The Ultrastructure of the Developing Leg Disk of Calliphora erythrocephala

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Summary. The ultrastructure of the imaginal leg disk of Calliphora has been studied at three stages of the third larval instar. Several cell types can be distinguished: epithelial cells of the disk proper, the peripodial membrane and the apodemes; cells of the new tracheal branch; promyoblasts and sense cells.

Between the different parts of the disk epithelium no structural differences are found. A preliminary morphometric study reveals that nuclear size differences do occur. The apodeme cells are very long and contain numerous microtubules. The promyoblasts are characterized by dilatations of the ER. The cells of the new tracheal branch resemble those of the disk epithelium but are larger and have less lipid vacuoles. Sense cells are found in a sense organ on the last tarsal segment, in a monoscolopale organ at the dorsal side of the disk and in a polyscolopale organ in the femurotibia region. They are partially enclosed by one or more sheath cells. In the scolopale organs the dendrites form a long cilium, which at the distal end is embedded in an extracellular substance, produced by a cap cell. The dendrites of the tarsal sense organ have a characteristic sheath and run through the peripodial cavity and the stalk to the hypoderm.

Key words: Imaginal disk — Insecta — Diptera — Sense organ — Ultrastructures.

Introduction

The ultrastructure of several Dipteran imaginal disks has already been described (See Ursprung, 1972). The leg disk of Calliphora has been given a limited study by Agrell (1968, 1969). For the leg disk of Sarcophaga (Chiarodo and Denys, 1968) and Drosophila (Poodry and Schneiderman, 1970), the ultrastructure of the epithelial and adepietal cells has been described. Still little is known of the ultrastructure of the other cell types which constitute the leg disks and have been described only light microscopically (Sprey, 1970a).

In these disks seven different cell types could be distinguished: 1. Epithelial cells of the disk proper, which will form the hypoderm of the adult leg and distinct thoracic parts; 2. Epithelial cells of the peripodial membrane, which prospective significance is not clear; 3. Cells of the apodemes; 4. Epithelial cells of the new tracheal branch; 5. Promyoblasts (adepetal cells), which are the precursors of the imaginal muscles; 6. Sense cells and nerve fibers; 7. Haemocytes. The haemocytes do not belong to the imaginal disk, but they form in the prepupal period a characteristic part and therefore are briefly mentioned.

In this paper a description is given of the fine structure of the mesothoracic leg disk of Calliphora.

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Materials and Techniques

The investigated tissue has been derived from third instar larvae and prepupae of Calliphora erythrocephala (Meig.). Nine leg disks were studied at three developmental stages: the early feeding period, the early post-feeding period, and the prepupal period.

The best results were obtained by fixation of the disks in situ for 10 min. at 0°C in a mixture containing 0.8% glutaraldehyde and 0.7% OsO₄ in 0.1 M cacodylate buffer pH 7.4 (Hirsch and Fedorko, 1968), to which 4% sucrose had been added. Post-fixation was performed in vitro for 1 h. at 0°C in 1% OsO₄ in the same buffer (see also Ursprung and Schabtach, 1972).

Following fixation the disks were dehydrated in cold ethanol, soaked for 5 min. in propylene oxide and embedded in an Epon mixture.

Thin sections were contrasted with uranyl acetate and/or lead citrate (Reynolds, 1963; Venable and Coggeshall, 1965). Thick sections (±1 μm) were stained with toluidine blue for light microscopy.

Results

The Disk Epithelium

The epithelial cells of the disk proper show a very close resemblance to those of the leg disk of Sarcophaga (Chiarodo and Denys, 1968) and Drosophila (Poodry and Schneiderman, 1970). Only those features will be described, which deviate from or lack in the description of the previous authors.

At their lateral surface the cells are connected by the normal cell junctions: zonulae adhaerentes, septate desmosomes and gap junctions. Moreover, a number of intercellular interdigitations occur. They are especially numerous in cells rounding off for cell division. During mitosis the dividing cell remains in contact with the other cells by means of septate desmosomes.

As in the leg disk of Drosophila (Poodry and Schneiderman) we found a number of cytoplasmic bridges of Calliphora. These bridges are mainly found in the apical half of the cells, in contrast to Drosophila, in which they occur in the basal half. They are cylindrical with a length of about 1.6 μ and a diameter of 0.55 μ. The cylinder-wall is formed by folding of the plasma membranes. Sometimes septate desmosomes are visible within the fold (Fig. 1). The outer layer of the cylinder (300–400 Å) is very electron dense. The inner layer (450–500 Å) is less dense and has a more granular appearance. Within the core of cytoplasm a cisterna of rough endoplasmic reticulum is found near the cylinder-wall, with ribosomes only on the surface to the centre (Fig. 2). Structures comparable with cytoplasmic bridges are sometimes found surrounded by myelin-like figures within the cytoplasm of a cell (Fig. 3). This suggests that the bridges do not form permanent cell contacts but are probably broken down by one of the daughter cells.

Many microtubules run in the long axis of the cell. A few run parallel to the apical surface along the intercellular border. Sometimes they are seen in connection with the zonula adherens. This is very reminiscent to the apical skeleton of microtubules described by Burnside (1971) for the newt neurula epithelium.

The epithelial cells of different parts of the leg disk have an identical cell structure. However, we found clear differences in nuclear size (see below). Only little structural differences exist between the three developmental stages examined. Of these a gradual increase of the number of vacuoles and apical microvilli and an increase of the endoplasmic reticulum are the most distinct. Contrary to the findings in Drosophila, the vacuoles are clearly membrane bounded (see also Fig. 12).