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Ultraviolet radiation of *Schistosoma mansoni*. I. Influence of pre-hatching radiation of eggs on hatchability of eggs and survival of miracidia

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Abstract

The hatchability of *Schistosoma mansoni* eggs exposed to ultraviolet (UV) radiation and the activity of the hatched miracidia were examined. Hatchability decreased with the increasing exposure to irradiation. The difference in hatchability of eggs irradiated for 15 and 30 minutes were highly significant ($P < 0.01$. $d\alpha = 3.07$ and 3.24) compared with hatchability of the non-irradiated eggs. The mean motility rates of the hatched miracidia were found to be radiation dose-dependent. There was a 21.4% reduction in motility compared with the motility rates in the non-irradiated miracidia. The life span of irradiated miracidia was shortened, only 19% of those exposed to UV radiation for 30 minutes survived for 3 hours as against 80% survival rate in the non-irradiated miracidia. There was no significant difference found in the ability of hatched miracidia to attach to the snails irrespective of the radiation dose exposure the eggs were initially subjected.

Introduction

Several authors have reported on the influence of such factors as temperature, pH, light, salinity and redox potential on hatchability of schistosome eggs (Bair and Etges, 1973; Donelly, Appleten and Schutt, 1984. Samuelson, Quinn and Caulfield, 1984). There is much uncertainty about the specific influence of climatic factors in parasite transmission route and development (Weihe and Mertens, 1991). Hatchability of schistosome could range from 21% to 82% depending on prevailing circumstances (Jordan and Webbe, 1969). In like manner Upatham Sturock and Cook, (1976) recorded hatching rates from 1.1% to 97.1%

Radiation influence on a variety of living organisms has been observed to vary from partial to total interference with normal developmental processes (Litchenberg and Sadun, 1963; Michaels and Kean, 1969; Farvar and Cember, 1969; Sharma, Razdan and Ansari, 1978). Most radiation studies on schistosomes reported the lethal effects of gamma and x-radiations, the development of resistance to re-infection and the immune responses in the host.

However, Prah and James, (1977) studied the effect of UV radiation, a constituent of natural sunlight, on the infectivity and survival of *S. mansoni* and *S. haematobium* miracidia. Urbach, (1989), observed a highly significant correlation between time and length of exposure to solar irradiation and skin cancers.

The Nairobi declaration on climatic change in 1990 confirmed a significant increase in trace gases which is responsible for a gradual erosion of the stratospheric ozone layer which is expected to lead to an increase of UV radiation at the earth's surface. An augmentation of incident biologically effective UV-B radiation wavelength band between 290 and 325 nm could be a

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serious risk factor in the future (Urbach, 1989, Weihe and Mertens, 1991).

In view of an expected change in radiation intensities associated with the mean annual global temperature increase, the present study therefore aimed at determining the possible influence of changes in UV radiation intensities based on length of exposure on the hatching of *S. mansoni* eggs as well as the attachability of miracidia obtained from UV irradiated eggs. The implications of these experiments in relation to transmission from man to snail or snail to man are discussed.

Materials and methods

Preparation of *S. mansoni* egg suspension: Eggs of *S. mansoni* were recovered from the livers of infected white mice, 12 weeks post-infection by homogenizing the liver tissue in approximately 50 ml cold normal saline. The number of eggs in 0.5 ml of the thoroughly mixed suspension was determined.

Irraddiation procedure: Five millilitres of thoroughly mixed suspension were placed in each of five 90 mm diameter dishes. Four of these were exposed to UV radiation from a lamp source emitting a wavelenth of 254 mm from a distance of 125 mm from the base of the containers to the radiation source. Radiation procedure was as detailed out by Ariyo and Oyerinde, (1990). Radiation exposure of the egg batches were for 0.5, 5.0, 15.0 and 30.0 minutes respectively.

Hatchability rate: The egg suspension in each of the 5 petri-dishes was diluted with 50 ml distilled water and exposed to light under a 60-watts bench lamp to enhance hatching. At time intervals of 15, 30, 60 and 90 minutes, a drop of 10% formalin was added to every 0.5 ml of egg suspension from each of the 5 petri-dishes to prevent further hatching. The total number of eggs that hatched in an average of 3 counts divided by the total number of egg count multiplied by 100 was used as the % hatchability.

Miracidia life span: Suspension of miracidia were obtained from batches of eggs irradiated at different radiation levels. The mean number of miracidia per 0.5 ml of the miracidia suspension was estimated. At 26°C. room temperature and at one-hourly intervals, the number of dead miracidia in 0.5 ml of the suspension was counted. Death of miracidia was determined by lack of motility. A mean number of dead miracidia and hence the percentage of survival was determined.

Motility rate: The time taken by miracidia to move across two fixed points of known distance on a plain glass slide was determined under the microscope using a stop clock. The mean motility rate of miracidia hatched from eggs radiated at different levels was recorded as distance moved in millimeters per second (mm/sec.). Ten freshly hatched miracidia were used to estimate mean motility rate.

Miracidia infectivity: *Biomphalaria glabrata* in the size class 4-6 mm (shell diameter) were selected. The snails were grouped into 5 batches of 10 snails per batch Each snail in a batch was placed in a universal bottle containing 5 ml of distilled water with 10 miracidia of particular radiation level. The other batches of snail were similarly exposed to miracidia of other radiation levels. The control batch was infected with non-irradiated miracidia. After 1 hour exposure time, the snails were removed and placed in separate culture bowls. The infecting water was examined with a microscope and the number of unattached miracidia were noted for each snail. The % infectivity rate was determined using the difference in total number of infecting miracidia in each batch (100 miracidia) and the total number of unattached miracidia divided by the number of snails, ten, in each batch multiplied by 100.

Results

Egg viability and hatchability: Microscopic examination of irradiated *S. mansoni* eggs did not reveal any drastic morphological changes. The proportion of viable eggs to non-viable eggs was approximately equal in all batches of eggs exposed to different radiation levels. In the batches of eggs exposed for 30 minutes, an insignificant numbr of eggs among the viable ones were observed to have become darkened in colour.

The cumulative % hatchability after two hours exposure to light showed that there was a decrease in the hatching rate of eggs exposed to irradiation as compared to result obtained in the control (Figure 1).

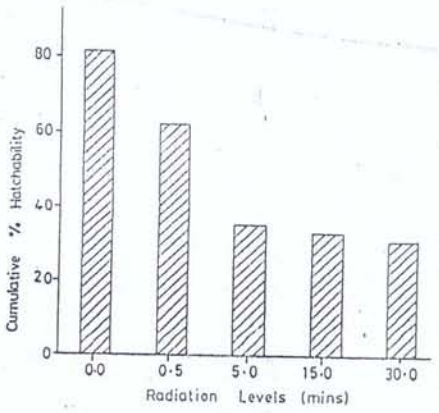

Fig. 1: Two-hour cummulative hatchability rate of irradiated *S. mansoni* eggs.

The difference in % hatchability compared with the control was not significant at 0.5 minutes irradiation level but was highly significant at 15.0 and 30.0 minutes

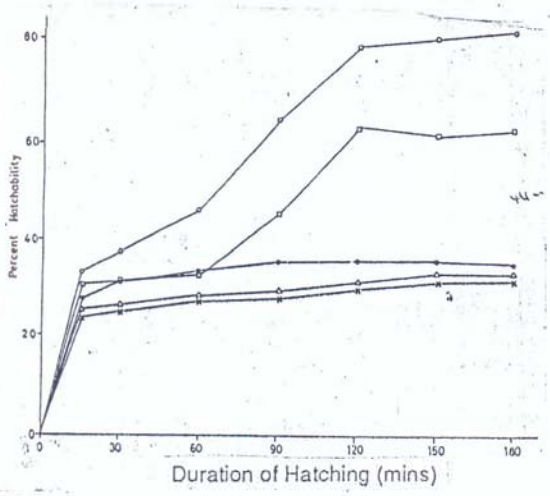

Fig. 2: Hatchability rates of eggs exposed to various levels of U.V. radiation.

Control (No radiation).
0.5 mins radiation.
5.0 mins radiation.
15.0 mins radiation.
30.0 mins radiation.

($p<0.1$, $d\alpha = 0.81$, 3.07 and 3.24 respectively). After 2 hours of exposure to light, no further hatching of eggs occurred in the irradiated eggs. An additional 2% of the eggs in the control batch hatched following a further one hour exposure time for hatching. As the number of eggs that hatched increased with the duration of hatching, the proportion of hatched eggs at a particular time interval varied with the radiation levels (Figure 2).

Table 1: Life span of *S. mansoni* miracidia hatched from UV radiated eggs.

Radiation Level (Mins.)	Time of Observation (hours) 0	1	2	3	4	5	6
0.0	100	100	86	80	49	14	0
0.5	100	80	67	50	30	0	0
5.0	100	75	39	17	8	0	0
15.0	100	69	31	15	8	0	0
30.0	100	69	44	19	0	0	0

Miracidia life span, motility and infectivity: The percentage survival rates (Table 1) show that under the experimental conditions, the control miracidia survived for 5 hours while only 19% of those exposed for 30 minutes survived for 3 hours. The cumulative mortality rate of *S. mansoni* miracidia increased with the age of miracidia recorded in hours as well as the radiation exposure level (Figure 3).

The activity (the rate of movement) was also radiation

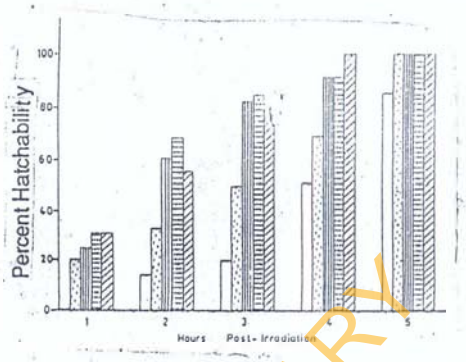

Fig. 3: Cumulative mortality rate of miracidia from *S. mansoni* eggs irradiated pre-hatching.

Control (No radiation).
0.5 mins radiation.
5.0 mins radiation.
15.0 mins radiation.
30.0 mins radiation.

level-dependent. There was a significant variation, $Y = 2.60 + (-0.02X)$, ($F = 60^*$), in the relationship between the rate of movement and the radiation dose level (Figure 4). At 30.0 minutes exposure level, a 21.4% reduction in motility compared with the motility of the control miracidia was recorded (Table 2).

Table 2. The rate of movement of *S. mansoni* miracidia from irradiated eggs.

Radiation. Level (mins.)	Mean motility rate• (mm/sec.) (S.D.)	Reduction in motility
0.0	2.67 (0.10)	0
0.5	2.50 (0.10)	6.4
5.0	2.50 (0.10)	6.4
15.0	2.31 (0.23)	13.5
30.0	2.10 (0.05)	21.4

*Each reading is an average of motility rate of 10 miracidia (i.e. time taken to cover 1 mm).

Table 3: The number of miracidia obtained from radiated eggs effecting infection per snail.

Radiation Levels (min.)	Snail number 1	2	3	4	5	6	7	8	9	10	Mean no of attached miracidia per snail (S.D.)
0.0	6	8	7	5	7	9	9	7	8	6	7(1)
0.5	7	8	9	7	6	8	6	8	9	6	7(1)
5.0	6	5	7	6	7	7	9	4	8	8	7(2)
15.0	8	7	6	7	8	10	7	6	9	8	8(1)
30.0	6	4	7	8	10	8	6	8	7	8	7(2)

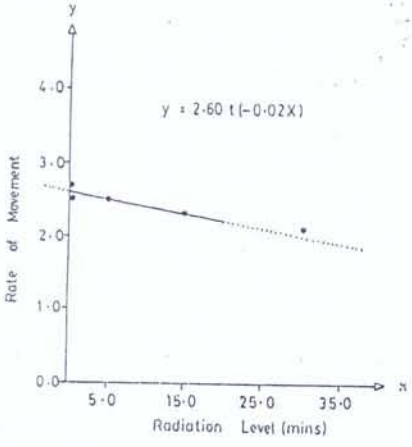

Fig. 4: The relationship between the mortality rate of miracidia from radiated eggs and radiation levels.

The results of miracidia ability to attach to snails are presented in Table 3. The regression analysis, $Y = 66.8 + (0.04X)$, showed that the variation due to regression is not significant, $F = 0.07$.

Discussion

The present study confirms previous findings that the development of schistosome larval stages is influenced by radiation. Inspite of unobserved drastic changes in the egg morphology or viability of the irradiated eggs, there was a significant interference with the hatching rate of eggs. The decreasing hatching rate with an increasing UV irradiation exposure is in line with the findings of Samuelson *et al.* (1984) who reported that hatching occured as a result of the activity of the miracidium. The decreased hatchability in eggs exposed to high level (30.0 minutes) of radiation might have been as a result of radiation damage to the miracidia and hence interference with miracidia activity or ability to induce hatching.

UV radiation cannot explain any inhibition in the build-up of internal osmotic pressure needd for hatching as reported by Kusel (1970) nor can UV support the proposed hatching mechanism by Bair and Etges (1973) that hatching may be induced by enzymatic degradation of the egg shell. No reports discussing the effect of UV radiation on the various type of enzymes reported to be present in *S. mansoni* eggs were encountered. Higgins-Optiz and Evers, (1983) observed that hatching of eggs occured as a result of the shell rupturing on one of the two lateral side of the egg surface. The observed inhibiting effects UV light had on hatchability in this study could have explained such distinctive longitudinal orifice but for localized damage or focal action by enzymes (Samuelson *et al.*, 1984) on the rupture line assuming they were present. Radiation could not have selectively affected particular location on the egg shell. On these line of thought, the decreased activity of miracidia exposed to high radiation exposure level, (30 minutes) might have been as a result of radiation damaged of the miracidia and hence the reduced hatchability rate with increased radiation exposure.

However, the most tenable assumption like Prah and James, 1977) suggested appears to be that the influence of UV radiation on metabolic processes in the miracidia may directly affect activity and hence survival and infectivity. Although the infectivity of the hatched miracidia exposed to UV radiation pre-hatching was not significantly affected by radiation of whatever dose level (Table 3), Ghandour and Webbe (1975) working on the development of gamma-irradiated cercariae found that, cercariae could not reach maturity though they were able to penetrate the host. One could therefore assume that the hatched miracidia that were not retained in the infecting water were capable of attaching. Moreover, the increased reduction in motility and survival rates could imply that the rate of further development or penetration would reduce with increase in UV radiation exposure.

This study did not establish the direct effect of natural UV radiation from sunlight on the miracidia which is positively phototropic, it however confirms that in the future, the increase in UV reaching the earth, if not controlled have a significant effect on organisms' activities positively or negatively. A reduction in the transmission level of schistosome could be forced.

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References

1. Ariyo, A.A. and Oyerindde, J.P.O., (1990). Effect of ultraviolet radition on survival, infectivity and maturation of *Schisosoma mansoni* cercariae. *Int. J. Parasitol. 20, No. 7*, 893-897.
2. Bair, R.D. and Etges, E.J, (1973). *Schistosoma mansoni:* factors affecting hatching of eggs. *Exptl. Parasitol. 33*, 155-167.
3. Donelly, F.A., Appleton, C.C. and Schutt, C.H.J. (1984). The influence of salinity on the ova and miracidia of three species of *Schistosoma. Int. J. Parasitol 70*, 321-331.
4. Farvar, M.A. and Cember, H. (1969). Lethal effects of gamma radiation on the schistosome larva carrier snail *Anstralorbis glabratus. Amer J. Pub. Hlth. 59*, 2077-2080.
5. Ghandour, A.M. and Webbe, G. (1975). The effect of gamma-radiation on cercariae of *Schistosoma mansoni, J. Helminthol. 49*, 161-165.
6. Higgins-Optiz, S.B. and Evers, P. (1983). Observations by scanning microscopy of miracidia hatching from

Schistosoma mansoni. J. Parasitol, 69, 432-433.
7. Jordon, P. and Webbe, G. (1969). *Human schistosomiasis*, Heinemann, London.
8. Kusel, J.R. (1970). Studies on the structure and hatching of the eggs of *Schistosoma mansoni. Parasitol. 60*, 79-88.
9. Lichtenberg, F. and Sadun, E.H. (1963). Parasite migration and host reaction in mice exposed to irradiated cercariae of *Schistosoma mansoni. Exptl. Parasitol. 13*, 256-265.
10. Michaels, R.M. and Kean, B.H. (1969). X-radiation of *S. Mansoni. In-vivo* and *in-vitro* effcts. *Amer J. Trop. Med. Hyg. 18*, 539-544.
11. Prah, S.K. and 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 ultraviolet light. *J. Helminthol. 51*, 73-85
12. Samuelson, J.C., Quinmn, J.J. and Caulfield. J.P. (1984). Hatching, chemokinesis and transformation of miracidia of *Schistosoma mansoni. J. Parasitol. 70*, 321-331.
13. Sharma, V.P.; Razdan, R.K. and Ansari, M.A. (1978). *Anopheles stephensi:* effect of gamma radiation and chemo-sterilants on the fertility and fitness of males for sterile male release. *J. Econ. Ent. 71*, 449-452.
14. *The Nairobi declaration on climatic change*. Nairobi, Kenya, Acts Press. African Centre for Technology Studies, 1990.
15. Upatham, E.S.; Sturrock, R.F. and Cook, J.A. (1976). Studies on the hatchability of *Schistosoma mansoni* eggs from a naturally infected human community on St. Lucia, West Indies. *Parasitol.* 73, 253-264.
16. Urbach, F. (1989). Potential effects of altered solar ultraviolet radiation on human skin cancer *Photochem. Photobiol. 50*, 507-514.
17. Weihe, W.H. and Mertens, R. (1991), Human well-being, diseases and climate *In*: Proceedings of the second World Climate Conference J. Jager and H.L. Ferguson (Eds.) Cambridge University Press, Cambridge. 345-359.