Propagated Spikes and Secretion in a Coelenterate Glandular Epithelium

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ABSTRACT The rete mirabile of *Hippopodius* (Cl. Hydrozoa, O. Siphonophora) is a sheet of giant endoderm cells penetrated by branches of the ventral radial canal. The cells appear to be highly polyploid. The rough ER is very richly developed and expanded ER cisternae containing amorphous material (presumably synthesized protein) are observed near the outer cell surface. The cells are electrically coupled, and are connected by gap junctions. The rete is electrically excitable and cell to cell conduction of action potentials at 10 cm/s is observed. The action potentials are all-or-none, positive-going events, showing amplitudes of about 70 mV and arising from a 44 mV resting potential. Slowly developing and decaying secondary depolarizations, capable of summing to the 20 mV level, are also observed. After passage of a train of impulses, the rete cells swell and secretion drops appear at the surface, these changes becoming apparent within a few seconds. In 15 mM Mn^{2+} the response fails to occur, and secondary depolarizations ("secretion potentials") are not seen. Spike propagation is not affected. In Na^+-free solutions the spikes are reduced and propagation eventually fails. It is suggested that the spikes are sodium-dependent events which trigger a calcium-dependent secretory process. The composition and biological activity of the secretion are uncertain, but indirect evidence suggests a possible defensive or repellant role for the response.

Impulse propagation in epithelia is known to occur in hydrozoan coelenterates and amphibian tadpoles and may occur in a number of other groups (see reviews by Mackie, 1970, and Spencer, 1974), to which must now be added pelagic tunicates (Bone and Mackie, 1975; Mackie and Bone, 1976). The analysis of these propagated events at the cellular level has been carried furthest in *Xenopus* tadpoles, where skin impulses are shown to be sodium-dependent events, and where the epithelial cells are found to be electrically coupled (Roberts and Stirling, 1971). Hitherto no equivalent information has been available for hydrozoan epithelia which are usually very thin and difficult to record from, but the presence of gap junctions between the cells in regions known to conduct supports the view that here too impulses probably spread by direct current flow from cell to cell.

The present work began in an attempt to obtain intracellular recordings from an excitable hydrozoan epithelium. The rete mirabile (hereafter, "rete") of *Hippopodius* was chosen for study because of the accessibility and large size of its cells (Chun, 1890, 1897; Münter, 1912). The preparation turned out to be suitable for microelectrode recordings. The rete also proved to have the wholly

unsuspected property of swelling up and producing secretion droplets when activated by passage of impulses. While the coordination of secretion has been suggested previously as a possible function for epithelial conduction (Mackie, 1970; Roberts, 1971), the phenomenon has not hitherto been demonstrated experimentally.

The tissue thus has a double interest: as the first reported case outside the Chordata of an excitable epithelium where the cells are large enough to permit routine penetration with microelectrodes (in which it offers scope for much more extensive work in the future) and as an apparently unique case of a glandular tissue which propagates the spikes which trigger release of the secretion product.

METHODS

Reference should be made to accounts by Chun (1897), Münter (1912), Totton (1965), and Carré (1968) for information on the anatomy and development of *Hippopodius* and to Mackie (1965) and Mackie and Mackie (1967) for behavioral physiology. The species in question is *H. hippopus* Forskal, specimens of which were collected in the Bay of Villefranche-sur-Mer with fair regularity during April and May, 1975. The experiments were carried out at the Station Zoologique, Université de Paris VI, at Villefranche-sur-Mer.

The rete is conspicuously located in the ventral radial canal of each nectophore, and beyond removing the nectophore and pinning it down on a Sylgard platform, no dissection was needed in order to prepare the gland for electrode work. The tissues are transparent, and the individual cells can be seen clearly with a dissecting microscope at a magnification of × 50. The only refinement to the viewing system worthy of note was a double substage mirror reflecting a light beam independently up each half of the microscope (Fig. 1).

Intracellular recordings were made with 30 mΩ glass microelectrodes filled with 3 M KCl, in conjunction with a Medistor A35 electrometer amplifier and an FET amplifier, based on a design by Lee Vernon, University of Oregon. The latter was equipped with a bridge arrangement permitting current injection, but it was not found possible to excite the cells by this means. Instead, stimuli were given by an external suction electrode placed adjacent to the rete. Shocks excite the endoderm sheet, of which the rete is a part, and impulses propagate directly to the rete on an all-or-none basis.

Polyethylene suction electrodes were used in some experiments to monitor passage of the impulse and to measure conduction velocity across the rete.

Amplified signals were displayed on a Tektronix 5103 storage oscilloscope and photographed with a Polaroid camera. The records included in this report have been photographically reversed, and the prints retouched where necessary to restore faint regions lost during processing.

For microscopic examination of the cells, thin pieces of the subumbrellar wall bearing the rete were cut out with scissors, mounted in seawater, and examined by phase contrast or Nomarski interference microscopy. Permanent whole mounts of fixed rete tissues were also made for general study. A Philips EM 300 was used for electron microscopic study of sections cut from Epon blocks of rete tissues fixed in 4% glutaraldehyde, post-fixed in 2% osmium tetroxide, both buffered in cacodylate buffer.

ANATOMICAL RELATIONSHIPS

(a) The Resting Condition

The rete, described by Leuckart (1854) as a "sinus," is actually an expanded region of the ventral radial canal and consists of a layer of large endodermal cells

which form the lateral walls of the branching canal system (Fig. 2 A). The outer walls of the canals are formed by smaller, nonglandular endodermal cells whose processes spread out over the gland cells, separating them in some places from the mesogloea.

The mesogloea is a layer of extracellular, fibrous material about 200 nm thick, perforated in places, allowing direct contact between the endoderm cells and those of the covering (ectodermal) epithelium. The latter shows the usual features of this layer (Mackie, 1965) including mucous granules. Nerves are absent from both ectoderm and endoderm in the region of the rete.

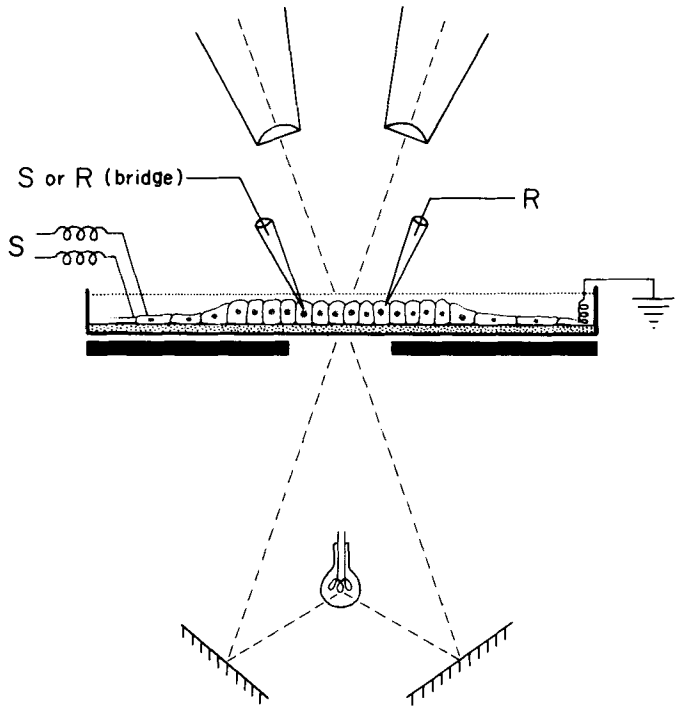
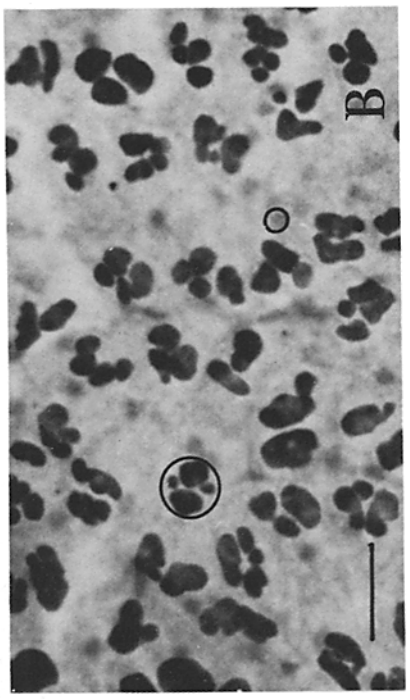


FIGURE 1. Recording arrangement. External stimulating (*S*) electrodes are used to evoke impulses in the excitable epithelium adjacent to the rete. These events propagate to the rete where they are recorded by intracellular recording (*R*) electrodes, one of which (*S* or *R*) is wired to allow simultaneous current injection. The preparation is mounted on the stage of a binocular microscope equipped with a double substage mirror.

The endodermal gland cells are much larger than other somatic cells in the animal. The nucleus is very large. Spherical in young nectophores, it becomes lobulated in older ones and may eventually separate into fragments (Chun, 1890; Münter, 1912). Chun (1890) likened the rete cells to giant cells in other animals where "intensive metabolic activity" was in evidence. The nuclei are strongly Feulgen-positive and are probably highly polyploid, like the giant secretory cells of insect larvae (Painter and Biesele, 1966) and larvaceans (Fenaux, 1971).

The most striking feature of the cytoplasm (Fig. 3 A) is the extremely rich and densely packed rough endoplasmic reticulum (RER). The cell is clearly special-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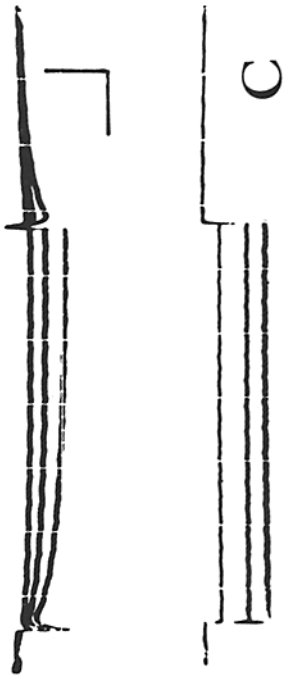
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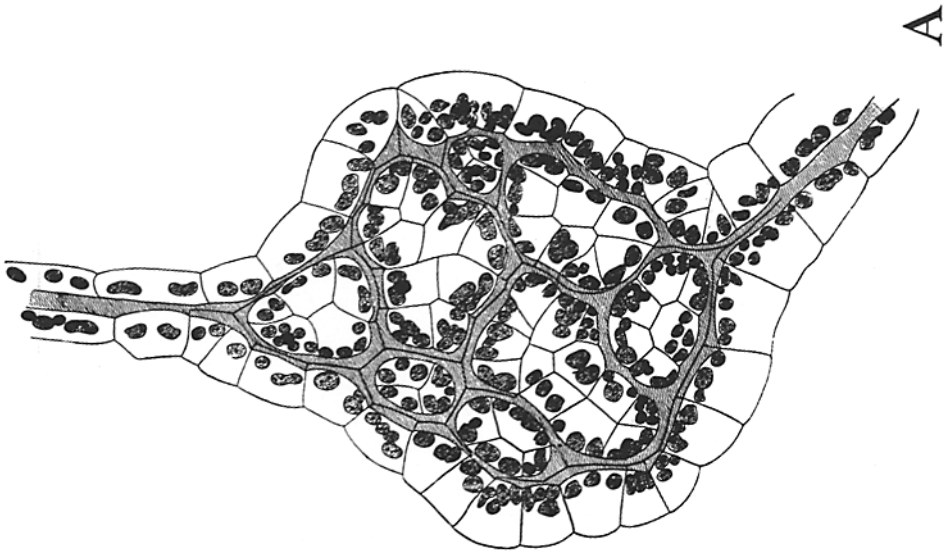
A

ized for synthesis of protein for external use (Palade, 1975), but in contrast to the process in most such cells the secretion product is not segregated in storage granules, but seems to lie within expanded cisternae of the RER near the outer surface (Fig. 4 A). The Golgi component, elsewhere associated with concentration and packaging of secretory products, is not especially prominent. An apparent absence of specific storage granules is also a feature of plasma cells (Zagury et al., 1970) but such cases are rather exceptional.

The gland cells are joined by septate desmosomes at their luminal borders. Elsewhere the applied membranes are some 30–50 nm apart, except for close contact points identifiable as gap junctions (Fig. 3 B) and resembling such junctions in other hydrozoan tissues (Hand and Gobel, 1972; Chapman, 1974).

The luminal surface of the gland cells bears microvillus projections and there are numerous vesicles in the adjacent cytoplasm, suggestive of pinocytosis. There is no indication of discharge of secretory matter into the canals or elsewhere in glands fixed while in the resting state.

(b) The Excited State

Electrical or mechanical stimulation of the excitable epithelium which covers the nectophore evokes propagated epithelial impulses which spread over the whole surface, and also enter the endoderm and invade the rete. In this respect the nectophore resembles medusae such as *Sarsia* and *Euphysa* (Mackie and Passano, 1968) where exumbrellar impulses spread into the subumbrellar endoderm. In *Hippopodius,* the rete can thus be excited by a stimulating electrode placed at a considerable distance from it, as the impulses are propagated without decrement.

The surface of the rete in the resting state is flat and smooth. Following repeated stimulation it undergoes a marked change in appearance. The surface of the gland develops swellings, giving it a berry-like appearance (Fig. 5). Stimulation at 2 Hz for 5 to 10 s usually produces a visible change, which becomes more striking the longer the stimulation is continued. The preparation shown in Fig. 5 B was stimulated for 20 s. It returned to the smooth, flat state again within 4 min of the end of the period of stimulation.

The outlines of these swellings are the same as the outlines of the gland cells prior to stimulation, so it is concluded that the gland cells are themselves increasing in volume so as to produce this bulging appearance at the outer surface. Unfortunately, not enough material was fixed in the excited condition to permit a description of the excited gland at the electron microscopic level, or to say what exactly happens during the swelling process.

Following strong stimulation, the surface of the rete appears not only swollen

FIGURE 2. A, rete of *Abyla* (Chun, 1897). The rete of *Hippopodius* is similar but larger. B, Feulgen-stained rete of *Hippopodius*. The clusters of nuclear lobules or fragments (e.g. in the large ring) represent nuclei of single gland cells. Diploid nuclei of ectoderm (e.g. in small ring) are much smaller and fainter. Scale, 50 μ, $\times$ 250. C, coupling of gland cells. Distance between cells penetrated was 280 μ, equivalent to a pathway crossing six membrane junctions. Horizontal scale, 50 ms; vertical scale, 20 mV (upper), 10^{-7} amp (lower).

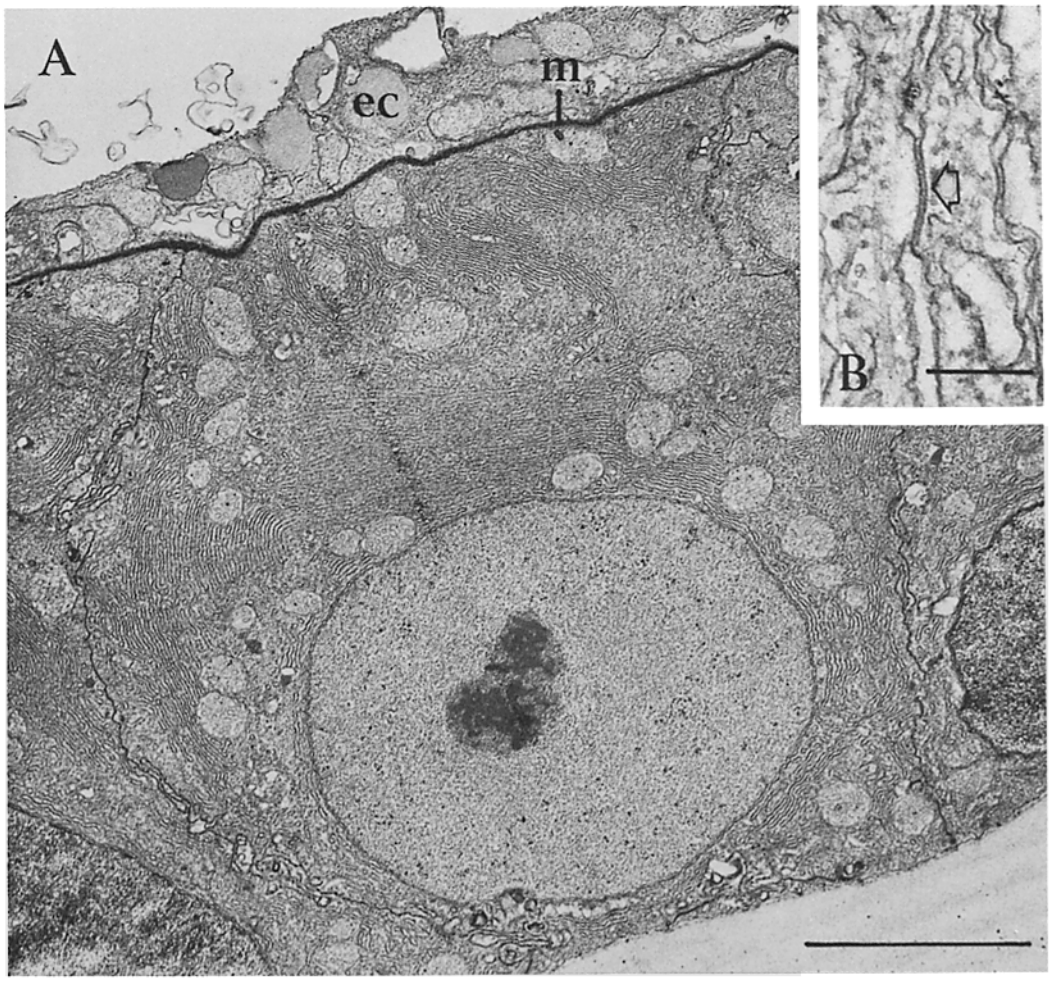

FIGURE 3. A, gland cell in endoderm of a young rete. The cell is separated from the ectoderm (*ec*) by a thin layer of mesogloea (*m*). Scale, 5 μ, × 6,000. B, gap junction (arrow) between two gland cells. Scale, 0.2 μ, × 70,000.

but frothy under phase contrast or Nomarski interference optics. Frothiness is due to the presence of small droplets at the surface of the gland cells. The droplets coalesce, forming larger pools of fluid. An hour or more after stimulation, long after the swelling has subsided, small residual pools of fluid can still be seen between the gland cells and the mesogloea (Fig. 4 B).

Setting up a rete for microscopic or physiological study involves dissection and pinning, which strongly excite the conducting epithelia, including the rete. Newly mounted preparations invariably show swollen retes. In practice, it is best to wait for at least half an hour before starting work with electrodes, to allow the gland to return to the resting state and secretion to disperse.

The changes in appearance of the gland accompanying excitation fail to occur when manganese ions are added to the seawater at a concentration of 15 to 20 mM.

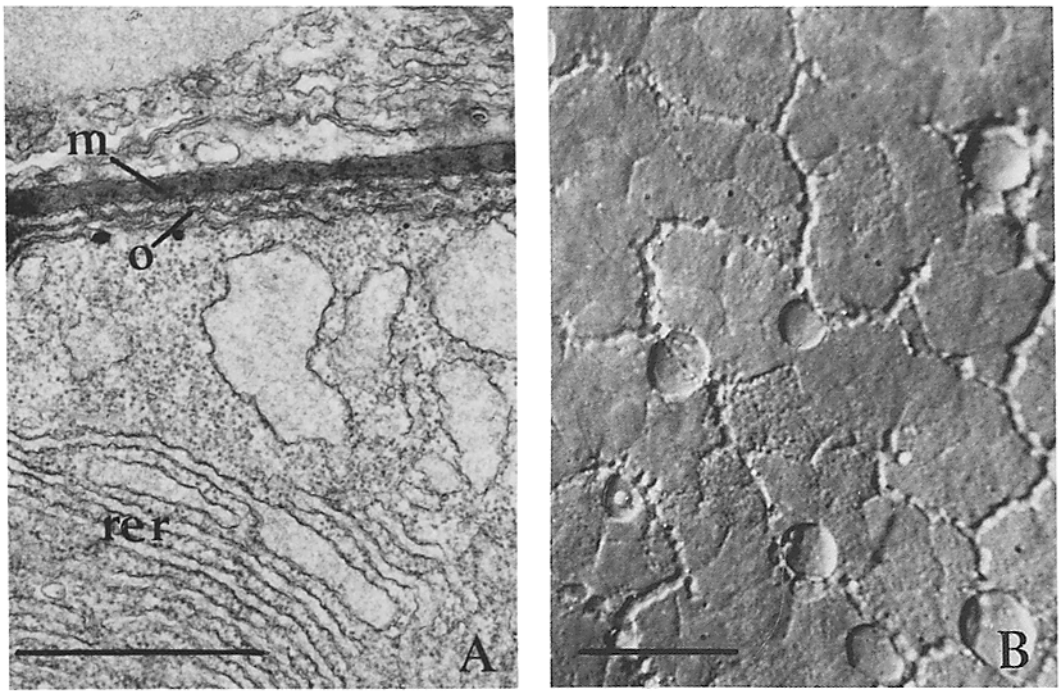

FIGURE 4. A, expanded cisternae of rough endoplasmic reticulum (*rer*) near outer end of a resting gland cell. A thin process from an outer canal wall cell (*o*) runs between the gland cell and the mesogloea (*m*) in this section. Scale, 1 μ, × 30,000. B, view of surface of rete in resting state, after recovery from the excited state. Secretion droplets lie over the gland cells. Outlines of rete cells and canals can be seen. Scale, 50 μ, × 2,000.

ELECTROPHYSIOLOGICAL RESULTS

(a) Intercellular Conduction and Coupling

The two dimensional structure of the rete allows conduction velocity to be measured by a simple linear arrangement of stimulating and recording electrodes placed upon its surface. Impulse conduction through the surrounding nonglandular epithelium is abolished by cutting around the rete with a scalpel. A mean conduction velocity value of 10 cm/s at 21°C was obtained in a series of measurements made across one rete.

Current pulses passed through a microelectrode placed in a rete cell produce corresponding voltage changes in neighboring cells (Fig. 2 C) showing that current can flow between cells.

(b) Wave Form of the Active Response

The active response of rete gland cells consists of two components (Fig. 6), an initial fast spike and a delayed, slow depolarization. The wave form characteristics are summarized in Table I. The resting potential is stable within the range shown, and shows no fluctuations when the gland is not stimulated. Single shocks usually evoke single spikes but repetitive firing is sometimes seen (Figs. 6 C and 7 D) as in other hydrozoan epithelia (Mackie and Passano, 1968).

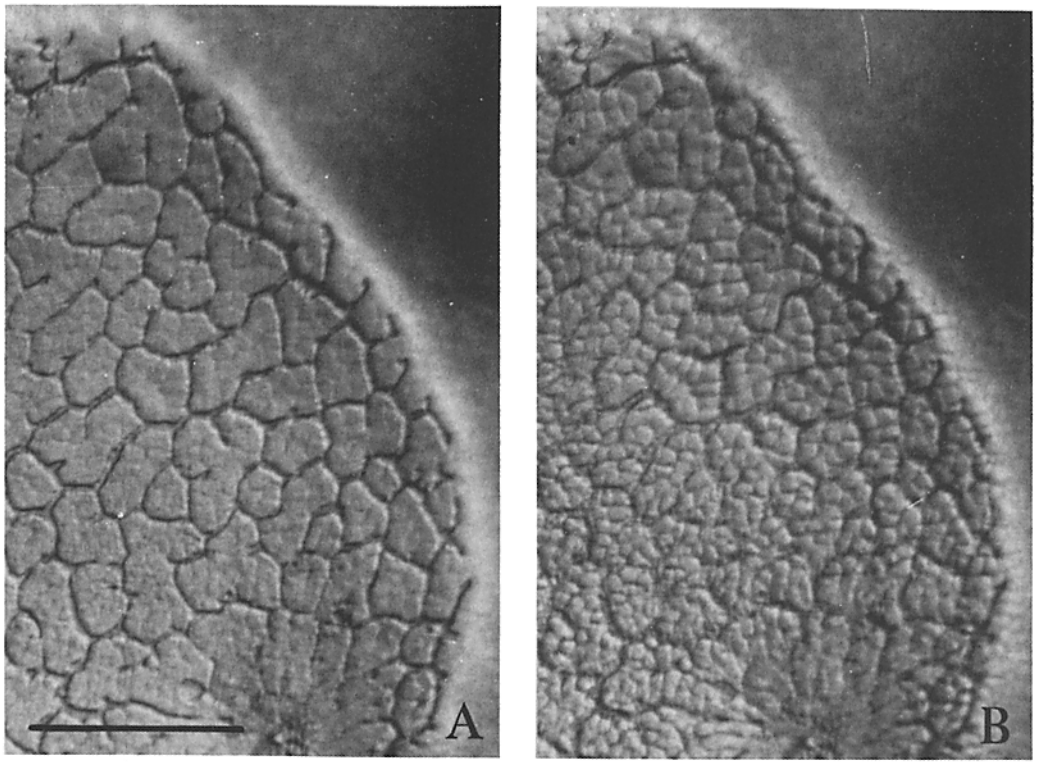

FIGURE 5. Resting (A) and excited (B) states compared. Scale, 500 μ, × 750 (see text).

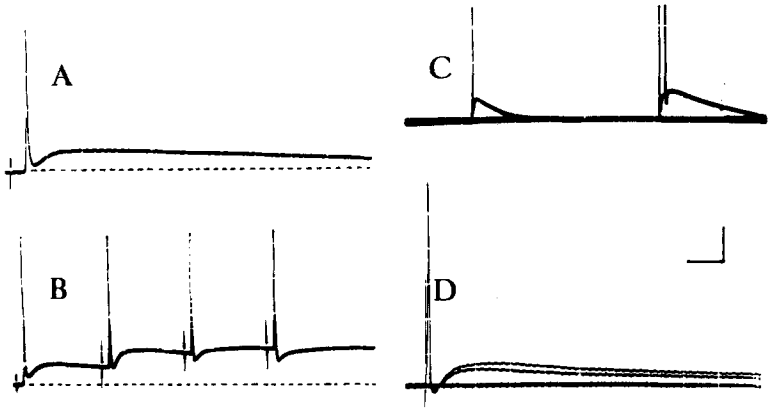

FIGURE 6. Intracellular recordings from rete cells. Stimuli were delivered on adjacent conducting epithelia. A, single spike followed by secretion potential. B, summing of secretion potentials to 20 mV level following series of shocks. C, spikes and secretion potentials at slower speed (several sweeps superimposed). D, two superimposed sweeps 5 s apart, showing facilitation of secretion potentials. Time scale represents 0.1 s in A, B, and D, 0.5 s in C. Amplification scale represents 20 mV in A, B, and C, and 10 mV in D.

TABLE I
WAVE FORM PARAMETERS

	Resting potential	Spike		Afterdepolarization	
		Amplitude	Duration	Amplitude	Duration
	mV	*mV*	*ms*	*mV*	*ms*
Seawater control	43.3±5.0	69.7±8.4	13.3±1.3	9.8±3.4	approx. 1000
Seawater plus 15 mM manganese	44.0±2.0	68.7±4.2	13.0±1.0	–	–

The secondary depolarizations show amplitudes within the range of 4 to 16 mV, the highest point being reached within 150 ms of the spike. Summation (Figs. 6 B and C) of secondary depolarizations occurs, the summating series reaching a maximum plateau of about 20 mV, with a correspondingly lengthened recovery time. Decay from the 20 mV level occurs with a time constant of about 2 s.

Facilitation is observed in the early steps of a series of responses, starting from the resting level. In the example shown (Fig. 6 D) the response to a second shock coming 5 s after the first is elevated by about 3 mV above the level of the first.

(c) Effects of Altering the External Ionic Milieu

In seawater containing 15–20 mM Mn^{2+} the slow depolarization is abolished. Manganese competes with calcium, suppressing calcium conductance in crustacean muscle (Hagiwara and Nakajima, 1966) and in other tissues. In some experiments, slight attenuation of the spike was noted, but spike height was never reduced below the level seen in normal cells in ordinary seawater (Table I) and the lowered response could have been due to a variety of causes. The spike now shows a hyperpolarizing afterpotential (Fig. 7 B) in place of the slow depolarization shown by the control (Fig. 7 A). The slow depolarization does not re-emerge even after a series of spikes which would suffice for summation to the 20 mV level in a control cell.

Artificial seawaters made up with choline chloride or Tris-HCl substituted for sodium chloride have the effect of reducing the spike height to below 40 mV and increasing its duration, as shown in Figs. 7 C and D. Spikes evoked by successive shocks 1 s apart showed further progressive attenuation (Fig. 7 C). Attenuated spikes are still propagated through the rete, but become rather hard to evoke on a one-to-one basis with stimuli and eventually fail. Secondary depolarizations following single spikes are small or absent, but can still be obtained after a series of spikes (Fig. 7 D).

A specimen placed in Na^+-free seawater with 15 mM Mn^{2+} initially shows small events like the "fatigued" spikes shown in Na^+-free water, but these become hard to evoke, and the tissue soon becomes unresponsive. A piece of tissue placed in Na^+-free seawater could probably carry a considerable amount of Na^+ in with it, enough to support electrogenesis for a while before it diffused away.

The effects of altered ion concentrations reported above are all reversible.

DISCUSSION

This investigation, though of a preliminary nature, establishes certain important facts about the rete gland cells. They are electrically excitable, forming a conducting epithelium in which all-or-none sodium-dependent impulses propagate from cell to cell, presumably electrically via gap junctions, triggering release of a secretion product, with calcium as the coupling ion.

While the main outlines of this picture are quite clear, the details remain unexplored and some particularly puzzling features may be singled out for special comment.

1. Storage of secretion product prior to release. Cells which produce large amounts of protein for export usually show conspicuous vacuoles in which the product is condensed and stored (Palade, 1975) but the rete cells show no such bodies and seem to store the product in the rough endoplasmic reticulum.

2. Swelling preceding release. It is hard to find a parallel for the gross swelling which the activated rete cells show a few seconds after stimulation.

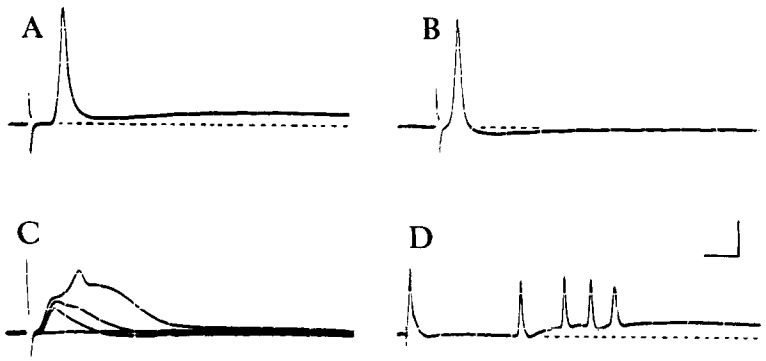

FIGURE 7. Experiments with ions. A, control in seawater. B, with 15 mM Mn^{2+} C, low Na^{+}, three shocks, 1 s apart showing declining response. D, production of secretion potential after series of spikes in low Na^{+}. Time scale represents 20 ms in A, B, and C, 100 ms in D. Amplification scale represents 20 mV in each case.

Swelling accompanies electrical activity in squid axons (Hill, 1950) but after 10,000 impulses the increase in radius only amounts to 0.05%. Gland cells often swell when activated owing to the intensive synthesis of secretion product, but this process typically takes hours rather than seconds to become apparent. Even in rapidly responding endocrine glands (Luborsky-Moore et al., 1975) there is no evidence of new protein synthesis until 5–10 min after stimulation. Microfilaments are absent or very scarce in the gland cells, so the swelling can hardly be due to contractile deformation of the cells. Perhaps the swelling represents water uptake and hydration of the raw secretion product. There might be a parallel here with mucocyst discharge in *Tetrahymena* which seems to involve solubilization and osmotic swelling of the product (Satir, 1974), a possibility which is being investigated.

3. Electrogenic events. The occurrence of propagated electrical events in this gland is not in itself surprising since other epithelia in the same animal conduct impulses, and several other effector responses are coordinated on the same

principle (Mackie, 1965; Mackie and Mackie, 1967). While the primary propagated event appears to be a conventional sodium spike, the secondary event (secretion potential) is blocked by manganese, suggesting that calcium ions are involved in the triggering step or that the secretion potential itself represents calcium current. However, potential changes might also be expected to accompany rapid water uptake, or exocytosis, and further analysis is obviously necessary. The long time course of the depolarization and the evidence of facilitation and summation appear to be without parallel in other gland cells which have been studied.

4. Role of Ca^{2+}. Calcium is well known to act as a coupling ion in many secretory processes (Rubin, 1970; Matthews, 1970), but the exact nature of its involvement is still being actively debated. Calcium may be involved not only in the triggering step but also in binding of the vesicles to the cell membrane, and the ions may be mobilized from intracellular storage sites, or flow in from outside (Dean, Matthews, and Sakamoto, 1975; Chandler and Williams, 1974). The *Hippopodius* cells, which are large and easy to keep electrodes in for long periods, seem to offer a potentially useful preparation for analysis of the electroionic events associated with the secretion process.

5. Biological role of the secretion. An earlier suggestion (Mackie, 1975) made before the results of electron microscopy were available referred to a possible role in ionic regulation, but the ultrastructural specializations of the rete cells point unmistakably to protein synthesis as the main function. Laboratory observations (Mackie, 1965; Mackie and Mackie, 1967) show that the excitable epithelia in this animal are never spontaneously active but respond only to external tactile stimulation. They constitute a transmission pathway mediating a variety of effector responses associated with protection and defense. The rete will be excited under the same conditions which evoke these defensive responses and therefore presumably functions in a defensive role itself. There are several examples of defensive secretions in marine invertebrates including predator repellants (Bakus, 1968; Edmunds, 1968) and alarm pheromones (Snyder and Snyder, 1970; Howe and Sheikh, 1975).

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