Reports

Intercellular Communication: Renal, Urinary Bladder, Sensory, and Salivary Gland Cells

Abstract. *In four epithelial cell systems (salivary gland, renal, urinary bladder, and sensory cells) cells are interconnected as far as much of their ion content is concerned. In the salivary gland and renal epithelia, all cells of the epithelium are interconnected; and communication between a given cell and any of its nearest neighbors is equally good. In the bladder and sensory epithelia, communication appears to be more restricted, manifesting itself in chains of connected cells in the former, and in small groups of connected cells in the latter. The permeability of the cell membrane at the junction between connected cells is several orders of magnitude greater than it is at the cell surface bordering the exterior of the cells. Each connected cell ensemble functions as a system with a fairly continuous cytoplasmic core bounded by a diffusion barrier which is continuous along the entire outer surface of the system. As a result, ions move rather freely from cell to cell, but not from cell interior to exterior. Intercellular communication in at least three epithelia is associated with the presence of certain close-junctional membrane complexes.*

I

It has recently been shown for the case of a salivary gland (*Drosophila*) that cells are interconnected as far as much of their ion content is concerned. At the junctions between these cells, membrane permeability is so high that ions diffuse rather freely from one cell to another (*1*). We have now examined the cell junctions of four other epithelia as to their ion communication: salivary gland and renal tubular cells of *Chironomus,* sensory epithelial cells of elasmobranchs, and urinary bladder cells of toad. We chose the first two because of their large size (cell diameter, 100 to 200 μ), transparency, and single layer distribution, which makes them well suited for electrophysiological analysis (Fig. 1). The latter two are in all these respects less advantageous. We chose them because we wanted to have samples of vertebrate material and of a sensory epithelium, and the toad bladder and elasmobranch epithelia were the best material we knew of at the time (*2*). We found that all four epithelia have interconnected cells.

Our method for demonstrating intercellular ion communication consists essentially of passing electric current between the interior of a given cell and the exterior, and measuring the resulting resistive voltage drops across the membranes bordering the exterior of this cell and of an adjacent one (Fig. 2, inset). (The cell membranes in all these epithelia show no signs of excitation and little or no rectification to a wide range of current.) The ratio of the voltages, V_{II}/V_{I}, hereafter referred to as the "coupling ratio," provides a convenient qualitative index of intercellular communication. (Voltage was measured roughly midway between cell junctions for this purpose.) Under favorable experimental conditions, the actual resistance of the junctional membrane surface may be determined (*3*).

Salivary gland cells. Figure 2 illustrates the result of a typical experiment with salivary gland cells of *Chironomus thummi,* fourth instar. The gland was isolated in a saline solution. Currents of a given intensity were passed along the interior of the epithelium from a microelectrode inside a cell, and voltage drops were measured within a series of cells in the epithelium. The coupling ratio is so high (0.83) that the electric field of the current can be recorded from cells a considerable distance away from the current source. The cells in this epithelium are clearly connected.

The coupling ratio changes with development of the animal. It averages 0.8 in the early prepupae and 0.04 in the late prepupae (see also Fig. 2*A*). The decrease in the coupling ratio is paralleled by a decrease in area of junctional membrane.

The epithelium consists of a single chain of gland cells. The effective resistance in the cell-to-cell direction along the cell interiors of the chain, "core resistance," found by linear core conductor analysis of voltage attenuation data, such as those of Fig. 2, is on the average 5×10^{6} ohm/cm in the fourth instar stage (*3*). This is equivalent to a continuous core of specific resistance of 140 ohm-cm. From this, the cell length, and the upper limit of junctional membrane area (2×10^{-4} cm^2) estimated from electron micrographs, one calculates a specific membrane resistance of 1 ohm-cm^2 for the junctional membrane surface. This estimate takes the entire contact area—that is, both the "convoluted" region and the tight "septate" junctional region (*3*) —as the electrical junction and attributes the core resistance entirely to junc-

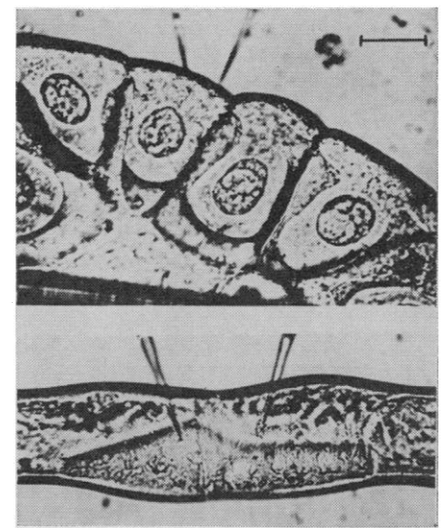

Fig. 1. Microscope views (brightfield) of living, unstained cell material. The tips of two microelectrodes (out of focus) are visible in the field. (Top) Salivary gland of *Chironomus,* fourth instar. (Bottom) Malpighian tubule of *Chironomus,* prepupa. Calibration, 50 μ.

tional barriers; it thus gives an upper limit of resistance. If one takes the "septate" junction to be the effective electrical junction, the specific junctional membrane resistance is 0.6 ohm-cm^2. By contrast, the specific resistance of the nonjunctional membrane surface bordering the exterior is at least 3 to 4 orders of magnitude greater (Table 1).

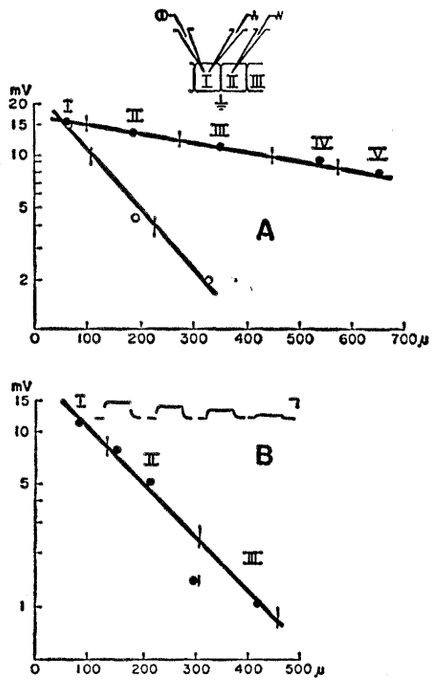

Fig. 2. Intercellular communication. (*A*) Salivary gland cell (*Chironomus*). Gland isolated in saline solution. Changes in transmembrane potential produced by outward current recorded at varying distance from the current source. Rectangular pulses of 10^{-7} amp and 100-msec duration were passed between a microelectrode inside cell I and the extracellular fluid (ground), and the resulting "steady state" voltages (ordinates) were recorded between the interiors of cells and the extracellular fluid. At zero current, the transmembrane potential was 30 mv, cell interior negative; the ordinates give the change in potential in this and following figures. The cells in this epithelium are arranged in a single chain; abscissa gives the distance between recording site and current source along this chain. Data from fourth instar (solid circles) and prepupa stages (open circles). Vertical lines on the curves mark the approximate location of cell boundaries (19°C). (*B*) Malpighian tubule cells (*Chironomus*). Tubule isolated in saline solution together with a piece of intestine. Experimental procedure and notation as in *A*. Current: 5×10^{-8} amp; pulse of 100-msec duration. Abscissa gives the distance along the axis of the tubule (19°C). Membrane potential at zero current, 40 to 50 mv. (Inset) Samples of oscilloscope records of membrane voltages from first four points. Calibration: 5 mv, 25 msec.

Malpighian tubular cells. Malpighian tubules of *Chironomus thummi* present another system with good intercellular ion communication (Fig. 2*B*). The average coupling ratio is 0.48. Because of the very favorable geometry of the system—a rather uniform cylindrical tube made of a single layer of cells (Fig. 1)—membrane resistance can be determined with a high degree of accuracy. Standard core conductor analysis, based on data for voltage attenuation, of the kind illustrated in Fig. 2*B*, and on estimates of surface area, as given by electron micrographs, gives core resistances ranging from 19×10^6 to 290×10^6 ohm/cm, specific core resistances from 420 to 5900 ohm-cm, and an upper limit of junctional membrane resistances of 30 ohm-cm^2. Here again the membrane resistance of the cell junctions is at least 2 orders of magnitude less than that of the nonjunctional membrane bordering the exterior.

II

Sensory epithelial cells. The sensory epithelium of Lorenzinian ampullae—the large sense organ of elasmobranchs—contains cells which are in close contact with one another. Some of these cells, in turn, are in close relation with nerve fibers (*4*). In the dogfish (*Mustelus canis*) of the waters off Cape Cod, and in the dogfish (*Mustelus mento*) and ray (*Raja psammobatis*) of the Chilean coast, the cells are sufficiently large (10 to 20 μ) to be explored by our technique. *Mustelus mento* and *Raja psammobatis* offered the more suitable material. We have recorded from cells of the central region in the ampullae. Some of them are electrically connected.

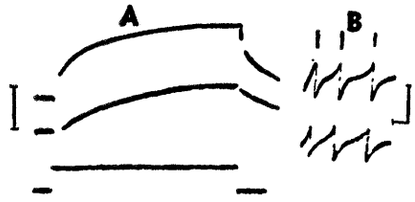

Fig. 3. Electrical connection in the sensory epithelium of a Lorenzinian ampulla (dogfish). (*A*) Current of 4.2×10^{-8} amp (lower oscilloscope beam) was passed with an electrode placed in a sensory epithelial cell, and the resulting changes in membrane potential in this cell (upper beam) and an adjacent one (center beam) are displayed. Electrode arrangement as in inset of Fig. 2. Calibration for upper and center beams, 20 mv. Duration of current pulse in lower beam, 132 msec; all beams have same velocity. (*B*) Nerve impulses recorded externally with microelectrodes from two sensory nerve fibers (two beams) at the base of two adjacent and electrically connected epithelial cells. Note the synchrony of impulses in the two fibers. Calibration: 200 μv, 20 msec.

The ampullae were isolated from the animals and trimmed down to their ampullar swellings (*5*), and their sensory epithelium was approached with microelectrodes from the tubular side. Figure 3*A* illustrates an example in

Table 1. Conductive characteristics of some epithelia.

Epithelium	Cell coupling ratio* V_{II}/V_I	Space constant* (10^{-4} cm)	Normalized space constant† $\lambda(p/a)^{1/2}$ (10^{-2} cm$^{1/2}$)	Lumped specific core resistance* R_i (ohm-cm)	Membrane specific resistance (ohm-cm^2)	
					Junctional maximum‡ R_m	Outer surface§ R_m
			Drosophila, 3rd instar ¶			
Salivary gland	0.93 ± 0.09	1100 ± 110	50–85	150 ± 30	0.3 –12	10,000
			Chironomus, 4th instar			
Salivary gland	0.8 ± 0.1	750 ± 150	180–290	140 ± 40	0.6 – 1	9000
			Chironomus			
Malpighian tubule	0.5 ± 0.2	260 ± 140	72–	420 – 5900	–30	3000
			Raja, Mustelus			
Sensory, Lorenzinian ampulla	0.08 to 0.5					
			Bufo			
Urinary bladder	0.4 to 0.8 #	18	16–33	180	0.02–~0.4	≲100

* Mean values with their standard deviations. † p and a are, respectively, perimeter and effective cross-section area of cell chain, not corrected for membrane infoldings. Lower and upper limits, based on an effective electrical-junctional area, equal, respectively, to the total cell cross-section and to the cross-section of the tight membrane junctions (septate junctions and *zona occludente*), are shown. ‡ Values calculated on the assumption that entire core resistance is due to functional membranes. Low and high values are based, respectively, on electrical junctional areas equal to the areas of the tight junctions and to the total junctional areas corrected for infoldings of membrane. § Corrected for infoldings of membrane. ¶ Data from reference *1*. # Range reflects uncertainty in location of cell boundaries.

which resistive voltage drops produced by a pulse of current were recorded in two adjacent cells of the sensory epithelium. In exploring the electrical field around a given cell, one finds electrical interaction to be restricted to few cell neighbors only. Apparently the epithelium is organized in groups of connected cells, the various groups being independent of one another. It is difficult to ascertain the extent of a group with the present technique because a negative finding, of course, does not necessarily mean absence of connection. From systematic mapping of electric fields, one gets the impression that there are very few connected cells, perhaps only two or three, to a group.

Occasionally our electrodes picked up action potentials in piercing through epithelial cells. These potentials were presumably from nerve fibers or their endings. When such action potentials happened to be recorded at the bases of two adjacent epithelial cells with demonstrable electrical connections, they were synchronous (Fig. 3*B*) (*6*).

III

Urinary bladder cells. The urinary bladder epithelium of the toad *Bufo marinus* offers another interconnected cell system. But the general pattern of connection differs from that in gland and renal epithelia described above in that it is directional.

The procedure was to isolate a piece of bladder in Ringers solution; to insert a microelectrode into a given cell of the epithelium; to pass rectangular current pulses of equal intensity between cell interior and exterior on the mucosal side; and, with a second intracellular microelectrode, to explore systematically the neighboring cells for resistive voltage drops across their membranes on the mucosal side (*7*). The epithelium is mostly a two-dimensional sheet of cells (*8*), and we expected to find an electric current roughly radially symmetric around the source. To our surprise we found that it follows only particular paths. In the example of Fig. 4, the voltages resulting from the current are recorded in a string of cells marked by *x*, while in other cells, marked by *o*, no voltage at all could be recorded, even close to the current source.

It appears, therefore, that ion communication is channeled here. Each channel consists of a chain of tightly connected cells; the chain is about one cell wide and branches at intervals. The full exploration in length of a chain was, in the present work, limited by the spatial decrement of the electric field. But from experiments such as the one illustrated in Fig. 3 it is clear that chains many cells long (100 μ) are common.

The epithelium evidently comprises many chain segments of this kind lying side by side (Fig. 4*C*). The relationship between chain segments is not clear. Contiguous segments which appear independent because no electrical coupling was detected between them may in fact be independent chains, or, alternatively, they may be connected at some distance. The connecting paths between chains, in the latter case, would simply have to be longer than the chains we explored, which were about 100 μ at the longest.

Within a given chain, cells clearly communicate with each other through low electrical resistance. Core conductor analysis leads to the values of 3.1×10^3 ohm-cm and 9.3×10^8 ohm/cm, respectively, for the surface resistance and core resistance of unit length. To get a rough idea of the specific resistances to which these correspond, we may assume the cell chain to be equivalent to a string of corrugated cylindrical cells 5 μ in diameter, 5 μ long, and joined base to base. The resulting specific resistance of the surface bordering the exterior is in the range 5 to 130 ohm-cm^2, depending on the correction made for corrugation as seen in electron

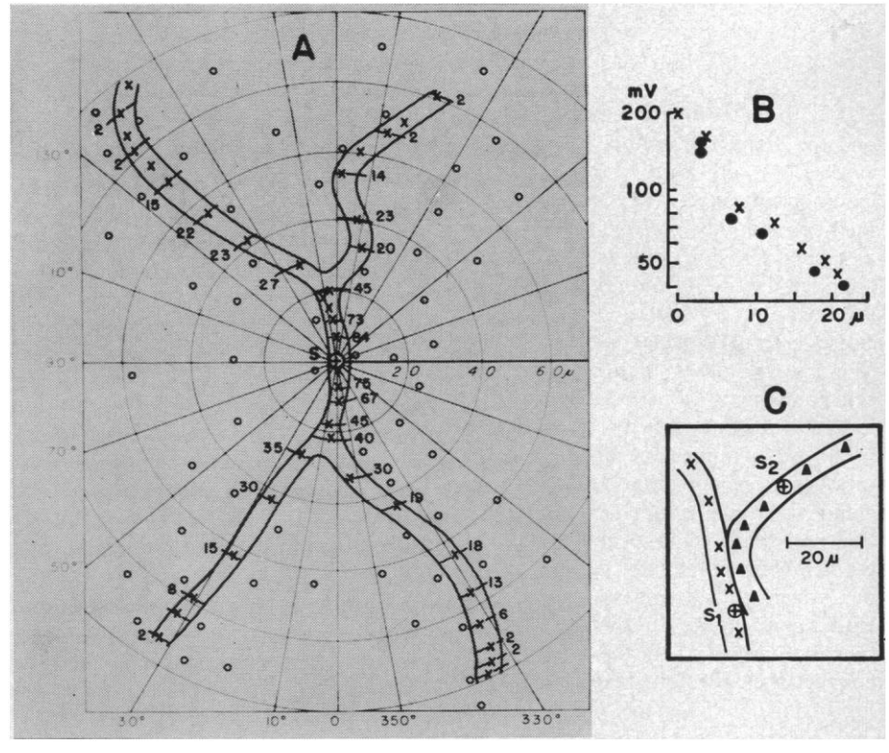

Fig. 4. Urinary bladder (toad). Rectangular pulses of current (1.5×10^{-7} amp; pulses of 100 msec duration) were passed with a microelectrode inside cell *s*, and the resulting changes in membrane voltage were recorded from surrounding cells (8°C). (*A*) Map of the transmembrane potential changes around the current source. Cells in which no voltage changes were detectable are marked by open circles; cells with recordable voltage changes, by crosses. The numbers give the respective voltage changes; boundaries of the cell chain are roughed in. Cell diameter (see text), approximately 5 μ. (*B*) Voltage in segment between branchpoints in *A* versus distance from *s*. Crosses and circles correspond to points above and below *s*, respectively. [In evaluating the space constant, the five points nearest the source were disregarded, since the electric field here is not yet coaxial with the cell chain (*14*).] (*C*) Two contiguous chain segments. Here the potential field of the bladder epithelium was explored for two successive locations, s_1 and s_2, of current source. Procedure and scale as in *A*. Crosses and triangles mark location of cells from which voltages were recorded when s_1 and s_2, respectively, were the current sources. The solid lines give the approximate boundaries of the chain beyond which no voltages were detectable. Most of the cells in the field, particularly those at the junction of the two chain segments, were explored twice for their electrical coupling: once with respect to s_1, and again, with respect to s_2. No electrical connections between cells of the two chain segments were detectable.

micrographs (*9*). If we attribute the core resistance entirely to the junctional membranes and take the area of electrical junction of a cell pair as 5 μ^2 (*10*), we obtain 0.02 ohm-cm^2 for the specific resistance of junctional membrane. The equivalent specific core resistance is 180 ohm-cm.

IV

General aspects. The specific core resistances along the chains of interconnected cells in the renal, salivary gland, and bladder epithelia are not substantially greater than those of cytoplasm alone. This underlines the absence of significant barriers to diffusion, of at least some ionic species, in the cell-to-cell direction along the chains. Diffusion in the interior-to-exterior direction, on the other hand, is limited by a strong barrier all along the surface of the cell chain, as is shown by the high surface resistances in these epithelia (Table 1).

The four epithelia described here, and the gland cell epithelium described earlier (*1*), all seem to conform to a common scheme: a cell system with a fairly continuous interior, at least as far as much of its ion content is concerned, bounded by a diffusion barrier which extends and is continuous along the entire external surface of the system. The system rather than its individual cell components constitutes the unit of ion environment. There are differences in spatial arrangement of the systems in the various epithelia. In the salivary gland and renal epithelia, communication between a cell and any of its nearest neighbors is equally good, and this one system thus comprises virtually the entire epithelium. In the bladder and sensory epithelia, on the other hand, connections are more restricted, resulting in tortuous channels in the former, with, perhaps, several such systems arranged in parallel.

At the structural level these epithelia have one feature in common: their cell membranes are closely joined at their surfaces of contact. In salivary gland (*11*) and Malpighian tubular cells (*12*), the membranes are joined periodically, and in bladder cells (*10*) they are joined at a certain region of their surfaces of contact (*13*). These junctional regions are likely to be the coupling elements which permit diffusion from cell to cell.

The general functional adaptation of intercellular communication is probably one that allows the cell system to operate in concert. Ions, metabolites, and, perhaps, hormonal and genetic information may conceivably flow from one cell to another, with economy of external nervous and humoral controls (*1*). A special variant shows itself in the sensory epithelium. Here intercellular communication appears to have adapted to a properly electrical role, namely, to transferring and synchronizing excitation over various sensory units. The connected system acts as a signal amplifier in this case.

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* Key to authorship of sections: Loewenstein, I to IV; Socolar, III, IV; Higashino, I, II; Kanno, III; Davidson, II.

References and Notes

1. W. R. Lowenstein and Y. Kanno, *J. Cell Biol.* **22**, 565 (1964).
2. We are indebted to Dr. G. Palade, Rockefeller Institute, who suggested to us the toad urinary bladder as a convenient material.
3. For details of technique, calculations, and definitions, see W. R. Loewenstein and Y. Kanno (*1*).
4. A. Barets and T. Szabo, *J. Microscop.* **1**, 47 (1962); V. B. Wigglesworth and M. M. Salpeter, *J. Insect Physiol.* **8**, 229 (1961).
5. W. R. Loewenstein, *Nature* **188**, 1034 (1960).
6. Part of this work was done at the Estación de Biología Marina, Universidad de Chile, Montemar. The University of Chile and the director of the Marine Biological Station provided most generous hospitality and facilities. We are indebted to them.
7. A major difficulty was the fragility of the cell membranes in the urinary bladder epithelia. The membranes often ruptured upon penetration. In such a connected system this, of course, ended the experiment. Hence the number of cell chains which could be identified, as well as the number of experiments which could be completed, was severely limited. Acceptable results were obtained only with electrodes of especially fine tips, and then only at the expense of a large number of trials. The bladder was kept at about 8°C to minimize smooth muscle movement. For reviews of physiological studies on the bladder, see A. Leaf, *Gen. Comp. Endocrinol.* **2**, 148 (1962); H. Frazier, *J. Gen. Physiol.* **45**, 515 (1962); and Peachey and Rasmussen (*8*).
8. L. D. Peachey and H. Rasmussen, *J. Biophys. Biochem. Cytol.* **10**, 529 (1961).
9. L. D. Peachey provided the electron micrographs used for surface estimates of bladder epithelial cells. We are indebted to him.
10. The "electrical" junction is taken here as a band of 0.5 × 10 μ which appears as tight intercellular junction (*zona occludente*) in electron micrographs of G. Palade (personal communication), of L. D. Peachey (personal communication) (see also *8*), and of J. K. Choi [*J. Cell Biol.* **16**, 53 (1963)].
11. J. Wiener, D. Spiro, W. R. Loewenstein, *J. Cell Biol.* **22**, 587 (1964).
12. I. Tsubo and P. W. Brandt, *J. Ultrastruct. Res.* **6**, 28 (1962).
13. Structural details of the junctions in the sensory epithelium of Lorenzinian ampullae are not known to us.
14. Compare, for example, P. Fatt and B. Katz, *J. Physiol.* **120**, 171, 177 (1953).

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Medial Neurosecretory Cells as Regulators of Glycogen and Triglyceride Synthesis

Abstract. *In the female mosquito, medial neurosecretory cells restrict synthesis of glycogen from sugar and stimulate triglyceride synthesis. Removal of these cells greatly increases the storage capacity for glycogen at the expense of triglyceride storage.*

When emerging female mosquitoes are starved, glycogen and triglycerides virtually disappear and subsequent feeding produces new pools of these products (*1*). In the experiments described here, we used this technique to study the role of the neurosecretory system in glycogen and triglyceride metabolism.

Three species, *Aedes taeniorhynchus*, *A. sollicitans*, and *A. aegypti*, were reared as described previously (*2*). In mosquitoes, two groups (12 to 15 cells each) of medial neurosecretory cells lie in a cluster on either side of the midline of the protocerebrum, easily distinguishable by their bluish appearance. Within 1 hour after emergence of the mosquitoes, these cells were surgically removed along with a small amount of surrounding tissue. The technique was the same as that described by Thomsen for the blowfly *Calliphora erythrocephala* (*3*), except that the head capsule was sealed by drying with air.

The medial neurosecretory cells do not affect the utilization of glycogen or fat, because, when experimental and control animals were starved, glycogen and triglycerides disappeared in the same time. Starved mosquitoes were fed, in a single dose, 2 μl of a solution of equal parts of glucose and fructose from a micropipette; the insects were