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SHORT COMMUNICATIONS

THE PRESENCE OF GAP JUNCTIONS IN THE SEPTATE DESMOSOMES OF THE SALIVARY APPARATUS OF THE COCKROACH, *NAUPHOETA CINEREA*

BY DAVID J. MAXWELL

From the Department of Veterinary Physiology, University of Edinburgh, Edinburgh, EH9 1QH Scotland

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The acini of the cockroach salivary apparatus consists of two morphologically distinct types of cell. These have been described by Bland & House (1971) and have been called peripheral and central cells. Ducts which convey the saliva to the hypopharynx are also associated with the acini. Electrophysiological experiments (Ginsborg, House & Silinsky, 1974) have demonstrated the presence of low-resistance junctions between the cells of the acini (coupling resistance of less than 0·2 MΩ). Septate desmosomes are a common feature of the acini and ducts and have been observed to link all of the cell types (Bland & House, 1971). Although in the past several authors suggested that septate desmosomes could act as low-resistance couplings between cells (e.g. Loewenstein, 1966; Gilula, Branton & Satir, 1970), it now seems probable that gap junctions are the sites of low-resistance pathways between cells (for reviews see: Sotelo & Korn, 1978; Peracchia, 1980). Gap junctions are often incorporated in septate desmosomes (Berridge & Oschmann, 1972; Satir & Gilula, 1973; Noirot-Timothée *et al.* 1978).

The present study demonstrates that intracellularly injected Procion yellow dye passes readily from cell to cell in an acinus and in a duct, thus indicating the presence of low-resistance pathways between these cells (see Payton, Bennet & Pappas, 1969). The study also shows that structures resembling gap junctions are present in the septate desmosomes of the acinar and duct cells and that these junctions are freely penetrated by extracellular lanthanum nitrate, a heavy metal salt that is known to enter the spaces in gap junctions (Revel & Karnovsky, 1967).

Electrophysiological experiments were performed on isolated preparations of the salivary apparatus of adult cockroaches, *Nauphoeta cinerea* (Olivier, 1789), according to the method of Ginsborg *et al.* (1974). Glass micropipettes containing 4% Procion yellow dye dissolved in distilled water were used to record potentials from the glands and as ionophoretic electrodes to eject dye into impaled cells (Stretton & Kravitz, 1968; see also House, 1975). When a successful impalement was made a hyper-

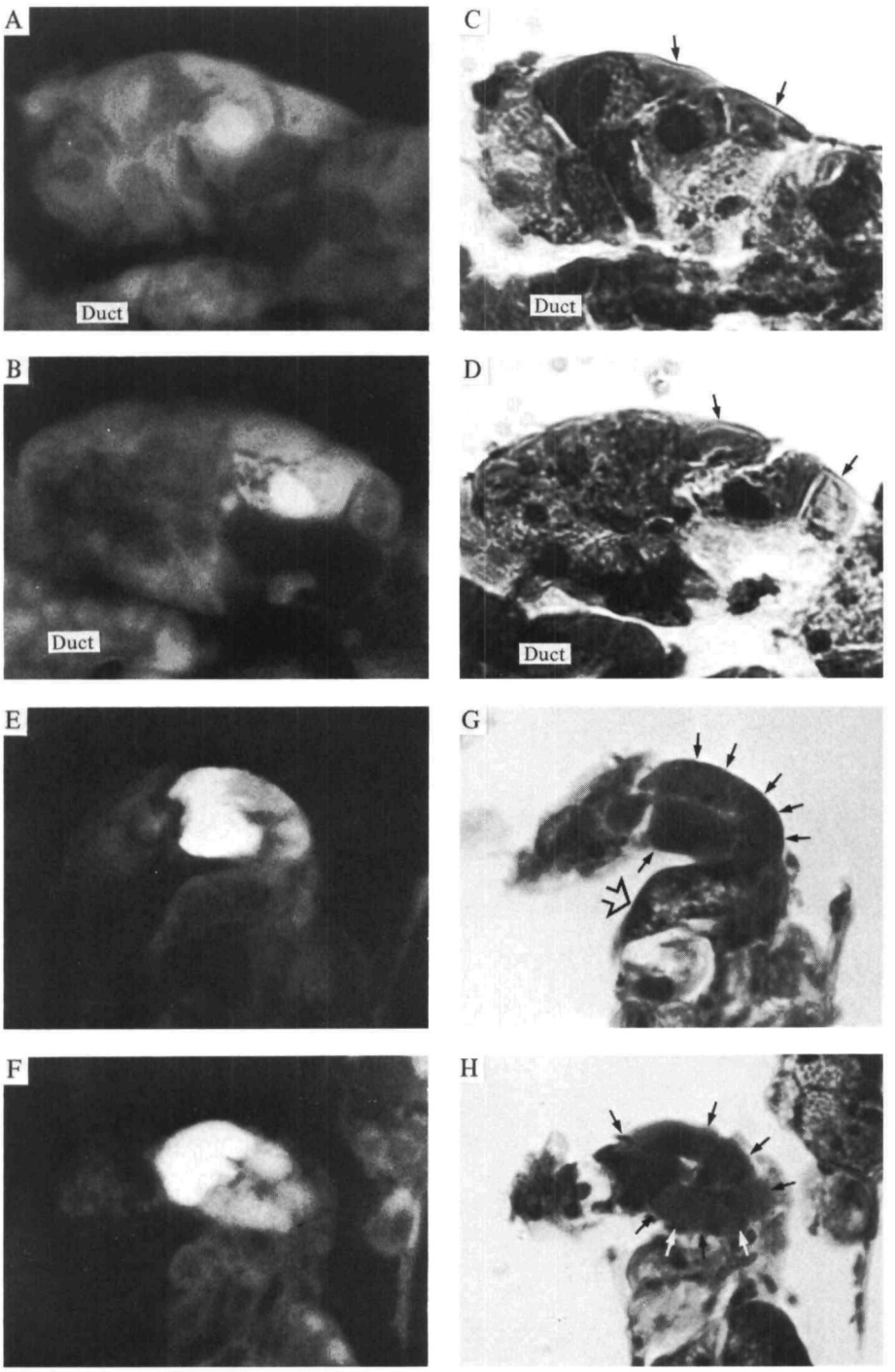
polarising secretory potential was evoked by electrically stimulating the salivary nerves (see Bowser-Riley & House, 1976). Procion yellow dye was then ionophoretically injected into the impaled cell using hyperpolarising current of 5×10^{-8} A. This current was generated by a Tektronix pulse generator (type 161) which produced square wave pulses of 100 ms duration at a frequency of 0·2 Hz. This procedure continued for 30 min during which secretory potentials were recorded at intervals of approximately 5 min to ensure that the electrode was still in position. At the conclusion of each experiment glands were fixed in 10% formol-saline and prepared for histological sectioning. Sections (20 μm thick) were viewed initially under u.v. light and then with phase-contrast microscopy to facilitate identification of injected cells. Some of the sections were rehydrated and stained with periodic acid–Schiff's reagent (P.A.S.) (Pearse, 1968) in order to differentiate between acinar cells and secretory duct cells; the latter react positively with this reagent (Bland & House, 1971). Tissue was fixed and prepared for electron microscopy according to the method of Maxwell (1978) except that some glands were post-fixed in a 1% solution of OsO_4 that contained 2% lanthanum nitrate in 0·1 M-s-collidine buffer at pH 7·4 (Revel & Karnovsky, 1967). Tissue that was treated with lanthanum was not subsequently contrasted with lead and uranium salts.

During the half hour period of injection, Procion yellow dye was found to diffuse rapidly between the acinar cells. Figs. 1 A and 1 B are adjacent sections through such an acinus. In u.v. light Procion dye is seen to fluoresce within a number of the cells. The cell types can be identified in Figs. 1 C and 1 D which is the same tissue, stained with P.A.S. In five such experiments both peripheral and central cells were found to contain dye. On one occasion a duct cell was impaled and injected with dye. In this case dye was also found to diffuse easily between the duct cells (Fig. 1 E, 1 F). Normal secretory potentials were recorded from this duct. Figs. 1 G and H show that the injected cells reacted in a positive manner with P.A.S. thus confirming that they are duct cells and not acinar cells.

Septate junctions were found to be a common feature between the cells of the acini and also between the duct cells. On a number of occasions, structures that had the appearance of gap junctions were seen to be present in these septate desomosomes (Fig. 2 A). The narrow spaces between the close membrane appositions of these structures were penetrated by lanthanum nitrate which was observed as an electron-dense deposit in uncontrasted tissue (Fig. 2 B).

The cell couplings of the cockroach salivary gland have been investigated in order to categorise the low-resistance pathways that were demonstrated by the electrophysiological experiments of Ginsborg *et al.* (1974). Procion yellow dye passes with ease

Fig. 1. A and B are fluorescence micrographs of 20 μm thick adjacent sections through an acinus of the cockroach salivary gland. Procion yellow dye was injected into one of the cells and has diffused into at least two others. The cells may be identified from plates C and D (which is the same tissue stained with P.A.S.) as two peripheral cells (arrows) and a central cell (dark nucleus). (A–D, × 435). E and F illustrate adjacent sections through part of an acinus and a duct. In this case a duct cell was injected with dye which can be seen to fluoresce in several cells. The duct cells may be identified in plates G and H which is the same tissue stained with P.A.S. Note the dense appearance of the duct cells (arrows) when compared with the rest of the acinus. In plate G a central cell is indicated for comparison (open arrow) (E–H, × 350).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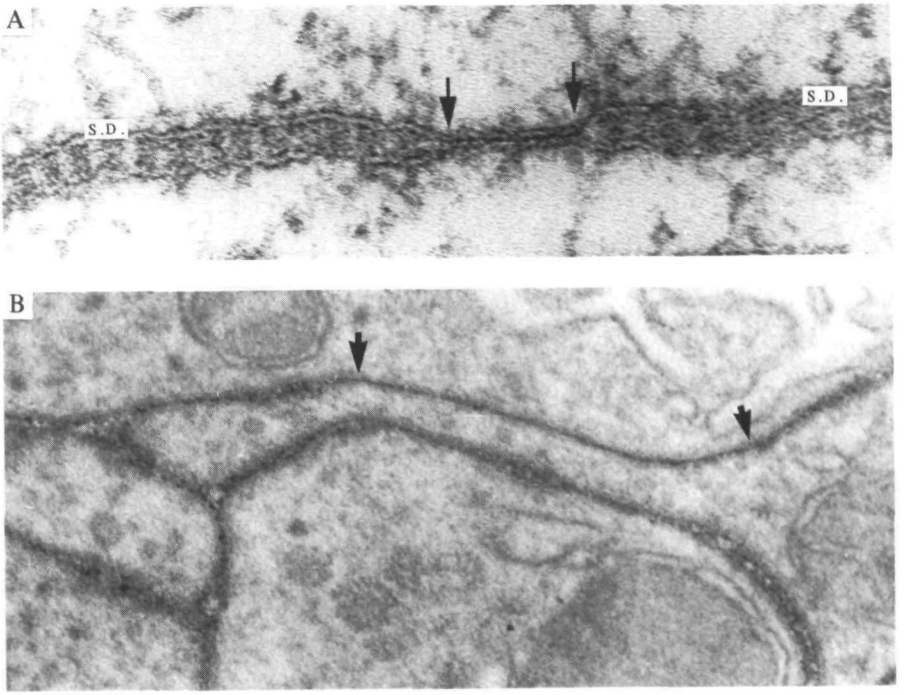

Fig. 2. A is a normally stained electron micrograph illustrating a presumed gap junction (between the arrows) that is situated in a septate desmosome (S.D.), located between acinar cells (×240000). B illustrates part of a duct in which a long gap junction (between the arrows) has been penetrated by lanthanum (×90000, uncontrasted).

from cell to cell when injected into a single acinar or duct cell. This observation supports the electrophysiological evidence that central and/or peripheral cells are linked by low resistance pathways and provides new evidence suggesting that this is also the case with the duct cells.

Payton *et al.* (1969) have shown that Procion yellow dye passes through gap junctions and in this study a number of structures resembling gap junctions have been observed in the septate desmosomes of the gland. Lanthanum was able to penetrate the close appositions of these structures showing that a gap is present; on the other hand true tight junctions do not permit the passage of extracellular tracers through the membrane appositions and hence are ascribed a barrier function (Brightman & Reese, 1969; Lane, 1972; Claude & Goodenough, 1973).

In conclusion it seems that the cells of the cockroach salivary gland, like those of other insects (e.g. see Loewenstein, 1975), communicate via low-resistance pathways. The sites of these pathways are probably gap junctions which are present in the septate desmosomes that link the cells. Septate junctions seem to play an important role in cellular cohesion and may also act as barriers to diffusion (Lord & DiBonia, 1976; Noirot-Timothée *et al.* 1978; Green, Bergquist & Bullivant, 1980), although some authors do not accept this secondary function (e.g. Lane, 1979).

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