International Journal for Parasitology Vol. 20, No. 7, pp. 893–897, 1990
Printed in Great Britain

0020–7519/90 $3.00 + 0.00
Pergamon Press plc

EFFECT OF ULTRAVIOLET RADIATION ON SURVIVAL, INFECTIVITY AND MATURATION OF *SCHISTOSOMA MANSONI* CERCARIAE

A. A. ARIYO and J. P. O. OYERINDE*

Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, P.M.B. 12003, Lagos, Nigeria

(*Received* 12 *January* 1989; *accepted* 23 *May* 1990)

Abstract—ARIYO A. A. and OYERINDE J. P. O. 1990. Effect of ultraviolet radiation on survival, infectivity and maturation of *Schistosoma mansoni* cercariae. *International Journal for Parasitology* **20**: 893–897. *S. mansoni* cercariae exposed to ultraviolet radiation for 1, 3, 5, 10 and 20 s as well as non-irradiated cercariae remained actively motile 30 min post-irradiation. Thereafter the activity decreased with increasing dose level of radiation and age of cercariae. There was no significant difference between the rates of attachment of the batches of cercariae. The recovery rates (0–49% of cercariae to which mice were exposed) of adult worms were, however, significantly different from the number of cercariae calculated to have attached to the mice (93.5–100% of cercariae to which mice were exposed). Maturation and penetration rates were dependent on radiation exposure levels. Numbers of eggs deposited in the liver of mice as well as hatchability rate of eggs varied significantly with the levels of exposure to radiation.

INDEX KEY WORDS: *Schistosoma mansoni*; cercariae; ultraviolet radiation; cercarial activity; cercarial attachment; cercarial survival; development and fecundity of irradiated cercariae.

INTRODUCTION

THE transmission dynamics of parasites which utilize two or more hosts are reported to be directly related to the average time spent by the parasite in any one developmental stage which could be determined by the expected life span of the developmental stage (Anderson, Mercer, Wilson & Carter, 1982).

The life span of schistosome cercariae is known to be dependent on their food reserve and it is also affected by a number of factors such as temperature and turbulence of the aquatic medium. The cercarial life span is roughly put at 8–20 h (Faust & Hoffman, 1934; Ansari, 1973), but the World Health Organization (1984) reported that cercariae can live up to 48 h. Also, factors such as temperature, pH and oxygen tension have been reported to affect cercarial infectivity as well as the survival of schistosome larval stages (Stirewalt & Fregeau, 1965; Purnell, 1966; Eveland & Ritchie, 1972; Upatham, 1972). As age of cercariae of *S. mansoni* increased from 0.6 to 3.6 h, their infectivity was reported to decrease from a range of 12.3 to 12.9% after 30.6 h (Oliver, 1966). He concluded that under natural conditions, cercariae do not survive so long.

Generally the rate of infectivity has often been correlated more with age of host and the duration of contact with the host (Chernin, 1968; Prah & James, 1977; Anderson *et al.*, 1982; Lawson & Wilson, 1983) than with environmental factors which include radiation. Most studies on the effect of radiation on the host–helminth system have focussed on the development of immunity (Cox, 1978; Ishii, Honda & Sano, 1986; Dean, Murrell, Shoutal & Mangold, 1983; Molony, Bickle & Webbe, 1985; Molony, Webbe & Hinchcliffe, 1987). Coggle (1971) showed from experimental studies that radiation can cause a reduction in life span, while preliminary experiments on the effect of ultraviolet radiation on the development of the schistosome have revealed a dose dependency.

Only a few reports are available, however, on the influence of low dose radiation on parasite transmission and morbidity. Recent reports on the increase of ultraviolet radiation due to decrease in the earth's protective ozone layer (IMOS, 1975) indicate the need for further investigation on its role in infectivity and fecundity of *S. mansoni*. We now report results of studies on the longevity and the rate of attachment of cercariae as well as the maturation of *S. mansoni* in the host.

MATERIALS AND METHODS

Cercariae. Cercariae of *S. mansoni* were obtained from infected *Biomphalaria glabrata* which were routinely maintained in the laboratory.

Irradiation. Ten millilitres of cercarial suspension were put into plastic containers, 70 mm in diameter by 35 mm in depth. They were exposed to u.v. radiation from a lamp source (A. P. W. Allen and Co. fluorescent lamp, ultraviolet emitter, Type A425, with fluorescent tubes, S/WG8T5) emitting a wave length of 254 mm from a distance of 125 mm from the

* To whom all correspondence should be addressed.

base of the containers to the radiation source. The radiation procedure,carried out in a dark room at a temperature range of 32–34°C, was as follows: the u.v. lamp was switched on for 15 min to allow maximum output from the radiation source. The cercarial suspension was passed under the lamp while it was on. Timing was done, using a stopwatch, by an assistant waiting outside the darkroom. The lamp was switched off immediately when a signal was received from the time keeper. Radiation exposure levels were for 1, 3, 5, 10 and 20 s after a 1 h shedding period in distilled water. In order to determine the survival rate of cercariae *in vitro*, 50 cercariae from each batch of irradiated cercariae were put in separate 90 mm diameter Petri dishes. The mean number of cercariae found to be actively motile, sluggishly motile, immotile but alive, or dead (the four criteria used by Oyerinde & Jaji (1986) to determine the cercarial activity scores) at 0.25, 0.5, 1, 4, 8, 11 and 20 h intervals was recorded and the percentage activity score was determined. Activity scores for the non-irradiated cercarial batch were also determined.

Cercarial attachment rate post-irradiation. Each of 16 inbred white mice in a group, aged 7–8 weeks, was percutaneously infected in 15 ml of tepid water for 1 h (at room temperature) with 200 cercariae irradiated for 1, 3, 5 or 10 s, using the paddling method. Prior to infection, the mice were placed in warm water for 1 h during which period they defaecated and urinated into the water. They were taken out of the water and rinsed in warm clean water before they were transferred to individual infecting jars. This procedure ensured that the cercariae would not be contaminated with faeces and urine which would have killed them. Each of 16 mice in another group was exposed to 200 non-irradiated cercariae by the same method. This group served as a control. The cercariae that were unable to attach after 15, 30, 60 and 90 min were counted under a dissecting microscope and the percentage of cercarial attachment was determined.

Development and fecundity of irradiated cercariae. Four mice from each group were sacrificed using the cervical dislocation technique at 4, 6, 8 and 10 weeks post-infection. The worms were recovered from the liver, lungs, intestinal mesenteries and spleen of infected mice by placing each of the organs into separate Petri dishes containing normal saline. The organs were meticulously teased apart to release the parasites into saline. The number of worms recovered from each of the organs was expressed as a percentage of the total worms recovered. One gram of the upper left lobe of the liver from each mouse was digested using methods outlined by Cheever (1970). Egg load in the digested liver was estimated at 6.8 and 10 weeks post-infection. At 10 weeks the percentage of eggs hatching from each batch of eggs obtained from the liver of mice infected with the different batches of cercariae was determined.

RESULTS

Survival rate

The differences in activity score of cercariae radiated for 1, 3, 5, 10 and 20 s and the non-irradiated cercariae, shown in Table 1, were insignificant ($P > 0.05$) within the first hour. After 4 h, activity decreased and mortalities increased from 0% in controls to 52% in cercariae radiated for 20 s. No mortality was recorded within the first 4 h in non-irradiated cercariae.

Cercarial activity decreased from active to sluggish and eventually they became immobile before death. Activity was noted to decrease as radiation levels as well as age of cercariae increased.

Cercarial attachment rate

The rates of attachment ranged from 93.5 (±0.32) to 100% in all batches of cercariae regardless of the radiation dose. The observed variation, which was due to differences in radiation level and age of cercariae, was not significant ($F = -4.00, -2.50$, at $P = 0.05$).

Worm recovery

Maximum worm recovery of adult worms was recorded at 6 weeks post-infection regardless of the radiation dose (Fig. 1). The peaks of worm recovery, when compared with the control, decreased significantly from 49% in the control to 37.5, 38.5, 27 and 21% as the radiation exposure dose increased from 1 s to 3, 5 and 10 s, respectively (1 s D = 4.28 for $P = 0.05$ and 15 degrees of freedom) (Fig. 1a–e).

Similarly, a significant reduction in the total number of worms was recorded as the dose of radiation increased. A radiation dose of 1 s duration yielded 77 and 81% of control worms recovered at 4 and 6 weeks post-infection, respectively, while only 11 and 43% of the worms harvested from the controls were recovered

TABLE 1—ACTIVITY SCORE (%) OF IRRADIATED *Schistosoma mansoni* CERCARIAE

Time post-irradiation (h)	No radiation (control)				Duration of exposure to radiation (s) 1				3				5				10				20			
	*	†	‡	§	*	†	‡	§	*	†	‡	§	*	†	‡	§	*	†	‡	§	*	†	‡	§
0.00	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0
0.25	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0
0.50	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0
1.00	100	0	0	0	96	4	0	0	100	0	0	0	97	0	0	3	100	0	0	0	93	0	5	2
4.00	69	0	31	0	7	59	7	27	17	28	22	33	14	33	20	33	3	79	3	15	9	5	34	52
8.00	14	33	25	27	0	12	15	73	0	20	11	69	7	3	0	90	0	0	0	100	0	2	5	93
11.00	0	11	11	78	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	4	96
20.00	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100	0	0	0	100

Key: * Actively motile; † sluggishly motile; ‡ immobile but alive; § dead.

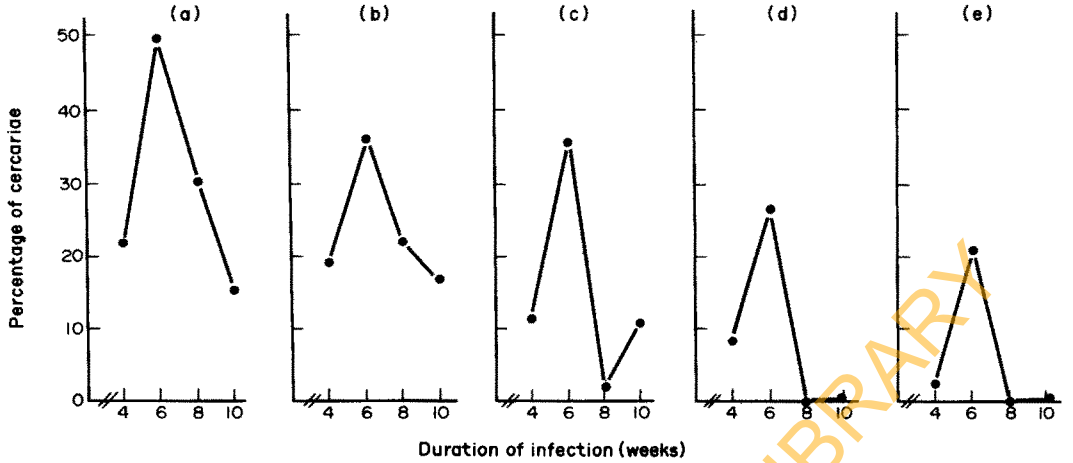

FIG. 1. Percentage recovery of worms in mice exposed to irradiated cercariae. (a) Control (no radiation); (b) 1 s exposure to u.v.; (c) 3 s exposure to u.v.; (d) 5 s exposure to u.v.; (e) 10 s exposure to u.v.

TABLE 2—PERCENTAGE OF WORMS RECOVERED FROM THE ORGANS OF MICE INFECTED WITH U. V. IRRADIATED *S. mansoni* CERCARIAE

Time of exposure (s)	Site of recovery	Duration of infection (weeks)			
		4	6	8	10
0 (control)	Liver	36	18	48	13
	Lungs	4	0	0	0
	Intestine	60	77	52	87
	Spleen	0	5	0	0
1	Liver	92	48	52	97
	Lungs	3	11	0	0
	Intestine	5	39	48	3
	Spleen	0	2	0	0
3	Liver	80	12	14	32
	Lungs	8	19	0	0
	Intestine	12	47	86	68
	Spleen	0	22	0	0
5	Liver	94	48	0	0
	Lungs	0	0	0	0
	Intestine	6	39	0	0
	Spleen	0	13	0	0
10	Liver	100	29	0	0
	Lungs	0	5	0	0
	Intestine	0	33	0	0
	Spleen	0	33	0	0

at 4 and 6 weeks post-infection, respectively, when cercarial exposure was for 10 s. None of the worms exposed to radiation for 5 and 10 s survived for 8 weeks in the mice.

The two major foci of infections were the liver and the intestine (Table 2). Fewer worms were recovered from the liver of mice at 4 and 6 weeks post-infection with non-irradiated cercariae, as compared with the numbers of worms recovered from the portal mesenteries of the mice. When the cercariae were

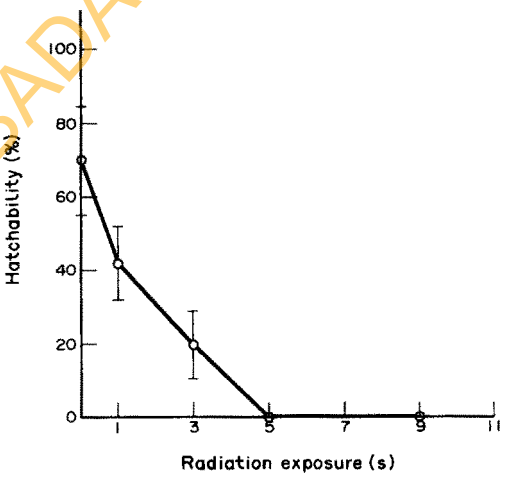

FIG. 2. Viability of eggs in the liver of mice 10 weeks post-infection with irradiated cercariae in relation to radiation exposure.

exposed to irradiation for 1 or 3 s the percentage worm recovery from the liver was highest (92 and 80%, respectively). Corresponding worm recovery from the intestine was 5 and 12% at 4 weeks post-infection. At both radiation levels, worm recovery from the liver decreased to 48 and 12% respectively, 6 weeks post-infection. These percentage recoveries increased again by the 8th and 10th weeks post-infection except for the 3 s radiation dose where more worms were recovered from the intestinal mesenteries than in the liver, from the 6th week to the 10th week, when the experiment was terminated. At high radiation doses (5 and 10 s) all worms were dead by week 8 post-infection (Table 2).

TABLE 3—EGG LOAD IN THE LIVER OF MICE INFECTED WITH IRRADIATED *S. mansoni* CERCARIAE

Duration of infection (weeks)	Radiation exposure (s)	epg ($\times 10^4$) ± S.D.	Whole liver wet weight (g) ± S.D.	Total number of eggs per mouse liver ($\times 10^4$) ± S.D.
6	0(control)	4.01* ± 1.45	1.624* ± 0.264	6.46* ± 2.01
	1	1.16 ± 0.31	2.079 ± 0.387	2.44 ± 1.08
	3	0.73 ± 0.19	2.774 ± 0.154	2.03 ± 0.56
	5	0.29 ± 0.07	1.450 ± 0.324	0.43 ± 0.19
	10	0.28 ± 0.05	1.704 ± 0.330	0.48 ± 0.16
8	0(control)	1.67 ± 0.16	2.658 ± 0.179	4.45 ± 0.71
	1	1.12 ± 0.17	1.647 ± 0.118	1.85 ± 0.30
	3	0.62 ± 0.12	1.595 ± 0.115	0.99 ± 0.20
	5	0.46 ± 0.09	5.024 ± 0.274	2.31 ± 0.43
	10	0.32 ± 0.07	3.360 ± 0.280	1.08 ± 0.29
10	0(control)	2.10 ± 0.25	2.144 ± 0.269	4.45 ± 0.83
	1	0.75 ± 0.11	1.768 ± 0.139	1.33 ± 0.20
	3	0.57 ± 0.10	1.814 ± 0.218	1.04 ± 0.28
	5	0.46 ± 0.19	1.645 ± 0.485	0.78 ± 0.60
	10	0.12 ± 0.03	2.305 ± 0.039	0.28 ± 0.11

* Each value represents a mean of four observations.

Worm productivity

The inhibiting effect of u.v. radiation on the fecundity of schistosomes is shown in Table 3. There was a significant ($P < 0.001$) decrease in the egg load of irradiated worms as compared with the egg load of control worms, Moreover, there was a decrease in viability of eggs produced by the worms as the level of u.v. radiation exposure increased (Fig. 2).

DISCUSSION

The results of this study showed that u.v. radiation has an inhibiting influence on the behaviour of cercariae of *S. mansoni*. This may reflect the possibility of a reduction in the number of parasites that will reach maturity and hence a limiting factor in the epidemiology or transmission of the parasite.

Results, similar to those obtained in this study, have been reported previously by Ghandour & Webbe (1975). They showed that there was no change in the activity of cercariae radiated for 0–20 s, but with radiation of 30–60 s, cercariae became sluggish immediately. However, these authors did not report on the activity or survival rate of the cercariae as a function of cercarial age (Oliver, 1966; Purnell, 1966; Lawson & Wilson, 1983). The effect of u.v. radiation on the demise of cercariae became progressively apparent after 1 h (Table 1). Sixty-nine per cent of the control non-irradiated cercariae were still actively motile after 4 h, compared with motility in only 7, 17, 14, 3 and 9% of cercariae irradiated for 1, 3, 5, 10 and 20 s, respectively.

Auto-radiographic tracking studies by Georgi, Dean & Chandiwanna (1982) and Dean, Mangold, Georgi & Jacobson (1984) have indicated that nearly all *S. mansoni* cercariae that penetrate the skin of mice eventually leave the skin and arrive in the lungs. In these studies, the radiation effect on cercarial attachment rate is so negligible that it could be ignored. In our study, the reduction in the percentage of cercariae recovered and egg count with increasing radiation level, and the significantly lower recovery rate of worms (0–49%) could imply that penetration by cercariae may be influenced by radiation, or, on the other hand, penetrating cercariae may have died while migrating to, from or within the liver. This supports the findings of Clegg & Smithers (1968) and Georgi *et al.* (1982).

Radiation exposures of 5 and 10 s apparently greatly affected the migratory behaviour of larvae and/or adult worms while 1 and 3 s exposure affected migration to a lesser extent (Table 2). The delayed effect was noticed at 8 weeks post-infection when the percentage of worms recovered in the liver was greater than the percentage recovered in the intestine. As expected the percentage worm recovery from the liver decreased as compared with the percentage recovery from the intestine in both the irradiated and the non-irradiated control cercariae, 6 weeks post-infection. It is noteworthy that in mice receiving cercariae irradiated for 1 s, the percentage recovery rate from the liver increased 8 and 10 weeks post-infection. Also the worms, resulting from cercariae irradiated for 5 or 10 s, were dead by 8 weeks post-infection. This migratory trend appears to be in consonance with the phenomenon of hepatic shift that precedes death of schistosomes as proposed by earlier workers such as Oyerinde (1976). Thus, it appears more likely that in mice infected with cercariae irradiated for 3 s and necropsied at 8 or 10 weeks post-infection, the higher

worm recovery rate obtained from the mesenteric veins than in the liver suggests that worms were dying at a faster rate in the liver than the rate at which they were being swept from the mesenteric veins to the liver.

The data clearly showed that penetration is dependent on radiation level and age of cercariae since reduction in degree of activity occurred within 4 h post-irradiation, the same as for control cercariae (Table 1). This may be due to the exhaustion of food reserve which is maximally utilized during penetration through the skin. However, it seems that u.v. radiation affects further development of cercariae in the host, independent of cercarial age, since a consistently low worm burden, egg count and viability were observed which decreased as a function of increasing radiation levels.

REFERENCES

ANDERSON R. M., MERCER J. G., WILSON R. A. & CARTER N. P. 1982. Transmission of *Schistosoma mansoni* from man to snail: experimental studies of miracidial survival and infectivity in relation to larval age, water temperature, host size and host age. *Parasitology* **85**: 339–360.

ANSARI N. 1973. *Epidemiology and Control of Schistosomiasis* (*Bilharziasis*). S. Karger, Basel.

CHEEVER A. W. 1970. Relative resistance of the eggs of human schistosomes to digestion in potassium hydroxide. *Bulletin of the World Health Organization* **43**: 601–603.

CHERNIN E. 1968. Interference with the capacity of *S. mansoni* miracidia to infect the molluscan host. *Journal of Parasitology* **54**: 509–516.

CLEGG J. A. & SMITHERS S. R. 1968. Death of schistosome cercariae during penetration of the skin. II. Penetration of mammalian skin by *Schistosoma mansoni*. *Parasitology* **58**: 111–128.

COGGLE F. E. 1971. *Biological Effects of Radiation*. Wykeham Publications, London.

COX F. E. 1978. Specific and non-specific immunisation against parasitic infections. *Nature* (London) **273**: 623–626.

DEAN D. A., MURRELL K. D., SHOUTAL X. & MANGOLD B. L. 1983. Immunization of mice with ultraviolet-irradiated *Schistosoma mansoni* cercariae: a re-evaluation. *American Journal of Tropical Medicine and Hygiene* **32**: 790–793.

DEAN D. A., MANGOLD B. L., GEORGI J. R. & JACOBSON R. H. 1984. Comparison of *Schistosoma mansoni* migration patterns in normal and irradiated cercariae-immunized mice by means of autoradiographic analysis. Evidence that worm elimination occurs after the skin phase in immunized mice. *American Journal of Tropical Medicine and Hygiene* **33**: 80–96.

EVELAND L. K. & RITCHIE L. S. 1972. Infectivity of cercariae of *Schistosoma mansoni* from snails on inadequate diets. *Parasitology* **64**: 441–444.

FAUST E. C. & HOFFMAN W. A. 1934. Studies on Schistosomiasis *mansoni* in Puerto Rico III. Biological studies I. The extra-mammalian phases of the life cycle. *Puerto Rico Journal of Public Health and Tropical Medicine* **10**: 1–47.

GEROGI J. R., DEAN D. A. & CHANDIWANNA S. K. 1982. Quantification of *Schistosoma mansoni* in mouse lungs by radioassay and autoradiography of ^{75}Se-labelled schistosomula. *Journal of Parasitology* **68**: 1092–1095.

GHANDOUR A. M. & WEBBE G. 1975. The effect of ultraviolet radiation on cercariae of *Schistosoma mansoni* and *Schistosoma haematobium*. *Journal of Helminthology* **49**: 153–159.

IMOS 1975. *Fluorocarbons and the Environment. Report of Federal Task Force on Inadvertent Modification of the Stratosphere* (IMOS). Council on Environmental Quality, Washington, D.C.

ISHII A. I., HONDA M. & SANO M. 1986. Infectivity and development of the gamma-irradiated first stage larvae of *Angiostrongylus cantonensis*. *Zeitschrift für Parasitenkunde* **72**: 331–334.

LAWSON J. R. & WILSON R. A. 1983. The relationship between the age of *Schistosoma mansoni* cercariae and their ability to penetrate and infect the mammalian host. *Parasitology* **87**: 481–492.

MOLONY N. A., BICKLE Q. D. & WEBBE G. 1985. The induction of specific immunity against *Schistosoma japonicum* by exposure of mice to ultraviolet attenuated cercariae. *Parasitology* **90**: 313–323.

MOLONY N. A., WEBBE G. & HINCHCLIFFE 1987. The induction of species-specific immunity against *Schistosoma japonicum* by exposure of rats to ultraviolet attenuated cercariae. *Parasitology* **94**: 49–54.

OLIVER I. J. 1966. Infectivity of *Schistosoma mansoni* cercariae. *American Journal of Tropical Medicine and Hygiene* **16**: 882.

OYERINDE J. P. O. 1976. The effect of 1: 7 bis (*P*-aminophenoxy) heptane on adult *Schistosoma mansoni* in mice. *Nigerian Medical Journal* **6**: 392–297.

OYERINDE J. P. O. & JAJI B. E. 1986. Laboratory transmission of *Schistosoma mansoni* in brackish waters: survival and infectivity of cercariae. *Tropical and Geographical Medicine* **38**: 240–243.

PRAH S. K. & JAMES C. 1977. The influence of physical factors on the survival and infectivity of miracidia of *Schistosoma mansoni* and *S. haematobium*. I. Effects of temperature and ultra-violet light. *Journal of Helminthology* **51**: 78–85.

PURNELL R. E. 1966. Host–parasite relationship in schistosomiasis. I. The effect of temperature on the infection of *B. tanganyiensis* with *S. mansoni* miracidia and of laboratory mice with *S. mansoni* cercariae. *Annals of Tropical Medicine and Parasitology* **60**: 90–96.

STIREWALT M. A. & FREGEAU W. A. 1965. Effect of selected experimental conditions on penetration and maturation of *Schistosoma mansoni* in mice. I. Environmental. *Experimental Parasitology* **17**: 168–179.

UPATHAM E. S. 1972. Effects of some physico-chemical factors on the infection of *Biomphalaria glabrata* (Say) by miracidia of *Schistosoma mansoni* Sambon in St. Lucia, West Indies. *Journal of Helminthology* **46**: 307–315.

WORLD HEALTH ORGANIZATION 1984. *World Health*, December 1984 (Schistosomiasis: New goals).