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Cell movement *in vitro* using mosquito tissues, *Anopheles stephensi* Liston[1]

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Numerous studies have been undertaken using *in vitro* system as a tool in an attempt to elucidate various developmental phenomenon that occur in the *in vivo* system. One of the areas of study is concerned with the locomotory behavior of the cells as they relate to the changes in the shape of the multi-cellular organism during morphogenesis. However, a survey of literature reveals that investigations deal mostly with the vertebrate tissues. Comprehensive, in-depth study of this phenomenon have been dealt with by Curtis ('67), Weiss ('67), Trinhaus ('69) and Abercrombie ('70).

Studies of the locomotory behavior during morphogenesis using insect tissues may elucidate certain problems in developmental biology from an invertebrate perspective. This paper reports the preliminary studies on the mechanisms of morphogenetic movements of insect cells *in vitro*. It is a point of interest here, that the locomotory behavior of the insect cells *in vitro* appear similar to that of the vertebrate cell movement as they are reported in the literature.

Postembryonic development in insects involves the morphogenesis of tissues functional in both the larval and imaginal, as well as those of strictly imaginal function. Locomotory activities of cells during morphogenesis have been demonstrated: epidermal cells move by means of long, contractile filopodia (Wigglesworth, 1959), larval oenocytia become associated with testicular rudiment and fat bodies (Horsfall and Ronquillo, 1970) and motile haemocytes have been implicated during the sculpturing of the appendages (Whilten, 1969).

Newly hatched larvae of male and female mosquitoes *Anopheles*

stephensi were cut into fragments, trypsinized for 30 minutes at 27°C, resuspended in the nutrient medium in glass V-bottom bellco culture flasks. Incubation took place at 27°C in the dark.

Attachment of cells to the glass substratum was observed within a 48 hour period. Tissue cells move readily over the substratum, after attachment whether as outgrowth from periphery of an explanted chunk of tissue or dissociated suspension of cells. Mitosis accounts for further proliferation of the various cells.

1. First group of slides will introduce certain motile cell types.
2. Second group of slides will illustrate their locomotory behavior.
3. A short film sequence will show the motility as seen through time lapse cinemicrograph camera.

*All photographs taken of unstained line cells.

CONCLUSION:

Concentrated efforts to accumulate information on the mechanism of locomotory behavior during morphogenesis *in vitro* may elucidate certain developmental phenomenon *in vivo*. To say that cell behavior *in vitro* is analogous in some respect to that *in vivo* is quite intriguing and must be arrived at with some caution. For instance, it appears that *in vitro* the capability of cells to move require a substrate for attachment. *In vivo*, this situation is not a requirement. However, in our culture, floating epithelial cells occasionally organize into fairly contiguous sheets. Perhaps, this organization without a need for a substrate is analogous to the *in vivo* system. This observation remains speculative, since it is not clear how much of what we learn from cells *in vitro* can be applied

in vivo. Presently, the objective is to learn as much as one can by *in vitro* means, with the hope that such studies *in vitro* will emerge fruitful for solving problems in cellular morphogenesis. When more is known about cellular properties: surface adhesiveness; surface deformability, ultrastructural changes in cells during motility; contact inhibition between cells and ability of cells to form locomotory structures such as ruffled membrane, lobopodia and filopodia, then *in vitro* system may reveal much about *in vivo* activities.

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ABSTRACT SUBMITTED TO MIDWEST REGIONAL DEVELOPMENTAL BIOLOGY CONFERENCE 1971

Cell movement *in vitro* using mosquito tissues, *Anopheles stephensi* Liston[1]

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A study of cell movement *in vitro* using insect cells is presently in progress. This report deals with some preliminary findings involving locomotory behavior of several types of cells: fibroblast-like cells, and large epithelial cells exhibit random gliding movement. A thin ruffled membrane, the lamella, appears at the leading edge of the advancing cell. Behavioral patterns of this lamella during contact with cells of the same or different types will be discussed. Monolayering of these cell types appears to be due to the presence of contact inhibition. Small epithelial cells exhibit short range oscillatory movement. Clustering of these cells is apparent as the culture ages. It is likely that such a morphological alteration is due to a loss of contact inhibition. Certain cell types, polygonal in shape, form a continuous cell sheet which moves in a cohesive fashion. The lamellae of the marginal cells of this sheet exhibit ruffling and contact inhibition.

A time lapse photomicrograph will be presented to illustrate the above locomotory behavior patterns.

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