Nuclear Secretory Particles Associated with the Calyx Cells of the Ichneumonid Parasitoid
Campoletis sonorensis (Cameron)*

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Summary. The present study is an ultrastructural investigation of the calyx region of the ichneumonid endoparasitoid Campoletis sonorensis. It appears that synthesis of electron-dense secretory particles occurs within nuclei of calyx cells. The particles consist of an ovo-cylindrical electron-dense inner core and a surrounding unit membrane. After their formation the particles pass from the nucleus by budding through both membranes of the nuclear envelope. The particles, along with fully developed parasitoid eggs concentrate within the lateral oviduct lumen. Feulgen histochemical studies suggest the presence of DNA within the calyx fluid. The possible function of the particles is discussed.

Key words: Parasitoid — Nuclear secretory particles — Calyx cells — Insect immune system.

Introduction

The exact mechanism(s) by which insect parasitoids escape haemocytic encapsulation while within their habitual hosts is still unresolved. Two general theories regarding the prevention of encapsulation have been proposed. Salt (1965) speculates that successful parasitoids avoid recognition as foreign bodies while within the host, and this ability depends upon a property of the parasitoid egg surface. Walker (1959) and Streams and Greenberg (1969) suggest that the parasitoid actively inhibits or interferes with the encapsulation process either by the secretion of an inhibitory substance by the egg or by chemical(s) released by the adult female during oviposition.

If haemocytic encapsulation is indeed actively inhibited or suppressed by secretions from the adult female, then one possible source of the substance(s) may be the calyx region. Salt (1973) and Vinson (1972a, 1974) have reported that components of the fluid of the parasitoid calyx appear to affect the immune response.
of the host. In addition, it has been shown that secretions from the calyx of several parasitoids modify the growth, behavior and development of their hosts (Guillot and Vinson, 1972). The primary objective of the present study was to investigate the ultrastructure of the calyx region of the ichneumonid endoparasitoid *Campeolitis sonorensis* (Cameron) for evidence of a secretory product and if possible determine its site of synthesis.

**Materials and Methods**

**Insect Cultures.** Adult parasitoids were fed on a solution of honey and water absorbed by a cotton pad. Both adult parasitoids and parasitized host larvae were kept at a temperature of 27°C in a 12:12 light: dark photochamber. Larvae of the host, *Heliothis virescens* (Fab.), were mass reared according to the method described by Berger (1963) and fed on an artificial medium developed by Vanderzant et al. (1962).

**Microscopy.** Several ovaries were dissected from the abdomens of adult *C. sonorensis* females for examination by electron microscopy. They were fixed for 1 hour in a solution of 3% glutaraldehyde at 4°C and postfixed in 2% osmium tetroxide for 1 hour at 4°C. The fixatives were buffered in either 0.1 M sodium cacodylate (pH 7.4) or 0.1 M S-collidine (pH 7.0) solutions. Tissues were then bulk-stained for 12 hours in 1% aqueous uranyl acetate and routine dehydration and embedding procedures followed. Thin sections were cut with an LKB ultramicrotome and doublestained in uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). For negative staining, calyces were gently minced with fine forceps in a small drop of saline. Particles were picked up on carbon-coated grids and stained with 2% aqueous potassium phosphotungstate. All material was examined with an Hitachi HU-11E electron microscope at 50KV. Procedures for Feulgen histochemistry were those described by Pearse (1960).

**Observations**

The parasitoid used in this study, *C. sonorensis*, is able to develop successfully within a variety of hosts, including *H. virescens* (Lingren et al., 1970). The reproductive system of the female *C. sonorensis* consists of 2 ovaries, each of which contains 12 ovarioles. The ovarioles join proximally and open into the calyx, the anterior-most region of the lateral oviduct (Fig. 1). The calyx epithelial cells are enveloped by a double sheath: an outer cellular layer and an inner non-cellular membrane. The cellular sheath is a multinucleated, tube-like covering containing a network of longitudinal and circular muscle fibers.

The calyx epithelial cells are usually conical in shape and have the general dimensions of 32 × 26 µm (Figs. 2 and 3). The apical surfaces of the cells possess microvilli which extend into the calyx lumen. Unlike the lateral oviduct the calyx has no cuticular lining, and as result, the plasma membranes of the microvilli are in direct contact with the calyx lumen.

The nuclei of calyx cells are large, irregularly-shaped structures which constitute a major portion of the cellular volume (Figs. 2, 3 and 4). Cellular organelles such as mitochondria, Golgi complexes and free ribosomes are abundant, but appear to be more highly concentrated within the apical region of the cells. Intercellular spaces of various sizes are commonly located between adjacent calyx cells. Microtubules, 180 Å to 200 Å in diameter are distributed throughout the cytoplasm, but are especially evident at cellular junction sites. The septate desmosome and zonula adherens are the two primary forms of cellular junctions between adjacent calyx cells (Fig. 5).