# Growth of Insect Cells in Tissue Culture. (31583)

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Since Grace reported(1) the establishment in tissue culture of strains of cells from ovarian tissue of the saturniid moth *Antheraea eucalpti* Scott, many unsuccessful attempts have been made to grow these cells in other laboratories. Since we have maintained these cells in monolayer culture for more than 2 years (with some 30 passages), a summary of our experiments may be helpful to others attempting to establish and maintain insect cells in tissue culture.

The cells initially obtained from Dr. Grace have been grown in monolayers in 1 × 6 inch glass tubes with teflon-lined screw-caps (Bellco Glass Co.) and in plastic flasks (Falcon Plastics Division, Becton, Dickinson Co.). Seven ml of Grace's medium(1) supplemented with 3% (v/v) of *Antheraea pernyi* sterile heat-treated hemolymph† was added to each 1 × 6 tube and 4 ml to each plastic flask (24 $cm^2$ surface). The initial cell count ranged from about $10^3$ to $10^4$ cells per ml. The tubes were placed on a slanting board (17° angle from horizontal) in an incubator at 28°-30°C. Medium changes have been routinely made on a weekly basis. Those cells found floating have been collected by centrifugation and used to start new tubes and flasks. Well developed monolayers covering the tube surface have usually been obtained within 3 weeks of starting the culture. As the monolayers are only loosely attached to the glass surfaces, care is needed in handling the tubes and flasks. The attached cells are aseptically scraped with a rubber policeman from the tubes once a month and these cell suspensions used to start new tubes and flasks. The glass tubes have not been specially washed or treated prior to use, and the sterile plastic flasks have been used as received from the manufacturer.

The cells grown under the above conditions have the morphology reported by Grace(1,2). Clumps of floating cells often disperse when transferred to new tubes and the cells adhere to the solid surface. Several experiments have been terminated due to yeast and bacterial contamination by organisms resistant to penicillin and streptomycin (the antibiotics present in Grace's medium(1)). Other antibiotics were not helpful in eliminating these contaminants(3).

Stock cultures of *A. eucalypti* cells maintained their viability for more than 9 months when stored at +4°C or at −185°C (vapor phase above liquid nitrogen). In this latter study the cells were suspended prior to freezing in Grace's medium(1) supplemented with 20% glycerol (v/v).

A major problem in our experimental program has been the difficulty in obtaining hemolymph. Since *Antheraea eucalypti* and the related *Antheraea pernyi* are not found in the United States, we tested hemolymphs (prepared by Dr. Grace's process) of a number of other pupae. Replacement of the *A. pernyi* hemolymph component of the medium with hemolymph from *Bombyx mori, Callosamia promethea, Hyalophora cecropia, Rothschildia orizoba,* or *Samia cynthia* resulted in eventual lysis of the cells. *Antheraea polyphemus* hemolymph was a satisfactory substitute for the *A. pernyi* hemolymph in experiments extending over a 6-month period.

Our experiments have shown that this line of insect cells can be maintained for indefinite periods in Grace's medium containing hemolymph of either *A. pernyi* or *A. polyphemus*. The techniques and equipment developed for culturing mammalian cells are adequate for the culture of insect cells.

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