

Electrical Properties of an Excitable Epithelium

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ABSTRACT The exumbrellar epithelium of the hydromedusa, *Euphysa japonica*, is composed of a single layer of broad (70 μm), thin (1–2 μm) cells which are joined by gap junctions and simple appositions. Although the epithelium lacks nerves, it is excitable; electrically stimulating the epithelium initiates a propagated action potential. The average resting potential of the epithelial cells is -46 mV. The action potential, recorded with an intracellular electrode, is an all-or-nothing, positive, overshooting spike. The epithelial cells are electrically coupled. The passive electrical properties of the epithelium were determined from the decrement in membrane hyperpolarization with distance from an intracellular, positive current source. The two-dimensional space constant of the epithelium is 1.3 mm, the internal longitudinal resistivity of the cytoplasm and intercellular junctions is 196 Ωcm , and the resistivity of both apical and basal cell membranes is greater than 23 $\text{k}\Omega\text{cm}^2$. Although the membrane resistivity is high, the transverse resistivity of the epithelium is quite low (7.5 Ωcm^2), indicating that the epithelium is leaky with a large, transverse, paracellular shunt.

INTRODUCTION

The nervous system is the usual but not the only channel used by animals for rapid communication between body parts. A few animals have excitable epithelia in addition to excitable neurons—epithelia which can conduct signals over wide areas without decrement. Epithelial or neuroid conduction has been demonstrated in hydrozoan coelenterates (Mackie, 1965, 1976; Mackie and Passano, 1968; Spencer, 1974 *a,b*, 1975, 1978; Campbell et al., 1976), in larval amphibians (Roberts, 1969, 1971, 1975; Roberts and Stirling, 1971; Spencer, 1974 *b*), in tunicates (Galt and Mackie, 1971; Bone and Mackie, 1975; Mackie and Bone, 1976), and in molluscs (Kater et al., 1978). Epithelial conduction probably occurs as well in ctenophores (Horridge, 1965; Tamm, 1973) and in anthozoan coelenterates (McFarlane, 1969, 1974; Shelton and McFarlane, 1976). Thus, epithelial conduction is apparently widespread in the animal kingdom and an important coordination mechanism in some animals. Moreover, epithelial conduction has been considered as the likely evolutionary precursor of neuronal conduction (Horridge, 1968; Mackie, 1970; Pavans de

Ceccatty, 1974). On these grounds epithelial conduction deserves a detailed investigation.

An obvious way to investigate the mechanisms of epithelial conduction is by monitoring the membrane potentials of epithelial cells with intracellular microelectrodes. Microelectrode recordings from excitable epithelial cells have been made from epitheliomuscular cells of the siphonophore *Nanomia bijuga* (Spencer, 1971); both the myocytes and endodermal cells of the hydromedusa *Polyorchis penicillatus* (Spencer, 1978); the large, secretory epithelial cells of the siphonophore *Hippopodius hippopus* (Mackie, 1976); dissociated epitheliomuscular cells of hydra (Kass-Simon and Diesl, 1977); skin cells of larval amphibians (Roberts and Stirling, 1971) and ascidians (Mackie and Bone, 1976); and salivary gland cells of gastropod molluscs (Kater et al., 1978). It is generally assumed that propagation of impulses in an excitable epithelium is a consequence of electrical current flow between electrically coupled, electrically excitable cells (Mackie, 1965, 1970; Horridge, 1968; Roberts and Stirling, 1971; Spencer, 1974 b; Josephson, 1974); and electrical coupling between cells of excitable epithelia has been demonstrated in larval amphibian skin (Roberts and Stirling, 1971), in the glandular epithelium of a siphonophore (Mackie, 1976), and in the salivary gland of a gastropod mollusc (Kater et al., 1978).

The exumbrellar epithelia of hydromedusae are particularly attractive tissues for studying epithelial conduction since these epithelia are often excitable and, in several cases, have been demonstrated to be nerve-free (Mackie, 1965; Mackie and Passano, 1968; Mackie and Singla, 1975). Such nerve-free tissue can be used to examine epithelial conduction without the complexities introduced by coexistent neuronal activity. Further, the exumbrellar epithelia of hydromedusae are composed of a single layer of thin ectodermal cells (Mackie, 1965; Mackie and Passano, 1968) so that analysis is not hampered by complex cell morphology or multiple cell layers. A disadvantage of exumbrellar epithelia is that the epithelial cells are small (usually on the order of 10 μm in diameter) and exceedingly thin (typically less than 3–5 μm) which makes intracellular recording with microelectrodes rather difficult. In this report techniques are described which have allowed intracellular recordings from cells of the nerve-free, exumbrellar epithelium of the hydromedusa *Euphysa japonica*.¹ The epithelial cells were found to be electrically excitable and electrically coupled, thus accounting for the all-or-nothing action potentials which can be propagated across the epithelium. Two-dimensional cable analysis has been used to determine the electrical properties of this excitable epithelium.

Some of the work described in this paper has been briefly presented elsewhere (Schwab and Josephson, 1978).

MATERIALS AND METHODS

Euphysa japonica is seasonally available (April–June) at the Friday Harbor Laboratories of the University of Washington. Most specimens were collected at night and kept in

¹ In the Puget Sound area, where this work was done, this species has been described as *Euphysa flammea* (Mackie and Mackie, 1963). The more correct name, however, is *E. japonica* (Kramp, 1928; Arai, M., and A. Brinckmann-Voss, manuscript in preparation).

the laboratory in small containers of seawater at 9–14°C, the ambient seawater temperature. Animals were used in experiments within 1 wk of capture.

The usual preparation was a rectangular strip of the swimming bell made by cutting off the margin and the apex of the bell and by slitting the resulting hollow cylinder up one side. In this preparation the exumbrellar epithelium is everywhere separated from the other tissues of the bell (the endoderm and the subumbrellar ectoderm) by a thick layer of mesoglea. The tissue was pinned, exumbrellar side up, to a layer of transparent resin (Sylgard, Dow Corning Corp., Midland, Mich.) on the bottom of a shallow chamber filled with natural seawater. Cooled water, flowing through a jacket surrounding the chamber, kept the seawater surrounding the preparation at 9–11°C. The chamber was mounted on the stage of a compound microscope whose condenser and objective (Nikon U20, Nikon Inc., Instrument Div., Garden City, N.Y.) had been modified for modulation contrast microscopy (Hoffman and Gross, 1975). The tissue was viewed at a total magnification of $\times 200$. The modulation contrast system made it possible to distinguish nuclei, cell boundaries, and nematocysts in living, unstained tissue (Fig. 1). The microscope stood on a heavy,

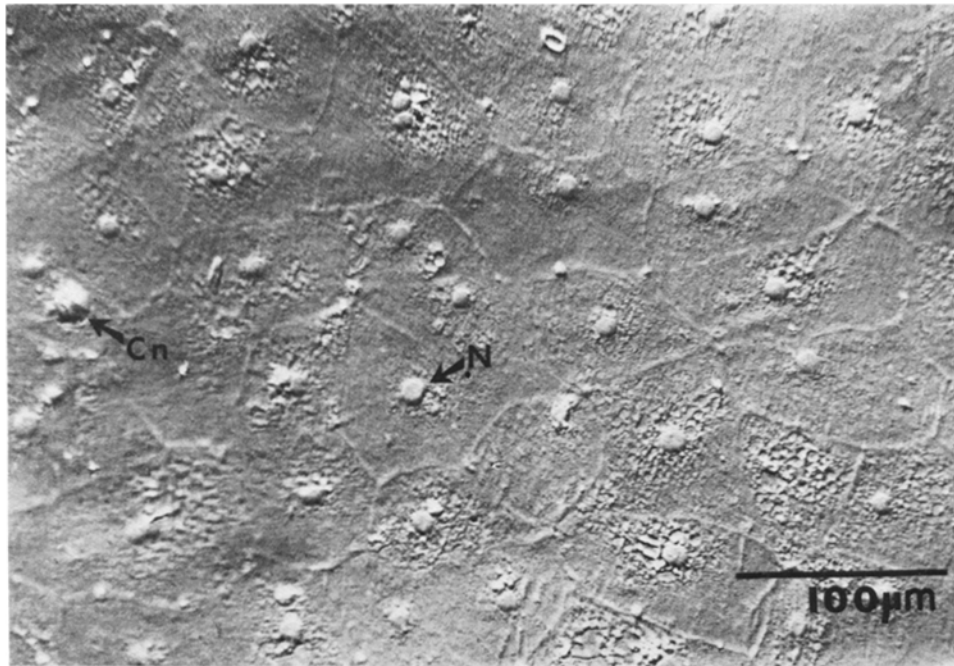


FIGURE 1. Surface of the exumbrellar epithelium of *E. japonica* as seen with modulation contrast optics. The cells are polygonal with a central nucleus. The granular appearance of the cell contents probably represents vacuoles and mitochondria. Cn, nematocyst; N, nucleus.

shock-mounted table to minimize vibration. Intracellular recordings were made with glass capillary microelectrodes filled with 3 M KCl. Useful electrodes had 45–90 M Ω impedance, electrodes toward the higher end of the impedance range being preferred. The epithelial tissue is extremely thin and the customary procedure for electrode penetration, slowly advancing an electrode while periodically tapping the manipulator

or table, was useless. A slowly advanced electrode dimpled the epithelium without penetrating it, and further advancing the electrode, or tapping, caused the electrode tip to pass completely through the epithelium and into the underlying mesoglea. This problem was overcome by using a piezoelectric drive (Chen, 1978) which allowed very rapid electrode acceleration over a short distance. The piezoelectric device was mounted on a stable manipulator (E. H. Leitz, Inc., Rockleigh, N.J.). In practice the electrode was advanced with the manipulator until its tip just touched the tissue. The piezoelectric drive was then activated with a 2-ms voltage pulse of polarity such that the electrode retreated on the onset of the pulse and advanced into the tissue at the end of the pulse. It usually took several voltage pulses to the piezoelectric drive, with slight repositioning of the electrode between the pulses, before penetration was achieved. Once an electrode had been used to penetrate a cell, it was difficult to penetrate another cell with the same electrode, so a new electrode was used in each penetration. Electrical stimuli were given to the tissue through a suction electrode placed on the exumbrellar surface.

Tissues were fixed for electron microscopy in 2.5% glutaraldehyde in 0.4 M phosphate buffer (pH 7.4) for 1 h at room temperature, postfixed in 1% OsO₄ at 4°C for 1 h, dehydrated in an ethanol series, and embedded in Epon (Shell Chemical Co., Houston, Tex.). The tissue was sectioned with a diamond knife and the sections were stained in 10% uranyl acetate in methanol followed by lead citrate (Reynolds, 1963). Sections were examined with either a Zeiss 9S2 (60 kV) or a JEOL JEM-100C (80 kV) electron microscope (Carl Zeiss, Inc., New York, JEOL USA, Electron Optics Div., Medford, Mass.). Additional techniques are described in pertinent portions of Results.

RESULTS

Structure of the Epithelium

The epithelium is composed of a single layer of broad, polygonal cells (Fig. 1). Each cell has a single, central nucleus, unlike the epithelia of some medusae in which the cells are multinucleate (Mackie, 1965). The nuclei are circular, as viewed from the surface, and are ~10 μm in diameter. The distance between adjacent nuclei is generally about 70 μm, but this varies considerably throughout the tissue. Occasional nematocysts in the epithelium stand out from the nuclei because of their larger size and birefringence. In cross section the epithelium is quite thin and the cells are seen to be rather vacuolated (Fig. 2 A). The tissue thickness through a nucleus is about 3–4 μm; elsewhere the tissue ranges from 0.4 to 2 μm thick. The average epithelial thickness is 1.4 μm (SE = 0.32 μm) as determined from 30 randomly selected, nonnuclear transects of the epithelium in electron micrographs from six cells.

The contact between adjacent epithelial cells is not a simple abutment; instead, one of the adjoining cells tends to overlap the other (Fig. 2 B). The intercellular junction is comprised of simple appositions and gap junctions. A section through the contact area between two cells is not perpendicular to the membrane faces throughout the whole width of the junction, thus it is difficult to determine exactly how much of the contact area is gap junction. In the section shown in Fig. 2 B, a single gap junction makes up about 20% of the junctional complex. The total width of the gap junction is about 15 nm

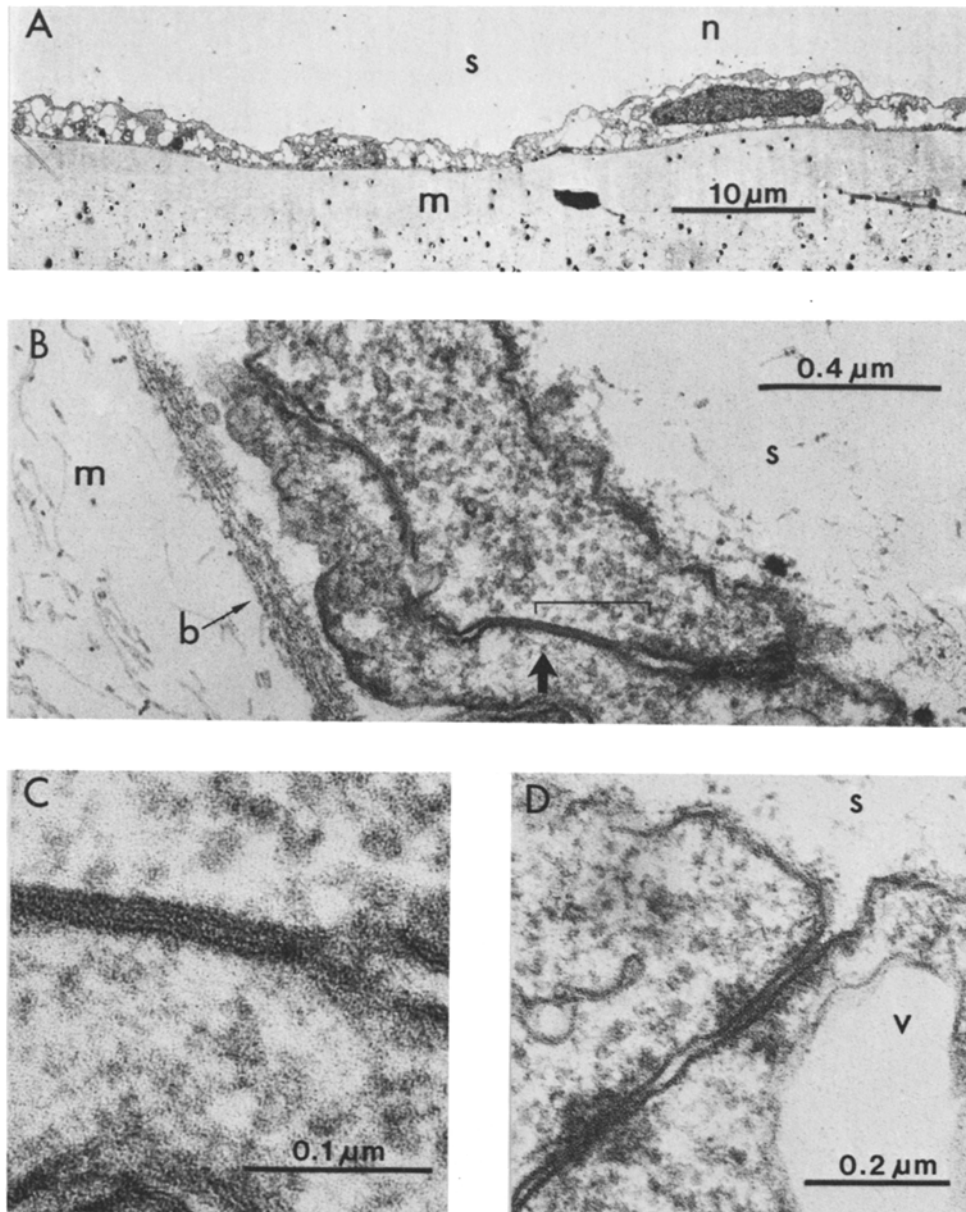


FIGURE 2. Electronmicrographs of the exumbrellar epithelium. (A) Low magnification cross section showing nuclear and nonnuclear portions of a cell. (B) Gap junction (arrow) between two epithelial cells. (C) Enlargement of the gap junction in the bracketed portion of B. (D) Apical portion of an intercellular junction. Note the absence of a tight junction and that a gap junction occurs near the surface of the epithelium. b, basement membrane; m, mesoglea; n, nucleus; s, seawater side of epithelium; v, vacuole.

whereas the gap itself is about 5.5 nm wide. No other types of intercellular specializations have been found. It is interesting that no tight junctions have been found between exumbrellar epithelial cells. Tight junctions occur between vertebrate epithelial cells and are there thought to function as transverse permeability barriers (Farquhar and Palade, 1963; Goodenough and Revel, 1970). Gap junctions have been found between electrically coupled cells (reviewed by Gilula, 1974; Loewenstein, 1975) and also between the cells of electrically excitable epithelia (Hand and Gobel, 1972; Mackie and Singla, 1975). It is assumed here that the gap junctions found in the epithelium of *E. japonica* are the structures responsible for the low resistance pathways between the epithelial cells (see below).

Resting and Action Potentials from Epithelial Cells

Stable resting potentials, -40 to -60 mV, can be recorded from exumbrellar cells of *E. japonica*, but in our experience only from the nuclear region of a cell. The epithelium is considerably thicker at the nucleus than elsewhere (Fig. 2 A) which probably accounts for the successful recordings from the nuclear region and not other parts of the cell. Further, the nucleus may stabilize the electrode somewhat, damping vibration of the tip. When an electrode is advanced into the nuclear region, the potential recorded usually appears in two steps, an initial, rapid voltage drop to -30 to -35 mV followed by a slower polarization reaching -40 to -50 mV. We interpret the slower voltage change as due to sealing of the surface membrane about the electrode. It is possible to record potentials from nonnuclear portions of the cell, but in our attempts, the recorded potentials were brief, ~ 20 – 60 ms, and always negative, ranging from a few to a few tens of millivolts. The low amplitude is probably because of insufficient time for membrane sealing about the electrode. Although the nonnuclear potentials recorded were not stable, it is clear that nonnuclear portions of the cell also have a negative resting potential. No resting potential is recorded if the electrode passes through the epithelium into the mesoglea, indicating that there is no significant transepithelial potential in this tissue.

We believe that the nuclear envelope does not contribute to the stable resting potentials recorded from the epithelium, but we have no unassailable evidence for this. The nucleus often sticks to an electrode tip and remains attached when the electrode is withdrawn from the tissue. In such instances significant potentials were never recorded from the isolated nuclei, indicating that there is no appreciable potential across the nuclear envelope. However, it could be argued that the nucleus is damaged during the withdrawal and the nuclear potential lost. For this we have no answer. It should be pointed out that in our recordings a nuclear potential would be in series with, and therefore additive to, the potential across the surface membrane. If there is a nuclear potential, it would not affect the recorded amplitude of action potentials generated by the surface membrane nor the amplitude of voltage changes due to current passed across the surface membrane, but it would affect the base line from which these voltage changes are measured.

Stimulating the epithelium with a suction electrode initiates an action potential which is propagated across the epithelium at ~ 10 cm per second (11°C). In an intact *E. japonica*, as in other hydromedusae (Mackie and Passano, 1968), action potentials in the exumbrellar epithelium evoke infolding of the margin of the swimming bell, a protective response known as crumpling. The action potential, as recorded from an impaled cell, is an all-or-nothing, positive, overshooting spike followed by a hyperpolarizing afterpotential (Fig. 3). The depolarizing portion of the action potential lasts about 11 ms; the total duration of the afterpotential can be nearly a second.

Hydromedusae of other genera were also examined (*Sarsia*, *Eutonina*, *Aglantha*, *Proboscoidactyla*, *Halistaura*, *Phialidium*, and *Stomotoca*) but *Euphysa japonica* was the only species from which stable resting potentials could be recorded from the exumbrellar epithelium. Resting potentials were briefly recorded from the other species examined, but these decayed rapidly, presumably because of cell damage during electrode insertion. Better elimination of vibration and off-axis movement of the electrode during insertion would likely allow successful penetration of epithelial cells in other species as well.

Electrical Coupling between Cells

Injecting current through an electrode in one cell changes the membrane potential in nearby cells, demonstrating that the epithelial cells are electrically coupled. The epithelium is an imperfect transmission line; in cells close to the current electrode, the voltage changes resulting from injected current pulses are larger, and the charging times are faster than in cells further away (Fig. 4). Since the cells are coupled, the epithelium can be treated as an extended sheet of cytoplasm interrupted by relatively conductive intercellular junctions and bounded above and below by relatively nonconductive cell membranes. In a sheet of coupled cells, current flows in two dimensions away from an intracellular current source rather than predominantly along a single axis, as is the case for elongate cells such as axons or muscle fibers; and standard, one-dimensional cable analysis is not applicable to a two-dimensional epithelium. The passive electrical properties to be expected from sheets of coupled cells have been analyzed by several authors (e.g., Woodbury and Crill, 1961; Eisenberg and Johnson, 1970; Shiba, 1971; Frömter, 1972; Jack et al., 1975). Before applying the two-dimensional cable analysis derived for sheets of cells to the *E. japonica* epithelium, it is necessary to show that the tissue is not unduly damaged by the multiple electrode penetrations necessary to gather the data, and that the electrical properties of the epithelium are linear in the current and voltage range used.

An indication of possible damage due to electrode penetration was obtained by monitoring the potential of one cell while a second microelectrode was inserted into another cell some distance away. Sometimes a penetrating electrode clearly damaged a cell and its nucleus as evidenced by shriveling of the nucleus. Excluding instances with visible tissue damage, there was little or no detectable change in the potential from one cell when a second electrode was inserted into the epithelium. In 16 cells, the average membrane potential

was -45.9 mV ($SE = 1.9$ mV) before a second electrode impaled an adjacent nucleus $10\text{--}100$ μm away from the first, and the average potential change during insertion of the second electrode was only $+0.2$ mV ($SE = 0.4$ mV). From judging by membrane potential stability, it appears that inserting an electrode into a cell does little if any damage to the epithelium.

The relation between the amount of current injected into one cell and the resulting voltage displacement in a nearby cell is linear for hyperpolarizing current up to at least $60\text{--}80$ nA, and for depolarizing current less than $20\text{--}30$ nA (Fig. 10). Stronger depolarizing current initiates regenerative, depolarizing responses which can lead to all-or-nothing, overshooting, propagated spikes identical to those evoked by a stimulating electrode on the epithelial surface (Fig. 5). Over a reasonable current range the epithelium is electrically linear, and a linear two-dimensional cable analysis can be applied.

The model of the epithelium to be considered is illustrated in Fig. 6. Current from an intracellular electrode flows to the grounded seawater surrounding the preparation through both the apical and basal cell surfaces. The basal

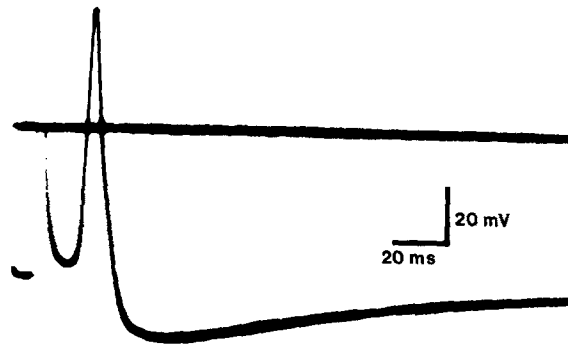


FIGURE 3. A typical all-or-nothing, overshooting, action potential recorded intracellularly from an epithelial cell. Note the long afterpotential. The upper trace is the zero potential level.

pathway includes the underlying mesoglea which contacts the seawater at the cut edges of the preparation. It is assumed that the resistance of the mesoglea is negligible, thus both apical and basal surfaces can be considered to be at ground potential. R_a is the resistivity of the apical membrane (Ωcm^2), R_b is the resistivity of the basal membrane (Ωcm^2) and r_x is the resistance of unit length and breadth of the epithelial interior (Ω). A more complete model would include membrane capacitance as well as resistance, but it will be assumed that the voltage is measured sufficiently long after the onset of a current pulse so that the membrane potential has reached its asymptotic value, therefore there is no capacitive current. The internal longitudinal resistance of the epithelium, r_x , depends on its thickness. If d is the thickness of the epithelium (cm) and R_i is the resistivity of the epithelial interior (Ωcm) lumping together the resistivity of the cytoplasm and that of intercellular junctions, $r_x = R_i/d$. R_a and R_b are parallel pathways for the flow of current from the cytoplasm to

the medium, and it is convenient to combine these to a single egress resistivity, R_z , where $1/R_z = 1/R_a + 1/R_b$.

The steady-state relation between injected current and voltage displacement in a two-dimensional sheet, such as a layer of coupled epithelial cells, is given by (Frömter, 1972; Jack et al., 1975):

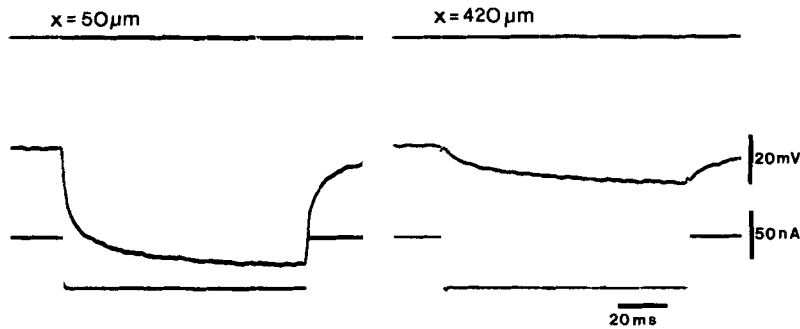


FIGURE 4. Electrical coupling between epithelial cells. The upper traces mark the zero potential level, the middle traces are potentials recorded with an intracellular electrode, and the lower traces monitor current pulses injected through a second microelectrode into another cell. x is the distance between the current electrode and the voltage recording electrode.

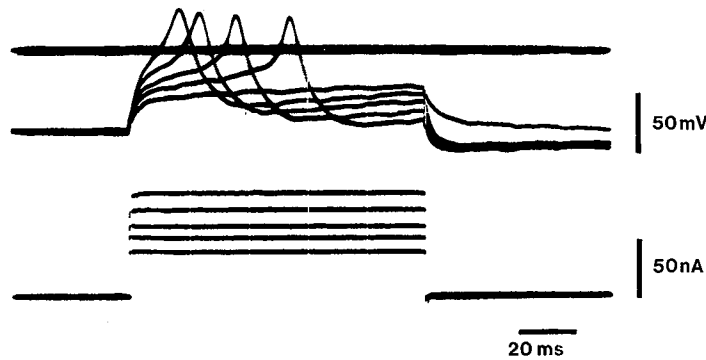


FIGURE 5. Action potentials initiated by depolarizing current pulses. The smallest current pulse failed to initiate an action potential. Above threshold, increasing current amplitudes evoked spikes with decreasing latencies. The upper trace is the zero potential level.

$$V = \frac{I_0 r_x}{2\pi} K_0(x/\lambda_2),$$

where V is the voltage displacement measured from the resting potential (volts), I_0 is the injected current amplitude (amperes), $K_0(x/\lambda_2)$ is a modified Bessel function of zero order with the argument (x/λ_2) , x is the distance between the current and voltage electrodes (centimeters), λ_2 is a two-dimensional space constant defined as $(R_z/\tau_x)^{1/2}$. Values of K_0 for a wide range of

arguments are tabulated, for example, in Abramowitz and Stegun (1965) which also gives asymptotic approximations for nontabulated values. In the *E. japonica* measurements, the distance between the current and voltage electrodes was always many times greater than the epithelial thickness so no correction is needed for three-dimensional current flow near the tip of the current electrode (Eisenberg and Johnson, 1970). If it is assumed that the apical and basal membranes are of identical resistivity R_m , i.e., $R_a = R_b = R_m$, then the two-dimensional space constant can be written as:

$$\lambda_2 = (R_{md}/2R_i)^{1/2},$$

which is the form given by Jack et al. (1975).

The cable parameters of the *E. japonica* epithelium were determined by measuring the voltage displacement in one cell of a pair while injecting positive current pulses of constant amplitude into the second cell. The measured values are plotted as a function of distance between current and

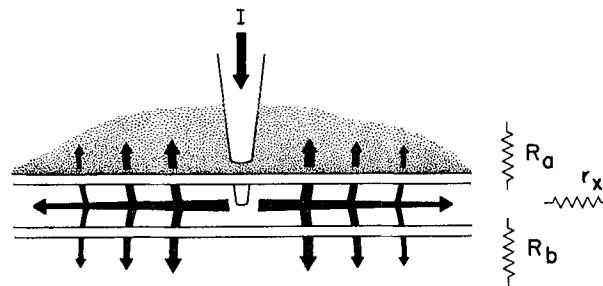


FIGURE 6. Current pathways in a sheet of coupled cells. R_a , the resistivity of the apical membrane; R_b , the resistivity of the basal membrane; r_x , internal longitudinal resistance of the cytoplasm and intercellular junctions.

voltage electrodes in Fig. 7. The current pulses were 5×10^{-8} A and the resulting potential change was measured 100 ms after the onset of the current pulse. The data in Fig. 7 is from 19 animals with an average of 3.6 current-voltage penetration pairs per animal. Points from the animal with the greatest number of successful penetrations are shown as open circles. The scatter for this one animal is similar to that for all animals combined, indicating that the inter-animal and intra-animal variability are similar. A Bessel function of the following form has been fit to the data:

$$V = AK_0(x/\lambda_2)$$

A is a constant. The initial values of A and λ_2 were arbitrarily selected. A computer routine calculated the expected value of V for each x and the selected values of A and λ_2 . The routine then determined the sum of the squared differences between the measured values of V and the calculated values of V . The program systematically varied the values of A and λ_2 to minimize the sum of squares error. The best fit was obtained with $A = 11.2$ mV and $\lambda_2 = 1.3$ mm. The resistance parameters of the epithelium were

calculated from these values ($r_x = 2\pi A/I_0$, $R_z = (\lambda_2)^2 r_x$) giving $r_x = 1.4 \times 10^6 \Omega$ and $R_z = 23 \text{ k}\Omega\text{cm}^2$. Since R_z is formed by R_a and R_b in parallel, neither R_a nor R_b can itself be less than R_z . Therefore R_a and R_b are both greater than $23 \text{ k}\Omega\text{cm}^2$.

Transverse Resistance of the Epithelium

In several epithelia the transverse electrical resistivity is far lower than can be accounted for on the basis of the resistivity of the cell membranes, implying the presence of a paracellular electrical shunt across the epithelium (e.g., Frömter, 1972; Reuss and Finn, 1974, 1975; Spenny et al., 1974). The

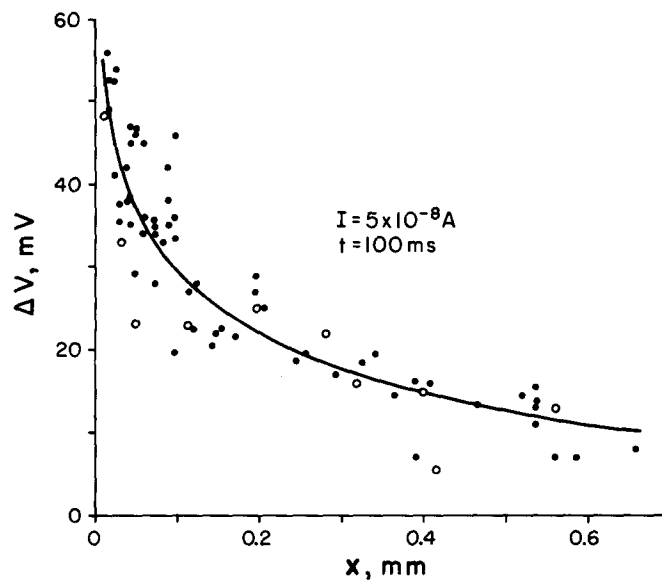


FIGURE 7. Voltage responses to injected current pulses as a function of the distance, x , between the voltage and current electrodes. The filled circles are data pooled from 19 animals; the open circles are points from a single animal. The curve is the best fit of a Bessel function to the data by the least squares criterion.

transverse electrical resistance of the *E. japonica* epithelium was measured as shown in Fig. 8 to determine if this tissue also has a significant paracellular shunt. The exumbrellar surface of an animal, usually demarginate, was held by light suction to a fire-polished tube, 1.6 mm i.d., mounted through the bottom of a dish containing seawater. The glass tube was connected by a seawater-filled, flexible tube to a beaker whose height could be adjusted to vary the suction. The transepithelial resistance was determined as the ratio of the voltage change across the epithelium to the amplitude of current pulses passed between a chlorided silver wire electrode in the beaker and a similar electrode in the seawater surrounding the animal. An operational amplifier used as a current-to-voltage converter monitored the current. The potential across the epithelium was measured between a chlorided silver wire in the

holding tube just below the animal's surface and an electrode in the bath surrounding the animal. It was assumed that the relatively large area of subumbrellar surface and the exposed mesoglea would offer negligible electrical resistance as compared to the small patch of exumbrellar epithelium across the opening of the holding tube, and that the measured resistance would be largely that of the patch. The current was delivered in 200-ms pulses of 2×10^{-7} A and the potential was measured just before the pulse termination.

The experimental sequence was as follows. First, the electrical resistance of the apparatus (the resistance of the fluid in the holding tube above the recording wire plus the resistance of the sea water in the dish) was measured as the ratio of voltage to current without the animal in place. Then the animal

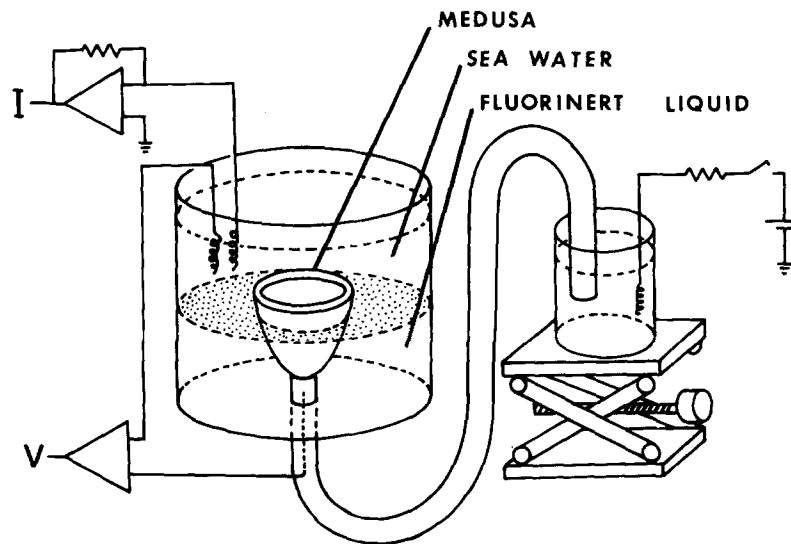


FIGURE 8. The apparatus used to determine the transverse resistance of the exumbrellar epithelium. Current was passed between the seawater in the beaker to the right and the seawater in the test chamber. The medusa was held by slight suction across the aperture of the tube joining the beaker and test chamber.

was put on the holding tube and the new resistance was measured. Finally, the resistance was measured after the bottom of the dish was filled with Fluorinert liquid FC-78 (3M Co., St. Paul, Minn.) so that the holding tube and approximately the lower 3 mm of the medusa were surrounded by the liquid. Fluorinert FC-78 is a nonmiscible, inert, dielectric liquid which is heavier than seawater so it sinks to the bottom of the dish. The Fluorinert liquid was used to minimize electrical leakage around the edges of the holding tube.

The results of these measurements were surprising. The resistance of the apparatus alone was $1,200 \Omega$. Putting an animal on the holding tube increased the resistance by an average of only 320Ω . ($n = 5$, $SE = 45 \Omega$). Adding the

Fluorinert increased the additional resistance by only 15%, to 376Ω (SE = 47Ω). Since the area of the holding tube is $2 \times 10^{-2} \text{ cm}^2$, this is equivalent to a resistivity of only $7.5 \Omega \text{ cm}^2$ for the patch of epithelium across the mouth of the holding tube. If the electrical pathway across the epithelium included two continuous sheets of membrane, with the resistivity determined in the coupling measurements ($>23 \text{ k}\Omega \text{ cm}^2$), the transverse resistivity should have been $>46 \text{ k}\Omega \text{ cm}^2$. There are two possible explanations for the low transverse resistivity measured for the epithelium. (a) The epithelium might be pierced by low resistance channels, channels either normally present or newly created by the slight distortion of the cell layer when the medusa is sucked to the holding tube. If there are channels present normally, they must not communicate with the cytoplasm, or the measured resistance from the cell interiors to the outside could not be so high. (b) The resistance measured was not that of the epithelium but rather that of a leakage pathway between the epithelium and the holder and, when Fluorinert was used, the resistance of a water film between the Fluorinert and the epithelium. The minimum thickness required for such an aqueous film can be estimated. The diameter of a medusa is about 5 mm. If one assumes the postulated aqueous film is a hollow cylinder, 3 mm tall (the depth of the Fluorinert liquid layer), and with a resistivity of $20 \Omega \text{ cm}$ (the resistivity of seawater), the cylinder thickness would have to be $>100 \mu\text{m}$ for its resistance to be 376Ω . This is an underestimate of the required thickness. Since the medusa bell is curved, its diameter near the holder is not nearly 5 mm; with smaller diameter the thickness of the aqueous layer would have to be correspondingly increased to give the same cross-sectional area and resistance. It seems unlikely that an aqueous layer $>0.1 \text{ mm}$ in thickness would remain between the Fluorinert and the epithelium. Further, some of the animals used in this series were intact and frequently beat vigorously. These swimming movements should have helped to squeeze out residual water around the medusa. Finally, as an additional check, the resistance was measured between the mesoglea and the outside seawater of several intact medusa with current and voltage microelectrodes inserted through the ex-umbrellar into the mesoglea. In these measurements with intact animals, as with isolated preparations, no resting transepithelial potential was detected. We estimate the total surface area of an *E. japonica* to be $4\text{--}5 \text{ cm}^2$. If the animal were covered entirely with an epithelium whose apical and basal membranes each had a resistivity of $23 \text{ k}\Omega \text{ cm}^2$, the resistance between the mesoglea and the outer bathing solution should be at least $9 \text{ k}\Omega$. In fact the resistance was too small to measure, certainly less than $100\text{--}200 \Omega$. Despite the high resistivity of the cell membranes, the transverse resistivity across the whole epithelium is quite low.

Decrease in Membrane Resistivity during Action Potentials

In Fig. 9 a series of voltage transients resulting from short hyperpolarizing current pulses is shown superimposed on a propagated action potential. This was done by initiating an action potential with a suction electrode placed on the epithelium some distance from both the current and voltage microelec-

trodes. The voltage transients are obscured during the depolarizing spike, but it is clear that those during the afterpotential are smaller and have faster rise and fall times than the voltage transients before, or long after, the action potential. The decreased amplitude of the voltage transients indicates that the input resistance ($r_{in} = V/I_0$) of the tissue has decreased; the faster rise and fall of the transients indicates that the time constant of the surface membrane has decreased. Together these changes indicate that the resistivity of the surface membrane has decreased. The change in input resistance is not great but, as pointed out by Jack et al. (1975), the input resistance of a two-dimensional cable is rather insensitive to changes in membrane resistivity, so a small change in input resistance signals a moderately large change in membrane resistivity.

Reduced input resistance during the afterpotential can also be demonstrated in another way. As indicated above, strong depolarizing current pulses initiate

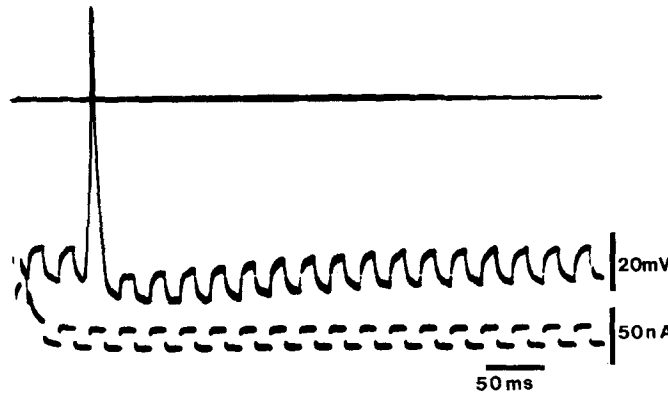


FIGURE 9. Voltage responses to imposed current pulses superimposed on an action potential. Note that the voltage transients just after the spike are smaller and less rounded than those before or long after the spike. The upper trace is the zero potential level.

action potentials. If the afterpotential occurs during the current pulse, the membrane potential reached during the afterpotential varies with the current intensity (Fig. 5). Plots of the peak afterpotential against current intensity have lower slopes, indicating a lower input resistance, than do plots for the resting membrane (Fig. 10). In the four animals thus examined, the input resistance during the afterpotential averaged 54% of the resting input resistance (SE = 4%, distances between current and voltage electrodes = 15–73 μm).

The egress resistivity from the cytoplasm, R_z , can be calculated from the input resistance (r_{in}) and the two-dimensional cable equation. Solving for R_z in the logarithmic approximation for the cable equation (see Appendix) gives:

$$R_z = (x^2 r_x / 1.25) \exp(4\pi r_{in} / r_x).$$

This relation can also be used to determine R_z during the afterpotential if it is assumed that the epithelium is briefly in steady state. This assumption

seems justified since during the afterpotential the membrane charges and discharges quite rapidly to imposed current (see Fig. 9) so that the membrane potential rapidly comes into equilibrium with imposed current. The value of r_x obtained in the coupling analysis ($= 1.4 \times 10^6 \Omega$) was used in solving for R_z . In the four preparations for which input resistance during the afterpotential was available, R_z at rest averaged $26.3 \text{ k}\Omega\text{cm}^2$ (SE = $9.7 \text{ k}\Omega\text{cm}^2$), whereas the average R_z during the afterpotential was $0.95 \text{ k}\Omega\text{cm}^2$ (SE = $0.25 \text{ k}\Omega\text{cm}^2$). On average the R_z during the afterpotential was 4.8% (SE = 1.3%) of that at rest.

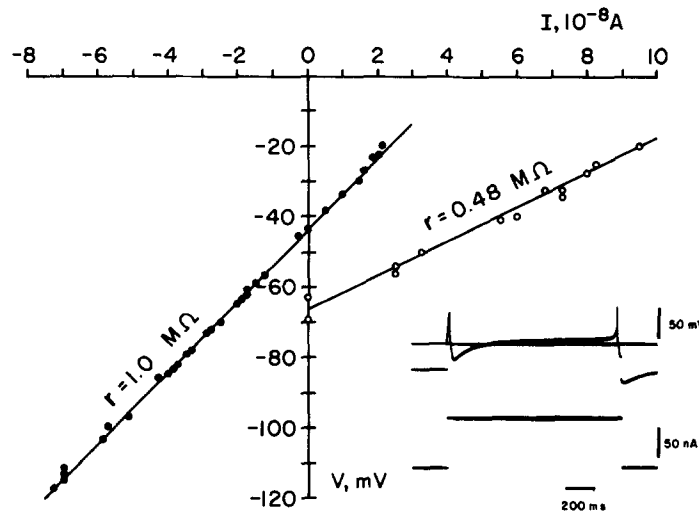


FIGURE 10. Current-voltage relation for the epithelium at rest and during the afterpotential. The closed circles are the steady-state potentials during current pulses which did not initiate action potentials. The open circles are from strong depolarizing current pulses which evoked action potentials (cf. Fig. 7) and indicate the potential at the peak of the after potential. Distance between the current and voltage electrodes = $24 \mu\text{m}$. The open circles on the zero current line are two instances, one of which is the second action potential of the inset, in which the afterpotential occurred after the termination of the current pulse. The inset, incidentally, is the only example we saw of multiple firing during a single current pulse.

Thus, the egress resistivity during the afterpotential falls to about one-twentieth of that at rest.

DISCUSSION

We have shown that adjacent cells in the epithelium of *E. japonica* are strongly coupled (Fig. 7), the propagated response is a large, overshooting depolarization of the membrane potential (Fig. 3), and a membrane depolarization considerably less than that likely to be created by an approaching action potential is sufficient to trigger a spike (Fig. 5). Together these leave no doubt that electrical current flow through the coupled cells of this epithelium is

adequate to account for the propagation of activity. The epithelial sheet may indeed be viewed as “a single giant axon spread out over the whole exumbrellar surface” (Mackie, 1965).

Resistive Parameters of the Resting Epithelium

The equivalent electrical circuit suggested by this study for the *E. japonica* epithelium is shown in Fig. 11. Here R_a and R_b represent the resistivity of the apical and basal membranes, r_x is the longitudinal resistance of the cytoplasm and intercellular junctions, and R_s is the resistivity of the transepithelial, paracellular shunt required to account for the low resistance across the epithelium. From the cable analysis, $r_x = 1.4 \times 10^6 \Omega$. r_x is the ratio of R_i , the resistivity of the contents of the epithelial sheet including both the cytoplasm and the intercellular junctions, and d , the thickness of the epithelium. Since d_2 is $\sim 1.4 \mu\text{m}$, $R_i = 196 \Omega\text{cm}$. This is on the order of, or only slightly greater

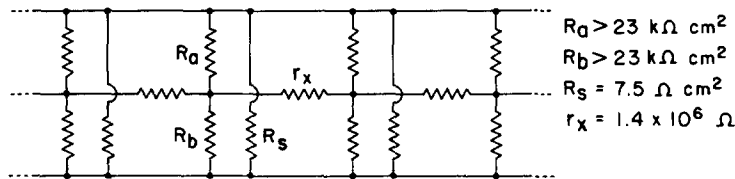


FIGURE 11. Equivalent circuit of the exumbrellar epithelium of *E. japonica*. R_a , the resistivity of the apical membrane; R_b , the resistivity of the basal membrane; R_s , the resistivity of the paracellular shunt; r_x , internal longitudinal resistance of unit length and breadth of epithelium (= resistivity of cytoplasm and intercellular junctions divided by the epithelial thickness).

than, specific resistivity values obtained for pure cytoplasm from several cells (e.g., squid axoplasm— $28 \Omega\text{cm}$, Cole, 1975; *Myxicola* axoplasm— $68 \Omega\text{cm}$, Carpenter et al., 1975; frog muscle cytoplasm— $169 \Omega\text{cm}$, Hodgkin and Nakajima, 1972; crayfish muscle cytoplasm— $150 \Omega\text{cm}$, Law and Atwood, 1971; katydid muscle cytoplasm— $250 \Omega\text{cm}$, Josephson et al., 1975). Clearly much, if not most, of the internal resistivity can be accounted for by the cytoplasm, and the intercellular junctions must pose very little barrier to the spread of current in the *E. japonica* epithelium. The only junctional specializations found between epithelial cells were gap junctions; it is presumably these which provide the low resistance pathways between cells. R_z , R_b , and R_s have not been measured directly, but limiting values can be given these from the data available. As discussed above, R_a and R_b in parallel form R_z , and therefore neither R_a nor R_b can be less than $23 \text{ k}\Omega\text{cm}^2$, the value determined for R_z . For transepithelial current flow, R_a and R_b are in series, and together form a pathway parallel to R_s . The transepithelial conductivity of R_a and R_b in series is less than $2.17 \times 10^{-5} \Omega^{-1} \text{cm}^{-2}$. The measured transepithelial resistivity is $7.5 \Omega \text{cm}^2$, equivalent to a transepithelial conductivity of $0.133 \Omega^{-1} \text{cm}^{-2}$. Thus, only a tiny fraction of the transepithelial conductance can be due to the cellular pathway of R_a and R_b , and essentially the whole of the measured conductivity is that of the shunt pathway, R_s . The value of R_s must be nearly that of the epithelium as a whole, that is, $7.5 \Omega \text{cm}$.

Several other epithelia have been the subject of analysis similar to that used here with *E. japonica*. Table I compares some of the electrical properties of these epithelia. Although the epithelium of *E. japonica* is the thinnest of the group, it has the longest space constant. This is a consequence of the very high egress resistivity, R_z , and the low specific resistivity, R_i , of the epithelial contents. *E. japonica* is a marine invertebrate, and the osmotic concentration of its cytoplasm is almost certainly greater than that of vertebrates or insects. A high internal ion concentration may account in part for the low value of R_i . The *E. japonica* epithelium is the only excitable epithelium in Table I, and it is tempting to regard the differences between this epithelium and the others as adaptations favoring the propagation of action potentials. But it must be

TABLE I
ELECTRICAL PROPERTIES OF SOME EPITHELIAL SHEETS

Tissue	λ_z	d	R_i	R_z	R_t	G_s/G_t	V_t
	μm	μm	Ωcm	$\text{k}\Omega\text{cm}^2$	Ωcm^2	%	mV
<i>Euphysa</i> exumbrellar epithelium	1300	1.4	196	23	7.5	>99	0
Newt gastric epithelium*	320-400	40	1,100-1,300	0.35-0.45**	—	—	—
Toad urinary bladder†	460‡	5	750	1.9**	3940	35	58
<i>Necturus</i> gallbladder§	443	30	2,547**	1.7	307	95**	2.5
<i>Tenebrio</i> larval epidermis, newly molted¶	850	16	450	2**	—	—	—
<i>Tenebrio</i> larval epidermis, intermolt¶	1,000	6	460	7.5**	—	—	—

λ_z , two-dimensional space constant; d , epithelial thickness; R_i , specific resistivity of cytoplasm and intercellular junctions; R_z , egress resistivity from cytoplasm; R_t , transepithelial resistivity; G_s/G_t , ratio of paracellular shunt conductivity to total transepithelial conductivity ($= R_t/R_z$).

* Shiba, 1971.

† Reus and Finn, 1974.

‡ Frömter, 1972. Similar values are given by Reus and Finn, 1975.

§ Caveney, 1974.

¶ Measured with nonconductive oil on mucosal surface, so the value is an overestimate for epithelium bathed on both sides with saline.

** Calculated from data given in the source.

pointed out that, excluding the epidermis of *Tenebrio*, the other epithelia are secretory-absorptive, so that comparison is between transporting epithelia and a probably nontransporting epithelium, as well as between nonexcitable tissues and an excitable cell layer. A larger sample is needed before differences between epithelia can be confidently identified as adaptations for excitability. Having given this caveat, let us point out that a long space constant as in the *E. japonica* epithelium promotes the spread of depolarization about a source which should facilitate propagation. Although the R_z is large in *E. japonica*, indicating high membrane resistivity, the transverse resistivity of the epithelium is unusually low (see also transepithelial resistivity values tabulated by Frömter and Diamond, 1972); the *E. japonica* epithelium is very leaky. This is surprising. In several hydromedusae the sulphate concentration of the mesoglea has been shown to be lower than that of the surrounding sea water (Denton and Shaw, 1961), and the epithelium surrounding the mesoglea has been shown to be a barrier to the movement of sulphate (Mackay, 1969). The paracellular shunt in *E. japonica* must be quite permeable to small ions, although possibly not to sulphate, to account for the low transepithelial resistivity. Paracellular shunts elsewhere have been shown to be ion selective

(Barry et al., 1971; Machen and Diamond, 1972), so it is not unreasonable to expect that the same might also be true in the medusa epithelium. The low transepithelial resistivity does have one consequence for the propagation of electrical activity. It has been suggested that the apical and basal membranes of a coelenterate epithelium might be separately excitable, so a single epithelial sheet could provide two rapid communication channels (Josephson, 1974). This would require high transepithelial resistivity, to prevent current flow across the apical membrane from completing the circuit by flowing across the basal membrane and back through the epithelium, and conversely, to prevent current flow through the basal membrane from also flowing through the apical membrane. With a low transepithelial resistivity, as in *E. japonica*, current flows readily across the epithelium, and if the resistivity of the apical and basal surfaces were similar, the current density through the apical and basal membranes should also be similar. Put another way, because of the low transepithelial resistivity, there will be no significant potential difference across the epithelium and so the membrane potential will always be similar across both the apical and basal faces of a cell, thus precluding independent activity of apical and basal membranes. It might be noted that a low transverse resistivity is neither theoretically nor empirically required for impulse propagation in a sheet of coupled cells. In fact, a counter-example exists. The hearts of tunicates are thin sheets of coupled myoendothelial cells which propagate impulses (Kriebel, 1967), and in which the transverse resistivity is moderately high (230–280 Ωcm^2 , Kriebel, 1968).

Epithelial Action Potentials in Different Tissues

The action potentials so far recorded with intracellular electrodes from excitable epithelia differ both in wave form and in ionic dependency. Table II compares resting and action potentials from epithelial cells of four coelenterates, *Euphysa japonica*, *Hippopodius hippopus*, *Nanomia bijuga*, and *Polyorchis penicillatus*; the tunicate *Dendrodoa grossularia*; larvae of the amphibian, *Xenopus laevis*; and the gastropod mollusc, *Helisoma trivolvis*. The resting potentials and the potentials described as action potentials from dissociated epithelial cells of hydra (Kass-Simon and Diesl, 1977) are quite variable and not readily summarized in tabular form. Epithelial action potentials may be short and spike-like (*E. japonica*, *H. hippopus*, *H. trivolvis*, *P. penicillatus* endoderm), or with plateaus on the depolarizing phase (*P. penicillatus* myocytes, *D. grossularia*, *X. laevis*). There may be a depolarizing afterpotential (*H. hippopus*, *P. penicillatus* myocytes) or a hyperpolarizing afterpotential (*E. japonica*, sometimes in *H. trivolvis*). In preliminary experiments, the action potentials in *E. japonica* were not blocked by TTX or by bathing the tissue in sodium-free seawater. Sodium-free solutions block action potentials in both *H. hippopus* and *X. laevis* (Mackie, 1967; Roberts and Stirling, 1971). TTX blocks action potentials in *X. laevis* (Roberts and Stirling, 1971), but not in *D. grossularia* (Mackie and Bone, 1976). From this sample, it is apparent that epithelial conduction is not a homogeneous phenomenon; several variants occur in different tissues.

Excitable Epithelia and Nerve Cells

One of the goals of this study was to determine if the mechanisms of epithelial conduction are sufficiently similar to those of neuronal conduction that epithelia could reasonably be regarded as candidates for the evolutionary precursors of nerve cells as has been suggested by several recent authors (Horridge, 1968; Mackie, 1970; Pavans de Ceccatty, 1974). The results from *E. japonica* and those obtained from other excitable epithelia demonstrate that epithelial and neuronal conduction are very similar indeed. Excluding dissociated hydra cells (Kass-Simon and Diesl, 1977), all cells from excitable

TABLE II
WAVE FORM PARAMETERS OF EPITHELIAL ACTION POTENTIALS

	Resting potential	Spike amplitude	Spike duration	Duration of hyperpolarizing or depolarizing after potential (to 50% recovery)
	mV	ms	ms	ms
<i>E. japonica</i>	-46.1 ± 1.0 (n = 45)	69.7 ± 8.4 (n = 23)	10.6 ± 0.7 (n = 10)	98.0 ± 4.2 (n = 10)
<i>H. hippopus</i> *	-43.3 ± 5.0	73.7 ± 3.3	13.3 ± 1.3	~1 s
<i>N. bijuga</i> †	-63	17-20	up to 3 s	—
<i>P. penicillatus</i> ‡ (myocytes)	-70	132	179	179
<i>P. penicillatus</i> ‡ (endoderm)	-55	92	57	—
<i>X. laevis</i> §	-90	130	120	—
<i>D. grossularia</i> ¶	-42	52	~300	—
<i>H. trivolvis</i> **	-73 ± 9	~80	~70	~300

Values given are means ± SE when available.

* Mackie, 1976.

† Spencer, 1971.

‡ Spencer, 1978.

§ Roberts and Stirling, 1971.

¶ Mackie and Bone, 1976.

** Kater et al., 1978.

epithelia which have been penetrated with intracellular electrodes have been found to have negative resting potentials and to produce positive, in most cases, overshooting action potentials (Table II). The action potentials from different epithelia differ considerably in waveform, but those from *E. japonica* are strikingly similar to action potentials from, for example, molluscan neurons, with an initial, rapid depolarizing spike followed by a hyperpolarizing afterpotential. In *E. japonica* the membrane resistance falls to about one-twentieth of its resting value during the afterpotential, suggesting that here, as in axons, the afterpotential is due to an increase in membrane conductance to some ion or set of ions whose equilibrium potential is more negative than the resting potential. Finally, available evidence strongly indicates that conduction of activity across an excitable epithelium, like conduction along an axon, is due to electrical currents generated about an area of activity. These many similarities strongly suggest that epithelial conduction and neural conduction are phylogenetically related; if not, they represent a remarkable instance of convergent evolution.

APPENDIX

Linear Approximations for Voltage Decrement at Short Distances

In a two-dimensional cable, the steady-state change in membrane potential declines with distance from a current source as a modified Bessel function (Jack et al., 1975). Fitting experimental data to a Bessel function is extremely tedious without a computer. A logarithmic approximation to a Bessel function can be used if the distances at which the potentials are recorded are short compared to the two-dimensional space constant.

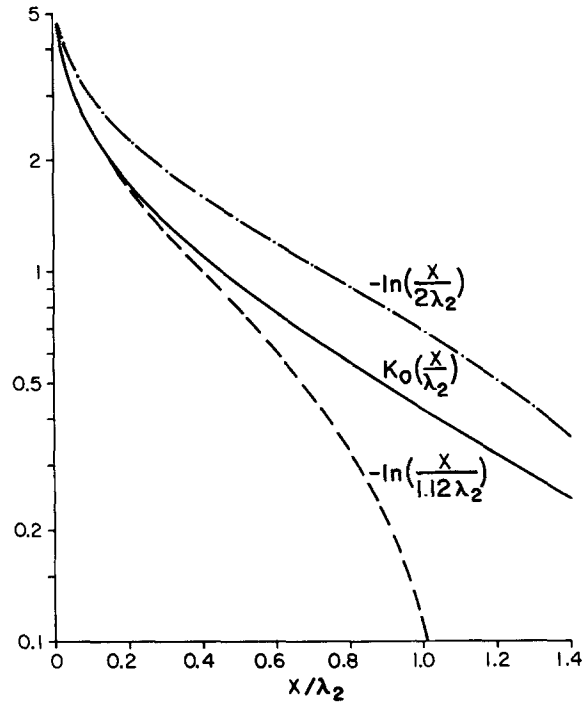


FIGURE 12. The zero order, modified Bessel function K_0 (solid line) and two logarithmic approximations to the Bessel function.

According to Jack et al. (1975, Eq. 5.18), if the distance, x , is short compared with the space constant, λ_2 :

$$V = \frac{I_0 R_i}{2\pi d} \left[-\ln \frac{x}{2\lambda_2} \right].$$

Here $-\ln(x/2\lambda_2)$ is used as an approximation for $K_0(x/\lambda_2)$. The terms used are defined in the text. Abramowitz and Stegun (1965) give the following as a limiting form of the modified Bessel function, K_0 , for small arguments (Eq. 9.6.13):

$$K_0(\alpha) = -[\ln(\alpha/2) + \gamma]I_0(\alpha) + \frac{1}{2}\alpha^2 + (1+1/2)\frac{(\frac{1}{2}\alpha^2)^2}{(2!)^2} \dots,$$

where I_0 is a second modified Bessel function and γ is Euler's constant. Ignoring all

terms but the first, given that I_0 is nearly 1 for small arguments, and, for consistency, letting $\alpha = x/\lambda_2$, this gives as an approximation for K_0 at small arguments:

$$K_0(x/\lambda_2) = -\ln(x/1.12\lambda_2)$$

The two approximations for $K_0(x/\lambda_2)$, $-\ln(x/2\lambda_2)$ and $-\ln(x/1.12\lambda_2)$, are plotted in Fig. 12. The error between the second approximation and the actual Bessel function is a consequence of neglecting the higher terms in the expression given by Abramowitz and Stegun. The second approximation clearly is a better fit when (x/λ_2) is small. The error for the second approximation is 12.8% at $x = 0.5\lambda_2$ and rapidly becomes smaller for smaller x . Therefore, the following is a reasonable approximation for voltage decrement with distance when $x < 0.5\lambda_2$:

$$V = \frac{I_0 R_i}{2\pi d} [-\ln(x/1.12\lambda_2)].$$

This gives a linear relation between V and $\ln x$ with a slope of $-(I_0 R_i/2\pi d)$ and an ordinate intercept of $(I_0 R_i/2\pi d) \ln(1.12\lambda_2)$. Further, the line intercepts the abscissa at $x = 1.12\lambda_2$. As an example of the use of this relation, the data of Fig. 7 is replotted in Fig. 13 using the logarithmic transform. For convenience x is plotted as \log_{10} rather than \ln . The least squares regression line for the data is:

$$V = -25.2 \text{ mV} (\log x) + 4.8 \text{ mV}.$$

From this, $\lambda_2 = 1.4 \text{ mm}$, $R_i/d = 1.4 \times 10^6 \Omega$, and $R_z = 26.4 \text{ k}\Omega\text{cm}^2$. These are satisfactorily similar to the values obtained with the least squares fit to a Bessel function: $\lambda_2 = 1.3 \text{ mm}$, $R_i/d = 1.4 \times 10^6 \Omega$, and $R_z = 23 \text{ k}\Omega\text{cm}^2$.

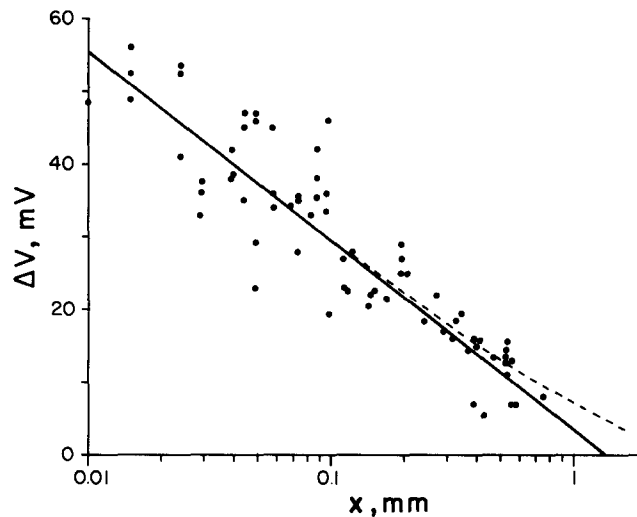


FIGURE 13. Voltage responses to injected current as a function of the distance between current and voltage electrodes. This is the same data as Fig. 7 but plotted semilogarithmically. The solid line is the least squares regression line for the data points; the dotted line is the fit to a Bessel function redrawn from Fig. 7.

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