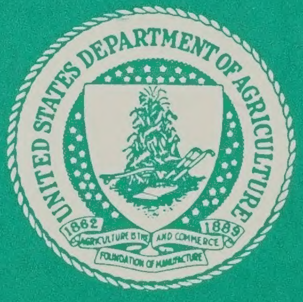
UNITED STATES DEPARTMENT OF AGRICULTURE
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AGRICULTURE IS THE FOUNDATION OF MANUFACTURE AND COMMERCE

FINAL TECHNICAL REPORT (MAY 1983-MAY 1985)

Utilization of Monoclonal Antibodies for Monitoring Regulation of Insect Reproduction

Specific Cooperative Agreement with the University of Maryland
No. 58-332U4-3-510

(1) Development of Monoclonal Antibodies for Monitoring Mosquito Vitellogenesis. - Monoclonal antibodies to a mixture of Aedes atropalpus and A. aegypti soluble yolk proteins were produced by fusing mouse splenocytes with myeloma cells in the presence of polyethylene glycol. Ascites fluid collected from mice inoculated with cloned hybridoma cells contained high specificity and affinity to the soluble yolk proteins of both Aedes species. A total of eight cell lines were produced. Monoclonal antibodies to these Aedes vitellogenins were characterized by a combination of gel electrophoresis and western blotting. An indirect double antibody sandwich ELISA was developed using a mixture of hybridoma antibodies for monitoring vitellogenin activity in individual mosquito hemolymph samples.

(2) Development of Hybridoma Antibody Production Protocol for Insect Neuropeptides. - An in vitro immunization procedure has been developed using murine thymoma cell EL-4 conditioned medium for the activation of antigen-primed B-cells. This improved protocol allowed the production of a panel of monoclonal antibodies to Drosophila yolk proteins using less than 1 nanomole of antigen. We believe this refinement will be valuable for the application of hybridoma technology to biologically active material that are hard to isolate and purify due to their low concentration in the biological fluids.

(3) Hormonal Basis of Adult Eclosion in the Gypsy Moth. - Eclosion hormone was found to control the stereotypic adult eclosion behavior of the gypsy moth. A bioassay for this neurohormone was developed utilizing pharate adult females, and comparisons were made with the Manduca wing assay. The distribution of eclosion hormone activity was confined to the central nervous system tissues including the protocerebrum, corpora allata/corpora cardiaca complex, thoracic and the last abdominal ganglion. Hemolymph ecdysteroid titers were determined daily throughout pupal adult development, and the peak activity period was found in 3-4 days pupae. Eclosion hormone activity in the brain, CA/CC complex started to increase when the ecdysteroid titer dropped to background levels. Eclosion hormone in the brain peaked in the pharate adult stage, was released in the hemolymph 1 hr prior to eclosion which coincides with the depletion of activity in the retrocerebral complex, and fell to undetectable levels after the adults emerged.

PUBLICATIONS

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