Relationship between Schistosomiasis and Bladder Cancer

M. H. MOSTAFA,[1] S. A. SHEWEITA,[1] AND P. J. O'CONNOR[2*]

*Institute for Graduate Studies and Research, University of Alexandria, Chatby 21526,
Alexandria, Egypt,[1] and Paterson Institute for Cancer Research,
Christie Hospital (NHS) Trust, Manchester M20 4BX,
United Kingdom[2]*

INTRODUCTION

Schistosomiasis has been endemic in Egypt at least since the time of the ancient Pharaohs, as indicated by the presence of calcified ova in the Egyptian mummies (180). Schistosomiasis, sometimes called bilharzia, is now a widespread endemic disease currently found in 75 countries. It is estimated that more than 200 million people residing in rural and agricultural areas are infected and that between 500 million and 600 million people are at risk of infection (225). Typically, schistosomiasis is a disease affecting agricultural communities, particularly those dependent upon irrigation to support their agriculture. The problem became much more significant in the 19th century, when the combination of new irrigation projects and population increases led to a higher probability of exposure to the parasite (12, 164). Four schistosome species, namely, *Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, and *S. intercalatum*, commonly infect humans. Infection with schistosomes does not always result in clinical disease, and many infections are asymptomatic. Symptoms associated with schistosomiasis include weakness, diarrhea, hepatosplenomegaly, and carci-

noma of the intestine, liver, uterus, and bladder (38, 40, 52, 59, 116, 193, 194, 199, 230).

Although *S. mansoni* infection is widespread in Africa, Eastern Brazil, and Central America and occurs commonly in mixed infections with *S. haematobium* in Egypt, no definitive reports have been made to link the parasite with the geographical occurrence of cancer. There are strong, but not definitive, indications that *S. japonicum* is a causative agent in the development of liver cancer in Japan and colorectal cancer in China (230). In schistosomiasis due to *S. haematobium*, the intensity of infection, or worm burden, is correlated with morbidity, the degree of hematuria and proteinuria, and the pathological changes observed in the urinary bladder and ureters (1, 88, 163, 192, 220), and malignancies of the bladder (230). Note that several terms are used in the literature to indicate the severity of schistosomiasis. In this article, the following terms are used: "worm burden," which is the number of worms present in the tissue and is determined either directly in animal studies or estimated from the rate of egg production in human studies, and "multiplicity of infection," which is the number of cecariae used to infect the host in animal studies.

Maintenance of the *S. haematobium* life cycle in rats and mice has generally proved difficult; it is somewhat less so in hamsters. However, only in monkeys can the disease be reproduced in a manner similar to that seen in humans (39). In experimental studies, therefore, infection of mice with *S. man-*

* Corresponding author. Mailing address: Paterson Institute for Cancer Research, Christie Hospital (NHS) Trust, Wilmslow Rd., Manchester M20 9BX, United Kingdom. Phone: 0161 446 3124. Fax: 0161 446-3109. E-mail: pjoconnor@picr.man.ac.uk.

soni is most frequently used as a model in which the development of the immature worms (schistosomulae) in the murine liver up to the adult stage is similar to the development of *S. mansoni* or *S. haematobium* in the human host. For these reasons, the mouse-*S. mansoni* model has been used extensively to study the effects of schistosomiasis on hepatic xenobiotic metabolism (see "Carcinogen activation").

Bladder cancer was one of the first cancers to be associated with an industrial process and exists in different histologic cell types. In industrialized countries (North America and northern Europe), bladder cancer occurs mainly as transitional cell carcinomas (TCC), with a peak incidence in the seventh decade of life (33, 127). More than 90% of bladder cancer patients in the United States have tumors of the transitional cell type (124). Other histological cell types, in decreasing order of frequency, are squamous cell carcinoma (SCC; often associated with chronic inflammation), adenocarcinoma, and rare histological types such as sarcomatoid carcinoma, small-cell carcinoma, and lymphoepithelioma (184). Multiple chemical and environmental exposures have been associated with TCC of the bladder. Epidemiological studies of urinary bladder cancer began in 1895 with a study of the excessive occurrence of bladder cancer among workers in the aniline dye industry (183); this was confirmed in 1954 by Case et al., (36). Since then, much evidence has accumulated to document the relationship of bladder cancer to certain industrial chemicals known to have carcinogenic effects. Case-control studies revealed that about 19 and 6% of bladder cancers in males and females, respectively, were related to occupational exposure to industrial carcinogens that are specifically implicated in the induction of bladder cancer, such as α- and β-naphthylamine, 4-aminobiphenyl, methylene dianiline, 4-chloro-*o*-toluidine and toluidine (102, 124, 188, 219).

Cigarette smoking is now recognized as a major cause of bladder cancer in developed countries, increasing the risk two-to threefold in North America and Europe and accounting for 50% of these cancers in males and 25% in females (229). Although much less information is available from developing countries, a recent study in Egypt indicated that smoking was strongly associated with bladder cancer in males and could account, at least in part, for 75% of these cancers. Thus, smoking may be an important part of the etiology of bladder cancer attributed to schistosomiasis in males. This was not the case for females, who also have a high incidence of schistosomiasis-related bladder cancer but a low prevalence of smoking (24).

The major histological cell type of bladder cancer associated with schistosomiasis of the urinary tract is SCC (114, 116, 117). Several biological factors such as bacterial infections and immunological status are implicated in predisposing individuals to bladder cancer (14, 230), and there are several well-documented relationships between infections with certain parasites and the development of cancer (15), in particular schistosomiasis and bladder cancer (14, 18, 193, 213) and *Opisthorchis viverrini* and *Clonorchis sinensis* infections with cholangiocarcinoma (100, 206). The evidence associating *S. haematobium* infection with the development of bladder cancer is, however, far greater than that for any other parasitic infection; it has been supported by several major studies in countries in Africa and the Middle East (14, 18, 38, 42, 87, 161, 193) and more recently confirmed as definitive (230).

The aim of this article is to review the relationship between schistosomiasis and bladder cancer with respect to the mechanisms of carcinogenesis and the roles played by microorganisms in the endogenous generation of carcinogens, their metabolism, DNA damage, and the genetic consequences of these events.

EVIDENCE SUPPORTING THE RELATIONSHIP BETWEEN SCHISTOSOMIASIS AND BLADDER CANCER

Epidemiological Evidence

With regard to the prevalence and intensity of infection, schistosomiasis heads the list of endemic parasitic diseases in Egypt. This infection has been extensively investigated by medical hospitals in Egypt in order to focus on, understand, and confront the health problems associated with the schistosomiasis. In spite of considerable efforts to eradicate the disease, the incidence of illness is still high: 60% of the Egyptian population is at risk of infection. Children of school age are especially at risk because of their daily contact with infected water in rural areas, leading to an overall disease prevalence of 37 to 48% (228). In the Nile Delta area, mixed infection with *S. haematobium* and *S. mansoni* is endemic, while *S. haematobium* is more prevalent in upper Egypt due to the greater abundance of the specific intermediate host snails in that area.

The association of bladder cancer with schistosomiasis seems to be related to the endemicity of the parasite (42). The consensus of available information strongly implicates an association between *S. haematobium* infection and the induction of bladder cancer. This neoplasm accounts for 30.8% of the total cancer incidence and is ranked first among all types of cancer recorded in Egyptian males and second only to breast cancer in females (101, 109). It was estimated that of 11,626 cancer cases of all types recorded at the Cairo Cancer Institute from 1970 to 1974, 27.6% were bladder cancer cases associated with schistosomiasis, and that of 2,500 new cancer patients at the Cairo Cancer Institute every year, 27% had cancer of the bladder associated with schistosomiasis (66). In another study, 28.8% of cancer cases were reported to be bladder cancer associated with schistosomiasis among 6,981 cancer patients recorded in Cairo hospitals during the period from 1978 to 1979 (3). Again, from 1970 to 1981, the incidence of bladder cancer in men and women ranked first (30.8%) among 25,148 cancer cases accessed by the registry of the National Cancer Institute, Cairo, Egypt. All of these observations support an association between schistosomiasis and bladder cancer. In other countries, such as Iraq (8), Malawi (137), Zambia (60) and Kuwait (9), where the endemicity of schistosomiasis due to either mixed or *S. haematobium* infestations is high, bladder cancer was also reported to be the leading malignant disease. In contrast, in schistosome-free countries such as Germany (23), the United States (51), the United Kingdom (170) and Turkey (26), bladder carcinoma ranks from the 5th to the 7th most common cancer in men and from the 7th to the 14th in women.

Experimentally Induced Schistosomiasis

Most of the earlier studies conducted on experimental animals were those in which clinical and pathological criteria were used to evaluate the carcinogenic effects of *S. haematobium*. Noninvasive papillary and nodular TCC of the urinary bladder were observed in a talapoin monkey (*Cercopithecus talapoin*), a capuchin monkey (*Cebus appella*), gibbons (*Hylobates lar*), and opossums (*Didelphys marsupialis*) (121–123) when infected with *S. haematobium*. These types of carcinoma were morphologically similar to those observed in human bladders (38), and such observations suggest that there is an association between *S. haematobium* and bladder cancer.

Heavy egg deposits in the bladder mucosa and submucosa were seen during the acute phase of *S. haematobium* infection in mice (4), monkeys (41, 123), baboons (92), and humans (see, e.g., references 38 and 43). Since these eggs can act as a

mechanical irritant to the urothelium, inducing chronic inflammatory lesions, several attempts were made to evaluate the carcinogenic potential of experimentally induced schistosomiasis. For example, chronic tissue injury could provide a promoting factor which acts to increase the rate of cell turnover via the induction of restorative hyperplasia and squamous metaplasia. At this stage, the proliferating cells are not neoplastic but are transitional and noninvasive; most of these focal hyperplasias are subsequently reversible (41). However, in some situations, hyperplasia and dysplasia may become irreversible, particularly during concomitant exposure to low (subcarcinogenic) doses of carcinogens e.g., *N*-nitroso compounds (92).

Infection with *S. mansoni* increased the risk of hepatic carcinoma associated with the administration of the liver carcinogen 2-amino-5-azotoluene in mice (56). Similarly, epithelial hyperplasia and metaplasia were found in the bladders of mice that had been infected with *S. haematobium* after pretreatment with an aromatic amine such as acetylaminofluorene (87) or following a mechanically induced *Escherichia coli* infection in combination with 2-naphthylamine treatment (6). Studies were also performed with schistosome-infected baboons treated with the specific bladder carcinogen *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BHBN) (92). In this case, the carcinogen was administered weekly for 30 months and bladder cancer was induced in 4 of 10 infected baboons (92). These observations suggest that schistosomiasis could supply the proliferative stimulus necessary to accelerate cancer growth from latent tumor foci that were induced by exposure to subcarcinogenic doses of a bladder carcinogen such as *N*-nitroso compounds (92). Exposure of the rat urothelium to *N*-methyl-*N*-nitrosourea (NMU) for periods up to 1 year or sequentially to NMU and BHBN for several weeks resulted in the development of high-grade, deeply invasive SCC from low-grade, noninvasive TCC (172, 191). In the human schistosome-infected bladder, high-grade SCC is usually well advanced at the time of diagnosis (59, 116).

Histopathological Findings Associated with Schistosome Infection

The histopathological entities of bladder cancer associated with schistosomiasis have certain distinct features which differ from those of bladder cancer found in Western countries (162). In many areas of endemic schistosome infection, a much higher proportion SCC of the bladder was seen compared to those occurring in Europe or North America. In Egypt, for example, SCC occurred in 10 of 1,000 adults infected with *S. haematobium* but only in 0 to 3 of 1,000 schistosome-free patients (84). Some changes in the pathological types of schistosomiasis-associated bladder tumors have been found over periods spanning two decades in the same institution. Comparing the periods 1962 to 1967 and 1987 to 1992, there was a decrease in the incidence of nodular tumours (83.4 to 58.7%) and of SCC (65.8 to 54.0%) but an increase in the incidence of papillary tumors (4.3 to 34.8%) and TCC (31.0 to 42.0%); all changes were statistically significant (116). The extent of *Schistosoma* infection apparently plays a significant role in the induction of different types of carcinoma, since SCC is usually associated with moderate and/or high worm burdens whereas TCC occurs more commonly in areas associated with lower degrees of infection (116). In other countries also (e.g., Iraq) a strong correlation between *S. haematobium* infection and SCC is maintained (8, 230). The proportion of SCC varied from 54 to 81% of all bladder cancer cases in different areas of endemic

infection, which contrasts to Western countries, where the frequency of SCC in bladder cancer cases is much lower (3 to 10%) (59). The predominance of SCC in human urinary bladder tissues in patients with schistosomiasis is probably related to the continuous exposure to the carcinogens, e.g., *N*-nitroso compounds, which were detected in larger quantities in the urine of patients with schistosomiasis than in patients without this infection (91, 92, 161, 213).

Age and Gender Ratios

In schistosome-free countries throughout the world, the peak incidence of bladder cancer is in the sixth or seventh decade of life (127) and is maximal between the ages of 65 and 75 years (33); only 12% of bladder cancer cases occur in people younger than 50 years (173). By contrast, in Egypt, Sudan, Iraq, Zambia, Malawi, and Zimbabwe, the mean age of the highest incidence of bilharzial bladder cancer is between 40 and 49 years (7, 59, 60, 76, 101, 137, 138), which clearly contrasts with the findings for nonschistosomal areas. The ratio of bladder cancer incidence (males to females) in countries with endemic infection was reported to be 5:1 (67, 104) but may vary within the range of 4:1 to 5.9:1 (101). The relatively higher gender ratio in the countries with endemic infection (c.f. 3:1 in countries of nonendemicity) has been suggested to be because in rural areas the main route for infection is through contact with infected waters during agricultural activities, which are normally done by men rather than women (3).

MECHANISMS OF BLADDER CARCINOGENESIS: ROLE OF HOST AND ENVIRONMENTAL FACTORS IN SCHISTOSOMIASIS

Inflammatory Cells

It has been reported recently that schistosome-induced chronic inflammation and irritation in the urinary bladder are associated with increased cancer initiation at the site of inflammation (186, 187). Inflammatory cells such as macrophages and neutrophils are important sources of endogenous oxygen radicals, which are also implicated in the formation of carcinogenic *N*-nitrosamines (141). Moreover, inflammatory cells induce genotoxic effects, such as mutations (224), sister chromatid exchanges (223), and DNA strand breaks (197). These toxic effects may result from the formation and release of hydroxyl radicals from the inflammatory cells (54). Inflammatory cells also participate in the activation of procarcinogens, such as aromatic amines and polycyclic aromatic hydrocarbons, to their ultimate carcinogenic metabolites (i.e., the final reactive form of the carcinogen) (168). Since the aromatic amines are an important group of bladder carcinogens, an increased number of inflammatory cells in the urinary bladder of schistosomal patients may enhance the carcinogenic potential of these agents by increasing their rate of activation (see "Carcinogen activation").

Microorganisms

The prevalence of bacterial infection as a consequence of urinary schistosomiasis has been assessed in different epidemiological, clinical, and experimental studies to determine if there is a link between the two conditions. Some 39 to 66% of hospitalized subjects with schistosomiasis were found to have a bacterial infection in the urinary tract (bacteriuria) (62, 130). However, since the relationship between schistosomiasis and

bacteriuria was based solely on data from hospitalized patients, community-based epidemiological surveys were also carried out to evaluate the strength of this association in different areas of endemic infection. Recently, bacterial counts were performed on urine samples collected from 76 patients in El-Fayoum Province, Egypt, where *S. haematobium* is endemic. These patients comprised three groups: controls (those not infected with *Schistosoma*; $n = 26$), those with schistosomiasis and treated with an antischistosomal agent ($n = 25$), and those with untreated schistosomiasis ($n = 25$) (156). Clean-catch, midstream urine samples were collected in autoclaved containers by the patients themselves, who were previously instructed by a physician to ensure proper sample collection from both control and schistosome-infected patients. The cases included males and females (not in equal numbers) and ranged in age from 6 to 50 years. In this study, 60% of the males in the untreated-schistosomiasis-patient group had bacterial counts of $>10^3$ CFU/ml. Although these counts were generally higher than in the uninfected control group (median values, 1.9×10^3 CFU/ml for untreated patients with *Schistosoma* infection versus 2.0×10^2 CFU/ml for controls), this difference was not considered significant due to the high variation (156). In the female patients, all of whom were young (<10 years), half of the patients infected with *S. haematobium* had low bacterial counts ($<10^3$ CFU/ml) whereas the females in the control group, as expected, all had bacterial counts of $>10^3$ CFU/ml. These female control subjects differed qualitatively from the control males in that the number of different bacteria isolated from females was greater than the number isolated from males, with mean values of 4.0 ± 1.1 and 1.8 ± 1.3 isolates, respectively ($P < 0.001$). Surprisingly, the situation was reversed in the female schistosomiasis group, where the number of isolates fell from 4.0 ± 1.1 in the controls to 1.3 ± 1.4 in the infected females ($P < 0.001$) (156). In light of these data, one can postulate that the interaction between schistosomiasis and bacterial infection may depend largely on gender.

In a separate study group, the number of patients with urine containing $>10^4$ CFU/ml was 16 (25.8%) of 62 patients infected with *S. haematobium* and 40 (83%) of 48 in patients infected with *S. mansoni* (156). This highly significant difference in the prevalence of bacterial infection is the opposite of the anticipated consequence of urinary schistosomiasis (caused by *S. haematobium*) rather than intestinal schistosomiasis (caused by *S. mansoni*). Bacteriuria with a prevalence of 10% was reported in Tanzania (72), 1 to 3.2% in Nigeria (112) and 6.6% in Gambia (226) among persons infected with *Schistosoma*. Although these values vary from one area to another and even from one report to another in the same country, they are generally much higher than those documented in areas with no endemic infection (125). With these relatively high levels of bacterial and *Schistosoma* infection, it seems possible that agricultural workers who are regularly exposed to contaminated water are occasionally simultaneously infected with both the schistosome parasite and pathogenic bacteria.

This association between schistosomal and bacterial infection could result from a relationship (possibly symbiotic) in which the bacteria either become fixed on the cutaneous surface of the worms in clearly defined places (175) or colonize the cecum of the parasite (171). In vitro and in vivo studies show that cocultivation or dual infection of worms with a specific bacterial strain (*Salmonella paratyphi*) yielded more bacterial growth than in the absence of the worms (147). This observation suggests the involvement of nutritional and possibly physical factors in this relationship.

Various strains of bacteria have been identified in the livers of animals (171), in human gastric juice (142), and urine (62,

TABLE 1. Concentrations of volatile and nonvolatile nitrosamines in urine[a]

N-Nitrosamine[b]	Concn (µg/day)[c] of nitrosamine in:		
	Uninfected controls ($n = 27$)	Schistosomiasis patients ($n = 27$)	Schistosomiasis patients with bladder cancer ($n = 23$)
Volatile			
NDMA	0.27 ± 0.47 [11]	2.74 ± 6.13 [24]*	1.31 ± 1.65 [20]**
NDEA	0.4 [1]	0.26 ± 0.71 [6]	0.17 ± 0.47 [4]
NPIP	0.6 [1]	0.21 ± 0.43 [10]**	0.09 ± 0.19 [4]
NPYR	0.7 [1]	0.26 ± 0.35 [12]*	0.14 ± 0.36 [5]
Total	0.32 ± 0.64	3.5 ± 6.4 [25]	1.7 ± 2.0 [20]
Nonvolatile			
NSAR	3.40 ± 5.91 [24]	6.01 ± 3.45 [27]*	6.24 ± 9.90 [23]
NPRO	7.16 ± 5.07 [27]	17.01 ± 7.14 [27]**	12.61 ± 3.37 [23]*
NTCA	11.69 ± 7.60 [27]	24.37 ± 9.27 [27]**	15.93 ± 3.78 [23]
NMTCA	9.08 ± 5.55 [27]	15.12 ± 6.81 [12]**	9.47 ± 4.60 [23]
Total	31.5 ± 22.5 [27]	65.9 ± 25.8 [27]*	46.0 ± 11.2 [23]

[a] Reprinted from reference 213 with permission of the publisher.
[b] Volatile nitrosamines: NDMA, *N*-nitrosodimethylamine; NDEA, *N*-nitrosodiethylamine; NPIP, *N*,*N*-nitrospiperidine; NPYR; *N*-nitrosopyrrolidine. Nonvolatile nitrosamines: NSAR, *N*-nitrososarcosine; NPRO, *N*-nitrosoproline; NTCA, *N*-nitrosothiazolidine-4-carboxylic acid; NMTCA; *N*-nitroso-2-methyl-thiazolidine-4-carboxylic acid.
[c] Values are mean $\pm$ SD of individual values; the number of positive samples is given in brackets. Comparison with the levels detected in the Egyptian control group: *, $P < 0.1$; **, $P < 0.05$.

161) in association with schistosome infection. Some of these organisms are thought to play a significant role in the endogenous formation of *N*-nitrosamines. Nitrate-reducing bacteria, including *Staphylococcus aureus*, hemolytic *Staphylococcus albus*, *Proteus mirabilis*, *Klebsiella* spp., and *E. coli* (95, 161), were isolated from the urine of *S. haematobium*-infected patients. Several of these organisms can mediate nitrosation reactions in vitro between secondary amines and nitrate under the conditions of physiological pH normally encountered in the urinary bladder (34). A correlation between schistosomiasis and bacterial infection of the urinary tract thus provides supporting evidence for the endogenous formation of *N*-nitroso compounds and the possible consequent induction of preneoplastic initiating events in schistosome-infected subjects. Consistent with these observations, high concentrations of *N*-nitroso compounds have been detected in the urine of *S. haematobium*-infected patients (91, 161, 213) (Tables 1 and 2).

Bacterial infection of the urinary tract per se has been reported to increase the risk of bladder cancer (111) in patients with chronic or repeated cystitis (178), paraplegia (146), or *S. haematobium* infection (94), as well as to increase the risk of all cancers (166). The mechanisms of initiation of these and many different types of cancer could be due to the nitrosation of secondary amines with ingested or metabolically derived nitrite that leads to *N*-nitrosamine formation (97). Many bacteria present in the urine reduce diet-derived nitrate to nitrite, which under mildly acidic or neutral conditions becomes a potent nitrosating agent. The production of *N*-nitrosamines by the nitrosation of amine precursors was detected in the urine of bacterially infected rats (98). Secondary amines are present in the diet and can also be produced by the intestinal microflora and excreted in the urine in significantly large amounts (63). Therefore, in addition to the *N*-nitrosamine exposure originating from the external environment, individuals with bacterial cystitis and schistosomiasis are potentially more exposed to nitrate and/or nitrite, which would then greatly in-

TABLE 2. Concentrations of *N*-nitrosamines, nitrate, and nitrite in saliva of control and schistosomiasis patients[a]

Parameter	Concn[b] in group infected with:		
	Uninfected control	*S. mansoni*[c]	*S. haematobium*[c]
N-Nitrosamine concn (μg/day)			
NDMA[d]	0.27 ± 0.47 [11/27]	2.9 ± 2.9 [64/65] ($P < 0.001$)	19.2 ± 21 [79/79] ($P < 0.001$)
NPIP[d]	0.6 [1/27]	0.4 ± 0.3 [40/65]	1.6 ± 2.3 [56/79] ($P < 0.001$)
NPYR[d]	0.7 [1/27]	0.9 ± 0.9 [59/65]	1.3 ± 1.9 [58/79] ($P < 0.1$)
Nitrate concn in saliva (ppm)	41 ± 52 [27/27]	60 ± 59 [61/64]	52 ± 48 [120/129]
Nitrate concn in urine (mg/day)	139 ± 82 [27/27]	249 ± 12 [65/65] ($P < 0.001$)	174 ± 176 [77/79] ($P < 0.005$)
Nitrite concn in saliva (ppm)	8.8 ± 9.0 [27/27]	7.8 ± 9.5 [61/64]	7.5 ± 7.9 [120/129]
Nitrite concn in urine (mg/day)	1.7 ± 0.3 [2/27]	3.2 ± 13 [5/65]	7.9 ± 24 [18/79]

[a] Reprinted from reference 161 with permission of the publisher.
[b] Values are mean ± SD of individual samples [number of positive samples/total number assayed].
[c] Comparison between the control group, *S. mansoni*-infected patients, and *S. haematobium*-infected patients by the Wilcoxon rank sum test.
[d] Volatile nitrosamines: NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; NPR, *N*-nitrosopyrrolidine.

crease the risk of in situ formation of carcinogenic alkylating agents, e.g., *N*-nitrosamines.

Genetic Changes

Oncogenes and tumor suppressor genes have been implicated in a variety of human cancers. It is suggested that their activation or inactivation, respectively, due to point mutations within the gene or deletions can play an important role in differentiation and tumor progression. Recent studies have attempted to identify molecular events associated with specific genes that underlie neoplastic progression in the development of schistosomal bladder cancer. These include the activation of H-*ras* (115), inactivation of *p53* (202), and inactivation of the retinoblastoma gene (105). Since the protein products of oncogenes are known to participate directly in cell cycle processes, any alterations of these genes or their proteins can alter their function, leading to uncontrolled cell growth and ultimately to tumor formation.

Among the most frequently activated oncogenes are the members of the *ras* gene family. These genes encode a low-molecular-mass (21-kDa) protein that mediates signal transduction between tyrosine kinase receptors and the nucleus, a process that can be altered by different mutations in various regions of the *ras* genes. For example, the activation of K-*ras* is an early event in a sequence of gene changes leading to the development of colon cancer (71). In contrast, the involvement of *ras* genes in bladder cancer is much less clear, since mutations in this gene family, except for H-*ras* are relatively rare (28). Several studies estimated the frequency of H-*ras* gene activation at between 7 and 17% in human urinary bladder cancer (209). The frequency of activating H-*ras* mutations and expression of the corresponding protein were similar for bladder cancers associated with schistosomiasis and those associated with other causes (11). However, in one study, 2 of 21 tumors harbored an H-*ras* codon 13 (Gly→Arg) point mutation which is rarely encountered in TCC (181). In experimental studies, early neoplastic lesions (papillomas) of the bladder induced in rats by BHBN showed only one exon 1 (codon 12) mutation of H-*ras* in 10 lesions (143). In contrast, in tumors of the esophagus that were induced by another *N*-nitroso compound, the expected point mutations of H-*ras* were detected in 48 and 58% of BDV1 and F344 rats, respectively (135). Experimental studies on *ras* mutations in vitro have also shown a cumulative effect with other genetic alterations, including those of c-*erbB-2* and c-*myc*, leading to more aggressive and invasive tumor properties (118). Overexpression of c-*erb-B-2* in

TCC has been associated with advanced disease and tumor recurrence (45).

To date, the *p53* gene is one of the most intensively investigated tumor suppressor genes in human cancer. It is located on the short arm of chromosome 17 and encodes a protein involved in the growth and regulation of cells (131); it also regulates multiple components of the DNA damage control response and promotes cellular senescence (35) by participating in a "fast-track" system responsible for death by apoptosis (231). Mutation of the coding sequence of the *p53* tumor suppressor gene is the most frequent genetic alteration in a variety of human malignancies (202). The resultant loss of the surveillance of DNA damage may enable the onset of genetic instability, thereby enhancing the development of malignancy (233). The pattern of point mutations in *p53* appears to reflect the site of tumor origin, with G-A transitions predominating in colon and lymphoid tissues and G→T transversions occurring more frequently in cancers of the liver and lung; most of the transitions occur in mutational hot spots at CpG dinucleotides (99). In bladder cancer, a high frequency of mutations and chromosome 17p allelic deletions were associated with invasive primary TCC (73, 202). Also, overexpression of *p53* appears to identify superficial bladder cancers in patients who are at risk of developing invasive, metastatic tumors (120). Allelic loss of chromosome 9 was also observed in both superficial and invasive bladder cancers (151), although this was less common in uncultured than in cultured tumors (205).

For Egyptian schistosomiasis-associated bladder cancers (83), it was reported that about 86% (six of seven) of these had *p53* mutations in exons 5, 6, 8, and 10 and that in a Japanese group (61 patients) the mutation frequency increased with the tumor grade. These findings are in agreement with a literature-based observation that the frequency of *p53* mutations varies with the different grades of schistosomiasis-associated bladder cancer (11). Thus, *p53* inactivation ranged from 0 to 38% at the early stage of the disease, as opposed to 33 to 86% in the advanced tumor stage (11). Habitual smoking in a group of Japanese bladder cancer patients did not increase the frequency of *p53* mutations, but an unusual AT:GC mutation pattern was observed (83). Loss of *p53* function has been shown to allow cells to become permissive for genetic changes such as DNA amplification (134).

Molecular changes in H-*ras* (see above) and *p53* mutations that were limited mainly to exons 7 and 8 were reported for 21 individuals with schistosomiasis-associated bladder cancer in South Africa (181). These are different from those reported for the seven Egyptian cases (83) (see above) and may indicate

alternative etiologies in different schistosomiasis-endemic regions. Multiple inactivation events were found at the *p53* locus in schistosomiasis-associated bladder cancer (181). These events might be caused by specific etiological agents, such as abnormal tryptophan metabolites (2) and/or *N*-nitrosamines (161, 181, 213), that are thought to be responsible for the neoplastic progression in schistosomiasis-associated bladder cancer patients. In rats, for example, about 89% of *N*-nitrosamine-induced *p53* mutations have a high incidence (75 to 100%) of G→A transitions (135).

Recently, Warren et al. (221) found that 30 of 90 Egyptian patients with a history of schistosomiasis had tumors with mutations in exons 5 through 8 of the *p53* gene: 17 of 53 of these mutations were in SCC, 8 of 23 were in TCC, 4 of 13 were in adenocarcinoma and 1 of 3 were in other tumors. Of 19 mutations in SCC, 16 were base pair substitutions (BPS), 2 were deletions, and 1 was an insertion. Of the BPS, nine were transitions at CpG dinucleotides and two were G→T transversions. All the mutations in TCC were BPS: four were transitions at CpG dinucleotides and three were G→C transversions. Of four adenocarcinomas with mutations, two had transitions at CpG dinucleotides. It was suggested in this report that the excess of transitions at CpG dinucleotides in schistosomiasis-associated bladder cancer results from nitric oxide produced by the inflammatory response provoked by schistosomal eggs. Nitric oxide causes such mutations directly by deamination of 5-methylcytosine or indirectly via its capacity to act as a nitrosating agent, leading to the formation of endogenous *N*-nitroso compounds which cause DNA alkylation and hence mutations in the *p53* gene (135). The promutagenic base O^6-alkylguanine in DNA thus leads to very high rates of G:C→A:T transitions (190). The DNA repair protein O^6-alkylguanine-DNA alkyltransferase (ATase), which is responsible for the repair of O^6-alkylguanine (see also "*N*-Nitrosamines"), is inducible in *p53*-expressing wild-type tissues but not in the tissues of *p53*-null mice (179). Alterations in the *p53* gene may therefore modulate the expression of genes that regulate relevant DNA repair processes as well as cell division and cell death by apoptosis (35).

Changes in cell cycle control are thought to be critically associated with cancer development (167). A family of enzymes called the cyclin-dependent kinases (CDKs) control progression from the G_1 to the M phase during the cell cycle. CDK activity is dependent on positive regulators called cyclins and is inhibited by a set of proteins termed CDK inhibitors. Cyclins are low-molecular-weight proteins whose function is markedly modulated during phases of the cell cycle. The role played by these proteins in human cancer has long remained unclear. However, in adenomas of the parathyroid, the promoter of the parathyroid hormone gene is fused to the gene encoding cyclin D_1, suggesting that cyclins could be directly involved in cancer development. In fact, cyclin D_1 is overexpressed in some cancers, including bladder cancer (30, 211). Seven CDK inhibitors, including p57, p21, p27, p19, p18, p16^{INK4}, and p15, have been characterized (211). Tamini et al. (211) found that a p16^{INK4} deletion was present in 23 of 47 samples from schistosomiasis-associated bladder cancer patients and that mutations were present in another 2 patients (in all, 53% of tumors exhibited *p16^{INK4}* gene alterations). They concluded that *p16^{INK4}* alterations are more frequent in schistosomiasis-associated bladder cancer than in other bladder tumors and may thus be associated with a specific etiology. p16^{INK4} binds specifically to CDK4 and CDK6 and inhibits these two kinases (196). Interestingly, cyclin D1 activates CDK4 and CDK6, so that p16^{INK4} acts as a specific regulator of cyclin D1-dependent kinases. It is

therefore likely that p16^{INK4} alterations, as a result of schistosomiasis, can also be involved in cancer development.

In another study, deletions in chromosome 9p, where the *CDKN2* gene is located (and without changes in 9q), were found in 92% of SCC (10 of 11) of Egyptian and Swedish origin, compared with only 10% of TCC (11 of 110) from a literature-based sample. Whereas the *p53* mutation frequency in SCC was similar to that reported for invasive TCC, there were differences in the type and position of these mutations between the two tumor types (79). Since the two forms of bladder cancer, SCC and TCC, thus differ in the frequency of these specific gene deletions, the presence of distinct, underlying genetic defects may explain, at least in part, the pathological differences between these two cancers.

The *Bcl-2* gene was discovered in chromosomal translocations identified in B-cell leukemias and follicular lymphomas. Expression of this gene results in extended viability of cells by overriding the program for cell death (apoptosis) induced under various conditions, thereby prolonging the life span and increasing the risk of acquiring genetic changes that may result in malignant transformation (144). *Bcl-2* can cooperate with viral or other cellular proto-oncogenes in transformation and tumorigenesis, both in vivo and in vitro (208). Importantly, its expression in a variety of hematological and epithelial malignancies has been reported (74). A positive correlation between *Bcl-2* expression and tumor progression was found in prostate and gastrointestinal epithelial carcinomas (31). Recently, it has been shown that *Bcl-2* was overexpressed in some schistosomiasis-associated bladder cancers (37). The high level of *Bcl-2* expression in malignant cells, but not in precancerous cells, suggests that the gene may be upregulated in the later stages of tumor progression. This could be of clinical significance, since *Bcl-2* was expressed at high levels only in SCC and adenocarcinoma, but not significantly in TCC, suggesting that *Bcl-2* expression may also be related to the tumor cell lineage. Mutated *p53* expression was also observed in a majority (73%) of these tumors, and *Bcl-2* expression was present in 32%: overexpression of both *p53* and *Bcl-2* in a subset of these cancers (13%) suggests that in these cases there may have been a breakdown of the normal reciprocal control mechanism for apoptosis attributed to these two genes (37).

It is evident that multiple genetic changes occur during the development of primary bladder cancer and that some of these changes may eventually be used to distinguish schistosomiasis-associated bladder cancers from those of different origins. The presumed role of various etiological agents in effecting these changes suggests that the detection of early genetic changes may have value in indicating individuals at higher risk. Recent developments in the use of microsatellite analysis, which is a sensitive procedure for the detection genomic instability, may also have the potential for the early detection of bladder cancer (139).

Diet

Significant differences in the incidence of specific types of cancer, including bladder cancer (90), in particular countries or regions of the world have directed attention to the possible influence of dietary components on the biological processes concerned with carcinogenesis. It has been estimated from statistics available in the United States that some 40 to 50% of cancers are attributable to diet (55). It is generally considered that the Western diet is deficient in fiber (215), and it was originally suggested that fiber protected against colorectal cancer. This concept was based on the low incidence of the disease in East Africa, where a high-fiber diet was consumed (32).

Evidence from both animal and experimental studies was used in support of the concept that a high-fiber diet protects not only against colorectal cancer (182) but also against other cancers, such as mammary cancer (185). In a case-controlled study on the effect of diet on breast cancer risk in Singapore and China, a quantitative food frequency questionnaire was used to evaluate the consumption of certain foodstuffs over the 12-month period prior to the interview of 200 Singaporean and Chinese women with histologically confirmed breast cancer and 420 matched controls. It was found that red meat intake (but neither total meat nor saturated fat) was a predisposing factor with regard to breast cancer risk (129). A study by La Vecchia et al. in 1988 (126) indicated that green-vegetable consumption was inversely related to the risk of breast cancer. For many years, interest has centered on the potential dangers of a diet high in fat content, especially with regard to breast cancer. Extensive information from experimental studies was offered in support of the concept that a high intake of dietary fat is a causative factor for breast cancer, but the evidence from epidemiological case-control studies is to some extent equivocal and inconclusive (145, 204). Indeed, reports from many epidemiological studies have concluded that there was little evidence to support the concept that a high intake of dietary fat is associated with increased breast cancer risk (68, 207, 227). For bladder cancer, although the role of dietary fat was consistently high on the list of associated factors, it was attenuated when other components of the diet were included (90). However, there is growing evidence that dietary lipids may play a significant role in the induction of cancer. Especially interesting are lipid constituents, principally of plant origin, such as cyclopropenoid fatty acids (CPFA). The major constituents of cyclopropenoids are malvalic acid and sterculic acid. They occur in association with plant triglycerides and may be present in human foods derived from cotton seeds or kapoic oil (20, 203). Different studies have shown that CPFAs can act as potent synergists or promoters in hepatocarcinogenesis induced in rainbow trout by aflatoxin B_1 (128), aflatoxin M_1 (203), and aflatoxin Q_1 (89).

Regarding the role of proteins in the process of carcinogenesis, Lee et al. (128) found that the incidence of aflatoxin B_1-induced hepatomas was significantly higher in rainbow trout given a diet containing 49% fish protein than in those given a diet containing 32% protein. The enhancing effect of a protein-rich diet on aflatoxin-induced hepatocarcinogenesis was shown to be even more dramatic with higher protein concentrations; the 9-month hepatoma incidences in rainbow trout fed 20 ppb of aflatoxin B_1 in a diet containing 40, 50, 60, or 70% protein were 33, 48, 68, and 90%, respectively.

Carcinogens associated with traditional foodstuffs have frequently been implicated as causative agents. In agreement with the previous studies, small amounts of N-nitrosamines, N-nitrosodimethylamine, N-nitrosopyrrolidine, and N-nitrosodiethylamine), within a range of 0.2 to 0.25 μg/kg of sample, were detected in Egyptian cheese samples stored for different periods (156). N-Nitrosodimethylamine was also detected in some traditional Egyptian foods such as fava (fresh fava beans boiled in water until soft), fried fava (softened fava beans mixed with vegetables, mainly onions, garlic, red peppers, and spices), and raw salted fish (sardines and mullet) with a range of 0.35 to 0.65 μg/kg of sample (156). These foods are a part of the staple diet of Egyptian farmers, since they are consumed almost daily. The presence of some N-nitroso compounds in traditional Egyptian foods, combined with the fact that these compounds may be formed from endogenous sources in the stomach or via bacterial synthesis in the bladder (34, 98), might play a significant role in the induction of schistosomiasis-associated bladder cancer in Egypt.

Carcinogen Metabolism During Schistosomiasis

Carcinogens are ubiquitous in the internal and external environment, and certain agents are deemed to play an important role in the initiation of bladder cancer. Most carcinogens are chemically inert and therefore have to be activated before they can initiate their short- and long-term biological consequences (148). For these reasons, the tissue-specific distribution of carcinogen-metabolizing enzymes is an important factor in the mechanisms of carcinogenesis.

Carcinogen metabolism takes place predominantly in the liver (113) and to a lesser extent in the bladder (5), esophagus, kidneys, lungs, and other tissues (149). Carcinogens are metabolized primarily by the microsomal cytochrome P-450 system, which is more abundant in the liver than in other organs, although there are some enzymes, such as those responsible for metabolizing the asymmetrical nitrosamines, which are more active in esophageal tissues and other tissues of the same embryological origin than in the liver (50). This enzyme system is therefore very important in the bioactivation and hence in the target specificity of chemical carcinogens. Similarly, the expression and distribution of enzymes involved in the detoxification of carcinogen and carcinogen metabolites are also major determinants in these processes. In this section, the effects of experimentally induced schistosomiasis on the processes of carcinogen activation are considered.

Carcinogen Activation. (i) Polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbons are ubiquitous in the environment, and some of them are believed to cause cancer in humans. The cytochrome P-450 system participates in the bioactivation of polycyclic aromatic hydrocarbons and other carcinogens to their reactive intermediates (81, 154, 159, 160, 201). An important and very extensively studied member of the polycyclic aromatic hydrocarbons is benzo[a]pyrene, which is metabolized mainly by the cytochrome P-450-dependent arylhydrocarbon hydroxylase (AHH) into various derivatives, including electrophilic epoxides (75, 81). These epoxides are thought to play a major role in determining the mutagenic and carcinogenic effects of the parent compound in various species and tissues (29, 75, 81, 222). The carcinogenic potency and the extent of binding of these metabolites (epoxides) to DNA and proteins are thought to be correlated with the induction of cytochrome P-450-dependent AHH (44, 80).

Our previous studies on mice clearly demonstrated that S. mansoni infection increased the activity of drug-metabolizing enzymes including P-450, cytochrome b_5, and NADPH-cytochrome c reductase at earlier stages (30 days) of schistosomal infection; at later stages of infection (75 days), these activities subsided again (200) (Table 3). In accordance with these findings, S. mansoni infection was associated with a marked decrease in P-450 content (2.6 ± 0.18 and 0.79 ± 0.10 nmol/mg of protein in liver samples from 5 normal and 15 infected patients, respectively [mean ± SE] [70% reduction; $P < 0.001$]), cytochrome b_5 (1.36 ± 0.34 and 0.58 ± 0.25 nmol/mg, respectively [52% reduction; $P < 0.05$]), and NADPH-cytochrome c reductase activity (85.3 ± 1.7 and 32.7 ± 1.3 nmol/mg/min, respectively [61% reduction; $P < 0.001$]) in human livers (82). From these studies, it is apparent that S. mansoni infection decreased the activity of drug-metabolizing enzymes in the livers of humans and experimental animals, especially at the later stages of disease. A decrease in such activities might be related to the development of liver fibrosis or to toxic metabolites produced either by the adult S. mansoni worms or

TABLE 3. Changes in carcinogen-metabolizing enzyme activities after infection of male mice for different periods with 100 to 120 cercariae of *S. mansoni*

Enzyme	Enzyme activity (% of control)[a] after infection for (days):				
	20	30	45	60	75
Cytochrome P-450[b]	+81	+161	−37	−49	−58
Cytochrome b_5[b]	+89	+49	NS	−38	−46
NADPH-cytochrome c reductase[b]	+61	NS	−46	−30	−26
Arylhydrocarbon hydroxylase[b]	+64	+92	−37	−48	−47
NDMA-N-demethylase I[c]	+39	+64	+35	NS	
NDMA-N-demethylase II[c]	+172	+162	+171	+175	
Glutathione[d]	NS	NS	−18	−31	−27
Glutathione S-transferase[d]	NS	NS	+45	NS	−30
Glutathione reductase[d]	+34	−39	NS	+35	+79

[a] The changes in activity are presented as activation (+), inhibition (−), or not significantly different from control (NS).
[b] Data are from reference 200.
[c] Data are from reference 157.
[d] Data are from reference 199a.

their deposited ova (13, 65). This reduction might therefore increase the exposure of other organs to the toxic, reactive carcinogenic intermediates, which could then lead to a higher incidence of toxicity and carcinogenicity at these sites. More recently, the expression of different cytochrome P-450 isozymes and epoxide hydrolase were studied in human transitional-cell bladder cancers (165). The cytochrome P-450 proteins 1A, 2C, and 3A were present in 68, 28, and 68% of tumors, respectively, and expression of CYP1A correlated with tumor grade; epoxide hydrolase was identified in 84% of tumors.

Another microsomal enzyme investigated in mouse liver infected with *S. mansoni* was AHH. This enzyme converts benzo[a]pyrene into various hydroxylated derivatives including phenols, dihydrodiols, quinones, and epoxides (195). Table 3 shows the ability of *S. mansoni* to induce the activity of AHH 20 and 30 days postinfection. However, the magnitude of induction varies according to the period of infection, reaching its maximum after 30 days (200). The increased hepatic AHH activity observed in infected mouse liver is believed to be important, since any alteration in the activity of this system might affect the carcinogenicity of benzo[a]pyrene and other polycyclic aromatic hydrocarbons (218). In the later stages of the disease, the activity of AHH was also markedly decreased (200). The depression in AHH activity during the later stages of the disease could be due to a release of unknown toxic metabolites from the schistosome worm or its ova (236).

(ii) *N*-Nitrosamines. *N*-Nitrosamines (NNA) are an important class of environmental carcinogens (152), which are known to cause cancer in a wide range of animal species (27). Their potential role as causative agents in the carcinogenesis of some human neoplastic diseases has been extensively reviewed (22, 97, 176, 178). There are two potential sources of human exposure to NNA. First, certain dietary items are known to contain NNA (212), and second, they can be formed endogenously from the interaction of nitrite with secondary amines (25, 61, 133, 177). Several studies (Tables 1 and 2) indicate that the levels of NNA in urine are higher in Egyptian schistosomal patients than in controls (93, 161, 213).

The activities of *N*-nitrosodimethylamine-*N*-demethylase (NDMA *N*-demethylase) and aminopyrene demethylase show a gradual increase within a relatively short time after infection of experimental animals with *S. mansoni* (Table 3) (58, 158, 160, 200). This induction was followed by a significant decrease

in these activities in the later stages of the infection (Table 3). In fact, there are two species of enzyme capable of the oxidative *N*-demethylation of *N*-nitrosodimethylamine (NDMA) through an oxidative *N*-demethylation reaction. These are the NDMA *N*-demethylases I and II, which operate at substrate concentrations of ∼4 and ∼200 mM NDMA, respectively (10, 153, 217). Following the *N*-demethylation of NDMA, a diazonium ion is produced, and this leads ultimately to the formation of carbonium ion, which can methylate DNA (190). The mutagenicity of NNA is therefore dependent on these P-450 activities (49, 70), and this would be especially so at the early stages of infection, when the demethylases are more active (157), possibly leading to the generation of high levels of carbonium ions and so to a higher incidence of liver damage and bladder cancer. The carcinogenic effect of the carbonium ion, which results from the activation of *N*-nitrosodimethylamine, toward the liver and possibly other organs might thus be increased. This is in keeping with our previous observations on the schistosomiasis-induced promutagenic methylation damage to the hepatic DNA of *S. mansoni*-infected animals and in human tissues from various populations, in particular those with bladder cancer associated with schistosomiasis (Table 4) (48).

(iii) Aromatic amines. The aromatic amines include some very important industrial chemicals used as intermediates in

TABLE 4. O^6-MedG content of DNA from human bladder tissues and from the livers of mice infected with *S. mansoni*

Type of tissue	O^6-MedG concn (μmol/mol of deoxyguanosine)
Human bladder tissues	
Egyptian schistosomiasis patients with bladder cancer[a]	
Uninvolved tissue[b]	
All	0.171 ± 0.11 [8/8]
Male	0.119 ± 0.07 [4/4]
Female	0.225 ± 0.13 [4/4] (P < 0.15)
Tumor tissues	
All	0.126 ± 0.10 [36/38] (P < 0.15)
Male	0.134 ± 0.11 [30/31] (P < 0.15)
Female	0.095 ± 0.07 [6/7] (P < 0.01)
All samples	0.134 ± 0.10 [44/46]
Egyptian uninfected patients with bladder cancer[a,c]	0.025 [2/5]
European (U.K.) non-bladder cancer[a]	0.046 ± 0.08 [4/12]
Liver tissues of mice[d]	
Control	ND [5][e]
Male infected	0.239 ± 0.147 [5][e]
Control	ND [2][f]
Female infected	0.152 ± 0.11 [2][f]

[a] Mean ± SD value [positive samples/number assayed] of individual samples. Student's t test was used, and only values of $P < 0.15$ are represented. Data are from reference 18.
[b] Normal tissue, close to the tumor site, removed at surgery.
[c] Data are from references 11 and 11a.
[d] Animals infected with 300 cercariae/mouse 30 days before sampling. Data are from reference 15.
[e] Mean of five experiments in each of which the DNA adduct level was determined from the pooled liver samples of five animals. ND, not detected.
[f] Mean of two experiments, otherwise as in footnote e.

the manufacture of dyes and pigments for textiles, paints, plastics, paper, and hair dyes; they also include drugs, pesticides, and antioxidants used in the preparation of rubber for the manufacture of tires and cables. Studies of bladder cancer among workers in the dyestuff industry and later among rubber workers hold an important place in the history of occupational bladder cancer. Epidemiological studies of the hazards to workers in the chemical industry established that benzidine and 2-naphthylamine are carcinogenic to humans (36, 124). It was shown that rubber workers also had an increased risk for bladder cancer, attributed largely to exposure to aromatic amines (198).

Most aromatic amines are initially activated by *N*-hydroxylation, mainly in the liver via a cytochrome P-450-catalyzed reaction (106, 108) and it is well known that the P-450 system changes under the influence of schistosomiasis (Table 3). The activity of aniline hydroxylase, like that of AHH activity (see above), was also depressed in the schistosome-infected liver (58, 236). Thus, effects on the metabolic activation of aromatic amines might be similar to those of benzo[*a*]pyrene, especially during the early stages of the disease.

Recent studies also show that the human urinary bladder contains acetyltransferases, which could serve as a further bioactivation step to form the highly reactive electrophilic *N*-acetoxy derivative (17, 225).

Enzymes of carcinogen inactivation. Carcinogens and their reactive metabolites may also be metabolized by alternative routes (e.g., by conjugation or by hydrolase activities) to relatively harmless intermediates that can be eliminated from the body, although in some cases these may be further transformed into highly reactive chemical species (148). As with the enzymes of carcinogen activation, those responsible for inactivation may play pivotal roles in the determination of the target tissue specificity of a particular carcinogen.

Following experimental schistosome infection in mice, both β-glucuronidase and sulfotransferase enzyme activities were increased markedly (64, 65). Since these two enzymes are present in lysosomes as well as microsomes, their increased activities might be due to a marked accumulation of lysosome-rich macrophages at the site of egg deposition in the liver (64). Murine peritoneal macrophages are stimulated in vivo during the course of *S. mansoni* infection, and it is now generally recognized that macrophages are capable of inducing nitrosamine formation under various physiological conditions (150) (see "Inflammatory cells"). It has also been suggested that the sulfation of certain chemical carcinogens under these conditions could lead to more toxic conjugates, which can cause cell necrosis (64). The increased hydrolase activities found in infected livers (96) might also be due to the activating effects of schistosomes on peritoneal macrophages.

In the TCC type of bladder cancer, the α, μ, and π forms of glutathione *S*-transferase were expressed in 56, 72, and 52% of tumors, respectively, while in the normal bladder, epoxide hydrolase and glutathione *S*-transferase π were the main enzymes expressed (165). These enzymes are important for the detoxification of carcinogens and may influence the response of bladder tumors to anticancer drugs.

A major fraction of the *N*-hydroxy derivatives of aromatic amines is converted to the glucuronide, which is then excreted in the bile and urine (103). However, the glucuronide also may be hydrolyzed to release the free *N*-hydroxy arylamine, which is a potent electrophile (107). In general, the biotransformation capacity of the liver is altered in schistosomiasis in favor of deconjugation pathways.

CARCINOGENS AND THE CONSEQUENCES OF DNA DAMAGE

Various hypotheses have been proposed to explain the process by which carcinogenesis is induced in the bladder by schistosomiasis (11, 14, 19). The previous section dealt with changes in the mixed-function oxidase systems involved in the metabolism of polycyclic aromatic hydrocarbons, *N*-nitroso compounds, and aromatic amines as a result of schistosome infection and with the potential of these agents for mutagenesis and carcinogenesis as a result of these increased activities. The polycyclic aromatic hydrocarbons, however, although widespread in the environment, are not generally implicated in the etiology of bladder cancer in association with industrial processes, life style, or schistosomiasis and so are not considered further in this section.

N-Nitrosamines

More concern has been directed toward the possible role of the *N*-nitroso compounds (95, 161, 213), an important class of chemical carcinogens, in the development of bladder cancer associated with schistosomiasis. Experimental studies identified several of these compounds which are bladder carcinogens for rodents, dogs, and primates, e.g. *N*-nitrosomethylurea, BHBN, and *N*-nitrosomethyldodecylamine (57, 92, 132). The biogenesis of bladder cancer was studied in experimental animals, and these studies showed that there is good evidence for a multistage process. This process involves early and late stages, which can be influenced by genotoxic and nongenotoxic carcinogens, respectively, acting sequentially on the target tissue and accelerating the development of bladder neoplasia (92, 95).

The urinary excretion of NNA was studied in different populations from widely separate regions of the world in order to predict their possible exposure to this group of chemical carcinogens. Several of these studies showed that subjects with a high risk of developing stomach, esophageal, colon, and urinary bladder cancers excreted higher levels of NNA and precursors in their urine relative to low-risk groups (110, 136, 213, 237). Significant amounts of volatile and nonvolatile nitrosamines, nitrite, and nitrate were detected in the urine of schistosome-infested patients (Tables 1 and 2). Interestingly, volatile nitrosamine concentrations were much higher in patients infected with *S. haematobium*. The exceptionally high concentrations of NNA found in the urine of schistosomiasis patients (161, 213) may be due to macrophage accumulation as a result of chronic bladder inflammation (210). It has been recently demonstrated that murine peritoneal macrophages are stimulated in vivo during the course of *S. mansoni* infection (69, 119). Because *N*-nitroso compounds may be formed endogenously by activated macrophages, the infected liver could be considered a site of endogenous nitrosation; DNA alkylation has in fact been observed in the schistosome-infected mouse liver DNA (Table 4) at levels proportional to the multiplicity of *S. mansoni* infection (15). Therefore, the presence of these compounds in urine could provide the origin of initiating events that are critical for the development of bladder cancer. To express their carcinogenic effects, however, these compounds require activation to generate the reactive chemical species that can alkylate tissue constituents, as described above (see "Carcinogen activation").

Methylation of DNA has been detected in various tissues of human populations (47, 48, 189, 216), especially in patients with bladder cancer associated with schistosomiasis (18), and in the livers of *S. mansoni*-infected mice, as noted above (15). The data in Table 4 show the levels of O^6-methyldeoxy-

guanosine (O^6-MedG) found in the bladder tissue DNA of Egyptian patients with bladder carcinoma and suffering from schistosomal infections compared with those found in European and Egyptian controls and those found in infected mice. O^6-Methylguanine is the major promutagenic base formed in DNA by environmental methylating agents (169, 190). Detection of O^6-MedG at high levels in these tissues suggests the presence of a continuous and prolonged exposure to alkylating agents of the kind found in relatively large quantities in the urine of schistosomiasis patients (Tables 1 and 2). Therefore, damage to the DNA of bladder tissue in schistosomiasis patients (Table 4) may well be responsible for the initiation of bladder cancer.

The persistence of O^6-alkylguanine in different tissues depends strongly on the capacity of the cellular DNA repair system, ATase, whose levels are correlated with the mutagenic and carcinogenic effects of the alkylating carcinogens (77, 140, 174). The constitutive level of ATase activity varies considerably among the cells and tissues of different mammalian species (78, 232, 234, 235). As indicated above, samples of bladder mucosa and tumor tissue from Egyptian patients infected with schistosomiasis showed relatively large numbers of these promutagenic DNA lesions (18). While the presence of these lesions may be due to the increased efficiency of carcinogen-activating mechanisms during schistosomiasis (see "Enzymes of carcinogen activation"), it may also reflect the inefficiency of the relevant repair system. Bladder tissue is known to have a lower capacity for the repair of O^6-MedG in DNA (16, 78, 85). Studies of the same samples from patients with schistosomiasis indicated an inverse relationship between the amount of O^6-MedG in DNA and the expressed level of ATase in the bladder tissue (16). The lower ATase activity and the consequent persistence of unrepaired DNA damage could enhance the incidence of bladder tumors. Although the source of promutagenic damage in human bladder DNA (i.e., whether it is of endogenous origin and derived from activated macrophages or whether it is derived from nitrosation reactions occurring in urine) is uncertain, it is worthy of note that the levels of DNA alkylation found in the livers of *S. mansoni*-infected mice are very similar to those found in human bladder DNA (Table 4) and were in fact proportional to the multiplicity of infection (15).

Aromatic Amines

It is well known that human populations come into contact with a variety of chemical carcinogens (55). Of these, the aromatic amines are a potent group of carcinogens that are widely present in the environment as a consequence of human activities. Occupational exposure to aromatic amines, most notably in the manufacture of dyestuffs and tires (86), is known to be an important cause of bladder cancer. After metabolic activation, aromatic amines react with cellular DNA to form aromatic amine-DNA adducts; these adducts have been linked to the mutagenic, toxic, and carcinogenic effects of the amines (53, 106, 148). As previously described, N-hydroxylation is the primary pathway of activation for most aromatic amines and occurs mainly in the liver. N-hydroxy derivatives can then enter the circulation and react with hemoglobin or can enter the bladder lumen and be reabsorbed into the bladder epithelium, where they may also be converted into the highly electophilic N-acetoxy derivative. This derivative could bind covalently to urothelial DNA (17, 225) and so initiate bladder cancer. In support of the role of aromatic amines in bladder cancer initiation, hyperplasia of the urothelium was reported to be induced in the bladders of mice infected with *S. haematobium* and pretreated with acetylaminofluorene (87) or 2-naphthylamine in combination with *E. coli* infection (6).

CONCLUSIONS AND PERSPECTIVES

A large and compelling body of evidence links schistosomiasis of the urinary tract to bladder cancer (230). The mechanisms involved are not well understood, and many different etiological factors could be involved. Schistosomiasis induces chronic irritation and inflammation in the urinary bladder, and this could facilitate changes in at least two stages of the development of the disease: first, initiation of premalignant lesions, and second, action as a promoting agent to increase the likelihood of the conversion of these lesions to the malignant state.

At the stage of initiation, activated macrophages induced at the sites of inflammation are implicated in the generation of carcinogenic NNA and reactive oxygen radicals that lead to DNA damage and subsequently to events such as mutations, DNA strand breaks, and sister chromatid exchanges. Inflammatory cells have also been shown to participate in the activation of other bladder carcinogens such as the aromatic amines. Various species of bacteria have been found in greater numbers in the urine of patients with schistosomiasis than in the urine of uninfected patients. These higher levels of infection probably result from the tissue damage caused in various parts of the urinary tract by the egg-laying activities of the worms. Several of these bacterial species can mediate the *N*-nitrosation of amines, thereby providing a source of carcinogenic NNA in addition to those from exogenous sources (for example, those present in the diet).

In experimentally induced schistosomiasis, increased levels of the enzymes responsible for the activation of carcinogenic *N*-nitroso compounds, aromatic amines, and polycyclic aromatic hydrocarbons have been found. This response, together with the increased generation of carcinogens, above those arising simply from exogenous sources, may increase and prolong the exposure of the bladder to DNA-damaging agents. Evidence of the interaction of carcinogens, with the genetic material of the bladder has been obtained by analyzing bladder mucosal DNA for the presence of the promutagenic base O^6-methylguanine. This modified DNA base was found in schistosomiasis patients at levels and frequencies higher than in either Egyptian or European controls or, indeed, in patients in other countries where a high incidence of cancers of the esophagus, stomach, and bowel is recorded (48). Moreover, in experimental schistosomiasis, levels of endogenously induced DNA methylation were not only similar to the levels found in human tissue but were also proportional to the multiplicity of infection (15).

Mutations of bladder DNA have been observed in oncogenes, tumor suppressor genes, and genes associated with cell cycle control. In particular, mutations in the tumor suppressor gene *p53* have been observed more frequently in patients with schistomiasis-associated bladder cancer than in patients with non-schistomiasis-associated bladder cancer. Changes in these and other genes and in microsatellite DNA, presumably arising as a result of carcinogenic insults, may lead to greater genetic instability (233) and hence to the probability of malignant conversion.

Physical damage to the urothelium and mucosa caused by the activities of the worms leads to restorative hyperplasia. This process would then provide the promoting stimulus to propagate cells in which the sequence of genotoxic (DNA-damaging) events leading to the initiation of the premalignant change is complete. After this stage, it will only be a matter of time, given the influence of further genotoxic events, before

the conversion to the malignant state occurs and a potential cancer arises.

In recognizing this sequence of events, it is fairly clear how prevention could be achieved (155). Elimination of the parasite through education, improved hygiene, and improved conditions for living and working are the obvious solutions, but the level of investment required for this is well beyond the resources of most of the countries where infection is endemic. A possible interim solution that requires less extensive financial resources is chemotherapy with effective agents such as praziquantel; however, this approach is realistic only when individuals move away from areas where the parasite is prevalent and hence from sources of reinfection. Other possibilities include modification of the diet to ensure a high consumption of antioxidants to preclude, as far as possible, the DNA-damaging events that are generally recognized as requirements for the initiation of cancer, or the use of biomarkers (gene changes and DNA damage) to identify individuals who are more susceptible to the development of cancer. However, these latter measures would be possible only with the development of inexpensive and effective methods to detect those at risk. Nevertheless, they would have applications for bladder cancer diagnosis generally, rather than just for cancers associated with schistosomiasis. A study of individuals with urinary incontinence indicated that the detection of DNA damage for example may be of value. In this study, urine containing bacteria with a capacity for the N-nitrosation of amines, a potential source of NNA, was prevalent (214). In patients in whom augmentation enterocystoplasties have been used as a surgical procedure to treat bladder cancer, iatrogenic cancers arising from the bladder epithelium at the site of anastomoses are a potential problem (21). Most recently, preliminary observations have indicated that preoperative levels of alkylation damage in bladder DNA are higher than those subsequently found postoperatively in any areas of the reconstructed organ: it is suggested that alkylation DNA damage, in conjunction with an immediate postoperative increase in cell proliferation, might be involved in the generation of these iatrogenic bladder cancers (46). It is evident, therefore, that such studies deserve to be pursued, since they may lead to measures which are equally applicable to schistosomiasis- and non-schistosomiasis-associated cancers and hence are of general relevance for the understanding of neoplastic bladder disease.

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