# Extracellular Action Potentials Recorded from the Interior of the Giant Esophageal Cell of *Ascaris*

J. del CASTILLO and T. MORALES

From the Department of Pharmacology, School of Medicine, San Juan, Puerto Rico

ABSTRACT Exploration of the cytoplasm of the giant esophageal cell of *Ascaris* with a recording microelectrode shows the existence of shallow spaces where the microelectrode tip becomes extracellular in spite of being in the interior of the cell. When the microelectrode penetrates into these spaces from the cytoplasm, the resting potential shifts to a different level or entirely disappears. At the same time the large intracellular spikes are replaced by small transients similar to extracellularly recorded action potentials. It is concluded that such spaces are in communication with the external solution, and separated from the cytoplasm by an electrically active membrane; i.e., able to generate action potentials. Measurement of the potential differences between the interior of the spaces and the external solution shows that although some are not polarized, many spaces have a resting potential of the same polarity as that of the cytoplasm. It is suggested that although they are of larger size these spaces may be equivalent to the tubular systems which in other muscle cells are known to be involved in the spread of excitation into the cytoplasm.

As described in the previous paper (del Castillo and Morales, 1967), if the cytoplasm of the esophageal cell of *Ascaris* is minutely explored with an intracellular electrode, shallow layers are revealed where the tip of the micropipette becomes extracellular, at least from an electrical viewpoint. Indeed, the large intracellular spikes disappear when the tip of the microelectrode enters these spaces; instead, smaller transients, similar to extracellularly recorded action potentials, follow the applied pulses.

The experiments described in this paper suggest that such deflections are generated by the flow of action currents within a system of spaces which communicate with the cell exterior by pathways which bypass the membrane which generates the resting and action potentials.

Since extracellular "action potentials" are actually voltage drops caused by the flow of action currents in the resistance of the external solution, we shall use the word "currents" as a synonym for "extracellular action po-

tentials." The term "current-spaces" will be employed to describe the spaces from which the extracellular action potentials are recorded within the cell.

## METHODS

All the experiments were performed in esophageal cells of *Ascaris lumbricoides* at 39°C. The previous paper (del Castillo and Morales, 1967) should be consulted for details on preparations, solutions, and electrical techniques. Although action potentials can be recorded from the esophagus with microelectrodes of very low tip resistance, to reproduce the results described below microelectrodes with resistances of at least 15 MΩ should be employed. Since higher resistances are usually accompanied by higher tip potentials, the microelectrodes were rejected if the DC level did not return to within ±2 mv of the zero line after withdrawing them from the cell.

## RESULTS

1. *The Extracellular Action Potential*  Fig. 1 *a* illustrates an intracellular negative spike recorded from a depolarized cell whereas record 1 *b* shows the transients observed when the microelectrode tip was outside, but close to, the surface of the cell. This record was selected because of the unusually large size of such transients, which rarely exceed a peak-to-peak amplitude of 2 mv. In good preparations the extracellular action potentials can be detected over the entire surface of the cell at distances of up to about one cell diameter from its surface.
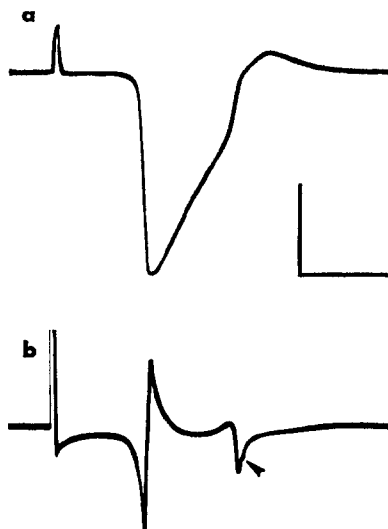


FIGURE 1. *a*, negative intracellular action potential recorded from a depolarized esophagus. *b*, extracellular action potential recorded from the same cell with a microelectrode tip placed just outside its surface (see text). Vertical calibration, 40 mv for *a* and 4 mv for *b*. Horizontal calibration, 100 msec.

Two parts can be distinguished in these transients: an initial biphasic deflection associated with the polarizing phase of the negative spike and a smaller ripple which coincides with the second and third stages of the depolarizing phase (del Castillo and Morales, 1967) and is also biphasic in some

cells. Occasionally, as indicated by the arrow (Fig. 1 *b*), a separate small negative wave is seen following the peak of the second deflection.

Despite its slower time course and polarity, the biphasic deflection associated with the negative spike resembles the extracellular action potentials recorded by Håkansson (1957) from isolated skeletal muscle fibers of the frog. The initial negative wave can be interpreted as the inward membrane current flowing in front of the oncoming action potential, whereas the sudden reversal in the direction of the current leading to a positive wave suggests that the membrane below the microtip becomes at this moment an active source of current.

The last part of the extracellular potential, a predominantly negative deflection, indicates that also at this moment the surface of the cell develops an emf (cytoplasm positive) behaving as an active current sink.

The extracellular action potentials recorded from polarized cells are similar to those just described, but the two components occur in the opposite order (Fig. 2).

2. *Extracellular Action Potentials Recorded with Microelectrodes in an Intracellular Position*   Each of the two columns of records of Fig. 3 shows the transients recorded, following stimulation, with a microelectrode tip placed just outside the surface of the cell and at three different points within its cytoplasm. Both *a* and *a'* were recorded when the microelectrode tip was outside the esophagus; *b* and *b'*, taken with a smaller gain just after impalement of the cell, show the large intracellular negative spikes.

If the microelectrode is now driven deeper into the cytoplasm, slowly and by very small steps synchronized with the sweeps of the cathode ray oscilloscope, shallow spaces can usually be discovered where the large action potentials disappear and are replaced by the smaller transients shown in *c* and *c'* (notice different gain). A sudden change in the DC level is observed simultaneously, but this will be discussed in a separate section. Further displacement of the microelectrode brings back both the initial resting potential and the intracellular action potentials (Fig. 3 *d* and *d'*). In many insertions only one of such layers of cytoplasm can be found. At other times, particularly if the direction of movement of the microelectrode is not strictly radial, two or even three layers are intersected.

If the micropipette is withdrawn from the cytoplasm, a reversed sequence of events is observed. In fact, it is easier to penetrate into these spaces when the electrode is being moved out of the cell, since when moving towards the lumen the microelectrode tip often overshoots the thin layers we are discussing.

A comparison of *c* and *c'* with *a* and *a'* (Fig. 3) and with Fig. 1 *b*, shows that although of larger amplitude, the transients recorded from the layers

where the large action potentials disappear closely resemble the extracellular action potentials.

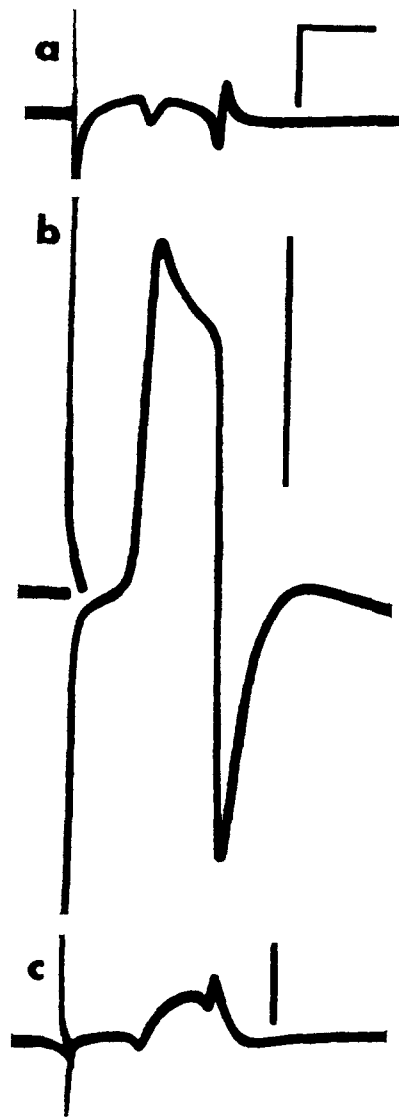The initial negative wave can be superimposed onto the foot and early



FIGURE 2.  a, extracellular action potential recorded from a polarized esophageal cell (resting potential about 30 mv).  b, intracellular positive (i.e. depolarizing phase first) action potential recorded from the same cell.  c, extracellular action potential recorded from a current-space within the cytoplasm (see text). Calibrations, vertical a, 1 mv; b, 30 mv; c, 2 mv. Horizontal, 200 msec for the three records.

part of the polarizing phase of the negative action potential (Fig. 4 a). A sudden reversal in the direction of the recorded current soon takes place. The peak of the resulting positive wave occurs about 5–10 msec before the peak of the negative spike (Fig. 4 b).

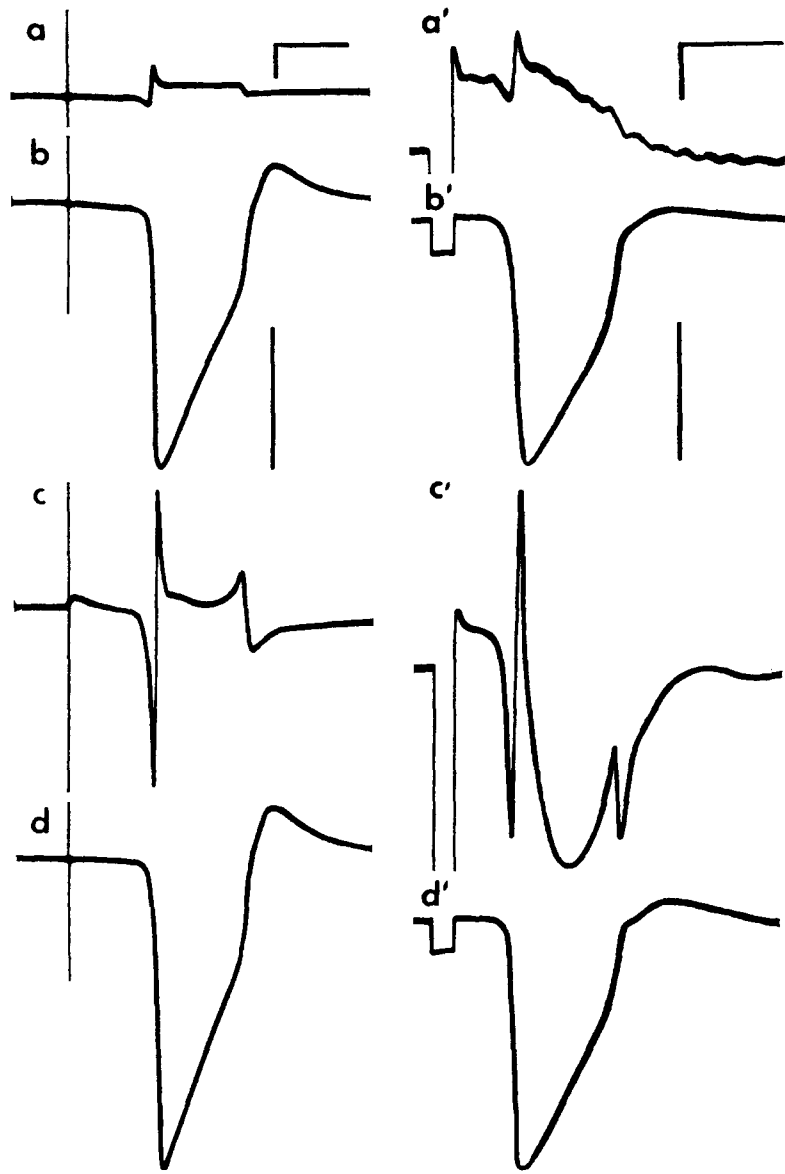The depolarizing phase of the negative spike is reflected in these records

Figure 3. Potential transients recorded, following stimulation, from two esophageal cells with the microelectrodes placed in different positions. Each vertical column corresponds to one cell. Records *a* and *a'* are the extracellular action potentials recorded with the microelectrode tips just outside the surface of the esophagus; *b* and *b'* are intracellular action potentials observed after cell impalement; *c* and *c'* are extracellular action potentials recorded when the microelectrodes penetrated into current-spaces within the cytoplasm. In *c* the electrode was in a nonpolarized current-space, whereas *c'* was recorded from a polarized one (see text). *d* and *d'* show again the intracellular action potentials observed when the microelectrodes reentered the cytoplasm after crossing the current-spaces. Calibrations, vertical, *a* and *a'*, 2 mv. Vertical bar in *b* is 40 mv for *b* and *d* and 4 mv for *c*. Vertical bar in *b'* is 50 mv for *b'* and *d'* and 5 mv for *c'*. Horizontal in *a*, 100 msec for all records in left column. Horizontal in *a'*, 100 msec for all records of the right column.

by another diphasic current (positive-negative) as shown in Fig. 3 c and c'. Occasionally, when a delay occurs between the second and third stages of the depolarizing process, two different negative waves are observed instead of the single biphasic deflection. This is shown in Fig. 4 a. The potential transients recorded from such spaces in polarized cells also resemble very closely the extracellular action potentials (Fig. 2 and upper records of Fig. 6).
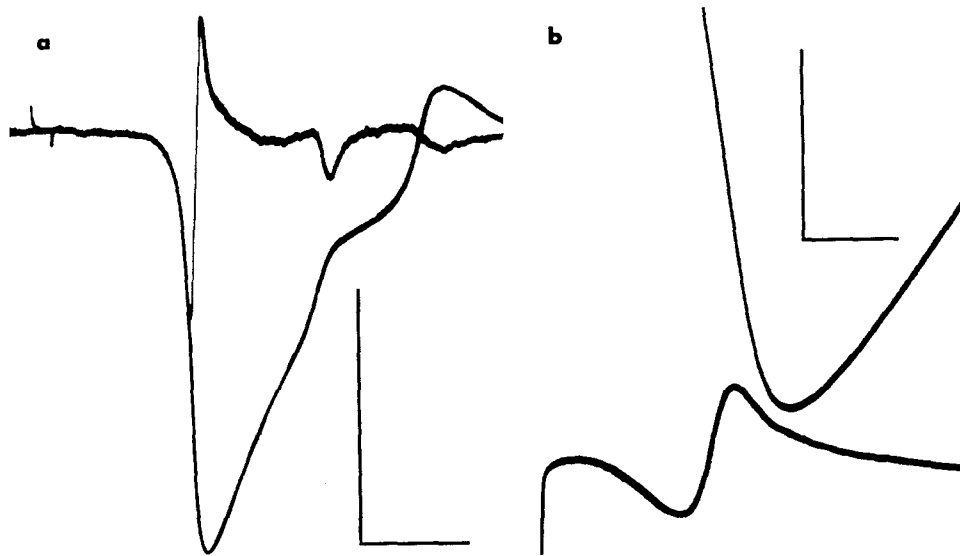


FIGURE 4. Records showing the time relations between intracellular and "extracellular" action potentials simultaneously recorded with two microelectrodes inserted in the cytoplasm of the esophagus at the same level along its longitudinal axis. In a, the two traces were photographically superimposed along the vertical axis. Notice similar time course of the initial part of both action potentials. b shows on a faster time base, the peak of the intracellular negative spike and the corresponding biphasic deflection recorded from a current-space. Calibrations, a, vertical, extracellular, 10 mv; intracellular, 50 mv; horizontal, 100 msec. b, vertical, extracellular, 10 mv; intracellular, 20 mv; horizontal, 10 msec.

These results can be explained by assuming that the potential transients recorded from the sites where the action potentials disappear, are due to the flow of action currents within spaces open to the external solution. They can be visualized, therefore, as a system of invaginations of the surface membrane of the cell.

The higher amplitude of the intracellularly recorded currents as compared to their external counterparts, suggests that the resistance between the microelectrode tip placed inside a current-space and the outside solution is larger than the convergence resistance encountered by the currents flowing around the tip of an extracellular micropipette at the surface of the cell.

3. *The DC Potential Level of the Current-Spaces*  If the current-spaces communicate directly with the outside solution, one would expect that in the absence of current flow no potential differences would be measured between their interior and the remote bath electrode. Nevertheless, as Fig. 5 shows,
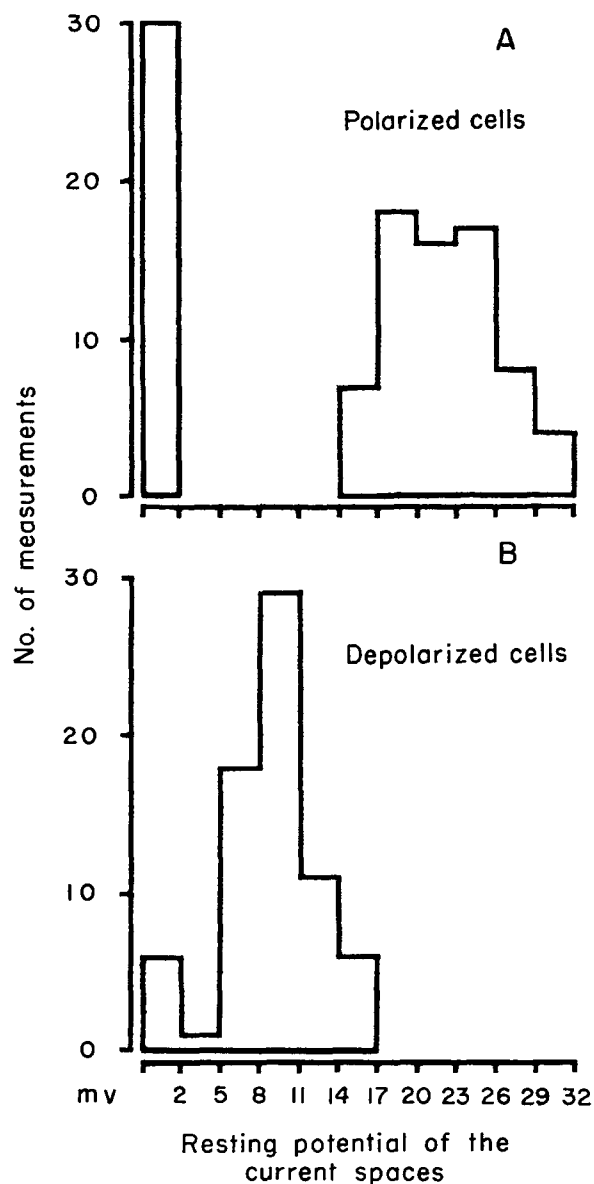


FIGURE 5. Histograms showing the distribution of the DC potentials recorded between the interior of the current-spaces and the bath electrode, in polarized cells (artificial perienteric fluid) A, and in depolarized cells (30% v/v sea water) B. See text.

only a fraction of these spaces are really at the same potential level as the bath solution. Histogram A represents the distribution of the DC potentials recorded from 100 current-spaces in 4 polarized cells. Only about one-third were isopotential with the bath solution. The interior of the other spaces showed potentials from 15 to 32 mv (inside negative).

The fact that a potential difference should exist between the interior of some spaces and the outside solution is not surprising, since the damage produced by the microelectrode to the boundary between space and cytoplasm would cause the flow of injury currents, and a voltage drop of the same polarity as the resting potential of the cytoplasm.

This hypothesis, however, is not supported by the fact that a wide gap (from 2 to 15 mv) exists in the histogram between polarized and nonpolarized spaces. Indeed, it would be difficult to explain why the flow of injury currents should not give rise to polarizations smaller than 15 mv. Furthermore, the potentials recorded from some spaces are actually larger than the resting potential of the cytoplasm, as illustrated in Fig. 6. The top records show the potential differences recorded between the microelectrode tip and the bath electrode before, during, and after impalement of a current-space with a potential about 4 mv higher than the resting potential of the cytoplasm. The effects of cell stimulation are seen on each trace.

Similar measurements in depolarized cells demonstrate, even more convincingly, that the polarization of the current-spaces is independent of the resting potential of the cytoplasm. Histogram B (Fig. 5) shows the distribution of the potentials of 71 spaces in 4 cells equilibrated with diluted sea water. Although the resting potential of the cytoplasm was lower than 5 mv the average potential of the current-spaces was 10.3 mv. As the histograms indicate, the spaces without a resting potential are more difficult to find than are polarized ones.

Each of the two lower records of Fig. 6 shows three traces: the zero base line, the resting potential of the cytoplasm, and the potential level of a current-space; the effects of cell stimulation are seen in the last two. The left side records show the impalement of a polarized space with a potential higher than that of the cytoplasm. The records at the right show the penetration into a nonpolarized space. For this reason, the currents are seen to be superimposed on the zero base line.

Although these results show that the potential of polarized spaces is not a consequence of the resting potential existing between the cytoplasm and the outside solution, histograms A and B (Fig. 5) show that current-spaces in polarized cells have higher average resting potentials than similar spaces in depolarized cells.

Such a difference could be explained in two different ways: either the potential of the spaces is influenced by the ionic composition of the external
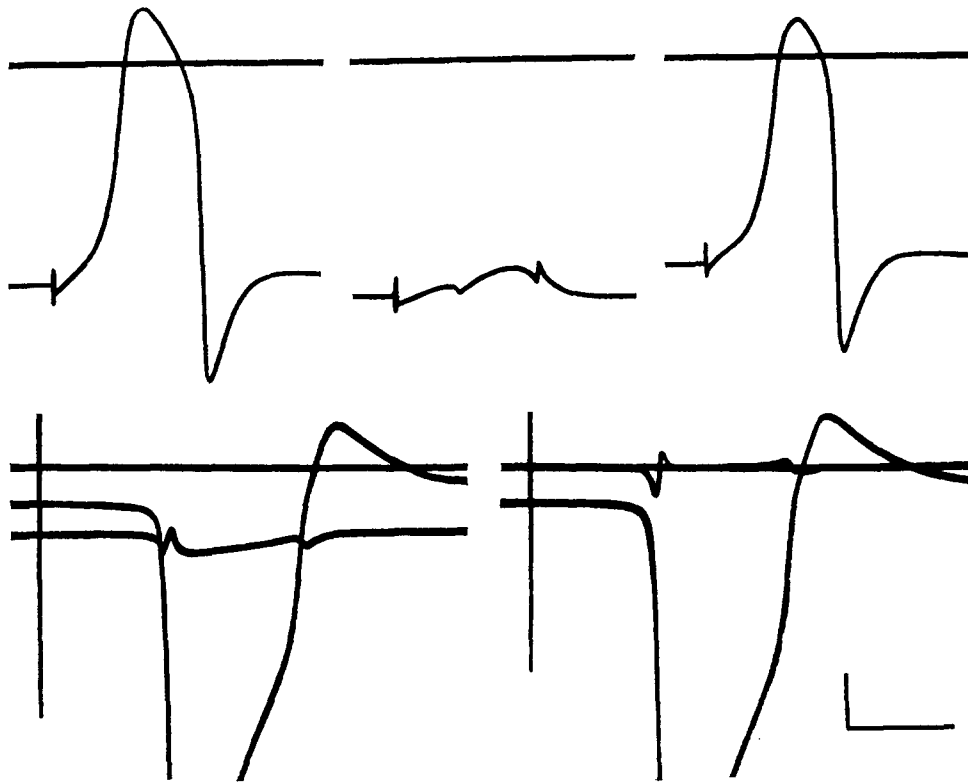
FIGURE 6.  *Upper records*, taken from a polarized cell, show from left to right the action potentials recorded before, during, and after crossing a polarized current-space with a microelectrode moving in an outward direction from the interior of the cell. Notice how the interior of the current-space is more negative than the cytoplasm with reference to the external remote bath electrode. The resting and action potentials at the record on the left are larger than those at the right as the microelectrode was more deeply and firmly inserted in the cytoplasm. The horizontal line through the three records is the zero base line. The *lower records* were obtained from a depolarized cell. Three traces were superimposed on each frame of film: the zero base line, the resting potential of the cytoplasm with an intracellular (negative) action potential, and the resting potential of a current-space, showing the extracellular transients which follow stimulation. In the left record the current-space was a polarized one; notice how its interior is more negative than the cytoplasm with reference to the outside solution. The current-space at the right is a nonpolarized one; the extracellular action potential appears superimposed on the zero base line. Notice also the difference between the shape of the extracellular transients recorded from the polarized and nonpolarized current-spaces. Calibrations, vertical, for both upper and lower records, 10 mv; horizontal, 200 msec for upper records, 100 msec for lower records.

solution, or the resting potential of the cytoplasm is reflected in the potential of the spaces. The second possibility seems to be the more likely one since it has been observed that when the membrane potential of the esophagus shifts
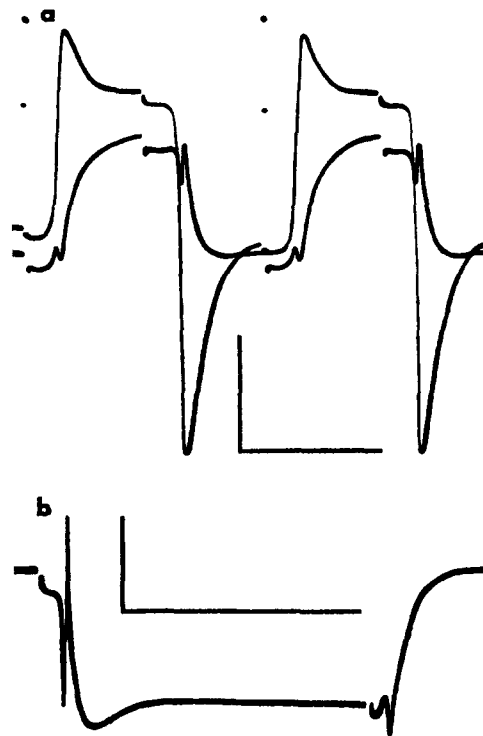


FIGURE 7.   The records in *a* were obtained, with two separate microelectrodes, from a cell being equilibrated with dilute sea water. One microelectrode tip was in the cytoplasm and the other in the interior of a polarized current-space. The resting potential of the cytoplasm shifted up and down upon stimulation, between the depolarized plateau and the reduced resting potential level (del Castillo and Morales, 1967, Fig. 15). The potential between the interior of the current-space and the external bath electrode is seen to follow the electrical changes across the cell surface membrane. Calibrations, vertical, 20 mv for the resting potential of the cytoplasm and 10 mv for the potential of the current-space. Horizontal, 0.5 sec. *b* illustrates in more detail the potential changes within a polarized current-space in another cell where the resting potential of the cytoplasm changed upon stimulation in the same fashion as in *a*. Vertical calibration, 4 mv; horizontal, 1 sec.

between two levels upon stimulation (del Castillo and Morales, 1967, Fig. 15), the potential of the polarized spaces tends to follow the resting potential of the cytoplasm (Fig. 7).

Polarized and nonpolarized current-spaces are often adjacent. It is possible sometimes to go back and forth from one type of space to the other by shifting the microelectrode slightly, without finding an interposed layer of cytoplasm.

4. *Relation between the Polarization of the Spaces and the Shape of the Current-Patterns*  Whereas some current-patterns look like amplified versions of the extracellular action potentials, those recorded from other spaces exhibit a different, although rather constant, shape. These two types are illustrated by records *c* and *c'* of Fig. 3

While in *c* the trace remains at approximately the base line level between the two diphasic deflections (cf. Figs. 2 *a* and 4 *a*), the pattern shown in *c'* exhibits a marked negativity during that period.

It soon became apparent that these two types of transients are associated with the DC potential recorded from the spaces. Thus, whereas the type seen in *c* is recorded from nonpolarized spaces, the development of negativity between the two diphasic deflections is observed, as a rule, in polarized current-spaces.

5. *Nature of the Membrane Binding the Current-Spaces*  The experiments described raise the question of the contribution of the membrane binding the current-spaces to the electrical activity of the cell; i.e., to the electromotive forces which cause the action currents to flow. We have no direct evidence on this problem; yet, we may consider two possible situations and see how their consequences would compare with actual observations.

The number, polarity, and shape of the transients recorded from current-spaces should be expected to reflect the properties of the surrounding membrane. If this is active, the currents flowing in its interior should closely resemble those recorded when the microtip is just outside the cell. On the contrary, if the spaces are lined by passive membrane, currents would flow only under the influence of electromotive forces developed at the surface of the cell. One should anticipate only a partial correspondence between intra- and extracellularly recorded action potentials.

As we have seen, a marked similarity exists between the extracellular action potentials and the transients recorded from nonpolarized current-spaces (cf. records *a* and *c*, Fig. 3). A closer comparison between both types of transients can be made by recording them simultaneously on a faster time base after equalizing their amplitudes on the CRO screen, as shown in Fig. 8, where the extracellular currents can be recognized by the greater thickness of the trace. The correspondence between both transients is extremely good, suggesting that the membrane separating the interior of the current-spaces from the cytoplasm is an electrically active one.

Although the over-all shape of the currents flowing in polarized spaces is different from the shape of those recorded from nonpolarized ones, the number and polarity of the main deflections are the same. One could surmise, therefore, that the membrane lining these spaces is, also, electrically active.

If the membrane surrounding the current-spaces were a passive one, i.e. if its electrical constants remained unchanged during the action potential,

it should be possible to reproduce the current-patterns recorded from the interior of the current-spaces by transforming the intra-cellular action potential with a passive circuit equivalent to the extracellular recording situ-
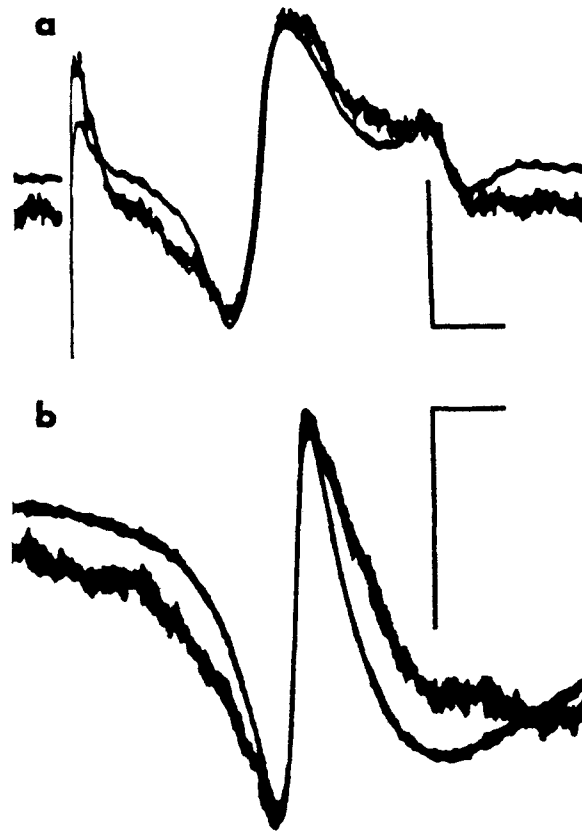


FIGURE 8. Transients simultaneously recorded with two microelectrodes from the same longitudinal level of the cell. In *a* one microelectrode was in a nonpolarized current-space within the cytoplasm and the other just outside the cell surface. In record *b* the tip of the intracytoplasmic micropipette was lodged within a polarized current-space; the other tip remained outside the cell. The amplitude of the signals in both beams was made equal by adjusting the gain of one of the recording channels. The true extracellular records can be recognized by the greater thickness of the tracings. Calibrations, *a*, vertical, 2 mv for the current-space record, and 200 $\mu$v for the external record; horizontal, 20 msec. *b*, vertical, 3 mv and 600 $\mu$v; horizontal, 10 msec.

ation, following the technique first employed by Freygang and Frank (1959). A circuit similar to that shown in their paper was employed in some of our experiments. However, the transients obtained in this manner, using a variety of values for the different components of the circuit, never matched the shape of those recorded from the interior of the spaces.

## DISCUSSION

The fact that extracellular action potentials are recorded with microelectrodes inserted into the esophagus of *Ascaris*, in an obviously "intracellular" position, demonstrates the existence, within this cell, of spaces which serve as a pathway for the flow of action currents into the depth of the cytoplasm.

It was suggested to us that the intracellular action currents might be recorded when the microelectrode tip is placed within the glandular cells. Indeed, if we assume that these cells have a low membrane resistance and are closely attached to the myoepithelial cells, their cytoplasm would behave as extracellular space for the contractile elements. Although this possibility cannot be ruled out, the size of the areas of glandular cytoplasm relative to the thickness of the esophageal wall is so large (see Fig. 1 of del Castillo and Morales, 1967) that one would expect to record action currents uninterruptedly as the microelectrode tip moves through wide regions of the cell, at least in some insertions. Instead, such action currents are consistently recorded from very shallow layers.

Preliminary observations with the electron microscope have shown the existence in the myoepithelial cells of both surface membrane invaginations and large membrane-bound spaces associated with the bundles of myofilaments (see Appendix) which provide a more likely morphological basis for our findings. Although the significance of these large spaces has not been elucidated it is tempting to assume that they are equivalent to those which in other muscle cells are known to couple the action potentials at the surface membrane with the contractile machinery.

Another problem is that of the structural relation between polarized and nonpolarized spaces. On the basis of the available evidence, it cannot be decided whether the polarized spaces are arranged in series or in parallel with the nonpolarized ones. The lack of a resting potential in the latter and the close similarity between the action potentials recorded from these spaces and those observed with microelectrodes placed outside the cell surface strongly suggest that such spaces are either invaginations of the surface membrane or, perhaps, interstices between the different cells which originally form the esophagus.

### APPENDIX

### L. R. OTERO-VILARDEBÓ

From the Department of Biochemistry, School of Medicine, San Juan, Puerto Rico

The electron micrograph reproduced in Fig. 9 illustrates the main structural features observed in the wall of the *Ascaris* esophagus. The tissue was fixed in 4 % formaldehyde in phosphate buffer (pH 7.0), postfixed in $OsO_4$ in 30 % (v/v) sea water, and

embedded in Araldite. Sections were cut on a Servall MT-2 microtome and stained for 15 min with lead citrate.

This section, parallel to the axis of the esophagus and close to its external surface, shows a narrow region of sarcoplasm wedged between two lobes of glandular cyto-
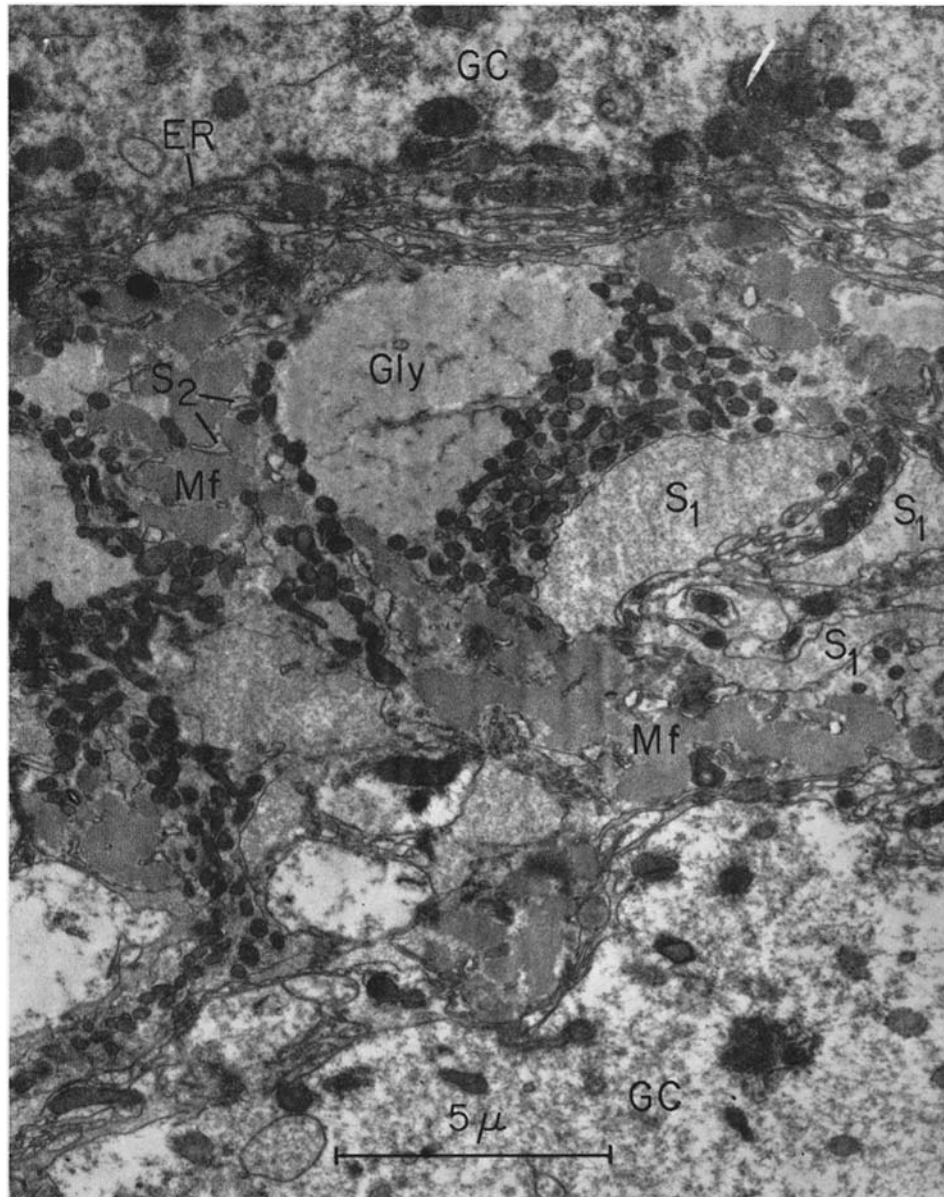


FIGURE 9

plasm (*GC*). The two types of cytoplasm are separated by a labyrinthine formation which results from the extensive interfolding of adjacent cell membranes. It is difficult to determine precisely the boundaries of a given cell and whether or not there exists cytoplasmic continuity between adjacent cells.

The glandular cytoplasm is distinguishable by the large amount of free, ribosome-like particles. At the peripheral regions, ribosomes are often found associated with a well organized endoplasmic reticulum (*ER*).

The sarcoplasm is characterized by myofibrils (*Mf*) (seen in cross-section in this figure) which at higher magnifications can be resolved into thin and thick myofilaments. Mitochondria are abundant and somewhat smaller than those of the gland cells. Some areas (*Gly*) continuous with the cytoplasmic matrix are relatively free of organelles and probably represent accumulations of glycogen.

A conspicuous feature of the sarcoplasm is the presence of membrane-bound spaces ($S_1$) as large as 10 $\times$ 10 $\mu$m in similar sections which may extend radially well over 20 $\mu$m. These spaces, which could easily accommodate the tip of a microelectrode, seem to be filled with a flocculent material and are lined by membranes free of ribosomes. Much smaller spaces ($S_2$), apparently continuous with the larger ($S_1$) ones, are also seen in close proximity to the myofibrils. Furthermore, sections through the external cuticle of the esophagus show the presence of invaginations of the surface membrane of the muscle cells, with a diameter up to 1 $\mu$m extending into the sarcoplasm.

As yet, it has not been established whether spaces $S_1$ and $S_2$ are or are not continuous with the invaginations of the surface membrane. Neither can we say whether these spaces are homologous to the transverse tubular system and sarcoplasmic reticulum of vertebrate skeletal muscle.

### REFERENCES

DEL CASTILLO, J., and T. MORALES. 1967. The electrical and mechanical activity of the esophageal cell of *Ascaris lumbricoides*. *J. Gen. Physiol.* **50**:603.

FREYGANG, W. H., and K. FRANK. 1959. Extracellular potentials from single spinal motoneurons. *J. Gen. Physiol.* **42**:749.

HÀKANSSON, C. H. 1957. Action potentials recorded intra- and extracellularly from the isolated frog muscle fibre in Ringer's solution and in air. *Acta Physiol. Scand.* **39**:291.