First publ. in: Mechanisms of carcinogenesis in risk identification / ed. by H. Vainio ...
Lyon, Internat. Agency for Research on Cancer, 1992, pp. 477-500

Species-specific mechanisms of carcinogenesis

J.A. Swenberg[1], D.R. Dietrich[1], R.M. McClain[2] & S.M. Cohen[3]

[1]*Departments of Environmental Sciences and Engineering and Pathology, The University of North Carolina at Chapel Hill, Chapel Hill, NC*; [2]*Department of Toxicology, Hoffman-La Roche, Inc., Nutley, NJ*; *and* [3]*Department of Pathology, The University of Nebraska Medical Center, Omaha, NE, USA*

Introduction

While there is excellent concordance between the results of animal and human studies on known human carcinogens, the data are much less consistent for known animal carcinogens. There could be many reasons for this. First, there may have been inadequate study or length and magnitude of human exposure to demonstrate human susceptibility. A second major factor is the difference in the proportion of genotoxic and nongenotoxic agents among known human and animal carcinogens: the vast majority of known human carcinogens are genotoxic. A recent survey by Shelby (1988) showed that 20 of 23 known human carcinogens were genotoxic. A similar proportion of the expanded IARC list of human carcinogens (IARC, 1987) is genotoxic (Shelby & Zeiger, 1990). In contrast, roughly half of the much larger number of rodent carcinogens are nongenotoxic (Ashby & Tenant, 1991). It is difficult to imagine that for only a few or none of these chemicals has there been adequate human exposure or epidemiological investigation to demonstrate human carcinogenicity. Unlike their genotoxic counterparts, the nongenotoxic agents usually induce neoplasia in rodents at maximum tolerated doses and rarely at doses one order of magnitude lower. Exceptions, such as tumour promotion by 2,3,7,8-tetrachlorodibenzo-*para*-dioxin, have been shown to work through a receptor-based mechanism.

A principal mechanism for several nongenotoxic carcinogens is increased cell proliferation. While it has long been recognized that cell proliferation is required for both mutagenesis and carcinogenesis, its role is beginning to be better understood. Cell proliferation is required to 'fix' DNA damage into mutations, whether that damage is chemically induced by a genotoxic agent or spontaneous. Such spontaneous DNA damage is common, with 50 000 to 250 000 such events occurring per cell per day (Loeb, 1989; US National Research Council, 1989). Most of these events are rapidly and efficiently repaired; when cell proliferation is increased, however, there is less time available for repair prior to DNA replication. Cell proliferation is also required for clonal expansion of initiated cells, a mechanism that enhances the probability of additional genetic events taking place. These

same mechanisms markedly enhance the carcinogenicity of genotoxic agents at doses that increase cell proliferation (Swenberg *et al.*,1990).

Enhanced cell proliferation increases the risk of cancer in rodents and humans (Preston-Martin *et al.*, 1990). The agents and mechanisms responsible for increased cell proliferation are often species-specific or limited to high exposures. Thus, accurate prediction of the risk to humans presented by these agents requires a detailed understanding of their mechanism and dose–response relationships. This paper presents current understanding of three species-specific mechanisms of carcinogenesis: α-2μ-globulin nephropathy and carcinogenesis, thyroid stimulationg hormone (TSH)-mediated thyroid tumours, and bladder carcinogens associated with crystal and calculi formation. Many other examples could be cited; however, these represent three of the best characterized models currently available.

α-2μ-*Globulin nephropathy and carcinogenesis*

α-2μ-Globulin nephropathy is a well characterized disease that occurs only in male rats: Female rats and mice, guinea-pigs, hamsters, dogs and monkeys of either sex do not develop this disease when exposed to agents that cause it in male rats (Halder *et al.*, 1985; Alden, 1986; Swenberg *et al.*, 1989; Borghoff *et al.*, 1990). Likewise, there are no data to suggest that these agents cause protein droplet accumulation in the kidneys of humans. Research from several laboratories has elucidated the mechanism responsible for this remarkable sex- and species-specific disease.

The acute disease is characterized by the accumulation of α-2μ-globulin in lysosomes of the P2 segment of the nephron, single-cell necrosis, formation of granular casts at the junction of P3 and the thin loop of Henle, compensatory cell proliferation and the presence of regenerative tubules (Halder *et al.*, 1985; Alden, 1986; Swenberg *et al.*, 1989; Borghoff *et al.*, 1990). Following chronic exposure to agents that cause α-2μ-globulin nephropathy, there is exacerbation of these lesions, linear mineralization of the renal pelvis and induction of a 0–25% incidence of renal-cell tumours. As mentioned above, neither female rats nor mice of either sex exhibit renal disease when exposed similarly.

α-2μ-Globulin is a low-molecular-weight protein of 18 700 Da that is synthesized under androgenic control by the hepatic parenchymal cells of mature male rats, secreted into the blood, freely filtered through the glomerulus, partially resorbed by the P2 segment of the nephron and excreted into the urine in large amounts (3-19 mg/day) (Roy & Neuhaus, 1966; Roy *et al.*, 1966; Vandoren *et al.*, 1983; Ekstrom & Hoekstra, 1984). α-2μ-Globulin is also found in the urine of female rats; however, the concentrations are 107 to 680-fold lower than that found in male rats (Vandoren *et al.*, 1983). This sex-specific difference is based on a lack of synthesis of the androgen-dependent hepatic form of α-2μ-globulin in female rats.

Mechanisms of α-2μ-globulin nephropathy and carcinogenesis

The chemicals known to cause this disease and the extent of mechanistic data available for each are listed in Table 1. These chemicals or their metabolites can bind reversibly to α-2μ-globulin (Lock *et al.*, 1987; Strasser *et al.*, 1988; Charbonneau *et al.*, 1989; Lehman-McKeeman *et al.*, 1989). Even though the agents fall into rather diverse chemical classes, molecular modelling studies have demonstrated strong structure-activity relationships (Borghoff *et al.*, 1991). Active chemicals fit deeply into a hydrophobic pocket of α-2μ-globulin. When hydrogen bonding between the chemical and protein can occur, the

Table 1. Data on chemicals that cause α-2μ-globulin nephropathy[a]

Substance/chemical	Protein droplets	Increased α-2μ-globulin	α-2μ-Globulin binding	Cell proliferation	Initiation/ promotion
d-Limonene	+	+	+	+	+
Unleaded gasoline	+	+	+	+	+
2,2,4-Trimethylpentane	+	+	+	+	+
1,4-Dichlorobenzene	+	+	+	+	NR
Isophorone	+	+	+	+	NR
3,5,5-Trimethyl hexanoic acid derivatives	+	+	+	NR	NR
Decalin	+	+	NR	+[b]	NR
Tetrachloroethylene	+	+	NR	+	NR
Pentachloroethane	+	+	NR	+	NR
C_{10}–C_{12} isoparaffinic solvent (saturated aliphatic hydrocarbons)	+	+	NR	NR	NR
Lindane	+	+	NR	NR	NR
BW540C	+	+	NR	NR	NR
BW58C	+	+	NR	NR	NR
Levamisol	+	+	NR	NR	NR
Gabapentin	+	+	NR	NR	NR
Tridecyl acetate	+	+	NR	NR	NR
Isopropylcyclohexane	+	+	NR	NR	NR
JP-5 jet fuel (mixed distillate hydrocarbons)	+	NR	NR	NR	NR
JP-4 jet fuel (mixed distillate hydrocarbons)	+	NR	NR	NR	NR
Diesel fuel, marine	+	NR	NR	NR	NR
JP-10 synthetic jet fuel (exohexahydro-4,7-methanoindan)	+	NR	NR	NR	NR
RJ-5 synthetic jet fuel (hydrogenated dimers of norbornadiene)	+	NR	NR	NR	NR
JP-7 distillate jet fuel	+	NR	NR	NR	NR
JP-TS distillate jet fuel	+	NR	NR	NR	NR
Stoddard solvent	+	NR	NR	NR	NR
Tetralin	+	NR	NR	NR	NR
Hexachloroethane	+	NR	NR	NR	NR

Table 1 (contd)

Substance/chemical	Protein droplets	Increased α-2μ-globulin	α-2μ-Globulin binding	Cell proliferation	Initiation/ promotion
Dimethyl methylphosphonate	+	NR	NR	NR	NR
Methyl isobutyl ketone	+	NR	NR	NR	NR
Methyl isoamyl ketone	+	NR	NR	NR	NR
Diisobutyl ketone	+	NR	NR	NR	NR
1,3,6-Tricyanohexane	+	NR	NR	NR	NR

[a]Adapted from Appendix 1, US Environmental Protection Agency (1991); NR, not reported
[b]Based on cell counts in urine

digestibility of α-2μ-globulin by proteases is inhibited, leading to accumulation of the male rat-specific protein in lysosomes of the P2 segment of the nephron (Lehman-McKeeman *et al.*, 1990). Hyaline droplets can also be produced by injecting female rats intraperitoneally with α-2μ-globulin (Ridder *et al.*, 1990). This accumulation of α-2μ-globulin is cytotoxic and results in single-cell necrosis. The exfoliated renal epithelium, which represents the nidus for granular cast formation, is restored by compensatory cell proliferation.

The increase in cell proliferation is localized in the P2 segment of the nephron and to a much lesser extent in the P3 segment (Short et al., 1986, 1987, 1989a). Increased cell proliferation can be demonstrated readily using pulse (Short *et al.*, 1986) or continuous (Short *et al.*, 1987, 1989a; Dietrich & Swenberg, 1991a) administration of ^{3}H-thymidine or bromodeoxyuridine; it can be detected as early as three days after exposure to α-2μ-globulin-inducing agents; and it has been demonstrated to remain elevated through at least 50 weeks of exposure (Short *et al.*, 1989a). The extent of this increase in proliferation is dose-related, maximum tolerated doses resulting in 5- to 12-fold greater numbers of labelled cells (Short *et al.*, 1987, 1989a). Clear no-effect doses can be demonstrated (Short *et al.*, 1986, 1987, 1989a). Of great importance is the demonstration that female rats that have been exposed identically have no increase in cell proliferation (Short *et al.*, 1989a). While this strongly suggests that the increase in cell proliferation requires the presence of large amounts of α-2μ-globulin, a recent comparison of cell proliferation in d-limonene-exposed Fischer 344 and NBR male rats showed that the protein is absolutely required (Dietrich & Swenberg, 1991a). NBR rats are the only identified strain that does not synthesize the androgen-dependent form of α-2μ-globulin (Chatterjee *et al.*, 1989). Whereas Fischer 344 rats exposed to d-limonene exhibited a five-fold increase in cell proliferation after 5 or 30 weeks of exposure to 150 mg/kg per day, NBR rats were unaffected (Dietrich & Swenberg, 1991a). Both strains metabolized d-limonene to the 1,2-oxide, the nongenotoxic metabolite that binds reversibly to α-2μ-globulin (Watabe *et al.*, 1980; Dietrich & Swenberg, 1991a). The increase in cell proliferation associated with α-2μ-globulin nephropathy is reversible. Following exposures of up to three weeks to 2,2,4-trimethylpentane or unleaded gasoline, proliferation rates returned to the control level within one week after cessation of exposure (Short *et al.*, 1989a). Longer-term exposures result in a slower return to control rates. Morphological evidence of regenerative tubules can still be identified four weeks after subchronic exposure ceases (Halder *et al.*, 1984).

This sustained increase in cell proliferation is capable of promoting spontaneously or chemically initiated cells of the proximal tubule to form preneoplastic and neoplastic lesions (Short *et al.*, 1989b; Dietrich & Swenberg, 1991a). The promoting activity is totally dependent on the presence of α-2μ-globulin. Concentration-related increases in the incidence of preneoplastic and neoplastic renal lesions were evident in male Fischer 344 rats initiated with *N*-nitrosoethyl-*N*-hydroxyethylamine (NEHE) and promoted with unleaded gasoline or 2,2,4-trimethylpentane (Short *et al.*, 1989b); no increase occurred in females. The promoting activity paralleled increases in cell proliferation (Short *et al.*, 1989a). When NBR rats were initiated with NEHE and promoted with d-limonene, no increase in the incidence of atypical tubules, atypical hyperplasia or renal adenomas occurred (Dietrich & Swenberg, 1991a). In contrast, Fischer 344 rats promoted with d-limonene developed more atypical tubules and atypical hyperplasia, while Fischer 344 rats initiated with NEHE and promoted with

d-limonene developed more atypical tubules, atypical hyperplasias and renal adenomas (Dietrich & Swenberg, 1991a). Promotion of preneoplastic or neoplastic lesions occurred only in groups that also exhibited increased cell proliferation. An important observation in this study was the presence of occasional preneoplastic lesions in the kidneys of control rats of both strains, as these lesions are thought to represent early forms of spontaneous kidney tumours. The incidence of these lesions was increased by exposure to NEHE in both strains and by exposure to d-limonene alone in Fischer 344 rats. These data strongly suggest that agents that cause α-2μ-globulin nephropathy induce renal tumours in male rats through sustained increases in cell proliferation. The higher rate of cell proliferation decreases the amount of time available to repair DNA damage, increasing the probability that mutations will lead to spontaneously initiated renal epithelial cells and promoting clonal expansion of such cells, thereby increasing the probability of neoplasia.

Additional evidence for the sex and species specificity of this syndrome comes from studies on levamisole. Levamisole, a drug used as an antihelminthic, in cancer chemotherapy and in the treatment of rheumatoid arthritis in humans, causes α-2μ-globulin nephropathy in male rats (Read *et al.*, 1988). No increase in the level of urinary *N*-acetyl ß-glucosaminidase, an indicator of nephrotoxicity, was seen in patients who received 150 mg levamisole per day for 26 weeks (Dieppe *et al.*, 1978). Levamisole has not yet been studied for carcinogenicity in animals or humans.

Several of the chemicals that induce α-2μ-globulin-related kidney tumours in male rats also cause tumours at other sites. 1,4-Dichlorobenzene, pentachloroethane, tetrachloroethylene and unleaded gasoline cause hepatocellular neoplasms in mice (US National Toxicology Program, 1983, 1986a, 1987a; IARC, 1989); administration of tetrachloroethylene was also associated with an increased incidence of leukaemia in rats (US National Toxicology Program, 1986a). The mechanisms responsible for the induction of tumours at these sites are not known at this time.

Not all agents that induce α-2μ-globulin nephropathy result in an increased incidence of kidney tumours in male rats. In some cases, this absence of effect has been due to inadequate length of exposure. For example, a series of hydrocarbons was evaluated in rats exposed for 90 days and held for an additional 19 months (Bruner, 1984, 1986). None of the hydrocarbons increased the incidence of renal tumours. When rats were exposed to hydrocarbons for one or more years, however, tumours were induced in the kidneys of males. The incidence of renal tumours induced by the α-2μ-globulin mechanism is much lower than that which can be achieved by genotoxic renal carcinogens administered at their maximal tolerated doses. Furthermore, the extent of mechanistic data available for different agents varies markedly (see Table 1). Thus, while the weight of evidence supporting the sex- and species-specific mechanism for this class of agents is very strong, the relevance of the mechanism for the carcinogenic activity of a specific chemical must be determined on a case-by-case basis.

Species differences in urinary proteins

Having established that the presence of α-2μ-globulin is mandatory for the formation of kidney tumours in male rats following treatment with α-2μ-globulin nephropathy-inducing agents, the question arises whether extrapolation of such data on carcinogenicity to other species, including humans, is warranted. Most compounds that are carcinogenic in animals are generally assumed to pose some risk to humans. In the case of α-2μ-globulin nephro-

pathy-inducing agents, the carcinogenic mechanism is clearly associated with the presence of a specific urinary protein (α-2μ-globulin) not found in humans or any other species. Several proteins that share some amino acid sequences with α-2μ-globulin have been identified in the serum and urine of various species, including humans (Pervaiz & Brew, 1987; Akerstroem *et al.*, 1987; Pevsner *et al.*, 1988), and the presence of such partially homologous proteins in humans raised concern that these low-molecular-weight proteins might interact with α-2μ-globulin nephropathy-inducing agents, thus putting into question the specificity of α-2μ-globulin nephropathy for male rats. Assuming that a homologous protein binds the aforementioned chemicals reversibly, the protein must be excreted into the plasma in large amounts, freely filtered by the glomerulus, readily reabsorbed into the proximal tubules and catabolized in the lysosomes of the proximal tubule epithelial cells at a slower rate than normal after binding one of the chemicals in order to induce lesions similar to those of α-2μ-globulin nephropathy. On the basis of the estimated daily average production of urine and the average body weights of rats and humans, Olson *et al.* (1990) 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-μ-globulin, myoglobin and β_2-μ-globulin. Of these four proteins, only α_1-acid glycoprotein and α_1-μ-globulin share amino acid sequences with α-2μ-globulin (Akerstroem *et al.*, 1987; Pervaiz & Brew, 1987; Pevsner *et al.*, 1988). α_1-Acid glycoprotein and α_1-μ-globulin are synthesized in the livers of rats and humans (Ricca & Taylor, 1981; Akerstroem & Landin, 1985; Gross *et al.*, 1987, 1988) and have been purified from the urine of rats and humans, as well as from the urine of rabbits and guinea-pigs in the case of α_1-μ-globulin (Akerstroem *et al.*, 1987; Pevsner *et al.*, 1988; Olson *et al.*, 1990); they can thus be compared directly with α-2μ-globulin.

If α_1-acid glycoprotein and α_1-μ-globulin reversibly bind α-2μ-globulin nephropathy-inducing chemicals and/or their metabolites with the same affinity as α-2μ-globulin, one would expect that female rats, male NBR rats, rabbits and guinea-pigs would also develop renal disease following treatment with these chemicals. Male NBR rats, female rats and guinea-pigs, however, do not accumulate protein in the renal cortex following treatment with α-2μ-globulin nephropathy-inducing agents and are thus refractory to this disease (MacEwen & Vernot, 1978; Gaworski *et al.*, 1980, 1981; Alden, 1986; Swenberg *et al.*, 1989; Ridder *et al.*, 1990; Dietrich & Swenberg, 1991a,b). In addition, mice, which excrete comparable amounts of the low-molecular-weight mouse urinary protein with the closest amino acid sequence homology to α-2μ-globulin (approximately 90%; Borghoff *et al.*, 1990), do not develop the protein-related nephropathy or renal tumours following chronic exposure to d-limonene and other α-2μ-globulin nephropathy-inducing agents (US National Toxicology Program, 1983, 1986a,b, 1987a,b; Short *et al.*, 1989b; US National Toxicology Program, 1990).

Recently, a sex-linked human protein of similar size was identified in urine from patients with renal disease (Bernard *et al.*, 1989, 1990); it was named urine protein 1 and has been called the human equivalent of α-2μ-globulin (Bernard *et al.*, 1989). This reference has led to considerable confusion and miscitation. Jackson and Turner (1988) purified and partially

sequenced human urine protein 1 and determined that it is related to rabbit uteroglobin, not α-2μ-globulin. Urine protein 1 does not bind d-limonene-1,2-oxide or 2,4,4-trimethyl-2-pentanol, metabolites of chemicals that bind to α-2μ-globulin. It is also present in human urine at concentrations four to five orders of magnitude lower than that of α-2μ-globulin in male rat urine (US Environmental Protection Agency, 1991).

Epidemiology

No systematic epidemiological study has been carried out on nephropathy in populations with elevated exposures to industrial chemicals that are known to induce binding to α-2μ-globulin. The only human situations studied in which exposure to these chemicals occurs are associated with gasoline. On the basis of the six available studies, an IARC working group classified the human evidence for the carcinogenicity of gasoline as 'inadequate' (IARC, 1989).

An important limitation of most of the epidemiological studies of renal cancer and exposure to gasoline is that exposures to hydrocarbon were not assessed quantitatively. In many of the studies, exposure assessment did not go beyond ascertaining that an individual had been employed by a petroleum company or in a refinery. An additional complication of judging the relevance of these studies to the α-2μ-globulin mechanism is that the exposures are to complex mixtures. Two of the studies (McLaughlin, 1984; Siemiatycki *et al.*, 1987) indicate small increases in relative risk for kidney cancer in subgroups potentially exposed to gasoline. A recently published study (Partanen *et al.*, 1991) found an elevated risk and an exposure-response relationship between kidney cancer and exposure to gasoline. These studies are summarized below.

McLaughlin (1984) found an elevated odds ratio (OR) for occupational exposure to 'petroleum, tar, and pitch products' (OR, 1.7; 95% confidence interval [CI], 1.0–2.9) in men, after adjusting for the confounding factors of age and cigarette smoking. In a subsequent, more detailed analysis of this grouping (McLaughlin *et al.*, 1985), no overall association (OR, 1.0; 95% CI, 0.7–1.4) was observed between renal-cell cancer and employment in a range of occupations with potential exposure to petroleum products. In a trend analysis, a slight risk among service station attendants was associated with duration of employment (OR, 1.2; 95% CI, 0.6–2.3). The most consistent finding in these studies (McLaughlin *et al.*, 1983; McLaughlin & Schuman, 1983; McLaughlin, 1984) was an association with cigarette smoking.

Siemiatycki *et al.* (1987) conducted a population-based case-referent study in Montréal on associations between cancer and estimated exposure to 300 chemical materials, of which 12 were petroleum-derived liquids. These mixtures included automotive and aviation gasolines and distillate jet fuel. No statistically significant risk for renal cancer was found with exposure to automotive gasoline (OR, 1.2; 90% CI, 0.8–1.6). Statistically significant increases were noted, however, at the 90% confidence level with exposure to aviation gasoline (OR, 2.6; 90% CI, 1.2–5.8) or to jet fuel (OR, 2.5; 90% CI, 1.1–5.4). Aviation gasoline differs in composition from its automotive counterpart by a high content of alkylate naphthas, constituted mainly of branched alkanes (Siemiatycki *et al.*, 1987). Six of the seven cases with exposure to aviation gasoline had also had exposure to jet fuel, making it difficult to distinguish a unique exposure. In-depth analyses of the two associations using logistic regression methods indicated, however, a greater role for aviation gasoline than for jet fuel.

Partanen *et al.* (1991) reviewed 672 cases of renal-cell adenoma for a case–control study. Owing to poor participation, only 338 sets of cases and controls were ultimately included for analysis—thereby limiting the interpretability of the findings. The investigators collected life-long job histories and translated them into indicators of industry, occupation and estimated occupational exposure. An elevated risk for kidney cancer was found to be associated with a history of employment in 'white-collar' occupations; the printing industry; the chemical industry; the manufacture of metal products, mail, telephone and telegraph services; and iron and metal work. An elevated risk (OR, 1.2; 95% CI, 0.6–2.5) was associated with exposure to gasoline, and an exposure-response relationship was observed for increasing exposure to gasoline. A potential confounder, discussed in the paper, is the fact that the gasoline used in Finland, especially in the past, contained tetraethyllead. Exposure to lead was associated with an increased risk for kidney cancer (OR, 2.9; 95% CI, 0.5–16.1). In addition, the sparsity of the estimates of exposure to gasoline meant that the investigators had to develop arbitrary exposure categories, which may have maximized the dose–response relationship.

Wong and Raabe (1989) conducted a quantitative meta-analysis by cancer site of employees in the petroleum industry in the USA, Canada, Europe, Australia and Japan, critically reviewing almost 100 published and unpublished epidemiological reports. Standardized mortality ratios observed for kidney cancer in the industry as a whole were similar to those for the general population. The results from the studies of refineries ranged from nonsignificant deficits to nonsignificant excesses; however, the possibility of an elevated risk for kidney cancer was raised for one specific group within the industry. Drivers among distribution workers in the United Kingdom had excess mortality from kidney cancer that is of borderline significance. The authors concluded that additional data, particularly on exposure to downstream gasoline, are needed to resolve the issue. Thus, while small risks cannot be excluded on the basis of specific job categories, the association between human kidney cancer and quantitative exposure to petroleum distillates, if there is one, must be small. Reviews by IARC (1989) and the US Environmental Protection Agency (1991) have come to similar conclusions.

In summary, a detailed understanding of the mechanisms involved in α-2μ-globulin nephropathy and renal carcinogenesis has been provided by investigations of several chemicals in various animal, biochemical and molecular modelling systems. All of the data are consistent with the hypothesis that reversible binding of chemicals or their metabolites to this abundant, male rat-specific protein is causally related to the induction of disease. No pathological or epidemiological data are available to suggest that humans are susceptible to a similar disease process. The equivocal data on human renal cancer and exposure to petroleum distillates do not address the hypothesis, since a causal association is doubtful and the exposures were to complex mixtures containing hundreds of chemicals. Our present understanding of this disease process strongly suggests that it is unlikely that nongenotoxic chemicals that have been shown to only induce renal tumours in male rats *via* this mechanism pose a carcinogenic risk to humans.

Role of carcinogens and goitrogens in the pathogenesis of thyroid gland neoplasia in rodents

Altered thyroid gland function and thyroid neoplasia

A basic understanding of the mechanisms of chemical induction of thyroid neoplasia was obtained during experimentation in the 1940s and 1950s. Two basic mechanisms were recognized, one of which was that of chemicals that exert a direct carcinogenic effect on the thyroid gland. Thyroid tumours have been produced by a variety of directly acting carcinogenic substances, such as polycyclic hydrocarbons (Esmarch, 1942; Gnatyshak, 1957), 2-acetylaminofluorene (Cox *et al.*, 1947), dichlorobenzidine (Pliss, 1959) and a variety of nitrosamines.

The second basic mechanism for the production of thyroid tumours is hormone imbalance. Kennedy and Purves (1941) found thyroid adenomas in rats fed a diet containing *Brassica* seeds, a naturally occurring goitrogen. Numerous studies have demonstrated that treatment with a variety of antithyroid substances (thiourea, thiouracil and their derivatives, and 3-amino-1,2,4-triazole) induces a high incidence of thyroid tumours in rats (Napalkov, 1976). Other substances that exert antithyroid effects in rats, such as some sulfonamides, also produce thyroid tumours (Swarm *et al.*, 1973). Although certain substances that produce hormonal imbalance, such as the polycyclic hydrocarbons, may also exert a direct carcinogenic effect, it is well established that hormone imbalance alone can increase the incidence of thyroid tumours in rodents and that many antithyroid (goitrogenic) substances known to cause thyroid cancer by hormonal imbalance have no direct carcinogenic effect.

A consistent mechanism, widely accepted by many investigators, to explain the pathogenesis of thyroid tumours in rats treated with antithyroid drugs has been described (Furth, 1959, 1969). Antithyroid drugs initially produce hormonal imbalance by interfering with thyroid hormone production or metabolism. A sustained increase in the synthesis and secretion of TSH occurs *via* the negative feedback system of the pituitary gland to stimulate thyroid function. TSH stimulation produces a variety of changes in the follicular cell and its function, including hypertrophy, hyperplasia and ultimately neoplasia. A sustained excessive level of TSH is considered to be the pathogenic factor responsible for thyroid tumour production under these conditions.

The fact that excessive secretion of endogenous TSH alone (in the absence of any chemical treatment) produces a high incidence of thyroid tumours has been clearly established by several experiments in which rats were fed diets deficient in iodine (Bielschowsky, 1953; Axelrad & Leblond, 1955; Leblond *et al.*, 1957; Isler *et al.*, 1958) or in which TSH-secreting pituitary tumours were transplanted into mice with normal thyroids (Furth, 1954). In rats, iodine-deficient diets are goitrogenic. Iodine-deficient diets induce simple hypothyroidism, thereby increasing TSH secretion and ultimately resulting in high incidences of thyroid tumours in rats. These effects can be reversed by iodine supplementation, thyroid hormone replacement or hypophysectomy. Goitrogenic substances or regimens are also powerful promoters of thyroid gland neoplasia after administration of directly acting carcinogenic substances (Bielschowsky, 1944; Morris, 1955; Hiasa *et al.*, 1982a). Under these conditions, pituitary tumours may also be observed, due to overstimulation of the pituitary thyrotropes.

Species differences in thyroid gland biochemistry and physiology

There are marked species differences in thyroid gland physiology that must be taken into account in an evaluation of species differences in the induction of thyroid gland neoplasia secondary to hormone imbalance. The most obvious difference between rodents and primates is that rodents, and some other species, lack thyroid-binding globulin (Dohler *et al.*, 1979). Thyroid-binding globulin is the predominant plasma protein that binds and transports thyroid hormone in the blood. Thyroxine (T_4) binds to three plasma proteins, thyroid-binding globulin, pre-albumin and albumin, with binding constants of 10^{-10}, 10^{-7} and 10^{-5}, respectively (Robbins & Rall, 1979). The lack of thyroid-binding globulin in the rodent, in which albumin and pre-albumin have three and five orders of magnitude less binding affinity for thyroxine, may be one of the more important factors responsible for the species differences in thyroid gland function.

The half-life of T_4 is 12 h in rats and five to nine days in humans, and the serum TSH level is 25 times higher in rodents than in man (Dohler *et al.*, 1979). The rodent thyroid gland thus has much higher activity than that of the primate, a conclusion that is also supported by the histological appearance of the thyroid gland. In primates, the follicles are uniformly large, with abundant colloid surrounded by relatively flattened follicular epithelial cells. In contrast, the rodent thyroid has large follicles only in the periphery of the gland, the interior of which is comprised of comparatively small follicles with small amounts of colloid surrounded by a more cuboidal follicular epithelium. If rats are given exogenous thyroid hormone, the follicles accumulate colloid and increase in size, and the epithelial cells assume a more flattened appearance. Both the physiological parameters and the histological appearance indicate that the rodent thyroid gland is markedly more active and operates at a considerably higher level with respect to thyroid hormone turnover as compared to the primate. It is important to note that many quantitative parameters of the rodent differ from those of the primate by orders of magnitude.

The incidence of 'spontaneous' thyroid gland neoplasia is also markedly different. The Fischer 344 male rat has about a 2% incidence of thyroid follicular cell neoplasia (0.8% carcinoma and 1% adenoma) (Haseman *et al.*, 1984), as compared to an average incidence of approximately 0.004% carcinoma, with a range of 0.001–0.016%, in humans (Sokal, 1953; Ron & Modan, 1982). The prevalence of occult thyroid carcinoma in humans at autopsy has been high in some studies; all or most of these were papillary carcinomas, a form rarely observed in rats (Ron & Modan, 1982).

The relative susceptibility of rodents and humans to thyroid neoplasia secondary to hormone imbalance or simple hypothyroidism can be assessed by comparing humans in iodine-deficient areas of endemic goitre to rats in the same areas or to rats treated with iodine-deficient diets. Over the years, there has been a slight, disputed association between endemic goitre and thyroid cancer in humans; however, endemic goitre has affected millions of individuals and, although relatively extensive epidemiological studies have been conducted, a clear etiological role in thyroid gland neoplasia has never been established (Saxen & Saxen, 1954; Pendergrast *et al.*, 1961; Doniach, 1970). In contrast, rodents in areas of endemic goitre (Wegelin, 1928) or those treated with iodine-deficient diets (Hellwig, 1935; Bielschowsky, 1953; Axelrad & Leblond, 1955; Leblond *et al.*, 1957; Isler *et al.*, 1958) exhibit a high incidence of thyroid gland neoplasia.

The marked species differences between rodents and primates in thyroid gland physiology, in the spontaneous incidences of thyroid gland neoplasia and in the apparent susceptibility to neoplasia secondary to simple hypothyroidism support the conclusion that thyroid gland neoplasia secondary to hormone imbalance is species specific. Rodents have an increased incidence of thyroid gland neoplasia in the presence of mild to moderate increases in TSH and may, in fact, be above an 'apparent threshold' for TSH-mediated neoplasia. In contrast, no clear etiological role for hypothyroidism has been established in human thyroid cancer, even though chronic hypothyroidism, in the moderate to severe range, occurs in people living in areas of endemic goitre (Doniach, 1970). Thus, it appears that the contribution of endemic goitre to human thyroid cancer is small at most.

Mechanisms for altered thyroid function

The functional unit of the thyroid gland is the follicle, which consists of a single layer of follicular epithelium surrounding an area of colloid, the storage form of thyroid hormone. The thyroid gland is unique among the endocrine glands in that hormone synthesis and storage are essentially extracellular processes, which occur at the apical surface of the cell membrane. Thyroid hormone synthesis involves active transport of iodine, peroxidase-mediated iodination and coupling of tyrosine residues on thyroglobulin to form thyroid hormone, which is stored as colloid until released (Degroot & Niepomniszcze, 1977; Elkholm, 1981).

The follicle and the follicular cell are highly organized, polarized structures. Thyroid hormone synthesis involves reactive biochemistry, and the organization of its structure and function serves to protect the cell from accidental iodination and cytotoxicity from oxidation products. The thyroid peroxidases appear to be active only at the apical surface of the cell membrane.

Thyroid hormone release involves pinocytosis of colloid by microvilli and digestion by lysosomes to release T_4 and T_3, which are secreted into plasma and bound to plasma proteins for transport to peripheral tissues. The most important aspect of the metabolism of thyroid hormone is monodeiodination to form T_3, the physiologically active form of thyroid hormone. The metabolism and excretion of thyroid hormone involve both T_3 and T_4. While T_4 is metabolized by glucoronidation, T_3 is predominantly sulfated. Both T_3 and T_4 are excreted in urine and bile (Robbins, 1981).

Thyroid hormone synthesis, release, transport and cellular uptake, conversion of T_4 to T_3, hormone metabolism and the regulation of these processes by the hypothalamic–pituitary–thyroid axis and autoregulatory processes in the thyroid gland itself is a complex process and provides many points at which chemicals can interfere with thyroid gland function. Regardless of the mechanism involved, the response to hypothyroidism is similar. The pituitary releases TSH as a compensatory response to stimulate the thyroid to produce more hormone. Chronic stimulation of the thyroid gland by TSH in rodents leads to a progression of follicular cell hypertrophy, hyperplasia and eventually neoplasia (Furth, 1959, 1969). Chemicals can intercede either intra- or extrathyroidally, or both. Intrathyroidal mechanisms can involve iodine uptake or hormone synthesis, and extrathyroidal mechanisms can involve effects on hormone metabolism or disposition. A few examples are discussed below.

Intrathyroidal mechanisms

Two classes of chemicals known to inhibit thyroid hormone synthesis are the thiourelyenes (thiourea, propylthiouracil, methimidazole) and the sulfonamides (sulfadiazine, sulfamethazine). The thiourelynes are considerably more potent in inhibiting thyroid hormone synthesis than the sulfonamides; however, sufficiently high doses of many sulfonamides are goitrogenic in rodents (Mackenzie *et al.*, 1941; Mackenzie & Mackenzie, 1943; Swarm *et al.*, 1973; Takayama *et al.*, 1986). Although the goitrogenicity of the sulfonamides has been known since 1941, only a few have been tested for carcinogenicity. Sulfamethoxazole at a dose of 50 mg/kg per day or more is goitrogenic and produced thyroid neoplasia in rats within 50 weeks of treatment. Sulfamethazine produced goitrogenic effects and thyroid neoplasia in rats at doses of 600 ppm or more and in mice at a dose of 4800 ppm (Heath & Littlefield, 1984; Fullerton & Kushmaul, 1987; Littlefield *et al.*, 1989, 1990). Sulfisoxazole, which is only weakly goitrogenic in rodents, did not produce thyroid gland neoplasia at doses up to 400 mg/kg per day in rats or at 2000 mg/kg per day in mice treated for two years (US National Cancer Institute, 1979). The various sulfonamides that have been tested have no apparent genotoxic effect, and the thyroid gland neoplasia observed after administration of goitrogenic dosages is considered to be secondary to hormonal imbalance.

While some species (including rats, mice, hamsters, dogs and swine) are sensitive to the goitrogenic effect of the sulfonamides, others, including chickens, guinea-pigs and monkeys, are not (Mackenzie & Mackenzie, 1943; Swarm *et al.*, 1973; Takayama *et al.*, 1986). No effect on thyroid function or morphology was observed in rhesus monkeys treated with sulfamethoxazole (Swarm *et al.*, 1973) for one year at doses up to 300 mg/kg per day or in cynomolgus monkeys treated for four weeks with sulfamonomethoxine (Takayama *et al.*, 1986) at doses up to 300 mg/kg per day. In humans, no clinically significant effect on thyroid function has been observed with sulfonamides given at therapeutic dose levels (Koch-Weser *et al.*, 1971). Although Cohen (1981) observed a small decrease in serum thyroid hormone values in patients treated with sulfamethoxazole or cotrimoxazole, no increase in TSH was observed. In addition, no abnormality of thyroid function was observed in a group of young patients receiving chronic treatment with cotrimoxazole (Smellie *et al.*, 1982, 1983).

Takayama and co-workers (1986) studied the species difference between rats and monkeys using propylthiouracil and sulfamonomethoxine. The effect of these compounds on thyroid gland function (*in vivo*) and their ability to inhibit thyroid gland peroxidase from rats and monkeys (*in vitro*) were assessed. Rats treated with either compound had decreased T_3 and T_4 values and markedly elevated TSH values, accompanied by follicular-cell hyperplasia and increased thyroid gland weight at doses of 30 or 300 mg/kg per day. In contrast, no effect on thyroid function or morphology was observed in monkeys receiving 300 mg/kg per day of either compound.

In vitro there was a marked difference in the inhibition of microsomal thyroid peroxidase by sulfamonomethoxine, depending on the source of the enzyme; the concentration (IC_{50}) required to inhibit monkey thyroid peroxidase was approximately 450 times greater than that required to inhibit thyroid peroxidase from rats. The IC_{50} of propylthiouracil required to inhibit monkey thyroid peroxidase was approximately 50 times greater than for the rat, indicating a quantitative difference between rats and monkeys for inhibition of thyroid function by propylthiouracil both *in vivo* and *in vitro*. With respect to the sulfonamide, the

difference is sufficiently great that it can be considered to be a qualitative species difference in antithyroid activity between rats and monkeys. The marked difference in inhibition of thyroid peroxidase by sulfonamide between rats and monkeys constitutes the biochemical basis for the observed species differences in the goitrogenic effects of sulfonamides. It explains why the sulfonamides are goitrogenic in rats at relatively low doses but do not produce effects in monkeys at very high doses or in humans at therapeutic doses.

Extrathyroidal mechanisms

Conversion of T_4 into the more active hormone triiodothyronine (T_3) occurs in many peripheral tissues and is mediated by a microsomal 5′-monodeiodinase which removes one iodine at the 5′ position in thyroxine (Robbins, 1981).

Various iodinated organic compounds, such as tetraiodofluoresceine (Ruiz & Ingbar, 1982), amiodarone (Burger *et al.*, 1976) and iodinated radio-contrast media (Burgi *et al.*, 1976) inhibit 5′-monodeiodinase and thus disrupt the conversion of T_4 to T_3. The decrease in serum T_3 values results in a compensatory increase in pituitary TSH. Prolonged treatment with tetraiodofluoresceine at very high doses results in a moderate increase in thyroid follicular neoplasia in rodents. Propylthiouracil and methimidazole not only inhibit thyroid peroxidase but also inhibit the monodeiodinases.

Chemicals acting on various aspects of thyroid hormone metabolism have an important impact on thyroid hormone economy in the rodent (Cavalieri & Pitt-Rivers, 1981). The monodeiodinases are quantitatively the most important path in the disposition of T_4. In addition, T_4 is glucuronidated and T_3 is sulfated and subsequently excreted in bile. Deamination, decarboxylation and cleavage of the ether link occur but are of quantitatively lesser importance (Robbins, 1981). Many chemicals are hepatic microsomal enzyme inducers at high doses and alter thyroid function in rodents by increasing the hepatic disposition of thyroid hormone (Oppenheimer *et al.*, 1968; Hill *et al.*, 1989). Decreased serum thyroid hormone results in a compensatory increase in pituitary TSH, which can exert a tumour promoting effect in initiation-promotion models (Hiasa *et al.*, 1982b) or an increase in thyroid gland neoplasia in two-year carcinogenicity studies (McClain, 1989). The finding that small amounts of T_4 block the tumour promoting effect of microsomal enzyme inducers such as phenobarbital supports the conclusion that this effect is secondary to hormone imbalance as a result of increased hepatic disposition of T_4 as opposed to a direct tumour promoting or carcinogenic effect in the thyroid gland (McClain *et al.*, 1988; McClain, 1989; McClain *et al.*, 1989).

Of the chemicals mentioned above, tetraiodofluoresceine produced only mild effects on thyroid function in humans at very large multiples of the allowable daily intake (Gardner *et al.*, 1987). Chronic exposure to anticonvulsant drugs, many of which induce enzymes at therapeutic doses, results in only mild changes in thyroid function (moderately decreased T_4 with normal T_3 and TSH values and a normal TSH response to administered thyrotropin releasing hormone) (Ohnhaus & Studer, 1983). These changes are not clinically significant.

Although neither inhibition of the monodeiodinase nor induction of thyroid hormone disposition following chemical exposure represents a species-specific effect, these responses must be considered in the context of the degree of hypothyroidism, if any, that is produced under the exposure conditions and of the likelihood that mild or moderate simple hypothyroidism presents an increased risk for thyroid neoplasia in humans.

Extrapolation

Chemicals can alter thyroid function and result in hypothyroidism through a variety of intra- and extrathyroidal mechanisms. Many of these chemicals cause thyroid hormone imbalance and neoplasia in rodents in two-year carcinogenicity studies. Chemicals that produce thyroid neoplasia in rodents secondary to simple hypothyroidism can be considered to be species specific, in that simple hypothyroidism in humans is not a known etiological factor for thyroid cancer. The degree of hypothyroidism that results from exposure to a chemical would present a major toxicological problem before the exposure entails an increased risk for neoplasia.

Because of marked species differences in thyroid gland physiology and apparent susceptibility to hypothyroidism, the rodent is an inappropriate model from which to extrapolate cancer risk to man for chemicals that operate secondary to hormone imbalance. Rodents are, however, very sensitive to the action of 'directly acting carcinogens'. Even in this case, all other factors being equal, the rodent model is likely to be conservative and to provide overestimates of risks for species with different thyroid gland function because of the strong promoting effect of high levels of TSH.

Urinary bladder carcinogenesis

Urinary bladder carcinogenesis has been associated with chemicals since the report by Rehn in 1895 of bladder cancer in workers in the German dye industry (Clayson & Cooper, 1970; Price, 1971). Several specific chemicals and mixtures have been identified as human bladder carcinogens, including cigarette smoke, 2-naphthylamine, benzidine, 4-aminobiphenyl, phenacetin, chlornaphazine and cyclophosphamide (Clayson & Cooper, 1970; Cohen *et al.*, 1987). In addition, urinary bladder carcinogens have been established in animal models. Similarities and differences in susceptibility and organ specificity have been identified between species (Cohen *et al.*, 1987; Cohen, 1981).

Urinary bladder carcinogens can be subdivided into those that act *via* a genotoxic mechanism (including all of those known to affect humans) and those that act by increasing cell proliferation over time, or by both processes (Cohen & Ellwein, 1990). It has become evident that increased cell proliferation also contributes to the carcinogenicity of genotoxic bladder carcinogens in susceptible species, including humans (Auerbach & Garfinkel, 1989; Cohen & Ellwein, 1990).

The most extensively studied urinary bladder carcinogens are the class of aromatic amines and amides, several of which are known human urinary tract carcinogens (Clayson & Cooper, 1970; Cohen, 1981). They are metabolically activated by several enzymes, resulting in some variation in organ specificity between species (Cohen, 1981; Dawley *et al.*, 1991). Considerable evidence indicates that, for humans, it is the amine that is involved in the induction of bladder cancer rather than the amide, and differences in ability to acetylate and/or deacetylate these compounds greatly influence whether the chemical affects the bladder in a given species (Cartwright *et al.*, 1982). Several genotoxic chemicals have been identified as urinary bladder-specific carcinogens in animal models, including nitrofurans, such as *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, and nitroso compounds, such as *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine (Clayson & Cooper, 1970; Cohen *et al.*, 1987). There is little difference in the organ specificity of these compounds between rats, mice and dogs, although there are some differences in potency and strain responsiveness.

In summary, genotoxic urinary bladder carcinogens have strikingly similar effects among species. The differences that exist can be partially explained by differences in metabolic activation and detoxication. Strain differences can occur secondary to metabolic differences, even among humans. For example, the carcinogenicity of aromatic amines is different in people who are fast acetylators and in those who are slow acetylators (Cartwright *et al.*, 1982).

Nongenotoxic bladder carcinogenesis

The differences between species in response to nongenotoxic chemicals that affect the urinary bladder are considerably greater than that in response to genotoxic chemicals. A common feature of nongenotoxic bladder carcinogens is their ability to produce sustained increases in cell proliferation of the urothelium, with a corresponding influence on the dose–response curve. They appear to induce bladder cancer only at high doses (Cohen & Ellwein, 1990).

Marked increases in urothelial proliferation in rats and mice can be brought about by inserting pellets of a variety of materials, including paraffin wax, cholesterol, glass and stainless-steel, into the lumen of the bladder (Clayson & Cooper, 1970; Price, 1971; Cohen *et al.*, 1987). This produces erosion and ulceration of the bladder epithelium, with consequent marked regenerative hyperplasia and eventually the formation of urinary bladder carcinomas. Variations in the roughness of the surface of these pellets alters the rate at which proliferation and tumours occur, but with any of these pellets tumours ultimately occur at a significant level of incidence.

Calculi are equivalents of pellets which can be generated by the administration of a variety of chemicals to various species (Clayson & Cooper, 1970; Clayson, 1974; Cohen, 1981). These chemicals have been studied most extensively in rats and mice and less frequently in guinea-pigs and rabbits. The production of bladder tumours following calculus formation has been reported only in rats and mice, and the responsiveness of the urothelium in the different species varies considerably. For example, administration of uracil as 3% of the diet to rats produces diffuse papillomatosis, with the appearance of carcinomas within 30 weeks of administration. Mice also respond to this level of uracil, with calculus formation but with nodular rather than papillary hyperplasia and with a longer time required for the generation of malignancies (Sakata *et al.*, 1988). Rats also appear to be more susceptible to the proliferative and tumorigenic effects of implanted pellets (Clayson & Cooper, 1970; Price, 1971). A response similar to that to uracil is seen in rats following the administration of melamine, which at high doses leads to the formation of melamine calculi, diffuse hyperplasia and eventually carcinomas (Melnick *et al.*, 1984). In mice, although formation of calculi and significant hyperplasia occur, tumours do not appear to form at significant incidences. Numerous other substances have been reported to produce calculi of various types when administered orally to rats or mice, including oxalates, ethylene glycol and orotic acid. If calculi persist and the animals survive long enough, bladder tumours arise (Clayson & Cooper, 1970; Price, 1971; Clayson, 1974). Again, for many of these chemicals, rats would appear to be more susceptible than mice.

The susceptibility of other species, including humans, to tumour formation after calculi is not well understood. In humans, there is little evidence to suggest that calculus formation leads to an increased incidence of bladder tumours, although there are case reports of

bladder tumours arising in patients with long-standing calculi (Clayson, 1974; Matanoski & Elliott, 1981). The usual type of bladder tumour that arises in humans is transitional-cell carcinoma, but the most frequent tumours reported in patients with long-standing calculi are squamous-cell carcinomas. This is also true for patients with other forms of chronic inflammation, such as schistosomiasis (El-Bolkainy, 1983). In all of these circumstances, the chronic inflammatory process involves persistent epithelial necrosis and regeneration (Cohen *et al.*, 1991).

Several chemicals appear to produce increased cell proliferation rather than calculus formation, at least in rats, by the formation of a variety of microcrystals (Cohen & Ellwein, 1990; Ellwein & Cohen, 1990). Representative of this class of compounds is sodium saccharin: Administration of sodium saccharin appears to result in the formation of silicate crystals and precipitates which are cytotoxic to the urothelium, resulting in regenerative hyperplasia and ultimately bladder tumour formation. For these silicates to form, several factors are required, including a urinary pH greater than 6.5 and large amounts of protein; increased sodium concentration increases the likelihood of their formation. Rats, particularly males, have large amounts of protein and particularly low-molecular-weight proteins such as α-2μ-globulin. Binding of the saccharin anion to these proteins seems to produce the necessary milieu for generation of the silicate precipitates and crystals, which appear to be necessary for the carcinogenicity of sodium saccharin.

Several other sodium salts have been identified that produce increased cell proliferation and bladder tumour formation in male rats, although their relationship to silicate formation has not yet been investigated (Cohen & Ellwein, 1990; Ellwein & Cohen, 1990). These include the sodium salts of ascorbate, erythorbate, citrate, glutamate, aspartate, bicarbonate and chloride. For those compounds that have been studied in detail, male rats are more susceptible than females, and mice, hamsters and monkeys appear to be refractory to their effects. Humans also appear to be unresponsive to the effects of sodium saccharin on urothelial proliferation or bladder tumour formation (Auerbach & Garfinkel, 1989). The resistant species do not appear to have the necessary physiological responses to sodium saccharin, even at high doses, to produce persistent cell proliferation or tumours.

A variety of other chemicals has been demonstrated to increase urothelial proliferation in specific species and to enhance bladder tumour formation (Cohen *et al.*, 1987; Ellwein & Cohen, 1990). Many of these compounds are antioxidants, including butylated hydroxyanisole, butylated hydroxytoluene and ethoxyquin, in addition to ascorbate. These antioxidants increase urothelial proliferation in male rats, but their mechanism of action is unknown. In the few instances in which they have been studied, mice again appear to be resistant to their effects. It is unknown what their effects are in humans, although there has been no reported instance of an effect of any of these antioxidants on the urothelium.

Nitrilotriacetate is a strong chelating agent for a variety of metals, including zinc (Anderson *et al.*, 1985). If administered at high doses to rats, it has several toxic effects in the urinary tract and eventually forms renal and urothelial carcinomas, but only at high doses and, so far, only in rats. Although not studied as extensively, it does not appear to have carcinogenic effects in mice.

In summary, the interspecies variation in response to a variety of nongenotoxic compounds is considerably greater than that for genotoxic urinary bladder carcinogens. This

is probably related to the large differences in physiological responses to these chemicals as well as in the responses to inflammatory and cell proliferative effects on the bladder epithelium. It would appear that mice and humans are much less susceptible than rats to the effects of such compounds, and, even in rats, very high doses are required, with an apparent threshold (Cohen & Ellwein, 1990).

In extrapolating the results of bioassays in animals to humans, it is essential to take into account metabolic, physiological and cell biological differences between the test species and humans. Many chemicals that are genotoxic carcinogens in animals appear to present a significance risk to humans; however, many nongenotoxic compounds, whether because of the mechanism involved or the dose-responsiveness, have not been associated with a risk to humans, in contrast to the findings in rats and/or mice (Cohen & Ellwein, 1990).

Conclusions

Three of the best-studied examples of species-specific mechanisms for carcinogenicity have been reviewed in some detail. All three involve a common etiological factor, i.e., a sustained increase in cell proliferation; however, the mechanism(s) by which the sustained increase in cell proliferation is induced varies from one organ to the next. For kidney tumours and bladder tumours, the increased cell proliferation is a result of site-specific cytotoxicity and the associated compensatory regenerative response; however, in the thyroid, the increased follicular cell proliferation is a response to hormonal imbalance. The existence of species- and sex-specific physiological and metabolic differences in rats (kidney, bladder and thyroid) and mice (thyroid), which are not present in humans, predisposes these animals to the development of kidney, bladder and thyroid tumours following chemical exposures.

The involvement of increased cell proliferation in the etiology of these tumours, does not, however, imply that all compounds that cause increased cell proliferation are species specific, nor does it imply that all agents that cause increased cell proliferation are carcinogens. Many additional examples of species-specific mechanisms of carcinogenesis could be cited, including prolactin-induced mammary tumours in rats, murine ovarian tumours following ovarian atrophy, and forestomach tumours induced by butylated hydroxyanisole in rodents. While for only a small number of chemicals are adequate data on mechanisms available at present that could be used for classification, modern research offers exciting possibilities for improved understanding of the mechanisms responsible for chemical carcinogenesis. It is important to note that human risk can be estimated accurately only by a thorough understanding of the mechanisms involved in the etiology of tumours. Incorporation of mechanistic data into evaluations of carcinogens will permit greater accuracy in estimations of human risk and will thereby ultimately lead to greater improvements in human health.

References

Akerstroem, B. & Landin, B. (1985) Rat alpha$_1$-microglobulin: purification from urine and synthesis by hepatocyte monolayers. *Eur. J. Biochem.*, **146**, 353–358

Akerstroem, B., Loedgberg, L., Babiker-Mohamed, H., Lohmander, S. & Rask, L. (1987) Structural relationship between α_1-microglobulin from man, guinea-pig, rat, and rabbit. *Eur. J. Biochem.*, **170**, 143–148

Alden, C.L. (1986) A review of the unique male rat hydrocarbon nephropathy. *Toxicol. Pathol.*, **14**, 109–111

Anderson, R.L., Bishop, W.E. & Campbell, R.L. (1985) A review of the environmental and mammalian toxicology of nitrilotriacetic acid. *Crit. Rev. Toxicol.*, **15**, 1–102

Ashby, J. & Tennant R.W. (1991) Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the US NTP. *Mutat. Res.*, **257**, 209–227

Auerbach, O. & Garfinkel, L. (1989) Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. *Cancer*, **64**, 983–987

Axelrad, A.A. & Leblond, C.P. (1955) Induction of thyroid tumors in rats by a low iodine diet. *Cancer*, **8**, 339-367

Bernard, A.M., Lauwerys, R.R., Noel, A., Vandeleene, B. & Lambert, A. (1989) Urine protein 1: a sex-dependent marker of tubular or glomerular dysfunction. *Clin. Chem.*, **35**, 2141–2142

Bernard, A.M., Roels, H., Cardenas, A. & Lauwerys, R. (1990) Assessment of urinary protein 1 and transferrin as early markers of cadmium nephrotoxicity. *Br. J. Ind. Med.*, **47**, 559–565

Bielschowsky, F. (1944) Tumours of the thyroid produced by 2-acetyl-amino-fluorene and allyl-thiourea. *Br. J. Exp. Pathol.*, **25**, 90–95

Bielschowsky, F. (1953) Chronic iodine deficiency as cause of neoplasia in thyroid and pituitary of aged rats. *Br. J. Cancer*, **7**, 203–213

Borghoff, S.J., Short, B.G. & Swenberg, J.A. (1990) Biochemical mechanisms and pathobiology of alpha$_{2u}$-globulin nephropathy. *Ann. Rev. Pharmacol. Toxicol.*, **30**, 349–367

Borghoff, S.J., Miller A.B., Bowen, J.P. & Swenberg, J.A. (1991) Characteristics of chemical binding to α_{2u}-globulin in vitro—evaluating structure-activity relationships. *Toxicol. Appl. Pharmacol.*, **107**, 228–238

Bruner, R.H. (1984) Pathologic findings in laboratory animals exposed to hydrocarbon fuels of military interest. In: Mehlman, M.A., Hemstreet, G.P., Thorpe, J.J. & Weaver, N.K., eds, *Advances in Modern Environmental Toxicology*, Vol. 7, *Renal Effects of Petroleum Hydrocarbons*, Princeton, NJ, Princeton Scientific Publishers, pp. 133–140

Bruner, R.H. (1986) Chronic consequence of alpha$_{2u}$-globulin nephropathy: review. *Proceedings, Toxicology Forum, Aspen, Colorado, July 15*, pp. 161–166

Burger, A., Dinichert, D., Nicod, P., Jenny, M., Lemarchand-Beraud, T. & Vallotton, M.B. (1976) Effect of amiodarone on serum triiodothyronine, reverse triiodothyronine, thyroxine. A drug influencing peripheral metabolism of thyroid hormones. *J. Clin. Invest.*, **58**, 255–259

Burgi, H., Wimpfheimer, C., Burger, A., Zaunbauer, W., Rosler, H. & Lemarchand-Beraud, T. (1976) Changes of circulating thyroxine, triiodothyronine and reverse triiodothyronine after radiographic contrast agents. *J. Clin. Endocrinol. Metab.*, **43**, 1203–1210

Cartwright, R.A., Rogers, H.J., Barham-Hall, D., Glashan, R.W., Ahmad, R.A., Higgins, E. & Kahn, M.A. (1982) The role of N-acetyltransferase in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, **ii**, 842–845

Cavalieri, R.R. & Pitt-Rivers, R. (1981) The effects of drugs on the distribution and metabolism of thyroid hormones. *Pharmacol. Rev.*, **33**, 55–80

Charbonneau, M., Strasser, J., Lock, E.A., Turner, M.J. & Swenberg, J.A. (1989) Involvement of reversible binding to α_{2u}-globulin in 1,4-dichlorobenzene-induced nephrotoxicity. *Toxicol. Appl. Pharmacol.*, **99**, 122–132

Chatterjee, B., Demyan, W.F., Song, C.S., Garg, B.D. & Roy, A.K. (1989) Loss of androgenic induction of α_{2u}-globulin gene family in the liver of NIH Black rats. *Endocrinology*, **125**, 1385–1388

Clayson, D.B. (1974) Bladder carcinogenesis in rats and mice: possibility of artifacts. *J. Natl Cancer Inst.*, **52**, 1685–1689

Clayson, D.B. & Cooper, E.H. (1970) Cancer of the urinary tract. *Adv. Cancer Res.*, **13**, 271–381

Cohen, S.M. (1981) Promotion of urinary bladder carcinogenesis. In: Langenbach, R., Nesnow, S. & Rice, J.M., eds, *Organ and Species Specificity in Chemical Carcinogenesis*, Vol. 34, New York, Plenum Press, pp. 253–270

Cohen, S.M. & Ellwein, L.B. (1990) Cell proliferation in carcinogenesis. *Science*, **249**, 1007–1011

Cohen, S.M., Ellwein, L.B. & Johansson, S.L. (1987) Bladder tumor promotion, In: Butterworth, B.E. & Slaga, T.J., eds, *Nongenotoxic Mechanisms in Carcinogenesis* (Banbury Report 25), Cold Spring Harbor, NY, CSH Press, pp. 55–67

Cohen, S.M., Purtilo, D.T. & Ellwein, L.B. (1991) Pivotal role of increased cell proliferation in human carcinogenesis. *Mod. Pathol.*, **4**, 371–382

Cox, A.J., Wilson, R.H. & DeEds, F. (1947) The carcinogenic activity of 2-acetylaminofluorene: characteristics of the lesions in albino rats. *Cancer Res.*, **7**, 647–657

Dawley, R.M., Zenser, T.V., Mattammal, M.B., Lakshmi, V.M., Hsu, F.F. & Davis, B.B. (1991) Metabolism and disposition of bladder carcinogens in rat and guinea pig: possible mechanism of guinea pig resistance to bladder cancer. *Cancer Res.*, **51**, 514–520

Degroot, L.G. & Niepomniszcze, H. (1977) Biosynthesis of thyroid hormone: basic and clinical aspects. *Metabolism*, **26**, 665–718

Dieppe, P.A., Doyle, D.V. & Burry, H.C. (1978) Renal damage during treatment with antirheumatic drugs. *Br. Med. J.*, **ii**, 664

Dietrich, D.R. & Swenberg, J.A. (1991a) The presence of alpha$_{2u}$-globulin is necessary for d-limonene promotion of male rat kidney tumors. *Cancer Res.*, **51**, 3512–3521

Dietrich, D.R. & Swenberg, J.A. (1991b) NCI-Black-Reiter (NBR) male rats fail to develop renal disease following exposure to agents that induce α_{2u}-globulin (α2u-G) nephropathy. *Fundam. Appl. Toxicol.*, **16**, 749–762

Dohler, K.D., Wong, C.C. & von zur Muhlen, A. (1979) The rat as a model for the study of drug effects on thyroid function: consideration of methodological problems. *Pharmacol. Ther.*, **5**, 305–318

Doniach, I. (1970) Aetiological consideration of thyroid carcinoma. In: Smithers, D., ed., *Neoplastic Diseases at Various Sites, Tumours of the Thyroid Gland*, Edinburgh, E. & S. Livingston

Ekstrom, R.C. & Hoekstra, W.G. (1984) Investigation of putative androgen like activity of alpha$_{2u}$-globulin in castrated and estrogen treated male rats. *Proc. Soc. Exp. Biol. Med.*, **175**, 491–496

El-Bolkainy (1983) Schistosomiasis and bladder cancer. In: Cohen, S.M. & Bryan, G.T., eds, *The Pathology of Bladder Cancer*, Vol. 1, Boca Raton, FL, CRC Press, pp. 57–89

Elkholm, R. (1981) Iodination of thyroglobulin. An intracellular or an extracellular process? *Mol. Cell Endocrinol.*, **24**, 141

Ellwein, L.B. & Cohen, S.M. (1990) The health risks of saccharin revisited. *Crit. Rev. Toxicol.*, **20**, 311–326

Esmarch, O. (1942) Deposition of methylcholanthrene in some organs of the rat. *Acta Pathol. Microbiol. Scand.*, **19**, 79–99

Fullerton, F.R. & Kushmaul, R.J. (1987) Influence of oral administration of sulfamethazine on thyroid hormone levels in Fisher 344 rats. *J. Toxicol. Environ. Health*, **22**, 175–185

Furth, J. (1954) Morphologic changes associated with thyrotropine-secreting pituitary tumors. *Am. J. Pathol.*, **30**, 421–463

Furth, J. (1959) A meeting of ways in cancer research: thoughts on the evolution and nature of neoplasms. *Cancer Res.*, **19**, 241–258

Furth, J. (1969) *Pituitary Cybernetics and Neoplasia* (Harvey Lectures), New York, Academic Press, pp. 47–71

Gardner, D.F., Utiger, R.D., Schwartz, S.L., Witorsch, B., Meyers, B., Braverman, L.E. & Witorsch, R.J. (1987) Effects of oral erythrosine (2′,4′,5′,7′-tetraiodofluorescein) on thyroid function in normal men. *Toxicol. Appl. Pharmacol.*, **91**, 299–304

Gaworski, C.L., Leahy, H.F. & Brunner, H.F. (1980) Subchronic inhalation toxicity of decalin. In: *Proceedings of the Tenth Conference on Environmental Toxicology* (AFAMRL-TR-121 [ADA086341]), OH, Wright Patterson Airforce Base, Aerospace Medical Research Laboratory, pp.

Gaworski, C.L., Haun, C.C. & MacEwen, J.D. (1981) A ninety day inhalation study of decalin. *Toxicologist*, **1**, 276

Gnatyshak, A.I. (1957) Experimental thyroid tumors and accompanying changes in some endocrine glands. *Vopr. Onkol.*, **3**, 659–665

Gross, V., Steube, K., Tran-Thi, T., Haeusinger, D., Legler, G., Decker, K., Heinrich, P.C. & Gerok, W. (1987) The role of N-glycosylation for the plasma clearance of rat secretory proteins. *Eur. J. Biochem.*, **162**, 83–88

Gross, V., Heinrich, P.C., vom Berg, D., Steube, K., Andus, T., Tran-Thi, T., Decker, K. & Gerok, W. (1988) Involvement of various organs in the initial plasma clearance of differently glycosylated rat liver secretory proteins. *Eur. J. Biochem.*, **173**, 653–659

Halder, C.A., Warne, T.M. & Hatoum, N.S. (1984) Renal toxicity of gasoline and related petroleum naphthas in male rats. In: Mehlman, M.A., Hemstreet, G.P., Thorpe, J.J. & Weaver, N.K., eds, *Renal Effects of Petroleum Hydrocarbons*, Princeton, NJ, Princeton Scientific Publishers, pp. 73–87

Halder, C.A., Holsworth, C.E., Cockrell, B.Y. & Piccirillo, V.J. (1985) Hydrocarbon nephropathy in male rats: identification of the nephrotoxic components of unleaded gasoline. *Toxicol. Ind. Health*, **1**, 67–87

Haseman, J.K., Huff, J. & Boorman, G.A. (1984) Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.*, **12**, 126–131

Heath, J.E. & Littlefield, N.A. (1984) Effect of subchronic oral sulfamethazine administration on Fischer 344 rats and B6C3F1 mice. *Environ. Pathol. Toxicol. Oncol.*, **5**, 201–214

Hellwig, C.A. (1935) Thyroid adenoma in experimental animals. *Am. J. Cancer*, **23**, 550–555

Hiasa, Y., Ohshima, M., Kitahori, Y., Yuasa, T., Fujita, T. & Iwata, C. (1982a) Promoting effects of 3-amino-1,2,4-triazole on the development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis*, **3**, 381–384

Hiasa, Y., Kitahori, Y., Ohshima, M., Fujita, T., Yuasa, T., Konishi, N. & Miyashiro, A. (1982b) Promoting effects of phenobarbital and barbital on development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)-nitrosamine. *Carcinogenesis*, **3**, 1187–1190

Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L. & Wilkinson, C.F. (1989) Review, thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.*, **12**, 629–697

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon

IARC (1989) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 46, *Diesel and Gasoline Engine Exhausts and Some Nitroarenes*, Lyon

Isler, H., Leblond, C.P. & Axelrad, A.A. (1958) Influence of age and of iodine intake on the production of thyroid tumors in the rat. *J. Natl Cancer Inst.*, **21**, 1065–1081

Jackson, P.J., Turner, R., Keen, J.N., Brooksbank, R.A. & Cooper, E.H. (1988) Purification and partial amino acid sequence of human urine protein 1. Evidence for homology with rabbit uteroglobin. *J. Chromatogr.*, **452**, 359–367

Kennedy, T.H. & Purves, H.D. (1941) Studies on experimental goitre. I. The effect of Brassica seed diets on rats. *Br. J. Exp. Pathol.*, **22**, 241–244

Koch-Weser, J., Sidel, V.W., Dexter, M., Parish, C., Finer, D.C. & Kanarek, P. (1971) Adverse reactions of sulfisoxazole, sulfamethoxazole, and nitrofurantoin. Manifestations and specific reaction rates during 2,118 courses of study. *Arch. Intern. Med.*, **128**, 399–404

Leblond, C.P., Isler, H. & Axelrad, A. (1957) Induction of thyroid tumors by a low iodine diet. *Can. Cancer Conf.*, **2**, 248–266

Lehman-McKeeman, L.D., Rodriguez, P.A., Takigiku, R., Caudill, D. & Fey, M.L. (1989) d-Limonene-induced male rat specific nephrotoxicity: evaluation of the association between d-limonene and $\alpha_{2\mu}$-globulin. *Toxicol. Appl. Pharmacol.*, **99**, 250–259

Lehman-McKeeman, L.D., Rivera-Torres, M.I. & Caudill, D. (1990) Lysosomal degradation of $\alpha_{2\mu}$-globulin and $\alpha_{2\mu}$-globulin-xenobiotic conjugates. *Toxicol. Appl. Pharmacol., 103*, 539–548

Littlefield, N.A., Gaylor, D.W., Blackwell, B.N. & Allen, R.R. (1989) Chronic toxicity/carcinogenicity studies of sulphamethazine in B6C3F1 mice. *Food Chem. Toxicol.*, **27**, 455–463

Littlefield, N.A., Sheldon, W.G., Allen, R. & Gaylor, D.W. (1990) Chronic toxicity/carcinogenicity studies of sulphamethazine in Fischer 344/N rats: two-generation exposure. *Food Chem. Toxicol.*, **28**, 157–167

Lock, E.A., Charbonneau, M., Strasser, J., Swenberg, J.A. & Bus, J.S. (1987) 2,2,4-Trimethylpentane (TMP)-induced nephrotoxicity. II. The reversible binding of a TMP metabolite to a renal protein fraction containing alpha$_{2\mu}$-globulin. *Toxicol. Appl. Pharmacol.*, **91**, 182–192

Loeb, L.A. (1989) Endogenous carcinogenesis: molecular oncology into the twenty-first century—presidential address. *Cancer Res.*, **49**, 5489–5496

MacEwen, J.D. & Vernot, E.R. (1978) *The Effects of Subchronic Exposure of Rodents to Inhaled Decalin Vapors* (AMRL 78-55 [ADA062138], Toxic Hazards Research Unit Annual Report 1978), OH, Wright-Patterson Air Force Base, Aerospace Medical Research Laboratory

Mackenzie, C.G. & Mackenzie, J.B. (1943) Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. *Endocrinology*, **32**, 185–209

Mackenzie, J.B., Mackenzie, C.G. & McCollom, E.V. (1941) Effect of sulfanilguanidine on thyroid of the rat. *Science*, **94**, 518

Matanoski, G. & Elliott, E.A. (1981) Bladder cancer epidemiology. *Epidemiol. Rev.*, **3**, 203–228

McClain, R.M. (1989) The significance of hepatic microsomal induction and altered thyroid function in rats: implications for thyroid gland neoplasia. *Toxicol. Pathol.*, **17**, 294–306

McClain, R.M., Posch, R.C., Bosakowski, T. & Armstrong, J.M. (1988) Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. *Toxicol. Appl. Pharmacol.*, **94**, 254–265

McClain, R.M., Levin, A., Posch, R.C. & Downing, J.C. (1989) The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicol. Appl. Pharmacol.*, **99**, 216–228

McLaughlin, J.K. (1984) Risk factors from a population-based case-control study of renal cancer. In: Mehlman, M.A., Hemstreet, G.P., Thorpe, J.J. & Weaver, N.K., eds, *Advances in Modern Environmental Toxicology*, Vol. 7, *Renal Effects of Petroleum Hydrocarbons*, Princeton, NJ, Princeton Scientific Publishers, pp. 227–244

McLaughlin, J.K. & Schuman, L.M. (1983) Epidemiology of renal cell carcinoma. *Rev. Cancer Epidemiol.*, **2**, 170–210

McLaughlin, J.K., Blot, W.J., Mehl, E.S., Stewart, P.A., Venable, F.S. & Fraumeni, J.F. (1985) Petroleum-related employment and renal cell cancer. *J. Occup. Med.*, **27**, 672–674

Melnick, R.L., Boorman, G.A., Haseman, J.K., Montali, R.J. & Huff, J. (1984) Urolithiasis and bladder carcinogenicity of melamine in rodents. *Toxicol. Appl. Pharmacol.*, **72**, 292–303

Morris, H.P. (1955) The experimental development and metabolism of thyroid gland tumors. *Adv. Cancer Res.*, **3**, 51–115

Napalkov, N.P. (1976) Tumours of the thyroid gland. In Turusov, V.S., ed., *Pathology of Tumours in Laboratory Animals, Vol. 1, Part 2, Tumours of the Rat* (IARC Scientific Publications No. 6), Lyon, IARC, pp. 239–272

Ohnhaus, E.E. & Studer, H. (1983) A link between liver microsomal enzyme activity and thyroid hormone metabolism in man. *Br. J. Clin. Pharmacol.*, **15**, 71–76

Olson, M.J., Johnson, J.T. & Reidy, C.A. (1990) A comparison of male rat and human urinary proteins: implications for human resistance to hyaline droplet nephropathy. *Toxicol. Appl. Pharmacol.*, **102**, 524–536

Oppenheimer, J.H., Bernstein, G. & Surks, M.I. (1968) Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J. Clin. Invest.*, **47**, 1399–1406

Partanen, T., Heikkila, P., Hernberg, S., Kauppinen, T. & Moneta, G. (1991) Renal cell cancer and occupational exposure to chemical agents. *Scand. J. Work Environ. Health*, **17**, 231–239

Pendergrast, W.J., Milmore, B.K. & Marcus, S.C. (1961) Thyroid cancer and thyrotoxicosis in the United States. Their relationship to endemic goiter. *J. Chronic Dis.*, **13**, 22–38

Pervaiz, S. & Brew, K. (1987) Homology and structure-function correlations between alpha$_1$-acid glycoprotein and serum retinol-binding protein and its relatives. *FASEB J.*, **1**, 209–214

Pevsner, J., Reed, R.R., Feinstein, P.G. & Snyder, S.H. (1988) Molecular cloning of odorant-binding protein: member of a ligand carrier family. *Science*, **241**, 336–339

Pliss, G.B. (1959) The blastomogenic action of dichlorobenzidene. *Vopr. Onkol.*, **5**, 524-533

Preston-Martin, S., Pike, M.C., Ross, R.K., Jones, P.A. & Henderson, B.E. (1990) Increased cell division as a cause of human cancer. *Cancer Res.*, **50**, 7415–7421

Price, J.M. (1971) Etiology of bladder cancer. In: Maltry, E., ed., *Benign and Malignant Tumors of the Urinary Bladder*, New York, Medical Examination Publishing Co., pp. 189–261

Read, N.G., Astbury, P.J., Morgan, R.J.I., Parsons, D.N. & Port, C.J. (1988) Induction and exacerbation of hyaline droplet formation in the proximal tubular cells of the kidneys from male rats receiving a variety of pharmacological agents. *Toxicology*, **52**, 81–101

Rehn, L. (1895) Ueber Blasentumoren bei Fuchsinarbeitern. *Arch. Klin. Chir.*, **51**, 588–600

Ricca, G.A. & Taylor, J.M. (1981) Nucleotide sequence of rat α_1-acid glycoprotein messenger RNA. *J. Biol. Chem.*, **256**, 11199–11202

Ridder, G.M., Von Bargen, E.C., Alden, C.L. & Parker, R.D. (1990) Increased hyaline droplet formation in male rats exposed to decalin is dependent on the presence of $\alpha 2\mu$-globulin. *Fundam. Appl. Toxicol.*, **15**, 732–743

Robbins, J. (1981) Factors altering thyroid hormone metabolism. *Environ. Health Perspectives*, **38**, 65–70

Robbins, J. & Rall, J.E. (1979) The iodine containing hormones. In: Gray, C.H. & James, V.H.T., eds, *Hormones in Blood*, 3rd ed., London, Academic Press, p. 576

Ron, E. & Modan, B. (1982) Cancer by tissue of origin—thyroid. In: Schottenfeld, D. & Fraumeni, J.F., Jr, eds, *Cancer Epidemiology and Prevention*, Philadelphia, W.B. Saunders, pp. 837–854

Roy, A.K. & Neuhaus, O.W. (1966) Proof of hepatic synthesis of a sex-dependent protein in the rat. *Biochim. Biophys. Acta*, **127**, 82–87

Roy, A.K., Neuhaus, O.W. & Harmison, C.R. (1966) Preparation and characterization of a sex-dependent rat urinary protein. *Biochim. Biophys. Acta*, **127**, 72–81

Ruiz, M. & Ingbar, S.H. (1982) Effect of erythrosine (2′,4′,5′,7′-tetraiodofluoresceine) on the metabolism of thyroxine in rat liver. *Endocrinology*, **110**, 1613

Sakata, T., Masui, T., St John, M. & Cohen, S.M. (1988) Uracil-induced calculi and proliferative lesions of the mouse urinary bladder. *Carcinogenesis*, **9**, 1271-1276

Saxen, E.A. & Saxen, L.O. (1954) Mortality from thyroid diseases in an endemic goitre area. Studies in Finland. *Doc. Med. Geogr. Trop.*, **6**, 335-341

Shelby, M.D. (1988) The genetic toxicity of human carcinogens and its implications. *Mutat. Res.*, **204**, 3-15

Shelby, M.D. & Zeiger, E. (1990) Activity of human carcinogens in the Salmonella test and rodent bone marrow cytogenetics tests. *Mutat. Res.*, **234**, 257-261

Short, B.G., Burnett, V.L. & Swenberg, J.A. (1986) Histopathology and cell proliferation induced by 2,2,4-trimethylpentane in the male rat kidney. *Toxicol. Pathol.*, **14**, 194-203

Short, B.G., Burnett, V.L., Cox, M.G., Bus, J.S. & Swenberg, J.A. (1987) Site-specific renal cytotoxicity and cell proliferation in male rats exposed to petroleum hydrocarbons. *Lab. Invest.*, **25**, 564-577

Short, B.G., Burnett, V.L. & Swenberg, J.A. (1989a) Elevated proliferation of proximal tubule cells and localization of accumulated $\alpha_{2\mu}$-globulin in F344 rats during chronic exposure to unleaded gasoline or 2,2,4-trimethylpentane. *Toxicol. Appl. Pharmacol.*, **101**, 414-431

Short, B.G., Steinhagen, W.H. & Swenberg, J.A. (1989b) Promoting effects of unleaded gasoline and 2,2,4-trimethylpentane on the development of atypical cell foci and renal tubular cell tumors in rats exposed to N-ethyl-N-hydroxyethylnitrosamine. *Cancer Res.*, **49**, 6369-6378

Siemiatycki, J., Dewar, R., Nadon, L., Gerin, M., Richardson, L. & Wacholder, S. (1987) Associations between several sites of cancer and twelve petroleum-derived liquids. *Scand. J. Work Environ. Health*, **13**, 493-504

Smellie, J.M., Bantok, H.M. & Thompson, B.D. (1982) Co-trimoxazole and the thyroid. *Lancet*, **ii**, 96

Smellie, J.B, Preece, M.A. & Paton, A.M. (1983) Normal somatic growth in children receiving low-dose prophylactic cotrimoxazole. *Eur. J. Pediatr.*, **140**, 301-304

Sokal, J.E. (1953) Occurrence of thyroid cancer. *New Engl. J. Med.*, **249**, 393-397

Strasser, J., Jr, Charbonneau, M., Borghoff, S.J., Turner, M.J. & Swenberg, J.A. (1988) Renal protein droplet formation in male F344 rats after isophorone treatment. *Toxicologist*, **8**, 136

Swarm, R.L., Roberts, G.K.S., Levy, A.C. & Hines, L.R. (1973) Observations on the thyroid gland in rats following the administration of sulfamethoxazole and trimethoprim. *Toxicol. Appl. Pharmacol.*, **24**, 351-363

Swenberg, J.A., Fedtke, N., Fennell, T.R. & Walker, V.E. (1990) Relationships between carcinogen exposure, DNA adducts and carcinogenesis. In: Clayson, D.B., Munro, I.C., Shubik, P. & Swenberg, J.A., eds, *Progress in Predictive Toxicology*, Amsterdam, Elsevier Science Publishers, pp. 162-164

Swenberg, J.A., Short, B.G., Borghoff, S.J., Strasser, J. & Charbonneau, M. (1989) The comparative pathobiology of $\alpha_{2\mu}$-globulin nephropathy. *Toxicol. Appl. Pharmacol.*, **97**, 35-46

Takayama, S., Aihara, K., Onodera, T. & Akimoto, T. (1986) Antithyroid effects of propylthiouracil and sulfamonomethoxine in rats and monkeys. *Toxicol. Appl. Pharmacol.*, **82**, 191-199

US Environmental Protection Agency (1986) Guidelines for carcinogen risk assessment. Part II. Fed. Regist., **51**, 33992-34003

US Environmental Protection Agency (1991) Alpha$_{2\mu}$-globulin: association with chemically-induced renal toxicity and neoplasia in the male rat (in press)

US National Cancer Institute (1979) *Bioassay of Sulfisoxazole for Possible Carcinogenicity* (Tech. Rep. NCI-CG-TR-138, DHEW Publication No. [NIH] 79-1393), Bethesda, MD, National Institutes of Health

US National Research Council (1989) *Drinking Water and Health*, Vol. 9, Washington DC, National Academy Press

US National Toxicology Program (1983) *Carcinogenesis Bioassay of Pentachloroethane in F344/N Rats and B6C3F1 Mice* (NTP Tech. Rep. Ser. No. 232), Research Triangle Park, NC

US National Toxicology Program (1986a) *Carcinogenesis Bioassay of Tetrachloroethylene (Perchloroethylene) in F344/N Rats and B6C3F1 Mice* (NTP Tech. Rep. Ser. No. 311), Research Triangle Park, NC

US National Toxicology Program (1986b) *Carcinogenesis Studies of Isophorone in F344/N Rats and B6C3F1 Mice* (NTP Tech. Rep. Ser. No. 291), Research Triangle Park, NC

US National Toxicology Program (1987a) *Carcinogenesis Studies of 1,4-Dichlorobenzene in F344/N Rats and B6C3F1 Mice* (NTP Tech. Rep. Ser. No. 319), Research Triangle Park, NC

US National Toxicology Program (1987b) *Carcinogenesis Studies of Dimethylmethylphosphonate in F344/N Rats and B6C3F1 Mice* (NTP Tech. Rep. Ser. No. 323), Research Triangle Park, NC

US National Toxicology Program (1990) *Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 5989-27-5) in F344 Rats and B6C3F1 Mice (Gavage Studies)* (NTP Tech. Rep. Ser. No. 347), Bethesda, MD, National Institutes of Health

Vandoren, G., Mertens, B., Heyns, W., Van Baelen, H., Rombauts, W. & Verhoeven, G. (1983) Different forms of $\alpha_{2\mu}$-globulin in male and female rat urine. *Eur. J. Biochem.*, **134**, 175–181

Watabe, T., Hiratsuka, A., Isobe, M. & Ozawa, N. (1980) Metabolism of d-limonene by hepatic microsomes to non-mutagenic epoxides toward Salmonella typhimurium. *Biochem. Pharmacol.*, **29**, 1068–1071

Wegelin, C. (1928) Malignant disease of the thyroid gland and its relationship to goitre in man and animals. *Cancer Res.*, **3**, 297–313

Wong, O. & Raabe, G.K. (1989) Critical review of cancer epidemiology in petroleum industry employees, with a quantitative meta-analysis by cancer site. *Am. J. Ind. Med.*, **15**, 283–310