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Electrophysiology of a Myoid Epithelium in *Chelophyes* (Coelenterata: Siphonophora)

B.M. Chain [1]*, Q. Bone [2], and P.A.V. Anderson [3]

[1] Department of Zoology, Downing Street, Cambridge, England
[2] The Marine Laboratory, Citadel Hill, Plymouth, England
[3] Department of Developmental and Cell Biology, University of California, Irvine, California 92717, USA

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Summary. The passive electrical properties, and the ionic basis of the action potential have been examined in the subumbrella myoid epithelium of the siphonophore *Chelophyes*. The myoepithelial cells are electrically coupled, and are 20 μm wide, some 1 mm long, and only 5 μm thick. Membrane constants determined by a 2-electrode study were: $\lambda = 280$ μm; $R_m = 0.11$ kOhm/cm^2; $R_i = 24$ Ohm/cm. Mean resting potential was −85 mV. The first action potential of a series (whether evoked by repetitive stimulation, or occurring in a natural unstimulated swimming burst) shows a rapid rise and fall with no afterpotential. The overshoot is small, but successive action potentials show a remarkable facilitation, overshooting by as much as 70 mV. They also show a plateau phase after the initial rapid rise, which is terminated by a rapid fall. Conduction velocity was 27 cm/s.

Changes in the external milieu, and the effects of Ca^{2+} blocking agents indicated that the action potentials are complex events. Although insensitive to TTX, the action potential is dependent on external sodium concentration, and is not abolished by Ca^{2+} blocking agents: in this respect it resembles the sodium-dependent action potentials of other siphonophore tissues.

The ionic basis of the facilitated action potentials is not yet clear, but it seems probable that a fast potassium conductance terminating the unfacilitated action potential is progressively inactivated during repetitive activity, and that the plateau phase of the facilitated action potential is maintained by a sodium conductance mechanism, to be terminated by a calcium-activated potassium conductance.

* Present address: Sloan Kettering Institute for Cancer Research, 410, East 68th St., New York, New York 10021, USA

Abbreviations: EGTA 1,2-bis-[2-di(carboxymethyl)-amino-ethoxyl]-ethane; *TEA* tetraethyl ammonium chloride; *TTX* tetrodotoxin

Introduction

The contractile responses of coelenterates range from localised and very slow postural movements like those of many anthozoans and hydrozoan polyps, to the rapid co-ordinated swimming movements of some medusae and siphonophores. Little is known, however, of the cellular events underlying any of these locomotor activities. This study describes some electrical and ionic properties of muscle cells in one of the most rapidly swimming of all coelenterates, the diphyid siphonophore *Chelophyes*. The cells form a myoid epithelium lining the inner surface of each nectophore, which is easily accessible, does not exhibit intrinsic spontaneous contractions, and despite its thinness, permits stable long-term intracellular recording. The tissue therefore provides an unique opportunity to examine the properties of a coelenterate myoid epithelium. The electrophysiology and ultrastructure of *Chelophyes* have been the object of a study by G.O. Mackie and D. Carré (in preparation), who made extracellular recordings of the potentials associated with contraction, and showed that the action potentials were propagated through the subumbrella ectodermal epithelium by myoid conduction. Our results extend their observations on the properties of this conduction system.

Materials and Methods

Chelophyes appendiculata (Eschscholtz) is a small calycophoran siphonophore consisting of two linked nectophores (Fig. 1) and a trailing fishing stem provided with nematocyst batteries. Fairly regular supplies of *Chelophyes* colonies were collected in plankton tows (from 0–30 m depth) in the Rade de Villefranche between March and May 1979; the colonies could be maintained for some days in bowls of seawater in a cold room.

Locomotion is by jet propulsion, the smaller posterior nectophore being used for low speed swimming, and the anterior nectophore for short bursts of rapid escape swimming (Totton 1954, p. 129). We used only the larger anterior nectophore, which consists

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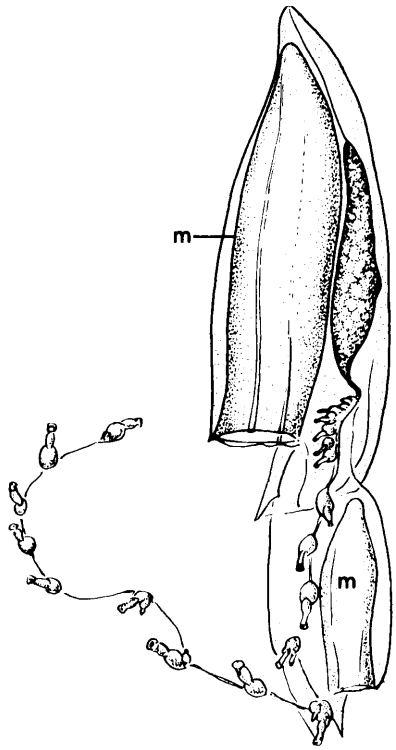

Fig. 1. Colony of *Chelophyes* (from photograph in Totton 1965), showing larger anterior nectophore and trailing stolon. The subumbrella myoepithelial sheet (*m*) lines the nectophores (Drawn by Mr J. Rodford, Dept. of Zoology, Cambridge University)

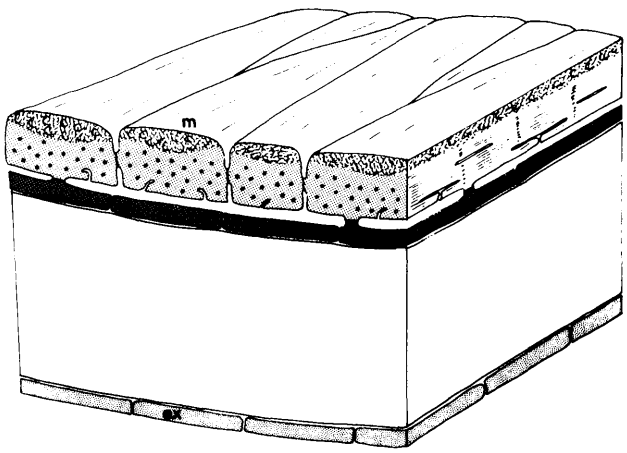

Fig. 2. Block diagram of portion of anterior nectophore based on ultrastructural observations by Dr. Carré and by the present authors. The subumbrella myoepithelial cells (*m*) are fusiform and interdigitate, and have an outer mitochondrial zone and an inner myofilamentous zone. They overlie (and are coupled to) the endodermal epithelium (*darker stipple*). The major thickness of the nectophore consists of elastic mesogloea (reduced in dimension in the diagram); this is covered externally by the exumbrella epithelium (*ex*)

of an elongate chamber lined with the ectodermal myoepithelial cells of the subumbrella. These are aligned circumferentially around the chamber; their contractions expel water from the nectophore base and drive the colony forwards. Refilling of the pressure chamber is entirely due to the elasticity of the mesogloeal walls, which oppose the subumbrellar muscle. Locomotion is described in detail by Bone and Trueman (in preparation).

Apart from two nerve rings at the base of the nectophore (around the aperture) nerves are absent from the nectophore walls. The structure of the wall is shown diagrammatically in Fig. 2. The myoepithelial cells lining the chamber are about 1 mm long, 20 μm wide, and at most, 5 μm thick. Each consists of an outer mitochondrial zone, and an inner zone of cross-striated myofibrils. The myofibrillar zone is penetrated by a regular tubular array which appears to be derived from the sarcolemma (thus having something of the same morphological relationships as the T-system of vertebrate muscle cells). The cells lie upon a thin mesogloeal sheet, which seems to penetrate inside them, via the 'T-tubules', so that the bases of the cells are much invaginated. Under the thin mesogloeal layer, there is a sheet of endodermal cells, and then the thick mesogloea forming the main bulk of the nectophore. The endodermal cells and the subumbrellar ectodermal sheet are coupled by numerous gap junctions between transmesogloeal processes. We are indebted to Dr. D. Carré for these ultrastructural details (which we have confirmed on our own thin sections); they are significant in understanding our electrophysiological observations.

Intracellular records were made by cutting the anterior nectophore open and pinning it out in seawater on a Sylgard base, thus exposing the subumbrella. Under these conditions, preparations remained active for many hours, often overnight, and showed intermittent short bursts of contractions. Such contraction bursts could also be evoked by light touch on the nectophore surface, or by electrical stimulation of the region containing the two nerve rings at its base. Spontaneous activity has been shown to be controlled by these nerve rings (Mackie and Carré, in prep.), and in some preparations was deliberately abolished by cutting the nectophore base away. 'Nerve-free' preparations of this sort showed no spontaneous activity, and could only rarely be fired repetitively by large stimuli. The action potentials were similar to those observed in intact preparations.

Conventional KCl-filled glass microelectrodes (tip resistances greater than 30 megohms) were used for recording; they were coupled to a storage oscilloscope via an FET amplifier, or to a pen-recorder via a transient store.

For experiments involving current injection, two microelectrodes were used, one connected to the recording equipment as usual and the other connected via a 20 MOhm resistor to a Digitimer stimulator. The potential of the current electrode was monitored through the second channel of the amplifier. The total current passed was measured by a current amplifier in the earth circuit. The lowest values of tip resistance which still permitted penetration were used for the current electrodes, in order to obtain maximum linearity and amplitude of the current pulses. Initial attempts to use only a single electrode, in conjunction with a Wheatstone bridge current injection circuit were unsuccessful because of the very large currents required. For extracellular stimulation, short pulses (2 10 ms) were delivered from an isolated Digitimer stimulator, via two 25 micron platinum wires placed on the surface of the epithelium. For the experiments involving ionic substitution, impalements were made in a small recording chamber moulded in Sylgard with a total volume of approximately 1 ml. Solutions flowed continuously into the chamber via a multiway non-return valve, and were removed at the other end by suction. Complete exchange of the solution in the bath took place in about one min. The control solution was fresh filtered sea water. Experimental solutions were based on an artificial sea water with a total salinity of 38‰ corresponding to analyses of local sea water. The solutions were buffered with 10 mM $NaHCO_3$ or, in bicarbonate-free solutions, in Tris/Tris Cl buffer at pH 8 8.2. All the chemicals used were

Table 1. Electrical properties of the epithelium in *Chelophyes* and of some other epithelia. λ space constant; d epithelial thickness; R specific intraepithelial resistivity; R_m membrane resistivity; RP resting potential

Tissue	λ (μm)	d (μm)	R_i(ohmcm)	R_m(Kohmcm^2)	C_m(μFcm^2)	RP (mv)
Chelophyes subumbrella extoderm	280	5	34	0.05 (f=1) 0.11 (f=2)		75–105
Euphysa[a] exumbrella ectoderm	1,400	1.4	196	23		40–60
Ciona[b] heart		10	1.6×10^3	0.19	1.6	
Chelyosoma[b] heart		10	2.8×10^3	0.23	1.7	

[a] Josephson and Schwab (1979)
[b] Kriebel (1968)

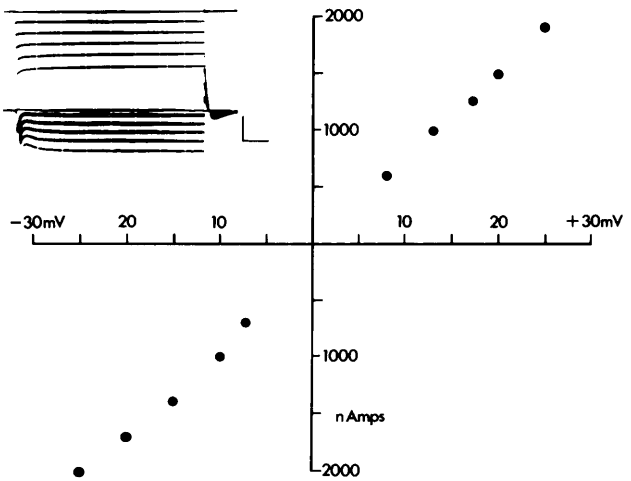

Fig. 3. A typical current-voltage relationship obtained by a two-electrode analysis of the subumbrella epithelium. The slope of the line gives the apparent input impedance of the epithelium. Inset: typical record from such an experiment. Scale bars: 20 ms, upper trace 1,000 nA, lower trace 25 mV

reagent grade. The calcium inhibitor D 600 was kindly given by Prof. P.F. Baker, F.R.S.

Results

A. Passive Electrical Properties

The passive electrical properties of myoid epithelia have received little attention, partly, perhaps because of the technical problems resulting from the thinness of conducting epithelial sheets. However, the ease with which stable penetrations could be obtained in *Chelophyes* made the subumbrella muscle very suitable for such an analysis and the results obtained are summarised in Table 1. The parameters were obtained by a 2-electrode study of the cable properties of the cells. Significant voltage changes could be recorded up to distances of several hundred microns from the current electrode (i.e. many times greater than the cell width). Current flow in the epithelium appeared to be non-directional (see Discussion), so that only interelectrode distances were considered when analysing the data. Over a wide range, the I/V relation was linear (Fig. 3); no evidence was obtained for significant rectification. The slope of the I/V relation can be taken as a measure of the apparent input impedance of the preparation. Figure 4 shows the results of a series of 37 such measurements at different distances between recording and current-injection electrodes, and illustrates the radial spread of current from a point source within the epithelium.

The structure of the subumbrellar myoepithelium appears to meet the criteria for the 'thin plane cell' model described by Eisenberg and Johnson (1970). The passive electrical properties of such an epithelium are well known, and can be described by the properties of the Heavyside Bessel cable (see Eisenberg and Johnson 1970; Shiba 1971; Jongsma and van Rijn 1972; Jack et al. 1975). For a square current pulse, the steady state solution for the voltage change induced at a distance D from a point source of current simplifies to: $V = nK_o(D/\lambda)$mV, where n=constant; K_o is a zero-order Bessel function; and λ is the space constant. The "best-fit" function to the data given in Fig. 4 was obtained graphically by the method of Jongsma and van Rijn (1972), and is shown by the solid line in Fig. 4. The specific membrane resistance (R_m) and intracellular resistance (R_i) can be derived directly from the Bessel function by: $R_i = 2\pi nd\Omega$cm, and $R_m = 2\pi nf\lambda^2\Omega$cm^2, where d=epithelial thickness, and f is between 1 and 2, representing the degree to which the second membrane is taken into account. Insufficient data were obtained for a complete analysis of the time-dependent voltage changes in the epithelium (which would in any case be a complex process).

Values for the membrane constants for the epithe-

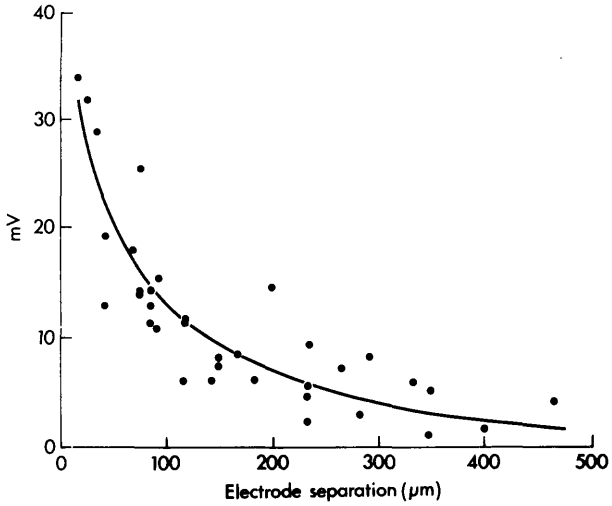

Fig. 4. Data showing radial current spread from a point source within the subumbrella epithelium. Each point represents the result of a series of measurements similar to those of Fig. 2, to obtain the apparent epithelial impedance measured at varying inter-electrode separation. This value (expressed as the voltage deflection which would be produced by a 1 μA current) is plotted against the inter-electrode distance. The curve represents the best-fitting zero-order Bessel function obtained as described in the text

lium calculated as above are shown in Table 1, where data from other excitable epithelia are given for comparison.

B. The Action Potential

a) Extracellular Stimulation

Quantitative details of the action potential are shown in Table 2. Their most prominent and unusual feature is the very pronounced facilitation which occurs on repetitive stimulation (Fig. 5). The first action potential of a burst is a short (approximately 5 ms) event consisting simply of a fast rising and fast falling phase. There is no after potential. The action potential has only a rather small overshoot of a few millivolts. Successive stimuli produce two effects: the fast rising phase reaches a greater amplitude (the rate of rise sometimes increasing as well) and a third completely new plateau phase appears which is terminated by a second fast repolarisation. The amplitude and slope of the plateau is very variable, depending critically on the rate of stimulation (Fig. 6). At the optimal rate (approximately 3/5 impulses/s depending on the preparation) the action potential facilitates rapidly to a maximum level which can overshoot by as much as 70 mV. The first rapid repolarisation disappears completely in this case. On continued stimulation the action potential defacilitates to an intermediate level, in which the plateau has a small negative slope. If

Table 2. Parameters of the action potential in *Chelophyes*

Parameter	Mean value	S.D.	Number of observations
Resting potential (mV)	85.4	2.0	20
1st action potential in a burst			
Amplitude (mV)	98.5	3.1	24
Max. rate of rise (V/s)	46.2	2.6	9
Duration (ms)	8.26	0.6	11
Action potential after maximal facilitation			
Amplitude (mV)	129	3.0	18
Duration (ms)	44	1.9	6
Rate of conduction (cm/s)	27 (range 15.8–41.2)	3.3	7
Intracellular triggering current (μA)	1.4	0.07	12

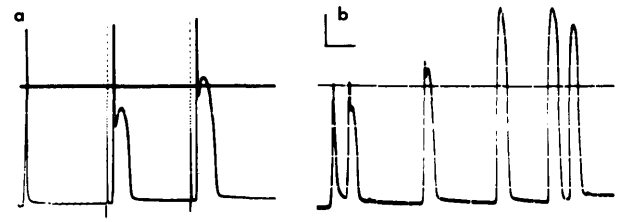

Fig. 5. a Three successive action potentials evoked by electrical stimulation of the basal nerve ring of the nectophore. Scale bars: 50 ms, 20 mV. **b** Series of action potentials in a natural swimming burst evoked by light tactile stimulation of the exumbrella surface. Scale bars: 100 ms, 20 mV

the rate of stimulation is faster, defacilitation is more rapid and extensive and complete block sometimes results. At slower rates facilitation is slower and much less marked. Each action potential, including the first unfacilitated one, is accompanied by a strong 'twitch' contraction of the swimming muscles. Isometric records of twitch tensions of the subumbrella muscle sheet during swimming bursts (Bone, unpublished observations) did not show any obvious facilitation, nor did records of pressures within the intact nectophore resulting from successive muscle contractions within a swimming burst (Bone and Trueman, in preparation).

During spontaneous swimming bursts in intact preparations (Fig. 5b), the action potentials fired at frequencies just above that giving the optimal regime for maximum facilitation observed above.

Conduction velocity was measured as the time between the stimulus artifact and the base of the action potential. It too sometimes showed slight facilita-

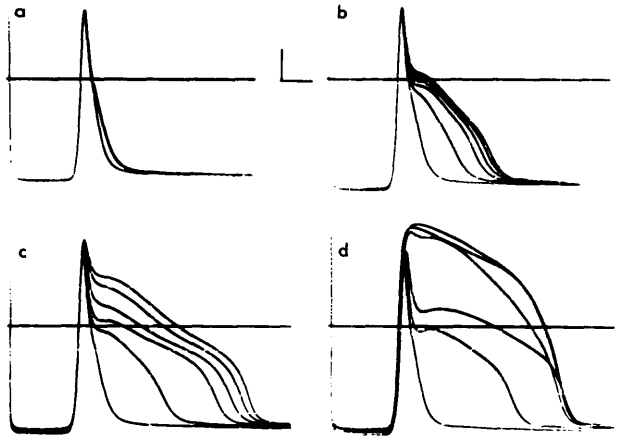

Fig. 6a–d. Superimposed sweeps of series of action potentials evoked by electrical stimulation of the basal nerve ring at different frequencies. **a** 0.2 Hz; **b** 0.5 Hz; **c** 1 Hz; **d** 3.3 Hz. All from same cell. Scale bars: 5 ms, 20 mV

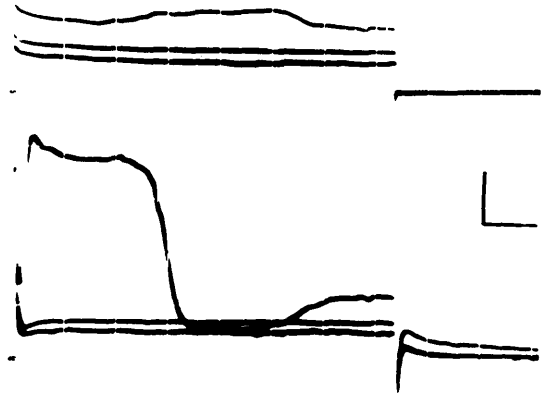

Fig. 7. Action potential evoked by intracellular current injection. Note that the action potential is partially facilitated although it has not been preceded by others. Upper trace, injected current. Scale bars: 20 ms, 25 mV lower trace, 1,000 nA upper trace

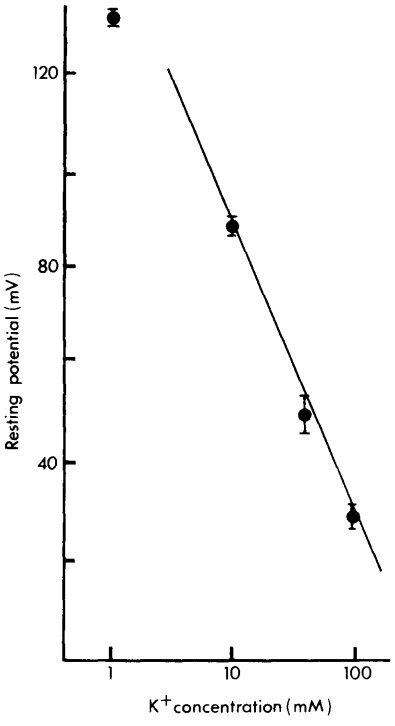

Fig. 8. Resting potential as a function of external K^+. Bars represent S.E. of mean ($n=5$)

tion. The value obtained is close to that of 35 cm/s given by Mackie (1965) (see Table 2).

b) Intracellular Stimulation

The epithelial system can be fired by current injection through a second electrode (Fig. 7). Very large currents are required and the threshold level of depolarisation at the current source is high. This high threshold phenomenon is well known in other epithelial tissues, where it is frequently impossible to fire the action potential with a point current source (see Tomita 1970). It is due to the steep drop in voltage around the current electrode owing to the two dimensional current flow into the epithelium, which makes it difficult to depolarise a sufficient minimal length of membrane. Once the action potential is initiated, cessation of current flow simply causes an equivalent drop in membrane potential but does not interrupt the action potential. Action potentials fired intracellularly show facilitation in the normal way, though repetitive firing is difficult to induce. The falling phase of the action potential is also more irregular in many cases and a positive after potential is usually present if the current pulse is continued after the duration of the action potential (Fig. 7). Contraction occurs in the usual way when an action potential is fired intracellularly. No visible 'subthreshold' contractions were ever observed, despite very considerable depolarisations of the active membrane (cf. potassium effects).

c) The Ionic Basis of the Action Potential

i. Monovalent Cations. Potassium: K^+ ions rapidly depolarise the membrane. The dependence of the resting potential on external K^+ concentration closely approximates to the theoretical behaviour of a Nernst K^+ electrode over the range 10–100 mM K with a slope of 60 mV per decade change in concentration (Fig. 8).

There is no evidence that K^+ depolarisation causes contraction of the muscle fibres. The effects of K^+ are all readily reversible. The rapidity of the

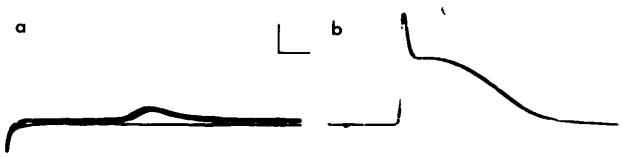

Fig. 9. a An action potential in 50% Na^+ seawater. Scale bar: 20 ms, 25 mV. **b** An action potential from a burst in Ca-free EGTA sea water. Scale bars: 20 ms, 25 mV

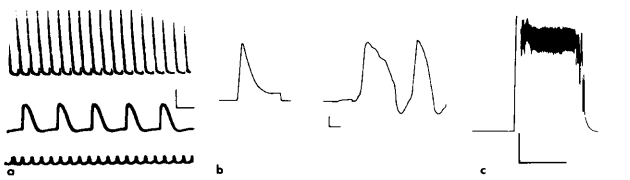

Fig. 10. a Succeeding stages in the effects of Ca^{2+}-free sea water (with EGTA) on the action potential. See text for full description. Scale bars upper record 200 ms, 25 mV, mid- and lower record: 100 ms, 25 mV. **b** The effects of Ba^{2+} on the action potential. Left: normal sea water, right: after 15 min in 20 mM $BaCl_2$. Scale bars: left: 5 ms, 20 mV, right: 50 ms, 20 mV. **c** The effect of Ca^{2+}-free Sr^{2+}-substituted sea water on the action potential. A single stimulus evokes first a normal action potential (not shown), followed by a series of repetitive small action potentials. Scale bars: 15 s, 20 mV

effects indicate (as expected) that no diffusion barrier is present between the excitable membrane and the external solution.

Sodium: Changes in external Na^+ did not affect the resting potential, but action potential generation is critically dependent on the external concentration of Na^+. In 25% or 10% Na^+ sea water (choline or tris-substituted) the action potential was completely blocked. With 50% sea water the effects are somewhat variable. The stimulus threshold becomes very high. On one occasion, the action potential was almost totally abolished and no facilitation occurred (Fig. 9a). In six other preparations the initial action potential of a burst was slightly attenuated, and the rate of rise lowered. Facilitation occurred to a maximum level of 101 mV (SE $\pm$6), 28 mV lower than in normal sea water. The relatively high Na^+ concentration at which propagation is blocked makes a precise analysis of Na^+-dependent changes of amplitude rather difficult.

Lithium: 90% of the Na^+ ions could be replaced by lithium with no effect on the action potential.

ii. Divalent Cations. Calcium: The effects of Ca^{2+}-free sea water (replaced by magnesium) on the preparation were complex (Figs. 9b, 10a). Three sequential stages of the response could be observed: i) an increase in excitability and in spontaneous swimming bursts; a regular 'bursting' pattern was sometimes evident. In nerve-free nectophores, a single stimulus resulted in increasingly long bursts of repetitive activity.

(ii) the epithelial cells began to fire continuously at steadily increasing frequencies. During this phase movement of the epithelium weakened and eventually ceased (Fig. 10a, upper trace).

(iii) the amplitude of the action potential began to decline as the firing rate became very high (10/s) (Fig. 10a, lower traces). The cells also began to depolarise at this stage. Ultimately action potentials were entirely blocked.

The whole sequence of events was reversible, provided the preparation was not left too long in Ca^{2+}-free solution after complete block. The addition of 5 mM EGTA as a Ca^{2+} chelator, which reduces the free Ca^{2+} concentration to less than 10^{-8} M, speeded up the events described considerably, completely blocking action potentials in 5–10 min. However, the basic pattern of the response was unaltered.

Barium: SO_4^- and HCO_3^- free sea water were used for all experiments with Ba^{2+} or Sr^{2+} to prevent precipitation. The slight effects of the absence of these anions are described below. The addition of 5 mM Ba^{2+} caused very pronounced changes in action and resting potential (Fig. 10b). The action potential was facilitated, and the cells were more prone to repetitive firing. However, the action potentials became gradually longer and ultimately became "giant" very long events, of a shape totally unlike those of a normal action potential. In 5 mM Ba^{2+}, the cells partially depolarised, but action potential and contractions could be evoked indefinitely. Higher concentrations blocked action potentials. These effects were slowly but completely reversible.

Strontium: Only one experiment was carried out on the effects of this ion, but the result was of sufficient significance to be described here. If all Ca^{2+} ions in sea water were replaced by Sr^{2+} rather than Mg^{2+} excitability of the membrane still increased but the responses obtained were entirely different (Fig. 10c). A stimulus fired a first action potential which was apparently entirely normal, though there was no contraction. This was followed by a second action potential whose falling phase was totally blocked. The membrane remained depolarised at -30 to -40 mV and small repetitive action potentials were fired rapidly and continuously from this level. At the end of these bursts, which became progressively longer, the membrane gradually repolarised to the normal resting potential. Excitability finally failed as the membrane

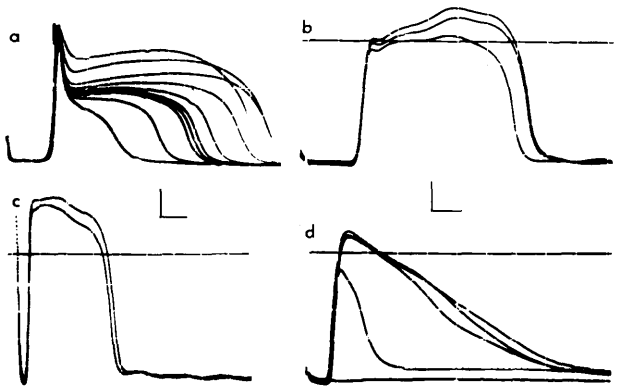

Fig. 11. a Action potential series in 10 mM Mn^{2+} solution. Scale bars: 10 ms, 25 mV. **b** Action potentials in 15 mM Co^{2+} solution. Scale bars: 5 ms, 20 mV. **c** Action potentials in 5 mM 4-amino pyridine solution. Scale bars: 20 ms, 20 mV. **d** Action potentials in D-600 solution. Scale bars: 10 ms, 20 mV

remained permanently depolarised, recovering only slowly on return to normal sea water. The most striking feature of these results was perhaps that the first action potential fired by a stimulus retained a more or less normal shape while the second was so profoundly affected.

Manganese and Cobalt: Extremely high concentrations (50 mM) of Mn^{2+} and Co^{2+} were necessary to block contraction despite the evident accessibility of the membrane to other divalent ions. Their action on the action potential itself, however, was apparent at concentrations of only 10–20 mM and was entirely different to those of Ca^{2+}-free solutions. Excitability of the membrane was much inhibited as shown by a very pronounced increase in the stimulus threshold. Repetitive firing in response to a single stimulus was never observed in the presence of Mn^{2+} ions. Facilitation on repetitive stimulation was much slower, and the maximum levels obtained in sea water were never attained, though the plateau reached the level normally observed during spontaneous swimming bursts. The most marked change, however, was the enormous increase in action potential duration, which could exceed 100 ms (Fig. 11a). Similar results were obtained with 10–20 mM Co^{2+} (Fig. 11b).

Lanthanum: 1 mM La^{3+}, a powerful Ca^{2+} blocker in many systems, had no noticeable effects on either contraction or excitability of the membrane.

iii. Anions. Chloride: Low Cl^- solution (in which all NaCl was replaced by $NaCH_3SO_4$) caused a small and transient hyperpolarisation of the membrane. The stimulus threshold rose very sharply, and the epithelium frequently fired only after two or three consecutive stimuli.

Sulphate: The absence of SO_4^{2-} (replaced by Cl^-) and HCO_3^- (replaced by Tris/Tris Cl buffer) appeared to have little or no effect on spike generation. Contractions seemed to be stronger and possibly longer in the absence of SO_4^{2-}, though no quantitative measurements were made.

iv. Pharmacological Agents. Tetrodotoxin had no effect on the action potential even after an hour at concentrations up to 3.5×10^{-5} M.

4-amino pyridine, and tetraethyl ammonium chloride (TEA), which are blockers of K^+ conductance, produced effects at relatively high concentrations (1 mM 4-amino pyridine and 5 mM TEA). On intact preparations the effect initially was to increase the amount of spontaneous activity which ultimately became continuous. The action potential became permanently facilitated (Fig. 11c) with the initial repolarisation phase either small or disappearing altogether even in response to a single stimulus. There was no clear evidence that the substances affected the final repolarisation. Ouabain (1 mM) had no effect on the preparation during periods of 15–20 min. D600, a well known blocking agent for Ca^{2+} channels in other preparations, produced action potentials of greatly increased duration (Fig. 11d), as did Mn^{2+} and Co^{2+} ions.

Discussion

Comparison of the passive properties of the *Chelophyes* myoepithelium with those of the non-myoid exumbrella ectodermal epithelium in the Hydromedusan *Euphysa* (see Table 1) shows large differences in the values for internal longitudinal resistance and specific membrane resistance.

It seems improbable that the low values for the resistances obtained in *Chelophyes* simply reflect damage due to electrode penetration. First, resting potentials measured both at recording and current electrodes were stable for long periods, and action potentials of normal amplitude and duration could be evoked by current injection, even after long periods of recording. Secondly, the characteristic steady state voltage distribution in the epithelium, which rather closely approximates to the theoretical Bessel-type function, suggests that most of the injected current actually flows into the epithelium.

But, Dr. Carré's ultrastructural observations have shown numerous gap junctions linking transmesogloeal processes of the epithelial layer, and the underlying endodermal layer (Fig. 2). If such junctions repre-

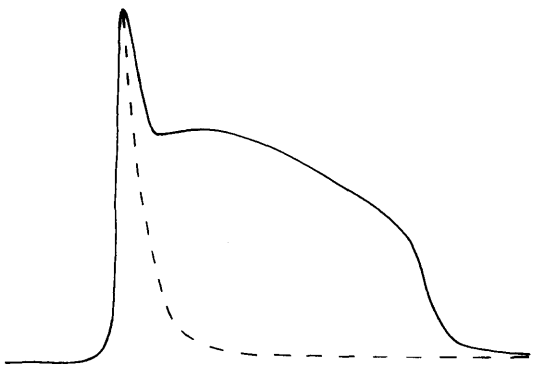

Fig. 12. Diagram of typical unfacilitated action potential (*dotted*) and facilitated action potential (*solid line*) showing four phases of the action potential discussed in text

sent low impedance pathways between ectoderm and endoderm, a significant fraction of the injected current would flow out of the endoderm into the underlying endodermal sheet, instead of radially in the ectoderm from the current source.

Nevertheless, values for R_m in *Chelophyes* are similar to those found for the conducting myoepithelium of the ascidian heart investigated by Kriebel (1968), which is the most similar tissue to the subumbrella that has been previously studied. Values for R_i in *Chelophyes* are very much lower than in the ascidian heart epithelium.

In the *Chelophyes* subumbrella, as in the ascidian heart, the cells are fusiform, and the tissue is thus not symmetrically arranged, so that different numbers of gap junctions are traversed by current flowing in the direction of the long axes of the cells, and in the direction across them. Voltage changes observed in response to current injection were similar, however the two electrodes were aligned with respect to the long axes of the cells. If current flow in the subumbrella is truly non-directional, this implies that the resistivity of the gap junctions coupling the cells can only contribute insignificantly to R_i as compared with cytoplasmic resistance, and there may therefore be a real difference in gap junction resistivity between coelenterate and ascidian epithelia. However, the existence of possible low resistance "shunts" between the subumbrella myoepithelium and the underlying endodermal sheet means that this possibility requires further investigation.

In our study of the passive electrical properties of the subumbrella, the values for membrane constants given in Table 1 represent a first attempt to characterise what is likely to be a complex situation, and it has not escaped our attention that the possibility of such coupling introduces uncertainties in these values.

The value for the length constant found in *Chelophyes* (280 μm) is lower than that found in *Euphysa*, but it is within the range of values found for other epithelia (Jongsma and Van Rijn 1972) and is in any case much larger than the width of the cells, indicating that action potential propagation along the subumbrella by local current flow from cell to cell is certainly feasible.

The action potentials generated in the epithelium of *Chelophyes* are clearly complex events, and it is difficult to be certain of the underlying ionic currents in the absence of voltage clamp data. However, a number of tentative conclusions emerge from the results obtained. These will be discussed in terms of the four distinct phases of the action potential which are shown schematically in Fig. 12.

a) The fast depolarisation (Phase 1). It seems likely that the rising phase of the action potential is produced by a fast inward sodium current, since it is critically dependent on the external sodium concentration.

Sodium dependent action potentials have also been found in the secretory epithelium of *Hippopodius* (Mackie 1976; Chain 1979) and the myoid epithelium of the gastrozooids of *Agalma* (Chain 1981). Siphonophores therefore appear to be unusual among invertebrates in possessing muscular tissue which is capable of propagating action potentials which are sodium dependent, and are conducted within the muscular tissue itself, rather than via a parallel neuronal conduction pathway. The sodium conductance mechanism in *Chelophyes*, in common with that of a number of other invertebrates, is insensitive to TTX. It seems to show normal voltage-dependent inactivation, since the rising phase is substantially slowed in partially depolarised preparations. Action potential propagation fails at sodium concentrations below 50% of normal sea water. This rather high threshold may be a direct consequence of the passive electrical properties of the epithelium (see above), which indicate that a very high current density is required to trigger an action potential.

b) The fast repolarisation (Phase 2). The fast repolarisation which normally terminates the first action potential of a burst is blocked by 4-amino pyridine and TEA. It seems probable that an unfacilitated action potential is terminated by the activation of a fast potassium conductance, which is progressively inactivated during repetitive activity. A model based on such a mechanism has been postulated in order to explain the increase in spike duration during repetitive activity in the neurons of the puffer fish (Nakajima and Kusano 1966). Fast potassium activation may in any case be rather poorly developed in *Chelophyes*, since there is no hyperpolarising after potential under normal conditions.

c) The plateau (Phase 3). The possible theoretical basis for plateau formation, in terms of underlying changes in the ionic conductances of the excitable membranes, has been extensively investigated, particularly in relation to the cardiac action potential (Brady and Woodbury 1960; Fitzhugh 1960; Noble 1962; McAllister et al. 1975). A number of possible models have been developed, and data on conductance changes during the action potential are required to distinguish experimentally between the alternatives. In *Chelophyes*, the magnitude of the plateau voltage depends critically on stimulus frequency, as has been found in a number of other preparations (Bennett et al. 1959; Anderson 1979). A model to account for this in terms of progressive potassium inactivation has been suggested above, but this phenomenon does not fully explain all the variation observed.

In 50% sodium sea water the maximum levels of the plateau are very substantially reduced. A substantial Na^+ current may, therefore, be involved in generating the plateau. However it is not clear whether the electrochemical gradient for Na^+ in the epithelium is sufficient to account for the very large overshoots which occur during the plateau. The complex effects of calcium-free solution are more difficult to interpret: in solutions containing manganese, the maximum levels of facilitation are rarely obtained, but since the kinetics of the facilitation process are very much slowed, this may not imply that there is any significant calcium current contributing to the plateau potential. A certain amount of calcium influx is presumably necessary for contraction: however, large contractions occur even after unfacilitated action potentials, and there is no obvious correlation between contraction and the size or duration of the plateau. There is no evidence for a voltage-dependent decrease in potassium conductance during depolarisation (anomalous rectification), which is an important contributor to plateau potentials in the cardiac impulse (see McAllister et al. 1975). In particular, the duration of the plateau is almost independent of the plateau potential. The most likely ionic mechanism for the plateau seems to be maintained increase in sodium conductance, either as a result of a second independent 'slow' sodium current, or delayed inactivation of the 'fast' current.

d) The second fast repolarisation (Phase 4). Barium ions, which block potassium channels in many preparations, very substantially prolonged the facilitated action potential (see Armstrong 1974). The normal action of strontium ions, which substitute for calcium in maintaining contraction, and are relatively ineffective in blocking potassium channels, appears to be reversed in *Chelophyes*. TEA and 4-amino pyridine do not affect the length of the facilitated action potential, and a second independent potassium conductance may therefore be involved in the final repolarisation. Manganese ions and D600, conversely, do not affect the unfacilitated action potential duration but prolong the plateau phase, after facilitation. Since manganese ions and D600 block calcium influx, it is possible that the late potassium conductance is calcium activated. Such calcium activated TEA-insensitive potassium conductances have now been reported from a large number of preparations (see Meech 1978).

Our results show that action potentials in this myoepithelium are complex processes, involving a whole range of interacting changes in the ionic permeabilities of the excitable membrane. The relationship between the electrical complexities of the action potential and the contraction process itself is as yet entirely unknown, but would seem to present an intriguing and difficult problem. It is hoped to continue the study of the properties of this epithelium, both by using the voltage-clamp technique, and by obtaining simultaneous voltage/tension recordings on the preparation. Preliminary voltage/tension recordings (Bone, unpublished) show no relation between tension and the degree of facilitation of the action potential, so that the functional role of facilitation in the contraction of the subumbrella remains enigmatic.

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Note Added in Proof

Further examination of simultaneous recordings of action potentials and tension in the subumbrellar myoepithelium has now shown that tensions *are* augmented by a.p. facilitation. This work will be reported in a subsequent communication.