

AN APPLICATION OF THE CONSTANT-FIELD THEORY TO THE BEHAVIOUR OF GIANT NEURONES OF THE SNAIL, *HELIX ASPERSA*

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INTRODUCTION

This paper describes some experiments on the resting potentials of giant neurones in the common snail, *Helix aspersa*. The central neurones of a number of gastropods represent, in terms of size and accessibility, an ideal subject for study by means of intracellular glass capillary microelectrodes. The suboesophageal ganglia of the common snail contain many neurones of diameters ranging from 40 to 200 μ . The majority of experiments so far carried out on them have, however, been mainly concerned either with their action potentials, or with the details of synaptic mechanisms and drug action. Such experiments as have been made specifically on the resting potentials (Kerkut & Walker, 1961; Gerasimov, Kostyuk & Maiskii, 1965 *a, b*; Kerkut & Meech, 1967) have served to show both that they are extremely variable and that their ionic dependence is far from simple.

The object of the present experiments was to investigate in detail the potassium-dependence of the resting potentials, particularly at concentrations near the physiological range, and to discover to what extent the 'constant-field' theory, as first formulated by Goldman (1943), can be used to describe the behaviour of the neurones. Equations derived from the theory by Hodgkin & Katz (1949) have been modified to form linear relationships which could be fitted to a relatively small amount of experimental data for each neurone. In this way it has been possible to test the application of the theory, and to make estimates of the intracellular potassium concentrations, and relative sodium and potassium permeabilities of the cell membranes, of a large number of snail neurones. The results have been applied to a statistical study of the behaviour of the cells, and in particular to the investigation of possible seasonal changes in their properties.

THEORY

The purpose of this section is to summarize the development of the equations used in this paper. The resting potentials of nerve and muscle are generally considered to be determined by the concentrations of sodium, potassium and chloride ions on either side of the cell membrane, and by the relative permeabilities of the membrane to these ions (e.g. Hodgkin, 1951). The most successful expression of this dependence has been that derived from the 'constant-field' theory of Goldman (1943), by Hodgkin & Katz (1949). As it stands, however, the equation derived by Hodgkin & Katz requires a large amount of experimental data for its application; in particular, in the case of

a single snail neurone, the intracellular ion concentrations are very difficult to measure directly, thus adding still more unknown quantities to the equation. The object of this analysis was to derive a modified form of the 'constant-field' equation, to express the relationship between the resting potential of a neurone, and the extracellular potassium concentration, in a form suitable for simple analysis. The notation used is the same as that of Hodgkin & Katz (1949), and is mainly self-explanatory.

The starting-point is the well-known equation of Hodgkin & Katz:

$$V = \frac{RT}{F} \ln. \frac{P_K[K_o^+] + P_{Na}[Na_o^+] + P_{Cl}[Cl_i^-]}{P_K[K_i^+] + P_{Na}[Na_i^+] + P_{Cl}[Cl_o^-]}, \quad (1)$$

where V is the resting potential of the cell, P_K etc., are the permeabilities of the cell membrane to the various ions, and R , T and F have their usual meanings. The subscripts $_o$ and $_i$ are used to distinguish the ionic activities outside and inside the cell, respectively.

For the purpose of simplification, this equation is first rearranged, to remove the logarithm

$$\exp\left(\frac{FV}{RT}\right) = \frac{P_K[K_o^+] + P_{Na}[Na_o^+] + P_{Cl}[Cl_i^-]}{P_K[K_i^+] + P_{Na}[Na_i^+] + P_{Cl}[Cl_o^-]}. \quad (2)$$

Two assumptions are then made. First, it is assumed (as is justified by experiment) that the potassium permeability of the cell membrane is much greater than that to sodium, and that the intracellular sodium concentration is small. The value of the term $P_{Na}[Na_i^+]$ is then much smaller than that of the term $P_K[K_i^+]$, and may be neglected.

Secondly, the contribution of chloride ions to the resting potential is assumed to be small. The justification of this latter assumption rests largely upon the experimental observation, that, following a change in the external potassium concentration, the resting potential always reaches a steady level within the period of a few minutes, which was allowed in the experiments, before taking readings. This implies, either that the chloride distribution rapidly reaches equilibrium, in which case chloride ions will not contribute at all to the resting potential, or that the approach to chloride equilibrium, by movement of potassium chloride across the cell membrane, is too slow to be detected, from measurements of the resting potential, in which case the contribution of chloride ions to the potential will be small. In either case, equation (2) can be simplified by omitting the chloride terms, so that it becomes

$$\exp\left(\frac{FV}{RT}\right) = \frac{[K_o^+]}{[K_i^+]} + \frac{P_{Na}[Na_o^+]}{P_K[K_i^+]}. \quad (3)$$

Assuming that the intracellular potassium concentration, and the relative permeabilities of the cell-membrane to sodium and potassium ions, remain constant (see below, under 'Discussion'), equation (3) represents a linear relationship between the resting potential and the extracellular potassium concentration. The slope of the line gives an estimate of $[K_i^+]$, and its intercept gives the quantity P_{Na}/P_K ($[Na_o^+]$ being known), which is a measure of the selectivity of the cell membrane for potassium ions.

METHOD

Experiments were carried out on giant neurones in the abdominal ganglia of the snail, *Helix aspersa*. The 'brain', comprising cerebral, pedal and abdominal ganglia and commissurae, was removed from a live, active snail, and mounted, dorsal surface uppermost, on a glass slide. The brain was held in position by rubber bands. The connective tissue was dissected away from the abdominal ganglia, each of which is enclosed in a thin tissue sac. This was torn away from each ganglion, as required, with fine forceps, exposing the neurones, which form a layer round a central neuropile. The general layout of the preparation is indicated in Fig. 1, together with the location

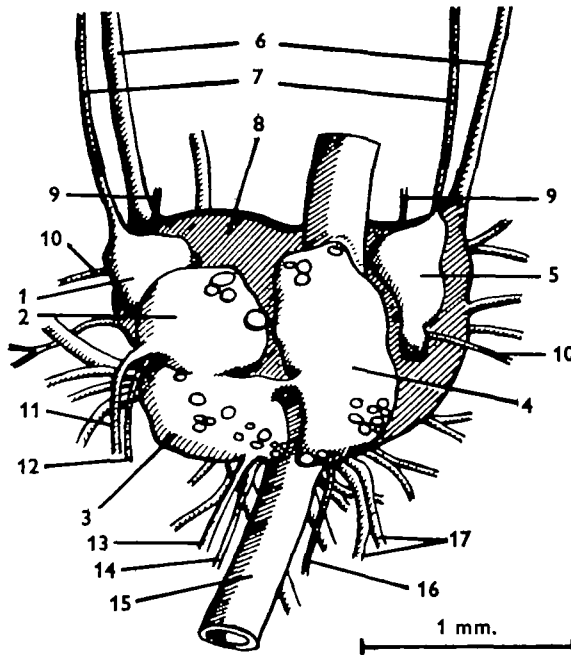


Fig. 1. Dorsal view of the abdominal ganglia; outer connective tissue removed to show principal nerves and location of some of the larger neurones:

- | | | | |
|---|-----------------------------|----|-------------------------|
| 1 | Left pleural ganglion | 10 | Columellar nerves |
| 2 | Left parietal ganglion | 11 | Pharyngeal nerve |
| 3 | Visceral ganglion | 12 | Cutaneous pallial nerve |
| 4 | Right parietal ganglion | 13 | Intestinal nerve |
| 5 | Right pleural ganglion | 14 | Anal nerve |
| 6 | Cerebropedal commissurae | 15 | Aorta |
| 7 | Cerebropleural commissurae | 16 | Aortic nerve |
| 8 | Pedal ganglia | 17 | Right pallial nerves |
| 9 | Pharyngeal retractor nerves | | |

of some of the larger cells. The cells used were mainly from the visceral and right parietal ganglia, and ranged in size from 40 to 200 μ . It was to some extent possible to identify particular cells, though their layout was not quite constant from one preparation to another, and no attempt was made to confine attention to any particular cells.

The 5 ml. Perspex chamber in which the slide was placed is shown in Fig. 2. The preparation was illuminated from behind, and viewed through the glass front of the

chamber. The purpose of the intermediate compartment was to eliminate junction potentials; the Ringer in the agar bridge and in the centre compartment should be kept the same as that in the main compartment, though for small changes in potassium content, this was not found to be necessary. The solution in the chamber could be changed by running through 50 ml. of the new solution. The flow rate was adjusted so that this took about 1 min.

The Ringer used was that employed by Kerkut & Thomas (1965). Different potassium concentrations were obtained simply by adding or leaving out potassium chloride, the resulting differences in osmotic pressure being small, over the range normally used.

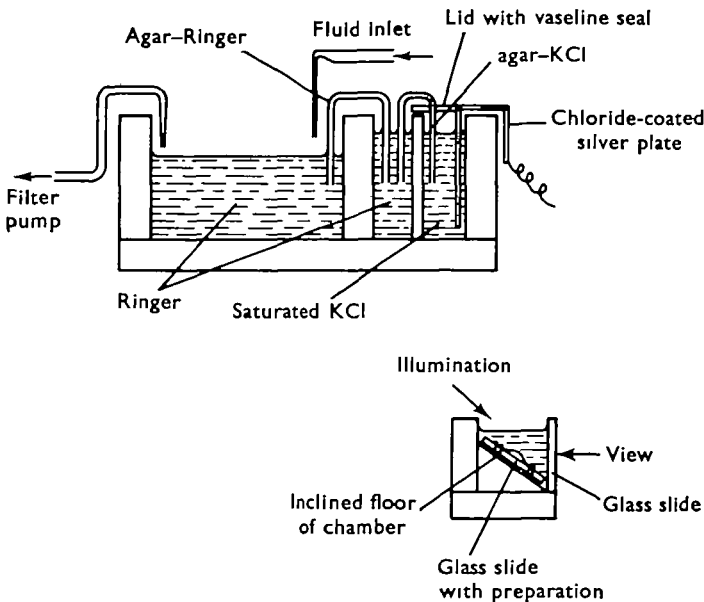


Fig. 2. Perspex chamber and fluid circuit: front view actual size, side view inset.

Resting potentials were measured with glass microelectrodes, which were pulled on a horizontal solenoid puller, and filled with 3 M potassium chloride solution by boiling under reduced pressure. The resistance of the electrodes ranged from 3 to 20 M Ω , measured in Ringer, and only electrodes with tip potentials of 5 mV. or less were accepted. The tip potentials were checked before and after each experiment. Connection to the electrodes was made through a chloride-coated silver wire, embedded in agar gel, saturated with potassium chloride.

The silver wire was connected to a conventional cathode-follower (Brimar 6BR7) and oscilloscope. Potentials were measured by comparison with a standardized calibrator, placed in series with the plate electrode. The potassium-dependence of the resting potential of each neurone was investigated by changing the concentration in the experimental bath in several steps, usually over the range 0–12 mM. Each new concentration was run in between readings in 4 mM. potassium, to allow correction for changes in the selectivity of the cell.

Readings of resting potential were taken about every minute; Fig. 3 shows the record

of a typical experiment, together with the readings obtained from it, before and after correction for changes in selectivity. In reading the values of the resting potential from the graph, the value selected was always that reached 1–2 min. after the change of solution was complete; this time was found to be sufficient for the attainment of a steady potential.

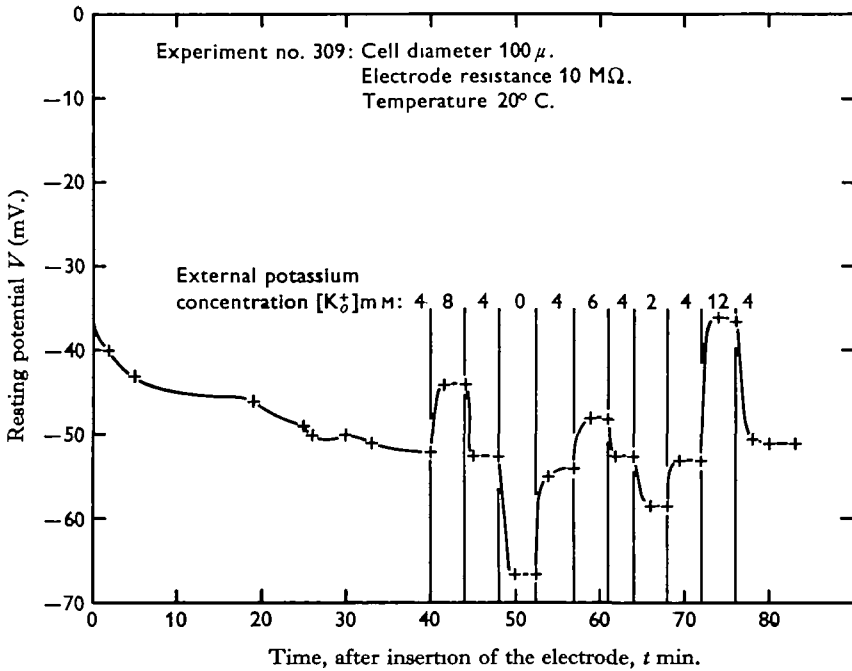


Fig. 3. Time course of an experiment to investigate the potassium dependence of the resting potential of a neurone. Readings obtained were as follows, before and after correction for change in selectivity of the cell (see also Fig. 4 (1)):

External potassium concentration, mM.	0	2	4	6	8	12
Uncorrected resting potential, mV.	66.5	58.5	52.7	48.0	44.0	36.5
Corrected resting potential, V mV.	65.3	58.4	52.7	47.6	44.3	36.9
exp (FV/RT)	0.081	0.097	0.121	0.149	0.170	0.229

Vertical lines on the graph indicate changes of solution.

RESULTS

(1) *Potassium dependence of the resting potential.* Altogether, successful experiments have been carried out on fifty-seven neurones. Figure 4 shows some typical results, chosen to illustrate the range of properties encountered. In Fig. 4A, the resting potentials have been plotted against the logarithm of the external potassium concentration, resulting in a series of curves, with slopes averaging 20–30 mV., per tenfold change in concentration. The small slopes, and large degree of curvature, are taken as indicating that the cell membranes have a relatively large permeability to ions other than potassium.

In Fig. 4B, the resting potentials of the same cells have been plotted according to equation (3) of the theoretical section—exp (FV/RT) against external potassium concentration. It will be seen that this method produces straight lines: except, in a few

cases, at very low potassium concentrations, this was found to be true for all the cells examined. Equation (3) thus provides a much better representation of the behaviour of the resting potentials of snail neurones, than does the Nernst equation.

Best straight lines have been fitted, by 'least square' regression analysis, to the experimental points in Fig. 4B. The slope and intercept of each line give estimates of the intracellular potassium concentration, and the selectivity of the cell membrane, respectively: the values obtained are shown in the legend. From the intracellular potassium concentration it is possible to calculate for each cell the resting potential given by the Nernst equation, as indicated by the broken lines in Fig. 4A.

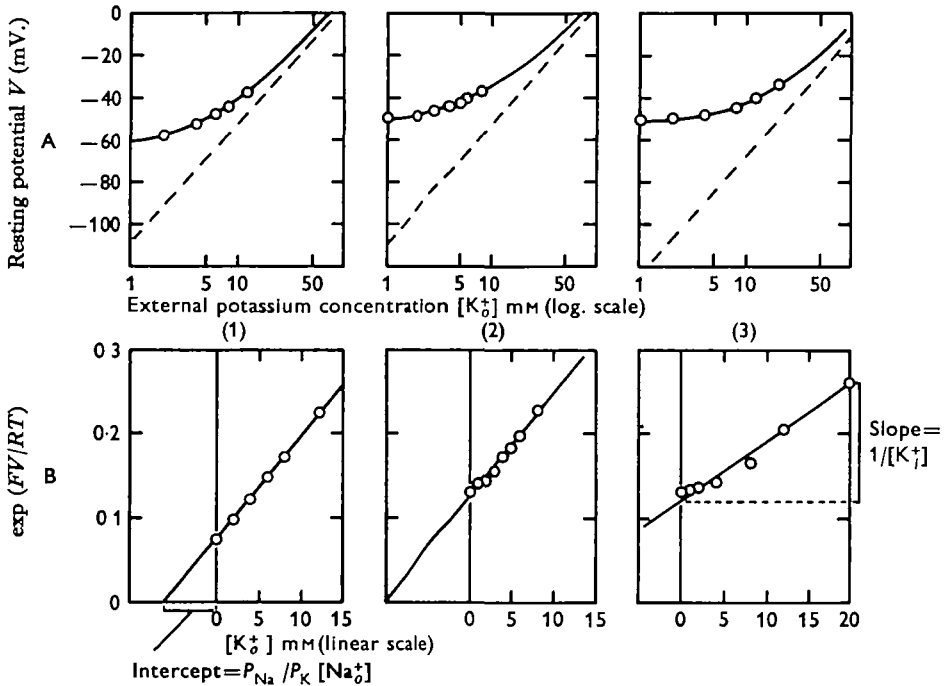


Fig. 4. Behaviour of the resulting potentials of three neurones, at low potassium concentrations. The results are plotted as follows:

A (upper): resting potential (V mV.) against logarithm of external potassium concentration ($[K^+]_o$ mM). The broken lines represent the potassium equilibrium potentials, as given by the Nernst equation; the solid curves represent the potentials given by the 'constant-field' equation, using the permeability ratios and intracellular potassium concentrations estimated from the straight lines of B.

B (lower): $\exp(FV/RT)$ against external potassium concentration. The 'best straight lines' were determined by regression analysis, and give estimates of the parameters of the cells, as follows (error = one standard deviation):

Figure	4 (1)	4 (2)	4 (3)
Experiment no.	309	200	297
$[K^+]_o$ mM	80.3 ± 5.2	82.0 ± 23.5	147.5 ± 22.6
V mV. ($[K^+]_o = 4$ mM)	-52.5	-44.0	-48.5
P_{Na}/P_K	0.074	0.127	0.217

Significant deviations from the type of behaviour represented by equation (3) were found in only ten of the experiments. In these cases the resting potentials of the cells were smaller, at very low potassium concentrations, than would be expected from the

equation. This deviation is probably to be attributed to a reduction in the potassium permeability of the cell membranes (see 'Discussion' below).

Figure 5 shows the distribution of the estimated intracellular potassium concentrations and selectivities (P_{Na}/P_K) of the cells examined, and also the distribution of the resting potentials, measured at an external potassium concentration of 4 mM. The mean values, with standard deviations calculated from the spread of the results, are as follows:

Resting potential, V	= $-(40.8 \pm 1.1)$ mV.
Intracellular potassium concentration, $[K^+]_i$	= (92.9 ± 4.3) mM.
Permeability ratio, P_{Na}/P_K	= 0.180 ± 0.015 .

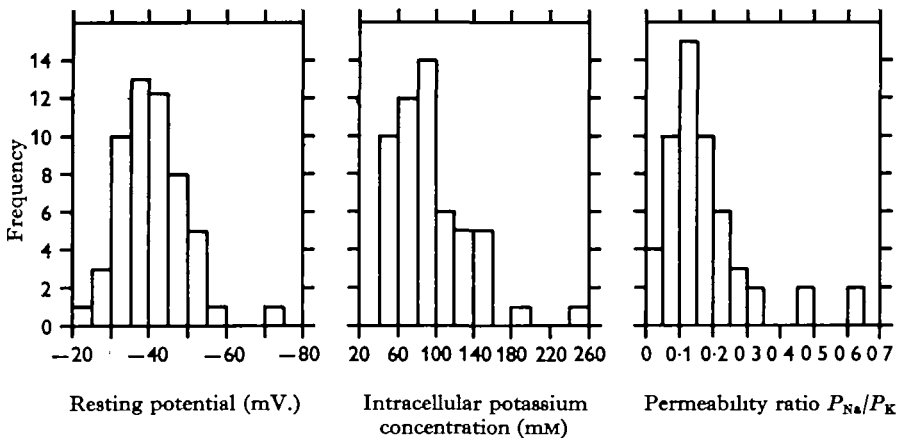


Fig. 5. Distribution of properties of the neurones.

(2) *Seasonal trends.* The results form a continuous record of the average properties of snail neurones, over a complete year. Since the snails were always used within a month of collection, and treated in the same way, the results can be used to give an indication of any seasonal trends in the behaviour of the neurones. For this purpose the experiments have been divided into groups, those in each group having been carried out over a 2-month period, and the results in each group have been averaged. Figure 6 shows the mean results, plotted against time; the standard deviations were calculated from the spread of the figures. It will be seen that, while the mean resting potentials of the neurones vary comparatively little throughout the year, the intracellular potassium concentrations, and the selectivities of the cell membranes, show quite well-marked, parallel seasonal changes. The potassium concentrations are highest, and the selectivities poorest, during the 4 months from February to May, inclusive; during July and October, the reverse is the case.

DISCUSSION

Points of interest arising from the results will be discussed in order. The resting potentials of snail neurones have been measured, and their dependence on the external concentration of potassium ions has been investigated. It has been found that, while the behaviour of the resting potential deviates considerably from that of a pure

potassium electrode, it can be successfully represented by an equation derived from the 'constant-field' theory. By fitting this equation to the experimental results, estimates of the intracellular potassium concentrations, and the selectivities of the cell membranes of a large number of neurones, have been obtained.

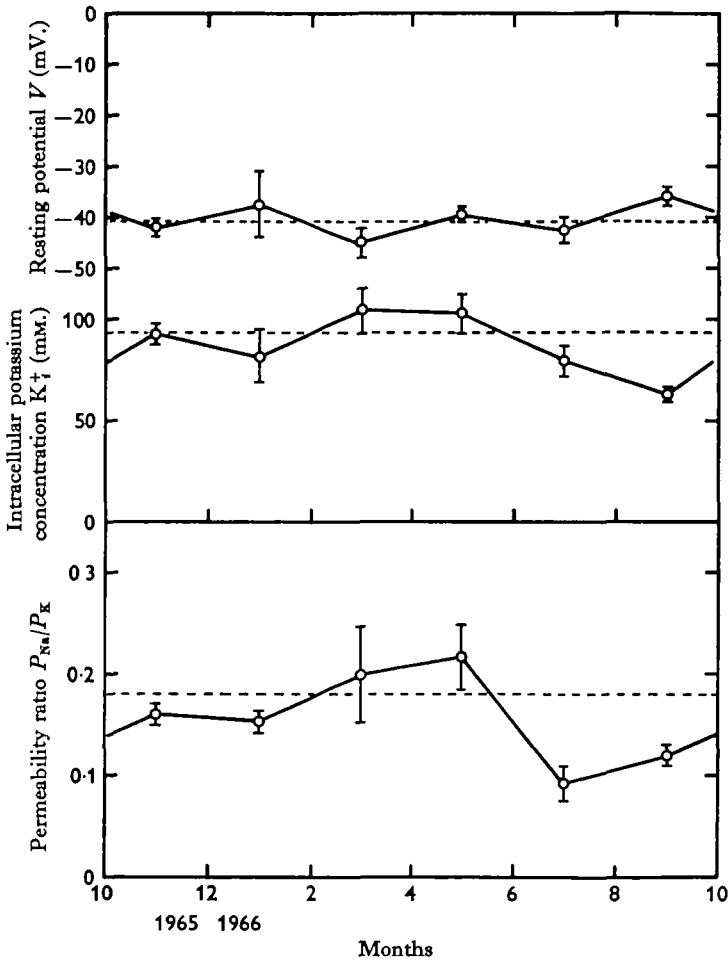


Fig. 6. Seasonal changes in the average properties of the neurones, over the period from October 1965 to October 1966. Limits of error are ± 1 standard deviation, as estimated from the spread of the results; broken lines give the overall mean values, throughout the year.

(1) *Potassium-dependence of the resting potential.* The resting potentials of snail neurones vary widely (Fig. 5). The values obtained are in the main higher than those found by Kerkut & Walker (1961) for active specimens of *Helix aspersa*, though in later experiments (e.g. Kerkut & Thomas, 1965; Kerkut & Meech, 1967) much higher resting potentials have been recorded. Neurones of *Helix pomatia* (Tauc, 1955; Maiskii, 1964) commonly give resting potentials of 30–50 mV. Most cells punctured showed a steady increase in resting potential over the first 10–20 min., following insertion of the electrode, the net increase varying from 5 to 30 mV. (see Fig. 3). This

increase could be due to an improvement in the selectivity of the cell, following damage caused by the puncture; Kerkut & Thomas (1965) observed only small increases in resting potential under similar conditions.

Over the range of external potassium concentrations from 0 to 12 mM., which is not very different from the physiological range (e.g. Arvanitaki & Cardot, 1932; Hughes & Kerkut, 1956), the 'constant-field' equation provides, in most cases, an accurate description of the behaviour of the resting potential. This is not, of course, a guarantee that the 'constant-field' hypothesis provides an adequate description of the passive properties of the cell-membrane: we have no independent evidence relating to the intracellular ion concentrations. Thus Hodgkin & Katz (1949) find that, for squid axons, the curve relating resting potential to external potassium concentration bends very sharply at low concentrations. Equations of the 'constant-field' type can be fitted to the behaviour at both high and low concentrations, but the sets of parameters required in each case are different. In particular, the values indicated for the internal potassium concentration differ by about 10%, that required to explain the behaviour at low concentrations being higher than the true value. This anomalous behaviour may be due to variation in the relative ionic permeabilities of the cell membrane.

Frog muscle fibres, on the other hand, will behave in accordance with the theory over a wide range of potassium concentrations, provided either that chloride ions are absent, or that the product $[K^+]_o[Cl^-]_o$ is kept constant (Adrian, 1956; Hodgkin & Horowicz, 1959). In the presence of chloride ions the situation is complicated by the movement of potassium chloride across the cell membranes (Boyle & Conway, 1941; Hodgkin & Horowicz, 1957, 1959; Adrian, 1960).

For lack of evidence to the contrary it will be assumed that the behaviour of snail neurones is also adequately represented. Where graphs such as those of Fig. 4B are linear, it is thus assumed that the relative ionic permeabilities of the cell membrane remain constant. The deviations found in a few cases, at low potassium concentrations, are attributed to a deterioration in the selectivity of the cell membrane (i.e. an increase in P_{Na}/P_K), so that reducing the external potassium concentration causes a smaller increase in the resting potential than is expected. Deterioration in the selectivity could be caused by a decrease in the potassium permeability, somewhat similar to that observed in frog muscle fibres under similar conditions (Hodgkin & Horowicz, 1959; Adrian, 1964). The observed behaviour could represent an automatic stabilization of the resting potential against changes in the cell's ionic environment, a theory which has also been suggested by Kerkut & Meech (1967).

(2) *Intracellular potassium concentrations.* It has been assumed that the slopes of graphs, such as those in Fig. 4B, give estimates of the intracellular potassium concentrations of the neurones. Three points should be considered.

First, the effect of chloride movements on the resting potential has been assumed to be negligible. This requires either that, following a change in the external potassium concentration, chloride equilibrium is restored within a few minutes, or that the chloride permeability of the cell membrane is small. In the former case, the amount of potassium chloride crossing the cell membrane can be shown, from the theory of Boyle & Conway (1941), to be sufficient to change the intracellular potassium concentration by not more than about 8 mM., over the entire range of extracellular

concentrations used. The intracellular potassium concentration could thus be regarded as constant. In the latter case the intracellular potassium concentration would, in fact, remain constant, but it can be shown that the estimate of its values, obtained from the slopes of graphs such as Fig. 4B, are affected by the chloride flux, being too large by an amount equal to the magnitude of the quantity $P_{\text{Cl}}[\text{Cl}_o^-]/P_{\text{K}}$. A limit to the value of this term can be deduced from the experimental behaviour of the resting potential: from the absence of any observable drift after the initial equilibration period (following a change in the extracellular potassium concentration), it appears that any chloride movement which is still taking place occurs very slowly, with a time-constant for equilibration of at least 30 min. This time-constant corresponds to the very low chloride permeability of about 5×10^{-7} cm. sec.⁻¹. The membrane resistance of *Helix* neurones is not exceptionally high (Maiskii, 1964; Moreton, in preparation), so that the potassium permeability presumably has the usual value for neurones, about 10 times this figure. The error introduced by neglecting the term $P_{\text{Cl}}[\text{Cl}_o^-]/P_{\text{K}}$ would thus not be serious.

The experimental results provide no means of distinguishing between the cases of high and low chloride permeability. There is a small amount of evidence (Gerasimov *et al.* 1965*a*; Kerkut & Meech, 1967) in favour of a low chloride permeability.

The second point, regarding the intracellular concentration of potassium, is that what is measured in the experiments is, in fact, the intracellular potassium activity, in terms of the activity in the bathing solution. In the Ringer, which is isotonic with 110 mM sodium chloride solution, the activity coefficient of potassium ions may be taken as approximately that given by Robinson & Stokes (1959) for 100 mM potassium or sodium chloride solutions: the two values are very similar, being about 0.77 at 25° C. The cytoplasm contains fewer ions, but more macromolecules, than the extracellular fluid. Sorokina (1966) has used cation-sensitive microelectrodes to measure the intracellular potassium activities in neurones of *Helix pomatia* and compares the results with the mean concentration, determined by flame-photometry, to obtain a mean activity coefficient of 0.78. This is quite close to the values obtained for frog muscle by the same technique (Lev, 1964), but somewhat higher than the values in some other excitable tissues (Hinke, 1959, 1961).

The activity coefficient of potassium ions in the cytoplasm of snail neurones thus appears to be very little different from that in the snail Ringer; since this implies that the ratio $[\text{K}_o^+]/[\text{K}_i^+]$ will be virtually unaffected by the substitution of concentrations for activities, it follows that the use of concentrations in equation (3) will give the correct value for the intracellular potassium concentration.

Thirdly, it has been assumed that the concentration of potassium ions immediately outside the cell membrane is the same as their concentration in the bulk of the external solution. If there were an unstirred layer around the cell, or a layer of fluid trapped in a restricted space between cells, changes in the concentration of potassium ions in the bulk of the surrounding solution might be only partially reflected at the cell surface. The resulting changes in the resting potential would then be small, and would give an overestimated value for the intracellular potassium concentration. As against this, in the experiments, the resting potential nearly always reached a steady value within 2–3 min. after changing the external solution, suggesting that potassium ions penetrate readily to the surface of the cell.

Following these considerations, the mean intracellular potassium concentration measured in the present experiments may thus be given as:

$$[K^+]_i = (92.9 \pm 4.3) \text{ mM/l.}$$

being the average of fifty-seven results. The quoted error of one standard deviation was estimated from the spread of the results; consideration of the experimental errors, obtained from the regression analysis used to estimate the intracellular potassium concentration, suggests that a large part of the spread of the results is due to experimental error, though not all of the variation can be accounted for in this way. The mean value is very close to that obtained by Sorokina (1966).

(3) *Selectivity of cell membranes.* The intercepts of graphs such as those in Fig. 4 B give estimates of the permeability ratio, P_{Na}/P_K , which is an indication of the degree of selectivity of the membrane for potassium ions; a high value of the ratio indicates that the cell membrane is relatively leaky. The values obtained, in the region of 0.1, may be compared with the value of 0.01 found for frog muscle (Adrian, 1956), and 0.04 for squid axons (Hodgkin & Katz, 1949). The snail neurones are generally rather less selective than these tissues.

(4) *Seasonal variation.* There appears to be a seasonal variation in the intracellular potassium concentrations of snail neurones; this variation is accompanied by a parallel change in the permeability ratio, P_{Na}/P_K , so that the resting potentials are comparatively little affected.

This result is in conflict with the differences between resting and active snails, investigated by Kerkut & Walker (1961), who found quite large differences in the resting potentials. It appears from the present results that the resting potentials of the neurones are to some extent stabilized, by adjustment of the selectivity, to balance any change in the intracellular potassium concentration. Examination of the individual results tends to confirm this; the correlation coefficient between permeability ratio and intracellular potassium concentration, calculated from all fifty-seven results, is 0.76, which represents a highly significant correlation ($P < 0.001$). This could be another example of the 'self-stabilising' tendency of snail neurones.

The behaviour of the intracellular potassium concentration differs from that found by Kerkut & Meech (1967), who found that the maximum value was reached in the autumn. Their results were, however, obtained from experiments on one particular cell, whose behaviour may not reflect that of the bulk of the neurones. Moreover, their estimates of the intracellular potassium concentration were based on extrapolation of the resting potential, according to the Nernst equation, and are extremely high (200–300 mM). From the present results (e.g. Fig. 4 A) it appears that, in the majority of cases, linear extrapolation of the 'resting potential versus log. potassium concentration' plot does not give a true value for the intracellular potassium concentration.

It is difficult to attach any specific interpretation to the seasonal behaviour; in combination with the results of Kerkut & Walker (1961), it suggests that the snails might be most active during the spring (March to May), and least so in autumn (August to October), which is reasonable. On the other hand, Hughes & Kerkut (1956) and Kerkut (1958) have suggested that increased activity of terrestrial molluscs may result from dilution of their haemolymph, in which case it would be expected to be accompanied by loss of potassium from the neurones.

SUMMARY

1. The resting potentials of giant neurones in the abdominal ganglia of the snail, *Helix aspersa*, have been measured, and their dependence on the extracellular concentration of potassium ions has been investigated.

2. The behaviour of the resting potentials differs considerably from that of a potassium electrode, as given by the Nernst equation. A modification of the equation derived from the 'constant-field' theory is described, which transforms the equation for the resting potential into a linear relationship; it is found that the experimental results can be fitted quite well by this equation, for potassium concentrations up to 12 mM. This is taken as evidence that the relative permeabilities of the cell membrane to potassium and sodium ions are independent of the external potassium concentration over the range of concentrations used.

3. By fitting 'best straight lines' to the experimental data, estimates can be obtained from the 'constant-field' equation of the intracellular potassium concentration, and the ratio of permeabilities of the cell membrane to sodium and potassium ions, P_{Na}/P_K . The average results from experiments on fifty-seven neurones are:

$$\begin{aligned} \text{intracellular potassium concentration, } [K^+]_i &= (92.9 \pm 4.3) \text{ mM.} \\ \text{ratio of permeabilities, } P_{Na}/P_K &= 0.180 \pm 0.015, \end{aligned}$$

the error in each case being one standard deviation, calculated from the spread of the results.

4. The average intracellular potassium concentrations of snail neurones show a distinct seasonal variation, being highest in the spring and lowest in the autumn. These changes are accompanied by changes in the selectivity of the cell membranes, as characterized by the ratio of permeabilities to sodium and potassium ions, so that the resting potentials of the cells are comparatively little affected. The changes could be connected with changes in the pattern of activity of the animals.

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