated male F344 rats, only the largest protein droplets could be detected with the M-H stain. Accumulations of multiple small protein droplets within the same epithelial cell resulted in diffuse staining of the whole cell (not shown). The higher resolution of the LMBBF stained slides is largely due to the 2- μ m-thin sections achieved by GMA embedding of the tissue (Short *et al.*, 1987, 1989a; Burnett *et al.*, 1989). Thus non- α_{2u} protein droplets could be detected with the LMBBF stain, corroborating earlier reports by Short *et al.* (1989a). Despite the lower resolution of the M-H stained specimens, they were capable of demonstrating the difference in protein accumulation between the different strains of rats and treatment groups.

FIG. 1. Kidney sections of a control F344 male rat (magnification $\times 65$). (A) $\alpha_2\mu$ immunostain. Note the smaller area and lower intensity of immunostaining in comparison to the lindane-treated F344 male rat (Fig. 3A). (B) LMBBF stain. Note the smaller size and the different shape of the protein droplets ($\rightarrow$) in comparison to the droplets observed in the lindane-treated F344 male rat (Fig. 3B).

TABLE 2

IMMUNO- AND HISTOPATHOLOGICAL RESULTS OF STAINED KIDNEY SECTIONS OF MALE NBR RATS TREATED WITH TMP, DCB, IP, UG, *d*-L, AND LINDANE AND OF MALE AND FEMALE F344 RATS TREATED WITH LINDANE

Compound/ control	Strain/sex	α_{2u} stain ^a	LMBBF ^b
TMP	NBR/male	—	+
Control	NBR/male	—	+
DCB	NBR/male	—	+
Control	NBR/male	—	+
IP	NBR/male	—	+
Control	NBR/male	—	+
UG	NBR/male	—	+
Control	NBR/male	—	+
<i>d</i> -L	NBR/male	—	+
Control	NBR/male	—	+
Lindane	NBR/male	—	+
Control	NBR/male	—	+
Lindane	F344/male	+++++	+++++
Control	F344/male	++	++
Lindane	F344/female	—	+
Control	F344/female	—	+

Note. Grade scale for ^a area and intensity of α_{2u} immunostaining or ^b area, number, and size of protein droplets observed in the LMBBF stain: — negative, + present, ++ mild, +++ moderate, ++++ severe, +++++ marked severe.

No α_{2u} was detected in the kidney sections of lindane-treated and control female F344 rats (Table 2), and no lesions were observed in any of the kidney sections stained with H&E or LMBBF (data not shown). However, as reported above for male NBR rats, very small protein droplets were detected in the sections stained with LMBBF, irrespective of whether they were control or treated animals (Table 2). M-H staining did not detect these protein droplets.

The GC-MS analysis of kidney low molecular weight protein extracts of male F344 rats treated with lindane revealed the presence of lindane and its metabolites 1,2,3-trichlorobenzene (TCB), 1,2,3,4-tetrachlorobenzene (TeCB), and hexachlorocyclo-1-hexene (data not shown). Only trace to nondetectable amounts of lindane, but no metabolites of lin-

dane, were found in the low molecular weight protein extracts of lindane-treated male NBR and female F344 rats.

DISCUSSION

The present study clearly shows that treatment of NBR male rats with compounds and doses known to cause renal disease in male F344 rats, as well as treatment with lindane, does not elicit any pathological response in the kidneys of these animals. Our findings on TMP, DCP, IP, UG, *d*-L, and lindane corroborate earlier findings by Ridder *et al.* (1990) on decalin. As expected, female F344 rats did not have renal disease following treatment with lindane. This lack of response is comparable to those reported following treatment of female rats with *d*-L, UG, TMP, and decalin (Webb *et al.*, 1989; Short *et al.*, 1987, 1989a,b; Stone *et al.*, 1987). Male F344 rats, on the other hand, responded to treatment with lindane with an exacerbated accumulation of α_{2u} in the proximal convoluted tubules of the kidney and the characteristic pathological changes that are associated with its nephropathy. These changes are comparable to the lesions reported for Sprague-Dawley or F344 rats following treatment with TMP, IP, DCB, UG, *d*-L, or decalin (Short *et al.*, 1986, 1987, 1989a,b; Olson *et al.*, 1987; Kanerva and Alden, 1987; Kanerva *et al.*, 1987a,b; Swenberg *et al.*, 1989). In addition, lindane and its metabolites (TCB and TeCB) were found in the low molecular weight protein extract of lindane-treated male F344 rats, while only trace to nondetectable amounts of lindane or metabolites were found in lindane-treated female F344 and male NBR rats. The shape, volume, and lipophilic characteristics of the isomers of TCB and TeCB, as well as those of lindane, are similar to structure activity relationships for other chemicals that cause α_{2u} -nephropathy (Borghoff *et al.*, 1991; Dietrich and Swenberg, 1990; Aaron Miller, personal communication). These observations lead to the conclusion that lindane exacerbates the accumulation of α_{2u} and in-

FIG. 2. Kidney sections of a control NBR male rat (magnification $\times 65$). (A) α_{2u} immunostain. (B) LMBBF stain. Note the very small droplets ($\rightarrow$).

FIG. 3. Kidney sections of a F344 male rat treated with 10 mg lindane/kg/day (magnification $\times 65$). (A) α_{2u} immunostain. Note the large area and the high intensity of immunostaining. (B) LMBBF stain. Note the large crystalline droplets ($\Rightarrow$), exfoliation of tubular cells ($\bullet$), various stages of cell necrosis ($\rightarrow$), and regeneration ($\blacktriangleright$) of epithelial cells in the P_2 segments of the proximal tubules.

FIG. 4. Kidney sections of a NBR male rat treated with 10 mg lindane/kg/day (magnification $\times 65$). (A) $\alpha_2\mu$ immunostain. (B) LMBBF stain. Note the very small droplets ($\rightarrow$).

FIG. 5. Kidney sections of a NBR male rat treated with 1000 mg isophorone/kg/day (magnification $\times 65$). (A) α_{2u} immunostain. (B) LMBBF stain. Note the very small droplets ($\rightarrow$).

duces renal disease in a similar fashion as was shown previously for TMP, DCP, IP, UG, decalin, and *d*-L.

NBR male rats, like female rats of all strains, do not synthesize the androgen-dependent α_{2u} that is present in large amounts in other strains (Chatterjee *et al.*, 1989). The lack of α_{2u} in kidneys of treated male NBR and female F344 rats confirms the necessity for the presence of large amounts of this protein for developing α_{2u} -nephropathy. The small protein droplets observed at the highest magnification in the GMA-embedded LMBBF-stained slides of the control and treated male NBR and female F344 rats represent normally occurring, non- α_{2u} protein droplets present in the cytoplasm of the P₂ segment epithelial cells of the proximal convoluted tubules that result from protein resorption via endocytosis (Maack *et al.*, 1985; Short *et al.*, 1989a). The lack of any pathological response, specifically the lack of any increase of non- α_{2u} protein droplets in treated male NBR and female F344 rats suggests that no other protein, including other members of the α_{2u} superfamily, is involved in the renal disease induced by TMP, DCB, IP, UG, *d*-L, decalin, and lindane. Similar conclusions were drawn in an earlier study after two-dimensional electrophoresis profiles of kidney cortex samples from decalin-treated NBR rats failed to demonstrate an accumulation of either α_{2u} or non- α_{2u} proteins (Ridder *et al.*, 1990).

The absence of α_{2u} in humans, primates, dogs, and mice (Alden, 1986) suggests that the chemically induced renal disease is unique to rats. Supporting this hypothesis, treatment of either sex of mice, hamster, guinea pigs, dogs, or nonhuman primates with compounds inducing α_{2u} -nephropathy has not elicited pathological responses in the kidneys of these animals and did not result in renal disease (Swenberg *et al.*, 1989). Furthermore, epidemiological studies of humans with prolonged exposure to petroleum-derived hydrocarbons, known to induce nephropathy in male rats (reviewed by Alden, 1986), do not show an

increase in morbidity or mortality due to renal pathology (Pitha *et al.*, 1987).

Several proteins sharing amino acid sequence homology with α_{2u} have been identified in the serum and urine of various species including humans (Pervaiz and Brew, 1987; Akerstroem *et al.*, 1987; Pevsner *et al.*, 1988). The presence of these partially homologous proteins in humans raised concern as to the possible interaction of these low molecular weight proteins with α_{2u} -nephropathy-inducing agents, thus questioning the male rat specificity of α_{2u} -nephropathy. Assuming that a homologous protein reversibly binds the aforementioned chemicals, the protein needs to be excreted into the plasma in large amounts, freely filtered by the glomerulus, be readily reabsorbed in the proximal tubules, catabolized at a slow rate in the lysosomes of the proximal tubule epithelial cells, and show an even lower catabolism rate upon binding one of the chemicals in order to induce lesions similar to α_{2u} -nephropathy. Olson *et al.* (1990) approached a part of this question by comparing the urinary proteins of the male rat and the male human and their urinary concentrations. Based on estimated daily average urine production and body weights of rats and humans, they showed that rats excrete approximately 90 times more total protein than humans. Of the total protein excreted, the predominant fraction in rats consisted of low molecular weight proteins (18 kDa), whereas a predominance of high molecular weight proteins (66 kDa) was found in humans. The small amount of low molecular weight protein excreted by male humans was identified as α_1 -acid glycoprotein, α_1 -microglobulin, myoglobin, and β_2 -microglobulin. Of these four proteins only α_1 -acid glycoprotein and α_1 -microglobulin share amino acid sequence homology with α_{2u} (Pervaiz and Brew, 1987; Pevsner *et al.*, 1988). α_1 -Acid glycoprotein (AGP) and α_1 -microglobulin (AMG) are synthesized in the livers of rats and humans (Ricca and Taylor, 1981; Gross *et al.*, 1987, 1988; Akerstroem and Landin, 1985), have been purified from the urine of rats and humans, as well as the

urine of rabbits and guinea pigs in the case of AMG (Akerstroem *et al.*, 1987; Olson *et al.*, 1990), and thus permit a direct comparison with α_{2u} .

If AGP and AMG reversibly bind the aforementioned chemicals and/or their metabolites with the same affinity as α_{2u} , then one would expect that female rats, male NBR rats, rabbits, and guinea pigs would also develop renal disease following treatment with these chemicals. However, as shown in this and in earlier studies, male NBR rats, female rats, and guinea pigs do not accumulate protein in the renal cortex following treatment with α_{2u} -nephropathy-inducing agents and thus are refractory to this disease (MacEwen and Vernot, 1978; Gaworski *et al.*, 1980, 1981; Alden, 1986; Swenberg *et al.*, 1989; Ridder *et al.*, 1990). In addition, epidemiological studies of humans with prolonged exposure to petroleum-derived hydrocarbons, known to induce nephropathy in male rats (reviewed by Alden, 1986), do not show an increase in morbidity or mortality due to renal pathology (Pitha *et al.*, 1987).

Thus it seems reasonable to assume that despite the presence of measurable amounts of low molecular proteins sharing amino acid sequence homology with α_{2u} , humans seem unlikely to develop chemically induced renal disease; and it would appear that the chemically induced renal disease, associated with α_{2u} accumulation, is a disease unique to male rat strains expressing α_{2u} .

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