# FORMATION OF TEMPORARY FLAGELLAR STRUCTURES DURING INSECT ORGANOGENESIS

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Cilia and flagella are rare in nongerminal tissues of arthropods, and are generally thought to be restricted to sperm and sensory cells in insects (2). Whitten (5) has reported the presence of kinetosomes at the base of microtrichia in the dipteran fly *Sarcophaga bullata*, but reports no evidence of the organization of fibrous elements characteristic of cilia or flagella. During an ultrastructural analysis of morphogenesis of the colleterial gland of the silk moth *Hyalophora cecropia*, we found the first example of paired flagella associated with an insect secretory cell. These structures are also unusual in that they serve a temporary role in morphogenesis and subsequently disappear at the terminal stages of differentiation.

## MATERIALS AND METHODS

Glands were dissected from female pharate pupae at specific stages of adult development, as ascertained from the timetable of Schneiderman and Williams (3), and were fixed at room temperature in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 90 min. Tissue was postfixed in 2% buffered osmium tetroxide for 30 min, dehydrated in acetone, and embedded in Epon-Araldite (Shell Chemical Co., New York, and Ciba Products Co., Summit, N.J., respectively) by the rapid infiltration method of Hayat (1). Sections were stained for 30 min in alcoholic uranyl acetate and for 4 min in lead citrate (4). Cuticular structures were prepared by boiling the gland in 10% KOH for a few seconds, rinsing with distilled water, and drying on Formvar-coated grids (Belden Mfg. Co., Chicago, Ill.).

## RESULTS AND DISCUSSION

The colleterial gland is an accessory component of the female reproductive apparatus, consisting of two long (12-cm) lobes with a common duct opening into the oviduct. It produces a viscous, glycine-rich, proteinaceous secretion that coats each egg as it is laid. In the pupa the cells that will form the gland are undifferentiated; in response to the molting hormone, ecdysone, they undergo a series of mitotic divisions. At the end of this mitotic period, centrioles are observed in the apical ends of the cells (Fig. 1 *a*). After mitosis, the cells realign themselves into clusters of three, with two outer cells wrapped concentrically about an inner one. The central cell then develops two flagella at the apical end (Fig. 1 *b*). When first formed, the flagella project from the apical surface of the central cell into the lumen of the gland. Each of the flagella contains a typical basal body and a second associated kinetosome (Fig. 2 *a*). The shaft of each flagellum contains nine doublet fibers in rather loose array (Fig. 2 *b*). The generally flaccid appearance of the shaft may indicate that active beating is not this organelle's primary function.

As differentiation progresses, the flagellar complex and the surrounding fibrous matrix are withdrawn into the central cell, leaving behind a cuticular duct surrounded by the two outer cells of the original cluster. The two flagella eventually disappear, leaving empty spaces in the lumen of the secretory cell (Fig. 2 *c*). The lumen of the gland eventually becomes lined with a thick cuticle, and at the site originally occupied by the flagellated cell, the cuticle is reflected inward to form a cuticular duct leading from the invaginated tip of the secretory cell into the lumen. The flagella thus serve as a mold around which the duct is formed. The isolated duct is shown in Fig. 2 *d*.

The structure of the kinetosome, with its nine triplet fibers surrounding a more complex central core and the 9 + 0 arrangement of doublet fibers in the shaft of the flagella, is similar to structures found in a variety of animal species. Thus, although insects have discarded motile cilia and flagella except for sperm movement, on the rare occasions when these organelles are found, they fit a well-established pattern. We know of no other reports of flagella acting as temporary aids in cellular differentiation, but the secretory cells of the colleterial gland resemble other insect secretory cells, and we anticipate that similar mechanisms may be found in secretory cell differentiation in other species.

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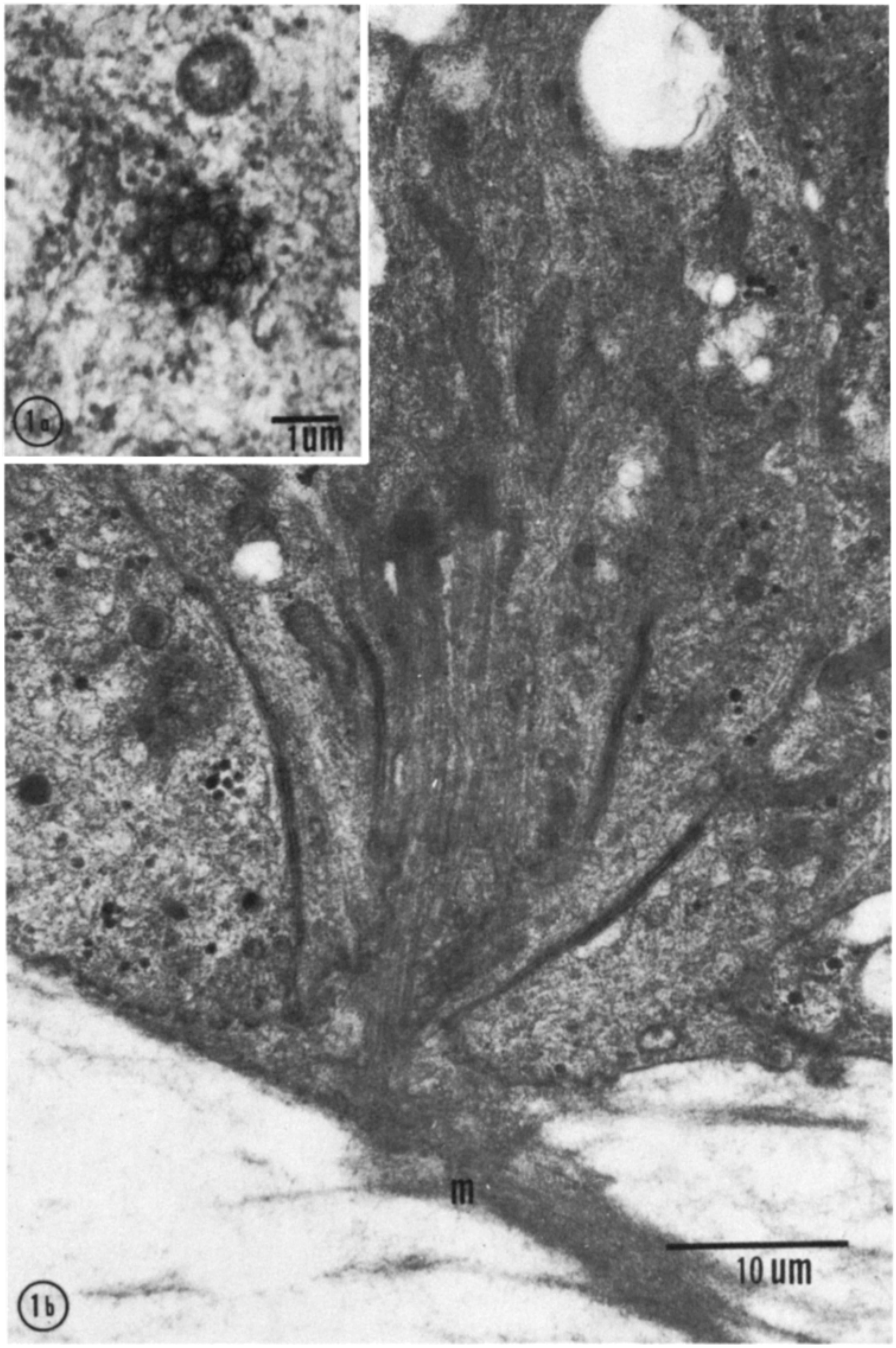


FIGURE 1 (*a*) Cross section of a centriole in the apical end of a collaterial cell at the end of the phase of active mitosis. × 92,000. (*b*) Apical end of a differentiating cell showing the basal bodies and a portion of the shaft of the two flagella. The lumen of the gland is at the bottom of the page, and the extracellular matrix (*m*) which surrounds the flagella is visible. × 26,000.

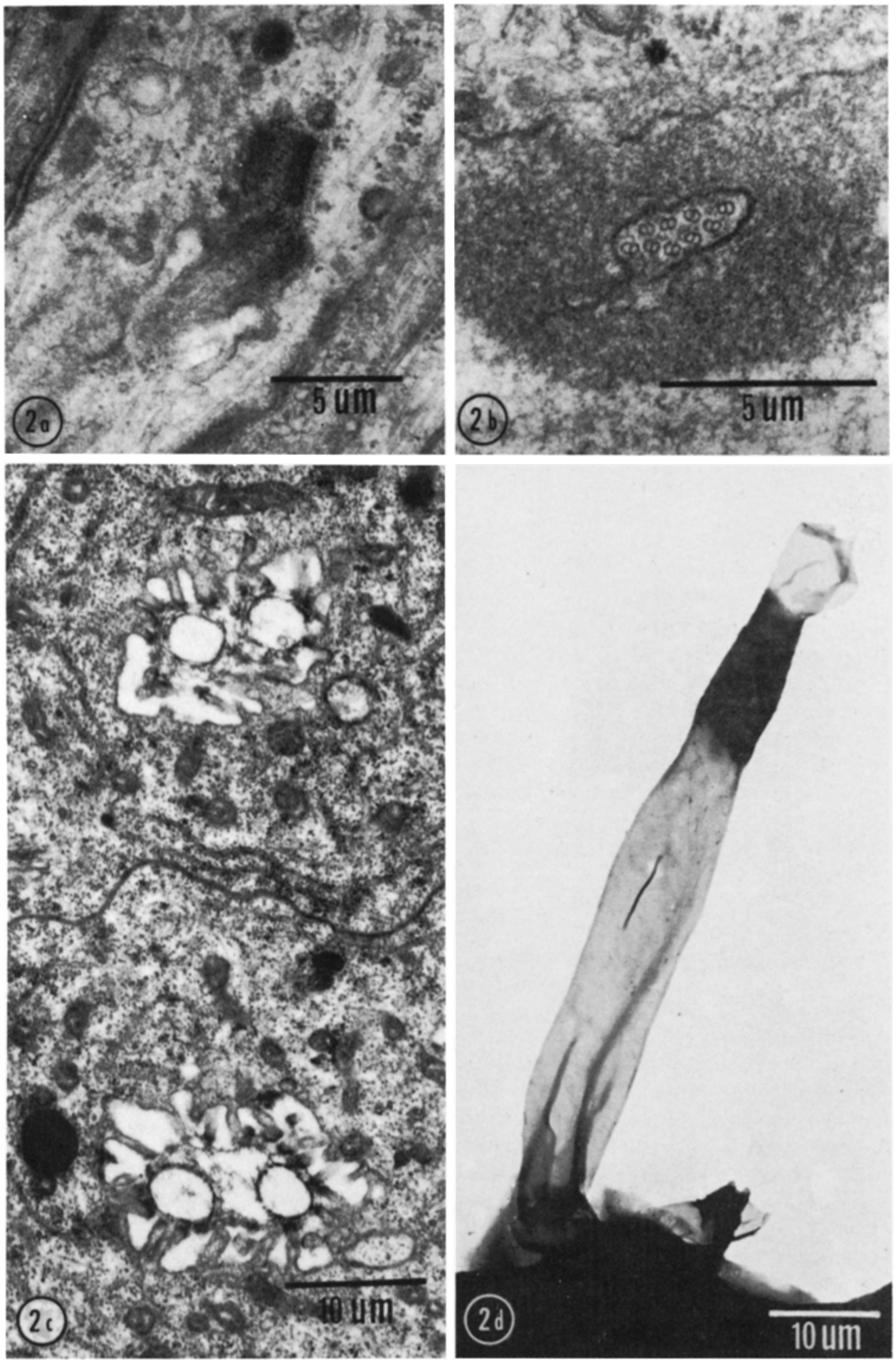


FIGURE 2 (*a*) Basal body and its associated kinetosome. × 38,000. (*b*) Cross section of one flagellum in the lumen of the gland. Note the nine doublet fibers. × 63,000. (*c*) Cross sections of the secretory apparatus of the two adjacent mature secretory cells, showing the spaces left by the withdrawn flagella. × 20,000. (*d*) A single secretory duct after the digestion of the cell with KOH. The cuticular lining of the gland is at the bottom of the photograph. × 20,000.

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