pneumonia (9 patients), and none were found in 72 serums from healthy individuals. These virus strains were found to be cytopathogenic in primary tissue cultures of various cell types. They caused complete destruction of cultures of human amniotic cells and about 50 percent destruction of monkey kidney cells, and they had a moderate cytopathogenic effect in cultures of fibroblasts from monkey testis and epithelial cells from monkey epididymus. No effect was seen on the following cell types in continuous culture: HeLa, two strains of human kidney epithelium (adult and fetal), human lung fibroblasts, strain KB (Eagle) (*1*), conjunctiva (*2*), human intestine (*2*), and human liver (*2*), mouse mammary carcinoma cells (strain Ma Ca, de Bruyn), and normal mouse kidney epithelium.

The cytopathogenic effect of all these strains was prevented by neutralization with rabbit antiserum ECHO type 9 (*3*). Conversely, two standard ECHO type 9 virus strains (*4*) were neutralized by several rabbit serums against Belgian strains and by serums of human convalescents.

Most of these strains, as infected tissue culture fluid, produced myositis in suckling mice at least up to the age of 10 days. Many caused paralysis and some even death. Several of these strains, however, did not cause histologically detectable myositis. The two aforementioned standard strains belonged to the last group.

When a mixture of infectious tissue culture fluid and anti-ECHO type 9 serum was injected into suckling mice, no myositis occurred, while controls without immune serum died or were paralyzed. In the complement-fixation test, antigen prepared from aqueous suspension of baby mice was even better antigen than tissue culture fluid.

On histological examination of infected baby mice, no lesions other than those of myositis were found. The virus content of 10-percent suspensions of different tissues was determined in tissue culture; muscle contained about 10^6 TCD_{50}/ml, while brain, liver, kidney, and spleen contained from 10 to 10^2 TCD_{50}/ml. Up to now, three strains tested were not pathogenic for adult mice.

Ten original fecal specimens from which virus was isolated in tissue culture and found pathogenic for suckling mice did not cause any clinical symptom on direct inoculation into these animals; lesions were, however, detectable, histologically. A Belgian prototype strain was not neutralized by Dalldorf's antiserums against Coxsackie types A_1 to A_{17} and A_{19} and B_1 to B_5.

Considering these facts, we feel justified in proposing that the viruses of ECHO type 9 should be removed from the ECHO group and reclassified, probably as a new member of the Coxsackie A group of viruses.

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Notes

1. From H. Eagle.
2. Supplied by Microbiological Associates.
3. Kindly sent by A. Sabin.
4. Received through the courtesy of H. v. Magnus and A. Svedmyr.

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Induced Copulation of Aedine Mosquitoes

Aedine mosquitoes that develop in floodwaters are difficult to maintain as colonies in cages because conditions conducive to copulation are rarely attained. An efficient procedure for stimulating copulation has been devised in our laboratory, and by this means viable eggs have been obtained from *Aedes stimulans* and *Aedes vexans*.

Copulation is induced by manipulating an immobilized female into contact with a decapitated male. From the literature we learned that decapitation of males of mantids removed the center for inhibition of the copulatory act (*1*). Female mantids often eat the heads from their suitors prior to or during copulation, yet the act goes forward normally. Roeder (*2*) has shown that the center of inhibition in mantids is in the subesophageal ganglion. Removal of the head of male mosquitoes removes the ganglion also.

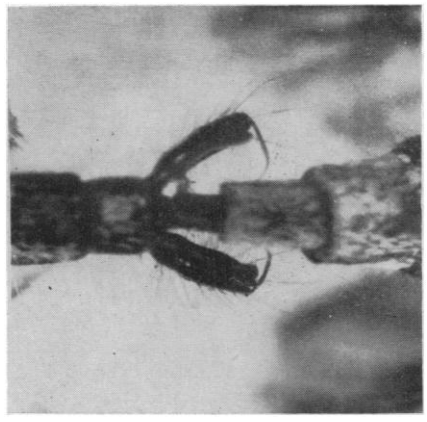

Fig. 1. Ventral view of male and female of *Aedes stimulans* in act of copulation (×18).

Mosquitoes that had emerged for a time greater than 72 hours were prepared for mating as follows. Females were anesthetized by exposure to atmospheres of either carbon dioxide or chloroform. The dorsum of the thorax of each was cemented to the tip of a fine needle. Several were prepared at each time and held until ready for use. Males were decapitated without prior anesthesia. Each of them was immobilized by cementing the dorsum of the thorax to a piece of white cardboard. Several of these could likewise be prepared at once before mating the series.

Contact between two immobilized mosquitoes was accomplished manually under a stereomicroscope magnifying about 20 diameters (Fig. 1). Each female was placed in mating position with its ventral side up and its head directed away from the male. Apexes of the two abdomens were then brought together. The position thus was end to end and venters uppermost. Insemination was usually completed within a few seconds.

The females were not injured by the treatment. The kind of cement used to attach them to the needles is not critical as long as it sets within a minute or two and can be loosened after copulation.

Techniques employed here permit matings at a rate of about 25 females an hour. This rate is reasonable for maintenance of even a large colony of one species or small colonies of several species. Since the eggs may be kept for months, they may be accumulated for large-scale experiments.

It was found that males would copulate with several females but that sperms were transferred dependably to only the first two. A large series of single matings resulted in insemination of 90 percent of the females. A comparable series in which males were mated a second time resulted in transfer of sperms to 85 percent of the females. When the males were mated a third time, only about half of the females were inseminated.

Attempts to inseminate *Culex pipiens* and *Anopheles quadrimaculatus* in this manner met with little success. A contributing factor seems to be differences in genitalia. Proper contact is difficult to establish, and the sperm mass rarely enters the genital opening of the female.

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