ESTABLISHMENT OF A DIPLOID CELL LINE FROM LARVAL TISSUES OF *AEDES VITTATUS* (BIGOT, 1861)

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ABSTRACT

A diploid cell line from larval tissues of *Aedes vittatus* has been established. The cells grew as sheet on the glass surface and also as multicellular hollow vesicles floating in the medium. So far (July 1970) the cells from the attached sheet have undergone 34 passages. The hollow vesicles, which are also maintained as a separate sub-line, have undergone 12 passages. Cells from attached sheet and hollow vesicles have been stored in liquid nitrogen and regenerated successfully.

FOLLOWING the establishment of the first mosquito cell line by Grace[1] from *Aedes ægypti* larvæ, several workers have succeeded in establishing cell lines from different mosquito species: *Aedes ægypti* by Singh,[2] Peleg[3] and Varma and Pudney[4]; *Aedes albopictus* by Singh[2]; *Aedes vexans* and *Culiseta inornata* by Sweet and Dupree[5] and *Anopheles stephensi* by Schneider.[6] The present communication reports the establishment of another diploid cell line from larval tissues of *Aedes vittatus*.

The techniques used for setting up of the primary culture and maintenance of the cell line of *Aedes vittatus* were essentially the same as described by Singh.[2] The eggs were obtained from mosquitoes colonized in the laboratory. As the eggs surface-sterilized with acetone failed to hatch, they were surface-sterilized by washing first with 70% ethanol for 2–3 minutes and keeping them immersed in White's solution[2] for about 10 minutes. These eggs were washed thoroughly with sterile glass-distilled water and kept for hatching in Rinaldini's salt solution[2] (RSS), under reduced pressure. Most of the eggs hatched within one hour. The freshly hatched larvæ along with 1–2 ml. of RSS were transferred to a small tube and were minced. Five ml. of 0·1% trypsin (1 : 250, Difco) in RSS was added to the minced larval tissues and the suspension incubated at 37° C. for 10 minutes. After incubation the larval cells and tissue fragments were washed once with RSS to remove the trypsin, resuspended in 1 ml. of the culture medium, and seeded into a Porter's flask. The culture was incubated at 30° C. in a stationary position.

The culture medium was similar to that used by Schneider[6] for growing *Anopheles stephensi* cells. The concentration of the various ingredients in 100 ml. of the medium was as follows: NaCl, 300 mg.; KCl, 110 mg.; $MgCl_2.6H_2O$, 114 mg.; $MgSO_4.7H_2O$, 40 mg.; $NaHCO_3$, 35 mg.; sucrose, 1,600 mg.; glucose, 70 mg.; trehalose, 50 mg.; cholesterol, 0·02 mg. (dissolved in 0·02 ml. 95% ethanol and 0·02 ml. 5% aqueous solution of tween 80); all the amino-acids (omitting L-tyrosin) of Grace's medium,[1] in the same quantity; 1 ml. of 10 × NCTC 135 medium (without amino-acids, inorganic salts and sugars); and 10 ml. inactivated fetal bovine serum. A few drops of 0·01% phenol red was added as pH indicator. The pH of the medium was adjusted to 6·9 before adding fetal bovine serum. The concentration of antibiotics used was, 100 units of penicillin G (Sodium) and 0·1 mg. of streptomycin sulphate per ml.

The cells were grown on coverslips in Leighton tubes for the study of their morphology. Living cells were studied both under dark phase and bright phase illumination. For detailed cytological studies the cells were fixed in methyl alcohol and stained with Wright's and Giemsa stains.

A large number of cells and tissue pieces were found attached to the glass surface by the second day after setting up of the culture. Small patches of cells growing on the glass surface and a large number of small bubble-like structures growing at the cut ends of floating larval fragments were observed by the fourth day. These bubbles further increased in number and size, and formed multicellular hollow vesicles (Fig. 1), similar to those observed in the cultures of *Aedes ægypti*.[7] On the thirteenth day, when a complete monolayer of cells was observed on the glass surface, the floating hollow vesicles, and the cells attached to the glass surface were subcultured separately, in the manner described for *Aedes ægypti* cultures.[2,7] Subsequent subcultures both from the cells attached to the glass surface and the floating hollow vesicles were made

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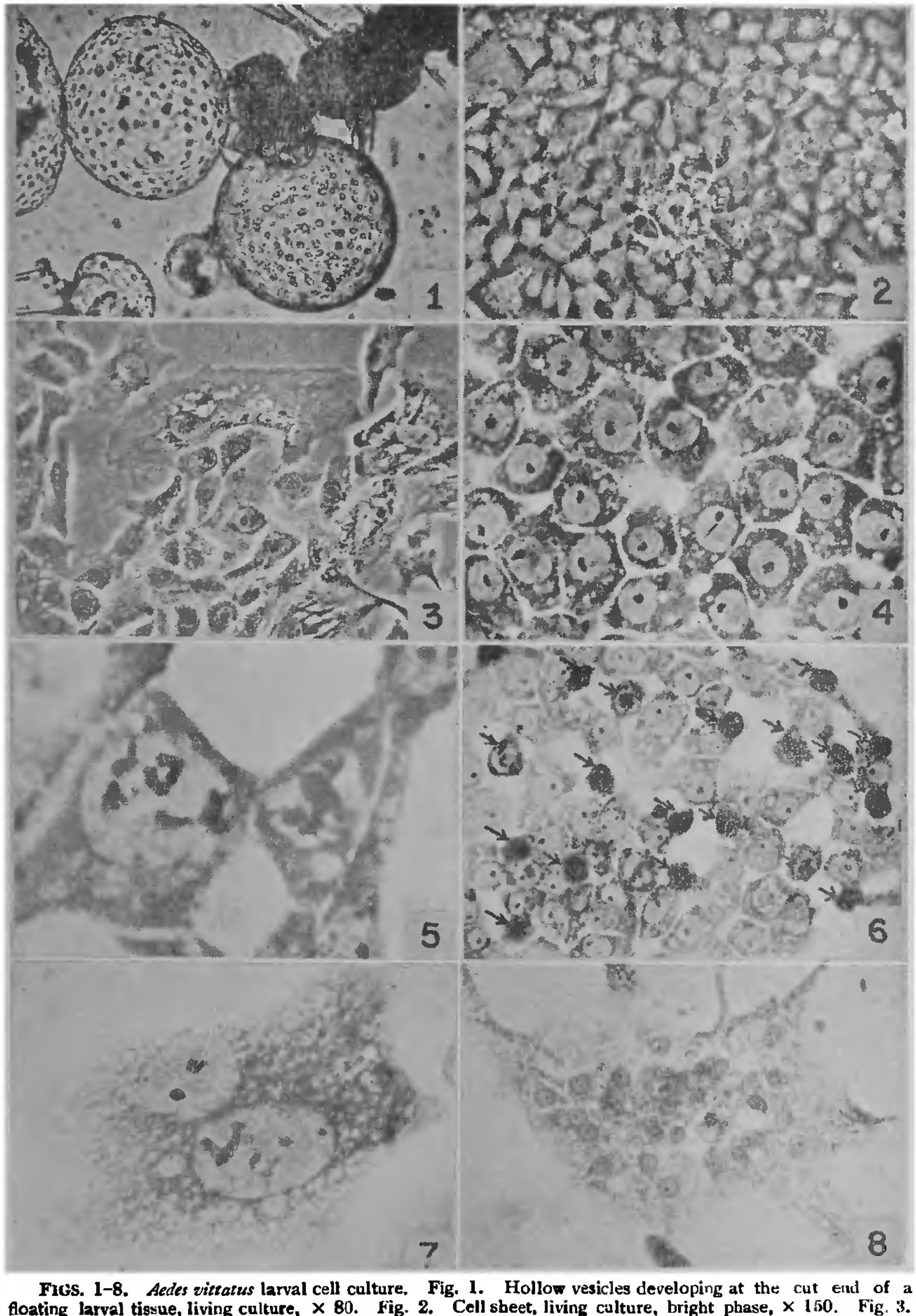

FIGS. 1–8. *Aedes vittatus* larval cell culture. **Fig. 1.** Hollow vesicles developing at the cut end of a floating larval tissue, living culture, × 80. **Fig. 2.** Cell sheet, living culture, bright phase, × 150. **Fig. 3.** Cell sheet, living culture, dark phase, × 200. **Fig. 4.** Type A, epithelial-like cells, stained with Wright's and Giemsa, × 600. **Fig. 5.** Type A, epithelial like cells showing mitosis, stained with Wright's and Giemsa, × 1,300. **Fig. 6.** Type A, epithelial-like cells, stained with Wright's and Giemsa, showing active mitosis, × 300. **Fig. 7.** Type B, epithelial-like cell, stained with Wright's and Giemsa, × 300. **Fig. 8.** Multinucleated giant cell, stained with Wright's and Giemsa, × 300.

once in six to eight days depending on the growth of the cells. The attached cells have so far (July, 1970) been subcultured 34 times. The hollow vesicles, were maintained as a separate subline. Their structure and development was observed to be similar to that of *A. ægypti*.[7] They were subcultured twelve times and then stored in liquid nitrogen at the thirteenth passage.

Three morphologically different cell types were observed to grow on the glass surface in the form of monolayers (Figs. 2, 3). Majority of these were epithelial-like cells and grew as compact sheet. These were of two types, smaller ones (Type A) and larger ones (Type B). The third type were multinucleated giant cells.

Epithelial-like Cells, Type A.—These were smaller, mononucleated, diploid ($2n = 6$) (Figs. 4, 5), and formed the major portion of the cell population both in the primary culture and in the established cell line. The cells were polygonal with well-defined cell boundaries, and measured from 10 to 20 μ in diameter. The cytoplasm was compact with uniformly distributed fine granules. A few broad pseudopodia-like ectoplasmic processes were observed in most of the cells. The nuclei were round, vesicular with well-defined nuclear membrane, and measured from 6 to 12 μ in diameter. The chromatin was thin and finely granular, with a distinct small nucleolus. Mitosis was frequently observed in these cells (Fig. 6).

Epithelial-like Cells, Type B.—These were few in number, both in the primary culture and in the established cell line, and were generally observed isolated or at the periphery of growing cell sheets. They were mono or multinucleated, round or polygonal, with ill-defined cell boundaries (Fig. 7). The cells were much larger in size and measured from 30 to 60 μ in diameter. The cytoplasm was densely granular, with a large number of small vacuoles usually distributed at the periphery of the cell. Many broad, pseudopodia-like ectoplasmic processes were observed in these cells. There were few, fairly large, bright bodies in the perinuclear region of the cytoplasm. Nuclei were round or oval, vesicular, with well-defined nuclear membrane. They measured from 14 to 25 μ in diameter. The chromatin was thinly granular with one or more round or irregular nucleoli. Mitosis was observed in these cells and the cells were generally tetraploid or polyploid.

Multinucleated Giant Cells.—These were very few in number both in the primary culture and in the cell line. They were syncytia-like in appearance with ill-defined mass of thin cytoplasm, having diffused boundaries (Fig. 8). They spread out from small masses of attached cells. The cytoplasm was thin, finely and sparsely granular, with a few vacuoles of varying size. A large number of round or oval, vesicular nuclei, measuring from 6 to 8 μ in diameter were found scattered in the cytoplasm. The chromatin was thin, and a distinct nucleolus was observed in each nucleus. Mitosis was often observed in the peripheral nuclei, and they had diploid chromosomes.

In addition to these three types, a few other types of cells, scattered in between the patches of epithelial-like cells, were observed only in the primary culture. Mitosis was not observed in any of these cells.

The cells from attached cell sheets as well as from floating hollow vesicles have been stored in liquid nitrogen in the culture medium containing 20% fetal bovine serum and 10% glycerol and successfully regenerated.

It was interesting to note that the general growth pattern of *A. vittatus* cells resembled closely with that of *A. ægypti*, though they were cultured in different media. In both the cultures epithelial-like cells formed the major portion of the cell population, which grew either in the form of monolayers on the glass surface or as spherical, multicellular hollow vesicles floating in the medium.

Attempts to culture *A. vittatus* larval cells in the medium in which several cell lines of other two closely related species, *A. ægypti* and *A. albopictus*, have been established by Singh,[2] were not successful.

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