Fox and Bayona (1972) noted that some malathion-resistant strains of *A. aegypti* have appeared in Puerto Rico. Thus, it is possible that alternative methods such as source reduction or other insectides should be considered for future *A. aegypti* control.

Abate has been shown to be an effective *A. aegypti* larvicide (Gould *et. al.*, 1971) and there is no indication of resistance to this compound in Puerto Rico. In this study, mosquito larvae were suppressed in 50-gallon drums by the application of Abate. However, since it was not feasible to treat small containers, many of these harbored *aegypti* larvae after heavy rains in October (Table 3). Effective source reduction is an obvious solution to the problem of *aegypti* breeding in small containers.

ACKNOWLEDGMENTS. We thank personnel from the Puerto Rico Department of Health and San Juan Tropical Disease Laboratories for aid in collecting mosquitoes.

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THE PERSISTENCE OF HEPATITIS B ANTIGEN IN *AEDES AEGYPTI*

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Hepatitis B (long-incubation hepatitis) is associated with an antigen (HBAg) which is known to be transmitted by parenteral injections or blood transfusions. The high incidence of HBAg in human populations in the tropics has suggested that blood-sucking arthropods might serve as vectors (Prince, 1970, and Shulman, 1970). Furthermore, since mosquitoes serve as efficient vectors of a variety of viruses, we believe that their possible role in transmission of this antigen should be investigated.

We initiated a study, the first phase of which was designed to determine whether or not hepatitis B antigen persisted, with or without multiplication, for a sufficient period of time for mosquito transmission to be a possibility. This report deals with the persistence of HBAg in *Aedes aegypti*.

MATERIALS AND METHODS. Batches of 40 *A. aegypti* 4–6 days of age, in half-pint ice cream containers with nylon netting glued to top, were placed on the anterior aspect of the forearm and/or abdomen of each of two volunteer chronic asymptomatic carriers of HBAg, known to have stable antigen titers. The containers from these and a normal (anti-

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Supported by National Institute of Health Grants DHEW 5P01 HE 10893-06, AI-LA09278-03, and the University of Texas Medical Branch McLaughlin Faculty Fund. Some of the studies were performed in the Clinical Study Center supported by Public Health Service Grant FR00073.

gen-negative) control subject were removed after 30 minutes.

The experimental and control batches of specimens were anesthetized with CO_2 and those showing evidence of a blood meal were killed with —20° C cold, homogenized, and suspended in 0.3 ml. of phosphate-buffered saline (pH 7.2) containing Tween 80 (1/20,000) and polyvinyl pyrrolidone (0.0025 percent). The suspensions were then aspirated into calibrated capillary tubes, centrifuged in a hematocrit centrifuge (maximum speed for 15 minutes) to eliminate cellular debris. The supernate was frozen and thawed repeatedly to hemolyze any intact cells, and tested for the presence of HBAg.

During each of the 3 days following this initial experiment, batches of mosquitoes were allowed to feed on the antigen carriers. The engorged specimens were maintained at a constant temperature and humidity (80° F and 80 percent RH) for 6 days, after which they were treated as previously described. These suspensions of mosquitoes fed on each volunteer were tested unconcentrated, and again after pooling and 3-fold concentration using polyacrylamide gel.

The serums both from the volunteers and the mosquito suspensions were tested for HBAg, using both a hemagglutination inhibition (HI) test and a reversed passive hemagglutination test (Sultan, personal communication). The tests were done in microtiter plates with V-shaped wells. The hemagglutination inhibition test was done using human O Rh-red cells sensitized with HBAg and four hemagglutinating units of human hepatitis B antibody obtained from a multiply transfused hemophiliac with an RBC hemagglutination titer of 1/20,000–1/25,000. The reversed passive hemagglutination test utilized reconstituted lyophylized stabilized human erythrocytes sensitized with guinea pig hepatitis B antibody. It was performed by making two-fold dilutions of the samples with microdiluters and adding one drop (0.025 ml) of HBAg sensitized cells to each well. The microtiter plates were then covered with sealing tape, mixed carefully for 10 seconds and incubated at room temperature, over a test reading mirror, for two hours. The endpoint was read as the highest serum dilution showing agglutination. A test was called negative when there was no evidence of agglutination. Positive tests in both methods were confirmed using control unsensitized red cells.

RESULTS AND DISCUSSION. The sera of the carriers were HBAg-positive daily by both tests throughout the experimental period (Table 1). However, their antigen titers were higher by reversed agglutination than with the HI test. Both the serum and the suspensions prepared from the mosquitoes fed on the control subject were antigen-negative, as were suspensions prepared from control mosquitoes fed on sugar water only.

TABLE 1.—HBAg titers obtained with human sera and mosquito supernates.

Sample	Hemagglutination inhibition test	Reversed passive hemagglutination test
HBAg-negative control serum	Negative	Negative
Unfed mosquitoes (control)	Negative	Negative
Fed mosquitoes (HBAg-negative subject)	Negative	Negative
Serum (HBAg-positive subject no. I)	1:128	1:12,000
Serum (HBAg-positive subject no. II)	1:64	1:6,000
Fed mosquitoes sacrificed immediately (subject no. I)	1:4	1:512
Fed mosquitoes sacrificed immediately (subject no. II)	Negative	1:256
Fed mosquitoes sacrificed after 6 days (subject no. I)	Negative	Negative
Fed mosquitoes sacrificed after 6 days (subject no. II)	Negative	1:8
Pooled—fed mosquitoes after 6 days (3X con) (subject no. I)	Negative	1:16
Pooled—fed mosquitoes after 6 days (3X con) (subject no. II)	Negative	1:16

The suspensions prepared from mosquitoes killed a few hours after feeding on subject I were positive by both methods, whereas those from subject II were positive by reversed passive hemagglutination only (Table 1). Again, titers were higher using the latter technique. Unconcentrated suspensions of mosquitoes fed on subject II and sacrificed 6 days after feeding were HBAg-positive to a titer of 1:8 when tested by reversed hemagglutination. Concentrated pooled suspensions of mosquitoes fed on subjects I and II, 6 days previously, were HBAg-positive to a dilution of 1:16 by this method.

These changes in titer are of such magnitude as to indicate a significant decrease 6 days after the initial feeding. However, it is important to note that Barker *et al.* (1970) have shown that the inoculation of diluted plasma in amounts too low to be detected by current techniques could transmit serum hepatitis or produce an antigen-carrier state. Inoculation of the highest plasma dilutions (10^{-5} to 10^{-7}) with a 1:10 HBAg complement fixation titer were associated with antigenemia without signs of hepatitis, while the subjects inoculated with lower dilutions could contract antigen-positive clinical hepatitis.

After our preliminary investigation was in progress, Smith *et al.* (1972) reported that when *C. p. fatigans* females were fed artificially on infected blood and serum, the antigen persisted in the lumen for 10 days, and after 3 weeks reappeared in the salivary glands. Their findings were based on immunofluorescent staining techniques, thus providing no information on antigen titers.

Inasmuch as HBAg in the human serum was diluted by the subject's red cells, mosquito tissue and saline, and depended to some extent on the number of engorged specimens, it is not appropriate to make more than a gross comparison between samples obtained from different volunteers or on different days. It can be concluded however that HBAg persisted for a sufficient period of time in *A. aegypti* as to permit transmission of hepatitis B either through interrupted feedings, or through single feedings provided the antigen is present in the saliva 6 days after the infectious (initial) blood meal.

SUMMARY. Adult, female mosquitoes (*A. aegypti*) were fed on volunteer, chronic, asymptomatic carriers of hepatitis-B antigen. These and control batches of mosquitoes were homogenized immediately or kept for 6 days and further processed for comparison of antigen titers using both the hemagglutination test (HI) and the reversed passive hemagglutination test. Both unconcentrated and concentrated mosquito suspensions were used, and the reversed passive hemagglutination consistently produced the highest titers. Hepatitis B antigen persisted for 6 days, which would allow transmission to humans either by interrupted feedings or by single feedings if the antigen is present in the saliva at 6 days.

ACKNOWLEDGMENT. We thank Linda Parker, Glen H. Stanbaugh, M.D., Harry E. Sarles, M.D., William C. Levin, M.D., Nina Gaddy and Diana Rougeau for their interest in and assistance with this study.

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