Etiology of Bladder Cancer

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SINCE the surgeon Dr Rehn[38a] first suggested in 1895 a role for certain chemicals in the etiology of bladder cancer. this is historically the neoplastic disease most strongly linked to occupational and environmental exposure to chemicals. Subsequently. several chemical carcinogens that could be responsibie for human biadder cancer in particular groups of people have been identified. However, in many cases no obvious chemical carcinogen involvement can be found to explain cancer development. although factors such as cigarette smoking, coffee drinking, use of analgesics. artificial sweeteners. bacterial and parasitic injections, bladder calculi. and anticancer chemotherapeutic agents have been indicated as causally related. In general, bladder cancer develops predominantly in males (two to five times more frequently in men than in women). No clear explanation has been made for this sex differences.

By means of molecular biological approaches, it has been shown that carcinogens produce lesions in the genome of urothelial cells that initiate the process of carcinogenesis. Multiple lesions in the genome are required to cause complete malignant transformation of cells. and abnormalities of chromosomes, as well as induction of oncogenes and deletion of cancer suppressor genes, have all been shown *to* play possible roles. This review describes the etiological factors thought to be involved in bladder cancer development and general concepts in carcinogenesis.

OCCUPATIONAL EXPOSURE

Bladder cancer is the first human malignancy that was speculated to be related to systemic exposure to chemical(s) in the working place. Thus. after Rehn first described three cases of bladder cancer in workers in the German chemical dye industry, pointing out a possible causal relationship. similar reports appeared in Switzerland and England in the early 1900s. This provoked large numbers of investigations on occupational hazards, but the available data now indicate that the **risks** are not of general importance because the proportion of people exposed to individual factors at work is low.' Nevertheless, recent studies have estimated that between 21% and 25% of bladder cancers in Caucasians and 27% in nonwhites are occupationally related.' Similarly. it has been estimated that occupational exposure accounts for one fourth to on: third of bladder cancer cases in the United States.[3] The latent period may be as long as to 50 years.[4] Hueper et al[4] demonstrated in 1938 that when fed to dogs. the industrial arylamine 2-naphthylamine could induce bladder carcinomas identical to human lesions. Contrary to original suspicions. however, aniline itself did not cause the disease. In the early 1950s Case and his associates performed an epidemiological investigation of bladder cancers in workers in British chemical industries and found that arylamine-exposed individuals had a 30 times greater risk than did the general population. The average latent period for the development of bladder cancer was about 18 years.[3,4] With selected exposure circumstances, as many as 100% of exposed workers developed bladder cancer,[6] benzidine and 3-naphthylamine being implicated as especially potent human bladder carcinogens.[5,6] At present. both compounds are classified as human-bladder carcinogens' (Table 1). The low **risk** of bladder carcinogenesis by 1-naphthylamine is considered to be caused by contamination with 2-naphthylamine. In 1955 a close association between 4-aminobiphenyl exposure and an increased risk of bladder cancer development was also reported from clinical findings.[8] Thus. by the mid-1950s, conclusive evidence was available implicating at least three industrial arylamines as potent human-bladder carcinogens.[6,9]

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Table 1. Carcinogens for Which There is Evidence of Urinary Bladder-Cancer Induction in Humans

Chemicals	Induction of Bladder Cancer in Experimentol Animals
4-Ammobiphenyl	Mouse, dog, rabbit
Benzidine	—
2-Naphthylamine	Hamster, dog, monkey
Cyclophosphamide	Rat
N,N-Bis(2-chloroethyl)-2-naphthylamine	—
Phenacetin	Rat

In 1975. it was shown that about 85% of hair dyes contain mutagenic arylamines and arylnitro compounds.[11] One of these. 2.4-diaminotoluene was demonstrated to induce liver and mammary gland tumors in rats whereas p-cresidine (2-methoxy-5-methylaniline). was found *to* be a bladder carcinogen in both rats and mice.[11] Similarly. 4,4'-methylene-bis(2-chloroaniline)(MOCA), used as a curing agent for epoxy resins since the early 1950s, was shown to cause bladder carcinomas in dogs.[12] Another agent. 3.3'-dichlorobenzidine, used in the dyestuffs industry for nearly 40 years and more recently used as a curing agent for polyurethane elastomers was also demonstrated to be a bladder carcinogen in dogs and rodents.[11] Thus. human exposure to arylamines is considered an important causative factor for bladder carcinoma development.

TOBACCO SMOKING

There is substantial evidence supporting a relationship between cigarette smoking and development of bladder cancer. with epidemiological studies showing that cigarette smokers have up to a fourfold higher incidence than nonsmokers.[13-15] The association of smoking with bladder cancer holds for both sexes and has been observed in many different geographical areas of the USA. Europe, and Japan. Similar results have been obtained in both case-control studies[15-17] and follow-up studies.[11,18] The risk correlates with the number of cigarettes smoked. the duration ofsmoking. and the degree of inhalation of the smoke.[15,16] exsmokers having a reduced incidence of bladder cancer as compared with current smokers.[18] Although an estimated one third of bladder-cancer cases may be related to cigarette smoking.[21,22] the specific chemical carcinogens responsible have not been identified. However, it has to be noted that cigarette smoke 'contains a number 'of carcinogenic nitroso compounds and many arylamines. such as 2-naphthylamines. toluidines. and 4-aminobiphenyl. Table 2 summarizes data on the concentration of arylamines in smoke from air-cured and flue-cured tobaccos[23] smoking the former type entailing exposure to a 2 to 2.5 times higher dose of arylamines. In fact. there are three reports suggesting that air-cured tobacco is more carcinogenic for the bladder than flue-cured tobacco.[14,21] Although a variety of chemicals in cigarette smoke are DNA reactive in the urinary bladder epithelium, some may increase proliferative response in the bladder epithelium as evidenced by hyperplasias in cigarette smokers." This affect of cigarette smoke may exert an enhancing effect on bladder carcinogenesis.

SPECIFIC ENVIRONMENTAL CHEMICALS

4-Aminobiphenyl

As mentioned above. the data on both occupational exposure and cigarette smoking strongly suggest that arylamines play important roles in human bladder carcinoma development. Among the different arylamines that may be involved. the aromatic amine 3-aminobiphenyl (4-ABP) must be taken into consideration. because it is experimentally one of the most potent bladder

Table 2. Amounts of Aromatic Amines in Condensates of Air-Cured and Flue-Cured Tobaccos

Aromatic Amines	ng per Cigarette: Air Cured (France)	ng per Cigarette: Flue Cured (USA)
Aniline	102	364
0-Toluidine	32.2	162
M-Toluidine	15.3	30.4
P-Toluidine	13.5	33.8
2-Ethylaniline + 2,6-dimethylaniline	14.9	54.2
2.5-Dimethylaniline	19.1	87.2
3-Ethyloniline - 2.4-dimethylaniline	14.0	56.7
4-Ethylaniline - 2.3-dimethylaniline	7.8	27.3
1-Naphthylamine	4.3	2.5
2-Naphthylamine	1.0	1.7
2-Aminobiphenyl	1.8	3.0
3-Aminobiphenyl	2.7	5.0
4-Aminobiphenyl	2.4	4.6
2-Methyl-1-naphthylamine	5.8	3.6

Note. Mainstream smoke condensates were analyzed. Data from Potrianakos C, Hoffmann D.[23]

carcinogens and because the urinary-bladder mucosae of cigarette smokers contain 4-ABP-DNA adducts identical to those found in the bladder epithelium of dogs exposed to 4-aminobiphenyl.[26,27] A known human and mouse-bladder carcinogen. it is present in tobacco smoke," and is a probable ubiquitous environmental contaminant[3]' because it has been detected in the blood of individuals with no obvious chemical exposure. A role for 4-ABP has been stressed by many investigators.[32,33] and increased exposure to cigarette-derived 4-ABP clearly correlates with an increased incidence of bladder cancer.[32,33] Cessation of smoking is associated with a dramatic drop in the risk of cancer development[14,24] irrespective of the type of tobacco, air cured or flue cured. strongly suggesting that tobacco smoking acts partly as a tumor promoter in bladder carcinogenesis.

N-Acetylation has been demonstrated to be involved in deactivation of some arylamines. including 4-ABP, and this reaction is mediated by N-acetyltransferase. Human populations show a characteristic genetically based polymorphism for the activity of this enzyme. with about one half of the subjects being "slow" acetylators and the other half "fast" acetylators. The slow acetylator thus possesses a lower capacity for detoxification as compared to the fast one. Lower et al[34] were the first to recognize the role of acetyltransferase phenotype in variable susceptibility to bladder cancer: they found patients from an urban population who were also phenotypic slow acetylators to be at increased risk of suffering from the disease. Cartwright et al[35] demonstrated that the proportion of slow acetylators was 57% among 207 controls and 67% among 111 bladder cancer cases. This proportion, however. was markedly increased when cancer cases were restricted to an occupationally exposed group (22 of 23 were slow acetylators, all suffering from transitional-cell carcinomas. The one exception of a fast acetylator had, in contrast, an adenocarcinoma). Thus the proportion of slow acetylators in occupational exposure cases was virtually 100%. this value being the highest reported in the literature.

Other hospital-based case-control investigations of bladder cancer have been reported in which methods used for phenotyping were based on metabolism of sulfadimidine or sulfamethazine." Data from 11 different places showed that there were no site-dependent differences in the proportion of slow acetylators and that. overall. a 30% to 50% increase over general population levels was demonstrated for this proportion by bladder cancer patients. There are also data clearly demonstrating that the concentration of 4-ABP-hemoglobin adducts is higher in slow acetylators." With regard to the biochemical mechanism underlying the association between the slow-acetylator phenotype and an elevated risk of bladder cancer. the possibility of reduced detoxification deserves particular consideration. Animal models suggest that metabolic conversion of carcinogenic arylamines to N-acetyl derivatives represents a deactivation step elevated in phenotypic rapid acetylators. and thus slow acetylators would be expected to suffer more exposure to active carcinogenic species. Thus would explain the excess bladder cancer cases.[34,38] Arylamines are also metabolized by additional acetyltransferase pathways such as that catalysed by *O*-acetyltransferase. whose activity has also been associated with a polymorphic inheritance pattern."

Heterocyclic Amines

A series of mutagenic and carcinogenic heterocyclic amines have been identified as food-pyrolysis products formed during the cooking or broiling of meat and fish." Of the variety of organs found to be their targets in rodents. the most common sites of tumor induction are the liver and intestine. Particular attention has been paid to 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) because it is the most abundant heterocyclic amine produced in cooking processes and it induces rat colon and mammary carcinomas, both of which are very common malignancies in populations who consume a large amount of meat. Of interest to this review, 3-amino-1-methyl-5H-pyridol[4,3-b]indol (Trp-P-2) has recently been shown to induce bladder tumors in rats." In this context. it is important that cigarette smoke has also been reported to contain several mutagenic and carcinogenic heterocyclic amines including PhIP and Trp-P-2,[42-45] as well as the 4-ABP described above. The amounts of these heterocyclic amines generated were measured to range from 0.25 to 260 ng/cigarette. Trp-P-2 was reported to be present at levels of 0.82 to 1.1 ng per cigarette. with a mean value of 0.95 ng/cigarette. Because carcinogenic heterocyclic amines are produced from meat and fish during

cooking, the fact of their presence in cigarette smoke suggests that they are also formed though combustion of tobacco. In fact. several heterocyclic amines have been found to be present in various environmental components such as airborne particles, rain water. cigarette smoke-polluted indoor air, and diesel exhaust particles.[46] These findings imply that carcinogenic heterocyclic amines in food and cigarettes could well be causative factors for bladder cancer in man.

N-Nitroso Compounds

Occupational hazards of N-nitro-N-methylurethane and dimethylnitrosamine were early recognized by Wrigley[47] and Barnes and Magee,[48] respectively. Extensive animal experiments have subsequently shown that nitrosamines produce many kinds of tumors in a variety of animal species. Under specific conditions. N-containing amines and amides may undergo N-nitrosation to yield N-nitroso compounds.[49] Furthermore, endogenous formation ofnitrosamine in the acid stomach from normal dietary intakes of nitrite and amino compounds has been demonstrated." Thus humans are exposed both to naturally occumng and endogenously produced N-nitroso compounds. An important point regarding N-nitrosamines is that they have multispecies and multiorgan targets. a property shared by most other human carcinogens identified in the International Agency for Research on Cancer (IARC) Monographs.[51] The cellular and molecular damage produced by nitrosamines is virtually identical in animal and human tissues" and time-dose animal studies performed by Druckrey et al[53] indicate that long-term or lifetime exposure to even low levels of certain carcinogenic nitrosamines may represent a cancer risk to man.

Concerning urinary bladder carcinogenesis, animal experiments have shown that many nitrosamines exert carcinogenicity in this organ of dogs and rodents.[54-58] Recently. it was found that endogenous nitrosation could also be mediated by bacteria" and by macrophages[60] in infected or inflamed organs. It was reported that as many as 30% of the strains of microorganisms isolated from human achlorhydric gastric juice and about 90% of strains from urinary tract infections possess nitrosating enzymes.[61] and Ohshima et al[62] and Tricker et al[63] detected N-nitrosamines in infected urinary bladders. Bacteria that can catalyze N-nitrosamine formation and reduce nitrates to nitrites could clearly be responsible for production of N-nitroso compounds in situ in individuals with urinary-tract infections. In fact, high levels of nitrosamines are detected in bacteria-infected bladders of patients suffering from Schistosoma hematobium as described below. Available data therefore point to a probable important role for nitrosamines in the etiology of urinary bladder cancer.

Medicines

Analgesic agents. A possible relationship between analgesic abuse and the development of malignant neoplasms in the urinary tract was first pointed out in Sweden.[64] This possibility has now also been recognized in other countries. and the occasional development of bladder carcinomas was also mentioned as a result of phenacetin abuse by the IARC.[65] Phenacetin is normally used alone or in combination with aspirin and caffeine: The usual dose is 300 mg, taken four to six times per day to give a total of no more than 2.4 g per day.[66] Bengtsson et al[67] surveyed 62 patients with phenacetin-related pelvic tumors and estimated average phenacetin consumption to be about 9 kg with a range from 1.5 kg to 2.7 kg.

Over a long period (more than 10 years), the exposure is thus considerable and an increased risk of bladder carcinoma development under these conditions appears clear.[68] Phenacetin was evaluated to have sufficient evidence for carcinogenicity to humans as analgesic mixtures." It has a chemical structure similar to that of aniline dyes and although it is itself not nephrotoxic, most of its metabolites and some related compounds have been found to cause kidney damage.[69] N-Hydroxy phenacetin. formed by hydroxylation of this arylamide, induces liver carcinomas in rats at high incidence but only few tumors of the urinary tract. probably because it is nearly all metabolized in the liver and very little reaches the kidney.

In 1979, Isaka et al[70] first showed experimentally that phenacetin could induce transitional cell carcinomas of the urinary tract and carcinomas of the nasal cavity of Sprague-Dawley rats. However, Nakanishi et al[71] had earlier reported that phenacetin could promote nitrosamine-initiated F344 rat urinary-bladder carcinogenesis.

Cyclophosphamide. Cyclophosphamide is an alkylating agent that has been used in the treatment of malignant neoplasms, and particularly,

lymphoproliferative and myeloproliferative diseases. It is acutely toxic to the bladder. mucosa and produces marked cellular abnormalities in the epithelium. Clinical administration of cyclophosphamide is associated with various degrees of hematuria.

In 1971 Worth reported the first two cases of bladder cancer related to cyclophosphamide therapy." Since that time. at least 39 urinary-tract neoplasms following chemotherapy with cyclophosphamide have been described in the literature." Hicks" described cyclophosphamide to act with N-methyl-N-nitrosourea as a cocarcinogen for induction of bladder cancer in rats and Schmähl and Habs[74] later provided the first demonstration that administration of cyclophosphamide could induce bladder carcinomas in rats. Patients treated with cyclophosphamide have an up to ninefold increased risk of bladder-cancer development, although a causative relationship has not yet been formally demonstrated in case-control epidemiological studies.[75] The cumulative risk is 3.5% after 8 years and 10.7% after 12 years.[76] Most of the tumors involved are of transitional-cell type and present as muscle-infiltrating lesions at the time of diagnosis." The latent period for neoplasia related to this drug is therefore relatively short, ranging from 6 to 13 years. IARC has evaluated cyclophosphamide as in the group 1 category.[29]

Cyclophosphamide is also an immunosuppressive agent, this effect being now well recognized as a contributing factor. It is unclear whether cyclophosphamide-induced urothelial malignancies are due to its immunosuppressive or inherent carcinogenic properties but probably the two influences work cooperatively. Of four known metabolites of cyclophosphamide, acrolein and phosphoamide mustard have been demonstrated to bind to DNA[78] and acrolein is known to be responsible for its bladder toxicity." It is also believed to be the prime cause of the associated urothelial malignancies."." 2-Mercaptoethane sodium sulfonate (Mesna) was first used successfully to prevent cyclophosphamide-induced hemorrhagic cystitis in 1984,[80] binding to the double bond of the acrolein molecule and thus detoxifying the metabolite. Intravenous administration of Mesna significantly alleviated the processes of ulceration and inflammation in the bladders of rats given cyclophosphamide," and Schmähl and Habs[74] further show that its application can reduce induction of bladder carcinoma by this clinically-important agent. Forced diuresis has also been shown to be associated with a significant decrease in the incidence of hemorrhagic cystitis in patients on a high-dose regimen of cyclophosphamide."

N,N-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine). Chlornaphazine was used as a therapeutic agent for polycythemia in mid 1990. It was reported that among 61 patients receiving chlornaphazine, 13 developed bladder carcinomas, and 8 had abnormal urinary cytology. Invasive carcinomas were observed in 4 of 5 patients treated with a cumulative dose of 200 g or more.[83] Although no evidence exists for bladder carcinogenicity to rodents. it was evaluated as a human carcinogen.[29]

Tryptophan

For many years, tryptophan and its metabolites have been suspected as being etiological agents in bladder carcinogenesis. For example, bladder-cancer patients were reported early to have increased levels of urinary tryptophan metabolites.'' and tumor recurrence rates are more frequent in patients with elevated excretion of metabolites derived from this amino acid.[85,86] Although administration of tryptophan to experimental animals does not induce bladder cancer, it was demonstrated that dietary tryptophan or its metabolites could enhance bladder carcinogenesis.[87,88]

However. more recent studies suggest that endogenous tryptophan metabolism does not contribute significantly to the development of bladder cancer.[89] On the other hand, a pyrolysis product of tryptophan. Trp-P-2, is currently known to induce bladder carcinoma in rats" as described in a previous section. It can be formed during cooking of'meat or fish[40] and is strongly mutagenic in *Salmonella typhimurium*.

DIETARY FACTORS

Artificial Sweeteners

Artificial sweeteners, particularly saccharin and cyclamate, have been shown to act as bladder carcinogens in rodents when given in very large doses.[90] The conclusion of complete carcinogenic potential is. however, controversial because of the extremely high exposure levels, and it has now been clearly demonstrated that both saccharin and cyclamate are strong tumor promoters in the

bladder. Recent extensive studies by Fukushima et al[91,92] showed that the promoting potential of sodium ascorbate for rat urinary bladder carcinogenesis is because of elevation of urinary pH and Na^+ concentration in combination. They found that many compounds that enhance tumor development in the bladder share this character[91] and in all cases of saccharin experiments. the Na^+ salt form had been used. It was later confirmed that sodium saccharin. but not the saccharin acid form. exerts promoting potential for bladder carcinogenesis.[94] In this context. it is of interest that cyclamate is also given as the sodium salt. Case-control epidemiological studies in humans. however, have provided little evidence for increased risk of bladder cancer in consumers of artificial sweeteners.[95,96] A proliferative and neoplastic response following oral administration of high doses of sodium saccharin has been seen only in the rat, generally with considerably greater effects in males than in females.[91] From the mechanistic view point. Ellwein and Cohen[97] therefore concluded that sodium saccharin has little effect on human urothelial cells at even the highest levels of human consumption.

Coffee and Tea Drinking

Coffee drinking has been implicated by some studies into the etiology of bladder cancer.[15,98] An increased **risk** of bladder cancer has also been reported in association with tea drinking.[98] Nevertheless. a recent review by IARC[99] stated that there is no consistent association in descriptive studies between coffee intake and cancer **risk** and that in case control studies. the observed weak positive relationship might be due to bias. In addition no association was concluded for tea consumption.

BLADDER INFECTION

A number of epidemiological and experimental studies have convincingly indicated that urinary tract infection is a strong risk factor for the development of bladder cancer.[100,101] The majority of bladder cancers linked with exposure to chemicals. **as** reviewed above. are transitional cell carcinomas with a variable biological potential. In contrast. the cancers related to chronic urinary infection are generally deeply invasive squamous-cell carcinomas that show a poor prognosis. This type of cancer has been reported to occur in patients with spinal cord injury, who inevitably develop chronic cystitis.[102,103] Several experimental investigations have also shown that bacterial infection in the urinary tract promotes bladder carcinogenesis in rats[104,105] and in this case a hyperplastic response of the bladder epithelium is thought to be responsible. However. the exact mechanisms whereby bladder cancers are induced in chronically infected human bladders is unclear. As discussed in the section on nitrosamines. in situ formation of carcinogenic nitroso compound(s) mediated by bacteria could play an important role.

In regions of North Africa and the Middle East. where infection with *Schistosoma hematobium*. a water-borne parasite. is endemic (schistosomiasis, bilharziasis). there is a very high incidence of bladder cancer. Known to have a lethal outcome despite aggressive surgical and chemotherapeutic treatment. bilharziasis-associated bladder cancer is characterized by a predominance of squamous-cell carcinomas.[100-108] El-Bolkainy et al histopathologically reviewed a series of 1095 Egyptian bladder-carcinoma patients treated by radical cystectomy and found that in 82.4% of cases. specimens were positive for schistosome eggs. and tumors developed at a younger age (46.7 years) in this group than in the 17.6% of egg-negative cases (53.2 years). However. they found no differences in the frequencies of various tumor stages or lymph-node metastases between the two groups. The etiology of bladder cancer in patients suffering from bilharziasis presumably involves many factors including carcinogen exposure and mechanical irritation by eggs. Secondary bacterial infection is once again suspected as an important factor,[109] the most commonly identified organisms being *E coli-Staphylococci*. and *Pseudomonas*.

Severity of bacterial infection correlates with elevation of urinary d-glucuronidase, which may activate potential carcinogenic compounds excreted from the liver as glucuronides.[110] Furthermore. the bacteria found can convert nitrate to nitrite. resulting in production of carcinogenic nitrosamines by reaction with amines.[110,111] and the appearance of urinary nitrite also appears dependent on the severity of bacterial infection. In fact. four nitrosamines have been identified in the urine of bilharzial patients.[110,111] and studies in Egypt showed the amounts of nitrosamines extracted from chemically nitrosated urine of

bilharzial patients with or without bladder cancer to be **70** times higher than in controls.[111]

Vitamin **A** deficiency may be an additional factor related to development of squamous cell metaplasia, and serum levels of β-carotene and vitamin **A** were demonstrated to be lower in bilharzial patients with squamous cell carcinoma than in those with transitional cell carcinoma.[111]

Urinary calculi. which are commonly associated with urinary tract infections. have also been linked to urothelial malignancies. In rodents calculi can induce severe urothelial hyperplasia and promote bladder carcinogenesis.[116-117] and their presence alone can result in development of bladder carcinomas in rats when exposure is prolonged.[118] Epidemiologically. Kantor et al[100] similarly found that bladder stones present an increased risk for bladder cancer. Furthermore, urothelial hyperplasia and dysplasia are commonly observed in association with stones.[111]

URINE

It was recognized early that the urine is important for the development of bladder cancer because it acts as a medium of exposure to carcinogenic substances.[12,121] The significance of urine-borne, rather than blood-borne, effective metabolites of bladder carcinogens was also shown by Ito et al,[122] who showed that unilateral ureter ligation resulted in induction of renal pelvic cancers, that normally do not develop, at the site of the ligation. Furthermore, Hashimoto and Kitagawa[123] reported that in vitro neoplastic transformation of rat urothelial cells by nitrosamine required the presence of urea in the tissue-culture medium. Oyasu et al[124] subsequently demonstrated the significance of urine for carcinoma development in **a** heterotopically transplanted urinary bladder system. They have found that a specific factor is responsible for urine-promotion of bladder carcinoma development, and recently, epidermal growth factor was shown to present in the urine.[111]

Kakizoe et al found that of 21 amino acids, L-leucine and L-valine increased Concanavalin **A** agglutinability of isolated rat-bladder cells in an in vivo-in vitro system, suggesting that both are possible tumor promoters.[126] Subsequently they demonstrated that dietary supplementation with both amino acids promoted bladder-cancer development in rats initiated with a bladder carcinogen, N-butyl-N-(4-hydroxybutyl) nitrosamine, and they speculated that the diet rich in protein, as in Western countries. is a risk factor.[111]

GENETIC FACTORS

From the above it is clear that the etiology of bladder cancer involves environmental factors. In addition. it has already been noted that a genetic predisposition to bladder cancer may exist. The incidence of cancer of the bladder in different areas of the world varies almost ninefold in males, and 190-fold in females. Although this international variation is practically the smallest among the common cancers.[111] race. sex. and geographical differences within the United States have also been documented.[111] Schulte[130] has reviewed the literature on the influence of genetic factors in bladder cancer. and summarized findings into four categories: (1) familial aggregation; (2) genetic polymorphism; (3) N-acetyltransferase phenotype: and (4) activated oncogenes and chromosomal changes. He stated that a role for genetic factors could not be conclusively ascertained, but he proposed that two different patterns of genetic involvement might be identified. One is the Mendelian pattern of autosomal dominant inheritance observed in a very small number of cases. and the other is the multifactorial pattern involving genetic and environmental interaction.

Since Fraumeni and Thomas[111] published the first report of familial occurrence of bladder cancer in a man and his three sons, many cases of bladder-cancer development in different family members have been documented.[130] Familial aggregation is however not necessarily confirmation of a genetic etiology but may be also caused by shared environmental factors or even freak random distribution. Nevertheless. Punilo et al[132] suggested that familial bladder cancer occurs rather more commonly than had been earlier thought, from their data on 13 cases of bladder cancer that occurred during a 2-year period in six unrelated families. **An** excess of the **A** gene of the ABO blood group in bladder-cancer cases was demonstrated by Herring et al[133] who have also investigated human leukocyte antigen (HLA) polymorphism and found two HLA genes, B5 and Cw 4, to be increased. However, other details regarding any association between HLA and this neoplasia are not available to our knowledge.

As described in a previous section. phenotypic variation in N-acetyltransferase activity has been found to be associated with bladder cancer, par-

ticularly in workers exposed to aromatic amines. This is an autosomal recessive trait and populations can be divided into two distinct phenotypes, slow and fast acetylators. The ratio between these two varies among racial and ethnic groups and Kadlubar et al[134] suggested that quantification of molecular adducts of aromatic amines might be useful to test genetic susceptibility to bladder cancer.

The concept of cancer as a genetic disease resulting from genetic changes in somatic cells is now well recognized. It is likely that multiple lesions are required to cause malignant transformation of any given cell. and bladder cancers demonstrate alterations in many kinds of oncogene (mutation, over expression and deletion) as with many other types of malignant tumor. Several oncogenes in the *ras* gene family have been investigated in bladder cancers.[135,136] The H-ras oncogene was found to be activated in 7% to 17% of human bladder cancer cases.[137,138] A number of workers have demonstrated experimentally-induced bladder carcinomas to similarly contain mutated or activated *ras* genes and show enhanced expression of p21.[139,140] The expression of c-*myc* oncogene was found to correlate with recurrence or invasion in superficial bladder cancer.[141]

A role for tumor suppressor genes in the development of human bladder cancer have recently been proposed by several laboratories. High frequencies of loss of heterozygosity (LOH) of chromosomes 9q, 11p. and 17p have been observed in bladder carcinomas.[142,143] LOH of chromosome 9 is seen in both low and high stage tumors whereas allelic losses of other chromosomes seem to be associated with tumor progression. It has therefore been postulated that inactivation of a tumor suppressor gene located on chromosome 9 is an early event in the development bladder cancer.[144,145] The isolation and characterization of this tumor suppressor gene are necessary to facilitate full understanding of this disease.

In contrast to the stage and grade independent loss of chromosome 9 in bladder cancers. losses of chromosomes 11p and 17p. where the p53 gene is located, are associated with high grade, high stage tumors.[143] The p53 tumor suppressor gene in human bladder cancers has been shown to contain frequent point mutations in the retained p53 allele.[145] Association of p53-gene mutations with invasive growth has also been reponed by Fujimoto et al.[146] and it has recently become clear that the kinds of mutations sustained in the p53 tumor suppressor gene might retlect exposures to different environmental carcinogens. Spruck et al[147] have compared the spectrum of mutations in the tumor suppressor gene in the tumors of smokers and nonsmokers. to determine whether differences exist in the kinds of mutations in these two groups. There were no obvious differences in the types or positions of mutations seen. However. there was a high frequency of double mutations seen in smokers only. suggesting that cigarette smoke may act to increase the extent of DNA damage. In the near future, these kinds of molecular approaches should help to disclose etiologically specific DNA changes within tumor suppressor genes that might give important clues to the mechanisms by which chemicals or other agents induce bladder carcinogenesis.

Retinoblastoma (Rb) tumor suppressor gene alterations have been frequently found in bladder cancer[148] and their role might also be important for neoplastic development in the bladder.

Understanding the molecular defects seen in human bladder tumors. and a delineation of the roles of specific tumor suppressor genes is likely to be achieved over the next few years. This molecular approach will undoubtedly add very significantly to our understanding of the mechanisms by which the urothelium becomes transformed during carcinogenesis.

GENERAL CONCEPTS IN CARCINOGENESIS

Multistage Carcinogenesis

The concept of initiation and promotion originally evolved from skin carcinogenesis experiments[149,150] has now been expanded to cover carcinogenesis in many organs including the urinary bladder. The "initiation" is the first event of the carcinogenic process in which irreversible or memorized genetic alteration(s) is induced in target cells by exposure to chemical or physical agents. A chemical. physical. or biological agent that is capable of inducing such alterations is termed an "initiator" or "initiating" agent. Repeated exposure induces additive effects in such genetic alterations. Initiated cells, however, are not readily identifiable. For the initiation event, cell replication could facilitate fixation of genetic alterations (DNA damage). Spontaneous

or fortuitous occurrence of initiated cells may also play a role. It is unlikely that initiation alone proceeds to formation of frank tumors.

Promotion is the second-stage process in which initiated cells grow clonally to form tumor masses. The process usually requires repeated exposure of cells to certain agents over long pe-nods. Biological characteristics of promotion are reversible increase in replication of the initiated cell population and reversible alterations in gene expression. Unlike initiators. promoters do not themselves directly react with the genetic materials. They exert more tissue and/or organ dependency than initiating agents" and continuous cell proliferation seems very important for promotion."' Many. but not all. promoting agents stimulate cell proliferation in target cells.

Recently. the concept of multistage carcinogenesis has increasingly come to include progression in addition to initiation and promotion. The progression was first proposed by Foulds[153] who described it as the process of development of malignant neoplasia that obtained higher degrees of autonomy and malignancy. Earlier promotion included all processes until malignant tumor development. Progression is a relatively late stage of the carcinogenic process in which genetic alterations such as oncogene activation, gene amplification in neoplasms associated with increased growth rate. invasiveness. metastatic potential and capability. morphologic characteristics. and/or hormone responsiveness are involved.

Molecular biological approaches are revealing roles for many kinds of genetic alteration (changes in oncogenes and antioncogenes), reflecting the multistage nature of neoplasia. Several genetic alterations are generally required for malignant transformation. These alterations evoke heterogeneity in tumor morphology and biology.

Characteristics of Carcinogenic Agents and Their Mode of Action

The majority of known carcinogenic agents possess the capacity for both initiation and promotion. They are termed "complete carcinogens"[152,155] and are capable of inducing DNA alterations as well as cell proliferation. Chemicals that possess only initiating activity are termed "incomplete carcinogens." A single or very short-term administration of complete carcinogen may practically act to drive the whole process, and tumor development may occur without subsequent administration of exogenous promoters, because there are many endogenous promoting factors or substances. Similarly. promoters could induce tumors without apparent application of an initiator. if they can stimulate growth of spontaneously-initiated cell populations.

Complete carcinogens can be categorized into several classes. according to their abilities for initiation and promotion.[156] Some have strong activities in both initiation and promotion stages, but some are strong initiators and weak promoters and vice versa. The overall carcinogenic activity of a chemical. therefore, may depend on the interplay between these activities.

Carcinogens can also be divided into two categories, genotoxic and nongenotoxic. although precise classification is sometimes difficult. Genotoxic carcinogens are DNA reactive. with or without dependency on enzymatic activation. Nongenotoxic (also called epigenetic) agents have reliable evidence of an inability to interact with genetic materials and exert other kinds of biological effects that appear to be the basis for their carcinogenicity.[157] Therefore. there is a wide variety of types of agents in this category with different modes of action. Many of them increase DNA synthesis, mitosis, and cell duplication rates.'" Some of them, however, could induce DNA damage through indirect mechanisms, such as producing oxygen radicals.[158] Because genotoxicity is at least partly irreversible and is additively accumulated, genotoxic carcinogens are generally effective for induction of tumors at low doses, whereas nongenotoxic chemicals need high doses, reflecting the presence of a threshold level to exert carcinogenicity. Strong promoters are nongenotoxic carcinogens, as in the case of phenobarbital in the liver. Precise distinction behveen promoters and nongenotoxic carcinogens is, however, sometimes difficult. If a promoter alone induced certain type of tumors with a statistical significance in a lifespan animal experiment. it would of necessity be classified as a nongenotoxic carcinogen.

Direct genotoxic effects are the result of interaction of chemicals themselves or the interaction of their electrophilic metabolites with DNA. Carcinogenicity of chemicals that require biotransformation to yield electrophiles depends on activities of activating and/or detoxifyin,, en-

zyme(s). which vary with organs. strains. species, and sex. These enzyme activities are furthermore easily influenced by many chemicals. nutrition. and other environmental factors. resulting in either inhibition or enhancement of carcinogenesis.

Additive or synergistic effects of two or more carcinogens are designated as "syncarcinogenesis." occurring when different agents acting on the same target organ are given together or in sequence. Another mode of carcinogenesis is cocarcinogenesis. by which cocarcinogen enhances the effectiveness (neoplastic conversion) of a carcinogen present at the same time. Promotion. in contrast. occurs subsequent to exposure to a carcinogen and only enhances neoplastic development of previously initiated populations.

Human Situation

Concepts of carcinogenic processes. including information relating to biological characteristics of each stage in tumor development. effective doses of carcinogens or modifying chemicals. species and/or organ specificities. and mode of interaction between two or more agents are essential in considering human cases. In the human bladder, initiation might occur because of the action of many kinds of genotoxic chemicals and drugs. including aromatic amines. benzidine, N-nitroso compounds. and cyclophosphamide and phenacetin. Promotion will occur because of exposure to aromatic amines. phenacetin. cytotoxic agents, chronic bladder infection. and mucosal injuries. Tryptophan metabolites may or may not be promoters. Cigarette smokes contains a variety of chemicals classified as genotoxic and nongenotoxic carcinogens and promoters. They might act synergistically and/or play a role in the cocarcinogenic mode. It is important to note that many of the specific steps in carcinogenesis are controlled and modified by numerous endogenous and exogenous factors. Among the exogenous elements. nutritional factors are very much involved.

SUMMARY

That the etiology of bladder cancer involves environmental factors is well documented. Most chemical carcinogens probably affect the urothelial cells via their presence in the urine. As an important cofactor. cell-proliferative activity may be increased by urinary bladder infection. Irritation by bladder stones or through the action of a variety of 'direct acting chemicals or agents. Among the known causative factors; avoidable major ones are occupational exposure to certain chemicals such as benzidine and 4-aminobiphenyl. cigarette smoking. and bilharzial infection. which could be eradicated by a Combination of praziquantel. antihelminth therapy, education. and improvements in social welfare. An anticarcinogenic drug. cyclophosphamide. used as an immunosuppressive agent also seems to be associated with a high risk of idiopathic induction of bladder cancer and physicians should therefore pay particular attention to its diverse effects when considering its prescription. In contrast to the above. the consumption of coffee and tea containing artificial sweeteners is now thought unlikely to be a major risk.

So far there is no good biochemical tool to predict individual esposure to bladder carcinogens/or relative risk of bladder-cancer development. However. acetylation capacity can be applied to assess susceptibility to carcinogenic amines in people exposed in their working environment.

Progress in molecular-biological analysis will hopefully bring to light etiology-specific DNA damage in bladder tumors. and it may prove useful for prediction of tumor behavior in the near future.

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