Influence of Some Ions on the Membrane Potential of *Ascaris* Muscle

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ABSTRACT The influence of several ions on the membrane potential of the somatic muscle of *Ascaris* has been investigated by changing their concentration in the surrounding solution. When $[K]_o$ is increased at the expense of $[Na]_o$ leaving $[Cl]_o$ constant, the membrane potential is first seen to increase. $[K]_o$ higher than 45 mM reduces the membrane potential with a slope of 23 mv for a tenfold change in $[K]_o$. However, when $[K]_o$ is increased keeping $[Na]_o$ and $[Cl]_o$ low and constant, the line relating the membrane potential with log $[K]_o$ has a slope of almost 50 mv. If $[Cl]_o$ is reduced in the absence of external Na, after the $[K]_o$ is increased to 45 mM, the membrane potential decreases with a slope of 59 mv per tenfold change in $[Cl]_o$ in close agreement with the Nernst equation. If Cl^- is replaced by SO_4^{2-}, a depolarization is produced, while chloride replacement by NO_3^-, Br^-, and I^- results in a hyperpolarization of the membrane. Removal of the external Na^+ ions increases the average membrane potential by 17 mv.

Microelectrode recordings from the somatic musculature of *Ascaris lumbricoides* reveal a relatively low resting potential interrupted by rhythmic graded spikes up to 45 to 50 mv in amplitude. These spikes are generated in a specialized region of the muscle cells in contact with the nerve cord. At this level, the terminal arborizations of the muscle cell *arms* interlace with one another forming a structure which exhibits functional properties similar to those of visceral smooth muscle of vertebrates.

The individual mononucleated, somatic muscle cells are electrically interconnected throughout that web by low impedance pathways; accordingly, this region of the muscle has been called the *syncytium* (DeBell, del Castillo, and Sanchez, 1963). Electron microscopy has shown areas in which the surface membranes of adjacent muscle arms come into close contact, with obliteration of the intervening extracellular space (Rosenbluth, 1963).

The membrane potential at the syncytium tends to oscillate showing waves of depolarization which, in turn, give rise to the graded spike potentials. These spikes are conducted both within the syncytium itself and away from it, along the muscle arms, to the contractile region of the muscle cells.

The work of Bülbring and her collaborators (*cf.* Holman, 1958; Burnstock and Straub, 1958; Kuriyama, 1963; Bülbring and Kuriyama, 1963 *a*, *b*) has shown that the ionic mechanisms involved in maintaining the membrane potential of visceral muscle differ quantitatively from those operating in vertebrate skeletal muscle. The contribution of Cl^- ions seems to be considerable and the surface membrane is highly permeable to extracellular sodium.

Therefore, it was interesting to see whether the functional resemblance between *Ascaris* muscle and vertebrate visceral muscle would also extend to the ionic mechanisms underlying the polarization of the cell surface membrane. This has been explored in the experiments described below. The influence of several ions on the membrane potential of *Ascaris* muscle was investigated by changing their concentration in the external solution. These experiments are interpreted from the viewpoint of the ionic theory, *i.e.* regarding the electrical potential difference between the cytoplasm and the outside solution as determined by the concentration gradients of K^+ and Cl^- ions across the cell surface membrane, as well as by the selective permeability of the membrane to these and other ions present.

Some of these results obtained were communicated in brief to the American Physiological Society (de Mello, del Castillo, and Morales, 1963).

METHODS

1. *Preparations* All the experiments were performed on specimens of *Ascaris lumbricoides*, var. *suum*. Fragments of the worms, 3 to 4 cm in length, were cut open along one of the lateral lines. After removal of the gut the tissue was pinned (cuticle side down) on a slab of teflon placed inside a double-walled Perspex chamber. See DeBell *et al.* (1963) for details on this technique.

2. *Solutions* Schopfer (1925) and Hobson, Stephenson, and Beadle (1952) showed that *Ascaris* tissues are approximately isotonic with a solution made by diluting 30 parts of sea water with 70 parts of distilled water. *Ascaris* preparations kept in this solution remain in good functional condition during reasonably long periods.

Since a certain amount of electrophysiological and pharmacological work has already been performed employing 30 per cent sea water (v/v) as extracellular solution (Jarman, 1959; DeBell *et al.*, 1963; del Castillo, de Mello, and Morales, 1963, 1964; del Castillo, Morales, and Sanchez, 1963), it seemed advisable to use the same as the "normal" or control saline for the present experiments.

Accordingly, an artificial sea water was prepared by mixing isotonic stock solutions of the different salts (*cf.* Hodgkin and Katz, 1949). When this artificial sea water was diluted to 30 per cent, a saline was obtained with the following ionic concentrations (mM): Na^+, 135; K^+, 3; Ca^{++}, 3; Mg^{++}, 15.7; Cl^-, 175.4, and HCO_3^-, 0.8.

Solutions with modified ionic content were also prepared. In some, the potassium was left out or increased at the expense of Na^+. In others, the concentration of Cl^- ions was reduced by replacing a part of the NaCl stock solution with an isosmotic solution of Na_2SO_4. Although *Ascaris* muscle is not very sensitive to a reduction in the external calcium concentration (del Castillo, Morales, and Sanchez, 1963), the presence of sufficient ionized calcium in solutions containing high concentrations of sulfate ions was insured by the addition of 8 mM of $CaSO_4$. The preparation of other modified solutions will be described below.

In a few experiments the membrane potential of the muscle cells was determined with the preparation immersed in the perienteric fluid from the same worm. To collect this fluid the worms were dropped from a height of about 12 inches onto a hard sur-

TABLE I

Comparison of the average membrane potential of somatic muscle cells of *Ascaris* in preparations surrounded by the perienteric fluid taken from the same worms (see text) and as measured after 30 minutes' immersion in artificial saline. Results are given in millivolts (± SE of the mean). Figures in parentheses show number of cells recorded.

Preparation	Perienteric fluid	30 per cent (v/v) artificial sea water
I	33.45 ± 0.99 (30)	34.16 ± 1.14 (30)
II	34.10 ± 1.17 (30)	34.20 ± 1.12 (30)
III	34.96 ± 0.91 (30)	32.13 ± 1.01 (30)
IV	35.60 ± 1.04 (30)	33.80 ± 1.15 (30)
Average membrane potential	34.5	33.5

face. This elicits a generalized muscle contraction which increases the internal hydrostatic pressure. If the caudal end of the worm is now sectioned, most of the perienteric fluid is ejected. Up to 1 ml can be collected from one single specimen, a sufficient amount to surround a preparation mounted in a small plastic container fitted inside the usual chamber. A thin layer of paraffin oil on the surface of the fluid was used to prevent evaporation.

According to Hobson, Stephenson, and Eden (1952) the ionic content of the perienteric fluid of *Ascaris* is (mM): Na^+, 129; K^+, 24.6; Ca^{++}, 5.9; Mg^{++}, 4.9; Cl^-, 52.7. The difference between the concentrations of chloride and total inorganic cations is largely due to the presence of volatile fatty acids of 1 to 6 carbon atoms.

3. *Electrical Recording* Muscle membrane potentials were measured at the nuclear bag or *belly*, largest and most accessible region of the cell, with conventional intracellular capillary electrodes. Before impalement of the cells, the preparations

were allowed to equilibrate for at least 15 minutes with the new surrounding solutions, which were renewed 2 or 3 times during this period. Only bellies close to the nerve cord were used.

The term "membrane potential" will be used in preference to "resting potential." In cells showing spike activity, membrane potential denotes the maximal level of membrane repolarization attained between the spikes.

All the experiments were performed with the bathing solution maintained at between 38° and 40°C by circulating warm water between the walls of the chamber.

4. *Statistical Procedure* The experimental results are given either in tables or as graphs. Each line in the figures represents the results obtained in a group of preparations each of which, with a few exceptions, was followed throughout the whole range of experimental conditions.

The initial point of each line represents a simple average of the pooled data from all the preparations. The standard errors attached to these initial points represent the pooled individual deviations from those averages.

To reduce scatter, the rest of the individual values for each line were transformed into deviations from the initial average membrane potentials of the individual preparations. From these deviations the average for each point and the corresponding standard error were calculated and plotted in the graphs in proper relation to the initial values.

RESULTS

1. *Comparison of the Membrane Potentials in Perienteric Fluid and Artificial Saline*

In preliminary experiments the average membrane potential of the muscle cells was determined with the preparation immersed in its own perienteric fluid and again after this fluid was replaced by artificial saline. The results obtained, see Table I, show no significant changes in spite of the different ionic composition of the solutions.

2. *Effect of Changing the External Potassium Concentration*

Fig. 1 shows two lines relating the average muscle membrane potential to the logarithm of the external potassium concentration.

Line *A* was obtained increasing $[K]_o$ at the expense of $[Na]_o$. In these experiments the relationship between $[K]_o$ and the membrane potential was found to be approximately linear for potassium concentrations higher than 45 mM. Between the concentrations of 3 and 35 mM the relation was not only nonlinear but the membrane potential increased instead of decreasing, when $[K]_o$ was raised.

Even when the potassium concentration is higher than 35 mM the slope of line *A* is considerably lower than expected from the Nernst equation. In fact, a tenfold increase in $[K]_o$, from 45 to 450 mM, results in a depolarization of only 23 mv instead of 61 mv (at 39°C). It should be emphasized that the

highest concentration of K could only be achieved by making the solution hypertonic.

The figures given in the legend of Fig. 1 show the changes in the concentration of Na^+ and Cl^- ions produced when $[K]_o$ is altered in the manner described. It can be seen that while $[Cl]_o$ remains constant up to a potassium

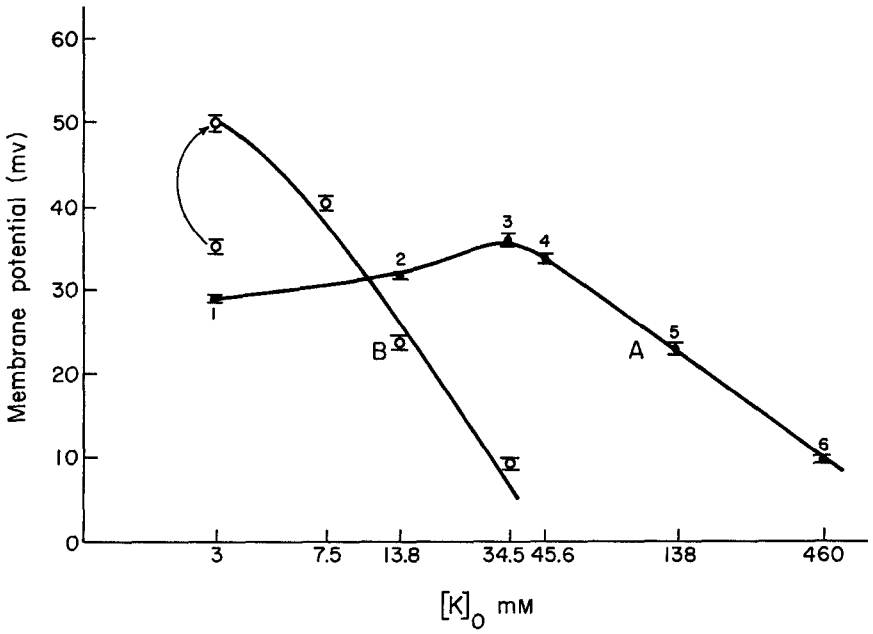

FIGURE 1. Influence of the extracellular potassium concentration (abscissae) on the membrane potential of *Ascaris* muscle cells (ordinates). Each point is based upon 60 cell impalements (three preparations). Vertical lines are 2× the SE. Deviations were measured from the average initial values appropriate to the individual preparations. Line *A* was obtained by replacing increasing amounts of NaCl by KCl (see text); the concentrations of sodium and chloride at each of the points are as follows (mM): *1*, Na 135.8, Cl 175.4; *2*, Na 125, Cl 175.4; *3*, Na 104.3, Cl 175.4; *4*, Na 93.2, Cl 175.4; *5*, Na 0.8, Cl 175.4; *6*, Na 2.7, Cl 584.6. In line *B* the potassium concentration was increased while keeping the concentrations of both Na and Cl low and constant (see text). Of the two points corresponding to a $[K]_o$ of 3 mM the lower one is the average membrane potential in the control solution (diluted artificial sea water) while the higher one is that determined after the NaCl stock was replaced by sucrose.

concentration of 138 mM, $[Na]_o$ decreases as $[K]_o$ increases, becoming practically zero at 138 mM K.

The increase in membrane potential when $[K]_o$ is raised from 3 to 35 mM could be explained by assuming a high conductance of the membrane to Na^+ ions. Furthermore, the comparatively small slope of the line in the region of low $[Na]_o$ could be accounted for if the contribution of Cl^- ions to the membrane potential was shown to be large.

To test these possibilities, the relationship between membrane potential and log $[K]_o$ was determined while the external concentrations of Na^+ and Cl^- ions were kept low and constant. This was done by preparing an artificial diluted sea water in which the NaCl stock solution was substituted by an equal volume of an isosmotic solution of sucrose. Thus, $[Na]_o$ was reduced to 0.8 mM, corresponding to the concentration of sodium bicarbonate, while $[Cl]_o$ was reduced from 175.4 to 40.4 mM; *i.e.*, that contributed by the K, Mg, and Ca chlorides. Increasing external concentrations of potassium were then obtained by replacing different amounts of the sucrose solution with equal volumes of an isosmotic K_2SO_4 solution.

The results obtained working with these solutions are shown by line *B* in Fig. 1. Of the two points corresponding to a $[K]_o$ of 3 mM, the lower one represents the average membrane potential in diluted artificial sea water, while the higher one is that measured after the preparations were equilibrated with the low Na and Cl solution described above. If the potassium concentration is now increased, it can be seen that the line relating membrane potential to log $[K]_o$ has only a small non-linear region, while its slope in the approximately linear region is nearly 50 mv per tenfold change in $[K]_o$. The difference between this value and the 61 mv slope predicted by theory can be accounted for by the relatively high concentration of chloride ions still present in the solution.

These results suggest that the internal resistance of the potassium battery is very high, since even when its E.M.F. is minimized by increasing $[K]_o$ above 45 mM (*cf.* line *B*, Fig. 1) the conductance of the membrane to K^+ ions is unable to short-circuit the E.M.F. of the chloride battery.

3. *Effects of Varying the External Concentration of Cl^- Ions*

The concentration of external Cl^- ions was changed in three different ways: (*a*) maintaining $[Na]_o$ and $[K]_o$ constant at their normal values, (*b*) in the absence of external Na but keeping the $[K]_o$ constant at its normal low value, and (*c*) in the absence of external Na but in the presence of a sufficiently high $[K]_o$ to minimize the contribution of the potassium battery.

Fig. 2 shows the results of experiments in which $[Cl]_o$ was altered in the presence of constant concentrations of Na^+ and K^+, as described under (*a*) above. The line relating the membrane potential to log $[Cl]_o$ has only a slope of 14 mv for a tenfold change in $[Cl]_o$.

Line *A* in Fig. 3 shows the relationship between the membrane potential and log $[Cl]_o$ in presence of low $[Na]_o$ (0.8 mM) and low $[K]_o$ (3 mM). Of the two values corresponding to 175 mM Cl the lower one (point *1*) represents the average membrane potential in normal diluted artificial sea water, while the higher one (point *2*) shows the membrane potential after removal of the sodium ions from the solution. This was achieved by replacing the NaCl stock by an equal volume of an isosmotic solution of choline chloride. Henceforth

$[Cl]_o$ was lowered by substituting increasing fractions of choline chloride for an isosmotic solution of sucrose. To prevent the activation of cholinergic synaptic receptors at the syncytium, atropine sulfate (10^{-3} w/v) was added to all the solutions containing choline chloride.

The average membrane potentials obtained in the solutions with reduced chloride content show a slope only slightly steeper than that of the line in Fig. 2, about 19 mv for a tenfold change in $[Cl]_o$. This relatively small change suggests that as the E.M.F. of the chloride battery is reduced by lowering $[Cl]_o$, the potassium battery gradually takes over control of the membrane potential.

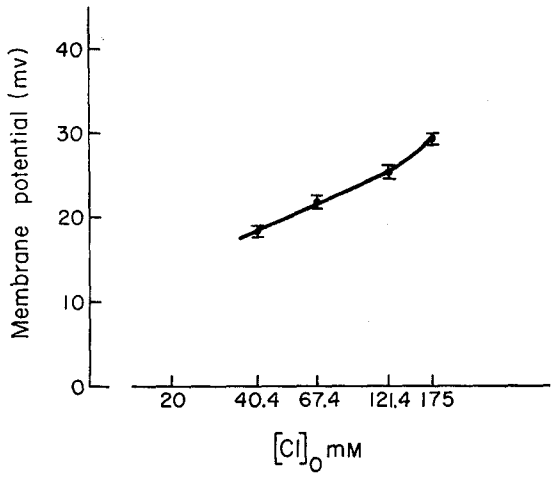

FIGURE 2. Line showing the influence of the external chloride concentration (abscissae) on the membrane potential of *Ascaris* muscle cells (ordinates). The $[Cl]_o$ was decreased while keeping the normal concentrations of Na^+ and K^+ ions constant. Each point is based upon 50 measurements in two preparations. Vertical lines are 2× the SE of the mean. Deviations were measured from the initial average values appropriate to the individual preparations at 175.4 mM Cl.

Line *B* in Fig. 3 shows the effects of reducing the external chloride concentration on the membrane potential after minimizing the E.M.F. of the potassium battery by increasing $[K]_o$. According to line *B* in Fig. 1 a concentration of 45 mM should be sufficient for this purpose. As described in the legend of Fig. 3, points *1* and *2* at a $[Cl]_o$ of 175.4 mM represent the pooled results from all the preparations, although only four of them were transferred from the "low Na–low K" solution (point *2*) to the "low Na–high K" solution (point *3*), a change which decreased the average membrane potential by just over 5 mv. Line *B*, relating the average membrane potential with log $[Cl]_o$ under these conditions, has a maximal slope of about 59 mv per tenfold change in the chloride concentration, a value in close agreement with that expected from

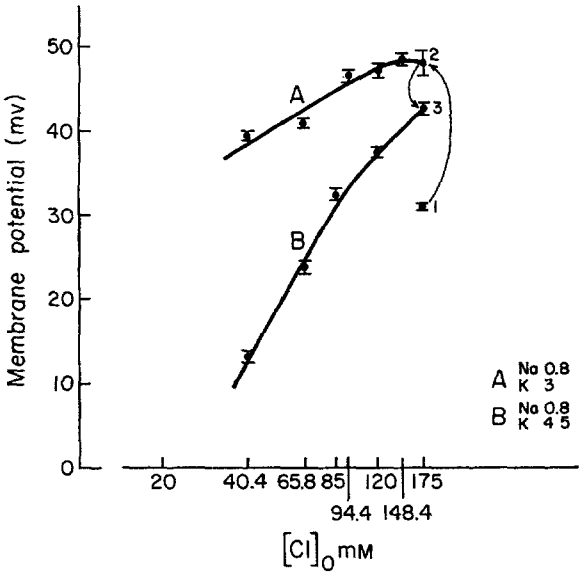

FIGURE 3. Influence of $[Cl]_o$ (abscissae) on the membrane potential of *Ascaris* muscle cells (ordinates). The extracellular concentration of chloride was changed in Na-free solutions containing 3 mM (line *A*) and 45 mM (line *B*) of potassium ions. Point *1*, at a $[Cl]_o$ of 175.4 mM, represents the average membrane potential of 140 impaled muscle cells in seven preparations immersed in artificial 30 per cent sea water (v/v). All the preparations were then transferred to a Na-free solution (see text) and the mean membrane potential was determined again in 140 cells (point *2*). Three of the preparations were then used to obtain line *A*, each point being based upon 60 measurements, while the four remaining preparations were employed for line *B*, each point being based on 80 measurements. Deviations for the values of line *A* were computed from the initial average values appropriate to the individual preparations (corresponding to point *2*), while those of line *B* were computed from the initial averages corresponding to point *3*. Vertical lines are 2× the SE of the mean.

the Nernst equation. These results fully confirm those shown in Fig. 1 and also indicate a low membrane conductance to potassium ions.

4. *Effect of Replacing the External Chloride by Foreign Inorganic Anions*

Since Cl^- ions appeared to play such an important role in the maintenance of the membrane potential of *Ascaris* muscle, it was interesting to test the influence of inorganic anions not normally present in the perienteric fluid. Kuriyama (1963) working with mammalian visceral muscle observed the occurrence of a depolarization when the extracellular Cl^- ions were substituted by either SO_4^{2-}, Br^-, NO_3^-, or I^-. The reduction in membrane potential was maximal with SO_4^{2-} decreasing in the order these ions have been listed.

The effects of anion replacement in our preparations are shown in Table II.

TABLE II

Changes in the average membrane potential of *Ascaris* muscle cells following partial replacement of extracellular chloride with foreign anions. Control solution, 30 per cent sea water (v/v), which contained 175 mM Cl; see Table I and text. Test solutions contained about 40 mM of chloride and 135 mM of either SO_4^{2-}, NO_3^-, I^-, or Br^-. Figures are given in millivolts (± SE of the mean). Figures in parentheses indicate number of cells impaled.

Exp.	Foreign anion	Control solution	Test solution	Difference	
1		25.20 ± 0.87 (20)	12.00 ± 0.47 (20)		−13.20
2	SO_4^{2-}	38.40 ± 1.08 (20)	22.05 ± 1.33 (20)		−16.35
3		32.20 ± 0.91 (30)	22.66 ± 0.96 (30)		−9.54
				Average	−13.03
1		26.75 ± 0.97 (20)	36.80 ± 1.07 (20)		+10.05
2	Br^-	31.00 ± 0.84 (20)	39.15 ± 1.15 (20)		+8.15
3		35.90 ± 1.01 (20)	43.10 ± 1.08 (20)		+7.20
				Average	+8.47
1		33.50 ± 1.11 (20)	45.00 ± 1.58 (20)		+11.50
2	I^-	30.55 ± 1.01 (20)	44.80 ± 1.40 (20)		+14.25
3		33.50 ± 0.79 (20)	44.40 ± 1.11 (20)		+10.90
				Average	+12.22
1		29.15 ± 0.53 (20)	52.55 ± 1.53 (20)		+23.40
2	NO_3^-	42.25 ± 1.28 (20)	61.50 ± 1.62 (20)		+19.25
3		24.65 ± 0.81 (20)	49.90 ± 2.03 (20)		+25.25
				Average	+22.63

The membrane potential decreased *only* when chloride was substituted by SO_4^{2-}, showing instead an increase in the presence of Br^-, I^-, and NO_3^-. These results suggest that these three anions permeate the muscle membrane more readily than chloride. Hyperpolarization was maximal, with an average value of 22.6 mv, with NO_3^-. Control experiments, not included in Table II showed that these effects were fully reversible.

5. *Effects of Removing the Extracellular Sodium Ions*

As described in preceding sections 2 and 3, removal of the extracellular Na^+ ions results in a hyperpolarization of *Ascaris* muscle membrane. The increase in membrane potential was maximal when the NaCl stock was replaced by choline chloride, thus leaving $[Cl]_o$ unchanged. In the experiments summarized in Fig. 3, the average membrane potential increased from 30.8 mv to 47.9 mv. The largest hyperpolarization observed in any one preparation was about 30 mv.

In experiments in which the NaCl stock was replaced by sucrose, the average hyperpolarization amounted to just over 10 mv. This value is an average from six preparations including those used to plot line *B* in Fig. 1. The decreased hyperpolarization can be accounted for by the simultaneous reduction of $[Cl]_o$.

DISCUSSION

The results of the experiments described previously resemble those obtained in mammalian visceral muscle. A chloride battery with a low internal resistance seems to be largely responsible for the maintenance of the membrane potential in *Ascaris* muscle, whereas the contribution of potassium appears to be limited by a low membrane conductance to these ions. As shown in Fig. 3 an increase of $[K]_o$ from 3 to 45 mM in the presence of the normal $[Cl]_o$ reduces the average membrane potential by only 5 mv. Nonetheless, when $[Cl]_o$ is low, a similar increment in the extracellular concentration of K^+ ions causes an almost complete depolarization of the membrane.

Both K and Cl batteries are shunted by a relatively large conductance to sodium ions. This seems to be a characteristic feature of cells which exhibit autorhythmicity and has also been observed in vertebrate heart (*e.g.* Trautwein and Kassebaum, 1961).

An interesting qualitative difference between *Ascaris* muscle and visceral smooth muscle is that concerning the effect of different foreign inorganic anions on the membrane potential.

The experiments summarized in Table I show that the organic compounds which represent about two-thirds of all the extracellular anions in intact *Ascaris* muscle, can be replaced by Cl^- ions without changing significantly the membrane potential. It is likely, therefore, that such organic anions play an important role in maintaining the polarization of the cell membrane. Changes in the permeability of the membrane to these ions are also believed to be involved in the paralyzing effect of piperazine on *Ascaris* muscle (del Castillo *et al.*, 1964).

Although the present experimental results appear rather clear-cut, one

should not overlook the fact that the surface membrane of *Ascaris* muscle cells is not a homogeneous one, since the ability to generate spontaneous spike potentials is restricted to the syncytial membrane. The ionic permeabilities of this region are likely to be different from those of the rest of the cell membrane. Inasmuch as our results were obtained from nuclear bags close to the nerve cord, they should be regarded as an average of the over-all properties of the cell membrane.

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REFERENCES

BÜLBRING, E., and KURIYAMA H., 1963*a*, Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli, *J. Physiol.*, **166**, 29.

BÜLBRING, E., and KURIYAMA, H., 1963*b*, Effects of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea-pig taenia coli, *J. Physiol.*, **166**, 59.

BURNSTOCK, G., and STRAUB, R. W., 1958, A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes, *J. Physiol.*, **140**, 156.

DEBELL, J., DEL CASTILLO, J., and SANCHEZ, V., 1963, Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*, *J. Cell. and Comp. Physiol.*, **62**, 159.

DEL CASTILLO, J., DE MELLO, W. C., and MORALES, T., 1963, The physiological role of acetylcholine in the neuromuscular system of *Ascaris lumbricoides*, *Arch. Internat. Physiol.*, **71**, 741.

DEL CASTILLO, J., DE MELLO, W. C., and MORALES, T., 1964, Mechanism of the paralyzing action of piperazine on *Ascaris* muscle, *Brit. J. Pharmacol.*, **22**, 463.

DEL CASTILLO, J., MORALES, T., and SANCHEZ, V., 1963, Action of piperazine on the neuromuscular system of *Ascaris lumbricoides*, *Nature*, **200**, 706.

DE MELLO, W. C., DEL CASTILLO, J., and MORALES, T., 1963, Ionic aspects of electrogenesis in the somatic muscle cells of *Ascaris lumbricoides*, *The Physiologist*, **6**, 167.

HOBSON, A. D., STEPHENSON, W., and BEADLE, L. C., 1952, Studies in the physiology of *Ascaris lumbricoides*. I. The relation of the total osmotic pressure, conductivity and chloride content of the body fluid to that of the external environment, *J. Exp. Biol.*, **29**, 1.

HOBSON, A. D., STEPHENSON, W., and EDEN, A., 1952, Studies in the physiology of *Ascaris lumbricoides*. II. The inorganic composition of the body fluid in relation to that of the environment, *J. Exp. Biol.*, **29**, 22.

HODGKIN, A. L., and KATZ, B., 1949, The effect of sodium ions on the electrical activity of the giant axon of the squid, *J. Physiol.*, **108**, 37.

HOLMAN, M. E., 1958, Membrane potentials recorded with high-resistance microelectrodes; and the effects of changes in ionic environment on the electrical and

mechanical activity of the smooth muscle of the taenia coli of the guinea-pig, *J. Physiol.*, **141**, 464.

JARMAN, M., 1959, Electrical activity in the muscle cells of *Ascaris lumbricoides*, *Nature*, **184**, 1244.

KURIYAMA, H., 1963, The influence of potassium, sodium and chloride on the membrane potential of the smooth muscle of taenia coli, *J. Physiol.*, **166**, 15.

ROSENBLUTH, J., 1963, Fine structure of body muscle cells and neuromuscular junctions in *Ascaris lumbricoides*, *J. Cell. Biol.*, **19**, 82A.

SCHOPFER, W. H., 1925, Recherches sur la concentration moleculaire des suc des parasites, *Parasitology*, **17**, 221.

TRAUTWEIN, W., and KASSEBAUM, D. G., 1961, On the mechanism of spontaneous impulse generation in the pacemaker of the heart, *J. Gen. Physiol.*, **45**, 317.