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EFFECT OF TEMPERATURE ON MEMBRANE POTENTIAL AND IONIC FLUXES IN INTACT AND DIALYSED BARNACLE MUSCLE FIBRES

BY REINALDO DIPOLO* AND RAMÓN LATORRE

From the Department of Physiology, Johns Hopkins University, School of Medicine, Baltimore, Maryland and Laboratory of Biophysics, NINDS, National Institutes of Health, Bethesda, Maryland, U.S.A.

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SUMMARY

1. The temperature-dependent component of the resting potential in intact, cannulated and dialysed fibres from the muscle of the barnacle *Balanus nubilus* was studied under a variety of different experimental conditions. A decrease in temperature from 22 to 12° C produced a mean depolarization of 10 mV.

2. Neither addition of strophanthidin, nor replacement of external sodium by lithium affect the voltage shift induced by temperature. However, the magnitude of the voltage shift depends on the external chloride and potassium concentration.

3. The dialysis technique was applied to measure the potassium, chloride and sodium fluxes as a function of temperature. The Q_{10} for the passive fluxes of these ions was 1·9, 1·7, and 1·4 respectively.

4. The temperature-dependent changes in the passive ionic fluxes combined with the inability of inhibitors of the sodium pump to alter the temperature dependence of the resting potential suggest that the change induced by temperature on the resting potential is primarily caused by a change in the passive permeability ratios, and is not related to active ion transport.

INTRODUCTION

The dependence of resting potential on ionic concentration for the squid axon is adequately described by the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949). This equation also predicts the temperature dependence of the resting potential via a multiplicative factor, RT/F,

* Present address: Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas, Venezuela.

where R is the gas constant, F the Faraday and T the absolute temperature. However, such simple temperature dependence is rarely observed.

In general, departures from the simple RT/F temperature dependence must be expected if some portion of the resting potential is caused by electrogenic sodium transport (as is the case for a variety of nerve cells (cf. Senft, 1967; Carpenter & Alving, 1968; Gorman & Marmor, 1970), or if there are differential temperature variations of the passive ion permeabilities.

Evidence for the last type of mechanism has been found in *Nitella translucens* by Hogg, Williams & Johnstone (1968). For this preparation, the voltage shift induced by temperature can be explained in terms of an increase in the ratio of sodium to potassium permeabilities with temperature.

For barnacle muscle fibres, Hinke & McLaughlin (1967) found that the resting potential increased by about 13 mV when the temperature was raised from 7 to 25° C, a factor four times greater than the constant field prediction of 3·4 mV. From the existing data, it is not known whether this phenomenon is caused by a change in the permeability ratio P_{Na}/P_K, or by changes in an electrogenic ion pump.

In this paper, we show that the temperature-dependent component of the resting potential is insensitive to inhibitors of the sodium pump. Moreover, the large size of the barnacle muscle fibre permits measurements of the ionic fluxes with radioactive isotopes by means of the dialysis technique (Brinley & Mullins, 1967; Mullins & Brinley, 1967). This technique provides the possibility of measuring ionic fluxes at different temperatures in a single fibre. In this manner, we avoid the error arising from the comparison of fluxes measured in different fibres. Another advantage of this technique is the possibility of controlling the internal ionic medium without much disruption of the cell. We have employed this technique and determined ionic fluxes at different temperatures. Our experiments indicate that the phenomenon under study is most probably related to a variation in the passive permeability ratios.

Some of this work has been already described briefly (Latorre & DiPolo, 1971).

METHODS

Maintenance of specimens and dissection of single fibres

Two different species of barnacle were used in this study, *Balanus nubilis* and *Balanus aquila*. All material was collected in Monterey Bay, California, by the Pacific Biomarine Company and transported by air to Baltimore, Maryland. Specimens were kept in an aquarium inside a cold room and they were supplied with recirculated filtered artificial sea water at 8–12° C. Under these conditions, the barnacles survived for several months.

Preparation of the muscle bundles was described by Hoyle & Smyth (1963). The depressor *scutorum rostralis* were used most frequently because these fibres presented a more circular cross-section. Each bundle was isolated with a fragment of scutum attached to the tendonous end and a piece of shell at the other end. Single muscle fibres were dissected in standard saline by cutting the tendon from the scutum and gently tearing the strands of connective tissue with a pair of scissors. The fibre was lifted up with forceps and cut in the air at the end closer to the shell. Cutting the fibre usually resulted in a slight contraction at the ends. In order to avoid further spreading of the contraction along the length of the fibre, the cut end was blotted by touching it briefly with filter paper and immersing a few millimetres in mineral oil. Finally, a fibre 3·5–4·5 cm in length was extended on a blob of petroleum jelly (Vaseline) well covered with saline, keeping both ends overlaid with Vaseline. After isolation, the fibre was left undisturbed for about 5 min before cannulation.

Dialysis procedure

The porous glass capillaries used here were supplied by Corning Glass Co., Corning, N.Y. The dimensions were as follows: length 12–14 cm, diameter 120–200 μ, wall thickness 50–75 μ and length of porous region 1·5–2·0 cm. The porosity of the capillaries as supplied by the manufacturer was further increased by soaking the capillaries in 60 mM-Na-EDTA (sodium diethylenediamine tetraacetate) (pH: 6·8) for 10–40 hr at room temperature. The degree of porosity was checked using Phenol Red as a marker. Porous capillaries were mounted in Leucite holders and stored in a solution of 20 % ethanol in water.

The efflux–influx chamber was patterned after the squid axon chamber of Brinley & Mullins (1967). Significant modifications in design were as follows.

(1) Two air gaps were created to avoid shunting of the membrane potential by the regions damaged by cannulation.

(2) The vertical motion of a micromanipulator holding the entire chamber was used to position the fibre in the central slot. Fig. 1 shows a schematic drawing of the apparatus used to hold the cannulated fibre during dialysis.

A single muscle fibre was placed in the dialysis chamber and cannulated at both ends using glass capillaries of 450 μ diameter (end cannulas). A few microlitres of calcium binding solution (Tris-EGTA: Tris (hydroxymethyl) amino methane; EGTA, ethyleneglycol-bis (β-aminoethyl ether) N, N^1-tetraacetic acid) was injected into the cut ends through the end cannulas. The blocking of the contraction mechanism in these regions permits the immersion of the whole fibre in saline during the insertion of the porous capillary without shortening of the fibre.

The porous capillary was introduced through the left-hand end cannula and slowly steered through the fibre, then through the right-hand end cannula until the porous region lay in the centre of the collection chamber. The internal structure of the fibre offers some mechanical resistance during the impalement; it was found that the injection of a few microlitres of perfusion solution along the centre of the fibre greatly reduced the resistance observed during the insertion. Apparently this procedure does not cause any distortion of the fibre and the increase in volume of the fibre was less than 10 %.

Following the insertion of the porous capillary, a second glass capillary (80–100 μ tip diameter) filled with 0·5 M-KCl for measuring the membrane potential was introduced through the right-hand cannula until the tip reached the middle of the porous region. After the fibre was positioned into the slot, a system of greased blocks converted the guard compartment into water-tight channels (see Fig. 1).

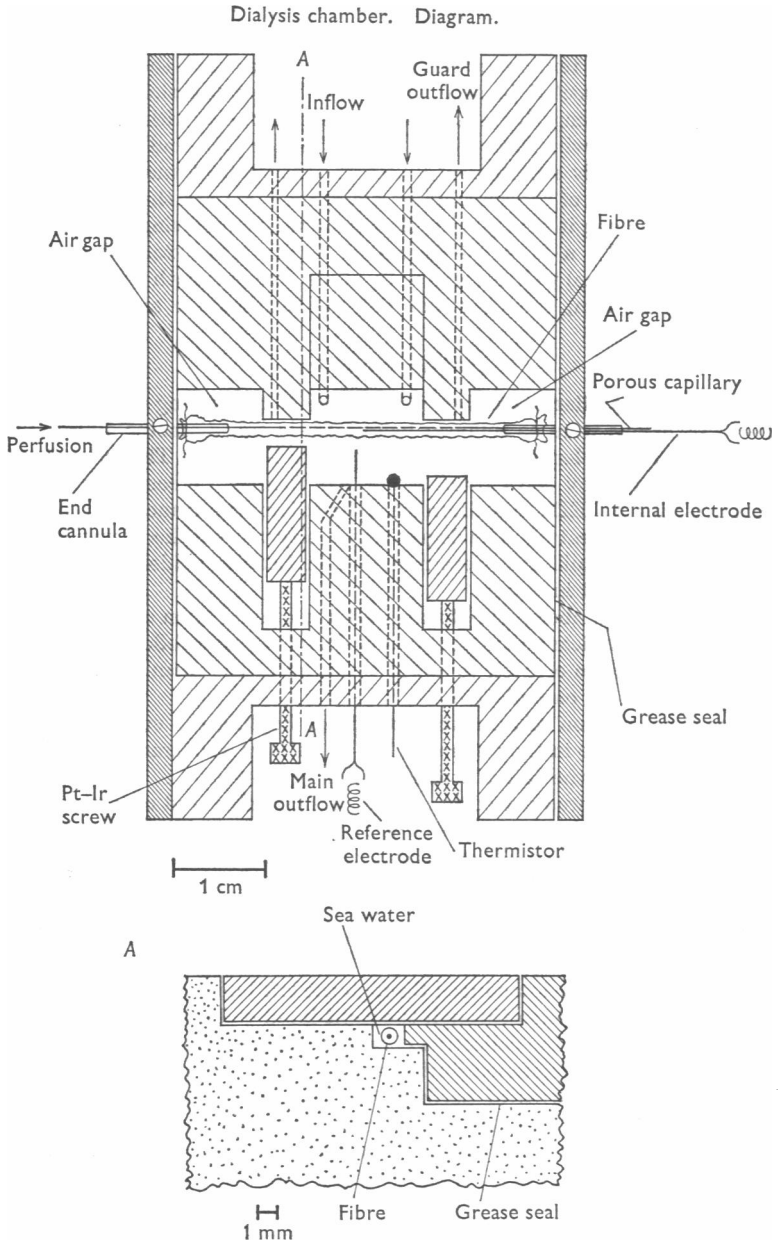

Fig. 1. Schematic diagram of the experimental chamber. *A* is a cross-section at the indicated level.

Membrane potential measurements

The membrane potential of non-cannulated fibres was recorded with glass micropipettes filled with 3 M-KCl, the electrode resistances were 5–10 MΩ. Micropipettes with more than 5 mV tip potential were discarded.

The fibre was tied at both ends with fine strings and placed in the slot of the chamber. The cut end was isolated from the centre compartment by an air gap. The insertion of the micro-electrode was done in the area closer to the tendon. The membrane potential was obtained as the potential difference between the micropipette inserted in the fibre and a small polyethylene tube containing 3 M-KCl–1 % agar just outside the fibre. Silver–silver chloride electrodes were used as reversible electrodes.

Membrane potentials in both cannulated and cannulated-and-dialysed fibres were recorded using a longitudinally inserted glass capillary of 90–100 μ in diameter filled with 0·5 M-KCl. The same system of reference electrodes was used.

Flux determinations

Efflux experiments. Effluxes were determined by replacing the internal dialysis fluid by a dialysis fluid containing a radioactive isotope of the ion and collecting the external solution flowing through the chamber. The internal dialysis solution was delivered to the porous capillary by a motor-driven syringe at a rate of 2–2·5 μl./min. The external solution was pumped to the centre compartment by means of a peristaltic pump at a rate of 1·2–3 ml./min. Efflux samples were collected by suction at regular intervals of time (3 or 5 min). Temperature was controlled to $\pm$ 0·5° C by flowing the solution delivered by the pump through a thermo-electric immersion cooler system before reaching the chamber. Washout of the chamber was about 95 % completed in 0·5 min. Efflux samples were collected in plastic test tubes, spread in planchets, dried in an oven, and counted in a low background gas flow counter.

Influx experiments. For influx experiments the same chamber was used and the entire porous length of the capillary was used to collect the isotope. The portions of the fibre out of the porous region were covered with thick grease. The solution in the centre compartment was replaced by a radioactive one and the perfusate was collected from the tip of the porous capillary by capillary action using glass tubes attached to a manipulator. Collection samples were taken usually every 5 min. Temperature control was achieved by circulating a mixture of ethylene glycol-water underneath the chamber from a thermal bath.

Solutions

The composition of the various external solutions used is given in Table 1. The pH of these solutions was adjusted to 7·5. Strophanthidin (Sigma Chemical Co.) was added directly to the saline solution at concentrations from 1×10^{-5} to 5×10^{-5} M. Radioactive sea water was made by adding solid [^{22}Na]NaCl [^{36}Cl]NaCl directly to the saline. The increase in Na or Cl content after the addition of the radioactive isotope was less than 5 %.

The standard dialysis solution used had the following composition (mM): K-isethionate or K-aspartate, 170; KCl, 25; Na-TES, 15 (TES, N-[2-hydroxy-1, 1-bis (hydroxymethyl) ethyl] taurine): Tris-EGTA, 10; $MgSO_4$, 4; sucrose, 550. The pH was adjusted to 7·0. Radioactive solutions were made adding solid [^{42}K]KCl or/and [^{36}Cl]KCl. All solutions were stored at $-90°$ C. In the dialysis technique, the internal perfusate must contain 10 mM-EGTA, in order to keep the amount of free calcium

TABLE 1. Composition of solutions used

Ref.	Solution	NaCl mM	LiCl mM	KCl mM	$CaCl_2$ mM	$MgCl_2$ mM	Na-MS* mM	K-MS* mM	Ca-P* mM	Tris-Cl mM	NaTES mM	pH
A	Standard saline	465	0	10	25	8	0	0	0	0	2	7·5
B	Li saline	0	465	10	25	8	0	0	0	10	0	7·5
C	K-free saline	465	0	0	25	8	0	0	0	0	2	7·5
D	Low Cl-saline**	0	0	0	0	8	465	10	25	0	2	7·5
E	High K-saline	435	0	40	25	8	0	0	0	0	2	7·5

* Na-MS, sodium methanesulphonate; K-MS, potassium methanesulphonate; Ca-P, calcium propionate.

below the threshold concentration for contraction. Portzehl, Caldwell & Rüegg (1964) have reported threshold calcium concentration in *Maia squinado* of the order of 6×10^{-7} to $1{\cdot}5 \times 10^{-6}$ M.

Calculations

The efflux (p-mole/cm² sec) was calculated according to the following expression:

$$J_e = \frac{\text{cpm collected in the external perfusate}}{\text{(specific activity of the dialysis solution) (surface area) (time)}}.$$

The influx (p-mole/cm² sec) was calculated according to the following expression:

$$J_e = \frac{\text{cpm collected in the internal perfusate}}{\text{(specific activity in the soak solution) (surface area) (time)}}.$$

The area of the fibre was taken as the area of a cylinder and no corrections were made for the well known invaginations of the sarcolemma. Selverston (1967) has estimated an increase in surface area of at least tenfold due to the giant clefts and the well developed transtubular system.

RESULTS

Experiments on intact fibres

Effect of temperature on the resting potential. Fig. 2 shows the effect of temperature on resting potential measured by means of micro-electrodes (filled circles) in a barnacle muscle fibre. The experimental points can be fitted by a straight line in the range of 22–11° C. The mean depolarization (sixteen fibres) in this range of temperature is $9{\cdot}5 \pm 2{\cdot}0$ mV, with an average resting potential of $-70 \pm 3{\cdot}5$ mV at 22° C. Taking the values for the internal potassium concentration reported by Brinley (1968) for the same species, the equilibrium potential for potassium would be $-67{\cdot}7$ mV at 22° C, a value which is very close to the experimental result given above and to previous values reported (McLaughlin & Hinke, 1966; Brinley, 1968).

The open circles of Fig. 2 show the resting potential as measured in a cannulated muscle fibre with a probe electrode inserted in the fibre through one of the cannulas. In this case, the average resting potential was -53 ± 3 mV at 22° C (thirteen fibres). The mean depolarization when temperature was varied from 22 to 11° C was 11 ± 3 mV. Comparing this last value with the 9·5 mV depolarization obtained for the same change in temperature in intact fibres we see that despite the fact that the absolute values of resting potential obtained with these two methods differ, the temperature-dependent component remains unaltered. The low values of resting potential obtained with cannulated fibres might be due to some damage produced upon insertion of the electrode into the fibre and/or to the liquid junction potential between the 0·5 M-KCl probe and the cytoplasm of the muscle fibre. The liquid junction potential between a 0·5 M-KCl micro-electrode and the axoplasm can be as high as 12 mV in the giant

axon of the squid (Cole & Moore, 1960). This potential difference could explain the difference in resting potential between cannulated and intact fibres shown in Fig. 2. However, there is no similar junction potential data for barnacle muscle fibres. Hence, we preferred not to make corrections for this type of potential, but rather to present the measured potentials.

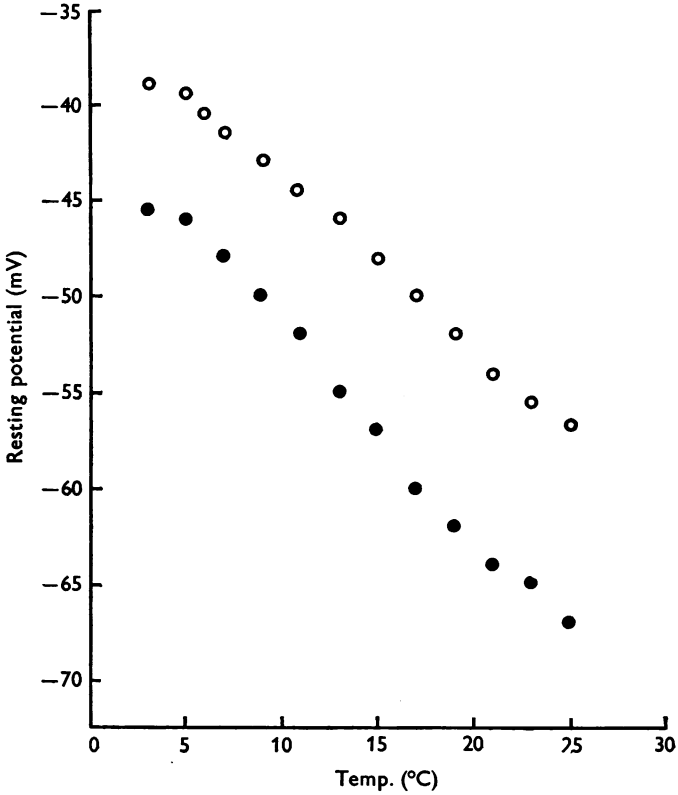

Fig. 2. Effect of temperature on the resting potential in an intact muscle fibre (●) in which the resting potential was measured with glass microelectrodes or in a cannulated fibre (○) in which a probe electrode was inserted axially through one of the cannulas.

Measurements of the temperature-dependent component of the resting potential in the presence of inhibitors of the sodium pump. To test the possibility that this large decrease in the resting potential with temperature was due to the presence of a metabolic component, the inhibitor strophanthidin was added to the external medium at concentration of 10^{-5}–5×10^{-5} M. Higher concentrations of strophanthidin were not used because

of the toxicity of this substance at 10^{-4} M (Brinley, 1968). Fig. 3 shows that the temperature effect on the resting potential remains unchanged even 40 min after the addition of the inhibitor.

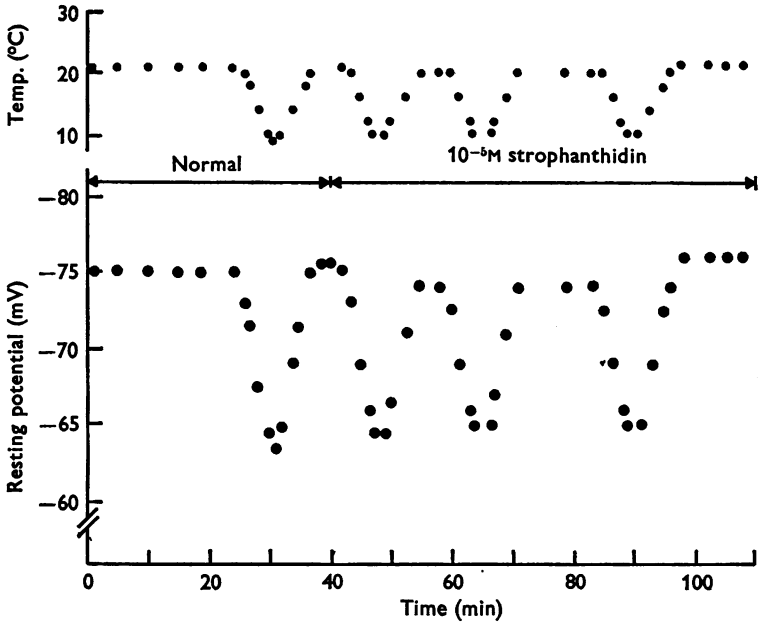

Fig. 3. Simultaneous recording of temperature and membrane potential in an intact muscle fibre immersed first in standard saline (normal) and then in saline with 10^{-5} M strophanthidin. The shift in potential induced by a change of temperature from 20 to 10° C was 8·5, 8·5, 8·0 and 7·5 mV respectively.

Replacement of the external sodium by lithium or removal of the external potassium ions also did not decrease the temperature effect in either intact or cannulated fibres. Furthermore, it was found that potassium-free solutions produced an increase in the voltage shift induced by temperature. The analysis of five experiments on intact fibres in potassium-free solutions gives an average of 15 ± 2 mV, in comparison with the control made in the same fibres that gave an average change of 9 ± 1 mV in the temperature range of 22–11° C. At a constant temperature, potassium-free solution produced a hyperpolarization ranging from 5 to 10 mV (five fibres). A less pronounced hyperpolarization, 2–5 mV, was found when the external sodium was replaced by lithium. Finally, strophanthidin had no effect on the resting potential.

Effect of external chloride concentration on the voltage shift induced by temperature. Hagiwara, Chichibu & Naka (1964) showed that in the

barnacle muscle fibres other ions in addition to potassium (mainly chloride and sodium) contribute in establishing the resting potential.

Fig. 4 shows the result of an experiment in which the normal saline was suddenly changed to a saline containing only 30 mM chloride, the rest of the chloride being replaced by the impermeant ion, methanesulphonate. In agreement with the results obtained by Hagiwara *et al.* (1964), a fast depolarization took place with a mean depolarization of 12 ± 2 mV (five fibres).

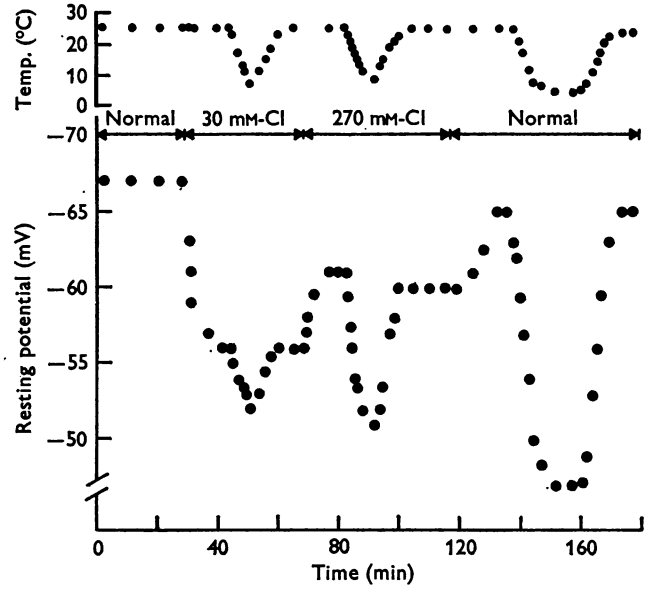

Fig. 4. Effect of temperature on resting potential in conditions of low external chloride concentration. The solutions were standard saline (*A*, Table 1), low chloride saline (*D*, Table 1) and a mixture of *A* and *D* in order to give 275 mM final concentration of external chloride. Intact muscle fibre.

This figure shows that the temperature-dependent component of the resting potential depends on the external chloride concentration. When the external chloride concentration is 30 mM, a decrease in temperature from 25 to 8° C produces a change in resting potential of only 4 mV. The same change in temperature with an external chloride concentration of 270 mM induces a voltage shift of 10 mV. When the muscle is returned to normal saline, there is an almost complete recovery of the initial resting potential value. A decrease in temperature from 25 to 8° C at this time induces a depolarizing voltage shift of 15 mV, which is completely reversible on warming.

The effect of temperature on resting potential and ionic fluxes in dialysed muscle fibres

Resting potential. The experimental value of the resting potential in dialysed muscle fibres was -59 ± 4 mV (sixteen fibres). The mean depolarization when the temperature was decreased from 22 to 11° C was 14 ± 3 mV. Also in dialysed fibres, the voltage shift induced by temperature is insensitive to inhibitors of the sodium pump.

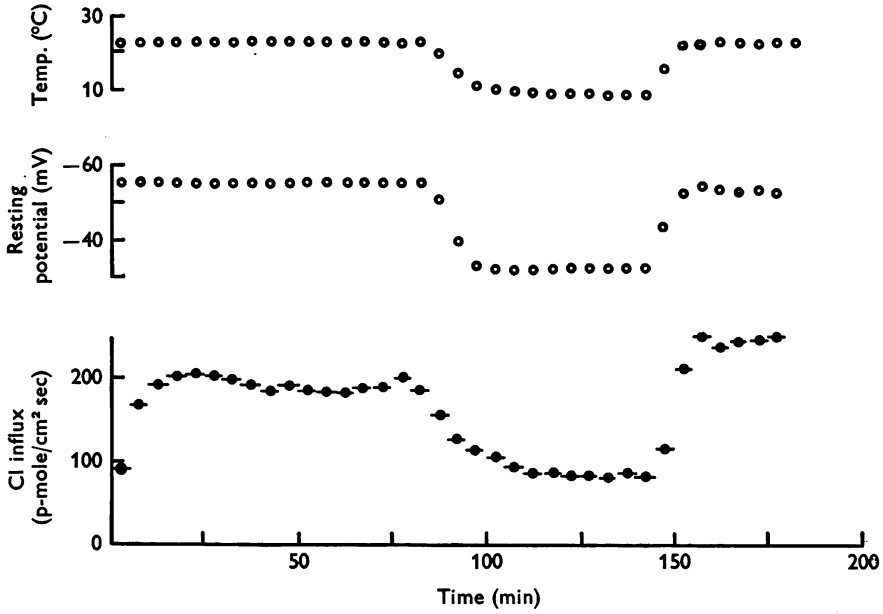

Fig. 5. Effect of temperature on the influx of chloride in a dialysed muscle fibre. Temperature and resting potential were recorded and chloride influxes were measured as a function of time at different temperatures.

Each point represents the average influx (p-mole/cm² sec) determined during intervals of 5 min, the horizontal bars through the experimental point being the collection time.

The fibre was bathed in standard saline with $6{\cdot}7 \times 10^{-3}$ cpm/p-mole ^{36}Cl. The composition of the dialysis fluid was as given in Methods.

Ionic fluxes. Fig. 5 shows the effect of temperature on the influx of chloride in a dialysed muscle fibre. The steady-state influx of chloride was reached 20 min after the addition of the isotope to the external medium. In steady-state the influx of chloride at 22° C was 191 p-mole/cm² sec. A decrease in temperature of 13° C produces a decrease in the chloride influx to 84 p-mole/cm² sec and a concomitant depolarization of 23 mV.

Simultaneous measurements of the effluxes of potassium and chloride are shown in Fig. 6. Resting effluxes for chloride and potassium were 162 and 135 p-mole/cm^2 sec at 23° C respectively and 39·5 and 56 p-mole/-cm^2 sec at 9° C.

The effect of partial removal of the external chloride from 541 to 30 mM on the chloride and potassium effluxes is also shown in Fig. 6. In these

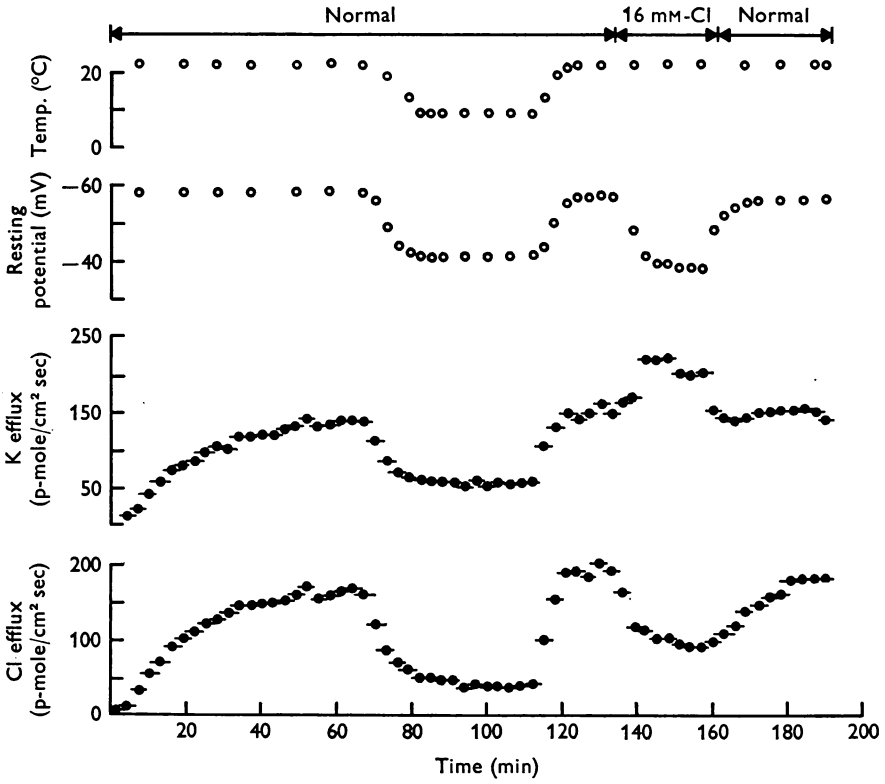

Fig. 6. Simultaneous determinations of the effect of temperature upon the resting potential and the effluxes of potassium and chloride as a function of time in a dialysed muscle fibre.

The fibre was bathed in standard saline (normal) and was dialysed with a solution as described in Methods plus 0·21 cpm/p-mole ^{42}K and 0·035 cpm/p-mole ^{36}Cl. The samples containing ^{42}K and ^{36}Cl were dried in planchets and counted immediately after the experiment. After complete decay of ^{42}K the samples were counted again obtaining the counts provenant from the ^{36}Cl. Subtraction between the first and second counting gave the amount of ^{42}K present. Appropriate corrections were made for the ^{42}K decay.

The effect of low external chloride concentration on resting potential and fluxes is also shown (solution *A*, Table 1, was replaced by solution *D*, Table 1).

conditions, the efflux of chloride was 92 p-mole/cm² sec and the efflux of potassium 209 p-mole/cm² sec. Collected data from four experiments gave a reduction of 50% in the chloride efflux and an increase of 25% in the potassium efflux, when the external chloride concentration was reduced to 30 mM.

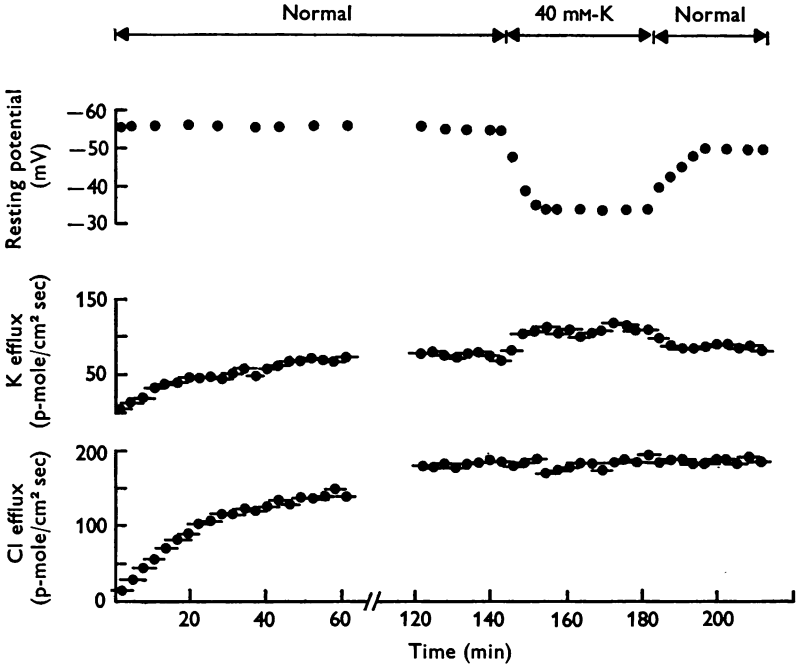

Fig. 7. Effect of high external potassium concentration on resting potential, potassium efflux and chloride efflux in a dialysed muscle fibre. The fibre was bathed in standard saline (normal) and was dialysed with a solution containing 0·02 cpm/p-mole ^{36}Cl and 0·03 cpm/p-mole ^{42}K. At the time indicated in the Figure, the standard saline was changed to one containing 40 mM-KCl (*E*, Table 1). Temperature 22° C.

Fig. 7 shows that a depolarization induced by high external potassium concentration does not alter the chloride efflux, but causes a change in the potassium efflux from 75 p-mole/cm² sec to 110 p-mole/cm² sec. Thus, the chloride efflux is not altered by the change in membrane potential brought about by the change in the potassium concentration. This result would be expected if a large fraction of the chloride flux is attributable to chloride-chloride exchange diffusion as has been shown by DiPolo (1971). Chloride efflux insensitive to changes in the external potassium concentration has also been found for muscle fibres of the crab *Maia squinado* (Richards, 1969).

The influx of sodium as a function of temperature and temperature-induced potential change is shown in Fig. 8. A decrease in temperature from 22 to 7° C produces a change in membrane potential from −53 to −37 mV and a change in the sodium influx from 56 p-mole/cm² sec to 29 p-mole/cm² sec.

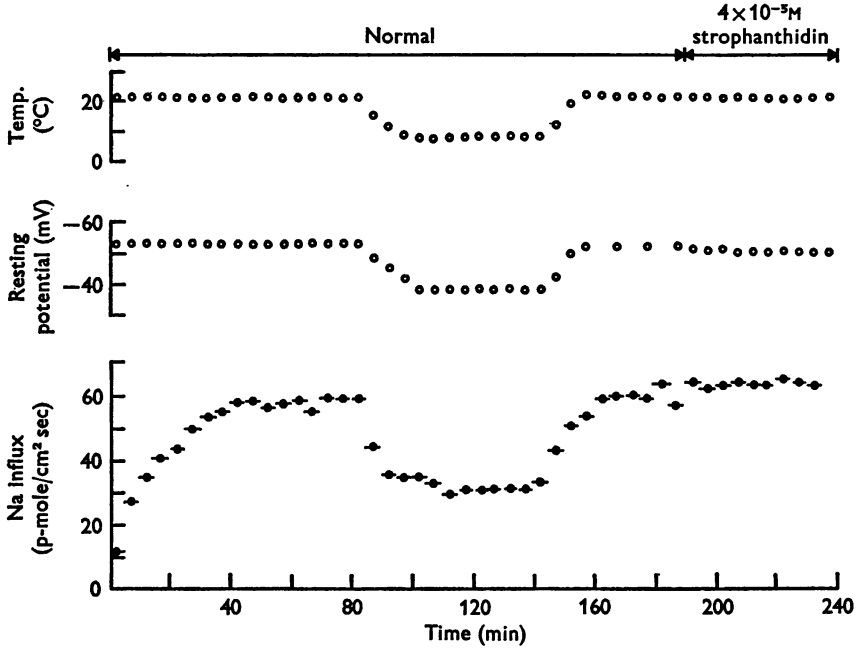

Fig. 8. Effect of temperature on the sodium influx in a dialysed muscle fibre. The fibre was bathed in standard saline with 0·025 cpm/p-mole ^{22}Na (normal). Strophanthidin (10^{-4} M) was added directly to the radioactive solution bathing the fibre to give the final concentration indicated in the Figure. The composition of the dialysis fluid was as given in Methods. Resting potential was also recorded.

Addition of strophanthidin at a concentration of 4×10^{-5} M has no effect on the sodium influx and indicates that there is not a substantial strophanthidin-sensitive sodium-sodium exchange component in the sodium influx.

The effect of temperature on the potassium, sodium and chloride fluxes is summarized in Table 2. It should be noted that both potassium efflux and chloride influx are more affected by temperature than the sodium influx.

TABLE 2. Temperature dependence of potassium, sodium and chloride fluxes in dialysed muscle fibres

Ion	Temperature (°)	Resting potential (mV)	Influx (p-mole/cm² sec)	Efflux (p-mole/cm² sec)	Q_{10}
K (4)	22	−59 ± 1	—	108 ± 27	1·9
	9	−40 ± 4	—	43 ± 8	—
Na (4)	22	−62 ± 1	52 ± 7	—	1·4
	8	−47 ± 2	28 ± 3	—	—
Cl (5)	22	−57 ± 5	144 ± 20	—	1·7
	9	−37 ± 3	65 ± 5	—	—
Cl (6)	22	−59 ± 2	—	146 ± 9	3·0
	9	−40 ± 3	—	37 ± 2	—

Values are given as mean ± S.D. The number of determinations is given in parentheses.

DISCUSSION

Non-electrogenic characteristics of the voltage shift induced by temperature

The present experiments have ruled out the possibility that the sodium pump is involved in the voltage shift produced by temperature in the muscle fibre of the barnacle. Addition of strophanthidin, removal of the external potassium, and replacement of the external sodium by lithium all strongly reduced the sodium efflux in barnacle muscle fibres (Brinley, 1968). However, none of these conditions affect the variation of resting potential with temperature. These results on the barnacle muscle fibre contrast with the results obtained in nerve cells (Senft, 1967; Carpenter & Alving, 1968; Gorman & Marmor, 1970). In nerve cells the conditions described above lead to a decrease in the temperature-dependent component of the resting potential to a value close to that expected from the thermodynamic factor, RT/F. Moreover, Rang & Ritchie (1968) have indicated that the post-tetanic hyperpolarization ascribed to an electrogenic pump in non-myelinated nerve fibres is increased by replacement of the external chloride by impermeable anions. In contrast, the removal of the external chloride in barnacle muscle cells produces a substantial decrease in the voltage shift induced by temperature. However, when chloride concentration is varied no transient change in resting potential is observed. This is true despite the effect of chloride on the temperature-dependent component of the resting potential because in barnacle muscle fibres Hagiwara, Gruener, Hayashi, Sakata & Grinnell (1968) have determined that at pH 7·7 the ratio of chloride to potassium conductance is about 1/7; this

value is much lower than that found in frog muscle by Hodgkin & Horowicz (1959).

The increase in the voltage shift produced by the removal of the external potassium is also in contradiction with what is expected when an electrogenic pump participates in the process

TABLE 3. Sodium and potassium fluxes in intact, micro-injected and dialysed muscle fibres of the barnacle *Balanus nubilus*

Na influx (p-mole/cm² sec)	K efflux (p-mole/cm² sec)	Temperature (° C)	Technique	Source
49	—	13–16	External loading	Brinley (1968)
—	60	13–16	Micro-injection	Brinley (1968)
35	71	15	Dialysis	Present results

Analysis of the potassium, chloride and sodium permeabilities

Since there has been no systematic study on the ionic fluxes in barnacle muscle fibres under dialysis perfusion conditions, a comparison between the values reported in this paper with previous studies in intact or micro-injected muscle fibres of the same species is given in Table 3.

Table 3 shows that there is a good agreement between the present results and those reported by Brinley (1968). Moreover, Brinley demonstrated that the magnitude of the effluxes of sodium and potassium obtained by micro-injection were within the range of the effluxes obtained by external loading. The potassium efflux in dialysed fibres is higher than that obtained by micro-injection. This discrepancy can be ascribed to the differences in internal potassium concentrations; 195 mM in dialysed fibres compared to 150 mM in the experiments reported by Brinley.

If the resting membrane potential is described by the equation (Goldman, 1943; Hodgkin & Katz, 1949):

$$V = \frac{RT}{F} \ln \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i},$$

where V is the resting potential, P_K, P_{Na}, P_{Cl} represent the permeability coefficient for each ion, $[\ ]_o$ indicate concentrations outside the fibre, $[\ ]_i$ are the concentrations inside the fibre. It seems reasonable to ascribe the observed changes in resting potential with temperature to passive effects arising from differential changes in the ion permeabilities.

If we assumed that both sodium influx and potassium efflux are entirely passive, then the permeability coefficients (P_{Na}, P_K) for both ions can be calculated at two different temperatures using the values obtained for the

fluxes at those temperatures by means of the equations (Hodgkin & Katz, 1949; Katz, 1966):

$$P_{Na} = J_{Na} \frac{RT}{F} \frac{1-\exp(FV/RT)}{[Na]_o}, \quad (1)$$

$$P_K = J_K \frac{RT}{F} \frac{1-\exp(-RT/FV)}{[K]_i}, \quad (2)$$

where V is the resting potential taken as the potential of the inside solution minus that of the outside solution, J_{Na} and J_K are the sodium influx and the potassium efflux respectively. In all the calculations $[Na]_o$ was taken

TABLE 4. Effect of temperature on the permeability coefficients (P) of sodium and potassium ions

Ion	temperature (° C)		$P \times 10^{-6}$ (cm/sec)
K	22		2·60 ± 0·10
	9		0·64 ± 0·06
Na	22		0·042 ± 0·004
	9		0·027 ± 0·003
P_N/P_K	22	0·016	—
	9	0·042	—

Values are mean ± S.D. of four experiments. P_{Na} and P_K were calculated using the values for potassium efflux and sodium influx given in Table 2.

as 465 mM and $[K]_i$ as 195 mM (concentration in the dialysis fluid). The values obtained in this way are shown in Table 4. According to this Table the permeability ratio P_{Na}/P_K increases 2·6 times when temperature is decreased from 22 to 9° C. However, the permeability coefficients are described by eqns. (1) and (2) only if the fluxes are truly passive. Although there is a good deal of evidence that sodium influx and potassium efflux do not have appreciable active components (Brinley, 1968), there may be errors in the measured fluxes coming from exchange diffusion or flux interaction.

The large chloride–chloride exchange diffusion component of the chloride flux (DiPolo, 1971) makes it difficult to estimate the chloride permeability. Experiments such as those shown in Figs. 6 and 7 indicate that at least 50% of the chloride efflux is due to exchange diffusion. However, the exact amount of this component is not yet known.

The present experiments indicate a temperature dependance of the P_{Na}/P_K ratio in the correct direction to explain the temperature-dependent shift in potential. However, the effect of changes in the external chloride concentration on the temperature-dependent component of the resting

potential remains unclear and needs further experimentation. Furthermore the effect of temperature on the P_{Na}/P_K ratio must be confirmed by electrical data.

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