Journal of the American Mosquito Control Association, 15(4):437–445, 1999

SITE OF PHOTOFRIN II® PHOTOSENSITIZATION IN LARVAE OF *ERETMAPODITES QUINQUEVITTATUS* THEOBALD

A. M. HELLECK AND W. K. HARTBERG

Baylor University, Department of Biology, PO Box 97388, Waco, TX 76798-7388

ABSTRACT. Photofrin II® (PII) accumulates and fluoresces in the alimentary canal, anal papillae, malpighian tubules, rectum, and gastric caeca at greater concentrations (>10 μg/ml) and accumulates (fluoresces) in (along) the alimentary canal only at lower concentrations (≤10 μg/ml). Initial experiments with larvae exposed to different osmotic conditions and PII indicated that cells of osmoregulation were most susceptible to life-threatening cell damage. Larvae that lacked anal papillae and were exposed to different osmotic conditions and PII indicated that cells of osmoregulation may not be the most deleterious site for cell damage. Larvae ligated around the neck and exposed to PII survived significantly longer than larvae ligated after the 4th abdominal segment and after the 6th abdominal segment while also being exposed to PII. Pupae are unaffected by PII at any concentration and they do not ingest during their development. These experiments indicate the midgut gastric epithelia could be the most deleterious site for cell damage caused by PII.

KEY WORDS Photosensitization, photoactive, photochemical, photosensitizer, photoreactive, osmoregulation, anal papilla

INTRODUCTION

This study examined the effects of Photofrin® II or Photofrin® porfimer sodium (PII) (a photosensitizer) on the mosquito *Eretmapodites quinquevittatus* Theobald. This species is native to subsaharan Africa and Madagascar (Gillett 1972) and may transmit arboviruses (Macnamara 1953, McIntosh et al. 1961, Gilotra and Shah 1967, Serie et al. 1968, Brottes et al. 1969). *Eretmapodites quinquevittatus* was chosen as the test organism because it is easily maintained in the laboratory and has been studied extensively by this investigator (Helleck et al. 1993, 1994, 1996, 1997) and others (Hartberg and Gerberg 1971; Hartberg and Johnston 1977, 1979; Johnston and Hartberg 1981; Hartberg and Faircloth 1983).

Resistance to and lack of effectiveness of insecticides on mosquitoes has been documented and is life-threatening, because mosquitoes are known vectors of disease (Georghiou and Lagunes-Tejeda 1991, Raymond et al. 1993, Reiss and James 1993, Pasteur and Raymond 1996). Although the problem of mosquito resistance to conventional insecticides has been avoided by varying the concentration and types of chemicals used, an effectiveness problem still exists. Photoactive compounds could provide effective and environmentally safe photoinsecticides for future use in insect control.

This research was conducted because of the increase in mosquito resistance to conventional insecticides, and to determine which tissues of mosquitoes are most affected by the destructive pathway(s) of PII. We have begun elucidation of the cell types most susceptible to photosensitization by PII in *Er. quinquevittatus*. Ascertaining which cell types are susceptible to photosensitization will be advantageous, because this information will assist researchers in determining which cell types readily absorb photosensitizer (PII). Knowledge of which cell types readily absorb photosensitizer could lead to understanding why certain cell types absorb photosensitizer. Answers to these questions could lead to methods for cell targeting of photosensitizers in cancer treatment. The same knowledge would be useful in selecting photosensitizers for research and use in mosquito control.

MATERIALS AND METHODS

Laboratory colonies of *Er. quinquevittatus* (EQ-MIXED) were maintained at the Medical Entomology Research Laboratory at Baylor University in a walk-in environmental chamber set at 25°C, 80 ± 5% relative humidity, and a 14:10 h light : dark photoperiod. The EQ-MIXED adult colony was provided sugar cubes for a carbohydrate source, and females were provided anesthetized mice every 2–3 days for blood meals. This colony was established by W. K. Hartberg from larvae collected from a tree hole in the Kisutu section of Dar es Salaam, Tanzania, in July 1969 (Hartberg and Gerberg 1971).

Eggs were collected from the EQ-MIXED colony by providing ovipositing females with moist, bleached, paper towels (oviposition or egg papers) lining the inside of 250-ml beakers or half-pint (279.3 ml) Ball™ jars placed inside of the colony cage. Collected eggs were a mixture of autogenous and anautogenous eggs, were not stored, and hatched ≈48 h after oviposition.

First-stage larvae were transferred to Nalgene® pans (VWR Scientific Products, Suwanee, GA, USA.) (32 × 26 × 6 or 26 × 16 × 6 cm) and reared to the 4th instar, or pupal stage. During development larvae were provided ≈0.25 ml of larva

food (Wardley's® fish food and water, 2:1 ratio) placed at the bottom of the Nalgene pans.

All 4th-stage larvae and pupae were photoirradiated with 8 15-W Philips® fluorescent light bulbs in parallel array (1.12 mW/cm², 350–800 nm), placed ≈25 cm above the larvae and pupae.

Toxicity of photoirradiated PII on larvae while in different osmotic conditions: Eggs were collected, hatched, and reared to early 4th instar (≈6.0 days of larval development) without PII exposure. Fifty larvae each were exposed to PII (3.75–30 μg/ml) (100 ml final volume) and larva food while in 3 different osmotic solutions: hypotonic (=distilled water), hypertonic (=1.1× *Aedes aegypti* saline solution), and isotonic (Hayes 1953) (=*Ae. aegypti* saline solution). *Aedes aegypti* saline at 1.1× the normal concentration of saline was used because it was the most hypertonic solution larvae could withstand. Larvae could not survive in 1.15× *Ae. aegypti* saline. Fifty larvae each were also exposed to larva food, a hypertonic solution containing no PII (control 1), and tap water (tH_2O) containing no PII (control 2). All 3 experimental groups and the 2 control groups were immediately placed in darkness for 16 h. After the dark period, all larvae were rinsed 7 times with deionized water (diH_2O) and placed back into containers with the same osmotic solution, and photoirradiated until larvae were moribund or until pupation. Rinsing was performed to remove any PII from the exterior of the larva, so that any effects encountered were only from internalized PII. Larva food was provided if larvae were not moribund after 24 h even though larvae may have ceased feeding by this time.

Toxicity of photoirradiated PII on larvae without anal papillae while in different osmotic conditions: Eggs were collected, hatched, and reared to early 4th instar without PII exposure. Larvae were exposed to 5% NaCl solution for 2–3 min and then placed in tH_2O for 2 days or until anal papillae disappeared (Wigglesworth 1933). After ≈2 days larvae without anal papillae were placed into 1 of 3 experimental and 2 control groups as described previously.

Toxicity of photoirradiated PII on larvae without anal papillae and with neck ligation while in different osmotic conditions: Eggs were collected, hatched, and reared to early 4th instar without PII exposure. Larvae were exposed to 5% NaCl solution for 2–3 min and then placed in tH_2O for 2 days or until anal papillae disappeared (Wigglesworth 1933). Those larvae without anal papillae were ligated around the neck and placed in 3 experimental and 2 control groups as described previously. All ligations were performed with a fine silk thread 1–2 min after larvae had been placed on a cold microscope slide.

Toxicity of photoirradiated PII on larvae with anal papillae and neck, 4th, and 6th abdominal segment ligation: Eggs were collected, hatched, and reared to early 4th instar without PII exposure. Fifty larvae with anal papillae and with neck ligation were exposed to PII (7.5 μg/ml) (20 ml final volume) and larva food while in isotonic solution. Fifty more larvae with anal papillae and with ligation between the 4th and 5th abdominal segments were exposed to PII (7.5 μg/ml) (20 ml final volume) and larva food while in isotonic solution. Fifty more larvae with anal papillae and with ligation between the 6th and 7th abdominal segments were also exposed to PII (7.5 μg/ml) (20 ml final volume) and larva food while in isotonic solution. Fifty additional larvae were exposed to larva food and isotonic solution only (control). All 3 experimental groups and the single control group were immediately placed in darkness for 16 h. After the dark period, all larvae were rinsed 7 times with diH_2O and placed back into containers of isotonic solution and then photoirradiated until larvae were moribund or until pupation. More food was provided if larvae were not moribund after 24 h even though larvae may have ceased feeding by this time.

Toxicity of photoirradiated PII on pupae: Eggs were collected and hatched, and larvae were reared to the pupal stage (≈9–10 days of larval development) without PII exposure. Fifty pupae each were exposed to 10, 30, 50, and 90 μg/ml of PII and isotonic solution. Fifty more pupae were exposed to isotonic solution without PII (control). All 4 experimental groups and the single control group were immediately placed in darkness for 16 h. After the dark period, all pupae were rinsed 7 times with diH_2O and placed back into containers of isotonic solution and then photoirradiated until pupae became moribund or developed into adults. No larva food was provided because pupae do not feed.

Statistical analysis: Times from onset of photoirradiation until larvae or pupae became moribund were determined for each larva or pupa. Survival analysis began by constructing life tables for each group of larvae and pupae. The log-rank (chi-square) test was used to detect any differences in survival between experimental or control groups of larvae and pupae (Anderson et al. 1980).

RESULTS

Toxicity of photoirradiated PII on larvae while in different osmotic conditions

Experimental larvae (in different osmotic conditions) exposed to 30 or 15 μg/ml of PII showed no significant differences in survivorship when the different groups were compared (Figs. 1 and 2) ($P > 0.05$). Control larvae exhibited significantly greater survivorship from all 3 salinities than all 3 groups of experimental larvae ($P < 0.0001$).

Experimental larvae exposed to lower concentrations of PII (7.5 and 3.75 μg/ml) exhibited significant differences in survivorship when the different groups were compared (Figs. 3 and 4). Larvae exposed to hypotonic solution and PII lived signifi-

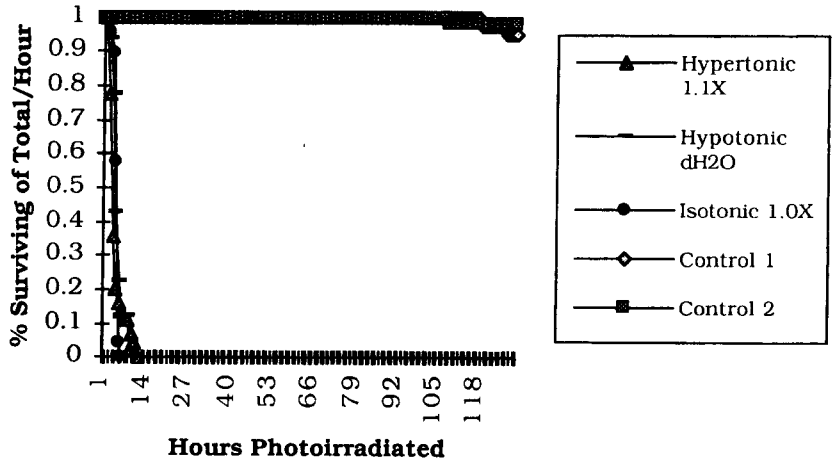

Fig. 1. Percent (%) survivorship of *Eretmapodites quinquevittatus* larvae exposed to 30 μg/ml of Photofrin II (PII) followed by photoirradiation in different osmotic solutions. Hypertonic 1.1× = 1.1 times *Aedes aegypti* saline and PII. Isotonic 1.0× = *Ae. aegypti* saline and PII. Hypotonic dH_2O = distilled H_2O (dH_2O) and PII. Control 1 = dH_2O, no PII. Control 2 = 1.1 times *Ae. aegypti* saline, no PII. n = 50.

cantly longer than larvae exposed to isotonic or hypertonic solution and PII (7.5 μg/ml of PII, $P < 0.0001$) (Fig. 3). Larvae exposed to isotonic solution and PII survived significantly longer than larvae exposed to hypertonic solution and PII (3.75 μg/ml of PII, $P < 0.01$). Larvae exposed to hypotonic solution and PII also survived significantly longer than larvae exposed to isotonic or hypertonic solution and PII (3.75 μg/ml of PII, $P < 0.001$) (Fig. 4).

Toxicity of photoirradiated PII on larvae without anal papillae while in different osmotic conditions

Experimental larvae, with no anal papillae, exposed to 7.5 μg/ml of PII showed significant differences in survivorship when the different groups were compared (Fig. 5). Larvae exposed to hypertonic solution and PII survived significantly longer than larvae exposed to hypotonic solution and PII ($P < 0.001$) (Fig. 5). Larvae exposed to isotonic solution and PII survived significantly longer than larvae exposed to hypertonic or hypotonic solution and PII ($P < 0.001$).

Toxicity of photoirradiated PII on larvae without anal papillae and with neck ligation

Experimental larvae cultured in different osmotic solutions without anal papillae and with neck ligation did not differ significantly in survival rate ($P > 0.05$). All experimental larvae (n = 50) and control larvae (n = 50) were dead within 10 h of exposure to PII in the dark.

Toxicity of photoirradiated PII on larvae with anal papillae and neck, 4th, and 6th segment ligation

Experimental larvae with neck ligation survived significantly longer than larvae with 4th ($P < 0.05$) or 6th ($P < 0.05$) segment ligations (Fig. 6).

Toxicity of photoirradiated PII on pupae

All experimental and control pupae survived and developed into adults. No differences were found in survivorship when comparing different concentrations of PII or when comparing experimentals and controls.

DISCUSSION

Microscopic observation of 4th instars of *Er. quinquevittatus* showed accumulation of fluorescent PII in the gastric caeca, alimentary canal (hindgut), rectum, malpighian tubules, and anal papillae at high concentrations (>10 μg/ml). All of these organs are involved in osmoregulation of developing larvae.

Fluorescence was observed in the alimentary canal when larvae were exposed to high or low concentrations of PII (5–400 μg/ml). The PII may adversely affect the absorptive or secretory function of cells of the midgut. If this is true, exposure of different sections of alimentary epithelia to PII might result in differences in larval survival.

The PII may disrupt tissues or cells involved in ionic and osmotic regulation, thus rendering the larvae unable to adapt to changes in water chemistry. If this is true, then exposure of larvae to PII under different osmotic conditions could result in differ-

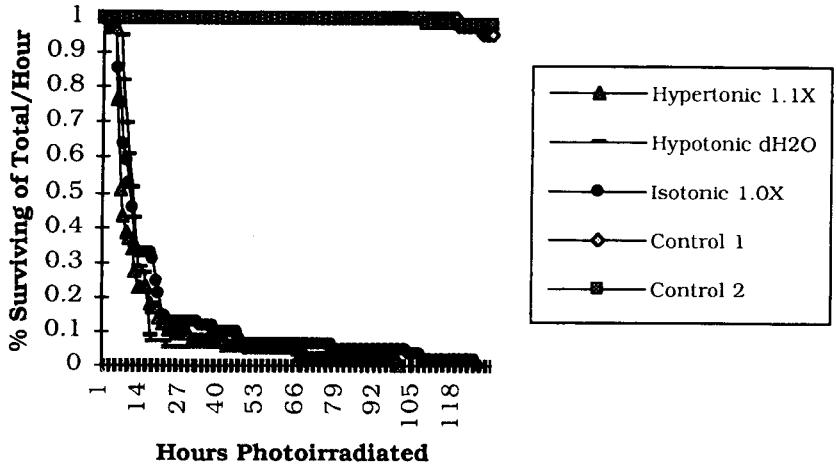

Fig. 2. Percent (%) survivorship of *Eretmapodites quinquevittatus* larvae exposed to 15 μg/ml of Photofrin II (PII) followed by photoirradiation in different osmotic solutions. Hypertonic 1.1× = 1.1 times *Aedes aegypti* saline and PII. Isotonic 1.0× = *Ae. aegypti* saline and PII. Hypotonic dH_2O = distilled H_2O (dH_2O) and PII. Control 1 = dH_2O, no PII. Control 2 = 1.1 times *Ae. aegypti* saline, no PII. n = 50.

ences in larval survival. Occurrences of differences in survival would indicate that the target area for PII action (photosensitization) is the cells of osmoregulation.

Photosensitizers are compounds requiring direct interaction with light and molecular oxygen to produce highly reactive oxygen species (primarily singlet oxygen). These compounds are toxic to all living things because singlet oxygen reacts with many types of biological molecules (membrane lipids, cytochromes, nucleic acids) (Kessel 1981, 1986). Natural and synthetic photosensitizers are equally destructive (Arnason et al. 1981, Sakurai and Heitz 1982, Kagan et al. 1987).

Our approach to photosensitizing cells involves providing a natural or synthetic photosensitizer (PII) to living organisms (via ingestion) in sufficient quantities so that when exposed to light, and molecular oxygen phototoxic products are produced and the organism is injured or killed. The widespread toxicity of these compounds toward all types of cells makes this approach a difficult choice in terms of pesticide or insecticide use and tumor therapy. Rebeiz and colleagues (1995) have found some interesting ways of using an organism's natural accumulation of photosensitizer by blocking molecules that quench photosensitizers. These quenching molecules are produced by all organisms that produce and use porphyric (heme) compounds (Rebeiz et al. 1995). Only organisms that have un-

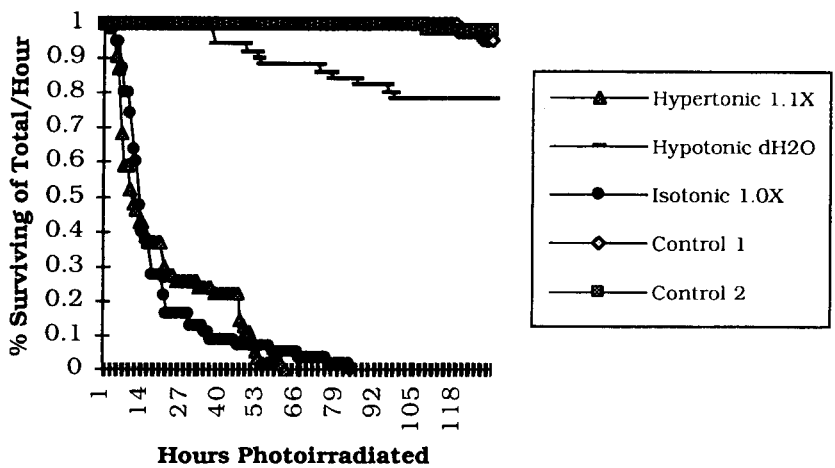

Fig. 3. Percent (%) survivorship of *Eretmapodites quinquevittatus* larvae exposed to 7.5 μg/ml of Photofrin II (PII) followed by photoirradiation in different osmotic solutions. Hypertonic 1.1× = 1.1 times *Aedes aegypti* saline and PII. Isotonic 1.0× = *Ae. aegypti* saline and PII. Hypotonic dH_2O = distilled H_2O (dH_2O) and PII. Control 1 = dH_2O, no PII. Control 2 = 1.1 times *Ae. aegypti* saline, no PII. n = 50.

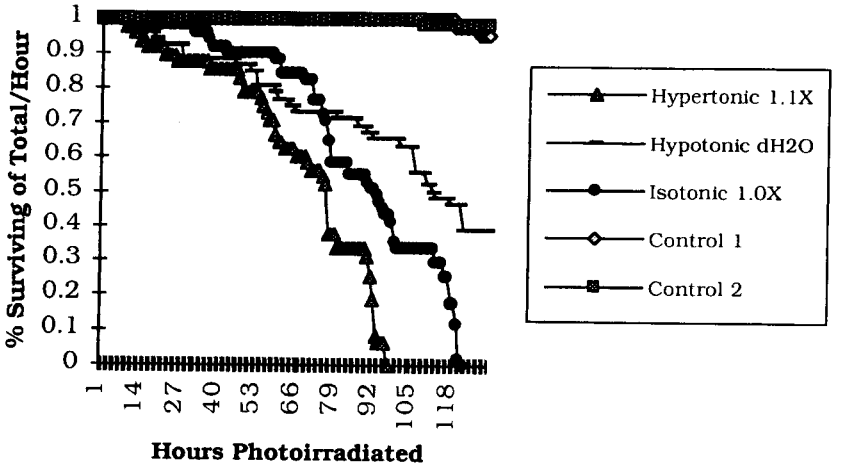

Fig. 4. Percent (%) survivorship of *Eretmapodites quinquevittatus* larvae exposed to 3.75 μg/ml of Photofrin II (PII) followed by photoirradiation in different osmotic solutions. Hypertonic 1.1× = 1.1 times *Aedes aegypti* saline and PII. Isotonic 1.0× = *Ae. aegypti* saline and PII. Hypotonic dH_2O = distilled H_2O (dH_2O) and PII. Control 1 = dH_2O, no PII. Control 2 = 1.1 times *Ae. aegypti* saline, no PII. n = 50.

checked accumulation of photosensitizer will be killed, because no exogenous distribution of photosensitizer occurs that could harm other organisms in close proximity.

Porphyrins or hematoporphyrin derivatives possess alternating double bonds around 4 pyrrole rings resulting in a conjugated 18 π electron system that provides high stability. The active component of PII seems to be a 2- to 3-unit oligomer that absorbs light in the 400- to 630-nm range of the spectrum (Dougherty 1987, Byrne et al. 1990). This oligomer, when activated by light in the presence of molecular oxygen, produces photoproducts detrimental to cells. The photosensitizer and its photoproducts are planar and lipophobic.

Photodynamic effects of porphyrins are due to their photoexcitable nature. The ground-state porphyrin is photoexcited to the singlet state. Next, intersystem crossing of an electron from the excited singlet state to the excited triplet state occurs. The excited triplet state has lower energy than the singlet state, but has a longer half-life. Now the excited triplet sensitizer may undergo 1 of 2 reactions, type I or II (Foote 1990). Indirect evidence indicates the primary pathway for cellular destruction is type II (Rebeiz et al. 1995). Type II reactions occur when triplet sensitizer interacts with ground-state oxygen to produce excited singlet oxygen (Foote 1990). Singlet oxygen is extremely reactive and will react with electron-rich substrates yielding

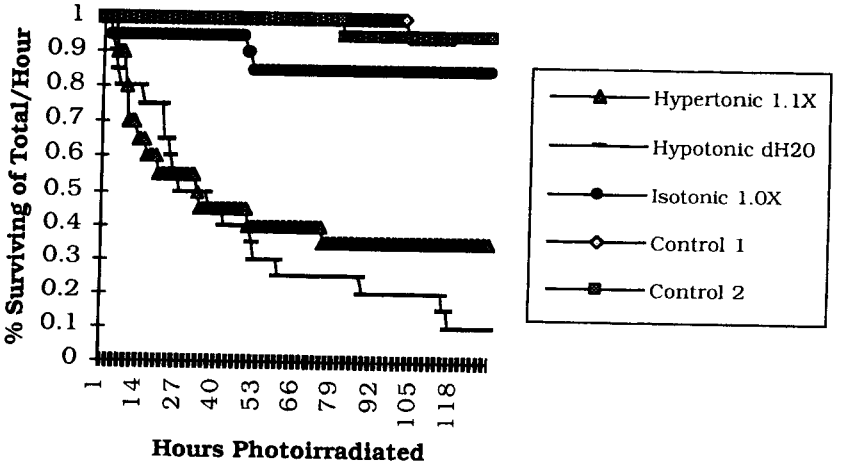

Fig. 5. Percent (%) survivorship of *Eretmapodites quinquevittatus* larvae without anal papillae exposed to 7.5 μg/ml of Photofrin II (PII) in different osmotic solutions followed by photoirradiation. Hypertonic 1.1× = 1.1 times *Aedes aegypti* saline and PII. Isotonic 1.0× = *Ae. aegypti* saline and PII. Hypotonic dH_2O = distilled H_2O (dH_2O) and PII. Control 1 = dH_2O, no PII. Control 2 = 1.1 times *Ae. aegypti* saline, no PII. n = 50.

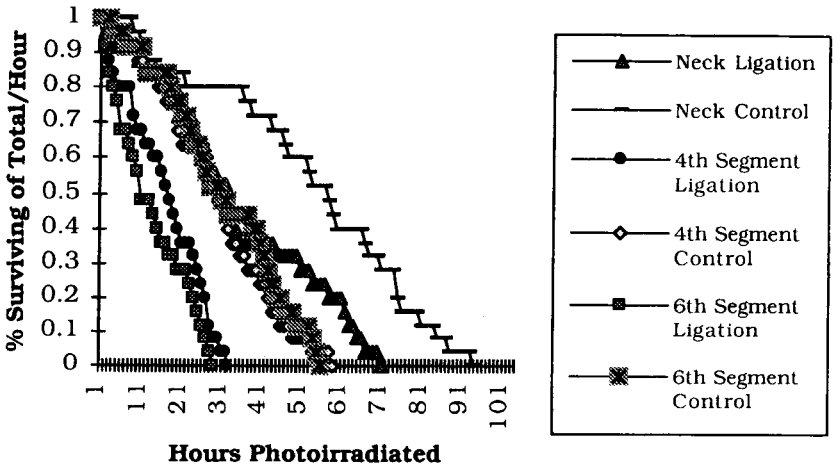

Fig. 6. Percent (%) survivorship of *Eretmapodites quinquevittatus* larvae with anal papillae and either neck, 4th-segment, or 6th-segment ligature exposed to 7.5 μg/ml of Photofrin II. $n = 50$.

peroxides and other oxidized species (Spikes 1985, Foote 1990). The effect of these oxidized species is disruptiom of metabolism, nucleic acids, and membranes (Foote 1990).

Barbieri (1928) demonstrated that aquatic larvae of *Anopheles maculipennis* (Meigen) were killed when exposed to halogenated fluorescein derivatives, erythrosin, and eosin, and then illuminated. Hartberg and Judy (1990) showed that *Er. quinquevittatus* larvae were killed by PII and that onset of effects was directly proportional to concentration of PII and length of exposure to PII. Helleck and Hartberg (unpublished) witnessed increased difficulty in larval and pupal extrication when larvae were fed PII, but were unsure whether this was due to improper chitin formation or reduced energy levels. For an excellent review of the history of insect photosensitization see Heitz and Downum (1987).

Toxicity of photoirradiated PII on larvae while in different osmotic conditions

In preliminary experiments, larvae were observed to be swollen or larger than normal at the time of moribundity. Microscopic observations showed PII fluorescence localized in the osmoregulation organs of the larva and the alimentary canal. The anal papillae, rectum, malpighian tubules, gastric caeca, and alimentary canal (gut) all fluoresced more intensely than surrounding tissue. The reason for increased fluorescence in the rectum and malpighian tubules is easily explained. These organs function to rid the larva of excess water (Clements 1992) and PII is entrained with water, so it may leave the larva with water just as easily. The intensity of fluorescence in the anal papillae, gastric caeca, and gut could be occurring because these areas are known to be active in ion regulation and conservation (Clements 1992). Some PII may be kept in or expelled because of its association with ions being regulated. The PII may simply get caught in the bulk flow of ions moving in or out of the larva, much as it could get caught in the bulk flow of water moving in or out of the larva. Areas such as the gut, caeca, and rectum will tend to conserve ions and larger molecules (Wigglesworth 1933, Ramsay 1950). This may affect the length of time and the amount of PII that remains in cells or tissues.

If all cells of a larva are acted on by PII at approximately the same rate, and if PII is toxic to larvae, then cells involved in water or ion regulation or absorption or secretion would be important to the survival of an aquatic larva. These are the 1st cells contacted by materials (PII) entering with water or food.

Two pieces of evidence support this conclusion. Fluorescence is observed in the anal papillae and the gut of the mosquito larva. Any disruption of the processes performed by osmoregulators would be life threatening to the larva. Any disturbance of absorptive and secretory processes performed by gastric epithelia would be equally life threatening. If the osmoregulators or gastric epithelia were damaged the larva would die.

Focus turned 1st to conditions that affect the osmoregulators. If these are most important to the larva, then differences in survivorship would be observed among experimental groups differing in solute concentration (hypotonic, hypertonic, isotonic). Larvae cultured in tH_2O (slightly hypotonic) will take up water and lose ions. The larva will compensate by excreting a dilute urine through the rectum and anal papillae and conserving ions with its rectum, gut, and gastric caeca. Most ions would be expected to be conserved by the larvae, yet PII is unlike any other ion. As stated before, PII is lipophobic and planar and has been shown by Kessel (1981, 1986) to be capable of oxidizing cell membranes. If PII has only a limited ability to damage

cell membranes this could provide the traversibility necessary for PII movement across the epithelia. If PII is able to do substantial damage to cell membranes then movement of PII and anything else could occur along the epithelia. We believe that this is the type of damage that is occurring and directly results in the death of the larvae. But larvae in hypotonic media do not die as quickly as those larvae in other osmotic conditions. We believe that the osmotic direction of ion movement precludes any damage by PII. Because PII is lipophobic and planar it could move easily through cells and into the excretion produced by the larvae. The excretion, containing PII, exits the larvae and little if any damage results due to PII. Larvae cultured in hypertonic solution (1.1× *Ae. aegypti* saline) will lose water and gain ions, so the osmoregulators must compensate by conserving water. The PII would be expected to remain in larvae and cause more damage to cells and tissues. The PII would not be entrained to move out of the larvae and would proceed with oxidation of various biological molecules including cell membranes. This would eventually lead to death of the larvae. Larvae cultured in isotonic solution (*Ae. aegypti* saline) will be in equilibrium with the outside solution. No net movement of water or ions in either direction would occur. The PII would be expected to remain inside larvae and cause more damage to cells and tissues. Larvae cultured in hypotonic solution (dH_2O) will gain large amounts of water and lose large amounts of ions, so osmoregulators must compensate by excreting more water and conserving ions. The PII would be expected to exit larvae in hypotonic solution and cause less damage to cells and tissues (Figs. 1–5).

Larvae cultured in different osmotic solutions (not including controls) with PII (15–30 μg/ml) showed no difference in survival. This could be because PII concentrations were high enough to prevent hypotonic larvae from pumping out enough PII before cell destruction occurred.

Larvae cultured in hypotonic solution and exposed to PII (7.5 or 3.75 μg/ml) had a significantly higher survival rate than larvae exposed to isotonic or hypertonic solution and did not differ significantly when compared to control 1 or 2 (Figs. 3 and 4). This is probably due to osmoregulation of larvae in hypotonic solution. The PII is retained for a shorter period of time in larvae exposed to hypotonic solution and PII than larvae exposed to other osmotic solutions. Osmoregulation in isotonic and hypertonic solutions retained PII for a longer period of time. Retention of PII for longer periods means more internal damage and more mortality as opposed to retention of PII for shorter periods.

Toxicity of photoirradiated PII on larvae without anal papillae while in different osmotic conditions

When viewing Fig. 5, larvae exposed to hypotonic solution and PII while simultaneously lacking anal papillae obviously became moribund or died at an increased rate when compared to the other 2 experimental groups. This is counter to what we observed in the same experiments in the same osmotic conditions when anal papillae were present (Figs. 1–4). Concluding that our earlier hypothesis concerning movement of ions, PII, and water by osmoregulation has gained more support is reasonable. Without the use of anal papillae, larvae in hypotonic solution might not be able to void water or any associated solutes (PII) as they did when possessing anal papillae.

Also obvious is that the anal papillae can be eliminated as the damaged tissue most deleterious to larval survival. Larvae can live without anal papillae, although more time is required for development. Although the anal papillae may still be damaged by PII they must not be the area critical to larval survival. Another critical piece of evidence is that pupae do not feed and they are unaffected by exposure to PII at any concentration. Pupae must also regulate ions and water the same as larvae. The rectum and/or the alimentary canal must be the areas where the most critical damage occurs.

Toxicity of photoirradiated PII on larvae without anal papillae and with neck ligatures

All larvae regardless of osmotic condition or presence or absence of PII were dead or moribund within 10 h of exposure to PII in darkness. All died at the same rate. The explanation for this is simple. These larvae had been rendered incapable of sequestering ions or water. They had no anal papillae and had been ligated about the neck so as to inhibit the flow of materials through the mouth and into the alimentary canal. Most organisms will die quickly when placed under these conditions and that is exactly what happened in this case. Interestingly, PII did not seem to be a factor. Although the larvae died at a much more accelerated rate than previously observed, not the slightest difference was found in survivorship when comparing experimentals to controls. This indicates that PII must have to make contact with alimentary epithelia to cause the most critical damage to larvae.

Toxicity of photoirradiated PII on larvae with anal papillae and neck, 4th, and 6th segment ligation

Larvae can survive without either the anal papillae or the alimentary canal, but not without both (Stobbart 1960). Larvae might be able to survive without the use of their rectum as long as the anal papillae are still intact. The anal papillae have been shown to aid in uptake of water. It stands to reason that larvae ligated at the neck only would survive longer than larvae ligated farther down the length of the abdomen The larvae are being deprived of

macromolecule (proteins, fats, carbohydrates, and so on) uptake and some ion uptake. The lack of nutrients will eventually kill the larva, but the anal papillae can compensate for the lack of ion uptake by the gut. Fourth-segment ligation of the larvae leaves most of the midgut exposed to contact by any PII entering through the mouth. The PII cannot contact the cells of the posterior midgut or the hindgut in this situation. This action of PII on the gastric epithelia may be the true cause of death in mosquito larvae. Larvae ligated after the 6th segment were expected to die at a rate similar to that of larvae ligated after the 4th segment.

Toxicity of photoirradiated PII on pupae

Pupae of *Er. quinquevittatus* are not affected by PII. This is probably because pupae do not ingest anything during this stage of development, yet they must continue to osmoregulate. This lends more support to our theory that PII's most active site is along the midgut. If interaction of active PII with gastric epithelia is necessary for cell damage that leads to death then noningesting organisms should not be affected by PII.

REFERENCES CITED

Anderson, S., A. Auquier, W. W. Hauck, D. Oakes, W. Vandaele and H. I. Weisberg. 1980. Statistical methods for comparative studies: techniques for bias reduction. John Wiley & Sons, New York.

Arnason, T., T. Swain, C.-K. Wat, E. A. Graham, S. Partington and G. H. N. Towers. 1981. Mosquito larvicidal activity of polyacetylenes from species in the Asteraceae. Biochem. Syst. Ecol. 9(1):63–68.

Barbieri, A. 1928. Sensibilizadaroes fluorescentes como larvicidas. Accion fortodynamica de la luz. Riv. Malariol. 7:456.

Brottes, H. A., P. Rickenbach, M. C. Bres, J. Williams, J. Salaun and L. Ferrara. 1969. A new arbovirus prototype Okola virus YM-50–64 isolated in the Cameroons from mosquitoes human new record—*Eretmapodites chrysogaster*. Ann. Inst. Pasteur. 116:543–551.

Byrne, C. J., L. V. Marshallsay and A. D. Ward. 1990. The composition of Photofrin II. Photochem. Photobiol. 6:13–27.

Clements, A. N. 1992. The Biology of Mosquitoes. Chapman and Hall, London.

Dougherty, T. J. 1987. Studies on the structure of porphyrins contained in Photofrin II. Photochem. Photobiol. 46:569–574.

Foote, C. S. 1990. Chemical mechanisms of photodynamic action. Proc. SPIE Institute. *In:* Advanced optical technologies on photodynamic therapy IS. 6:115–126.

Georghiou, G. P. and A. Lagunes-Tejeda. 1991. The occurrence of resistance to pesticides in arthropods. An index of cases reported through 1989. FAO, Rome, Italy.

Gillett, J. D. 1972. Common African mosquitoes. John Swain and Co., London.

Gilotra, S. K. and K. V. Shah. 1967. Laboratory studies on transmission of Chikungunya by mosquitoes. Am. J. Epidemiol. 86:379–385.

Hartberg, W. K. and W. H. Faircloth. 1983. Mitotic chromosomes of the mosquito *Eretmapodites quinquevittatus* Theobald. Mosq. Syst. 15:325–329.

Hartberg, W. K. and E. J. Gerberg. 1971. Laboratory colonization of *Aedes simpsoni* and *Eretmapodites quinquevittatus*. Bull. WHO 45:850–852.

Hartberg, W. K. and K. W. Johnston. 1977. Red-eye, a sex-linked mutant in the mosquito *Eretmapodites quinquevittatus* Theobald. Mosq. News 37(4):725–728.

Hartberg, W. K. and K. W. Johnston. 1979. White-eye, a mutant in the mosquito *Eretmapodites quinquevittatus* Theobald. Mosq. News 39(2):371–373.

Hartberg, W. K. and M. M. Judy. 1990. Photosensitization of mosquito larvae with DHE. Proc. 1990 South Central Regional Mosquito Control Conference 34th Annual Meeting 22.

Hayes, R. O. 1953. Determination of physiological saline solution for *Aedes aegypti*. J. Econ. Entomol. 46:624–627.

Heitz, J. R. and K. R. Downum (editors). 1987. Light-activated pesticides. ACS Symposium Series. American Chemical Society, Washington, DC.

Helleck, A. M., W. K. Hartberg and D. Vodopich. 1993. Daily survivorship and life span of the mosquito *Eretmapodites quinquevittatus* Theobald (Diptera: Culicidae) under laboratory conditions compared to *Aedes aegypti, Aedes albopictus,* and *Aedes bahamensis*. Bull. Soc. Vector Ecol. 18(2):109–113.

Helleck, A. M., W. K. Hartberg and D. Vodopich. 1994. Variations in development times of *Eretmapodites quinquevittatus* Theobald (Diptera: Culicidae). Bull. Soc. Vector Ecol. 19(2):100–103.

Helleck, A. M., W. K. Hartberg and R. S. Baldridge. 1997. Photosensitization of *Eretmapodites quinquevittatus* Theobald (Diptera: Culicidae) eggs and larvae with Photofrin II®. J. Am. Mosq. Control Assoc. 13:292–295.

Helleck, A. M., W. K. Hartberg and D. Vodopich. 1996. Fecundity, fertility, and development stage survivorship of *Eretmapodites quinquevittatus*. J. Am. Mosq. Control Assoc. 12:137–141.

Johnston, K. W. and W. K. Hartberg. 1981. Polygenic inheritance of variation in scale pattern of the abdominal sterna of *Eretmapodites quinquerittatus* (Diptera: Culicidae). J. Med. Entomol. 18(4):300–312.

Kagan, J., E. D. Kagan, S. Patel, D. Perrine and V. Bindokas. 1987. The phototoxicity of alpha-terthienyl in eggs, larvae, and pupae of the mosquito *Aedes aegypti*. J. Chem. Ecol. 13:593–604.

Kessel, D. 1981. Transport and binding of hematoporphyrin derivative and related porphyrins by murine leukemia L1210 cells. Cancer Res. 41:1318–1323.

Kessel, D. 1986. Sites of photosensitization by derivatives of hematoporphyrin. Photochem. Photobiol. 44:489–493.

Macnamara, F. N. 1953. The susceptibility of chicks to Semliki forest virus (Kumba strain). Ann. Trop. Med. Parasitol. 47:9–12.

McIntosh, B. M., R. H. Kokernot, H. E. Paterson and B. De Meillon. 1961. Isolation of Spondweni virus from four species of culicine mosquitoes and a report of two laboratory infections with the virus. S. Afr. Med. J. 35: 647–650.

Pasteur, N. and M. Raymond. 1996. Insecticide resistance genes in mosquitoes: their mutations, migration, and selection in field populations. J. Hered. 87:444–449.

Ramsay, J. A. 1950. Osmotic regulation in mosquito larvae. J. Exp. Biol. 27:145–157.

Raymond, M., E. Poulin, V. Boiroux, E. Dupont and N. Pasteur. 1993. Stability of insecticide resistance due to amplification of esterase genes in *Culex pipiens*. Heredity 70:301–307.

Rebeiz, C. A., L. J. Gut, K. Lee, J. A. Juvik, C. C. Rebeiz and C. E. Bouton. 1995. Photodynamics of porphyric insecticides. Crit. Rev. Plant Sci. 14(4):329–366.

Reiss, R. A. and A. A. James. 1993. A glutathione *S*-transferase gene of the vector mosquito, *Anopheles gambiae*. Insect Mol. Biol. 2:25–32.

Sakurai, H. and J. R. Heitz. 1982. Growth inhibition and photooxidative toxicity in the house fly, *Musca domestica* L., caused by xanthene dye in larval growth medium and after injection. Environ. Entomol. 11:467–470.

Serie, C. L., J. Andral, M. C. Casals, P. Williams, M. C. Bres and P. Neri. 1968. Studies on yellow fever in Ethiopia. 5. Isolation of viral strains from arthropod vectors. Bull. WHO 38:873–877.

Stobbart, R. H. 1960. Studies on the exchange and regulation of sodium in the larva of *Aedes aegypti* (L). II. The net transport and the fluxes associated with it. J. Exp. Biol. 37:594–608.

Wigglesworth, V. B. 1933. The function of the anal gills of the mosquito larva. J. Exp. Biol. 10:27–37.