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Lethal and Sub-lethal Effects of UVB on Juvenile *Biomphalaria glabrata* (Mollusca: Pulmonata)

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Abstract

Although *Schistosoma mansoni* occurs mainly in the tropics, where intense levels of solar radiation are present, the impact of ultraviolet (UV) light on schistosome transmission is not known. The purpose of this study was to investigate potential effects of UVB (290–320 nm) on juvenile *Biomphalaria glabrata*, the snail intermediate host of *S. mansoni*. Albino and wild type snails were exposed to doses of UVB from UV-fluorescent lamps, and the following were measured: survival, photoreactivation (light-mediated DNA repair), effects on feeding behavior, and morphological tissue abnormalities. Irradiation with UVB is lethal to *B. glabrata* in a dose-dependent manner. Exposure to white light subsequent to UVB irradiation enhances survival, probably by photoreactivation. The shell offers some, but not complete, protection. Experiments in which UVB transmittance through the shell was blocked with black nail polish suggest that injury to both exposed (headfoot) and shell-enclosed (mantle and visceral mass) tissues contributes to mortality in lethally-irradiated snails. Wild-type (pigmented) snails are less susceptible to lethal effects of UVB than albino snails, and they may be more capable of photoreactivation. UVB exposure inhibits snail feeding behavior, and causes tentacle forks and growths on the headfoot. Thus, UVB may influence the life cycle of *S. mansoni* by both lethal and sub-lethal damage to the snail intermediate host. However, the ability of snails to photoreactivate may mitigate these effects.

Keywords

Biomphalaria glabrata; ultraviolet (UV); ultraviolet-B (UVB); photoreactivation; shell; branched tentacles

1. Introduction

Approximately 200 million people, mainly in the tropics and subtropics, are afflicted with schistosomiasis, a debilitating parasitic disease caused mainly by three species of digenetic trematodes in the genus *Schistosoma* (Ross et al., 2002). Multiple parameters affect transmission of schistosomiasis, among them the life expectancy of the snail intermediate hosts, in which schistosome larvae undergo asexual reproduction and development to the human-infecting cercaria (Anderson and May, 1985). Consequently, deleterious effects of adverse environmental conditions, e.g., water quality, temperature, and solar irradiation, on the snail hosts would be expected to interfere with the transmission of the parasite to humans. Relative

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to solar irradiation, ultraviolet B (UVB) radiation (290–320 nm) is of particular interest, because these are the shortest solar wavelengths to reach the earth's surface, UVB can penetrate to biologically significant depths in natural waters, and incident UVB levels are increasing due to ozone depletion (Karentz et al., 1994; Morris et al., 1995; Williamson, 1996).

UVB has a major impact on organisms in aquatic ecosystems, both directly by affecting organic components of cells, and indirectly by photochemical reactions occurring in the surrounding water (Karentz et al., 1994; Helbling and Zagarese, 2003). Among such potential direct and indirect effects are mutagenesis, carcinogenesis, and lethality (Weber, 2005).

Apart from lethality of high doses, relatively little is known of the effects of UVB on juvenile and adult gastropods. Wong and Wagner (1956), using *Oncomelania nosophora* and *O. quadrasi*, and Nabih and Abd El Hamid (1984) using *Biompalaria alexandrina*, showed that UV from a lamp caused mortality among exposed snails, and that mortality was proportional to the amount of time snails were irradiated. Similarly, Thomas and Tait (1984) reported that albino *B. glabrata* crawling upside-down on the water surface were killed by exposure to a UV lamp. McNamara and Hill (1999) exposed the prosobranch snail *Elimia clavaeformis* and the pulmonate *Physella gyrina* to up to $26 \text{ kJ} \cdot \text{m}^{-2}$ UVB at a fluence of $2 \text{ W} \cdot \text{m}^{-2}$, and observed that *E. clavaeformis* survived all doses used, whereas small (1 mm shell length) *P. gyrina* showed decreased survival at 5 days post-exposure to 13 and $26 \text{ kJ} \cdot \text{m}^{-2}$. Rankin and Harrison (1979) reported mortality of hatchling and juvenile *Physa marmorata* exposed to direct sunlight for 6 hr, whereas Hill et al. (1997) found no effects on densities of *E. clavaeformis* from ambient levels of solar UV, and they hypothesized that the shell and pigmentation of the headfoot protected the snail from UV damage. Among sublethal effects, *B. alexandrina* snails exposed to a UV lamp showed elevated alanine aminotransferase activity (Nabih and el-Ansary, 1991), and increased levels of thymine dimers (Nabih and Abd El Hamid, 1984). Finally, Wong and Wagner (1956) reported morphological abnormalities, i.e., branched tentacles, in *O. nosophora* and *O. quadrasi* exposed to a UV lamp.

Based on what is known about the effects of UV on organisms, we can speculate that the above effects are due, in part, to DNA damage. Solar radiation in the UVB waveband causes significant damage to DNA (Weber, 2005), as well as affecting other biologically relevant molecules such as proteins, lipids and chromophores (Häder and Sinha, 2005). The major DNA lesion caused by UVB is dimerization of adjacent pyrimidine bases, resulting in two principal photoproducts: cyclobutane-pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) (Mitchell and Karentz, 1993; Häder and Sinha, 2005), primarily the former (Weber, 2005). This type of bulky distortion can halt both DNA and RNA polymerase activity, potentially resulting in physiological impairment, mutation, or cell death (Kao et al., 2005).

Organisms repair CPDs and 6-4 PP's by two major mechanisms: nucleotide excision repair and photoreactivation (Sancar, 1994a,b). The latter process is catalyzed by the light-dependent enzymes, CPD photolyase and (6-4) photolyase (Li et al., 2006). Photoreactivation occurs in many aquatic and terrestrial prokaryotic and eukaryotic organisms (Häder and Sinha, 2005), but not in placental mammals (Weber, 2005). Photolyases have been found in some archaeobacteria, and are considered to be ancient repair proteins; therefore, photoreactivation may be one of the oldest and simplest forms of DNA repair (Häder and Sinha, 2005). As reviewed by Sancar (1994b), CPD photolyase is a structure-specific DNA-binding flavoprotein in which catalysis is initiated by light. Initially, the enzyme binds the CPD lesion in a step that is independent of light. However, in the presence of 350–450-nm photons, photolyase initiates the splitting of the cyclobutane ring by electron transfer to restore the dipyrimidine. Although this DNA repair mechanism is widespread among aquatic organisms, it is not known whether *B. glabrata* undergoes photoreactivation.

In this study we investigate effects of UVB on juvenile *B. glabrata*, the snail intermediate host of *Schistosoma mansoni*, and document decreased survival of albino and wild type juvenile *B. glabrata*, their ability to photoreactivate, as well as sub-lethal effects on feeding behavior and soft tissues.

2. Materials and methods

2.1. Snails

Juvenile specimens of the NIH albino strain (Newton, 1955) of *B. glabrata*, which is susceptible to infection with *S. mansoni*, and specimens of the schistosome-nonsusceptible, pigmented Salvador strain (Paraense and Correa, 1963) were used in this study. All snails measured 2–5 mm in shell diameter. Snails were raised in aerated aquaria containing artificial pond water (Malek and Cheng, 1974) and fed fresh leaves of Romaine lettuce (*Lactuca saliva*) *ad libitum*.

2.2. UV Exposure

UVB exposures were conducted using 5 12-inch fluorescent lamps (BLE-8TB02, Spectronics corp., Westbury, New York) mounted in a plexiglass exposure chamber as described previously (Karentz et al., 1991). The lamps emitted wavelengths ranging from 275–350 nm, with a peak at 313 nm, and a cutoff filter was used to exclude wavelengths above 320 nm. Fluence was $5.78 \text{ W}\cdot\text{m}^{-2}$. Snails were placed on a shelf 22.9 cm below the lamps, and dose was regulated by an electronic shutter, which controlled the duration of exposure.

2.3. Dose-Response

Groups of 10 snails (5 NIH albino and 5 Salvador) were exposed to the following doses of UVB: $250 \text{ J}\cdot\text{m}^{-2}$, $500 \text{ J}\cdot\text{m}^{-2}$, $750 \text{ J}\cdot\text{m}^{-2}$, $1000 \text{ J}\cdot\text{m}^{-2}$, and $1500 \text{ J}\cdot\text{m}^{-2}$. Exposure times ranged from 43.3 sec to 4 min, 19.5 sec. Controls were not exposed to UVB. During UVB exposure, snails were placed in the center of a plastic petri dish in approximately a 1-ml drop of water. To prevent overheating, the petri dish rested on crushed ice. Following UVB exposure, snails, along with a 13 x 24-mm piece of index card indicating the dose, were placed in 55-mm diameter glass bowls, which were covered by plastic screening secured with a rubber band. Bowls were submerged in approximately 2 L of pond water in covered rectangular plastic containers (34.4 x 21.4 x 11.5 cm) and held in the dark (to prevent repair of DNA damage by photoreactivation) for 5 days at 27°C. Snails were examined after 5 days for mortality with a dissecting microscope, and those undergoing decomposition or lacking a heartbeat were counted as dead.

2.4. Photoreactivation

To determine the ability of albino and wild-type strains of *B. glabrata* to repair damaged DNA through the light-dependent repair process of photoreactivation, snails were exposed to $1500 \text{ J}\cdot\text{m}^{-2}$, a dose causing >90% mortality in the dose-response study. Immediately following exposure, snails were placed in glass bowls, as described above, and submerged in 2 L of pond water in a plastic container covered by a clear acrylic lid under a 60-W white fluorescent lamp positioned approximately 8 cm above the water surface. Snails were examined 5 days later for mortality.

2.5. Protection by Shell

Two separate experiments were conducted in order to assess the UVB-protective role of the shell. In the first study, snails were induced to retract into their shell by prodding the headfoot. The aperture was then sealed using rubber cement (Elmer's Products, Inc. Columbus, OH), and once the cement had dried, forming a thin diaphragm over the opening, snails were exposed

to 2000 J·m⁻² UVB as described above. Two groups of controls were employed. One control group was exposed to 2000 J·m⁻² without having been sealed in the shell, and the second control group consisted of snails that were sealed, but not exposed to UVB. Subsequent to exposure, the rubber cement was removed with watchmaker's forceps, the snails were placed in the dark for 5 days, and then all groups were examined for mortality.

In the second study, snail shells were covered with a single layer of Wet'n'Wild Black Crème nail polish (Markwins Beauty Products, City of Industry, CA). Once the polish had dried, snails were exposed to 2000 J·m⁻², placed in the dark, and examined for mortality as previously described. Controls were treated similarly, but were not irradiated.

Absorbance of the black nail polish was estimated by applying a single layer to the outside surface of a plastic disposable UV-transparent cuvette prior to performing a scan of absorbance from 250–500 nm with a spectrophotometer. Additionally, methanol extracts of lyophilized shells and soft tissues of both snail strains were screened for the presence of UV-absorbing mycosporine-like amino acids (MAAs) by the method of Karentz (2001). Briefly, approximately 0.01g of lyophilized material was extracted overnight with 1.0 ml 80% methanol, centrifuged, and the supernatant was scanned in a spectrophotometer through the UVB and UVA wavelengths.

2.6. Effects on Feeding

Because we had observed in preliminary experiments that snails grazed upon the surface of index card paper, we decided to measure the effect of UVB dose on this feeding behavior. We used the snails from the dose-response study, exposed to the doses listed in section 2.3, as well as the snails from the shell-protection study that had been sealed in their shells with rubber cement and exposed to 2000 J·m⁻² (section 2.5). The dry weights of the index card labels, used to denote dose, as explained above in section 2.3, were measured prior to the 5-day incubation with irradiated or control snails. After 5 days the pieces of paper were removed, placed in a 54°C incubator for 24 hr to dry, and then re-weighed. The difference in pre- and post-incubation dry weights represented paper consumption. To correct for the normal loss of mass of paper immersed in water, we incubated 13 pre-weighed index card labels in glass bowls without snails. The mean normal loss of dry weight ($0.00122 \pm \text{S.E.M. } 0.00014 \text{ g}$) was then subtracted from the paper consumption values obtained for each group of experimental or control snails. Finally, to correct for differing numbers of surviving snails in different treatment groups, this value was then divided by the number of surviving snails (except in cases where 100% mortality occurred), based on the observation that lethally-irradiated snails consumed little if any paper before dying. No distinction was made between NIH albino and Salvador strains.

2.7. Morphological Abnormalities

Groups of 10 Salvador snails measuring approximately 5 mm in shell diameter were exposed to 500 J·m⁻² (the highest non-lethal dose for this strain at 5 days post-exposure), and they were subsequently kept in the dark at 27°C, with food. All snails were examined weekly for abnormalities in the headfoot and tentacles for 6 weeks post-exposure. Samples of abnormal tentacles were fixed in Bouin's fluid and examined histologically in 7 µm-thick serial sections. Additionally, a group of five snails, measuring 12–13 mm, was exposed, right side up, to 1000 J·m⁻², and their right tentacle was excised and fixed in Bouin's fluid 24 hr post-exposure. The right, rather than the left, tentacle was selected because *B. glabrata* typically crawls with the right side of its shell facing upward when in shallow water, causing the right tentacle to be more directly exposed to overhead UVB. Tentacles from five non-exposed snails served as controls. Fixed tentacles were transected midway along their length, so that both longitudinal and cross serial sections could be obtained.

2.8. Statistical Analysis

Decreased mean survival of NIH albino or Salvador snails exposed to each UVB dose was tested for statistical significance (relative to 100% control survival) using the one-tailed Student's *t* test. Additionally, survival of the two snail strains was compared at each UVB dose using the two-tailed Student's *t* test (MS Excel).

To compare survival curves for the two snail strains, D_{37} values were calculated by regression analysis of the linear portion of the dose-survival data, using DeltaGraph (Red Rock Software, Salt Lake City, UT). Calculation of the D_{37} is a standard statistical method in radiobiology for analysis of survival curves (Fabrikant, 1972; Jagger, 1976). When applied to cell cultures, the D_{37} identifies the UV dose that causes an average of one lethal event per cell (reviewed by Karentz et al., 1991).

In the photoreactivation study, survival of snails exposed to UVB and subsequently placed in the dark was compared to that in irradiated snails placed under white fluorescent light, with the two-tailed Student's *t* test. In the shell protection study, enhanced survival of UVB-exposed experimental snails sealed in their shells or painted with nail polish was compared to that in exposed controls (which showed 0% survival) by the Student's one-tailed *t* test. Paper consumption in UVB-exposed snails was compared at each dose to that in unexposed controls with the Student's two-tailed *t* test. Finally, The proportion of UVB-irradiated snails developing tentacle abnormalities was compared to controls with the use of a Chi-square test (Sokal and Rohlf, 1981). For all statistical tests, differences were considered significant at $P < 0.05$.

3. Results

3.1. Dose-Response

Survival decreased with an increase in UVB dose (Fig. 1). No mortality occurred among control snails or at a dose of $125 \text{ J}\cdot\text{m}^{-2}$, whereas fewer than 10% of snails of both strains survived at $1500 \text{ J}\cdot\text{m}^{-2}$. Mean survival in NIH albino snails was significantly lower than control survival at doses of $500 \text{ J}\cdot\text{m}^{-2}$ and above, whereas mean survival in Salvador snails was significantly lower than control survival at doses of $750 \text{ J}\cdot\text{m}^{-2}$ and above ($P < 0.05$, Student's one-tailed *t* test). At all doses, survival was higher among Salvador snails than in NIH albino snails, and these differences were statistically significant at $500 \text{ J}\cdot\text{m}^{-2}$ and $750 \text{ J}\cdot\text{m}^{-2}$ ($P < 0.05$, Student's two-tailed *t* test). In agreement with these results, the D_{37} of Salvador snails was greater than that of NIH albino snails, 1010 and $756 \text{ J}\cdot\text{m}^{-2}$, respectively.

3.2. Photoreactivation

When snails of both strains were placed under white light after exposure to $1500 \text{ J}\cdot\text{m}^{-2}$ UVB to allow for photoreactivation, significantly greater survival occurred than in snails that were exposed to $1500 \text{ J}\cdot\text{m}^{-2}$ and then kept in the dark (Fig. 2, $P < 0.05$, Student's two-tailed *t* test). The difference in mean survival was not statistically significant between NIH albino and Salvador pigmented strains kept in the dark following irradiation with $1500 \text{ J}\cdot\text{m}^{-2}$. However, among snails undergoing photoreactivation, Salvador snails showed significantly higher survival than did NIH albino snails ($P < 0.05$, Student's two-tailed *t* test).

3.3. Protection by Shell

Survival increased when NIH albino and Salvador snails were sealed inside their shells during exposure to UVB (Fig. 3). Specifically, when snails that were not sealed in their shells were exposed to $2000 \text{ J}\cdot\text{m}^{-2}$, none survived. However, when snails were sealed in their shells and exposed to $2000 \text{ J}\cdot\text{m}^{-2}$, survival was significantly greater ($P < 0.05$, Student's one-tailed *t* test). Although increased survival occurred in both strains when protected by their shell, survival

was significantly lower than in sealed controls (non-UVB-exposed), and was also significantly lower in NIH albino versus Salvador snails ($P < 0.05$, Student's two-tailed t test). Some mortality occurred in control snails in which the shell aperture was sealed, but it was not significantly different from that in non-tampered controls, in which no mortality occurred ($P > 0.05$, Student's one-tailed t test).

Survival of snails exposed to $2000 \text{ J}\cdot\text{m}^{-2}$ also increased significantly when shells had been blocked with black nail polish ($P < 0.05$, Student's one-tailed t test). Protection was not absolute, inasmuch as survival among blocked, irradiated snails was significantly lower than that in blocked unirradiated controls (Fig. 3, $P < 0.05$, Student's two-tailed t test). These blocked controls showed no significant difference in survival relative to non-tampered controls. No significant differences occurred between NIH albino and Salvador snails in this experiment (Fig. 3, $P > 0.05$, Student's two-tailed t test).

UV absorbance of a single layer of nail polish applied to the surface of a cuvette was uniform across the UVB and UVA range. The polish increased UV opacity by approximately 30%. Methanol extracts of lyophilized shells and soft tissues of NIH albino and Salvador *B. glabrata* showed no UV absorbance, suggesting the absence of MAAs.

3.4. Effects on Feeding

As shown in Fig. 4, paper consumption, as indicated by loss of pencil markings from the surface of labels, over a 5-day period was inversely proportional to dose. Specifically, there was no loss of pencil markings from labels in bowls containing snails exposed to the highest dose, or in bowls without snails, whereas, markings were almost completely removed by controls (non-irradiated snails), and snails exposed to the lowest dose. A comparison of the dry weights of the labels showed that paper consumption decreased significantly, relative to that in unexposed snails, following exposure to $375 \text{ J}\cdot\text{m}^{-2}$ and higher doses (Fig. 5, $P < 0.05$, Student's two-tailed t test). No significant difference in consumption occurred in non-irradiated controls temporarily sealed in their shells relative to non-tampered controls. Sealing the snails in their shells with rubber cement did not prevent inhibition of feeding by UVB, since sealed snails exposed to $2000 \text{ J}\cdot\text{m}^{-2}$ showed significantly less consumption than controls ($P < 0.05$, Student's two-tailed t test).

3.5. Morphological Abnormalities

Among 80 snails initially exposed to $500 \text{ J}\cdot\text{m}^{-2}$, 10 out of 65 survivors displayed a single fork in the right tentacle at three weeks post-exposure (Fig. 6A). Additionally, six of the UV-exposed snails exhibited raised, transverse folds along the dorsal posterior margin of the headfoot, despite having normal tentacles (Fig. 6B). Conversely, among 155 stock, non-irradiated Salvador snails, none showed any morphological abnormalities. The observed differences were statistically significant ($P < 0.05$, Chi-square test).

Histologically, although more thin-walled, the tentacle branch is similar to a normal tentacle (see Pan, 1958). Like a normal tentacle, the branch is a gradually tapering cylinder surrounded by epithelium bearing dense cilia (Fig. 6C). A core of connective tissue, with a central artery and peripheral hemolymph sinus, passes through the root to the tip of the branch (Figs. 6C, D). Unlike the normal tentacle, which contracted when fixed in Bouin's fluid, the tentacle branch shown in Fig. 6C remained flaccid. However, we have observed that the branches contract when stimulated mechanically (unpublished).

Histological serial sections of tentacles from 3 of 5 NIH albino snails that had been exposed to $1000 \text{ J}\cdot\text{m}^{-2}$ showed exfoliation of epithelial cells at 24 hr post-irradiation, resulting in

exposed basement membrane (Figs. 6E, F). Among 5 non-irradiated controls, a single area of damaged epithelium was found in one tentacle.

4. Discussion

4.1. Dose-Response

As anticipated, survival of *B. glabrata* is dependent on dose of UVB. The shoulder (flat region) at the initial portion of the survival curve indicates that at low doses, repair mechanisms are effective. However, as dose increases, repair becomes insufficient to prevent mortality.

The greater UVB resistance of Salvador snails, as shown by a higher D_{37} , may be a result of several factors; e.g., black, presumably melanin-like (Lemos, 1999), pigmentation, more efficient repair pathways, or greater overall robustness, since Salvador snails are a wild type field isolate, whereas NIH albino snails are a highly inbred, laboratory strain. Although the pigmentation of the Salvador snails may provide a UV-screening function and contribute to the observed greater survival, there are a number of colorless UV-protective compounds (mycosporine-like amino acids, MAAs) found in a large variety of aquatic invertebrates that could play a major role in UV-protection (Karentz, 2001). There was no indication of MAAs in the shells and soft tissues of laboratory-reared Salvador and NIH albino snails. However, these compounds are bioaccumulated through food sources and were not available in the laboratory diet provided during these experiments. Therefore, it is not possible to speculate on whether or not these compounds occur in field populations of *Biomphalaria* spp. or how much UV protection they might provide for these snails in nature.

4.2. Photoreactivation

Survival increased when *B. glabrata* were placed under white fluorescent light following UVB exposure, showing that these snails are capable of photoreactivation. The magnitude of this increase suggests that most of the mortality in non-photoreactivating snails results from formation of CPDs and/or 6-4 PPs in DNA, rather than from other types of DNA lesions or damage to proteins and lipids, repair of which would be unaffected by light. Moreover, these results indicate that photoreactivation is a primary mechanism for repair of UVB-induced pyrimidine dimers in *B. glabrata*, since “dark repair” (Häder and Sinha, 2005) alone, e.g., by nucleotide excision repair, results in very low survival. A significant difference between NIH albino and Salvador strain survival at a dose of $1500 \text{ J}\cdot\text{m}^{-2}$ resulted only when snails were placed under photoreactivating light post-irradiation. These results suggest that the basal levels of DNA damage at the higher doses of UVB are probably similar in both strains, but that the Salvador strain may possess innately greater capacity for photoreactivation.

4.3. Protection by shell

As hypothesized by Hill et al. (1997) and demonstrated by Karentz (1994), the shell can provide substantial protection against UVB damage by blocking exposure of vital tissues and organs. We also observe significantly higher survival in both strains when the headfoot is sealed inside the shell. This result also implies that UVB damage to tissues and organs of the normally exposed headfoot (i.e., surface epithelium, musculature, hemolymph, mucus glands, central nervous system, and the anterior structures of the digestive tract) (see Pan, 1958) accounts for a significant proportion of the observed mortality. On the other hand, the mortality observed in snails that had been sealed within their shells indicates both that UVB can penetrate through the shell, and that damage to internal tissues and organs of the mantle and visceral mass, which are always enclosed within the shell, may also be important.

These conclusions are supported by results of experiments in which shells were covered with UVB-blocking black polish. Specifically, increased protection of internal organs with black

polish resulted in increased survival relative to that in unprotected, irradiated snails, all of which were killed. Taken together, these results suggest that mortality in lethally-irradiated snails results from additive damage both to the headfoot and internal organs. In addition, survival was quite similar in NIH albino and Salvador strains when shells were blocked with nail polish, whereas survival was significantly lower in NIH albino snails when snails were simply sealed inside their shells during irradiation. Therefore, we conclude that the headfoot tissues of both strains show comparable susceptibility to UVB, but that the mantle and visceral mass of NIH albino snails are more vulnerable. It should be noted that the shells of juvenile *B. glabrata*, like the snails used in this study, are quite transparent to visible light, whereas the thicker shells of large adults are more opaque. Therefore, shells of adult snails may offer significantly greater protection against UVB.

4.4. Effects on Feeding

Paper consumption per surviving snail decreased in proportion to UVB exposure. Such a sub-lethal effect could be of some importance in nature, by affecting the ability of UVB-exposed snails to repair tissue or shell damage, grow, reproduce, and perhaps resist parasitic infection, all processes that presumably are dependent to some degree on nutrition.

4.5. Morphological Abnormalities

The forked tentacles observed in *B. glabrata* in our study differed somewhat from those reported by Wong and Wagner (1956) in *Oncomelania* spp. Specifically, these authors described 2–6 branches in each tentacle at 14 wk post-exposure. However, we observed a single branch occurring on the right tentacle usually within 2 wk post-exposure, although in more recent studies we have seen occasional bilateral branching (unpublished). Wong and Wagner (1956) showed an increase in the degree of branching and number of snails with branched tentacles over the time course of the experiment, which involved daily irradiation of snails for approximately 1 year. However, snails in our study were exposed only once, and no new branches formed between 3 and 6 wk post-irradiation.

The mechanism by which UVB induces soft-tissue abnormalities in the tentacles and headfoot is unknown. Richards (1974) reported that forked tentacles spontaneously occurring in laboratory *B. glabrata* are due to a recessive mutation in the *Ta* allele. Possibly, UVB disrupts expression of *Ta* in cells of the tentacle, resulting in the formation of a fork. However, in view of the well-known ability of snails to regenerate tentacles, an alternative explanation is that the tissue damage caused by UVB induces inappropriate regeneration of a new tentacle at the site of damage. Histological evidence of damage to the epithelium of tentacles of UVB-irradiated snails lends support to this hypothesis (Figs. 6E, F).

In summary, UVB is deleterious to both NIH albino and Salvador juvenile *B. glabrata*, causing decreased survival at higher doses and sub-lethal effects at lower doses. Albino snails are significantly more susceptible to lethal effects. Among sub-lethal effects are decreased food consumption, abnormal tissue growths, and loss of tentacle epithelium. The shell offers partial protection against UVB, and injury to tissues of both the headfoot and mantle/visceral mass contribute to mortality in lethally-irradiated snails. Moreover, based on our results, we conclude that photoreactivation occurs in *B. glabrata*, and may be more efficient in wild-type snails. Photoreactivation would mitigate some of the DNA damage caused by UVB in nature.

With respect to the relevance of our experimental system to natural conditions in areas endemic for *S. mansoni*, it should be noted that the wavelength spectrum of these UV lamps does not match that of sunlight [see Fig. 1 in Karentz et al. (1991) for the lamp emission spectrum]. Therefore, since biological responses are wavelength-dependent, experimental doses cannot be directly correlated with ambient sunlight exposure. The purpose of this investigation was

not to simulate natural conditions, but rather to quantify and compare responses of albino and wild-type juvenile *B. glabrata* to controlled UVB exposure, as an initial study of the UV-photobiology of this snail.

B. glabrata is typically found in shallow water tropical habitats (Kloos et al. 2001) and therefore is exposed to the consistently high levels of UVB that occur at lower latitudes (Johnson et al., 1976; Kerr et al., 2003; Madronich et al., 1998). Our results suggest that UVB stress could potentially influence the prevalence of schistosome infection by affecting the survival and health of the snail intermediate host.

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Fig. 1. Mean $\pm$ SE survival (log scale) of NIH albino (open circles) and Salvador (closed circles) *Biomphalaria glabrata* as a function of UVB dose. N = 10–12 groups of 5 snails of each strain.

Fig. 2. Effect of photoreactivation in snails exposed to 1500 J·m⁻² UVB, and maintained in the dark (1500 dark; data from Fig. 1), or in light (1500 light). Bars indicate mean $\pm$ SE survival of UVB-exposed NIH albino (unshaded bars) and Salvador (shaded bars) *Biomphalaria glabrata*. N = 10–12 groups of 5 snails of each strain.

Fig. 3.

UV-protection by shell. Bars indicate mean $\pm$ SE survival of UVB-exposed ($2000 \text{ J}\cdot\text{m}^{-2}$) NIH albino (unshaded bars) and Salvador (shaded bars) *Biomphalaria glabrata*. Sealed, shell aperture plugged with rubber cement. Blocked, shells painted with black nail polish. CON = $0 \text{ J}\cdot\text{m}^{-2}$. N = 10–12 groups of 5 snails of each strain.

Fig. 4.

Photographs of index card labels showing paper consumption by snails, as indicated by loss of pencil markings from the surface. NON-SNAIL, label incubated in water in the absence of snails; CON, label incubated with non-irradiated snails; 250, 500, 750, 1000, labels incubated with snails irradiated at indicated doses ($\text{J}\cdot\text{m}^{-2}$). Note decreased consumption at increasing doses.

Fig. 5.

Mean $\pm$ SE of consumption of index card labels by snails as a function of UVB dose. Sealed, shell aperture plugged with rubber cement. CON = 0 J·m⁻². N = 10–12 groups of 10 snails.

Fig. 6.

Tissue abnormalities in Salvador *Biomphalaria glabrata* irradiated with UVB. A. Branched tentacle at 35 days post-irradiation with 500 J·m⁻². Shell aperture has been cut back for photography. B. Transverse ridge (R) on the dorsal surface of the headfoot at 50 days post-irradiation with 500 J·m⁻² UVB. P, posterior tip of headfoot. Shell aperture has been cut back for photography. Scale bars for A and B are approximate. C. Longitudinal section of a branched tentacle from a snail irradiated with 500 J·m⁻² UVB. Note the central core (arrows) of connective tissue and flaccid condition of the branch, as shown by its smooth surface in comparison to the ruffled surface of the primary tentacle. D. Cross-section of a tentacle branch from a snail irradiated with 500 J·m⁻² UVB. Note the central artery (A), surrounding

hemolymph sinus (S), and ciliated epithelium (arrow). E. Longitudinal section near the curved tip of a tentacle at 24 hr post-irradiation with $1000 \text{ J}\cdot\text{m}^{-2}$ UVB. Note exfoliating epithelium (arrowhead), and completely denuded area, with exposed basement membrane (arrow) along one side, presumably the side facing the UV lamps. F. Cross section of a tentacle at 24 hr post-irradiation with $1000 \text{ J}\cdot\text{m}^{-2}$ UVB. Note exfoliating epithelium (arrowhead) along one side, presumably the side facing the UV lamps.