4. Other Data Relevant for Evaluation of Carcinogenicity and its Mechanisms

4.1 Pathology of infection

4.1.1 *Humans*

(a) *Schistosoma haematobium*

Many of the most severe pathological manifestations of schistosomiasis are due to a large extent to a physical and immunological response of the host to the eggs (Parra *et al.*, 1991). A periovular area of granulomas surrounded by an exudative cellular reaction consisting of many polymorphonuclear leukocytes, lymphocytes and eosinophilic cells is found to occur in most granulomatous areas (Nawar *et al.*, 1992; Lukacs *et al.*, 1993).

Clinical and pathological evidence for 'early stage of infection' (haematuria and dysuria) is seen in the majority of infected children and young adults (King *et al.*, 1988). In contrast, 'late stage of infection' may be less symptomatic but associated with structural urinary tract diseases. Asymptomatic infection may still be associated with urinary tract lesions (Abdel-Salam & Ehsan, 1978).

da Silva Lopes (1984) reported a pathological study of 210 malignant tumours (206 carcinomas) of the bladder in Luanda, Angola. Of the 164 carcinomas associated with schistosomiasis, 122 were of the 'spinocellular' type, 15 were 'urothelial', 13 were 'urothelial plus epidermoid metaplasia', 8 were adenocarcinomas and 16 were undifferentiated carcinomas. Of the 42 carcinomas not associated with infection, 30 were 'urothelial', 6 were 'urothelial plus epidermoid metaplasia', 3 were 'spinocellular' and 3 were undifferentiated carcinomas.

(i) *Early stage of infection*

The most significant pathophysiological disease sequelae of the early stage of *S. haematobium* disease occur in the ureters and urinary bladder. Eggs are deposited in particularly large numbers at the lower ends of the ureters. Ureteric lesions result in anatomical or functional stenosis, leading to hydroureters and hydronephrosis. At the site of egg deposition in tissues, circumoval granulomas, fibrosis and muscular hypertrophy may be demonstrated histologically. The same pattern of tissue involvement is seen in the urinary bladder (Smith *et al.*, 1974).

Two major autopsy studies—one in Ibadan, Nigeria (Edington *et al.*, 1970) and the other in Egypt (Smith *et al.*, 1974)—contributed significantly to our appreciation of the pathological changes in schistosomiasis caused by *S. haemotobium*. Edington *et al.* (1970) studied 673 unselected cadavers in Nigeria and found *S. haematobium* in 20%; 183 of the autopsies were performed on individuals under 19 years of age. In Egypt, Smith *et al.* (1974) examined specimens taken at 190 consecutive autopsies and found evidence of *S. haematobium* infection in 117 (61.6%).

The morphological findings in early active *S. haematobium* disease comprise polypoid granulomatous lesions surrounding the parasite eggs. In the urinary bladder, the pathological manifestations are polyposis and/or ulceration. *S. haematobium*-induced bladder polyps consist of large inflammatory masses containing schistosome eggs. The deposition of eggs may be apicocentric, basocentric or diffuse. Apicocentric ova deposition usually occurs at the apex and dome of the urinary bladder, whereas basocentric deposition occurs predominantly in the trigone and lower posterior wall (Christie *et al.*, 1986b). Bladder polyposis is responsible for the haematuria seen in the early stages of infection and in obstructive disease. The other major morphological lesion is bladder ulceration, which may be due to polyp sloughing. Histological examination of bladder tissue in the early stage of infection demonstrates hyperaemia, granulomas around nests of schistosome eggs and early fibrosis and hypertrophy of muscle. Urethelial hyperplasia, metaplasia and dysplasia were significant in all stages of the disease in the series of Smith *et al.* (1974), hyperplasia occurring in 38% of autopsied cases and 21% uninfected controls, metaplasia in 31.6% cases and 11.5% controls and dysplasia in 27.2% cases and 8.5% controls.

(ii) *Late stage of infection*

The change from early-stage to late-stage schistosomiasis caused by *S. haematobium* occurs with age, decrease in parasite load (as determined by urinary egg excretion) and diminished manifestations of acute inflammatory disease, e.g. haematuria. Morphologically, urinary bladder disease in late-stage infection manifests as schistosomal ulcers or sandy patches (Smith *et al.*, 1974). Chronic schistosome-related bladder ulcers usually occur in individuals with previous heavy infection. They are located mainly in the posterior part of the bladder. Sandy patches occur late in infection, most frequently in the trigone area and are covered by irregularly thickened or atrophic mucosa. Histologically, old granulomas may be found in the submucosa and muscularis surrounding disintegrating or calcified ova. In many instances, fibrosis and scanty round-cell infiltration may be seen. Differences between the early and late stages of infection are summarized in Table 8. The eggs of *S. haematobium* tend to calcify and to remain in tissues longer than those of *S. mansoni* (Cheever *et al.*, 1978).

Table 8. Differences between early and late stages of infection with S. haematobium

Feature	Early stage of infection	Late stage of infection
Adult worm pairs	Commonly present	Commonly absent
Oviposition	Commonly present	Commonly absent
Urinary egg excretion	Commonly present	Commonly absent
Important in transmission	Yes	No
Granulomatous host response	Present	Absent
Polypoid lesions	Present and possibly obstructive	Very rare
Sandy patches	Present in late active disease	Present and possibly obstructive
Schistosomal obstructive uropathy	Due to obstructive inflammatory polyps	Due to sandy patches obliterating ureteral muscle
Schistosomal ulceration	Uncommon	Frequent
Treatment	Antischistosomal chemotherapy	Surgical repair

Adapted from Smith & Christie (1986)

The concordance of lesions of chronic infection with those of urethelial cancer has been known for over a century. In a series of 1095 patients with urinary bladder cancer in Egypt, *S. haematobium* eggs were found in 82.4% of cases (El-Bolkainy *et al.*, 1981). Well-differentiated squamous-cell carcinomas of the bladder were seen predominantly in patients with eggs and at an earlier mean age than transitional-cell carcinomas. The morphological changes in the urinary bladder associated with the late stage of infection included a spectrum of hyperplasia, squamous metaplasia, dysplastic changes and predominance of squamous-cell carcinoma. Of the 798 squamous-cell carcinomas, 691 occurred in *S. haematobium*-positive samples and 107 in patients with no eggs. Of the 148 cases of transitional-cell carcinoma, 103 were in patients with eggs and 45 in those without.

Similarly, urethelial hyperplasia and squamous metaplasia have been associated with urinary schistosomiasis. Squamous-cell metaplasia of the bladder occurs at increased frequency in schistosomiasis patients and in young people in populations at high risk of squamous-cell carcinoma (Khafagy *et al.*, 1972). Although granulomas occur in both the ureter and bladder, carcinomas occur predominantly in the bladder. In 30 patients with bladder carcinoma in Egypt, the tissue surrounding the tumours usually contained a higher concentration of *S. haematobium* eggs than other areas in the bladder. The egg burden in tissue surrounding the tumour was almost twice the mean in the remainder of the urinary bladder (Christie *et al.*, 1986a).

Further pathological sequelae of *S. haematobium* infection can be seen almost anywhere in the body. The infection may also be associated with other clinical conditions, such as bladder calcification, urolithiasis and pyelonephritis. Most of these lesions are thought to be related to the inflammatory and subsequent fibrotic responses that follow egg deposition in tissues (Cheever *et al.*, 1978).

(b)   Schistosoma mansoni

Infection with *S. mansoni* is often asymptomatic. In studies of populations in endemic areas, morbidity due to *S. mansoni*-induced schistosomiasis was found to be associated with

intensity of infection, particularly in the young (Arap Siongok *et al.*, 1976). Older individuals with light or no parasitologically demonstrable infection may also present with chronic sequelae of disease. Other factors, besides the age of the host, that may play an integral role in the pathogenesis of disease include the geographical strain of parasite, the genetic make-up of the host (Abdel-Salam *et al.*, 1986), water contact and other infectious and nutritional changes.

Disease due to schistosomiasis caused by *S. mansoni* may be classified according to the natural history of infection: cercarial invasion and dermatitis, maturing worms and acute schistosomiasis (Katayama fever) or established infection and intestinal–hepatic disease. Disease may also be classified into mild and severe forms according to its association with intensity of infection and the immunopathological responses of the host.

(i) Early stage of infection

Clinical and pathological changes during the acute phase of infection may manifest as cercarial dermatitis, Katayama fever and established intestinal, hepatosplenic and other features of morbidity. Cercarial dermatitis is a sensitization due to invasion of the skin by cercariae. Morphologically, the lesions are maculopapular eruptions, with oedema and round-cell and eosinophil infiltration. In most circumstances, cercarial dermatitis is self-limiting. Early-stage schistosomiasis may occur four to eight weeks after exposure, usually in infected individuals with a high worm load. Early infection is usually found in individuals with no prior exposure to schistosomes. Disease manifestations resemble those of serum-sickness syndrome and are characterized by hepatosplenomegaly, fever, lymphadenopathy and peripheral blood eosinophilia. The pathological features are nonspecific. Katayama syndrome is self-limiting in most circumstances; severe cases may be associated with heavy infection and may be fatal.

The morphological features that characterize acute established infection are related to the severe inflammatory response around mature eggs in tissues. Large periovular granulomas with prominent necrotic–exudative features are seen. Microscopically, mature eggs are surrounded by round-cell and eosinophilic infiltrations with necrosis and the development of fibrosis (Cheever *et al.*, 1978).

(ii) Late stage of infection

Hepatic disease is the best characterized feature of the late stage of *S. mansoni* infection (Kamel *et al.*, 1978). Granulomas around schistosome eggs first cause obstruction of the finest portal radicles at the periphery of the liver. With progression of inflammation, increased intrahepatic portal pressure occurs, leading to the opening up of fine collaterals around the main portal branches. Simultaneously, fibrosis follows inflammation, and the classical clay-pipe-stem fibrosis becomes the dominant feature, with its haemodynamic sequelae.

Colonic inflammatory pseudopolyposis [the Working Group noted that these lesions are not neoplastic] was described in 30 men in Egypt who were infected with *S. mansoni*, *S. haematobium* or both. Most of the pseudopolyps occurred in the rectosigmoid colon. Microscopically, the lesions contain mononuclear cells and eosinophils; the colonic glands show proliferation and distortion but no adenomatous change. Ulcers are frequently reported on the surface of colonic polyps (Smith *et al.*, 1977).

(c)   *Schistosoma japonicum*

The pattern of infection and disease due to *S. japonicum* infection in general follows closely the sequence of events in schistosomiasis caused by *S. mansoni*: swimmers' itch (cercarial dermatitis), Katayama fever and progression of the disease, leading to established infection (Domingo *et al.*, 1980; Warren *et al.*, 1983). The major differentiating feature is the morphology of the host granulomatous response around the eggs. Granulomas around *S. japonicum* eggs usually occur around nests rather than isolated eggs. In early-stage infection, the lesions look like abscesses with central necrosis. Early-stage acute granulomas consist of eosinophils, lymphocytes and a few histiocytes. At the late stage, histiocytes become more prominent, with the formation of multinucleated giant cells phagocytosing pieces of egg shell. The end result is a fibrotic lesion with a certain degree of hyaline degeneration (Kurniawan *et al.*, 1976).

Chen *et al.* (1980) compared 289 cases of colorectal carcinoma associated with schisto-somiasis with 165 cases not associated with the parasite in China. Well-differentiated adeno-carcinomas accounted for 91.6% of the malignant tumours in patients with schistosomiasis and 69.1% in patients without schistosomiasis. Benign adenomatous and papillary polyps were found in 6.4% of patients with schistosomiasis and in 29% of patients without schistosomiasis. The same group of investigators (Chen *et al.*, 1981) conducted a retros-pective review of specimens taken by colectomy from 60 patients with schistosomiasis. They described 36 lesions as dysplasia, which occurred in the flat mucosa, in pseudopolyps or in regenerative epithelium at the edges of ulcers. The incidence of dysplasia in the colon was not reported for people not infected with schistosomes. Another study from China (Yu *et al.*, 1991) included the results of mass screening for colorectal carcinoma, which led to the taking of 754 biopsy specimens from patients over 30; 320 polyps were studied histologically and were found to be distributed about equally between fibrous, mixed and epithelial polyps. Sialomucins and carcinoembryonic antigens were found more frequently in epithelial than in other types of polyps. [The Working Group noted that the terminology used is confusing and the relevance to carcinogenicity is uncertain.]

4.1.2   *Experimental systems*

(a)   *Schistosoma haematobium*

Repeated attempts have been made to infect several species of experimental animals with *S. haematobium* (Kuntz *et al.*, 1972), but no satisfactory model that reproduces infection and disease as it occurs in humans has yet been described. Webbe *et al.* (1974) demonstrated that infection of baboons (*Papio anubis*) results in passing of viable eggs in urine and faeces. Macroscopic bladder lesions have been reported to vary from pinhead discoloured elevation of mucosa to gross polypoid masses. Eggs have been seen scattered throughout subepithelial layers and surrounded by a predominantly eosinophilic infiltrate. No evidence of malignant transformation was reported. Similar lesions were seen in the ureters. The pathophysio-logical sequelae included distorted ureters, hydronephrosis and ureteric calculi.

(b)   *Schistosoma mansoni*

Animal models have made it possible to study the pathogenesis of granuloma formation and fibrosis due to this species of schistosome. For example, *S. mansoni* infection in mice

results in granuloma formation and disease in the intestines and liver. It was estimated that 63% of ova produced by the schistosome in the porto-mesenteric system were retained in the murine host. Egg deposition was followed by a delayed hypersensitivity granulomatous response which is central to the pathogenesis of disease in the intestine and liver (Warren, 1973). Hepatic egg granulomas are located in all the presinusoidal areas and result in hepatomegaly and destruction of portal blood flow. The haemodynamic consequences lead to portal hypertension, splenomegaly and oesophageal varices, which may bleed. Granulomas are finally replaced by fibrous tissue in the liver, resulting in a unique form of liver fibrosis (Olds *et al.*, 1989) in which hepatic parenchyma and perfusion are retained for a long time.

The regulation of granuloma formation has been carefully studied in the murine model (Warren, 1973; Henderson *et al.*, 1991, 1992). Parasite ova lodge in the small pulmonary vessels, and the host reacts to their presence by forming delayed hypersensitivity granulomas. These isolated lesions can be studied with respect to their composition, the basis of their induction and regulation and immunological reactions. The granuloma is made of lymphocytes, mononuclear phagocytes and eosinophils, but this rich cellular infiltrate is later replaced by scar tissue, with a marked reduction in cellularity. Several cytokines have been shown to be involved in the induction of granuloma, including interleukins 2 and 4 and interferon-γ (Henderson *et al.*, 1991, 1992; Lukacs & Boros, 1993). Granulomas that form in animals with chronic infection are smaller than those seen during the acute phase. This down-regulation or modulation of granuloma formation has been shown to be immunologically regulated and to be dependent on the interaction of Th1 and Th2 subsets of lymphocytes (Lukacs & Boros, 1993).

In baboons and chimpanzees infected with *S. mansoni*, the disease sequence closely resembles the features seen in infected humans (Warren, 1973).

(c)   *Schistosoma japonicum*

Several species of subhuman primates and rodents exhibit a host–parasite relationship similar to *S. japonicum* infection in humans (Cheever, 1985).

4.2   Other observations relevant to the interpretation of carcinogenicity and mechanisms of carcinogenesis

4.2.1   *Humans*

Numerous explanations have been offered for the proposed association between schistosomiasis and human cancers. Generally, these can be categorized as involving: exogenous and endogenous agents which induce DNA damage (Abdel-Tawab *et al.*, 1968a,b; Fripp & Kean, 1980; Hicks, 1982; Gentile, 1991) or possible tumour promoting activity (Ishii *et al.*, 1989); altered host metabolism (El-Aaser *et al.*, 1982; Gentile, 1985; Gentile *et al.*, 1985); pathological changes leading to increased cell proliferation (Ishak *et al.*, 1967; Brand & Brand, 1980a,b; Rosin *et al.*, 1994); and immune reactions (Raziuddin *et al.*, 1991, 1992, 1993; Gentile & Gentile, 1994).

Endogenous agents may be introduced into schistosome-infected organs in several ways. For example, quantitatively altered tryptophan metabolism in *S. haematobium*-infected

patients results in higher concentrations of certain metabolites (e.g. indican, anthranilic acid glucuronide, 3-hydroxyanthranilic acid, L-kynurenine, 3-hydroxy-L-kynurenine and acetyl-L-kynurenine) in pooled urine (Abdel-Tawab *et al.*, 1966a, 1968b). Some of these metabolites have been reported to be carcinogenic to the urinary bladder in implantation experiments (Allen *et al.*, 1957; Bryan *et al.*, 1964; Bryan, 1969; Röhl *et al.*, 1969).

Other endogenous agents may be involved in secondary bacterial infection. Bacterial urinary tract infections such as those that occur subsequent to the late sequelae of *S. haematobium* infection may play an intermediary role in the genesis of squamous-cell carcinoma. Secondary bacterial infection of *Schistosoma*-infected bladders is a well-documented event (Laughlin *et al.*, 1978; Hill, 1979; El-Aaser *et al.*, 1982; Hicks *et al.*, 1982).

Nitrosamines formed by bacterial catalysis (or via urinary phenol catalysis) of the nitrosation of secondary amines with nitrites have been detected in urinary bladders from *S. haematobium*-infected patients; they may be carcinogenic to bladder mucosa (Hicks *et al.*, 1977, 1978, 1982; Tricker *et al.*, 1989, 1991). Mostafa *et al.* (1994) also demonstrated the presence of nitrates and nitrites in the saliva and increased concentrations of *N*-nitroso compounds in the urine of *S. mansoni*- or *S. haematobium*-infected people who were not on controlled diets. The etiological significance of these findings is, however, unclear in the light of the finding that urine from schistosomiasis patients is not mutagenic (Everson *et al.*, 1983).

Nitrosamines have been detected in the urine of paraplegic patients with urinary tract infections due to urinary stasis (Hicks *et al.*, 1977, 1978).

In a US case–control study in which 2982 urinary bladder cancer patients (97% with transitional-cell carcinomas) were compared with 5782 controls (Kantor *et al.*, 1984), odds ratios of 1.5 (95% CI, 1.3–1.8) in males and 1.2 (0.9–1.5) in females reflect an association with one or two past urinary tract infections, and odds ratios of 2.0 (1.6–2.6) in males and 2.1 (1.6–2.7) in females reflect an association with three or more such infections. For the 39 patients with squamous-cell carcinomas, odds ratios of 1.9 (0.7–4.8) for having had one or two infections and 4.8 (1.9–11.5) for three or more infections were found for the two sexes combined. Adjustments were made for race, age, smoking and, for squamous-cell cancer, sex.

On follow-up of 6744 British paraplegic patients (who are subject to frequent urinary tract infections), 25 urinary bladder cancers were identified (El Masri & Fellows, 1981). On the basis of information for an otherwise comparable population, 1.6% of these would have been expected to be of squamous origin, whereas 44% actually were (estimated relative risk, 49; 95% CI, 20–119). In Uganda, squamous-cell bladder cancers are commonly seen in the absence of *S. haematobium* infection but in the presence of other urinary tract abnormalities (Anthony, 1974).

One of the prevalent theories for the association between schistosomal infection and cancer is that elevated levels of the enzyme β-glucuronidase in the host could increase the release of carcinogenic metabolites from their glucuronides. No data are available at present to confirm this association, although schistosome-infected humans are known to have elevated β-glucuronidase activity in urine (Fripp, 1960; Abdul-Fadl & Metwalli, 1963; Fripp, 1965; Abdel-Tawab *et al.*, 1966b, 1968a; Norden & Gefland, 1972; El-Sewedy *et al.*, 1978;

El-Zoghby *et al.*, 1978; El-Aaser *et al.*, 1979). The cause of the increase in β-glucuronidase activity in individuals suffering from schistosomiasis is unknown.

Several studies provide evidence for genetic damage in schistosomiasis patients. Sister chromatid exchange and micronucleus frequencies are increased slightly in peripheral blood lymphocytes harvested from schistosomiasis patients (Shubber, 1987; Anwar, 1994), and micronuclei were more frequent in urothelial cells from chronic schistosomiasis patients than in controls (Rosin & Anwar, 1992). The mean frequency of micronuclei was reduced significantly after treatment with praziquantel, which may indicate that infection is involved in chromosomal breakage in the urothelium (Anwar & Rosin, 1993).

No mutation was detected at codon 12 of the H-*ras* oncogene in nine squamous-cell carcinomas associated with schistosomiasis (Fujita *et al.*, 1987). Mutations of the *p53* tumour suppressor gene were detected in six of seven squamous-cell carcinomas associated with *S. haematobium*; no specific pattern of mutation emerged, in contrast to the pattern seen in transitional-cell carcinomas related to tobacco smoking (Habuchi *et al.*, 1993). O^6-Methyl-deoxyguanosine was detected in DNA from 44 of 46 Egyptian samples of bladder tissue, 38 from tumour tissue and eight from uninvolved bladder mucosa, and in 4 of 12 normal samples of bladder of European origin (Badawi *et al.*, 1992a).

4.2.2 *Experimental systems*

(*a*) *Schistosoma haematobium*

Capuchin monkeys (*Cebus apella*) and African baboons (*Papio cynocephalus*) were exposed to 500–3000 cercariae, which produced active schistosomiasis and associated pathological manifestations (Brown *et al.*, 1976). Analysis of urine samples collected when the infection was declared (5–8 months after infection) showed accumulation of high levels of 3-hydroxykynurenine and 3-hydroxyanthranilic acid, indicating altered tryptophan metabolism in the host.

Syrian hamsters infected with 200 cercariae of *S. haematobium* had elevated β-glucuronidase activity, and their livers had reduced competence to metabolize the urinary bladder carcinogen 3,3′-dichlorobenzidine. The mutagenic potential of this chemical to bacteria was significantly enhanced in the presence of urine from the infected animals, liver enzymes and β-glucuronidase (Gentile *et al.*, 1985).

(*b*) *Schistosoma mansoni*

The modified metabolic profiles of xenobiotics in parasite-infested hosts have been studied extensively (for a general review of altered xenobiotic metabolism in parasitic diseases, see Tekwani *et al.*, 1988). In most of these studies, mice were used as hosts and exposed to 100–200 cercariae. The xenobiotics studied include lindane (Mostafa *et al.*, 1984), *N*-nitrosodimethylamine (Mostafa *et al.*, 1984), 2-acetylaminofluorene (Siwela *et al.*, 1990) and aflatoxin B_1 (Daneshmend, 1984). The evidence suggests that alterations in the carcinogen metabolizing capacities of the liver of mice infected with *S. mansoni* lead to a decreased capability to process xenobiotics. Infected hosts also have enhanced enzymatic activity for some other enzymes, such as β-glucuronidase. O^6-Methyldeoxyguanosine was found in DNA of the liver (but not of other organs) of *S. mansoni*-infected mice, again implying an abnormal metabolic profile in infected livers (Badawi *et al.*, 1992a,b,1993).

(c) *Schistosoma japonicum*

Sequence homologies to the *env* gene of mouse ecotropic and xenotropic retroviruses were detected in the DNA of adult worms (Tanaka *et al.*, 1989). Iwamura *et al.* (1991) made similar findings in adult worms and in DNA isolated from eggs. Host (mouse)-related DNA sequences were identified in the subtegumental layer and inner tissues of adult *S. japonicum* by in-situ hybridization with ^{32}P-labelled probes (Irie & Iwamura, 1993).

Reduced levels of cytochrome P450 have frequently been reported in infected animals (see Tekwani *et al.*, 1988, for a review). These results were confirmed in mice infected with *S. japonicum* (Matsuoka *et al.*, 1989), and the same authors demonstrated that liver homogenate from *S. japonicum*-infected mice had a reduced mutagen activating potential for 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). Hepatic fractions from infected mice had a lower mutagen-activating capacity than hepatic fractions from uninfected mice when Trp-P-2 was used as the substrate (Arimoto *et al.*, 1992). A similar observation was made with aflatoxin B_1: microsomes from infected mice were less effective at producing ^{3}H-AFB$_1$ covalent binding than microsomes from uninfected animals (Hasler *et al.*, 1986).

S. japonicum-infected mice, however, maintain higher levels of serum Trp-P-2 given intravenously than uninfected mice treated in the same way, suggesting that although infected animals have lowered metabolism increased retention of the mutagen can occur (Aji *et al.*, 1994). This persistence could result in Trp-P-2 complexes with haem *in vivo* (Arimoto *et al.*, 1980; Arimoto & Hayatsu, 1989).

The mutagenicity of the parasite itself was investigated in bacterial bioassays; extracts of neither eggs nor adults were mutagenic to *Salmonella typhimurium* or *Escherichia coli* (Ishii *et al.*, 1989).