

Hepatitis B Surface Antigen in Laboratory Reared Mosquitoes

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OBJECTIVE: To determine the duration of carriage of hepatitis B surface antigen (HB_sAg) by laboratory reared mosquitoes fed on a HB_sAg carrier.

BACKGROUND: This study was reported in the SEATO Medical Research Laboratory Progress Report 1973—1974. This report concerns the completion of radioimmune assay testing of mosquitoes following feeding on an antigen positive donor.

DESCRIPTION: All mosquitoes used in this study were reared from eggs in the laboratory. After the adults emerged they were held for 48 hours and were deprived of fluids for 12 hours prior to use. Mosquitoes were fed on a known carrier of HB_sAg/adr with a constant complement fixation titer of 1:512. Engorged mosquitoes were then removed and unfed mosquitoes discarded. A sample of 10 fed mosquitoes were quick-frozen and stored at -70°C; the remainder were placed in cages and allowed to feed on sugar water. Samples of 10 mosquitoes were withdrawn from the cages at 1, 3, 5, 7, 10, 15 and 21 days after feeding, quick-frozen and stored at -70°C.

All mosquitoes were tested by radioimmune assay (RIA, Ausria I, Abbott Laboratories) simultaneously for each mosquito species. Pools of 10 mosquitoes were triturated in 0.5 ml of 0.01 M Tris buffered saline pH 7.4 and centrifuged at 2000 rpm; 0.1 ml of the supernatant solution was placed in each of two Ausria tubes. Following this the test was run according to the directions provided with the Ausria kit. Included in each experiment was a pool of 10 unengorged mosquitoes of each species. Also one mosquito species, *Aedes aegypti*, was allowed to bite a non-antigenemic individual. These mosquitoes were followed in the same way.

PROGRESS: Seven mosquito species, *Aedes aegypti*, *Aedes albopictus*, *Anopheles balabacensis*, *Anopheles maculatus*, *Anopheles minimus*, *Armigeres subalbatus*, and *Culex quinquefasciatus*, were tested in the above manner (Figure 1). RIA results of all unengorged mosquito controls fell within one standard deviation of the mean of the negative sera controls. Further, all samples of *Aedes aegypti* fed on a non-antigenemic individual also were found to fall within one standard deviation of the negative control mean. For all seven species the first pool of ten was taken immediately after feeding on the HB_sAg positive volunteer. HB_sAg was detected in all mosquito species in the first sample. However, HB_sAg as determined by RIA, had disappeared by 24—72 hours after feeding. All mosquito species were followed for 21 days or longer. In all species HB_sAg did not reappear; the RIA counts per minute on these mosquito pools remained within the limits of the unfed mosquitoes.

DISCUSSION: These data indicate that disappearance of HB_sAg from the mosquito pools was simultaneous with the digestion and elimination of the blood meal. In the seven mosquito species followed for 21 or more days HB_sAg did not reappear after its initial disappearance; however, the presence of antigen in mosquitoes for 24—72 hours might allow them to serve as mechanical vectors if they refeed within this period of time.

The laboratory work on this study is now complete and the data is being analysed in preparation for publication.

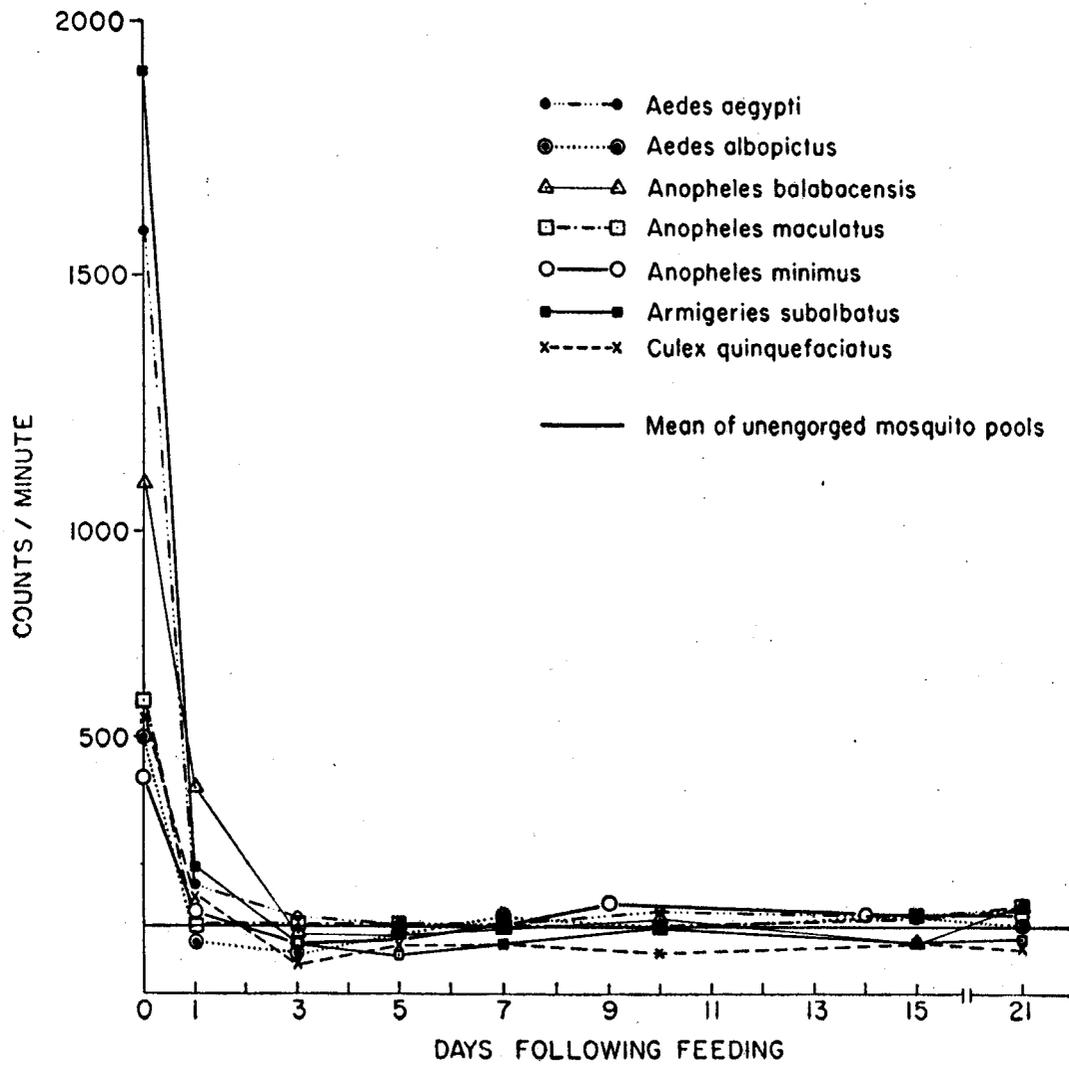


Figure 1 Radioimmuno assay for HB_sAg (Ausria I) on pools of 10 mosquitoes collected following feeding on an infectious HB_sAg positive volunteer.