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# THE RESTING MEMBRANE POTENTIAL OF THE SOMATIC MUSCLE CELLS OF *ASCARIS LUMBRICOIDES*

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SUMMARY

1. The resting membrane potential of *Ascaris* muscle fibres, which is normally relatively insensitive to ion changes in the medium, has been measured under a wide variety of conditions.

2. The results have been interpreted in terms of a form of the constant field equation containing additional terms for the contribution of ions and charged groups other than potassium, sodium and chloride.

3. Normally the contribution of the additional terms is large and tends to outweigh the contributions of potassium, sodium and chloride.

4. The contribution of the additional terms is considerably reduced in the absence of sodium and in the presence of $\gamma$-amino butyric acid and acetylcholine.

5. It is suggested that the additional terms may represent the contribution of an electrogenic active transport mechanism to the factors determining the membrane potential.

## INTRODUCTION

The resting membrane potential of the somatic muscle cells of the pig roundworm, *Ascaris lumbricoides*, appears to be remarkably insensitive to changes in the concentrations of ions in the bathing medium (Brading & Caldwell, 1964; del Castillo, de Mello & Morales, 1964*a*). The insensitivity of the potential to the extracellular concentration of potassium ions is perhaps its most remarkable feature. In saline media with compositions based on *Ascaris* haemolymph the resting potential of about $-30$ mV has been found to decrease by only 1·5 mV when the potassium concentration in the media is increased tenfold (Brading & Caldwell, 1964), this very small change being in marked contrast to the decreases of up to 40–50 mV

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usually found in the resting potentials of excitable tissues under these conditions (see, for example, Curtis & Cole, 1942; Fatt & Katz, 1953; Adrian, 1956). The resting potential of *Ascaris* muscle was found to be more sensitive to changes in the extracellular chloride but even in this case the increases observed in the potential when the extracellular chloride concentration was increased were equivalent to an increase of only 13 mV for a tenfold increase in the chloride.

Various rather tentative suggestions have been put forward to account for the insensitivity of the resting potential of the *Ascaris* muscle membrane to ion changes. These include the possibility that compensatory changes in the permeability of the membrane towards ions occur which counteract the effects of the changes in ionic concentration (Brading & Caldwell, 1964) the operation of a sodium ion shunt (del Castillo *et al.* 1964*a*) and the operation of an electrogenic pump (Brading, 1965). In the work described in this paper, which includes new data on the effects of ion changes on the membrane potential of *Ascaris* muscle, a quantitative approach to this insensitivity based on the Goldman constant field equation (Goldman, 1943; Hodgkin & Katz, 1949) has been tried. One of the reasons for the adoption of this approach has been that work on the exchange of labelled ions in *Ascaris* muscle (Caldwell & Ellory, 1968) has indicated that the insensitivity of the membrane potential to ion changes is not due to the occurrence of compensatory changes in membrane permeability.

## METHODS

### *Materials*

Worms were collected at a slaughter house and were taken directly from the small intestine of the pig and placed in a thermos of warm medium. They were used on the day of collection and the following day. The muscle cells of the central field only were used. These were exposed by slitting the worm open anterior to the vulva, along the dorsal nerve cord, and pinning the animal through the two halves of the dorsal muscle field thus leaving the ventral field undamaged. The intestine was removed by peeling it off with fine forceps. This exposes the belly region of the muscle field. The preparation was submerged in a medium at a constant temperature of 37° C.

### *Solutions and drugs*

Stock solutions were kept of the compounds needed and were diluted in the correct proportions when required. The composition (in mM) of the normal medium (new medium of Brading & Caldwell, 1964) was: $Na^+$ 130, $K^+$ 20, $Mg^{2+}$ 10, $Ca^{2+}$ 6, $Cl^-$ 54, $SO_4^{2-}$ 35, $CH_3COO^-$ 58, sucrose 82. Experimental media were made up in which only one of the ions, $K^+$, $Na^+$, $Ca^{2+}$ or $Cl^-$ was altered, with compensatory changes in $Mg^{2+}$, $SO_4^{2-}$, acetate and sucrose to maintain the ionic balance and total molarity constant. It had previously been found that increasing the concentrations of $MgSO_4$ or magnesium acetate had no significant effect on the resting potential, indicating that the terms for these ions in the constant field equation must be very small.

γ-Amino butyric acid (GABA) was obtained as a solid from British Drug Houses and was made up in distilled water as a stock solution of $5 \times 10^{-2}$ M. This was diluted to $5 \times 10^{-5}$ M with the desired solution. Acetylcholine was made up daily from capsules containing 100 mg acetylcholine chloride from Roche Products. The contents were dissolved in 100 ml. distilled water and diluted to $10^{-5}$ (w/v) with the required solution. Adrenaline was supplied by British Drug Houses as a $10^{-3}$ (w/v) solution and was diluted with the desired solution. Nicotine was supplied by British Drug Houses.

*Micro-electrodes*

Micro-electrodes were pulled from 1 mm thin walled Pyrex glass tubing with a Palmer micro-electrode puller. They were filled with approx. 3 M-KCl. The majority of electrodes had a resistance of 2–14 MΩ and were probably a little under 1 μ in external diameter at the tip.

*Measurement of resting potential*

The membrane potential of the muscle cells was usually recorded in the nuclear bag or bulge region which is about 300 μ in diameter. The location of this region is shown in Fig. 1 which shows the structure of a typical *Ascaris* muscle cell.

For the purpose of these experiments the resting potential is defined as that potential recorded across the cell membrane with a Vibron electrometer (which does not respond to the fast changes in potential occurring with the spontaneous depolarizations). The resting potential was first determined by penetration of ten cells in a standard solution.

For each experimental solution ten cells were penetrated in the new solution, and then ten further cells after returning to the standard solution. Never more than three different media were used on the same worm, and for each solution results from four or five worms were used. Each set of ten readings were averaged and if the two standard solutions gave similar means, the difference between the mean of the standard solution and the mean of the experimental results was found and plotted as a single point of a graph. Increases in the resting potential, which is negative, have been plotted in the Figures as positive changes while decreases have been plotted as negative changes. Unless otherwise stated the changes in potential plotted in the Figures are relative to the potential in the normal medium.

*Measurements of internal ionic concentrations*

Worms used for these estimations were collected 'dry' in a heated thermos, and thus did not come in contact with any artificial media. They were opened and pinned out on a cork sheet, and the exposed bulges were washed three times in isotonic sucrose at 38° C, and blotted dry as quickly as possible, to remove any traces of haemolymph. A large number of the bulges were dissected off using fine forceps and placed on a weighed slide in a Petri dish covered with a damp filter paper to reduce loss of weight by evaporation. The slide was reweighed to determine the weight of muscle and the bulges thus dissected were used to estimate Na, K and Cl concentrations and also to estimate the amount of cell water. Na and K were determined by extraction in distilled water and flame photometer analysis. Chloride was determined by Conway's method and also by the silver iodate method described by Milton & Waters (1949). Dry weight was estimated by drying to constant weight at 50° C and the sucrose solution adhering after washing was estimated with $^{14}C$ sucrose.

*Theoretical treatment of results*

If the constant field theory as outlined by Goldman (1943) and Hodgkin & Katz (1949) is applicable to *Ascaris* muscle fibres, then the following equation should give the resting potential, $E$

$$E = \frac{RT}{F} \operatorname{Log}_e \frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i + x}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o + y} \tag{1a}$$

$$= \frac{RT}{F} \operatorname{Log}_e \frac{\theta}{\phi} \tag{1b}$$

where the terms $p_K[K]_o$, etc. are products of permeability constants and ionic concentrations and $x$ and $y$ represent the contributions of ions other than $K^+$, $Na^+$ and $Cl^-$. Here $x$ and $y$ are usually taken to be constant under a given set of conditions.

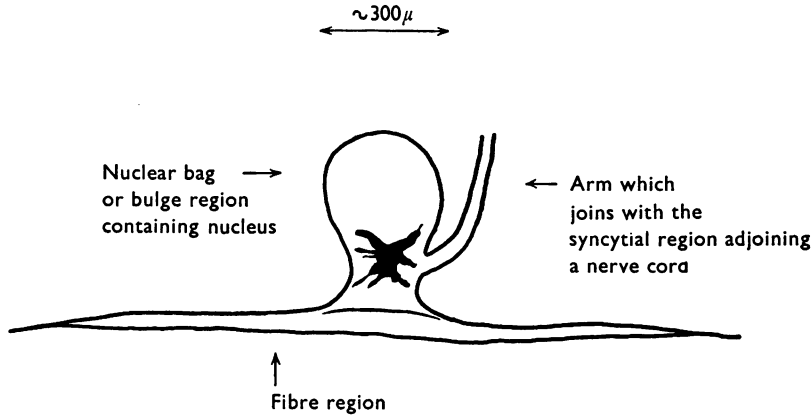


Fig. 1. Diagram to illustrate the structure of a typical *Ascaris* muscle cell. The membrane potential was normally recorded by penetration of the nuclear bag or bulge region containing the nucleus.

If eqn. (1$a$) is differentiated partially with respect to $[K]_o$, $[Na]_o$ and $[Cl]_o$, the following equations are obtained

$$\frac{\partial E}{\partial [K]_o} = \frac{RT}{F} \frac{p_K}{\theta}, \tag{2}$$

$$\frac{\partial E}{\partial [Na]_o} = \frac{RT}{F} \frac{p_{Na}}{\theta}, \tag{3}$$

$$\frac{\partial E}{\partial [Cl]_o} = \frac{RT}{F} \frac{p_{Cl}}{\phi}, \tag{4}$$

If $p_K$ is taken as 1 (cp. Hodgkin & Katz, 1949) and if particular values of $[K]_o$, $[Na]_o$ and $[Cl]_o$ are taken, then a value for $\theta$ can be calculated from the variation of $E$ with $[K]_o$ around the particular value of the latter by means of eqn. (2). $p_{Na}$ can then be calculated from the value for $\theta$ and the variation of $E$ with $[Na]_o$ around the particular value of the latter by means of eqn. (3). A value can be obtained for $\phi$ from the value of $\theta$ and the value of $E$ at the particular values of $[K]_o$, $[Na]_o$ and $[Cl]_o$ by

means of eqn. (1*b*). A value can then be obtained for $p_{Cl}$ from the value of $\phi$ and the variation of $E$ with $[Cl]_o$ around the particular value of $[Cl]_o$ by means of eqn. (4). Finally values of $x$ and $y$ can be calculated from the values of $\theta$, $\phi$, the permeability constants and the intracellular and extracellular concentrations of $K^+$, $Na^+$ and $Cl^-$.

Values of $p_{Na}$, $p_{Cl}$, $x$ and $y$ have been calculated in this way from the experimental data which have been obtained. These values (including $p_K = 1$) have been inserted into eqn. (1*a*) to see how far this approach can provide an explanation of the comparatively small changes in resting potential with ion concentrations which have been found with *Ascaris* muscle.

## RESULTS

### *Estimations of internal ion concentrations*

The concentrations of potassium, sodium and chloride in the muscle cells were calculated as millimoles per litre of cell water, having been corrected for the effects of extracellular fluid adhering after blotting and for

TABLE 1. Concentrations of ions in muscle cells from *Ascaris lumbricoides*

| Ion | Mean concentration (mM) | S.E. | No. of determinations |
|---|---|---|---|
| $K^+$ | 99·4 | 2·8 | 27 |
| $Na^+$ | 48·6 | 2·4 | 27 |
| $Cl^-$ | 13·7 | 1·0 | 20 |

the dry weight. The average results are summarized in Table 1. For convenience the values 99, 49 and 13 have been used for these concentrations of potassium, sodium and chloride in the calculations.

### *Effects of changes in the extracellular potassium concentration*

The mean resting potential in the normal medium ($[K]_o$ = 20 mM) is negative and was found from observations on 500 cells (ten cells in each of fifty animals) to be 32·85 ± 0·13 mV. Fig. 2 shows the changes in this resting potential which were observed when the extracellular potassium concentration in the medium was altered from that in the normal medium. These changes are very small indeed and illustrate the insensitivity of the membrane of *Ascaris* muscle to changes in the extracellular potassium concentration. In the region of $[K]_o$ = 20 mM, $\partial E/\partial[K]_o$ is found to be about 0·05 mV/mM. If this value is substituted into eqn. (2) and $p_K$ taken as 1, a value of about 530 is obtained for $\theta$. If this value for $\theta$ is taken and substituted into eqn. (1*b*) and the normal resting potential is taken as 33 mV, then a value of about 1830 is obtained for $\phi$.

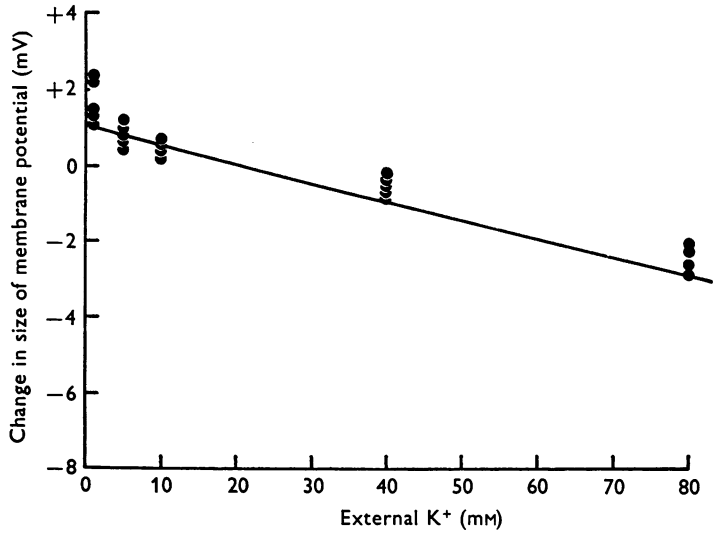


Fig. 2. Changes in membrane potential brought about by variations in external potassium concentration. The continuous line has been calculated from eqn. (1*a*) with $p_K = 1$, $p_{Na} = 1$, $p_{Cl} = 7$, $x = 290$, $y = 1300$.

*Effects of changes in the extracellular sodium concentration*

Fig. 3 shows the changes observed when the extracellular sodium concentration was altered from that in the normal medium (130 mM). The changes are small and similar to those observed when the extracellular potassium is altered, the value obtained for $\partial E/\partial[Na]_o$ near $[Na]_o = 130$ mM being about 0·05 mV/mM. Substitution of this value and the value of 530 for $\theta$ into eqn. (3) leads to a value of 1 for $p_{Na}$.

*Effects of changes in the extracellular chloride concentration*

Fig. 4 shows the changes observed when the extracellular chloride concentration was altered from that in the normal medium (54 mM). The changes correspond to a change of about 12·3 mV for a tenfold increase in chloride, agreeing well with the 14 mV found by del Castillo, de Mello & Morales (1964*a*). The value of $\partial E/\partial[Cl]_o$ in the region where $[Cl]_o = 54$ mM is about $-0{\cdot}1$ mV/mM and substitution of this value into eqn. (4) together with the value of 1830 for $\phi$ gives a value of about 7 for $p_{Cl}$.

Estimates can now be made of $x$ and $y$ and a value of about 290 is obtained for $x$ and a value of about 1300 for $y$. These quantities are large and $y$ is considerably larger than the largest product of permeability constant and concentration for a known ion ($p_{Cl}[Cl]_o = 378$). Consideration of the possible significance of these large values of $x$ and $y$ is deferred until

the Discussion. Substitution of these values of $x$ and $y$ into eqn. (1$a$), together with the permeability constants and concentrations of the known ions, enables calculations to be made of the resting potentials under the experimental conditions used in Figs. 2, 3 and 4. The calculated changes in resting potential are shown as continuous lines in these Figures and it will be seen that there is a good agreement with the experimental results.

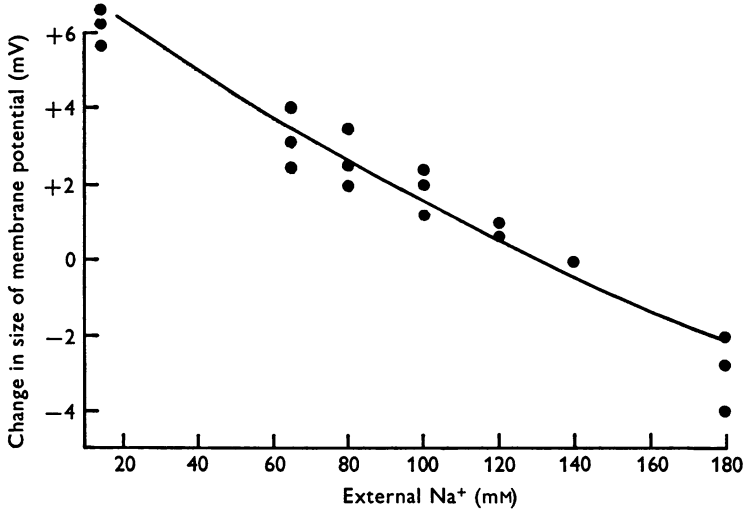


Fig. 3. Changes in membrane potential brought about by variations in external sodium concentration. The continuous line has been calculated for the same conditions as in Fig. 2.

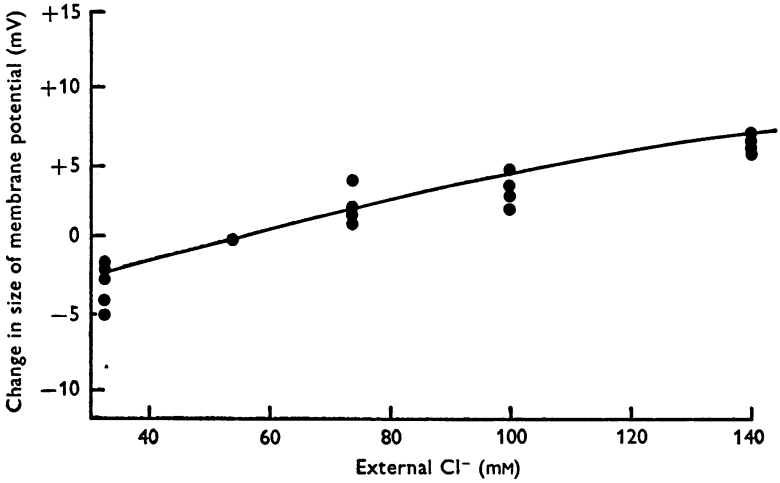


Fig. 4. Changes in membrane potential brought about by variations in external chloride concentration. The continuous line has been calculated for the same conditions as in Fig. 2.

*Effects of changes in extracellular potassium concentration in the presence of low extracellular chloride and of the changes in extracellular chloride in the presence of high extracellular potassium*

The changes in resting potential with extracellular potassium in the presence of a lower extracellular chloride concentration of 32 mM were investigated and the results are shown in Fig. 5. The changes in resting potential with extracellular chloride in the presence of a higher extracellular potassium concentration of 45 mM were also investigated and the

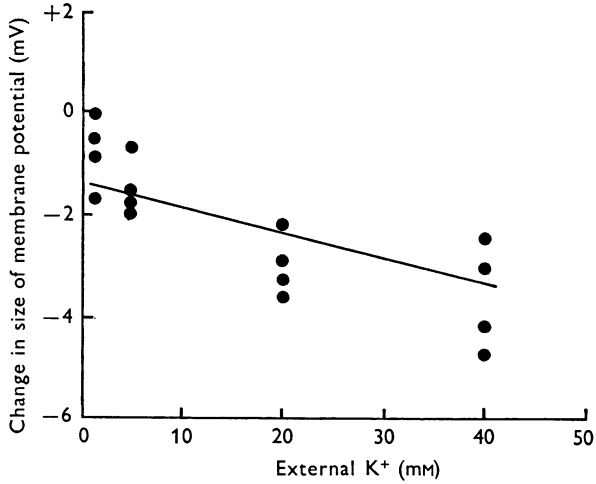


Fig. 5. Changes in membrane potential brought about by variations of external potassium at a low external chloride concentration (32 mM). The continuous line has been calculated for the same conditions as in Fig. 2.

results are shown in Fig. 6. In both cases theoretical values for the changes have been calculated with the values for the permeability constants and for $x$ and $y$ derived previously. These are shown as continuous lines in Figs. 5 and 6 and agree well with the experimental values.

*Effects of changes in extracellular potassium and chloride concentrations in sodium free media*

The changes found in resting potential with extracellular potassium concentration in the absence of extracellular sodium and in 32 mM extracellular chloride are shown in Fig. 7 (relative to the membrane potential when $[K]_o$ = 45 mM) and the changes found with extracellular chloride concentration in the absence of sodium and in 45 mM potassium are shown in Fig. 8 (relative to the membrane potential when $[Cl]_o$ = 32 mM). The theoretical values for these potential changes calculated using the

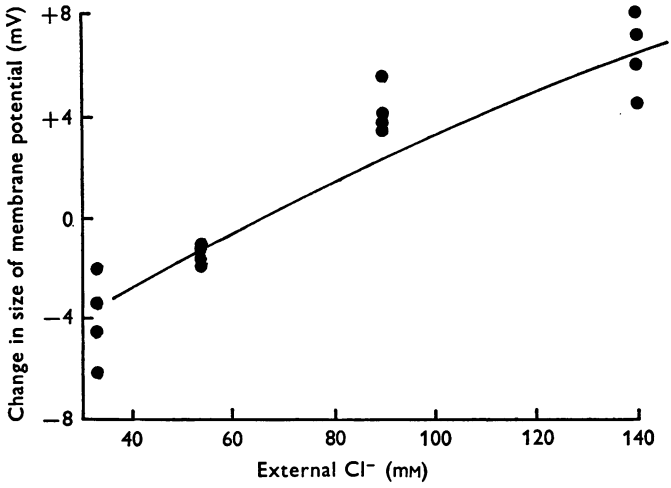


Fig. 6. Changes in membrane potential brought about by variations in external chloride at a high external potassium concentration (45 mM). The continuous line has been calculated for the same conditions as in Fig. 2.

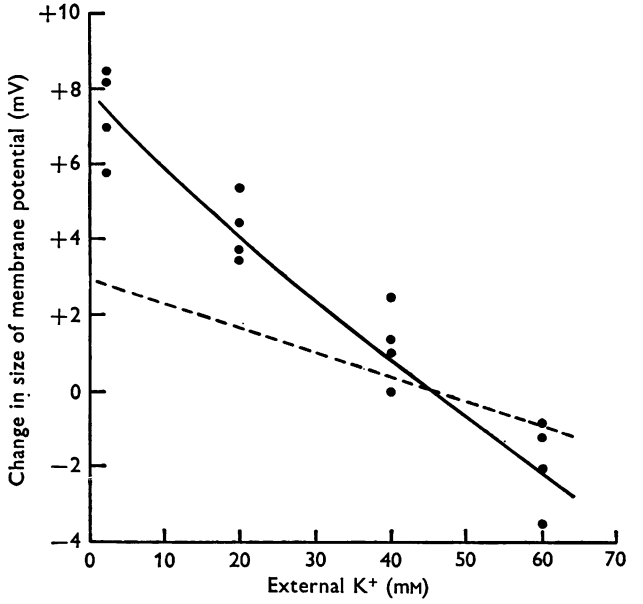


Fig. 7. Changes in the membrane potential (relative to $[K]_o = 45$ mM) brought about by variations in external potassium in the absence of sodium at a low external chloride concentration (32 mM). The dashed line has been calculated for the same conditions as in Fig. 2. The continuous line has been calculated from eqn. (1*a*) for $p_K = 1, p_{Na} = 1, p_{Cl} = 6{\cdot}6, x = 45, y = 224$.

same values for the permeability constants and $x$ and $y$ used previously are shown as dashed lines in both Figures and it will be seen that the slopes of the theoretical curves do not agree very well with the experimental values. In addition the resting potential is calculated to be about 36·5 mV when $[K]_o$ = 45 mM and $[Cl]_o$ = 32 mM, whereas the observed values are in the region of 32 mV. An analysis of the curves by the methods already

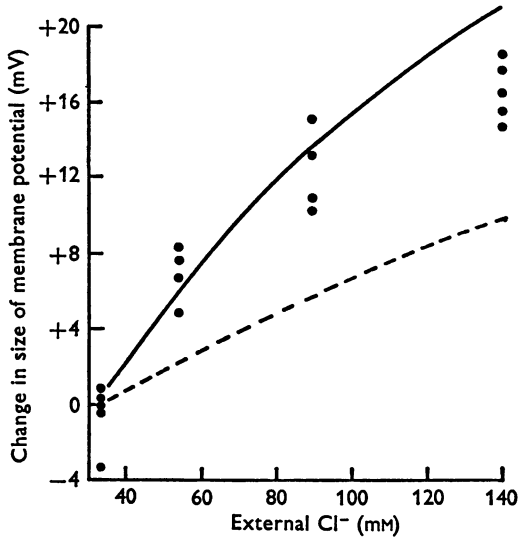


Fig. 8. Changes in the membrane potential (relative to $[Cl]_o$ = 32 mM) brought about by variations in external chloride in the absence of sodium at a high external potassium concentration (45 mM). The dashed line has been calculated for the same conditions as in Fig. 2. The continuous line has been calculated from eqn. (1*a*) for $p_K = 1$, $p_{Na} = 1$, $p_{Cl} = 6{\cdot}6$, $x = 45$, $y = 224$.

described for Figs. 2, 3 and 4 was carried out. In Fig. 7 the value of $\partial E/\partial[K]_o$ in the region of $[K]_o$ = 45 mM is 0·15 mV/mM. If this value is substituted into eqn. (2) and $p_K$ is taken as 1, a value of about 176 is obtained for $\theta$. The resting potential at $[K]_o$ = 45 mM under the conditions in Fig. 7 is about 32 mV and if the value of $\theta$ is inserted into eqn. (1*b*) a value of about 583 is obtained for $\phi$. In Fig. 8 the value of $\partial E/\partial[Cl]_o$ in the region of $[Cl]_o$ = 32 mM is about $-0{\cdot}3$ mV/mM and if this value is substituted into eqn. (4) a value of 0·0113 is obtained for $p_{Cl}/\phi$ which leads to a value of 6·6 or $p_{Cl}$ if the value just calculated for $\phi$ is taken. Values of $x$ and $y$ can now be calculated from these values of $\theta$, $\phi$, $p_K$ and $p_{Cl}$ if it is assumed that $p_K = 1$, the value for $x$ obtained being 45 and that for $y$, 224. Theoretical changes in membrane potential for these values of $p_K$, $p_{Na}$, $p_{Cl}$, $x$ and $y$ have been calculated and these are shown as continuous lines in Figs. 7

and 8. These give a rather better fit with the experimental data than the dashed lines, particularly in Fig. 7, and suggest that the greater rates of change of membrane potential with external ion concentration found in the absence of extracellular sodium are due to a substantial decrease in the terms $x$ and $y$ in eqn. (1*a*) and not to changes in the relative values of $p_K$ and $p_{Cl}$.

*Effects of changes in calcium concentration*

The changes in resting potential found when the calcium concentration in the medium is altered are shown in Fig. 9, while the effects of different calcium concentrations on the changes found when the sodium concentration is altered are shown in Fig. 10. The changes in potential cannot be due to a contribution of calcium to $x$ and $y$ in eqn. (1*a*) since an increase in external calcium makes the membrane potential more negative, not more positive. However, the data in Fig. 10 makes possible an interpretation of that in Fig. 9. Comparisons of the values of $\partial E/\partial[\mathrm{Na}]_o$ in Fig. 10 gives, from eqn. (3), the following values for $p_{Na}$: Ca = 2 mM, $p_{Na}$ = 1·6; Ca = 6 mM (i.e. the normal medium), $p_{Na}$ = 1·0; Ca = 11 mM, $p_{Na}$ = 0·8. The continuous lines in Fig. 10 are calculated from eqn. (1*a*) using these values for $p_{Na}$. These values for $p_{Na}$ and calcium concentration suggest that $p_{Na}$ varies as $1/[\mathrm{Ca}]_o^{\frac{1}{2}}$, where $[\mathrm{Ca}]_o$ is the calcium concentration. The value for $p_{Na}$ at any calcium concentration (in mM) should therefore be equal to $6/[\mathrm{Ca}]_o^{\frac{1}{2}}$, 6 being the normal medium calcium concentration for which $p_{Na}$ = 1. The continuous line shown in Fig. 9 shows the potential changes calculated from eqn. (1*a*) on the assumption that $p_{Na}$ is equal to $6/[\mathrm{Ca}]_o^{\frac{1}{2}}$. and it will be seen that this gives a satisfactory interpretation of the effects of calcium concentration on the resting potential.

*Effects of γ amino butyric acid on the resting potential*

A concentration of $5 \times 10^{-5}$ M-γ-amino butyric acid (GABA), when applied to the preparation in the normal medium, caused a reversible hyperpolarization of about 4 mV, (4·18 mV ± 0·19, from twenty worms), The changes in resting potential found when the external potassium, sodium and chloride are changed in the presence of this concentration of GABA are shown in Fig. 11. It will be seen from Fig. 11 that the large hyperpolarizations found by Jarman (1964) and del Castillo *et al.* (1964*b*) were obtained at the higher chloride concentrations used by these workers. Analysis of the results in Fig. 11 in terms of eqns. (1)–(4) gives the following values for the various parameters: $p_K$ = 1; $p_{Na}$ = 0·1; $p_{Cl}$ = 6·8; $x$ = 55; $y$ = 245. The curves which have been drawn in Fig. 11 have been calculated from these parameters and eqn. (1). It will be noted that the effect of GABA on $x$ and $y$ is similar to that of sodium-free conditions.

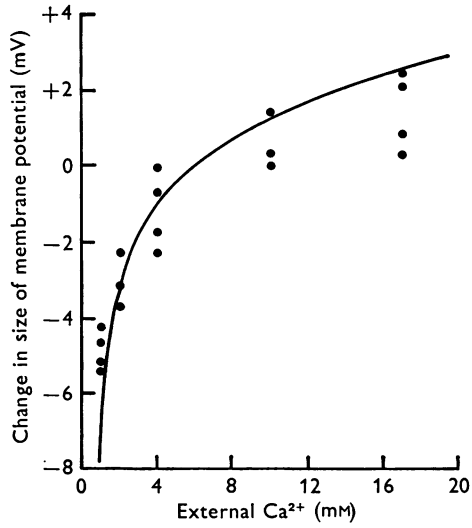


Fig. 9. Changes in the membrane potential brought about by variations in the external calcium concentration. The continuous line has been calculated from eqn. (1) for $p_K = 1$, $p_{Cl} = 7$, $x = 290$, $y = 1300$ on the assumption that $p_{Na} = 6/[Ca]_o^{\frac{1}{2}}$ (see text).

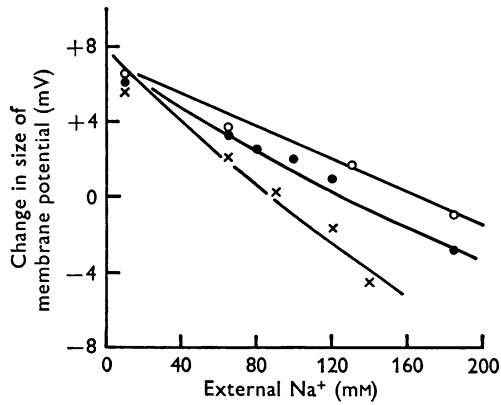


Fig. 10. Changes in the membrane potential brought about by variations in the external sodium concentration at three different external calcium concentrations (× 2 mM; ● 6 mM; ○ 11 mM). Continuous lines calculated from eqn. (1) for $p_K = 1$, $p_{Cl} = 7$, $\times = 290$; $y = 1300$ on the assumption that $p_{Na} = 6/[Ca]_o^{\frac{1}{2}}$ (see text).

*Effects of acetylcholine on the resting potential*

Application of acetylcholine, $10^{-5}$ w/v, in the normal medium caused an immediate reversible depolarization of about 19 mV ($18{\cdot}98 \pm 0{\cdot}38$ from twelve worms). The changes in resting potential found when the external

potassium, sodium and chloride are changed in the presence of this concentration of acetylcholine are shown in Fig. 12. It will be seen that at higher chloride concentrations smaller depolarizations nearer to those found by del Castillo, de Mello & Morales (1963) were obtained. Analysis

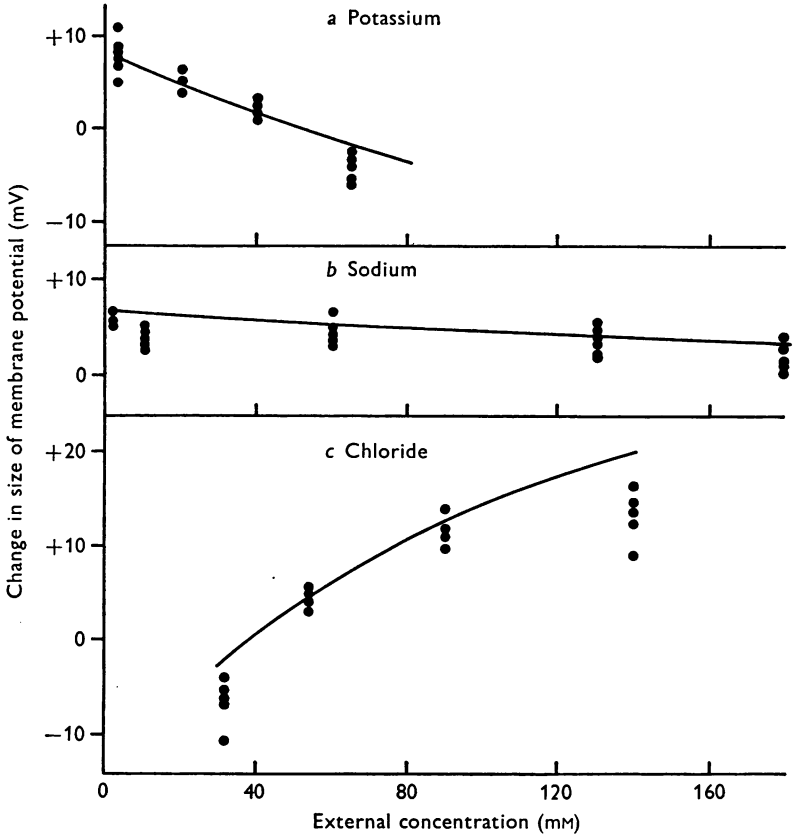


Fig. 11. Changes in the membrane potential brought about by variations in (*a*) external potassium concentration, (*b*) external sodium concentration and (*c*) external chloride concentration in the presence of $5 \times 10^{-5}$ M-$\gamma$-amino butyric acid (GABA). Continuous lines calculated from eqn. (1*a*) for $p_K = 1$, $p_{Na} = 0{\cdot}1$, $p_{Cl} = 6{\cdot}8$, $x = 55$, $y = 245$.

of the results in Fig. 12 in terms of eqns. (1)–(4) gives the following values for the various parameters: $p_K = 1$, $p_{Na} = 0{\cdot}7$, $p_{Cl} = 1{\cdot}0$, $x = 141$, $y = 260$. The curves in Fig. 12 have been calculated from eqn. (1) using these parameters.

*Interaction of GABA and acetylcholine*

A few experiments were carried out on the effects of these two drugs when applied separately and together. In one case a depolarization of

19 mV was observed on the addition of acetylcholine, but this depolarization was virtually abolished on addition of GABA. Similarly in a second case, a hyperpolarization of about 5 mV in GABA was abolished by the addition of acetylcholine. It seems therefore that GABA and acetylcholine can suppress each other's effects.

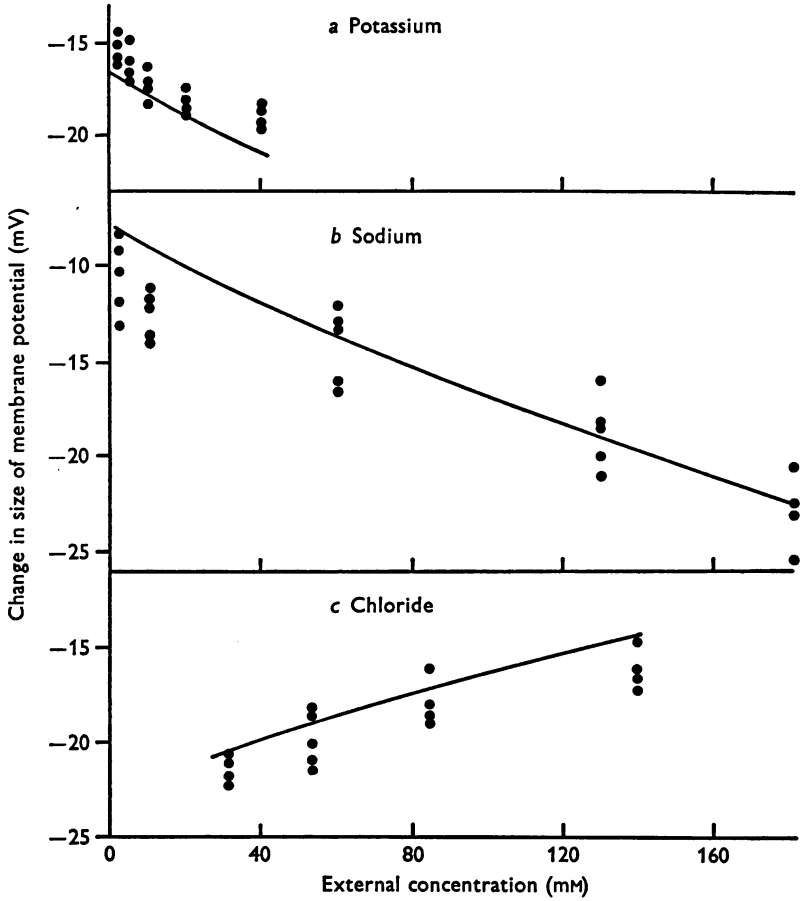


Fig. 12. Changes in the membrane potential brought about by variations in (*a*) external potassium concentration, (*b*) external sodium concentration and (*c*) external chloride concentration in the presence of acetylcholine ($10^{-5}$ w/v). Continuous lines calculated from eqn. (1*a*) for $p_K = 1, p_{Na} = 0{\cdot}7$, $p_{Cl} = 1$, $x = 141$, $y = 260$.

*Effects of adrenaline, $10^{-5}$ (w/v) on the resting potential*

It was found that a fairly high concentration of adrenaline ($10^{-5}$ w/v) was needed to give a measurable effect and this was an average hyperpolarization of 1·5 mV ± 0·17 in twelve worms. Fig. 13 shows the effects

of changes in external potassium, sodium and chloride on the resting potential in the presence of adrenaline, $10^{-5}$ w/v. Analysis of the results in terms of eqns. (1)–(4) gives the following values for the various parameters: $p_K = 1$, $p_{Na} = 0{\cdot}4$, $p_{Cl} = 3{\cdot}6$, $x = 146$, $y = 648$ and the curves

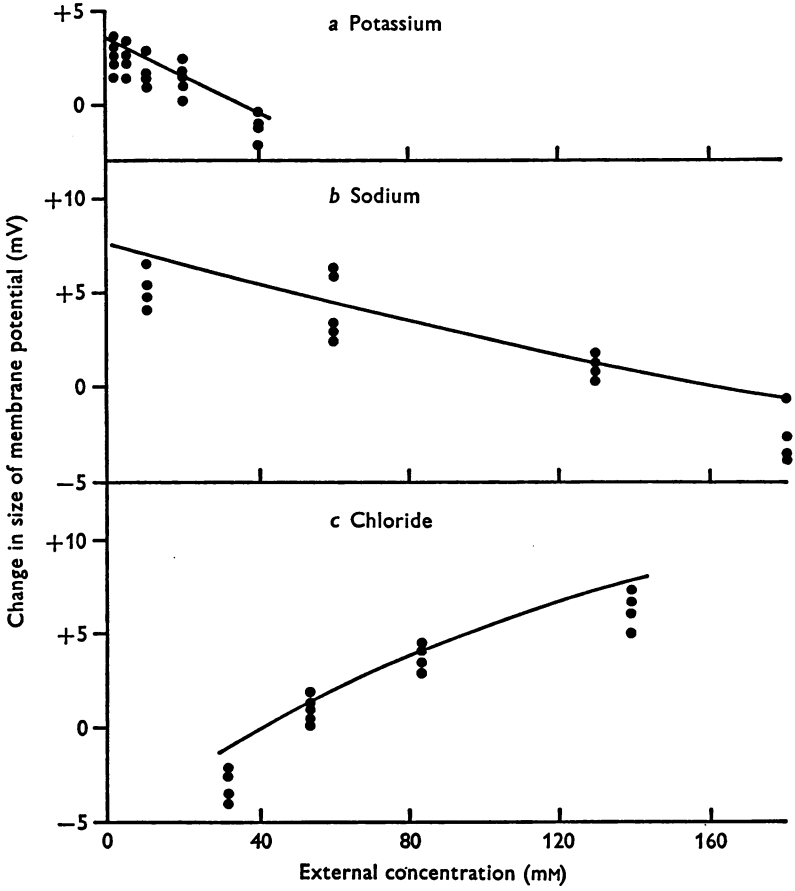


Fig. 13. Changes in the membrane potential brought about by variations in (*a*) external potassium concentration, (*b*) external sodium concentration and (*c*) external chloride concentration in the presence of adrenaline ($10^{-5}$ w/v). Continuous lines calculated from eqn. (1*a*) for $p_K = 1$, $p_{Na} = 0{\cdot}4$, $p_{Cl} = 3{\cdot}6$, $x = 146$, $y = 648$.

in Fig. 13 have been calculated using these. It might be noted that these values are equivalent to a doubling of $p_K$, the other parameters remaining similar to those in the normal medium ($p_K = 2$, $p_{Na} = 0{\cdot}8$, $p_{Cl} = 7{\cdot}2$, $x = 292$, $y = 1296$).

*The effects of nicotine*

In some preliminary experiments nicotine ($10^{-4}$–$10^{-5}$ w/v) depolarized the membrane to the same extent as acetylcholine. This depolarization was however irreversible and it was not studied in detail.

*The site of action of the drugs*

Del Castillo *et al.* (1963, 1964*b*, *c*) have used the technique of allowing drugs to be released from a micropipette by electrophoresis, and have concluded that the site of action of acetylcholine and piperazine is at the syncytial membrane and that the bulges are insensitive. A simpler technique was tried to determine whether or not the bulges contain receptor sites for the action of transmittors and drugs and this was to cut the arms linking the syncytial region to the muscle bulges. This was achieved with a longitudinal cut adjacent to the nerve cord which went right through the cuticle. The effect of cutting the arms was to reduce the resting potential of the bulges to about 18–20 mV. This potential was however affected by GABA, acetylcholine and nicotine in the same way as the potential of bulges with intact arms, that is with a hyperpolarization in GABA and a depolarization in acetylcholine and nicotine, although the absolute size of the charges was smaller. GABA caused a hyperpolarization in a group of muscle cells which had been removed from the worm and which contained neither lateral nerve cord nor ventral nerve cord. It would thus seem that the muscle bulges can respond to drugs when they are not connected to the muscle syncytium and that they contain receptor sites. It is probable, however, that the receptor sites are more abundant at the syncytium.

## DISCUSSION

The analysis of the resting membrane potential of *Ascaris lumbricoides* muscle presented in this paper suggests that there is a major factor determining this potential other than the contributions of the identified ions. This factor is expressed as the large additional components $x$ and $y$ found when the potential is analysed in terms of the Goldman constant field equation (eqn. (1*a*)). Since the contributions of potassium, sodium and chloride are allowed for in the analysis, it seems most unlikely that the unusual behaviour of the resting membrane potential can be ascribed to a low resistance of the chloride battery in the membrane or a high sodium conductance as has been done by del Castillo *et al.* (1964*a*). The analysis used here might be questioned on various grounds but there is one factor which indicates that it is valid. This is that the relative permeability constants of 1 for $p_K$, 1 for $p_{Na}$ and 7 for $p_{Cl}$ agree reasonably well with the

relative values of 1 for $p_K$, 0·8–3·1 for $p_{Na}$ and 7·9 for $p_{Cl}$ obtained from radioactive tracer movements (Caldwell & Ellory, 1968).

A fairly obvious explanation for the additional components is that they represent the effects of some electrogenic ion transport mechanism. The effects of such a mechanism on the Goldman constant field equation have recently been discussed by Geduldig (1968) and by Moreton (1969). Moreton has assumed that an electrogenic sodium pump would contribute an outward current to the currents flowing across the membrane and on this basis he has derived the following form of eqn. (1*a*)

$$E = \frac{RT}{F} \log_e \frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i + RTM_a/FE}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o + RTM_a/FE}, \qquad (5)$$

where $M_a$ is the net efflux of cations brought about by electrogenic transport mechanisms. If eqn. (5) were applicable to *Ascaris* muscle cells and was equivalent to eqn. (1*a*) then the terms $x$ and $y$ in eqn. (1*a*) would be equivalent to the term $RT\,M_a/FE$ and should be equal to each other. This is not so and eqn. (5) cannot be applied to the membrane potential of *Ascaris* muscle.

The form of constant field equation exemplified in eqn. (1*a*) with $y$ much greater than $x$, which has been used in the present analysis, is on the other hand compatible with the type of electrogenic sodium pump mechanism put forward by Cross, Keynes & Rybová (1965). In this mechanism sodium is transported outwards in combination with a negatively charged carrier $Y^-$ which is converted to a negatively charged form $X^-$ on the outside of the membrane which does not interact with sodium although it is considered as being able to interact with potassium. $X^-$ can, however, return across the membrane on its own bringing a negative charge into the cell and as a result generating an electrogenic potential. The movement of this carrier can be represented in the Goldman constant field equation in the same way as the movement of the other ions in terms of the concencentrations of $X^-$ ($[X^-]_o$ and $[X^-]_i$) on the outer and inner surfaces of the membrane and a permeability constant $p_X$. $x$ in eqn. (1*a*) then becomes $p_X[X^-]_i$ and $y$ becomes $p_X[X^-]_o$ to give

$$E = \frac{RT}{F} \log_e \frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i + p_X[X^-]_i}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o + p_X[X^-]_o}. \qquad (6)$$

On this interpretation it is then found that under many conditions the resting membrane potential of *Ascaris* is determined largely by the terms $p_X[X^-]_i$ and $p_X[X^-]_o$, these terms being a measure of the gradient of the carrier $X^-$ which is near to electrochemical equilibrium. The carrier postulated in this interpretation need not necessarily be involved in the transport

of sodium. There are very large fluxes of carboxylic acids across the *Ascaris* muscle membrane (Ellory, 1967) some of which are greater than the sodium, potassium and chloride fluxes. A positively charged carrier involved in carboxylic acid uptake which could move in with a negatively charged carboxylic acid anion and then move out in a non-carrier positively charged form could also give rise to the additional terms $x$ and $y$ in eqn. (1*a*), $x$ becoming $p_X[X^+]_o$ and $y$, $p_X[X^+]_i$.

Some of the values which have been calculated for $x$ and $y$ under different conditions are summarized in Table 2 and it will be seen that normally $x$ and $y$ have large values of about 290 and 1300. In either the absence of sodium or in the present of GABA the values of $x$ and $y$ are considerably reduced.

TABLE 2. Values of the parameters $x$ and $y$ needed to describe the resting membrane potential of muscle cells from *Ascaris lumbricoides* in terms of eqn. (1*a*) under different conditions

| Conditions | Value of $x$ | Value of $y$ |
|---|---|---|
| Normal saline, different $Ca^{2+}$ concentrations, low $Cl^-$, high $K^+$ | 290 | 1300 |
| $Na^+$-free saline | 45 | 224 |
| Normal saline + $\gamma$-amino butyric acid | 55 | 245 |
| Normal saline + acetylcholine | 141 | 260 |
| Normal saline + adrenaline | 146 | 648 |

(The values of $x$ and $y$ are those obtaining when $p_K = 1$ and the concentrations of $K^+$, $Na^+$ and $Cl^-$ are expressed in mM.)

The change in GABA reflects a decrease in $x$ and $y$ rather than an increase in $p_K$ and $p_{Cl}$ since Ellory (1967) has found that the movements of radioactive potassium and chloride are hardly affected by GABA. The effect on $p_{Na}$ is less clear cut. Ellory (1967) found that sodium movements were virtually unaffected by GABA whereas the analysis of the membrane potential changes in terms of eqns. (1)–(4) suggests that $p_{Na}$ is reduced relative to $p_K$ and $p_{Cl}$. These two findings would still be consistent if GABA had the dual effect of reducing the passive movement of sodium and increasing the carrier mediated exchange diffusion of sodium. The main action of GABA could therefore be to remove or block the electrogenic carrier $X^-$ discussed earlier.

In acetylcholine there seems to be a tendency for a general removal of ion selectivities, with $x$ and $y$ approaching similar values. Tracer experiments (Ellory, 1967) suggest that $p_K$ rises slightly and that $p_{Cl}$ decreases. The effect on $x$ and $y$ would be compatible with an interaction of acetylcholine with the electrogenic pump component $X^-$ discussed earlier to form a neutral complex able to cross the membrane. Such a situation would

lead to reduced roughly equal values of $[X^-]_o$ and $[X^-]_o$ and hence of $p_X[X^-]_o$, $p_X[X^-]_i$, $x$ and $y$.

No tracer data are available for *Ascaris* muscle exposed to adrenaline but the changes in $x$ and $y$ would be compatible with either a halving of these quantities or a doubling of $p_K$. The latter possibility is not unlikely since $p_{Na}$, $p_{Cl}$, $x$ and $y$ would remain virtually unchanged if $p_K$ doubles and a doubling of $p_K$ would mean that the action of adrenaline on *Ascaris* muscle cells was the same as its effect on smooth muscle (Bülbring & Tomita, 1969).

It would appear therefore that the unusual behaviour of the resting membrane potential of *Ascaris* muscle fibres can be understood in terms of the Goldman constant field equation if an additional factor making a major contribution to the membrane conductance under certain circumstances is postulated. This could be part of an electrogenic active transport system and the evidence which has been obtained suggests that there is a direct action of acetylcholine and $\gamma$-amino butyric acid on this system.

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