appeared after 24–48 hrs., the cultures were sometimes saved by changing the medium every day for 4 successive day, providing plating had occurred. For initiating the primary culture, 3.0 cc of medium containing antibiotics are pipetted into a sterile 2-inch Petri dish. If available, both glass and plastic Petri dishes should be used for comparison of growth and differentiation of cells upon the different surfaces. Subsequently 30 cc Falcon T-flasks may be used following the plastic Petri dishes, or glass prescription bottles following the glass Petri dishes.

As an example of how the press is used, the dissociation of uncontaminated mosquito larvae will be described. From one to six larvae can be conveniently crushed in a 1.0 cc syringe, depending on size. A sterilized syringe with bolting cloth sieve is prepared as already described. The plunger is removed and placed into a small sterile beaker for easy grasping; or, the plunger can be packaged and sterilized separately. A larva is picked up with a sterile pipette from the sterile distilled water or sterile culture medium in which it is being held, and is dropped upon sterile filter paper in a sterile Petri dish. After the water has been absorbed, the larva is lifted up with a fine needle or forceps, and deposited into the syringe as far down as possible. This procedure is repeated for as many larvae as one wishes to crush. The plunger is inserted, and the larva is gradually pushed down the barrel of the syringe until it is deposited upon the disk. The snout of the syringe is then placed into tissue culture medium in a Petri dish, and 0.2 cc of medium is drawn up. If a 5.0 cc syringe is used, 0.5 to 1.0 cc of medium is drawn up. The snout is then placed just above the surface of the medium in the Petri dish, and the plunger is pushed forward, crushing the larva while turning the plunger alternately to the right and left. The larval contents will be forced through the sieve into the medium. One can then examine for cellular dissociation immediately with an inverted microscope. If the disk has been cut properly, it will remain in place. The operation may be repeated, using other syringes. The disks, being easy to make and inexpensive, should be discarded; however, if nylon disks are used, they are more durable, and may be thoroughly washed, resterilized, and reused.

Newly-formed pupae and newly-emerged adults are so soft that they may easily be crushed in the same manner; however, it would be better to nick the pupal and adult integument with number 27 hypodermic needles (Burton, 1971), and extract the contents in large pieces prior to placing them into the syringe. The adults should have wings and legs removed, and be separated into head, thorax, and abdomen beforehand. When this procedure is used with eggs, the contents must be first extruded with needles, pooled, and dropped upon the mesh disk. If it is desired to utilize only individual organs, they should be dissected out, pooled, deposited upon the sieve, and crushed in a 1.0 cc syringe. The primary cultures should be incubated at 28 degrees C; however, they have been kept satisfactorily at 23 to 26 degrees, although growth is somewhat slowed at 23 degrees. Results should be compared with and without an atmosphere of 5 percent carbon dioxide, if a CO_2 incubator is available.

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RECOVERY OF *AEDES ALBOPICTUS* FROM USED TIRES SHIPPED TO UNITED STATES PORTS

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The importance of international traffic in the dispersal of mosquitoes and other arthropod vectors

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of diseases of public health significance has been well documented (Hughes and Porter, 1956; Joyce, 1961). Used tires are especially high risk cargo items. Pratt *et al.* (1946) reported the discovery of mosquito larvae in tires in 3 of 12 ships carrying old tires and other salvage from combat areas of the Pacific to the United States. Seven species of mosquitoes not indigenous to the United States were recovered, several of which are disease vectors. Used tires containing water are attractive oviposition sites for a number of mosquito species: even though dry on arrival at U. S. ports, such tires might well contain viable mosquito eggs.

Since about 1966, large quantities of military material, called retrograde cargo, have been moved from the Republic of Vietnam to the United States. Used tires have been transported as retrograde by both aircraft and surface vessels. Retrograde tires are U. S. Government property and are shipped in U. S. owned or contract ships and aircraft. Existing regulations require that government property being shipped out of Vietnam be treated and processed so that it is free of soil deposits and plant and animal life of concern to the public health and to agriculture. Used tires are to be treated with a mosquito larvicide.

No difficulty has been experienced with retrograde tires entering quarantine at United States ports. However, large quantities of used tires from automobiles, trucks, aircraft and earthmoving equipment are being declared surplus in Vietnam. Civilian contractors are purchasing these tires for resale to United States firms and shipping them to the United States in commercial vessels. There are no requirements that these privately owned tires be treated with mosquito larvicide prior to leaving Vietnam.

A number of partial shiploads of surplus tires have been inspected by Public Health Service quarantine officers at the ports of Oakland and Los Angeles during the Vietnam conflict. Prior to the case described here, no recoveries of mosquitoes were made. The tires were usually dry, although an occasional one was encountered which contained water.

A ship entered quarantine at Oakland April 2, 1971, with 460 short tons of surplus, earthmoving equipment tires consigned to a tire dealer in Los Angeles. A few of the tires contained water. Two larvae and three pupae of *Aedes albopictus* were discovered in one of them. The ship was remanded to Los Angeles where the tires were unloaded under the supervision of Public Health Service quarantine officers. Two additional tires containing several *Ae. albopictus* larvae and pupae were found.

Ae. albopictus is widely distributed in the Oriental and Indomalayan regions, where it has been implicated in the transmission of dengue viruses (Chan *et al.*, 1966; Gould *et al.*, 1968). It is a common mosquito in Vietnam, breeding in both natural and artificial containers in close association with man. The species was introduced and became established in Hawaii in the 19th century (Joyce, 1961).

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A STUDY OF OVIPOSITION OF *AEDES* MOSQUITOES

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The biological peculiarities of the *Aedes* (*Stegomyia*) species of mosquitoes and the role of some amongst them as agents of human diseases is now well recognized (Christophers, 1960). However, even at present there seem to be enough lacunae in the various facets of their ecology (Macdonald, 1965) and in particular their oviposition behaviour. A number of workers have reported on various influences on oviposition (Beckel, 1955; Fay and Perry, 1965; Petersen and Rees, 1966; Snow, 1971), but no studies on the role of different timbers appear to have been carried out. The object of the present investigation is to describe the preferential role of *Aedes* (*Stegomyia*) species in ovipositing on timbers in the laboratory.

Fifteen different types of timbers were selected for oviposition studies. An enamel bowl containing clean tap water and filled to ¾th its height was kept in the centre of the colony cages containing 50 gravid females of *Aedes aegypti*, *Aedes albopictus* and *Aedes vittatus*, each kept separately. The mosquitoes were fed on rabbits and utilised on the 4th day after feeding for egg laying. The timber pieces were floated on the

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