Scanning electron microscopical studies on the tegument of adult worms of *Schistosoma mansoni* originating from ultraviolet-irradiated and non-irradiated cercariae

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Abstract

The surface topography and ultrastructural changes of *Schistosoma mansoni* worms developed from ultraviolet-irradiated cercariae were compared with those from non-irradiated cercariae. The tegument of worms developed from irradiated cercariae showed a variety of changes including the occurrence of oedemata and most of the tubercles being torn at the tip and having lost their spines on the dorsal surface of some male worms. In females, parts of the tegument were devoid of spines and surface lesions were present. The range and extent of these changes differed not only among individual worms but also between different regions of individual worms resulting in generalized deformities in those worms originating from irradiated cercariae.

Introduction

For the past two decades, irradiated cercariae have been used in the vaccination of experimental animals against reinfection with schistosomes as an alternative strategy to chemotherapy. As a gamma ray source requires specialist and expensive facilities, an ultraviolet source of radiation has been widely used by several authors as a tool for the attenuation of schistosome cercariae (Standen & Fuller, 1959; Ghandour & Webbe, 1975; Murrell *et al.*, 1975; Dean *et al.*, 1983; Moloney *et al.*, 1985, 1987; Ariyo & Oyerinde, 1990; Shi *et al.*, 1993; Mostafa, 1995). Scanning electron microscopy (SEM) has become a useful tool for studying the surface structure of many helminths, particularly schistosomes (Silk *et al.*, 1969, 1970; Miller *et al.*, 1972). Little attention has been paid to ultrastructural studies on schistosomes developed from irradiated cercariae. However, many studies have been conducted on the ultrastructural damage induced by schistosomicides on male and female schistosomes (Moore, 1977; Becker *et al.*, 1980; Sabry, 1983; Irie *et al.*, 1989; Awad & Probert, 1991; Shalaby *et al.*, 1991; Shalaby, 1994; Fallon *et al.*, 1996).

The aim of the present study is to investigate the surface topography and ultrastructural changes of *S. mansoni* tegument originating from ultraviolet-irradiated cercariae for comparison with those from non-irradiated cercariae.

Material and methods

An Egyptian strain of *Schistosoma mansoni* was maintained in *Biomphalaria alexandrina* and CD_1 mice as intermediate and final hosts, respectively. The snails and mice were supplied by the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute, Cairo. An S-68 Mineralight lamp (Ultraviolet Products, Inc.), rated to deliver 95% of its output at 254 nM, was used as the source of ultraviolet radiation.

Mice were divided into two groups, one group being exposed to 500 non-irradiated cercariae per mouse (as controls) and a second group exposed to 500 irradiated cercariae per mouse for 2 min (after Dean *et al.*, 1983). The tail immersion method (Olivier & Stirewalt, 1952) was used for exposure of mice to cercariae in both groups. Adult worms of *S. mansoni* were recovered from mice by

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the perfusion technique (Smithers & Terry, 1965). Each group of worms was then fixed in 4% gluteraldehyde in sodium cacodylate buffer for 2 h, washed in the same buffer at a pH of 7.4, dehydrated with ethanol and critical point dried. Specimens were mounted on stubs, coated with carbon and gold and examined with a Joel JEM-1200 EXII electron microscope .

Results

Scanning electron microscopy of the tegument of male *S. mansoni* developed from non-irradiated cercariae revealed the presence of numerous large tubercles with a concentration of spines. In the areas between the tubercles, spines were evenly dispersed, and the surface was composed of circular foldings containing with minute pores (fig. 1). The tegument of the gynaecophoric canal differed in structure from the rest of the worm, being characterized by several folds and numerous spines (fig. 2). However, the surface of the female *S. mansoni* developed from non-irradiated cercariae lacked tubercles and was relatively smoother than that of the male, but the spines were numerous and uniformly distributed (fig. 3). The tegument of adult *S. mansoni* developed from irradiated cercariae showed a variety of structural changes in both male and female worms. In males, the tegumental damage was pronounced, with an extensive loss of spines from the tubercles. Parts of the dorsal surface, particularly between the spined tubercles, were devoid of sensory spines (fig. 4). In other specimens, and in different regions of the same specimen, the spined tubercles showed extensive surface oedemata and lesions, while erosion of the surface layer and loss of spines from the tubercles were extensive (fig. 5). The swelling (oedema) of the tubercles occurred on the dorsal surface of some males with most of the tubercles being torn at the tip and having lost their spines (figs 6 and 7). The tegument of the gynaecophoric canal also lost some of its spines and in some male worms varying degrees of swelling and lesions were also observed (fig. 8). In female worms, tegumental changes were not as prominent or as extensive as those in males from mice infected with irradiated cercariae. Parts of the female tegument were devoid of spines (fig. 9). In other worms surface swellings were widespread and lesions were also present (fig. 10).

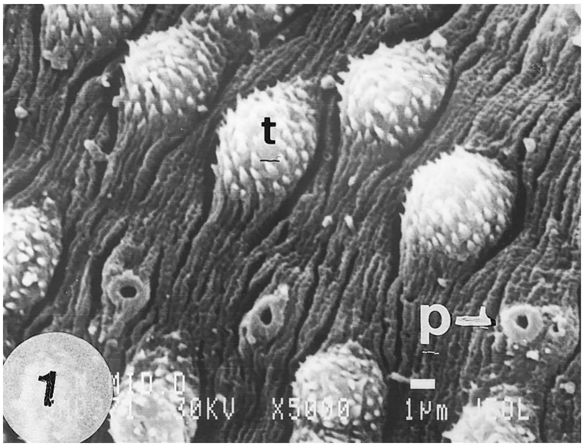

Fig. 1. Dorsal surface of male *Schistosoma mansoni*, developed from a non-irradiated cercaria, showing numerous tubercles (t) with a concentration of spines. Note the presence of pores (p) in between the tubercles.

Discussion

The cercaricidal properties of ultraviolet radition have previously been studied by a number of authors (Ghandour & Webbe, 1975; Dean *et al.*, 1983; Moloney *et al.*, 1987; Shi *et al.*, 1993 and Mostafa, 1995) especially as the ultraviolet source of radiation is simpler and less expensive than gamma radiation or X-radiation sources. The end product of radiation in a cell is the result of a

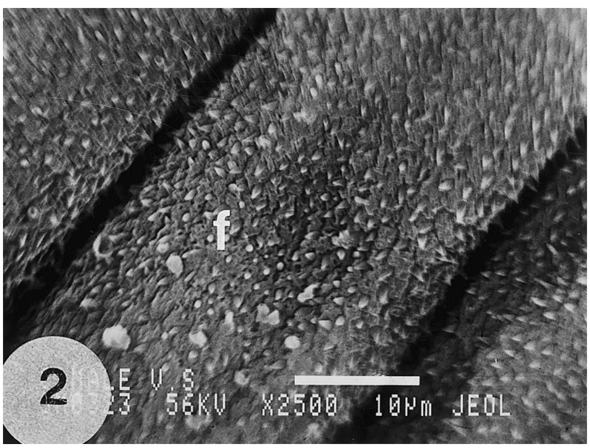

Fig. 2. Ventral surface of male *Schistosoma mansoni*, developed from a non-irradiated cercaria, showing several folds (f) in the region of the gynaecophoric canal.

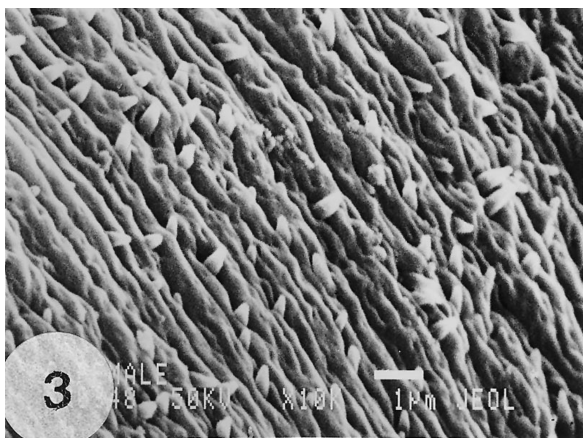

Fig. 3. Posterio-dorsal surface (without tubercles) of female *Schistosoma mansoni*, developed from a non-irradiated cercaria, without tubercles.

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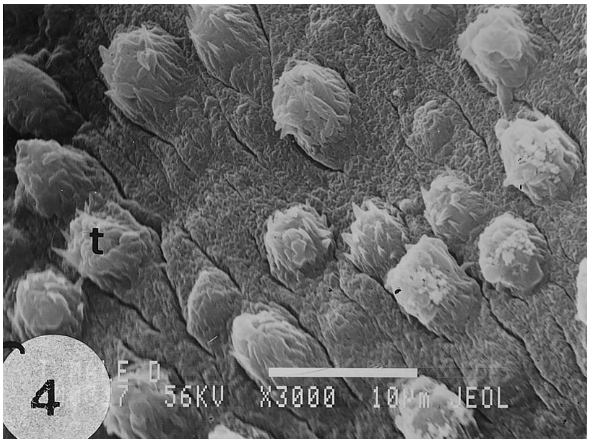


Fig. 4. Dorsal surface of male *Schistosoma mansoni*, developed from a 2-min ultraviolet-irradiated cercaria, showing the tubercles (t) without spines.

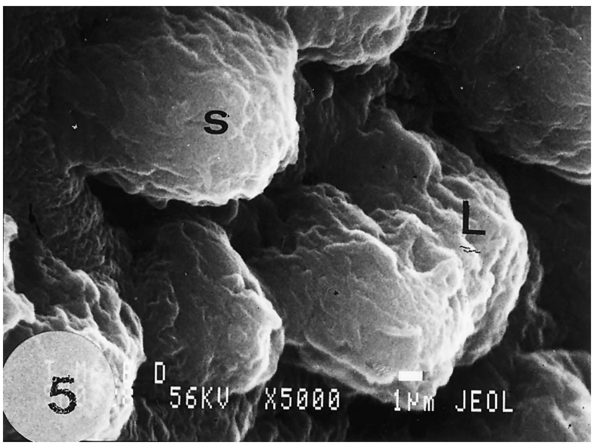
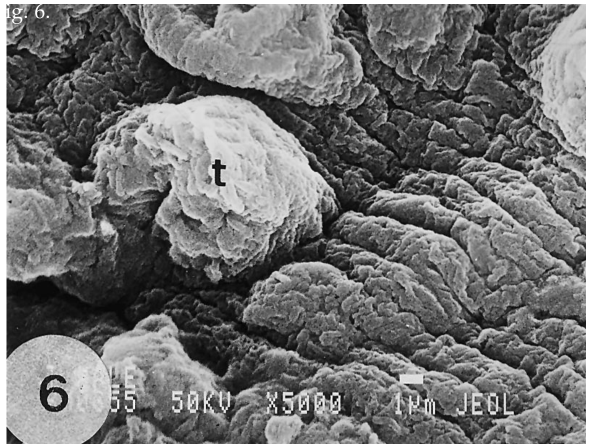


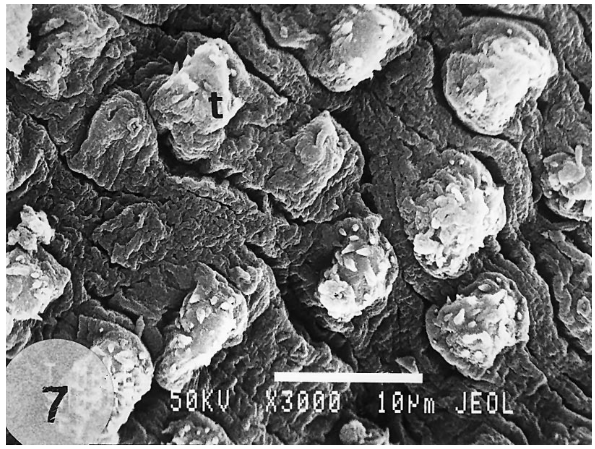
Fig. 5. Dorsal surface of male *Schistosoma mansoni*, developed from a 2-min ultraviolet-irradiated cercaria, showing extensive surface swellings (s) and lesions (L).

chain of events terminating in detectable effects and can be formalized according to Latarject & Gray (1954) as follows:

Absorption of radiation energy → Radiochemical events → Metabolic events → observable effects.

Harrop & Wilson (1993) reported that gamma-attenuated *S. mansoni* larvae exhibited random constriction of circular muscle fibres at intervals along the length of the body. They suggested that these abnormalities accounted for the persistence of attenuated larvae in the skin draining lymph nodes and lung, resulting in the delay of migration of the attenuated larvae and induction of protective immunity. Thirty years earlier, Lichtenberg & Sadun (1963) showed that stunted young adults of *S. mansoni* worms developed from gamma-irradiated





Figs 6 and 7. Dorsal surface of male *Schistosoma mansoni*, developed from a 2-min ultraviolet-irradiated cercaria, showing swelling (oedema) with most of the tubercles (t) being torn at the tip and devoid of spines.

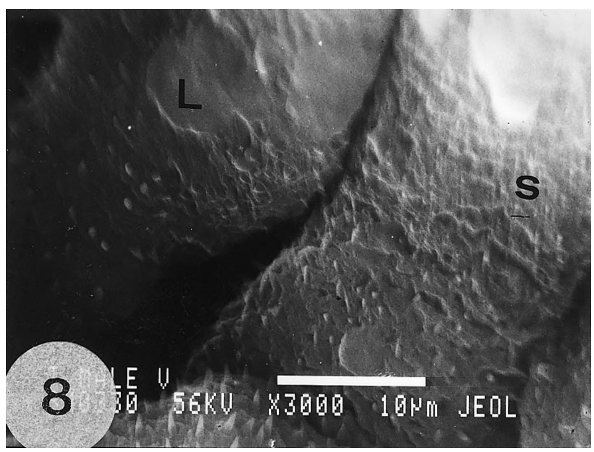


Fig. 8. Varying degrees of swelling (s) and lesions (L) on the ventral surface of male *Schistosoma mansoni*, developed from a 2-min ultraviolet- irradiated cercaria.

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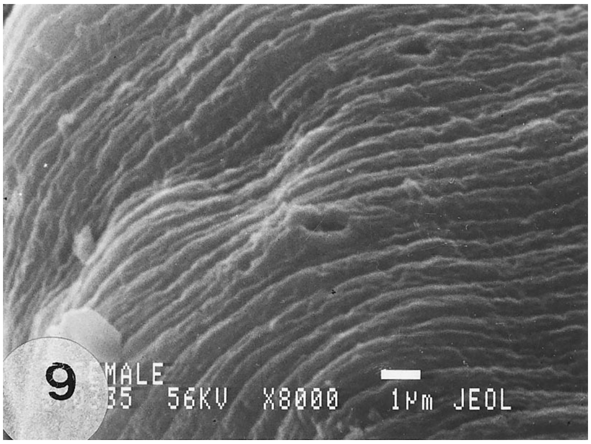

Fig. 9. Posterio-dorsal surface (devoid of spines) of female *Schistosoma mansoni*, developed from a 2-min ultraviolet-irradiated cercaria.

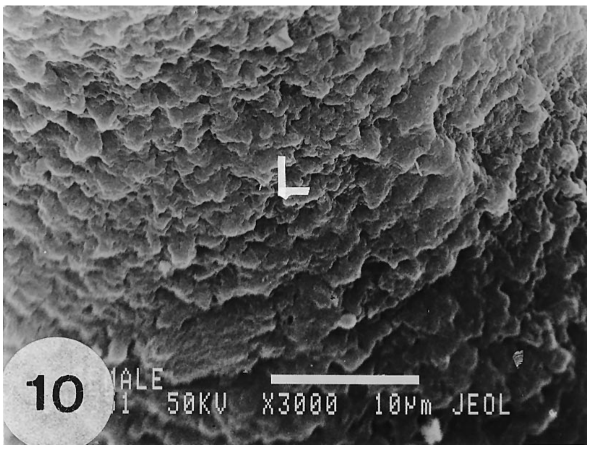

Fig. 10. Lesions (L) on the dorsal surface of female *Schistosoma mansoni*, developed from a 2-min ultraviolet-irradiated cercaria.

cercariae. Worms of *S. mansoni* originating from X-irradiated cercariae showed morphological changes which could be related to radiation damage; the worms were stunted and the majority of males showed an abnormal gut (Perlowagora-Szumlewicz , 1964).

Ultraviolet radiation, as in the case of ionizing radiation, causes a delay in cell-division and the effects are dose dependent (Claus, 1958). One-minute ultraviolet irradiation of cercariae leads to sexual anomalies in male worms and a delayed effect upon the development of the reproductive system, leading to the appearance of sterile males and females. Furthermore, the tegument of male and female worms observed from 2-min ultraviolet-iradiated cercariae is thrown into narrow folds (Mostafa, 1996).

The present study has shown that the tegument of adult *S. mansoni* worms, originating from ultraviolet-irradiated cercariae, exhibits abnormal features in both sexes of worms with signs of damage, especially in the males. The range and extent of these changes were different not only between different worms but also between different regions of individual worms. Ultraviolet radiation of different wave lengths causes severe damage to a wide range of living organisms. This may be associated with changes in normal physiological processes and, at high levels of radiation, with cytological interference and eventual death (Ghandour & Webbe, 1975). Moreover, ultraviolet radiation inhibits the action of many enzymes including those concerned with oxidation and digestion (Ellis & Wells, 1941; Dean, 1983; Wales & Kusel, 1992).

Urbach (1969) observed retardation of division in ultraviolet irradiated ciliate protozoans and this was often characterized by little or no delay in the first post-irradiation division (sometimes two divisions occur) and then a long delay (called stasis) before other divisions could occur. He also suggested that the immediate post-irradiation divisions were possible because of accumulated reserves. Once these were exhausted and the synthetic activities in the cell were suspended by ultraviolet treatment, growth and cell division were impossible. It is likely that in the present study schistosome cercariae may be similarly affected by ultraviolet radiation, thus explaining their development into morphological abnormal worms.

From previous studies (Urbach, 1969; Ghandour & Webbe, 1975; Ellis & Wells, 1941; Dean, 1983; Wales & Kusel, 1992), it could be concluded that the morphological abnormal worms originating from gamma and X-ray irradiated cercariae can be attributed to changes in normal physiological processes, cytological interference, inhibition of many enzymes, and/or retardation of cell division. However, the various degrees of tegumental damage inflicted on adult schistosomes that developed from irradiated cercariae, might be attributed to either the direct effect of the ultraviolet radiation on the cercariae or to the host's immunogenicity induced by *S. mansoni* larvae attenuated by ultraviolet irradiation. Further investigations are in progress.

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