3. Studies of Cancer in Animals

3.1 Infection with *Schistosoma haematobium* alone

3.1.1 *Mouse*

Groups of 6–10 male C3H mice, two months old, received single subcutaneous injections of 1.5–10 mg of lyophilized *S. haematobium* eggs or worms in saline or tricaprylin. Another group received a single intraperitoneal injection of 4 mg of egg material, while a further group received two subcutaneous injections of 3 or 4 mg of egg material at an interval of three months. Animals were examined for the presence of tumours 13, 17 and 20 months after injection. The experiment was terminated at 20 months. No tumours were seen at the injection site. Of the 20 mice killed at termination, five had pulmonary tumours and three had hepatomas. The authors noted that the frequency of pulmonary and liver tumours was similar to that in historical controls (Shimkin *et al.*, 1955). [The Working Group noted the limited reporting and that an infectious agent was not employed.]

As part of an experiment on *S. haematobium* in combination with 2-acetylaminofluorene (see below), a control group of 20 Swiss mice [sex and age unspecified] was repeatedly treated by subcutaneous injection with cercariae [schedule unspecified] and kept for 44 weeks. Urinary bladder epithelial hyperplasia beginning as early as three weeks was observed in the majority of the mice. Hyperplasia was not observed in 100 untreated control animals (Hashem & Boutros, 1961). [The Working Group noted the absence of ova or worms in the bladder and the short duration of the study.]

A group of 30 male BALB/c mice, three to four weeks of age, received subcutaneous implants of ligated urinary bladder cysts from donor mice. Bladder cysts were prepared by distending bladders with 0.15 ml mineral oil containing 1000 lyophilized *S. haematobium* ova before ligation. The mice were observed for 44 weeks. A control group of 29 mice received donor bladder cysts containing 0.15 ml mineral oil alone. Hyperplasia of the bladder cyst epithelium was seen in 4/30 mice and 5/29 controls. One transitional-cell tumour and one squamous-cell tumour in the implanted bladder cyst [tumour pathology not described] were reported in the experimental group but not in controls (Al-Hussaini & McDonald, 1967). [The Working Group noted the unusual design of the experiment and the lack of significance of the tumour response.]

3.1.2 *Rat*

In a combination experiment, a group of 100 white rats [sex, strain and age unspecified], weighing 140–160 g, were exposed to water containing 2000 *S. haematobium* cercariae per

litre. The urinary bladders from half of the rats were examined after 12 months, and the remaining rats were examined at 24 months. No malignant change was reported; a few rats had bladder lesions reported as 'sessile polyps' (Gawish, 1975). [The Working Group noted the inadequate reporting and found no compelling evidence for sustained bladder infection.]

3.1.3 *Hamster*

In a combination experiment reported in a proceedings volume (James *et al.*, 1974), a group of 50 hamsters [sex unspecified] were exposed to 80 *S. haematobium* cercariae. No adverse pathological finding in the urinary bladder was reported in the 56-week experiment. [The Working Group noted the inadequate reporting on e.g. the presence of infection.]

A group of 18 male hamsters, eight weeks of age, was exposed to *S. haematobium* by immersion in water containing 250 cercariae for 1 h. Exposure was repeated three months later. The animals were killed 7–11 months after the first exposure, and the viscera were examined histopathologically. Eleven animals developed manifestations of schistosomal cystitis. In four animals, epithelial hyperplasia of the urinary bladder was related to sites of submucosal reaction to ova. In four other animals, the bladder epithelial changes consisted of both hyperplasia and squamous metaplasia (El-Morsi *et al.*, 1975). [The Working Group noted that hyperplasia of the bladder is unusual in untreated hamsters and that the duration of observation was short in comparison with the lifespan of the animals, so that tumours might have developed in the animals if they had been allowed to live longer.]

3.1.4 *Opossum*

Eight opossums (*Didelphis marsupialis*) were exposed to 1000–2000 cercariae of *S. haematobium* on the shaved skin for 30 min. Between 18 and 53 weeks after infection, two animals were reported to have mucosal fibrous plaques in the urinary bladder. A third animal had multiple epithelial lesions of the bladder that were variably described as hyperplastic, papillomatous, polypoid or tumourous. The presence of eggs was associated with the lesions in two animals, and all three animals had evidence of infection (Kuntz *et al.*, 1971).

3.1.5 *Nonhuman primate*

Young adult primates, including one talapoin (*Cercopithecus talapoin*), seven capuchins (*Cebus apella*), seven squirrel monkeys (*Saimiri sciureus*) and 11 African baboons (*Papio cynocephalus*), were exposed percutaneously to 1000–2000 cercariae of *S. haematobium* and observed for up to 24 months. Epithelial lesions of the urinary bladder, reported to be papillary transitional-cell carcinomas, were found in a talapoin monkey which died 21 weeks after infection and in a capuchin that was killed 56 weeks after infection. An epithelial lesion reported as a papilloma of the ureter was associated with the presence of schistosomal eggs in an African baboon killed one year after infection (Kuntz *et al.*, 1972).

In a further experiment, nine capuchin monkeys (*Cebus apella*) were exposed via the skin to 1000–2000 cercariae of *S. haematobium* and were examined for pathological changes in the urinary bladder by laparotomy and cystotomy 94–164 weeks after infection. Six of nine animals showed papillary hyperplasia with or without nodular hyperplasia. In two animals, only focal nodular hyperplasia was seen (Kuntz *et al.*, 1978).

In a study to detect C-type viral particles in tumours, it was reported that four of six capuchin monkeys that had been infected experimentally with *S. haematobium* developed lesions described as papillary carcinomas of the urinary bladder during periods of observation of 109–111 weeks. Three of the animals also had squamous metaplasia of the bladder epithelium (Kalter *et al.*, 1974). [The Working Group noted the lack of experimental details and of documentation of the pathological findings.]

Kuntz *et al.* (1975) described the pathological findings and parasitological and radiological observations in two gibbons (*Hylobates lar*) infected by skin application with 1000 cercariae of *S. haematobium*. Both animals developed evidence of infection, the most striking change being extensive calcification of the eggs in the urinary bladder. One animal had evidence of papillary and nodular transitional-cell hyperplasia of the bladder, and the other had similar lesions in the ureter. The lesions were described as morphologically similar to the grade-I and grade-II papillary transitional-cell carcinomas that are seen in the bladders of humans. [The Working Group noted the small number of animals and the equivocal diagnoses of the lesions.]

In combination experiments with baboons (*Papio* sp.) (Hicks *et al.*, 1980; Hicks, 1982), five animals were infected by an abdominal pouch method with 1000 cercariae of *S. haematobium* and kept for 2.5 years. Four animals had polypoid hyperplasia of the urinary bladder and one had endophytic papillary hyperplasia of the ureter. None of these lesions was considered to be a tumour.

3.2 Infection with *Schistosoma haematobium* in combination with administration of known carcinogens

3.2.1 *2-Acetylaminofluorene*

Mouse: Two groups of 20 Swiss mice [sex and age unspecified] were administered 0.2 ml of a 1.5% suspension of 2-acetylaminofluorene (2-AAF) in olive oil by stomach tube three times a week [duration unspecified]. One of the groups was repeatedly infected with *S. haematobium* cercariae by subcutaneous injection [dosing schedules and duration unspecified]. Animals were observed up to 44 weeks, at which time survivors were killed. A third group of 20 mice was infected with *S. haematobium* alone. Epithelial hyperplasia of the urinary bladder was observed in *S. haematobium*-infected mice. One of the 2-AAF-treated mice developed a benign villous papilloma of the bladder after 43 weeks. Four of the carcinogen-treated animals infected previously with *S. haematobium* developed bladder neoplasms at 36–44 weeks; one had an anaplastic infiltrating carcinoma and three had papillomas, two of which had malignant areas (Hashem & Boutros, 1961). [The Working Group noted the short duration, the lack of verification of infection and inadequate documentation of experimental details.]

Rat: A group of 100 white rats [sex, strain and age unspecified], weighing 160 g, were exposed to water containing 2000 *S. haematobium* cercariae per litre; 45 days after exposure, the rats received intraperitoneal injections of 50 mg/kg bw 2-AAF three times per week for four weeks, followed by a diet containing 0.06% 2-AAF and 1.6% indole for one year. A control group of 100 rats received the carcinogen alone. Ten rats were killed every two months and the bladders examined microscopically. In 80 rats in the combined group killed

after six months, all but five had transitional-cell carcinomas of the urinary bladder, as did 7 of the 10 control animals treated with 2-AAF and killed after 10 months (Gawish, 1975). [The Working Group noted the inadequate experimental design, the lack of verification of infection and the fact that the results for the two groups did not differ statistically.]

3.2.2 ortho-*Aminoazotoluene*

Hamster: In a study reported in a proceedings volume (James *et al.*, 1974), groups of 50 hamsters were exposed to 80 *S. haematobium* cercariae. Ten weeks later, 0.02 or 0.1% *ortho*-aminoazotoluene was incorporated into the diet. Administration of the carcinogen alone caused hyperplasia of the urinary bladder epithelium. In the combined group at the 0.1% dose level, malignant changes were seen in the bladder within 24 weeks . [The Working Group noted the inadequate reporting.]

3.2.3 N-*Nitrosamines*

Hamster: In a combination study reported as an abstract (Hicks *et al.*, 1977), groups of hamsters received a single intravesicular instillation of *N*-methylnitrosourea and were infected with *S. haematobium*. Urinary bladder tumours developed in 5/16 hamsters receiving the combined treatment, 0/26 uninfected controls [$p < 0.001$; Fisher exact test], 0/28 infected animals and 0/19 hamsters treated with *N*-methylnitrosourea alone. In groups of hamsters treated with *N*-nitrosobutyl-4-hydroxybutylamine (NBHBA), bladder tumours developed in 9/24 infected hamsters and 5/30 uninfected controls [$p = 0.057$; Fisher exact test]. [The Working Group noted the inadequate reporting.]

Nonhuman primate: In an experiment designed to simulate the possible proliferative stimulus of *S. haematobium* infection on cancer growth due to exposure to low doses of *N*-nitrosamines in humans, small groups of baboons (*Papio* sp.) were either infected through an abdominal pouch with 1000 cercariae of *S. haematobium* alone (five animals); received intramuscular injections of 5 mg/kg bw (two animals) or 50 mg/kg bw (three animals) NBHBA per week up to the end of the experiment; or were infected with *S. haematobium* and administered 5 mg/kg bw NBHBA per week throughout the experiment (10 animals). All surviving animals were killed after 2.5 years. No urinary bladder tumour was found in animals receiving either *S. haematobium* or NBHBA alone, but three of the baboons receiving the combined treatment had adenomatous lesions of the urinary bladder described by the authors as 'early or latent adenocarcinomas' and a fourth had a papillary carcinoma. Three baboons had papillary growths in the ureter (Hicks *et al.*, 1980; Hicks, 1982). [The Working Group had difficulty in interpreting some of the diagnostic terms used in these reports.]

3.3 Infection with *Schistosoma mansoni* alone

3.3.1 *Mouse*

Groups of eight male C3H mice, three months old, were injected subcutaneously with one, six or 10–16 lyophilized, immature worms of *S. mansoni* and were examined for palpable tumours at the injection site every two weeks until termination of the experiment at 21 months. No tumours were found at the injection site. The numbers of survivors were

19/24 at 12 months, 11/24 at 18 months and 9/24 at termination. Of the nine mice killed at termination, three had hepatomas and one had a single pulmonary tumour. The authors reported that the frequency of pulmonary and liver tumours in this strain of mice was similar to that in historical controls (Shimkin *et al.*, 1955). [The Working Group noted that an infectious agent was not employed.]

In several combination experiments in mice (Domingo *et al.*, 1967; Haese *et al.*, 1973; Haese & Bueding, 1976; Bulay *et al.*, 1977; El-Aaser *et al.*, 1978; Kakizoe, 1985) in which control groups of untreated mice or mice infected with *S. mansoni* only were used, no increase in the frequency of liver tumours was reported. Some of the experiments lasted less than 50 weeks (see section 3.4).

As part of an experiment to study the carcinogenic potential of hycanthone, groups of female Swiss-Webster mice, four weeks of age, were infected by intraperitoneal injection with 40 or 80 cercariae of *S. mansoni*. Eighteen months later, the incidences of livers with nodules were 15/60 [$p < 0.001$; Fisher exact test] in the group given 40 cercariae and 1/49 [not significant] in that given 80 cercariae. No nodule was found in uninfected paired groups of 61 and 54 animals (Yarinsky *et al.*, 1974). [The Working Group noted that histological examination was not performed.]

3.3.2 *Mastomys natalensis*

A group of 200 *Mastomys natalensis*, about three weeks of age, were injected intraperitoneally with 100 *S. mansoni* cercariae and maintained until death (up to 2.5 years). Infection was confirmed by examination of faeces for ova during life and examination of liver, gut and mesentery for ova and adult worms after death. At the end of the experiment, 106 animals with evidence of infection were available for evaluation. The incidence of adenocarcinomas of the glandular stomach (23/106) did not differ significantly from that expected in controls (~20%). [The common stomach tumours in *M. natalensis* were then described as adenocarcinoma but are now recognized as carcinoids.] In contrast, hepatomas were observed in 22 infected animals; such tumours had not been observed in several hundred historical controls. Two animals also developed reticulum-cell sarcomas of the ileum and colon, respectively, associated with schistosomal granulomas (Oettlé *et al.*, 1959).

3.3.3 *Hamster*

Groups of 35 male and 35 female Syrian golden hamsters were infected by intraperitoneal injection of 15 cercariae of *S. mansoni*. No increase in tumour incidence was observed over that in uninfected hamsters within 73 weeks (Bulay *et al.*, 1977). [The Working Group noted the lack of verification of infection.]

3.3.4 *Nonhuman primate*

One case report of a hepatocellular carcinoma in a 12-year-old female chimpanzee (*Pan troglodytes*) has been published. The animal had been captured in the wild in Sierra Leone when two years of age and had no hepatitis B surface antigen, no antibodies to hepatitis B surface or core antigens and no viral RNA of hepatitis C on arrival at the laboratory, although granulomatous inflammation was seen. After 10 years in captivity, during an

intervention before the start of a study of hepatitis, a firm white nodule was discovered in the liver which, upon histological examination, was found to be a well-differentiated hepatocellular carcinoma. No cirrhosis was present, but a severe granulomatous inflammatory reaction was apparent, with remnants of schistosomal egg capsules. On the basis of morphological examination, the eggs were considered to be *S. mansoni* (Abe *et al.*, 1993).

3.4 Infection with *Schistosoma mansoni* in combination with administration of known carcinogens

3.4.1 *2-Amino-5-azotoluene*

Mouse: A total of 410 female CBA mice, two months of age, were divided into four groups as follows: 80 untreated, uninfected animals, which served as controls; 95 mice that each received subcutaneous injections of 10 mg 2-amino-5-azotoluene in glycerol once a month for nine months; 100 mice that received a single subcutaneous injection of 30 cercariae of *S. mansoni*; and 135 mice that were infected with *S. mansoni* and received 2-amino-5-azotoluene eight weeks later. Between 24 and 52 weeks, six animals from each group were examined periodically for pathological changes in the liver; the remaining animals were maintained until death and were examined for gross liver tumours. At 24 weeks after the beginning of the study, the numbers of animals alive in the four groups were 69/80, 80/95, 31/100 and 35/135, respectively. The authors noted that the high mortality in infected animals was due to the infection. No hepatoma was observed in control animals or in those infected with *S. mansoni* alone. At 52 weeks of age, the incidence of hepatomas was 1/80 in the group treated with 2-amino-5-azotoluene alone and 13/35 in the group given the combined treatment [$p < 0.001$; Fisher exact test] (Domingo *et al.*, 1967; Liu *et al.*, 1969).

3.4.2 *2-Naphthylamine and 2-acetylaminofluorene*

Mouse: Groups of female Swiss albino mice, six to eight weeks of age, were divided at random into the following groups: one group of 45 mice served as untreated controls; one group of 46 mice was infected with *S. mansoni* by immersion [technique unspecified] for 1 h in water containing 20–30 cercariae per millilitre; one group of 20 mice received 1% 2-naphthylamine in the diet; a group of 20 mice received 0.06% 2-AAF in the diet; one group of 17 mice was infected with *S. mansoni* and treated with 1% 2-naphthylamine; and a further group of 22 mice was infected with *S. mansoni* and treated with 0.06% 2-AAF. Administration of the carcinogens was terminated after 30 weeks owing to severe toxicity. The other experimental groups were continued up to 70 weeks. No liver or bladder tumour was observed in any of the groups. All mice infected with *S. mansoni* showed granulomatous areas in the portal tracts of the liver and had ova in the faeces (El-Aaser *et al.*, 1978).

A total of 109 female ddY mice, four weeks of age, were divided into three groups: 45 mice received an intraperitoneal injection of 20 *S. mansoni* cercariae and four weeks later were fed a diet containing 0.03% 2-AAF; 32 mice were infected with *S. mansoni* and fed normal diets; and 32 uninfected mice were fed normal diet for four weeks and subsequently fed a diet containing 0.03% 2-AAF. A number of animals from each group were killed every 10 weeks for interim examination. The experiment was terminated after 40 weeks. No liver

tumour was found in the group infected with *S. mansoni* only. In the group fed 2-AAF only, the incidence of hyperplastic nodules in the liver was 2/32 (6.3%) at 40 weeks. In the group that was both infected and fed 2-AAF, the incidence of hyperplastic nodules was 9/45 (20%) [$p = 0.005$; Fisher exact test]. Hepatocellular carcinomas were found in 12/45 mice in the combined treatment group at weeks 29–40 and 0/32 in the group infected with *S. mansoni* [$p < 0.001$; Fisher exact test] (Kakizoe, 1985).

3.5 Infection with *Schistosoma mansoni* in combination with administration of compounds used or evaluated in the past as antischistosomal agents

A variety of studies were undertaken to determine the effects of hycanthone (Haese *et al.*, 1973; Yarinsky *et al.*, 1974; Haese & Bueding, 1976), niridazole (Bulay *et al.*, 1977) and SQ 18506 (Haese *et al.*, 1973; Dunsford *et al.*, 1984) on tumour induction in uninfected and *S. mansoni*-infected mice and hamsters. These chemicals were used in the past (hycanthone and niridazole) or evaluated for possible use (SQ 18506) as antischistosomal agents; none are currently in use clinically. The agents were studied by various methods and schedules of administration, and various non-tumour and tumour end-points were evaluated, including hyperplastic nodules of the liver, hepatomas, tumours of the stomach and other tumours. Both higher and lower tumour incidences were found with combined treatment than in animals only infected with *S. mansoni*. The lower tumour incidences were presumably due to lowering or elimination of infection. [The Working Group noted that hycanthone was previously categorized in Group 3 and niridazole in Group 2B (IARC, 1987).]

3.6 Infection with *Schistosoma japonicum* alone

Mouse: A group of 395 female SPF ddY mice, four weeks of age, were exposed after anaesthesia with phenobarbital to five or six cercariae of *S. japonicum* on the shaved abdomen; 163 were found to be infected 8–10 weeks after exposure, as shown by the presence of eggs in the faeces. More than half of the infected animals had died within 30 weeks after exposure, and 70 survived to the end of the experiment (50 weeks). Of a control group of 61 females undergoing anaesthesia only, 60 survived to the end of the experiment. Upon autopsy, 9/70 infected mice showed no presence of eggs in the liver or intestine and were excluded from the analysis. Of the 61 remaining treated animals, 48 were found to have hepatomas, whereas none were found in the surviving controls [$p < 0.001$; Fisher exact test] (Amano & Oshima, 1988).

3.7 Infection with *Schistosoma japonicum* in combination with administration of known carcinogens

3.7.1 *Dimethylaminoazobenzene*

Mouse: Three groups of mice [initial numbers, sex and age unspecified] were either infected with *S. japonicum* and received no further treatment; were uninfected and fed a diet containing 20 ml 3% dimethylaminoazobenzene in corn oil mixed with 1 kg of rice powder; or were infected with *S. japonicum* and, 60 days later, fed the diet containing dimethylamino-

azobenzene. Groups of mice were killed at various intervals up to 150 days. The authors reported that the mice that received the combined treatment developed severe liver cirrhosis and had faster hepatic cancer formation than the uninfected, carcinogen-treated mice (Shigefuku, 1943). [The Working Group noted the limited reporting of this early study].

3.7.2 *2-Acetylaminofluorene*

Mouse: Female ddY mice, four weeks of age, were divided at random into two groups. The first group (77 animals) was infected by immersion of the tail in water containing 40 *S. japonicum* cercariae and four weeks later were fed a diet containing 0.03% 2-AAF for 40 weeks; the second group (86 animals) was fed basal diet followed four weeks later by a diet containing 0.03% 2-AAF for 40 weeks. Interim killings of animals were made between weeks 9 and 40 of 2-AAF administration. The first liver tumours were observed 16 weeks after administration of 2-AAF in the infected group and at 37 weeks in the uninfected group. At 40 weeks, the incidence of liver tumours was 24/77 in carcinogen-treated, infected mice and 6/86 in carcinogen-treated, uninfected mice ($p < 0.005$, χ^2 test). The tumour types in the two groups, respectively, were: hyperplastic type 1 nodules, 6 and 4; hyperplastic type 2 nodules, 10 and 2 ($p < 0.01$, χ^2 test); and hepatocellular carcinomas, 8 and 0 ($p < 0.005$, χ^2 test) (Miyasato, 1984).