ELECTROGENESIS IN THE EURYHALINE OSMOCONFORMER *CORDYLOPHORA LACUSTRIS* (HYDROZOA)

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SUMMARY

The electrical signals propagated through the ectodermal epithelium of *Cordylophora lacustris* (the Josephson pulses) were recorded as trans-epithelial action potential-like events. Experiments on the ionic basis of electrogenesis of these action potentials suggested that they result from an outward flow of chloride ions from the ectodermal cells into the enteron. Further evidence for this hypothesis came from measurements of the ionic concentrations in the tissues of *Cordylophora*, which showed that these cells have unusually high levels of chloride. Chloride dependent electrogenesis allows this excitable system to function in media of low and variable ionic strength, which are a typical feature of this organism's natural environment.

INTRODUCTION

Cordylophora lacustris is one of the only two species of coelenterate which are found primarily in estuarine and brackish waters (Kinne, 1971). Its natural distribution ranges from fresh water up to salinities of about 10‰ and it often grows in tidal regions where there may be large daily fluctuations of the ionic milieu (Percival, 1929). The mechanisms of cellular excitability in this animal must therefore be able to function both in media of very low ionic strength, and in media of rapidly changing and unstable ionic composition. Two studies have been carried out on the electro-physiology of *Cordylophora* (Josephson, 1961; Mackie, 1968). This organism, unlike many hydroids, seems to be normally electrically silent, but during, and for a short period after the feeding response, a number of types of electrical signal can be recorded, some of which can also be elicited in the silent animal by electrical or tactile stimulation (Mackie, 1968). The response with which this study will be concerned was first described by Josephson (1961), and the pulses were subsequently named 'Josephson pulses' (JP). They can be recorded with extracellular electrodes placed at any point on the surface of the animal as large (up to 15 mV) events which are generated in the ectodermal epithelium and propagate throughout the colony at speeds of 2-3 cm/s. They can be triggered by strong stimuli at any point of the organism. The arrival of

a JP at a hydranth triggers a sudden withdrawal response, consisting of a backward flexing movement of the tentacles, and a slight but rapid contraction of the body wall. Nothing is known of the ionic basis of electrogenesis in *Cordylophora*, except that the JP is insensitive to TTX (Mackie, 1968). Nevertheless this excitable system is of particular interest to electrophysiologists, since the passage of a JP from one hydranth to another through the coenosarc, from which nerves and muscles are absent (Jha & Mackie, 1967) must reflect the presence of a conducting epithelial tissue. The cells are unsuitable for intracellular recording, and the techniques used by Josephson and Mackie can give little information on these problems. However the parallels both functionally and electrophysiologically between the JP system in *Cordylophora* and the Contraction Pulse (CP) system in *Hydra* (see Passano & McCollough, 1964), suggested that it might be possible to study the former by the more quantitative methods developed for *Hydra* (Josephson & Macklin, 1969). In the present study, therefore, the Josephson pulses were investigated by recording the potential changes across the whole epithelial wall of a hydranth, by an electrode inserted through the mouth into the enteron (cf. Josephson & Macklin, 1969); under these conditions the pulses appear as large and constant action potential-like events, whose parameters and ionic dependence could be quantitively investigated. In addition a number of experiments were carried out on the ionic concentrations in the tissues of *Cordylophora*; though these were of a rather preliminary nature they serve as further support for the conclusions drawn from the electrophysiological results.

METHODS

Colonies of *C. lacustris* were obtained from a disused jetty, Cotehele Quay, south of Caldstock on the river Tamar (Devon). The high water salinity at this point of the river is about 10‰, but considerable variation occurs during the tidal cycle (Percival, 1929; Smith, 1956). The animals are uncovered for about one-sixth of each cycle (personal observation), and the water in the microenvironment immediately surrounding exposed colonies probably undergoes more marked fluctuations in salinity than the river as a whole. The animals were kept in aquaria at room temperature and in the dark to prevent algal growth. Two cultures were kept in the laboratory: one in the artificial medium devised by Fulton (1960) with a total ionic strength equivalent to 43 % sea water and the other in a 40× dilution of this medium. Both cultures were fed several times each week on freshly hatched *Artemia nauplii* and could be maintained for many months in the laboratory. The two cultures showed marked differences in morphology, presumably related to the differing salinities. The colonies grew best at the lower salinities, and the individual hydranths were larger and had more tentacles. At the higher salinity, which is well above the natural distribution limit, growth was extremely slow and the hydranths looked much less vigorous. These results are similar to those described by Kinne (1958).

The electrophysiological methods used were in principal similar to those developed for *Hydra* (see Chain, 1980). Electrical recordings were made by inserting a broken microelectrode previously filled with culture fluid through the mouth of an isolated hydranth (Fig. 1). A Ag/AgCl wire (connected to the recording equipment) was inserted into the barrel of the electrode. The potential within the enteron was there-
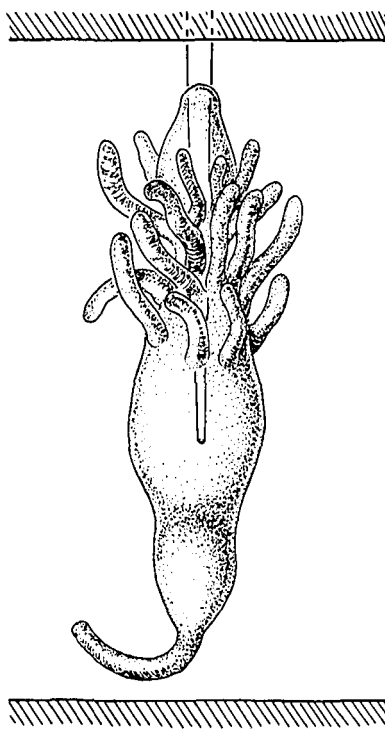
Fig. 1. Drawing showing the arrangement used to record from an isolated hydranth of *Cordylophora*. The broken-off microelectrode is inserted through the mouth, and is used both to record from the enteron and stabilize the preparation. (Drawing by Mr J. Rodford.)

fore recorded directly by this 'holding' electrode. The recorded signal was passed through a DC amplifier and displayed on a Telequipment DM 63 storage oscilloscope. The amplified signal was also passed through a transient store and recorded on two Servoscribe penrecorders, running at fast and slow speeds. The body wall of *Cordylophora* is not very contractile and the arrangement shown is sufficiently stable to maintain continuous recordings for long periods. The hydranth was stimulated to contract via two fine platinum wires, placed on the epithelium near the cut end of the hydranth stalk. Stimuli were delivered from an isolated Digitimer stimulator, driven by an electronic timer to produce stimulating pulses every 2 or 3 min. Faster stimulus rates fired a slow contraction system in the hydranth, and the consequent movement tended to alter the amplitude of the pulses.

Recordings were made in a small perspex chamber (approx. 200 μl). Fresh solution flowed continuously over the preparation, through a multi-way non-return valve, and was removed by suction. The time required for the fluid in the bath to exchange was approximately 30 s. Internal perfusion of the enteron proved to be technically difficult because of the small size of the hydranths. In practice it proved unnecessary. Preliminary experiments using a Ag/AgCl wire introduced through the holding electrode into the gut as a crude chloride electrode, indicated that changes in external solutions produced equivalent changes in the contents of the enteron within a period of 1-3 min.

The hydranth therefore appears to be 'leaky' though the leakage pathway has not yet been identified.

The measurement of ionic concentrations in the tissues of *Cordylophora* is particularly difficult because of the small size of these animals (in the order of 10-100 nl) and the presence of a relatively large quantity of 'extracellular fluid' in the enteron. Attempts at weighing individual or several hydranths were not successful, because of the very large errors caused by evaporation and extracellular water. The total ionic contents and the intracellular water content were therefore measured in two separate samples of hydranths, and the average values obtained were used to estimate intracellular ionic concentration. Such an approach results in a large variation in the data, which includes the natural variation of hydranth size within a colony, but the values obtained were sufficiently reproducible to give some estimate of intracellular ionic concentrations.

Sodium and potassium levels were measured by emission spectroscopy; the hydranths were prewashed in isotonic saline solution for 3 min to remove extracellular ions. Sodium levels were also measured by isotopic flux studies, and the same technique was used to measure chloride levels. Individual hydranths were 'loaded' overnight in culture medium containing the radioactive isotopes ^{22}Na and ^{36}Cl and then effluxed with 'cold' culture medium. The efflux procedure was similar to that described by Willmer (1977) (see Chain, 1979 for details) and allowed a wide variation in the rate of perifusion. The results were plotted in terms of percentage radioactivity remaining in the hydranth at successive times during the efflux, and were interpreted, using the graphical methods of compartmental analysis, as a number of superimposed logarithmic effluxes (Solomon, 1960). The time constants and total counts corresponding to each phase could then be calculated. The water contents of individual hydranths were calculated by three methods:

(*a*) The total volume of individual hydranths was calculated by approximating their shape to a regular cylinder or a series of cylinders, and measuring the length and average radius from photographs.

(*b*) The intracellular water was measured by a double labelling technique (cf. Horn, Rogers & Zierler, 1973; MacKnight & Leaf, 1977). The hydranths were left for 1 h in culture medium containing tritiated water. They were then transferred for 5-10 m to fresh medium containing an equal quantity of tritiated water and, in addition, ^{14}C-labelled sucrose, which has been shown in a number of studies to permeate the cell membrane only slowly (see MacKnight & Leaf, 1977; Willmer, 1977). The cellular water was estimated as the difference between total 'tritiated water' volume and the 'sucrose' volume. This approach yielded consistent results with the larger 1 % animals, but results obtained with the smaller 43 % hydranths were so variable as to be almost meaningless (the range of values obtained was from 2 to 25 nl).

(*c*) Intracellular water was measured by compartmental analysis of tritiated water efflux, by the same methods as were used for ionic measurements. It was assumed that water concentration and activity in all compartments was equal.

RESULTS

Electrophysiology

Josephson pulses could be recorded as monophasic negative transepithelial events, similar to Contraction Pulses recorded across the body wall of *Hydra* (Josephson & Macklin, 1969; Chain, 1979). Some parameters of these electrical changes (which will be referred to as action potentials) are shown in Table 1 and their typical shapes in 1% and 43% animals are shown in Figs 2 and 5.

The transepithelial resting potential is very small in both 1% and 43% animals, and the properties of this potential were not investigated further.

Table 1. *Parameters of the 'Josephson pulses'*

Salinity (% s.w.)	Amplitude (mV)	Duration (ms)	No. of measurements
1	36·6 [2·2]	133·1 [5·6]	75
43	16·0 [2·2]	160·6 [11·4]	35

Figures in brackets are the standard errors of the mean.

(1) *Osmotic experiments*

The excitable system in *Cordylophora* is able to withstand large and rapid changes in the ionic and osmotic milieu. Fig. 3 shows the effects on the action potential of 43% animals of a 40-fold dilution of the external medium. Fig. 4 gives a quantitative description of the changes in AP amplitude measured in five different preparations. The response consists of a rapid increase and subsequent decrease in the amplitude, stabilizing at a level close to the initial value. In a few preparations the system becomes blocked at this point. In most, however, the AP-generating mechanism continues to function, and the amplitude of the AP subsequently either increases slightly or remains the same. The force of the accompanying contractions decreases markedly during dilution and recovers only slowly.

Only a few experiments were performed on the reverse phenomenon, namely the effects of a 40-fold increase in the ionic concentrations of the medium on electrogenesis of 1% animals. The response seems to consist of an immediate block of the action potentials, which recover over a period of about 30 min to amplitudes typical of 43% animals. A 40-fold dilution of the ionic concentrations of the medium, while maintaining the total osmotic strength constant by the addition of mannitol, produced a maintained increase in spike amplitude (Figs. 5, 6a). A smaller but qualitatively similar effect is produced by replacing all the sodium chloride in the medium by mannitol (Fig. 6b). These two effects are rapidly reversible on return to normal solution.

(2) *Ionic effects*

(a) *Monovalent ions.* As mentioned above, removal of sodium chloride from the medium causes an increase in spike amplitude. However, if the sodium chloride is replaced by Tris/Tris chloride, keeping the chloride concentration constant, the amplitude of the JPs is unaltered.
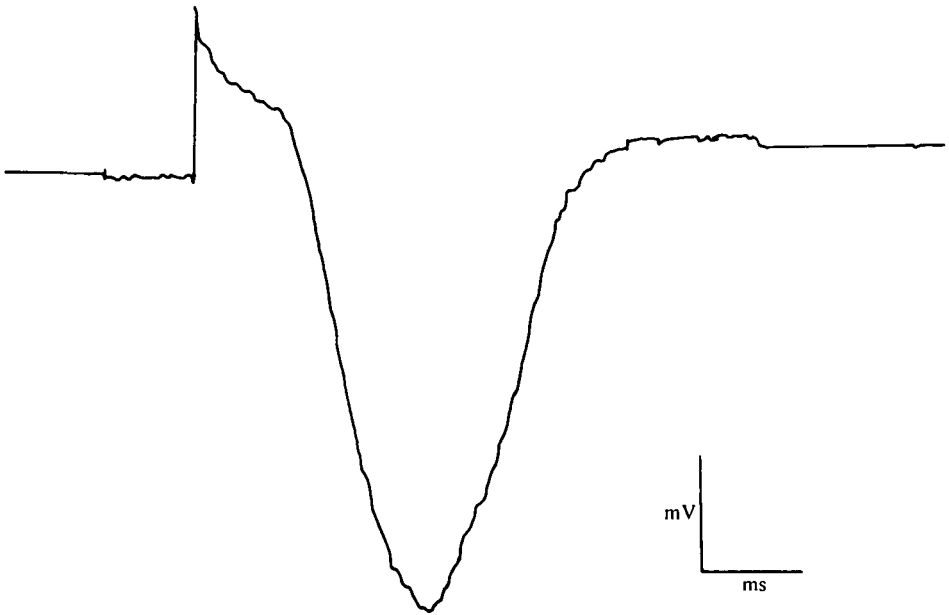
Fig. 2. A typical 'Josephson Pulse' from a 1 % sea-water hydranth, recorded through a transient recorder, on to a Servoscribe pen-recorder running at fast speed. More negative potentials are shown downwards in all figures. Scale: 10 mV, 50 ms.
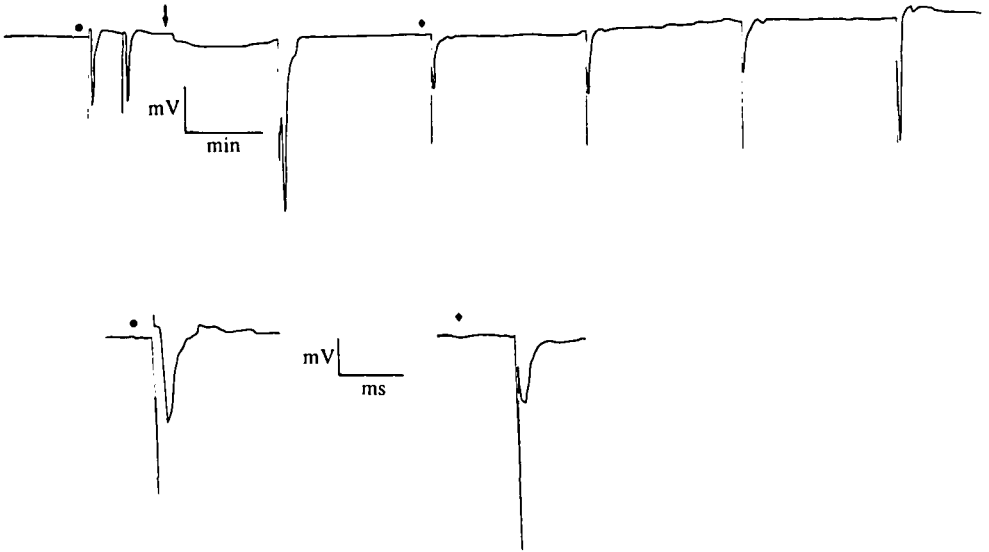

Fig. 3. The effects of a sudden dilution of the medium from 43 % s.w. to 1 % s.w. on the JP's of 43 % s.w. hydranths. The solutions were changed at arrow. Lower trace shows enlarged time scale recordings of selected JP's. Scale: upper trace 10 mV, 3 min; lower trace 10 mV, 250 ms.
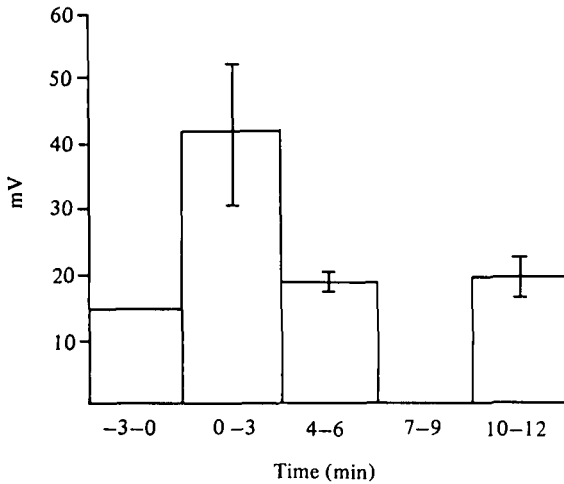
Fig. 4. Quantitative changes in JP amplitude of 43 % s.w. hydranths after sudden dilution of the medium to 1 % s.w. The measured amplitudes are given relative to the amplitude in 43 % s.w., which is arbitrarily taken as 15 mV. The abscissa represents successive time intervals before, and after, the change of solutions (at time zero). No readings were taken between 7 and 9 min. The graph shows the average values (±s.e.m.) obtained in five separate experiments.
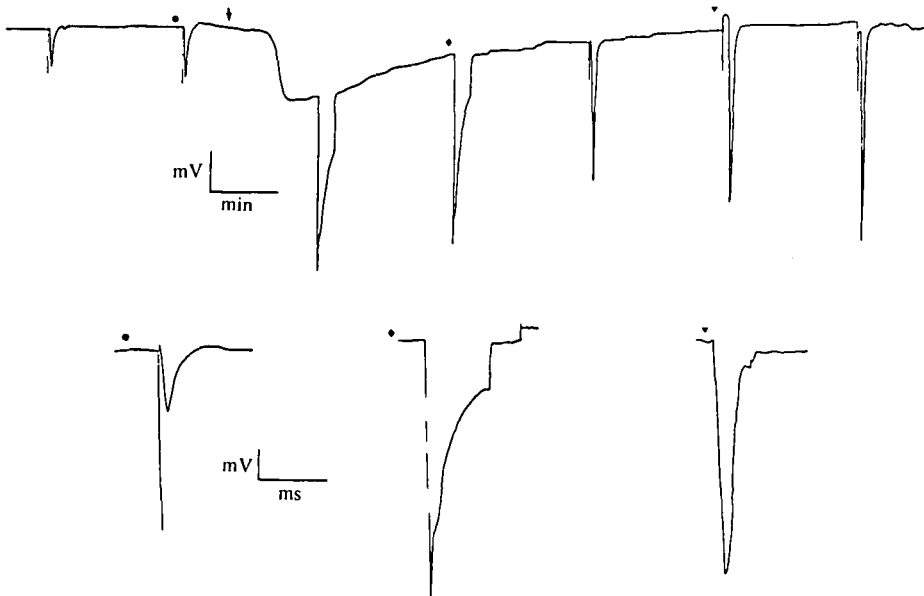

Fig. 5. The effect of a sudden ionic dilution from 43 % s.w. to 1 % isotonic s.w. (with the osmotic strength maintained at 43 % by the addition of mannitol) on the JPs of 43 % s.w. hydranths. The solutions were changed at arrow. The lower trace shows an enlarged time scale recording of selected JPs. Scale: upper trace 10 mV, 3 min. lower trace 10 mV, 300 ms.
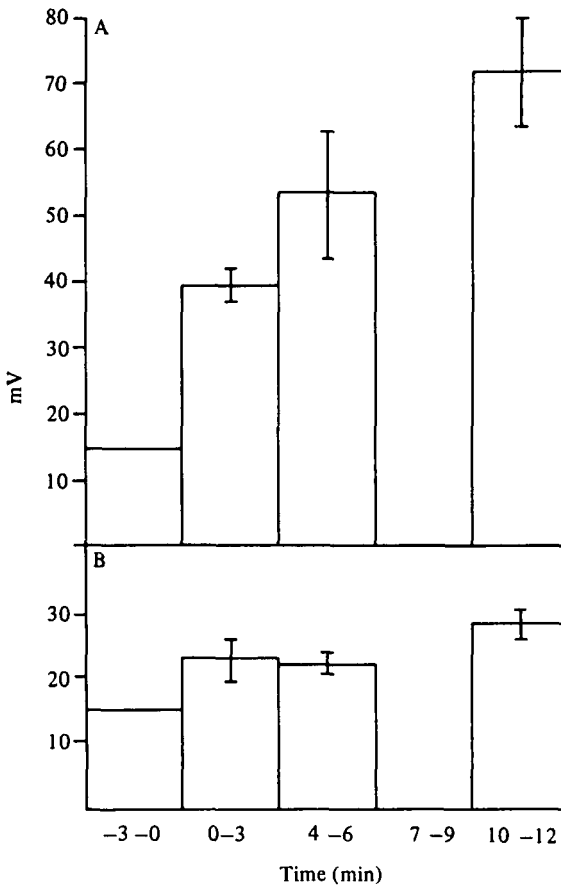
Fig. 6. (a) Quantitative changes in JP amplitude after sudden dilution of 43 % s.w. to 1 %
isotonic s.w. For explanation of graph see Fig. 4 (average of three experiments). (b) Quantitative
changes in JP amplitude produced by sodium chloride-free 43 % SW (mannitol substituted).
For explanation of graph see Fig. 4 (average of five experiments).

Potassium blocks the APs in both 1 % and 43 % animals. In the former 1 mM sub-
stantially inhibits and 4 mM totally blocks excitation. In 43 % animals the action
potential is blocked in 40 mM potassium (Fig. 7).

The concentration of chloride ions profoundly affects the amplitude of the action
potentials in both 1 % and 43 % animals. These effects are shown in Fig. 8a, b). In
these experiments chloride was replaced by the impermeant $MeSO_4^-$ anion. High-
chloride solutions were made up by the addition of sodium chloride or tris chloride to
the standard medium (the two cations gave apparently identical results) and were
therefore hyperosmotic. The amplitude varied linearly and inversely with the
logarithm of the external chloride concentration over the range 78–418 mM for 43 %
animals, and 8–48 mM for 1 % animals. The slope was 21 mV per decade change in
concentration in both cases.

(b) *Divalent and polyvalent cations.* Tenfold changes in external Ca^{2+} and Mg^{2+}
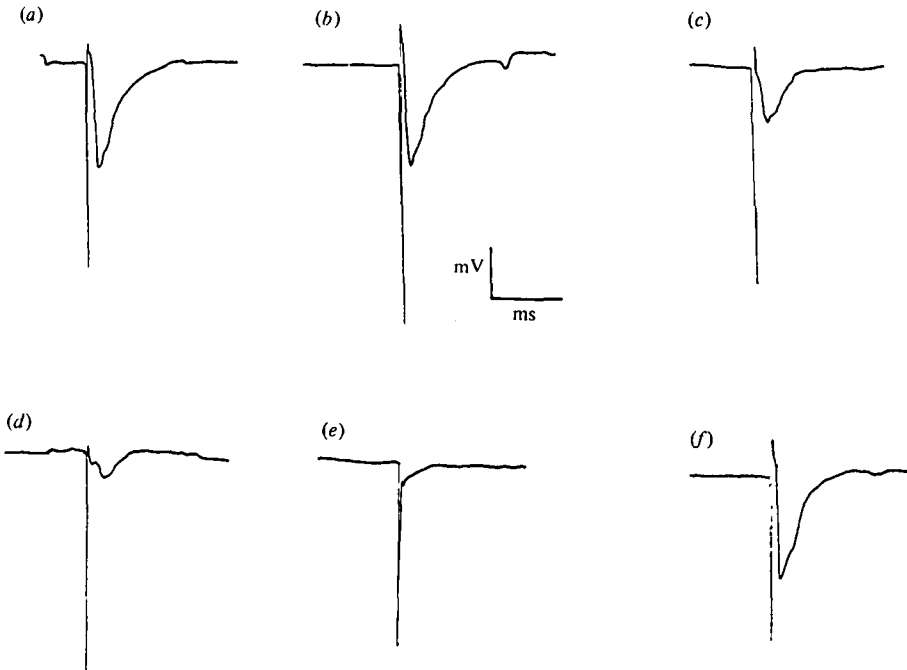concentrations did not affect the AP amplitude.

Fig. 7. The inhibition of JP in 43 % hydranths by 40 mM-KCl. (*a*) Normal s.w., (*b*) 1 min after solution change, (*c*) 3 min after solution change, (*d*) 5 min after solution change, (*e*) 10 min after solution change, (*f*) 5 min back in normal s.w. Scale 10 mV, 200 ms.
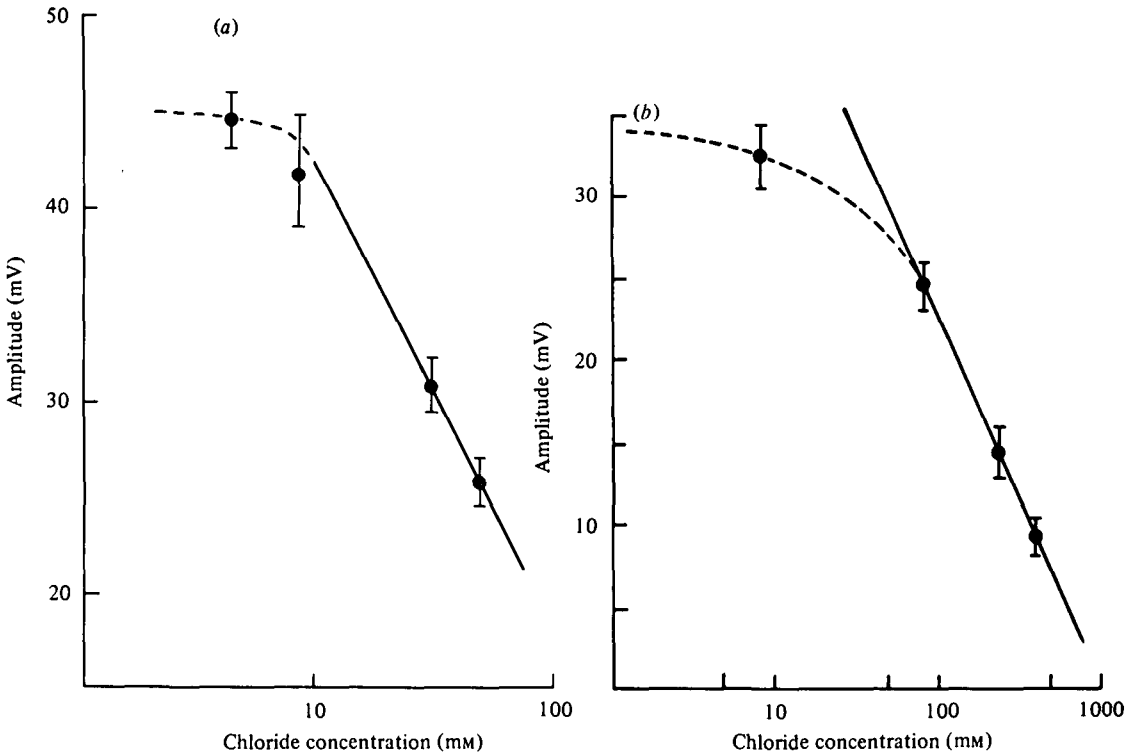

Fig. 8.(*a*) JP amplitude as a function of chloride concentration (1 % hydranths). Bars represent ±S.E.M., *n* = 5. (*b*) JP amplitude as a function of chloride concentration (43 % s.w. hydranths). Bars represent ±S.E.M., *n* = 5.
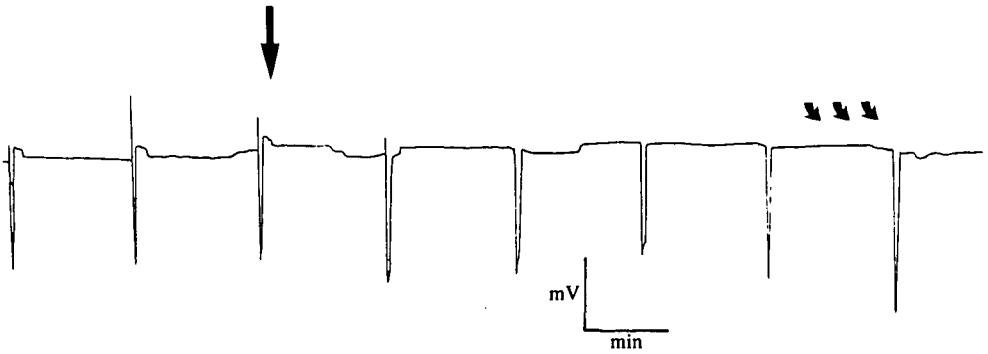
Fig. 9. The effect of 1 mM lanthanum chloride on 43 % s.w. hydranths. Solutions changed at single arrow. At multiple arrows all contraction is blocked, but JP amplitude is unaltered. Scale: 10 mV, 2 min.
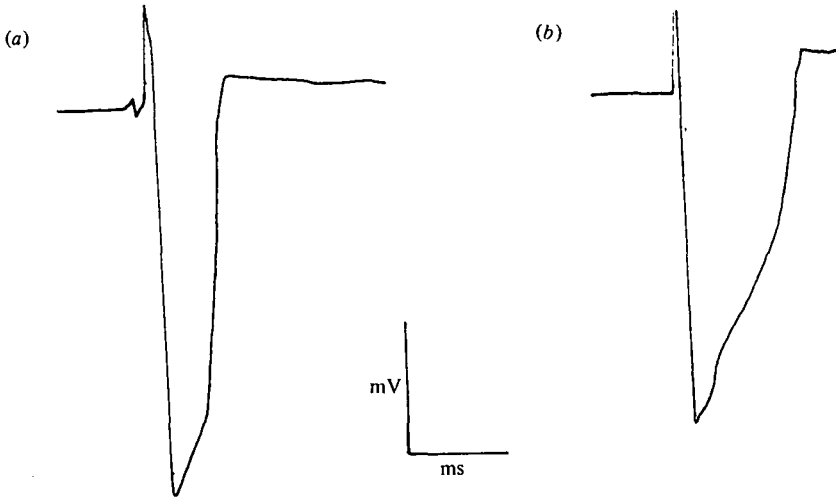

Fig. 10. The effect of 1 mM 4-aminopyridine on the JP. (a) normal solution. (b) after 10 min in 1 mM 4 AP. Scale 5 mV, 200 ms.

Ten mM manganese ions inhibited contraction but did not affect the AP in 43 % animals, except that they increased the threshold for stimulation. In 1 % animals, 10 mM-$MnCl_2$, blocked excitation; 10 mM-$MnNO_3$, however, only slightly reduced the amplitude of the AP.

One mM lanthanum, a powerful calcium blocking ion in many preparations (e.g. Ashcroft, 1978), rapidly blocked contraction in both 1 % and 43 % animals. AP's, however, could be recorded with normal amplitudes for long periods after all movement was blocked (Fig. 9). The effects of lanthanum were irreversible and toxic, and ultimately not only stopped electrical activity but killed the preparation.

(c) Pharmacological agents. 4-Amino pyridine substantially increased spike duration in Cordylophora (Fig. 10).
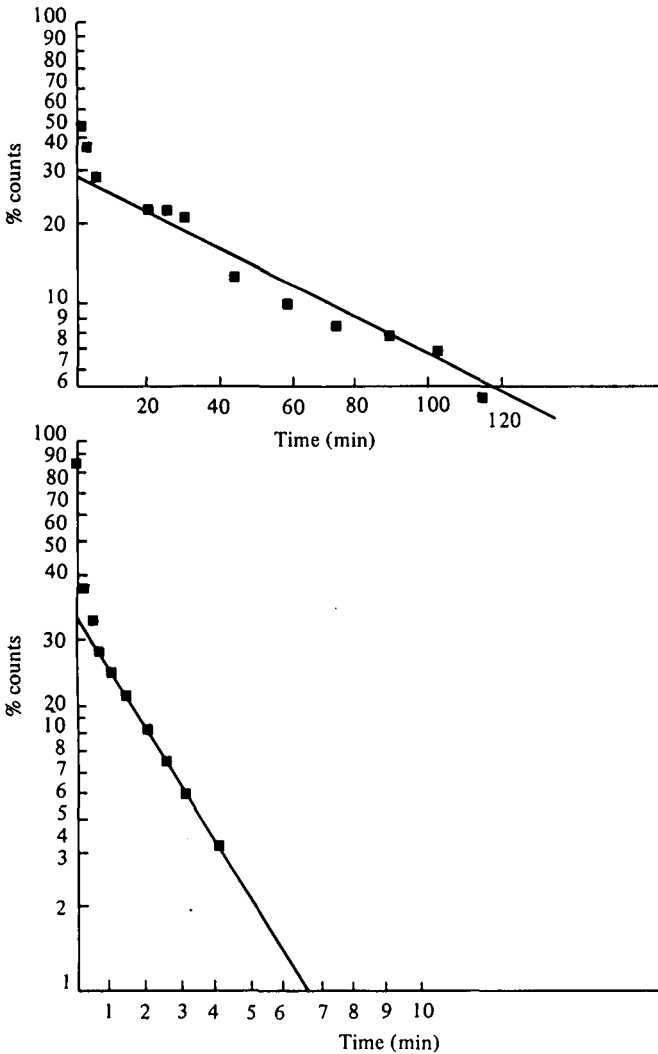
Fig. 11. A typical radioactive sodium efflux experiment. The points in the top graph show actual percentage counts obtained. The data in the lower graph has been recalculated by the graphical methods of compartmental analysis (Solomon, 1960) so as to take account of the slow component of the efflux, and to reveal the linear component underlying the data obtained within the first few minutes of the efflux. The solid lines in the upper and lower graphs were fitted to the data by inspection, and represent two hypothetical independent sodium compartments with slow and medium efflux rate constants respectively.

Ionic concentrations and fluxes

Typical sodium and chloride efflux curves are shown in Figs. 11 and 12, together with the individually calculated compartmental effluxes. The curve could be completely analysed in terms of three main efflux compartments. The most rapid was not adequately resolved by the techniques used, but had a half-life of less than 10 s. It is assumed that this fraction represents solution on the outer surface of the hydranth,
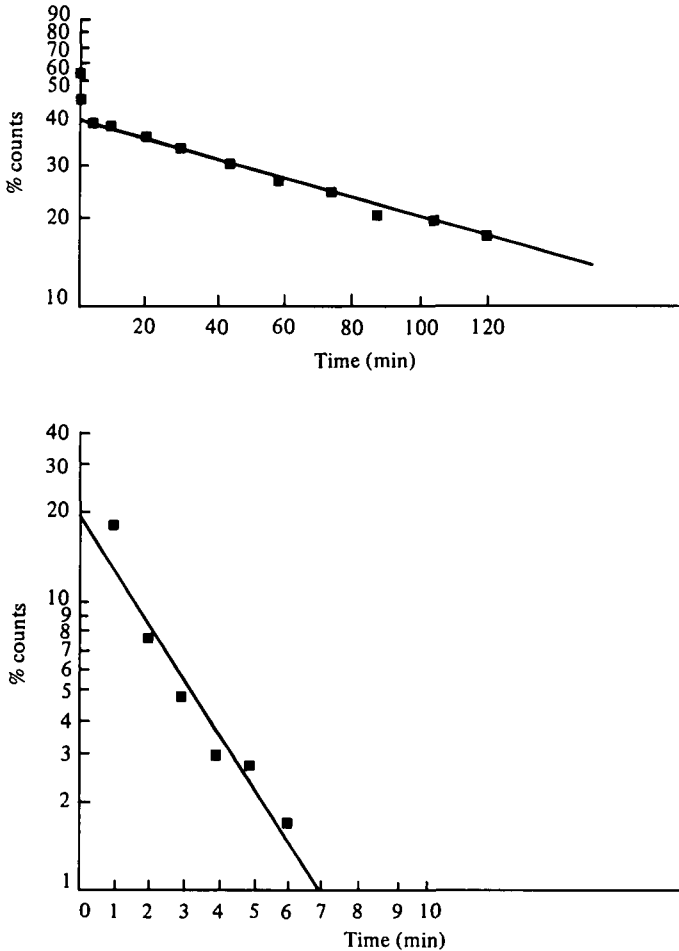
Fig. 12. A typical radioactive chloride efflux experiment. For explanation see Fig. 11.

which was not totally removed before effluxing, and it will not be considered further. The percentage ionic contents and half-lives of the two remaining fractions for sodium and chloride are given in Tables 2 and 3. Interpretations of such compartmental analysis is, in general, a complex problem that depends on the structure of the tissue involved. In the present case the presence of only two components with very widely differing efflux rates suggests a relatively straightforward interpretation. It is suggested that the faster of the two effluxes ($t_{\frac{1}{2}} = 1 \cdot 5$–4 min) represents solution caught in the extracellular spaces of the tissue and in the enteron. The rather slow rate of efflux from this compartment is presumably due to the complex system of extracellular paces within the epithelium (Schulze, 1871) and the presence of the enteron. The slow fraction for both sodium and chloride is presumed to represent efflux of intracellular ions. The values from this compartment are used in calculating intracellular ionic concentrations. The rate of sodium efflux is very considerably faster than that of chloride, suggesting that chloride permeability of the membrane is low. A few preliminary experiments indicated a very marked reduction in the rate of

Electrogenesis in C. lacustris

Table 2. Sodium efflux data for C. lacustris

Animal	No. of expts	Slow fraction		Medium fraction		Total sodium (M^{-10})	Sodium content of slow fraction (M^{-10})
		%	t_i (min)	%	t_i (min)		
1 %	5	23 [3·7]	31·9 [3·4]	18·8 [1·2]	1·5 [0·2]	12·8 [2·9]	2·9 [0·7]
43 %	7	3·1 [1·4]	22·4 [1·5]	5·3 [1·0]	2·3 [0·5]	220 [70]	7 [1·2]
1 % at 12 °C	3	7·2 [1·2]	69 [8]	18 [1·3]	2·3 [0·3]	11·4 [1·5]	0·8 [0·05]

Figures in brackets are the standard errors of the means.

Table 3. Chloride efflux data for C. lacustris

Animal	No. of expts	Slow fraction		Medium fraction		Total chloride (M^{-10})	Chloride content of slow fraction (M^{-10})
		%	t_i (min)	%	t_i (min)		
1 %	6	23·8 [4]	115 [8·3]	20 [3·8]	2·3 [0·2]	180 [30]	43 [10]
43 %	4	35·2 [3·9]	100 [11·2]	12·5 [1·2]	4·0 [0·4]	24·1 [3·0]	8·5 [1·6]

Figures in brackets are the standard errors of the means.

Table 4. *Total ionic contents for* C. lacustris

	Na$^+$ (10^{-10} M)	K$^+$ (10^{-10} M)	Cl$^-$ (10^{-10} M)
1 % hydranths			
by flame photometry	8·7 [1·7] (5)	21 [2·3] (5)	—
by efflux measurement	3 [0·7] (5)	—	8·5 [1·6] (6)
43 % hydranths			
by flame photometry	27·2 [4·3] (9)	24·4 [4·3] (9)	—
by efflux measurement	7 [1·2] (7)	—	43 [10] (4)

All figures are given with standard errors, and number of measurements.

Table 5. *Cordylophora hydranth volume*

	Volume measurements from:		
	Water efflux data (nl)	Double-labelling technique (nl)	Photographic measurements (nl)
1 % hydranths	63 [11] (5)	64 [14] (5)	92 [7·5] (7)
43 % hydranths	15 [2·4] (5)	2–30	28 [9·8] (8)

All figures are given with the standard error of the mean, and the number of measurements.

sodium efflux at 12 °C (Table 2), suggesting that the mechanism involved in sodium movement is not one of simple diffusion.

The ionic contents (per hydranth) calculated from flame photometry and efflux experiments are shown in Table 4. As can be seen the variation is considerable. Nevertheless a number of features emerge. K$^+$ levels remain rather constant in the two sets of animals suggesting a degree of K$^+$ homeostasis. In view of the large changes in the intracellular potassium concentrations which are discussed below, the significance of this regulation in unclear. Na$^+$ levels measured by flame photometry are very much larger than those found by sodium efflux experiments; however, the preliminary wash used to remove extracellular sodium before flame photometry was only 3 min. Since the half-life for extracellular sodium efflux is about 3 min a very considerable fraction of this compartment presumably remains even after washing, and would explain the wide discrepancy between the two sets of results.

The chloride levels are much higher than sodium in both 1 % and 43 % animals, and even exceed potassium in the latter. Unlike many other tissues, chloride therefore appears to be a major anion in the cells of *Cordylophora* – a situation which is found rather rarely in other animal tissues (Prosser, 1973). Figures for cellular water content and total hydranth volume are shown in Table 5. Water efflux from the hydranth can also be analysed in terms of three fractions. The slow fraction, however ($t_{\frac{1}{2}} = 8·1$ min), is not only rather slow for free cell water but represents only 1-2 % of the total water volume, and gives values of water content of only a few nanolitres. Ionic concentrations calculated from these figures were also very high (e.g. greater than 1 M). This fraction may therefore represent bound water within the cell. The faster fraction was therefore taken to represent free intracellular water. The efflux rate ($t_{\frac{1}{2}} = 20$–40 s) agrees with the value obtained by Benos and Prusch (1972) on *Hydra* (40 s).

Intracellular ionic concentrations, calculated from the data of Tables 4 and 5, are shown in Table 6. No figures are given for error margins since the variations

Table 6. *Estimated intracellular ionic concentrations in* C. lacustris

		Na+ (mM)	K+ (mM)	Cl− (mM)
1 % hydranths	Tissue	5 (14)	33	13·4
	Medium	4	0·5	6
43 % hydranths	Tissue	47 (180)	160	290
	Medium	170	3·5	240

The figures in parentheses for sodium concentration are obtained from flame photometry results.

volume and ionic content are clearly not independent variables. The values are approximate and may vary by as much as 20–30 %. Nevertheless the figures emphasize that the levels of Na+ and K+ vary in a manner qualitatively similar to that found in most other animal cells: in particular K+ is accumulated within the cell, while sodium, at least above a lower minimum value, is excluded. The potassium levels in a very wide range of cell types within the animal kingdom have been shown to be remarkably constant, with a plateau of around 140 mM in all but very dilute external surroundings (Steinbach, 1962; Schmidt-Nielsen, 1975). The close agreement to this general pattern shown in Table 6 provides some confirmation of the volume measurements discussed above. The chloride levels are very much more unusual. Assuming that the cells in *Cordylophora*, in common with those of virtually every other living cell, have a negative resting potential, chloride ions must be actively transported into the cells against an electrochemical gradient. The resting chloride levels must therefore be maintained at levels considerably removed from equilibrium – a hypothesis supported by the rather slow rates of chloride efflux.

DISCUSSION

The principal factor emerging from the results is that chloride ions play the dominant role in the generation of the initial downward portion of the action potential. The unusual polarity of the action potential has been discussed at length elsewhere (Chain, 1979). Because the excitable cells in this preparation form part of an epithelium, the solution outside the excitable membrane (namely the enteron fluid) is isolated from the external medium. Therefore while in most electrophysiological preparations the region outside the excitable membrane is grounded, and voltage changes are registered by an electrode inserted into the cell, in *Cordylophora* the cells themselves are grounded via the outer ectodermal membrane and voltage changes are recorded by an electrode in the enteron, a region immediately outside the cell. The inverted action potentials arise therefore from a reversal of normal recording techniques, and do not reflect fundamental changes in the underlying ionic currents. The initial portion of the AP is therefore produced by an inward positive current, as usual. The evidence from the electrophysiological experiments and ionic measurements suggests that this current is produced by a transient increase in the chloride permeability of the excitable membrane (the inner ectodermal membrane) that allows an outward flow of anions from the cell into the enteron to take place. This hypothesis is based on three main experimental findings:
(*a*) the amplitude of the AP varies linearly with the logarithm of the external

chloride concentration, increasing with decreasing chloride. The small slope of the
plot (only 21 mV rather than 59 mV as expected from a Nernst equation) could
result either from attenuation of the spike amplitude or a partial involvement of some
other ionic species. Below a minimum value (about 70 mM for 43 % and 6 mM for
1 % animals) the spike amplitude is rather insensitive to chloride concentration. Such
a 'saturation' effect at high transmembrane ionic ratios is found in a number of other
preparations, and could be due either to the background 'leak' conductance of the
membrane, or the onset of the repolarization process.

(b) The negligible effect of large changes in external concentrations of calcium and
sodium. This could, of course, merely reflect the presence of substantial ion stores in
the vicinity of the membrane. However, the calcium inhibitors manganese and
lanthanum are also rather ineffective in blocking the AP in 43 % animals, though their
rapid action on muscular contraction indicates that these ions penetrate readily to the
site of electrogenesis.

(c) The high intracellular chloride concentrations. The distribution of chloride ions
in many excitable cells approximates to that described by the Gibbs–Donnan equili-
brium (see Boyle & Conway, 1941): therefore, because of the presence of non-
diffusible anions within the cell, chloride concentration is below that of the external
medium. When changes in chloride permeability have been evoked to account for
certain electrophysiological phenomena, it has normally been assumed that an in-
crease of chloride permeability would favour an influx of chloride ions, and a con-
sequent hyperpolarization of the cell membrane (see Fig. 13b). Such a phenomenon
seems to exist in heart muscle (Dudel *et al.* 1967), frog skeletal muscle (Warner, 1972),
and a number of inhibitory synaptic junctions (see Eccles, 1964). In order therefore
to be able to generate depolarizing action potentials that are chloride-dependent, this
situation must be reversed (Fig. 13a). Chloride must be accumulated actively within
the cell, and the resting permeability to chloride ions should be rather low. Such
a situation is well known in some giant algal cells (see Gutknecht & Dainty, 1968;
MacRobbie, 1970) that produce chloride-dependent action potentials (Gaffey &
Mullins, 1958; Hope & Findlay, 1964). The results given above suggest that a similar
non-equilibrium chloride distribution may exist in the ectoderm of *C. lacustris*. If one
assumes a fairly typical value of 40 mV for the resting potential it is possible to
calculate from the values for chloride concentrations given in Table 6 that the cells
could generate action potentials with a maximum amplitude of about 45 mV for
43 % animals and 60 mV for 1 % animals: both these values are large enough to
account for the actual measured amplitudes.

The combined evidence from these three experimental findings suggests that action
potentials in *Cordylophora* are chloride-dependent events.

Potassium ions rapidly block APs in 1 % and 43 % animals: both the rising and
falling phases become slower during inhibition. Such an effect suggests a conventional
potassium-dependent resting potential, which is presumably decreased in high
potassium solutions. The resulting inhibition of the AP suggests that the spike-
generating mechanism becomes inactivated during depolarization – a phenomenon
common to many other excitable systems. The potassium blocking agent, 4-amino-
pyridine, though active only at high concentrations, broadens the AP. This action may

A $\mu_{in}^{Cl} > \mu_{out}^{Cl}$ B $\mu_{in}^{Cl} > \mu_{out}^{Cl}$
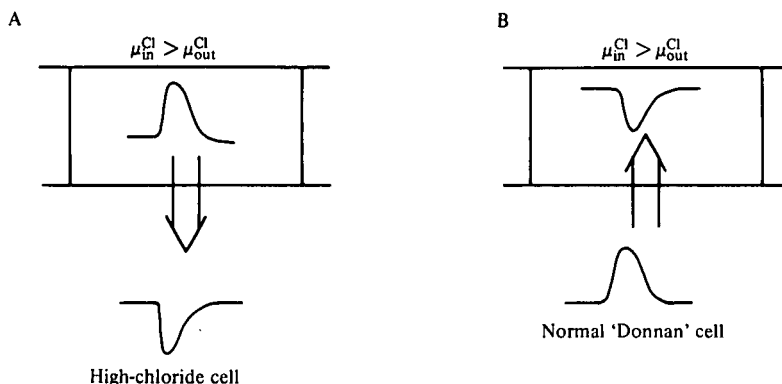
Normal 'Donnan' cell

High-chloride cell

Fig. 13. Diagram showing chloride distribution in two types of cell. The potential changes which would result from an increase in chloride permeability are shown, both as measured intracellularly and as they would appear if measured extracellularly as in Cordylophora. (A) High-chloride cell. Increase in chloride permeability leads to efflux of chloride, and a depolarization of the cell. (B) Normal 'Donnan' cell. Increase in chloride permeability leads to an influx of chloride, and a hyperpolarization of the cell.

reflect the presence of a potassium-dependent repolarization mechanism in this excitable membrane as has been shown in other preparations (Pelhate & Pichon, 1974; Gillespie & Hutter, 1975; Meves & Pichon, 1977).

The effects on electrogenesis of changes in the total ionic and osmotic content of the medium described above can be at least partly explained according to the ionic mechanisms outlined above. The increase in spike amplitude during iso- or hypo-osmotic dilution is presumably due to the decreased external chloride concentration. It should be noted, however, that the increase during a 40-fold iso-osmotic dilution of 43 % hydranths is greater than that produced by decreasing the chloride concentration to an equivalent level: a secondary process (e.g. membrane hyperpolarization) may therefore also effect the amplitude during gross dilutions of the ionic milieu. An additional effect, caused only by changes in total osmotic strength, must presumably be involved in the delayed inhibition of the action potential which occurs during hypo-osmotic dilution (Fig. 3). Such a phenomenon could be a direct effect of osmotic stress in the excitable membrane itself, leading to at least a temporary attenuation of the action potential. Indeed it is perhaps surprising that a 40-fold dilution should have such relatively slight effects on the preparation. The mesogloea and extra-cellular outercoat may act as a sort of supporting structure to the cells, to resist the large and sudden changes in intracellular osmotic pressure. Alternatively, hypo-osmotic solutions may trigger the release of chloride from the cells and thus regulate the chloride levels during changes in the ambient salinity. In any case, the combined effects of the two opposing influences, namely decreased chloride concentration and hypo-osmotic stress, seem to result in a sort of 'homeostasis' of the mechanisms of excitability. Such a system would seem ideally suited to the conditions of low and variable salinity that are the principal features of the environment of *Cordylophora lacustris*.

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