The Electrical and Mechanical Activity of the Esophageal Cell of *Ascaris lumbricoides*

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ABSTRACT   The esophagus of *Ascaris* is a syncytial muscle organ of tubular shape in which the myofibrils are arranged radially between the lumen and the external surface. A resting potential of almost 40 mv (cytoplasm negative) is maintained by the extracellular organic anions (volatile fatty acids) found in the perienteric fluid. Replacement of these anions by Cl^- ions results in a large depolarization. The resting potential is also decreased when the external pH is lowered. The leading phase of the action potential with a positive over-shoot of about 18 mv elicits contraction of the myofibrils, development of negative pressure within the lumen, and suction of liquid and food particles. The mechanical energy stored in the elastic components of the cell is released when the myofibrils relax, thus injecting the contents of the lumen into the intestine. A fast and synchronous relaxation is elicited by a regenerative membrane polarization, a negative spike with a peak value of up to 108 mv produced by an increase in the permeability of the membrane to K^+ ions. Cells completely depolarized in "chloride" saline are still able to generate such large potassium spikes.

As in other nematodes, the initial and only motile segment of the digestive system of *Ascaris* is a stout hollow organ called the esophagus or pharynx. Its vigorous contractions transfer liquid and suspended food particles from the surrounding medium, the intestinal contents of the host, to its own intestine, a long and flattened tube consisting of a single layer of cylindrical cells.

Although the esophagus is made up of 30 odd cells of 3 different types, the light microscope (even when working with 1 μm thick, plastic embedded sections, see Fig. 1) fails to reveal any obvious cellular boundaries between the external and internal cuticles. Furthermore, no appreciable barriers to the spread of electric current within the esophageal cytoplasm can be detected. For these reasons, we find it convenient to refer to the *Ascaris* esophagus as a single giant cell.
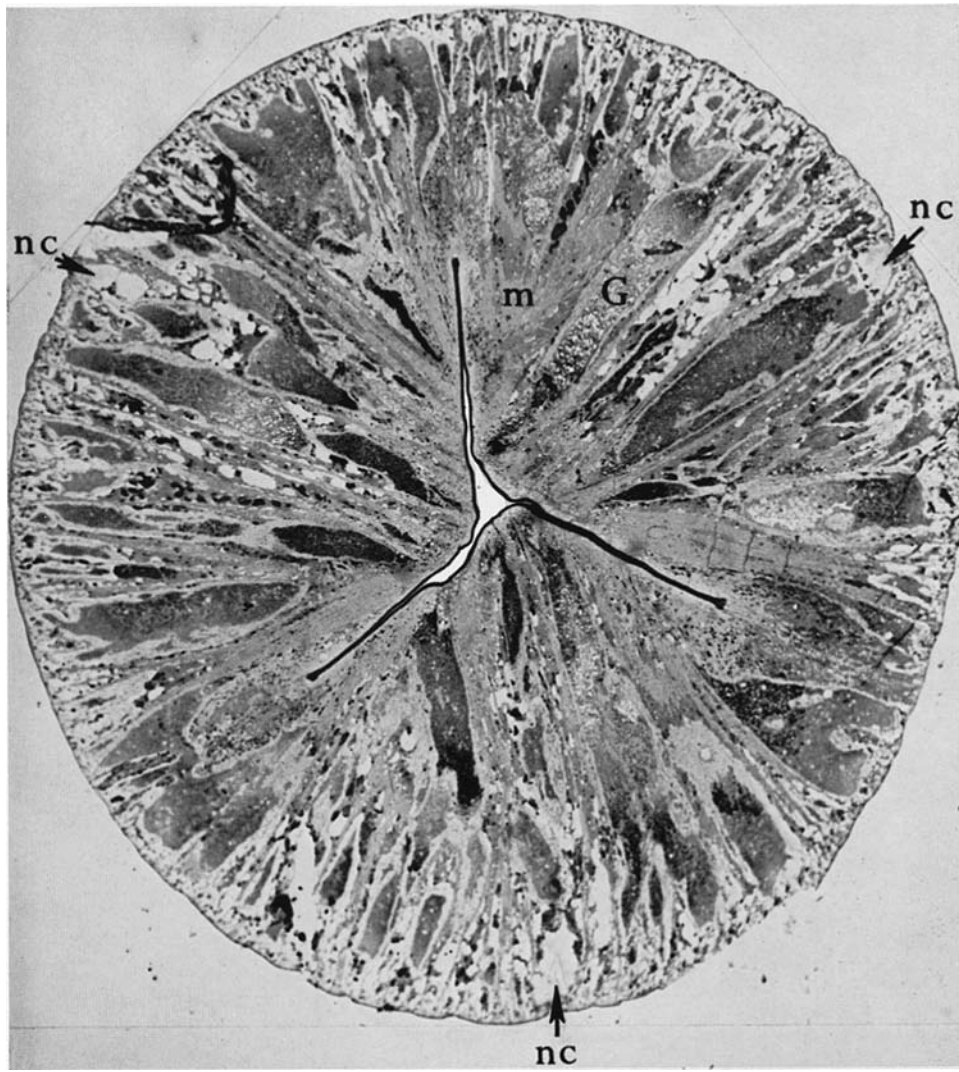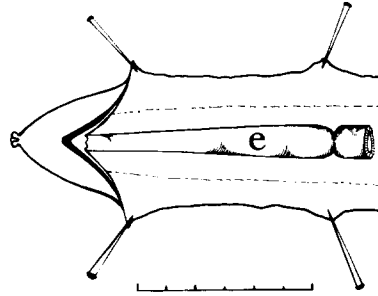
The electrical activity of this cell was first recorded from preparations, set up to study the somatic musculature of the anterior end of the worm, which were equilibrated with 30% (v/v) sea water. Under these conditions, resting potentials of only a few millivolts are recorded from the esophagus. Yet, these depolarized cells respond to externally applied stimuli with the negative, i.e. hyperpolarizing, action potentials of over 100 mv in amplitude described in a previous paper (del Castillo, de Mello, and Morales, 1964 a).

When a more systematic study of the esophageal cell was undertaken, its activity was examined in preparations surrounded by natural and artificial perienteric fluid. In contrast with the initial observations, cells kept in these solutions have a resting potential of nearly 40 mv and respond to applied stimuli with depolarizing action potentials which often show a long plateau and end by a large hyperpolarization which may exceed the resting potential level by more than 50 mv.

This paper describes the electrical behavior of the esophageal cells in both types of solutions as well as experiments carried out to study the ionic basis of the resting potential. The relation between electrical activity and pressure changes in the lumen is also briefly discussed here.

ANATOMY

The papers by Bastian (1866), Looss (1896), Goldschmidt (1904), Martini (1922), Kulmatycki (1918), Hsü (1929), Chitwood and Chitwood (1937), and the recent one of Mapes (1965) should be consulted for a description of the morphology of the nematode esophagus as studied with the light microscope.

Externally, the esophagus is a roughly cylindrical organ measuring up to 1 cm in length and more than 1 mm in diameter at its bulkiest point. A cross-section is shown in Fig. 1. The thick wall, the narrow triradiate lumen, and the cuticles which surround the external and internal surfaces of the cell are well illustrated in this photomicrograph.

The cells which initially form the esophagus are grouped like the seg-

FIGURE 1. The top drawing illustrates the anterior end of *Ascaris lumbricoides* with the body wall cut open along one of the lateral lines. The long tapering organ, separated from the intestine by a constriction, is the esophagus, a cross-section of which is shown in the photomicrograph below (kindly provided by Dr. L. Otero). The cell was fixed for 30 min in 2% glutaraldehyde followed by 60 min in 1% osmium tetroxide, both dissolved in 30% (v/v) sea water, and embedded in Durcupan. Sections were cut with a glass knife to a thickness of 1 μm and stained with the PAS method followed by methylene blue–Azur II. Each division of the scale equals approximately 1 mm for the diagram and 30 μm for the photograph. Symbols, *e*, esophagus; *nc*, nerve cord; *m*, muscular cytoplasm; *G*, glandular cytoplasm.

ments of an orange around the lumen. Six of them, *Kantenzellen* of Goldschmidt, are arranged in three pairs one at the end of each arm of the lumen. Their cytoplasm produces connective fibrils which anchor the corners of the lumen to the external cuticle. The myoepithelial cells contain bundles of myofibrils which extend in an approximately radial direction between the external and internal surfaces of the esophagus. Finally, the glandular cells are interspersed between the myoepithelial cells.

As mentioned above, light microscopy studies do not show clean-cut cell boundaries within the wall of the esophagus. However, observations with the electron microscope (Otero, unpublished data) have shown the existence of complex interdigitating membrane systems which separate "muscular" from "glandular" cytoplasmic areas. On the basis of the available evidence, it cannot be said whether or not cytoplasmatic continuity between adjacent cells occurs at this level. No "tight" junctions or membrane fusions have so far been observed.

The esophagus is innervated by the "pharyngeal-sympathetic nervous system" (Goldschmidt, 1910) formed by a nerve ring around its posterior end, from which three nerve strands embedded in the cytoplasm (Fig. 1) depart towards the oral opening. The dorsal nerve, which according to Goldschmidt contains two nerve cells, is shorter than the subventral ones which have four nerve cells each.

The radial arrangement of the myofibrils suggests that, mechanically, the esophagus functions like a diaphragm pump. As Bastian (1866) pointed out, contraction of the myofibrils converts the narrow lumen into a wide triangular canal with a consequent reduction in internal pressure and suction of liquid through the oral opening. Relaxation, on the contrary, would be followed by an increased intraluminal pressure and the injection of the contents of the lumen into the intestine. Two valves, one at each end of the lumen, ensure the unidirectional flow of material.

METHODS

1. *Preparation.* All the experiments were performed on the esophageal cells of fresh specimens of *Ascaris lumbricoides*, var. *suum*, collected in a local slaughterhouse. The anterior end (2 to 3 cm) of the worm was cut and pinned to a platform with stainless steel entomological pins. With the preparation immersed in saline, the esophagus was exposed by cutting open the body wall along one of the lateral lines (see Fig. 1).

2. *Solutions* The platform supporting the esophagus was placed in a thermostatically controlled chamber maintained at 39°C. The ionic composition of the solutions employed is given in Table I.

3. *Electrical Recording and Stimulation* The electrical activity of the esophagus was usually recorded with intracellular microelectrodes filled with 3 M KCl. These electrodes had resistances of between 15–30 MΩ showing occasionally large and vari-

able tip potentials. However, after the first few impalements through the external cuticle of the esophagus, their resistance decreased to about 10 MΩ. At the same time the tip potentials were greatly reduced and became steady. The observed DC potential returned consistently to the zero base line after the microelectrode tip had been withdrawn from the cell. In some experiments recording was done between the bath electrode and an Ag-AgCl electrode connected to the lumen of the organ by a saline-filled glass cannula (O.D. of the tip, about 0.3 mm). These electrodes were connected via a high-input impedance amplifier (Bioelectric Instruments, Inc., Hastings-on-Hudson, N. Y.) to a Tektronix Type 502 cathode ray oscilloscope. The cells were stimulated with either external platinum electrodes or by injecting current into the cytoplasm with a micropipette.

TABLE I

IONIC COMPOSITION OF THE MAIN SOLUTIONS

No.		Na+	K+	Ca++	Mg++	Cl-	HCO₃-	Organic Anions	Sucrose
1	30% (v/v) sea water*	135.8	3.0	3.0	15.7	175.4	0.8	—	
2	30% (v/v) sea water, without bicarbonate	135.0	3.8	3.0	15.7	176.2	—	—	—
3	Natural perienteric fluid	129.0	24.6	5.9	4.9	52.7	—	122.5‡	—
4	Artificial perienteric fluid (chloride)	129.0	24.0	5.5	4.5	173.0	—	—	—
5	Artificial perienteric fluid (acetate)	129.0	24.5	5.9	4.9	50.0	—	125.1§	7.5

* Hodgkin and Katz (1949).
‡ Volatile fatty acids of 1 to 8 carbon atoms. Those of 5 and 6 carbons amount to 60-90% of the total followed, in order of quantitative importance, by acetic and propionic acids. Butyric acid and a fraction of 7-8 carbon acids amount to only 1-2% of the total. Small quantities of formic acid are also present (Bueding, 1953; Bueding and Yale, 1951; Ellison, Thomson, and Strong, 1960; Hobson, Stephenson, and Eden, 1952; Moyle and Baldwin, 1952).
§ The molality for the acetate concentration was taken from Brading and Caldwell (1964).

4. *Mechanical Recording* The pressure changes within the lumen associated with contraction were recorded with a Statham Model P23-De Transducer (Statham Transducers, Inc., Hato Rey, P. R.). The plastic dome of the transducer was connected to the lumen by a glass cannula. The entire system was filled with saline which had been boiled to minimize formation of air bubbles. The transducer was mounted in one of the arms of a Zeiss sliding micromanipulator and the tip of the attached cannula was introduced under microscopic control into the posterior end of the lumen after sealing the anterior end with a silk ligature. The output of the transducer was connected directly to one of the channels of the cathode ray oscilloscope.

RESULTS

I. *Membrane Potential and Extracellular Ions*

1. PERIENTERIC FLUID An average resting potential of 38.9 ± 0.31 mv (SE), cytoplasm negative with reference to the outside solution, was measured in 26 cells (370 separate insertions) immersed in natural peri-

enteric fluid. The membrane potential of cells in artificial perienteric fluid. (see Table I, No. 5) is lower; an average of 33.8 ± 0.15 mv (SE) was found in 52 cells (1172 separate insertions).

A minute exploration of the esophageal wall with the recording micro-pipette shows the existence of shallow layers or spaces where the resting potential is greatly reduced or absent, as shown in Fig. 2. If the cell is stimulated when the microelectrode tip is in such positions, the recorded signals resemble the small extracellular action potentials observed when the micro-electrode is just outside the surface of the esophagus. Other spaces are also
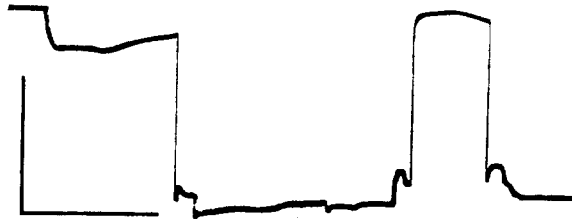

FIGURE 2. Potential recorded between a microelectrode tip and the bath electrode as the former is driven at an approximately constant rate from the external solution into the wall of the esophagus. The straight part of the trace at the upper left corner is the zero level when the microelectrode was outside the cell. A potential of 7–8 mv appeared as its tip crossed the surface of the esophagus and increased suddenly to about 35 mv, the resting potential of the cell. As the microelectrode tip moved on towards the lumen, a layer of cytoplasm was intersected which was at the same potential level as the external solution. This layer, presumably a membrane-bound space in direct communication with the extracellular solution, corresponds to one of the nonpolarized current-spaces described in the following paper (del Castillo and Morales, 1967). Calibrations, vertical, 25 mv; horizontal, 5 sec.

found with a resting potential higher than that of the cytoplasm (del Castillo and Morales, 1967).

2. DILUTED SEA WATER Hobson, Stephenson, and Beadle (1952) showed that *Ascaris* tissues are isosmotic with a solution made by mixing 30 parts of sea water with 70 parts of distilled water. Jarman (1959) and De Bell, del Castillo, and Sanchez (1963) employed this solution with good results for electrophysiological experiments. In fact, somatic muscle cells of *Ascaris* immersed in 30% (v/v) sea water remain functional for many hours; furthermore, it has been shown (del Castillo, de Mello, and Morales, 1964 *b*) that the average resting potential of these cells does not differ significantly from that measured when the same preparations were surrounded by natural perienteric fluid.

But, in contrast to those results, esophageal cells equilibrated with 30% (v/v) natural sea water become depolarized; the average resting potential measured in 16 cells (93 separate insertions) was only 5.6 ± 0.31 mv (SE).

3. TIME COURSE OF DEPOLARIZATION IN DILUTED SEA WATER   The de-
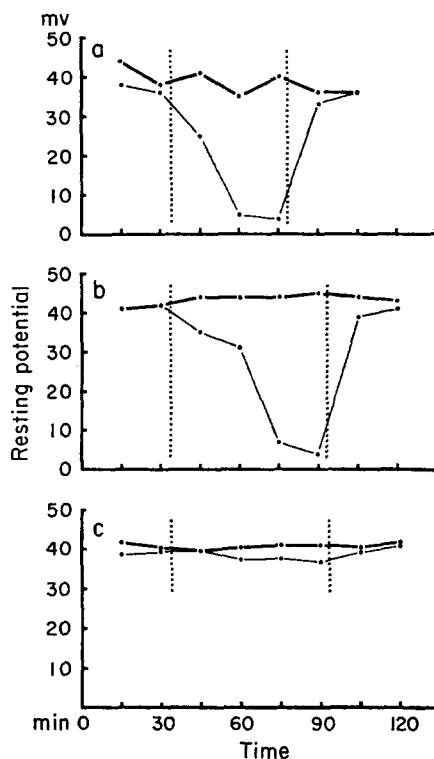polarization of the esophageal cells in diluted sea water is a slow process

FIGURE 3.   Diagrams showing the time course of the depolarization of the esophageal
cell when natural perienteric fluid is replaced with solution 1 of Table I in *a* and solution
4 in *b*. Two cells, placed in two small separate chambers within the usual bath, were used
in each experiment. One cell, used as a control, was kept throughout in natural peri-
enteric fluid (thick line). The other cell (thin line) was transferred to the test solution
during the interval marked by the two broken vertical lines. Each point is the average
resting potential of two experiments (six separate impalements). The repolarization of
the cell can take from 15 to 45 min when it is transferred to the natural perienteric
fluid. Diagram *c* shows how the resting potential is maintained in solution 5 of Table I
in which acetate accounts for about two-thirds of the anions. Each point in this diagram
gives the average resting potential of nine insertions in three different cells. Three other
cells were used as controls and kept throughout in natural perienteric fluid (thick line),
see also Fig. 6 (upper curve). Ordinates, resting potential in millivolts; abscissa, time
in minutes.

which takes from 30 to 60 min, as shown by the experiments summarized in
Fig. 3 *a*. This figure shows also how the cells repolarize when transferred
back to perienteric fluid.

4. EXTRACELLULAR ANIONS AND MEMBRANE POTENTIAL   A comparison
of the ionic composition of natural perienteric fluid with that of diluted sea

water (Table I, Nos. 1 and 3) reveals important differences regarding the concentration of potassium, calcium, and magnesium ions. Furthermore, whereas in diluted sea water chloride is the main anion (175 mM), perienteric fluid contains only 52 mM of Cl^- ions with the rest made up of organic anions, volatile fatty acids. Each of these differences was considered as a possible cause for the depolarization.

The decreased membrane potential in diluted sea water could not be explained on the basis of the lower potassium concentration. It seemed unlikely, also, that the differences in the concentration of divalent cations

TABLE II

COMPOSITION OF THE SOLUTIONS USED IN THE
EXPERIMENTS SUMMARIZED IN FIGS. 4 AND 14

	$[K^+]_o$	Na^+	Ca^{++}	Mg^{++}	Cl^-	Acetate	Sucrose	Choline	HCO_3^-
	2.9	129	5.9	4.9	51.5	125.1	7.5	23.1	
	5.1	129	5.9	4.9	51.4	125.1	7.5	20.8	
	9.6	129	5.9	4.9	51.1	125.1	7.5	16.0	
A	17.4	129	5.9	4.9	50.5	125.1	7.5	7.6	
Fig. 4	24.5*	129	5.9	4.9	50.0	125.1	7.5		
	33.3	120.2	5.9	4.9	50.0	125.1	7.5		
	65.8	87.7	5.9	4.9	50.0	125.1	7.5		
	149.6	3.9	5.9	4.9	50.0	125.1	7.5		
	3‡	135.8	3	15.7	175.4				0.8
	8.4	130.8	3	15.7	175.4				0.8
B	15.2	123.6	3	15.7	175.4				0.8
Fig. 14	24.6	114.2	3	15.7	175.4				0.8
	32.7	106.1	3	15.7	175.4				0.8
	51.6	87.2	3	15.7	175.4				0.8

* Solution 5 of Table I.
‡ Solution 1 of Table I.

should cause such depolarization. Even so, the membrane potential of cells in perienteric fluid was compared with that of cells immersed in solution No. 4 of Table I, with a concentration of K^+, Ca^{++}, and Mg^{++} almost equal to that of natural perienteric fluid, but with a chloride content similar to that of diluted sea water. As shown in Fig. 3 b this solution failed to prevent depolarization.

We were led to suspect, therefore, that the depolarization in diluted sea water might be due to the different external anions, a possibility that was tested by comparing the membrane potential of cells kept in natural perienteric fluid with that of others in solution No. 5 of Table I. These experiments (Fig. 3 c) showed that the resting potential is only slightly reduced when the cells are transferred from natural perienteric fluid to an "artificial perienteric fluid" containing 50 mM of chloride and 125.1 mM of acetate.

5. INFLUENCE OF THE EXTRACELLULAR POTASSIUM CONCENTRATION ON THE MEMBRANE POTENTIAL   To find out the relative importance of potassium in maintaining the resting potential this was measured in solutions containing increasing concentrations of K^+ ions (see Table II, A).

These experiments are summarized in Fig. 4 which shows the relation between the average membrane potential and log $[K^+]_o$ (25 cells). It can be seen that an increase in $[K^+]_o$ from 3 to about 30 mM does not change substantially the membrane potential which decreases only at concentrations higher than 30 mM. The slope of the line above this level is about 28 mv for a tenfold
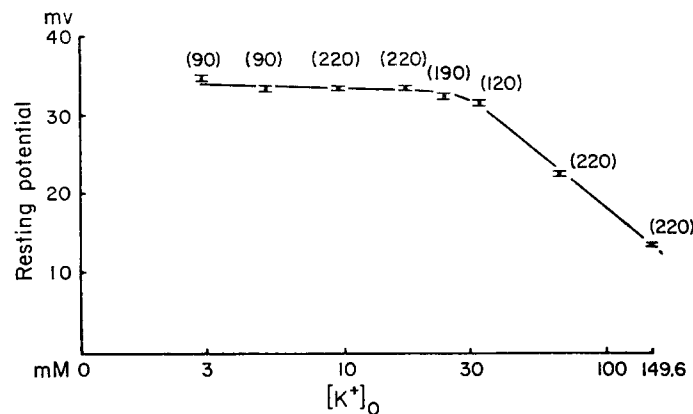
FIGURE 4.   This graph shows the relation between the extracellular concentration of K^+ ions and the average resting potential of 25 esophageal cells. Figures in parentheses indicate number of separate impalements. The distance between the two parallel lines at each point equals twice the SE of the mean. The ionic composition of the solutions used in these experiments is given in Table II.

change in $[K^+]_o$, instead of the almost 62 mv expected from the Nernst equation (at 39°C).

6. INFLUENCE OF pH ON THE MEMBRANE POTENTIAL   Although the resting potential of frog skeletal muscle is not markedly influenced by the external pH (Ling and Gerard, 1949; del Castillo, Nelson, and Sanchez, 1962), it is known that lowering the external pH increases the transverse resistance of its surface membrane by decreasing the permeability to Cl^- ions (Brooks and Hutter, 1962). It was interesting, therefore, to see whether pH changes would influence a resting potential controlled by external anions.

Fig. 5 shows the effect of changing the pH of artificial perienteric fluid from 7 to 8 (upper curve) and from 7 to 6 (lower curve). Whereas raising the pH to 8 increased the resting potential by about 4 mv, lowering it to 6 caused a depolarization of about 20 mv, changes which suggest the existence of a relation between pH and anionic permeability similar to that observed in frog muscle.

These results raised the question of whether the depolarization of the eso-phageal cells in diluted sea water might have been due to pH changes since this solution had been prepared with a distilled water with a pH of 6. To test this, the resting potential of cells kept in artificial perienteric fluid at pH 7 was compared with that of others in diluted artificial sea water also adjusted to pH 7. The HCO₃⁻ ions were left out of the latter solution to avoid sources of error in the measurement of pH (Clark, 1920).

The upper curve in Fig. 6 shows the membrane potential of cells main-
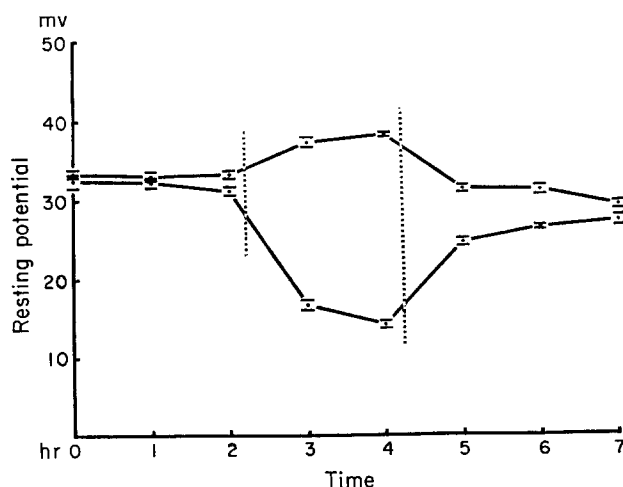
FIGURE 5.   Influence of pH on the resting potential of esophageal cells. Each line gives the average resting potential of 10 cells (60 separate impalements at each point). The pH of the solution (artificial perienteric fluid; No. 5, Table I) was changed from 7 to 8 and back to 7 (upper curve) and from 7 to 6 and back to 7 in the lower curve at the times indicated by the vertical lines. Distance between the two horizontal lines drawn at each point is twice the SE of the mean. Ordinate, resting potential in millivolts; abscissa, time in hours.

tained for 7 hr in artificial perienteric fluid (pH 7.0). The lower curve is that for cells in 30% (v/v) sea water also at pH 7.0; their decreased membrane potential became steady at 13 instead of 6 mv. Upon returning to the peri-enteric fluid, after 7 hr in the solution, it increased by more than 10 mv. The middle curve in this figure shows the effect of changing from artificial peri-enteric fluid to diluted sea water and back at a constant pH of 7.0.

It appears, therefore, that the depolarization of the cells in our diluted sea water was due to both the changes in external anions and to the simul-taneous decrease in pH, responsible for lowering the resting potential below 13 mv.

Incidentally, the lack of HCO₃⁻ ions in the artificial sea water used in the experiments summarized in Fig. 6 cannot be regarded as the reason for the
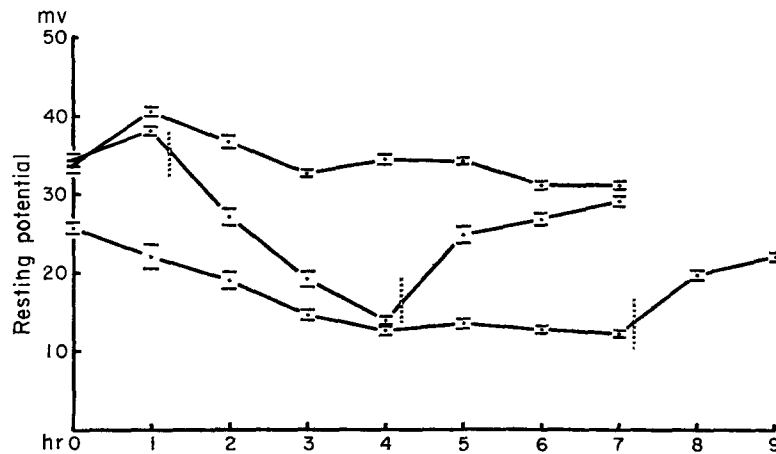
FIGURE 6. Effect of replacing the acetate contained in artificial perienteric fluid by Cl⁻ ions at a constant pH. Upper curve, resting potential of 10 cells maintained during 7 hr in artificial perienteric fluid at pH 7.0. Lower curve, resting potential of 10 cells kept in diluted artificial sea water without bicarbonate, also at pH 7.0. After 7 hr (vertical line) the cells were transferred to artificial perienteric fluid (pH 7.0). Middle curve shows the depolarization produced when 10 cells were transferred from artificial perienteric fluid to diluted sea water (both at pH 7) between the vertical lines. Ordinate, resting potential in millivolts; abscissa, time in hours. The two horizontal lines at each point show twice the SE of the mean. All the cells were allowed to equilibrate for 1 hr with the artificial solutions before any measurements were taken; this explains the different initial average resting potential of the lower curve. Each point is the average of 60 separate impalements.
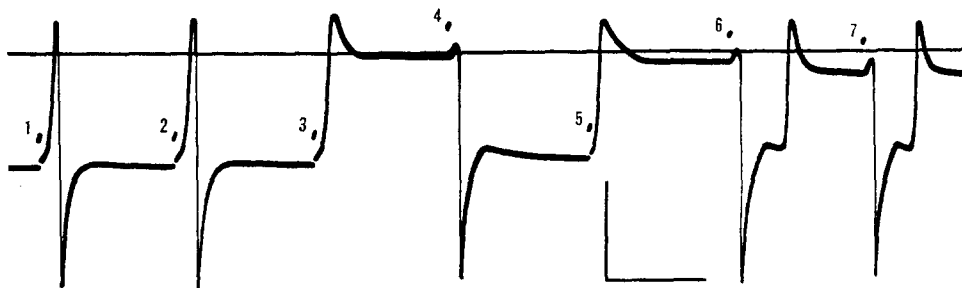

FIGURE 7. Action potentials elicited in a polarized esophageal cell by stimuli applied with external electrodes. The horizontal line is the zero reference level. Resting potential was 46 mv. Stimuli 1 and 2 gave rise to diphasic action potentials. In the action potential which follows stimulus 3, the positive phase was continued by a depolarized plateau. This was interrupted by stimulus 4 which caused a negative spike, followed by a hump due to reexcitation. The prolonged action potential which follows stimulus 5 was interrupted twice (Nos. 6 and 7) by negative action potentials. Calibrations, vertical, 40 mv; horizontal, 1 sec.

smaller depolarization observed in those cells, since solution No. 4 (Table I) also without bicarbonate, but with a pH of 5.9, causes an almost complete depolarization.

II. Electrical Activity of Polarized Cells

1. ACTION POTENTIALS   Fig. 7 shows intracellular action potentials recorded from a cell with a resting potential of about 46 mv. The first two
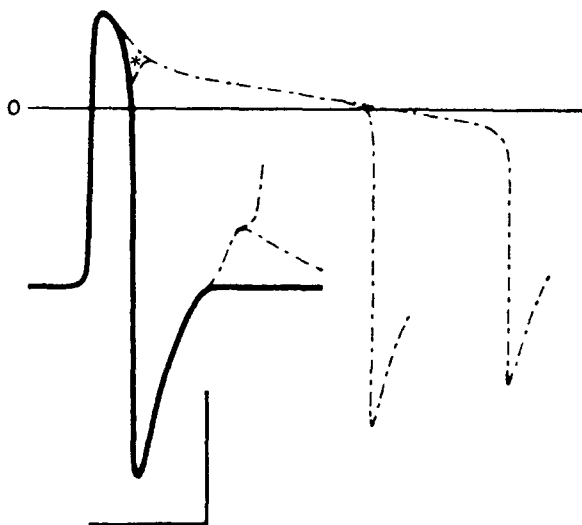

FIGURE 8. The diagram in thick line shows the average amplitude and time course of the action potentials recorded from 31 polarized cells. The thin horizontal line is the zero level. This diagram averages only the recorded diphasic action potentials, though tracings of two other action potentials showing a long depolarized plateau have been superimposed. The notch which often separates the positive peak from the plateau is also shown (see asterisk), as well as the hump produced by reexcitation at the end of the action potential which often gives rise to another positive action potential. Calibrations, vertical, 25 mv; horizontal, 100 msec.

stimuli gave rise to diphasic spikes in which the membrane potential underwent an oscillation almost symmetrical with reference to the resting level.

The depolarizing phase is accompanied by an overshoot with an average value (in 31 cells) of 17.7 ± 1.0 mv (SE). The over-all average amplitude of this phase in the same cells was 50.0 ± 1.8 mv (SE). The maximal rate of potential change observed was only 9 v/sec at 39°C.

The repolarization takes place at a faster rate and instead of slowing down as the potential approaches the resting level it is continued into a large "undershoot" or negative spike. The average amplitude of this peak was 67.7 ± 3.3 mv (SE), measured from the zero base line. Maximal peak-to-peak value of the diphasic action potential was 130 mv. Fig. 8 illustrates the average am-

plitude and time course of the action potentials in 31 cells surrounded by artificial perienteric fluid.

The positive peak is often separated from the negative spike by a plateau lasting up to more than 1 min. In some instances, a dip is seen between the positive peak and the plateau (see asterisk, Fig. 8).

In many cells, the size of the negative spike was inversely related to the membrane potential at the moment when the repolarization began. This explains the large standard error found for the average size of these spikes in polarized cells and suggests that membrane depolarization activates the
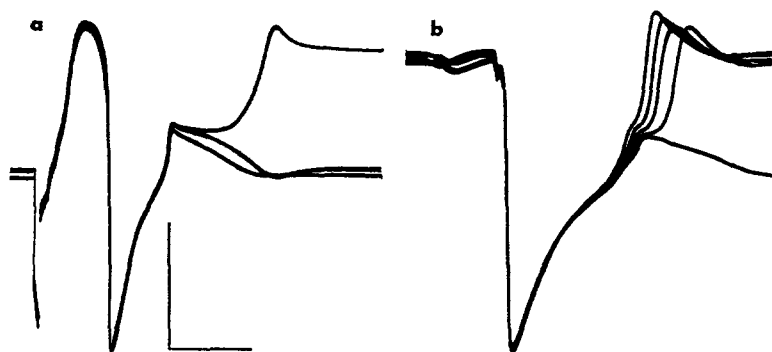

FIGURE 9. Intracellular records illustrating the process of cathodic reexcitation following negative spikes; as part of diphasic action potentials (*a*) and those elicited by stimuli applied during the depolarized plateau (*b*). Reexcitation after two of the negative spikes at *a* and one of those at *b* failed to cause a positive overshoot. Calibrations, vertical, 30 mv; horizontal, 100 msec.

mechanisms involved in the generation of the negative spike in much the same manner that the sodium mechanism is activated by membrane polarization.

Electrical stimuli applied to the cell during the depolarized plateau interrupt the action potential and cause the firing of a negative, or hyperpolarizing spike, as seen in Fig. 7 following stimuli 4, 6, and 7.

2. REEXCITATION   From the peak of the spike, the membrane potential decays with a time course which suggests a passive dissipation of the charge stored in the membrane. Often, when the potential reaches the resting level, an acceleration in the rate of depolarization takes place giving rise to a small hump (see stimulus 4, Fig. 7). A new depolarizing spike can be produced (stimuli 6 and 7, Fig. 7).

This is illustrated in more detail by Fig. 9. Fig. 9 *a* shows three superimposed diphasic action potentials. In one of them the process of reexcitation caused a new positive spike which flared up about 50 msec after the first small peak or hump, and was continued by a plateau. In Fig. 9 *b*, four negative spikes were followed by reexcitation and the return to the plateau level.

These records indicate that reexcitation following the negative spike involves two different processes. One is triggered directly by the decreasing membrane potential; this, in turn, elicits the second one leading to the overshoot.

3. STIMULATION OF POLARIZED CELLS WITH INTRACELLULAR MICRO-ELECTRODES If intracellular micropipettes are used to stimulate, the effective direction of the current is seen to depend upon the prevailing membrane
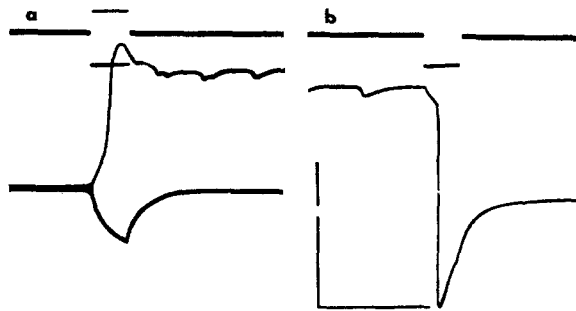

FIGURE 10.   Records illustrating cell stimulation with currents delivered from an intracellular micropipette at different membrane potential levels. The upper tracing monitors the applied current (inward membrane current down). The lower trace is the potential across the membrane. In a, two current pulses were applied to the resting, polarized membrane. The inward current resulted only in an anelectrotonic potential, whereas the pulse of outward current elicited an action potential. However, as shown in b, inward current applied during the plateau caused the firing of a negative spike. Calibrations, vertical, 40 mv; horizontal, 0.5 sec; the single vertical bar is the current calibration, 10 μa.

potential. A positive peak can only be elicited from the resting potential level by an outward current. Contrariwise, negative spikes are produced during the depolarized plateau by inward currents which increase the negativity of the cell interior (Fig. 10).

4. SUBTHRESHOLD EXCITATION IN POLARIZED CELLS If the intensity of the depolarizing stimuli applied to a polarized cell is gradually increased, three different effects are observed: (a) catelectrotonic potentials, (b) long lasting depolarizations, (c) finally, humps were observed in a few instances resembling the subthreshold responses recorded from other excitable cells. These effects are illustrated in Fig. 11 b and c.

III. Electrical Activity of Depolarized Cells

1. ACTION POTENTIALS In most excitable cells, depolarization of the surface membrane results in the abolition of all electrical activity. Yet,

esophageal cells depolarized in diluted sea water are still able to generate large hyperpolarizing spikes.

Fig. 12 shows the average amplitude and time course of the action potentials recorded from 39 depolarized cells in diluted sea water. The polarizing phase leads with a maximal rate of change of about 18 v/sec to a negative peak of 89.0 ± 1.5 mv (se); over 20 mv greater than that recorded from polarized cells.

Three different stages can be distinguished in the depolarizing phase: (a) a slow, presumably passive, depolarization, (b) at a level of between −31 and −27 mv, an increase in the rate of depolarization occurs, as shown by an inflection point (upward concavity), suggesting that excitation is now caused by the decreasing membrane potential (c) finally, at a level of between about −13 and −9 mv the depolarization is again interrupted, as shown by two more inflection points which can be separated in time by up to several seconds; as a result, a plateau or shelf often occurs at this level. These stages are particularly well illustrated in Fig. 13 (see also Fig. 2 of del Castillo et al., 1964 a).

2. EXCITATION OF DEPOLARIZED CELLS WITH INTRACELLULAR MICRO-ELECTRODES   Negative action potentials are elicited in depolarized cells only by inward membrane currents. The process of anodic excitation by pulses of increasing amplitude is shown in Fig. 11 a. Whereas small inward currents result in anelectrotonic potentials, stronger pulses give rise to humps superimposed on the decaying electrotonus. Despite their polarity and slow time course, they are very similar to the local subthreshold responses first described by Katz (1937) and Hodgkin (1938). When the amplitude of such subthreshold responses reaches threshold level (about −20 mv), negative spikes are fired. As in nerve fibers, spikes elicited by stimuli of just threshold strength may fire with considerable latency, more than 30 msec, after the end of the pulse.

If repetitive stimuli are applied to the cell, the intensity of the current needed to stimulate is seen to depend upon the frequency. Thus, stimuli of subthreshold strength if applied singly elicit spikes if delivered in succession, even at frequencies which do not result in electrotonic summation. Furthermore, if the stimulation is now maintained at a constant rate, the strength of the pulses can be decreased well below their initial subthreshold value without causing changes in the amplitude of the spikes.

3. EFFECTS OF THE EXTRACELLULAR POTASSIUM CONCENTRATION ON THE NEGATIVE SPIKES   As mentioned, negative spikes in polarized cells are smaller than in the depolarized ones; a difference which can be explained by assuming that these spikes are due to a regenerative increase in the permeability of the membrane to K^+ ions. Accordingly, the spikes in diluted sea water (3 mM K^+) should be larger than in perienteric fluid (24 mM K^+).
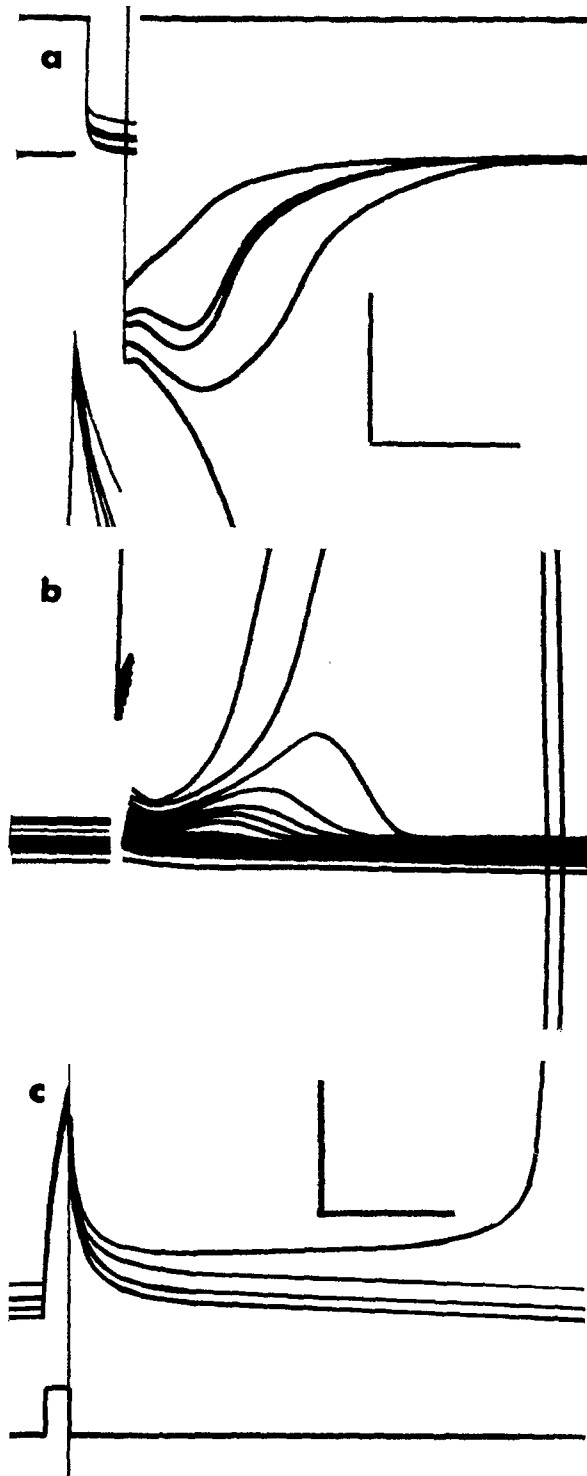
This possibility was tested by measuring the amplitude of the spikes in "chloride" solutions containing increasing concentrations of K^+ ions (Table II, B). Fig. 14 summarizes the results of 14 experiments. In six cells, the spike was abolished at $[K^+]_o$ higher than 24.6 mM; and only three cells were excitable, for a brief period, at 51.7 mM.

In contrast to the slow depolarization observed when the extracellular concentration of Cl^- ions is increased, changes in external potassium have an almost immediate effect on the negative spike. In the experiments summarized in Fig. 14 the size of the negative spikes was measured at 30, 60,
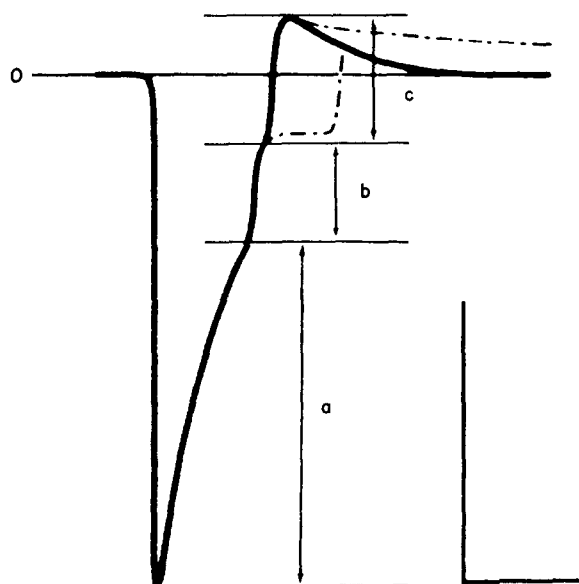

FIGURE 12. The thick line shows the average amplitude and time course of the action potentials recorded from 31 depolarized cells. The three stages *a*, *b*, and *c* in the depolarizing phase (see text) are marked by the line segments. The shelf produced by a delay between the second and third stages is also represented by a dot-dash line. The dot-dash line above the zero base line shows a particularly slow decay from the positive overshoot. Calibrations, vertical, 50 mv; horizontal, 100 msec.

FIGURE 11. Effect of subthreshold and just threshold stimuli applied to polarized and depolarized esophageal cells. *a* shows anelectrotonic potentials and subthreshold negative responses elicited by pulses of inward membrane current of increasing strength; one of them resulted in a negative spike. *b* and *c* show the effects of outward current (see text): (i) catelectrotonic potentials (*c*), (ii) long lasting depolarizations seen as a shift of the trace with each applied pulse, and (iii) depolarizing action potentials sometimes preceded by subthreshold responses (*b*). Calibrations, vertical, 10 mv; horizontal, 100 msec. Current, 2 μa in *a* and 7 μa in *c*. Records in *a* and *b* were superimposed on the same frame of film. *c* shows tracings from separate frames.

and 120 sec after the bath solution was replaced. Although their amplitude
soon became steady in the lower concentrations of K^+ ions, it declined con-
tinuously at 51.7 mM. The three points for this concentration (Nos. 1, 2, and
3, Fig. 14) give, respectively, the spike amplitude 30, 60, and 120 sec after
changing the solutions. The spike disappeared after the third measurement;
however, the first point was in agreement with the steady values measured
in the lower concentrations. These four points can be joined by a straight
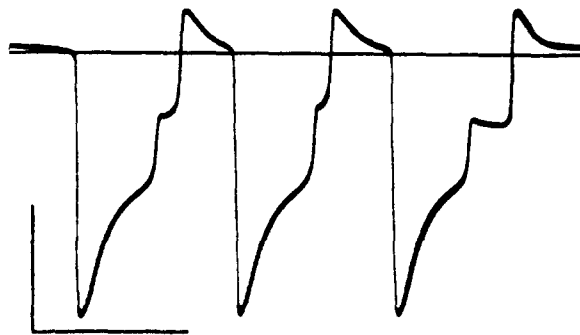line with a slope of 65 mv for a tenfold change in $[K^+]_o$.

FIGURE 13. Spontaneous negative action potentials recorded from a depolarized cell
illustrating relatively long delays between the second and third stages of the depolarizing
phase (see text). The horizontal line is the zero reference level. Calibrations, vertical,
40 mv; horizontal, 0.5 sec.

IV. *Electrical Activity during Equilibration with Diluted Sea Water*

The most immediate change observed after immersing the cells in diluted
sea water is an increase in the size of the negative spike, which occurs within
20 or 30 sec after changing the solution. As a consequence, probably, of the
increased membrane polarization the cells show an enhanced tendency
towards reexcitation. Furthermore, the duration of the depolarized plateau
of the action potential is seen to increase considerably.

Often, during this period the transmembrane potential can remain in-
differently at either the reduced resting potential level or at the depolarized
plateau switching from one level to the other whenever a stimulus is applied
(Fig. 15).

V. *Additional Observations*

1. SPONTANEOUS ACTVITY   All the types of electrical activity so far
described have also been observed in the absence of external stimulation.
Single action potentials are fired occasionally in both polarized and de-
polarized cells, but more often they occur as trains or volleys initiated at a
discrete pacemaker area whose position often changes during recording.

Fig. 16 *a* shows spontaneous negative spikes recorded from a pacemaker region. Each spike is preceded by a slow polarization; a mirror image of the depolarizing prepotentials observed in neural and muscular pacemakers.
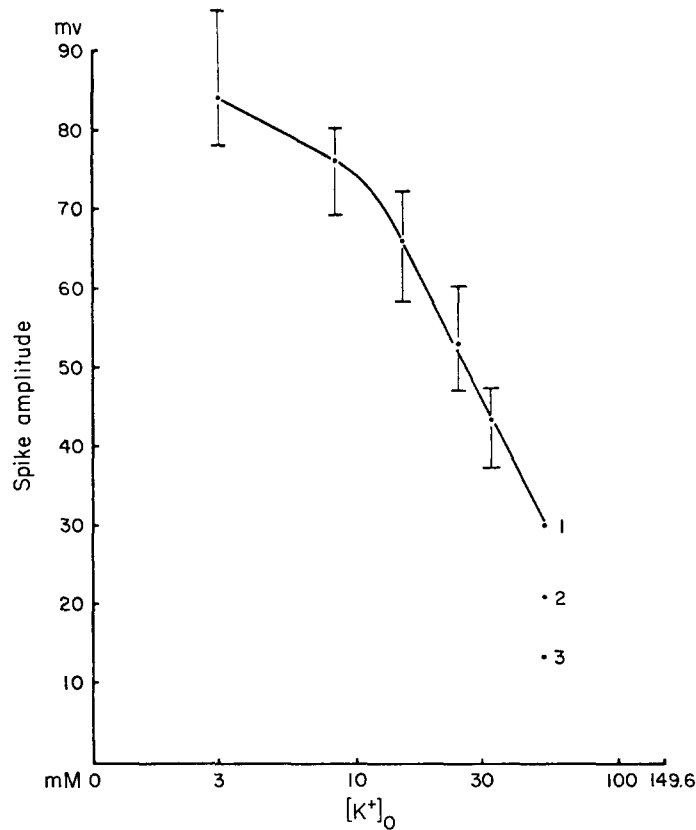

Figure 14. The line represents the relation between $[K^+]_o$ and the average amplitude of the negative spikes in 14 depolarized cells. The solutions (see composition in Table II) were changed without removing the microelectrode from the cells. In only eight experiments the cells were excitable at a $[K^+]_o$ of 32.7 mM and only three cells gave action potentials for a brief period at a $[K^+]_o$ of 51.6 mM. At this concentration three points (numbered 1, 2, 3) are given, which are, respectively, the average amplitude of the negative spike 30, 60, and 120 sec after beginning to change the solution (see text). The vertical lines indicate the range of variation.

As shown in Fig. 16 *b*, the frequency of the impulses may show sudden changes. Similar observations have been made in polarized cells.

2. SMALL REPETITIVE POTENTIALS In about one-half of all cells examined, small repetitive potential transients have been observed superimposed on both resting and action potentials (Fig. 17). They were particularly obvious in depolarized cells and during the depolarized plateau of positive

action potentials. Most often these potential changes had a negative polarity. Maximal amplitude was about 15 mv. Double and treble peaks have also been observed (Fig. 17 *a*). In a few instances relatively large positive transients have been seen superimposed on the resting potential of polarized cells (Fig. 17 *b*).

When the membrane potential shifts from the depolarized plateau to the resting level, the amplitude of the small potentials always changes, usually decreasing very markedly. In most instances, their polarity is also seen to reverse, as shown in Fig. 17 *b* and *c*.
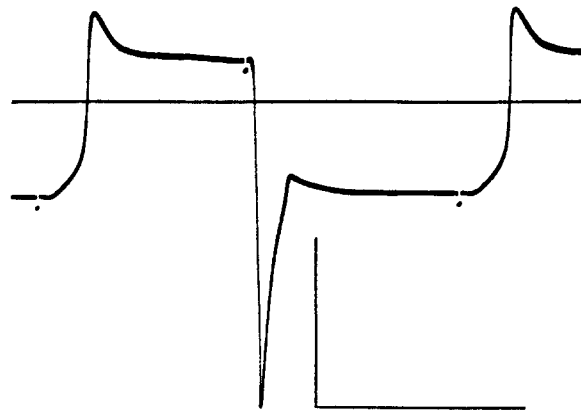

FIGURE 15. Effects of stimulation with external electrodes during equilibration of a cell with diluted sea water. The horizontal line is the zero reference level. The potential between cytoplasm and external solution shifted, upon stimulation, between two levels: the depolarized plateau, in which the cytoplasm was about 10 mv positive with reference to the external solution, and the decreased resting potential (see text). Calibrations, vertical, 40 mv; horizontal, 1 sec.

The frequency of these small potentials may remain constant for long periods, particularly in the absence of action potentials. However, when spikes occur, their frequency changes, usually reaching a maximum just after the positive overshoot (Fig. 17 *c*). Frequently, the first effect of applied stimuli is the appearance of small voltage activity before any action potentials are produced.

3. ACTION POTENTIALS RECORDED FROM THE LUMEN   Action potentials similar to those observed with intracellular microelectrodes are recorded between the bath electrode and an electrode connected to the lumen by means of a saline-filled cannula (see Methods). The size of these potentials depends on the shunt resistance between both electrodes. Maximal amplitude, about one-half of the intracellular action potentials, can be obtained by closing the anterior end of the esophagus with a ligature.
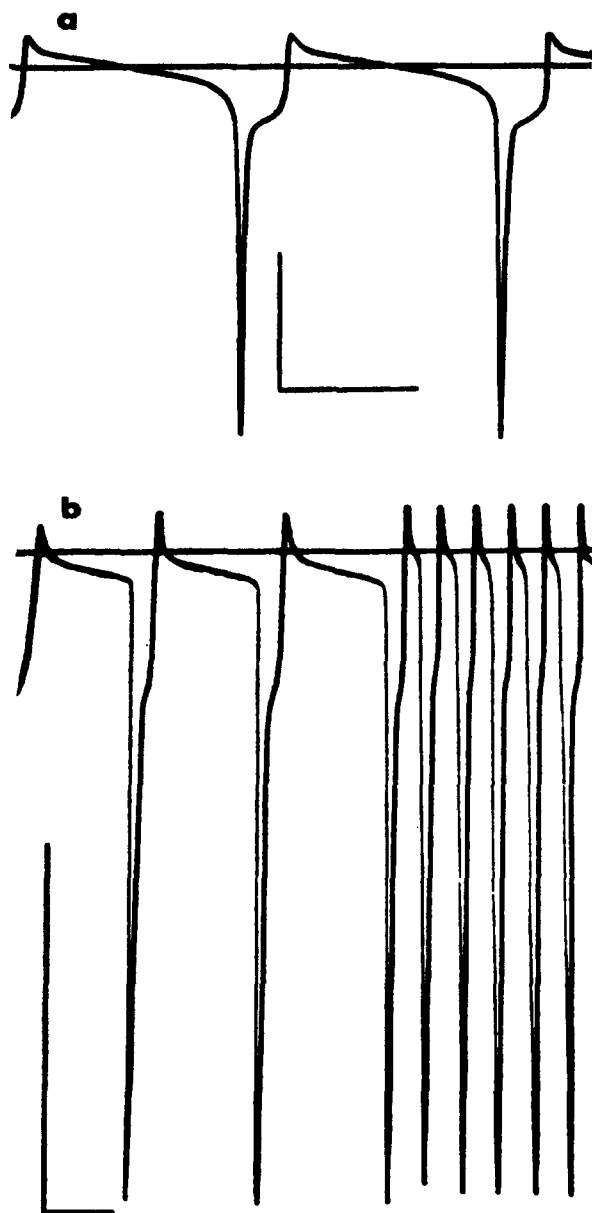
FIGURE 16. Examples of spontaneous rhythmic negative action potentials recorded from a depolarized cell. In *a*, the intracellular microelectrode was placed at the pacemaker region. Each negative spike is preceded by a prepotential. Calibrations, vertical, 20 mv; horizontal, 1 sec. In record *b* the pacemaker area was some distance away from the recording microelectrode as shown by the more abrupt initiation of the spikes. A sudden increase in the frequency of firing is seen. Calibrations, vertical, 50 mv; horizontal, 1 sec.

VI. *Relation between Electrical and Mechanical Activity*

Action potentials and intraluminal pressure changes were recorded simultaneously as described in Methods. Since the diaphragm of the pressure transducer is electrically floating, action potentials could easily be recorded between the solution filling the transducer system (and the lumen) and the
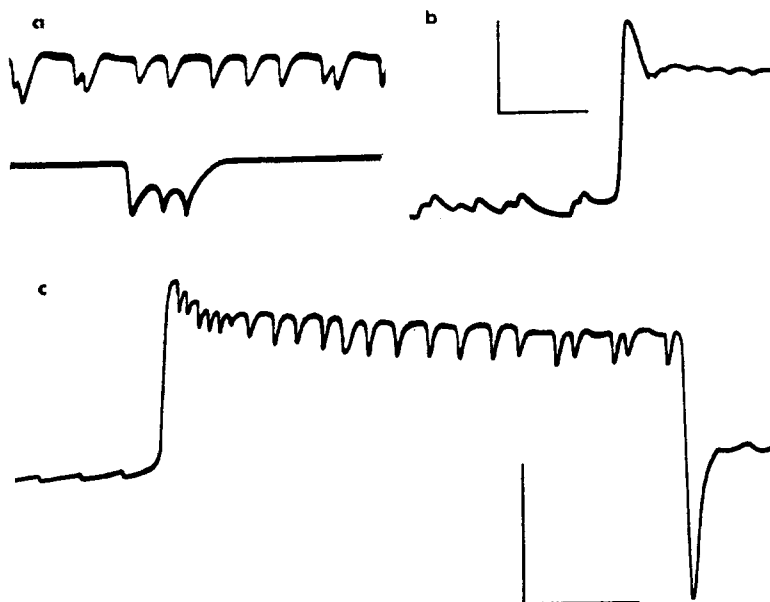

FIGURE 17. Small voltage oscillatory activity superimposed on resting and action potentials. Record *a* shows negative transients recorded from two different depolarized cells (without reference to a common DC level). Record *b* illustrates exceptionally large positive transients superimposed on the resting potential level; notice how their polarity is reversed and the amplitude is decreased when the potential shifts to the depolarized plateau level. Record *c* shows a long spontaneous action potential and superimposed transients in a polarized cell. Calibrations in *b*, vertical, 4 mv for *a* and 20 mv for *b*; horizontal, 0.4 sec for upper trace in *a*; 0.2 sec for lower trace in *a* and 1 sec for *b*. Calibrations in *c*, vertical, 30 mv; horizontal, 0.5 sec.

bath electrode. Fig. 18 shows the electrical activity of a cell recorded in this manner and the pressure changes within the lumen. Negative pressure, caused by contraction of the myofibrils, is shown as a downward displacement of the trace.

At the beginning of record 18 *a* the resting potential (upper trace) and the reference (zero) pressure level within the lumen (lower trace) are seen. Membrane depolarization was accompanied by the development of negative pressure which persisted with no appreciable relaxation during the plateau. Maximal negative pressure recorded was nearly 2 cm Hg. This value falls

short of the figures anticipated from the work of Harris and Crofton (1957) (see Discussion) probably because of leaks or dead spaces in the recording system. The negative spike caused the immediate relaxation of the cell.

Records taken on a faster time base (Fig. 18 *b*) show that the relaxation elicited by the negative spike occurs very rapidly whereas contraction follows
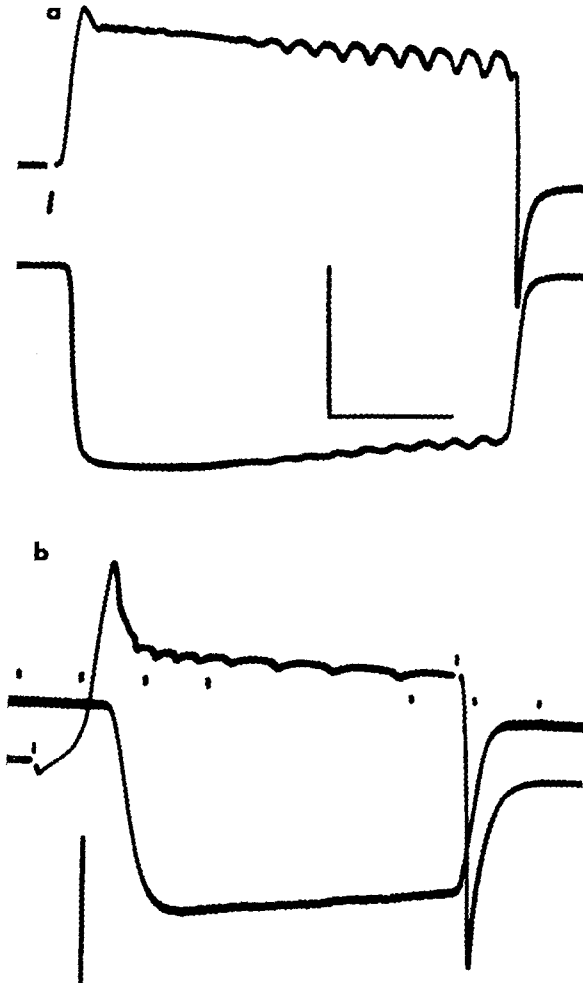

FIGURE 18. Simultaneous recording of the action potential and associated intraluminal pressure changes in polarized esophageal cells. In *a*, a positive action potential with a long depolarized plateau was elicited by an externally applied stimulus (upper trace); the lower trace is the pressure within the lumen; negative pressure (downward) is maintained throughout the depolarized plateau, reflecting the smaller potential oscillations. The negative spike causes relaxation. Calibrations, vertical, 25 mv and 1.4 cm Hg; horizontal, 1 sec. Traces in *b* were taken with a higher sweep speed (time signals 50 msec). Vertical calibration, 25 mv and 0.6 cm Hg.

membrane depolarization with a marked delay. Thus, whereas the negative pressure is reduced to about one-third or more of its initial value when the hyperpolarizing spike reaches its peak, the intraluminal pressure is barely beginning to decrease when the positive overshoot reaches its maximal value.

DISCUSSION

Most of the experiments described above should be regarded as preliminary. Furthermore, no mention has been made of other interesting aspects of the electrophysiology of these cells, such as those concerning the passive electrical properties of their surface membranes, which will be described in another publication.

Resting Potential In common with the somatic muscle cells the surface membrane of the esophagus appears to be sparingly permeable to K+ ions, the resting potential being determined mainly by the extracellular anions. Yet, in contrast to the former cells, chloride cannot substitute for the organic anions present in the perienteric fluid. Furthermore, the resting potential of the esophagus is very sensitive to changes in pH.

Evidence has been obtained (del Castillo and Morales, 1967) suggesting that the surface membrane of the esophagus forms a widespread system of invaginations into the cytoplasm. The slow rate at which the membrane potential decreases in diluted sea water can be explained by a restricted process of exchange of the anions contained in this system of invaginations due to either fixed charges or the presence of transverse membranes. Even if the resting potential generated by the more directly exposed areas of the membrane should cease when the external solution is changed, cell polarization could be maintained by the anions still in contact with the "hidden" areas.

The fast abolition of the negative spike when the extracellular potassium is increased can be explained by its immediate effects on the "exposed" membrane, since the increased K+ conductance at this region, caused by membrane hyperpolarization, would short-circuit any emf's which might still be developed at the membrane forming the invaginations.

The time course of the changes in resting potential elicited by changing the external pH has not been studied in detail, but it seems to be also rather slow. More experimental work is needed, however, before the mechanism of such delayed effects can be explained.

Action Potential An interesting feature of the esophageal action potentials is the occurrence of two different regenerative, all-or-nothing processes: one is responsible for the positive overshoot, whereas the other, a potassium spike, brings the potential to the resting level through an intense membrane hyperpolarization.

The occurrence of a long plateau between these two processes is not an exclusive feature of the esophageal cell since it has been observed also in other muscle cells under various conditions (Tasaki and Hagiwara, 1957; Fatt and Ginsborg, 1958; Hagiwara and Naka, 1964). A more typical feature of the *Ascaris* esophagus is the fact that a depolarization of the membrane does not prevent the generation of negative spikes.

The mechanisms involved in the depolarizing phase of the action potential have not yet been fully investigated. However, the three stages observed in the depolarizing phase which follows the negative spike suggest that two separate ionic processes or two areas of membrane, either functionally or anatomically different, are involved.

The electrical activity of the esophagus of other nematodes has not been studied with intracellular microelectrodes. However, the small potentials, synchronous with the esophageal contractions, recorded from the intact *Ancylostoma caninum* by Roche, Martinez-Torres, and Macpherson (1962) may be equivalent to some of the action potentials described in this paper.

Repetitive Small Potential Transients The nature and possible function of the small repetitive potentials described above are unknown. Oscillations of membrane potential resembling such transients have been described in frog muscle fibers immersed in solutions in which the Cl^- ions were replaced by ferrocyanide (Falk and Landa, 1960). Similar oscillatory activity has also been observed during the prolonged action potentials recorded from barnacle muscle fibers (Hagiwara and Naka, 1964). Whether or not such resemblance is only coincidental cannot be ascertained on the basis of the available evidence.

Mechanical Activity The relation between electrical activity and contraction is conventional in the sense that shortening of the myofibrils is caused by membrane depolarization. Yet, the fact that they remain contracted for as long as the depolarization persists is not a common feature of muscle cells; though it seems to occur also in the slow muscle fibers of the frog (Pauschinger and Brecht, 1961) which do not generate action potentials.

A more exclusive feature of the electrical control of contraction in the *Ascaris* esophagus is the occurrence of a relatively fast process of relaxation elicited by the negative, hyperpolarizing spike.

Both the long lasting shortening of the myofibrils and the negative action potential seem to be responsible for the efficiency of the esophagus as a pumping device. Indeed, its ability to eject a volume of liquid against the considerable hydrostatic pressure prevailing in the body cavity of *Ascaris*, up to 7 cm Hg (Harris and Crofton, 1957), appears to be made possible by the sudden onset and relatively high speed of the relaxation process, by which the energy developed during contraction is suddenly transformed into external work.

One would expect that the rate at which negative pressure is developed is not so important for the function of the esophagus as are the efficient energy storage by the elastic components of the cell and the rate at which the stored energy is released. It is not surprising, therefore, that special mechanisms in the electrical processes which control the contractile machinery have been evolved to meet such conditions, in the form of an amplified version of the positive afterpotential, or undershoot, observed in many excitable cells.

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