TRANSFER OF RADIOACTIVITY TO INDIVIDUAL EGGS BY FEMALE *AEDES AEGYPTI* TREATED AS LARVAE WITH ^{32}P

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ABSTRACT. Fourth stage larvae of *Aedes aegypti* were exposed (24–48 hr) to 4 concentrations (0.005–0.05 μCi/ml) of ^{32}P in distilled water. Radiolabeled females deposited ^{32}P into their eggs in sufficient quantities so that individual eggs were identifiable with a gas-flow proportional counter. The average radiolabeled egg contained about 0.4% of the radioactivity measured in the female after oviposition. Females retained 65% of their radioactivity after 2 days of radioactive decay and oviposition of the first batch of eggs. They retained 34% of their radioactivity after 6 days of radioactive decay and the oviposition of 2 egg batches. The females from the ^{32}P treatments produced about the same number of eggs as the control but the hatch was lower in eggs from the treated females. Eggs from the second oviposition had 59% of the radioactivity of eggs in the first oviposition. Eggs containing about 60 counts/min were easily separated from eggs laid by nonradioactive females when deposited together on the same oviposition substrate.

Radioisotopes such as phosphorous-32 (^{32}P) are useful tools in studies of mosquito biology, physiology and dispersal. Quraishi et al. (1966) and Smittle and Patterson (1970) produced females that transferred enough radioactivity to be detected in a single batch of eggs by *Aedes vexans* (Meigen) and *Culex quinquefasciatus* Say, respectively. Radiolabeled egg rafts from *Cx. quinquefasciatus* have been used in several field release programs to study survival, dispersal, reproductive behavior, mating competitiveness, overwintering and oviposition behavior (Weidhaas et al. 1973, Smittle et al. 1973, Lowe et al. 1973, Lowe et al. 1974, Smittle et al. 1975). Recently, Focks et al. (1981) and Focks et al. (1982), have demonstrated the need for monitoring the population densities of *Toxorhynchites rutilus rutilus* (Coquillett) for the control of *Aedes aegypti*. Since *Ae. aegypti* and *Tx. r.rutilus* lay eggs individually, a method to identify single eggs in natural populations would be advantageous. This study was conducted to investigate the transfer of radioactivity to individual eggs of *Ae. aegypti*.

MATERIALS AND METHODS

A preliminary test was conducted by exposing late third or early fourth stage larvae of *Ae. aegypti* for 72 hr to distilled water containing 0.005 and 0.05 μCi of ^{32}P per ml of water. The ^{32}P used was carrier free H_3PO_4 in 0.02 N HCL. Other tests exposed fourth stage larvae for 24–48 hr to concentrations of 0.005, 0.01, 0.025 and 0.05 μCi/ml. The 0.025 μCI-ml test was replicated 2 additional times. The volume of the treatment solutions ranged from 500 to 800 ml with 4 to 5 ml per larva. The larvae were fed a mixture of desiccated hog liver and dried brewer's yeast after they were in the treatment solution for 4 hr, and daily thereafter. Pupation of the females occurred 24 to 48 hr after treatment except in the preliminary test, where they pupated after 72 hr. The pupae were rinsed with tap water to remove external radioactivity and placed in cages for emergence. After emergence, the adults were allowed to mate, take a blood meal from guinea pigs and were provided with 10% sugar water. The gravid females were placed in individual vials with moist filter paper for oviposition.

After oviposition, the females and eggs were assayed for radioactivity using a windowless gas-flow proportional counter having about 45% efficiency for ^{32}P. During assay of radioactivity, the live females were confined in gelatin capsules which were centered in aluminum planchets. Single eggs and groups of 10 eggs were counted in aluminum planchets. This assay method allowed the eggs to be hatched after assay of radioactivty and did not kill the females. Radioactivity is reported as counts per minute minus background.

The number of eggs produced per female and egg hatch from females at the different concentrations were compared with untreated females. To evaluate radioactivity loss, females were assayed before and after the first and second ovipositions. The radioactivity of the eggs from the first and second ovipositions were compared.

Two groups of radioactive females (one group had 12,000–13,000 counts/min, the other had 20,000–25,000 counts/min) were caged with nonradioactive females and males, given a blood meal and provided moist filter paper for oviposition. The eggs were individually assayed to see if the eggs deposited together on the filter paper could be identified on the basis of radioactivity. This test was conducted to see if any surface contamination would occur when eggs were deposited on the same oviposition substrate as might occur in field experiments.

RESULTS AND DISCUSSION

The radioactivity of females and their eggs is shown in Table 1. In the preliminary 72 hr tests, the 0.05 μCi/ml concentration was detrimental as only a few females survived and they produced only a few eggs with some eggs deformed. No detrimental effects on survival or oviposition were observed in any of the other tests. At the 0.005 μCi/ml concentration, the preliminary 72 hr treatment produced females that had over 3 times the radioactivity of females from the 24 to 48 hr treatment. All treatments resulted in individual eggs having enough radioactivity to detect in a gas-flow proportional counter. The mean radioactivity of individual eggs ranged from 0.347 to 0.432% of the female radioactivity after oviposition. This compares favorably with the 0.369% reported for *Ae. vexans* by Quraishi et al. (1966). The radioactivity of individual eggs from a female was consistent (standard error of the mean ranging from 2.3 to 4.2) for eggs having a mean of 37 to 66 counts/min. The number of eggs the female laid had little if any effect on the amount of radioactivity deposited in each egg as evidenced by the correlation between female and egg radioactivity. Also, females having similar amounts of radioactivity produced eggs with similar amounts of radioactivity even when one female laid twice as many eggs as the other.

The females from all concentrations of the 24 to 48 hr treatments produced approximately the same number of eggs as the control (Table 2). The percentage hatch of the 0.05 μCi/ml concentration was 23% lower than the control but the other concentrations were within 8% of the control. The eggs were not subjected to vacuum or other hatch enhancing procedures which could have produced higher hatches in all tests.

The 0.025 μCi/ml concentration was chosen for additional tests on loss of radioactivity by females. The results discussed below indicate the radioactivity retained by the females, followed by the amount (shown in parentheses) that would be present based only on radioactive decay; the difference is due to biological elimination. A group of females from one of the 0.025 μCi/ml tests was assayed for radioactivity before oviposition and 2 days after oviposition. These females retained 65% of their radioactivity after oviposition (2 days decay = 91%). They were given another blood meal and allowed to oviposit a second batch of eggs and were assayed again for radioactivity 4 days after the first oviposition. These females retained 54% (4 days decay = 82%) of the radioactivity they had before the second oviposition and 34% (6 days decay = 75%) of the radioactivity they had before the first oviposition. The eggs from the second batch of eggs had 59% as much radioactivity as the first batch of eggs. At this concentration, the eggs from the second oviposition averaged over 35 counts/min and were easily detected.

In the test where radioactive females were put in cages with nonradioactive females and males and allowed to oviposit on the same moist filter paper, the eggs from the 12,000 to 13,000 counts/min group of females averaged 62 counts/min (range 50–75) above the nonradioactive eggs. The eggs from the 20,000 to

Table 2. Mean number of eggs and percent hatch from mosquitoes exposed to various ^{32}P concentrations 24–48 hr (10♀ per test).

Concentration (μCi/ml)	Mean no. of eggs/♀ ± S.D.	% hatch ± S.D.
0.005	105.8 ± 9.8	73.8 ± 22.5
0.01	98.1 ± 24.1	67.5 ± 21.1
0.025	116.2 ± 10.3	73.1 ± 20.2
0.05	95.6 ± 15.5	52.4 ± 28.1
Control	110.8 ± 34.2	75.1 ± 24.7

Table 1. Radioactivity of females and eggs of *Aedes aegypti* following exposure as larvae to various ^{32}P concentrations.

^{32}P concentration (μCi/ml)	Treatment time (hr)	No. of ♀♀	Mean counts/min: ♀ after oviposition ± S.D.	Mean counts/min: 1 egg ± S.D.	Egg % of ♀[a]
0.005	>72	15	10,679 ± 2,639	37.1 ± 11.6	0.347
0.05	>72	6	125,304 ± 16,188	528.8 ± 12.0	0.422
0.005	24–48	12	3,283 ± 1,297	12.0 ± 4.5	0.366
0.01	24–48	12	6,210 ± 3,221	22.8 ± 12.8	0.367
0.025	24–48	48	15,083 ± 5,533	63.2 ± 19.7	0.419
0.05	24–48	10	21,619 ± 12,296	93.5 ± 37.1	0.432

[a] Single egg % of ♀ = $\frac{\text{Mean counts/min egg}}{\text{Mean counts/min ♀ after oviposition}} \times 100.$

25,000 counts/min group of females averaged 104 counts/min (range 88–118) above the nonradioactive eggs. All eggs from radioactive females are radioactive and all eggs from nonradioactive females approximate background, thus it was easy to separate the radioactive eggs.

These tests indicate that it is possible to label individual eggs from females of *Ae. aegypti* by exposing larvae to ^{32}P solutions 24 to 48 hr before pupation. The labeled eggs can be easily differentiated from nonradioactive eggs on the same oviposition substrate which makes this technique useful for behavior studies in natural habitats. Eggs from second egg batches can be identified as they have only ~ 60% as much radioactivity as first batch eggs. Since the radioactivity of eggs is directly related to the radioactivity of the female, one can predict how radioactive the female must be to detect her individual eggs. This can be done with this species using the following formula:

$$\text{♀ counts/min} = \frac{\text{egg counts/min} \div 0.004 \text{ (proportion/egg of ♀ counts/min after oviposition)}}{0.65 \text{ (♀ retention after oviposition)}}$$

Thus, if 50 counts/min is required for each egg, the gravid females would need ~ 20,000 counts/min. This labeling technique provides a method for labeling individual mosquito eggs and may be useful for labeling the eggs of other insects.

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