Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Active Ion Transport and Resting Potential in Smooth Muscle Cells

R. Casteels, G. Droogmans and H. Hendrickx

Phil. Trans. R. Soc. Lond. B 1973 **265**, 47-56
doi: 10.1098/rstb.1973.0008

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1973 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B. **265**, 47–56 (1973) [47]
Printed in Great Britain

Active ion transport and resting potential in smooth muscle cells

By R. Casteels, G. Droogmans and H. Hendrickx
Laboratorium voor Fysiologie, Universiteit Leuven, Belgium

The diffusion potential in taenia coli cells calculated from the intra- and extracellular ion concentrations and from the calculated membrane permeabilities is less negative than the measured membrane potential. This discrepancy could be due to a continuous contribution of an electrogenic ion pump to the membrane potential. This hypothesis is supported by the finding that the sum of the active ion fluxes is sufficiently large to generate a potential of −20 mV across the membrane. Moreover, an unequivocal electrogenic component of the membrane potential has been demonstrated during maximal activation of the ion pump in K-depleted cells. The importance of this electrogenic component depends on the membrane resistance, a parameter which is very much affected by the experimental procedure. The active Na–K exchange is stimulated by $[Na]_i$ and $[K]_o$ and is inhibited by $[Na]_o$. Furthermore, it has been observed that 47 % of the Na-exchange of Na-enriched tissues is due to Na exchange diffusion.

Introduction

Active ion transport can affect the membrane potential of excitable cells in two ways. If there is no net transfer of electrical charge across the membrane, the ion pump is called neutral and by its function it generates and maintains the concentration gradients for the various permeant ions and thereby a diffusion potential E_{diff} which is determined by the passive ion movements through the membrane. If, however, the ionic pump is able to produce a net transfer of electrical charges across the membrane, it generates a current i_p, which by its passing through the membrane resistance (R_m) directly affects the membrane potential. Under steady-state conditions and in the presence of such an electrogenic sodium pump the net passive ionic current across the membrane will be different from zero, because the sum of the passive ion fluxes must now balance the flow of a charge caused by the pump.

In cells in which we have a diffusion potential and an electrogenic potential, the total resting potential (E_m) can be considered as a sum of both

$$E_m = E_{diff} + i_p R_m.$$

If the total ionic concentrations at both sides of the membrane are the same and if there is only one anion involved in the system, the diffusion potential will not vary with current flow (Finkelstein & Mauro 1963). This diffusion potential can then be calculated by the Goldman equation

$$E_{diff} = \frac{RT}{F} \ln \frac{P_{Na}[Na]_o + P_K[K]_o + P_{Cl}[Cl]_i}{P_{Na}[Na]_i + P_K[K]_i + P_{Cl}[Cl]_o}.$$

The equation $E_m = E_{diff} + i_p R_m$ can only give a rough estimate of the steady-state membrane potential because of the many simplifying assumptions involved in its derivation and in its application to living cells. It seemed, however, worth while to investigate the resting potential of taenia coli cells from this point of view.

The hypothesis that the membrane potential in smooth muscle cells can be affected by an electrogenic Na pump is not new. Burnstock (1958) and Bülbring (1962) have proposed such a mechanism to explain the hyperpolarization of these smooth muscle cells by adrenaline. In

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

this review we present some evidence in favour of the hypothesis that in smooth muscle cells the sodium pump is electrogenic, also under steady-state conditions and is therefore partly responsible for the maintenance of the resting potential.

Ion distribution and ion fluxes in taenia coli cells

The fraction of the membrane potential which is determined by the diffusion potential can be calculated by the Goldman equation. The intracellular concentrations of K, Na and Cl have been determined by analytical procedures and by compartmental analysis (Casteels 1969). The membrane permeabilities for these ions have been calculated from the steady-state flux values by the constant field equations given by Hodgkin & Katz (1949). From these data which are given in table 1, a diffusion potential of -37 mV has been calculated. If the remaining 15 to 20 mV of the resting potential would be due to an electrogenic Na extrusion, the cells would have to produce an ionic current $i_p = -20\ \text{mV}/25\ \text{k}\Omega\ \text{cm}^2 = -0.8\ \mu\text{A cm}^{-2}$. The membrane resistance of 25 kΩ cm^2 is the slope resistance given by Tomita (1966) for taenia coli cells. Because the current–voltage relation of the membrane is nearly linear in the range of -130 to $+10$ mV for taenia coli cells (Kumamoto & Horn 1970) the slope resistance is a good estimate for the chord resistance, which determines the electrogenic potential.

Table 1. The ion concentrations, the calculated equilibrium potentials, unidirectional fluxes and membrane permeabilities of taenia coli cells during steady-state conditions (Casteels 1969)

	Na	K	Cl
extracellular concentration/mmol l^{-1}	137	5.9	134
intracellular concentration/mmol l^{-1}	13	164	58
equilibrium potential/mV	+62	−89	−22
unidirectional flux/pmol cm^{-2} s^{-1}	6.0	5.4	9.4
membrane permeabilities/10^{-8} cm s^{-1}	1.8	11	6.7

The net passive fluxes of K, Na and Cl have been calculated from the equation given by Ussing (1949). They are represented together with the corresponding ionic current in table 2. In a steady state these net passive fluxes must be balanced by equal active fluxes of opposite direction, and one may therefore deduce the existence in taenia coli cells of an active ionic current of $-0.8\ \mu\text{A cm}^{-2}$, a value which agrees with the expected value of $-0.8\ \mu\text{A cm}^{-2}$.

Table 2. Equilibrium potentials and unidirectional flux values from which by the equation of Ussing (1949) the net passive fluxes and the corresponding ion currents have been calculated (Casteels *et al.* 1971*b*)

	Na	K	Cl
equilibrium potential/mV	+62	−89	−22
unidirectional flux/pmol cm^{-2} s^{-1}	6.0	5.4	9.4
net passive flux (Ussing equation)/pmol cm^{-2} s^{-1}	+6	−4	−6
ionic current/μA cm^{-2}	+0.6	−0.4	+0.6

These experimental data indicate that a net active outward current exists in these cells. The high membrane resistance results in an important contribution of the electrogenic pump to the total membrane potential.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Inhibition of the active transport and the electrogenic sodium pump

If an electrogenic component exists a sudden inhibition of the active transport should result in an immediate depolarization of the cell to the value of its diffusion potential, on condition that all other cell characteristics are maintained. This latter condition is not fulfilled for taenia coli cells. An inhibition of the Na–K exchange by ouabain causes a rapid gain of Na and loss of K and Cl, resulting in a change of the diffusion potential. Some evidence for an electrogenic pump is, however, obtained from the fact that the concomitant depolarization occurs more rapidly than the K depletion (Casteels 1966). Metabolic inhibition by 2,4-dinitrophenol and monoiodoacetic acid or by anaerobic and substrate-free conditions not only cause a decrease in the concentration of the energy-rich compounds, but also increase the membrane permeability of smooth muscle cells (Casteels, van Breemen & Wuytack 1972). K-free solutions do not result in a K-free medium at the edge of the membrane, because of the considerable leakage of K out of the cells. Moreover, this experimental condition causes an increase of the K-permeability of the membrane (Casteels, Droogmans & Hendrickx 1971*a*). These secondary effects of the different inhibitory procedures make it difficult to study by means of them the contribution of the electrogenic pump to the membrane potential. We have therefore tried to obtain other evidence for an electrogenic Na pump by producing experimental conditions which stimulate the Na pump and which increase the membrane resistance.

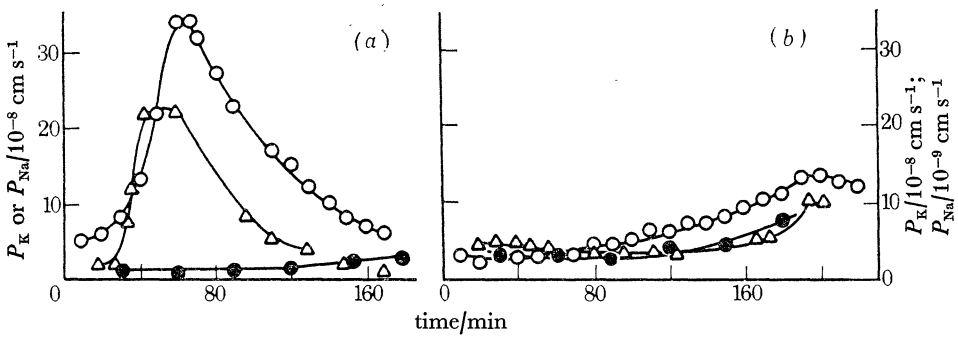

FIGURE 1. The changes of the total membrane conductance (△) in arbitrary units as calculated from the electrotonic potential, of the K-permeability (○) and of the Na-permeability (●) during K-depletion of taenia coli cells. (*a*) represents the changes occurring in a solution containing chloride, (*b*) the changes in a solution containing propionate. (From Casteels *et al.* 1971*a*.)

Change of the membrane potential and membrane conductance during K accumulation by K-depleted cells

The pump activity of the cells can be increased by augmenting the intracellular Na concentration. Such a modification of the intracellular ion content has been obtained by exposing the cells of taenia coli for 4 h to a K-free solution. During this period the cells exchange all their intracellular K for Na. This rapid and complete exchange is due to the large surface/volume ratio in these cells and to the increase of the K-permeability of the cells exposed to a K-free solution containing permeant anions as Cl or NO_3. This increase of P_K does not occur if Cl has been replaced by a slowly penetrating anion as propionate or benzene-sulphonate (figure 1). The simultaneous increase of the transmembrane K-gradient and of P_K explains the transient hyperpolarization of the cells bathed in a K-free chloride solution (Casteels *et al.* 1971*a*)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

If such K-depleted cells are again exposed to a solution containing 5.9 mmol/l K, the intracellular Na is rapidly exchanged for K and at the same time the cells hyperpolarize. The changes of the intracellular ionic concentrations are represented in figure 2. Similar observations have been made on Na-enriched striated muscle cells by Kernan (1962), Frumento (1965), Adrian & Slayman (1966) and others.

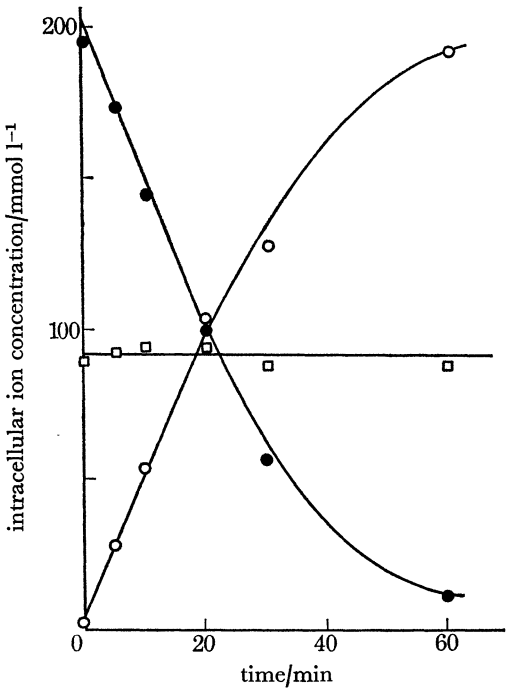

FIGURE 2. Changes of the intracellular ion concentrations of K-depleted taenia coli cells during exposure to a Krebs solution containing 5.9 mmol/l K. The experimental points for $[K]_i$ (○) can be fitted by the equation $[K]_i = 2.0+5.51t-0.039t^2$ and for $[Na]_i$ (●) by $[Na]_i = 200-6.2t+0.05t^2$. The intracellular chloride concentration (□) does not change during the first 60 min.

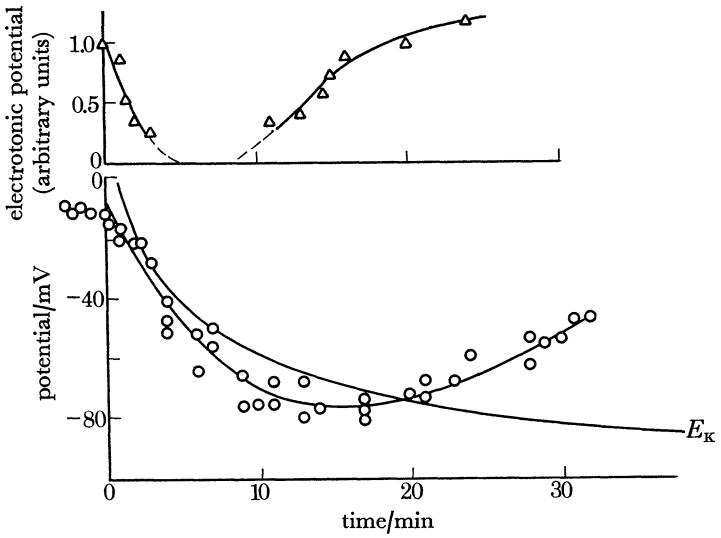

FIGURE 3. Changes of the membrane potential (○) and of the E_K of K-depleted taenia coli cells during K-accumulation from a solution containing 5.9 mmol/l $[K]_o$. The curve representing E_K has been calculated by the Nernst equation from the changes of $[K]_i$. The changes of the amplitude of the electrotonic potential are represented by triangles (△) and expressed in arbitrary units (after Casteels *et al.* 1971*b*).

Downloaded from rstb.royalsocietypublishing.org

The generally accepted criterion for sodium pump electrogenicity is that the membrane potential becomes more negative than E_K, because E_K is mostly more negative than the diffusion potential.

Figure 3 represents the changes of the membrane potential, of the calculated E_K and of the electrotonic potential during the activation of the Na-pump in K-depleted cells. The electrotonic potential measured close to the stimulating electrode can be considered proportional to the square root of the membrane resistance (Hodgkin & Rushton 1946) and has been measured as described by Abe & Tomita (1968). The membrane potential and E_K follow a similar time course during the first 5 min of exposure to a K-containing solution, but later on the membrane potential becomes more negative than E_K. The difference $|E_m - E_K|$ reaches a maximal value of 12 mV after 15 min and later on decreases again to reach values which are more positive than E_K as in the normal steady-state conditions. The membrane resistance, estimated from the electrotonic potential initially decreases to values which can no longer be measured by this technique. The resistance then increases progressively to values higher than the control value in K-free solution. The membrane potential becomes only more negative than E_K when the membrane resistance begins to increase.

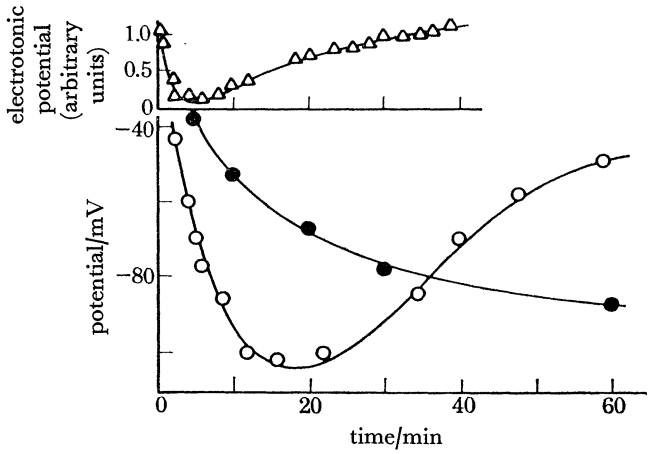

FIGURE 4. Changes of the membrane potential (○) and of the E_K (●) of K-depleted taenia coli cells during K-accumulation from a solution containing 5.9 mmol/l $[K]_o$ in which Cl is replaced by propionate. E_K values have been calculated by the Nernst equation from $[K]_i$. The changes of the amplitude of the electrotonic potential are represented by triangles (△) (after Casteels *et al.* 1971*b*).

The rather small value of the transient difference between the membrane potential and E_K during the initial uptake of K could be due to systematic or experimental errors, and for this reason it was important to study the potential changes in cells, in which the membrane resistance is increased by substituting propionate or other large anions for chloride. This procedure not only eliminates the contribution of chloride ions to the total membrane conductance but also reduces the potassium conductance to about half of its control value (Casteels 1971). The time course of potential changes in such a solution is represented in figure 4. The Na-extrusion and the K-uptake proceed at a similar rate as in the chloride solution, indicating that the pump activity is not affected. The hyperpolarization is, however, much larger and lasts longer. The changes of the electrotonic potential are similar to the ones observed in chloride solution, but never reach the low values observed in the presence of chloride ions.

Comparing the data obtained in chloride and in propionate solutions and taking into account the decrease of P_K in propionate solution one has to conclude that a neutral pump in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

association either with pericellular K-depletion by this pump or with diffusion limitation for K, cannot explain the hyperpolarization. The finding that the hyperpolarization proceeds to a maximal value and thereafter decreases to a value which is less negative than E_K, is probably due to an interaction of different factors. Because initially g_K has a high value as shown in figure 5, the diffusion potential is largely determined by E_K. The electrogenic component remains small in spite of a high i_p because of the low membrane resistance. An increase of the membrane resistance not only increases the potential generated by a given ionic current, but it also implies a decrease of the potassium conductance, so that the diffusion potential moves to a more positive value, further away from E_K. This latter change is probably the main reason for the decrease of the membrane potential after the first 20 min of K accumulation. At this stage the Na extrusion probably proceeds at its maximal rate because $[Na]_i$ is still higher than 50 mmol/l as shown in figure 2.

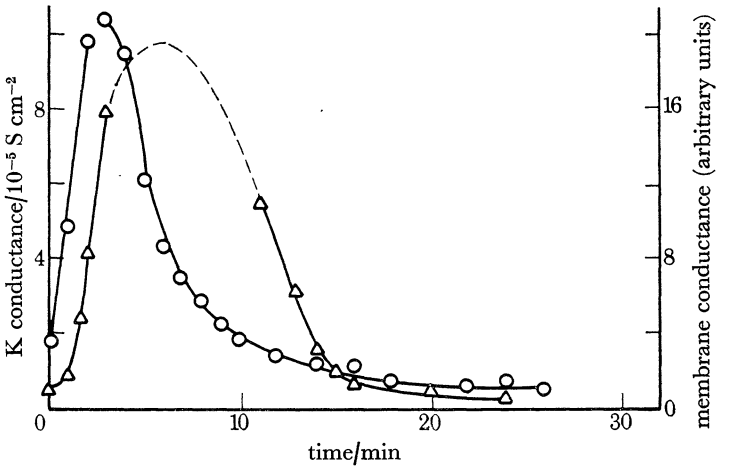

FIGURE 5. The changes of the membrane conductance (△) in arbitrary units (right ordinate) and of the K-conductance (○) during K reaccumulation in a solution containing chloride and 5.9 mmol/l K. The pecked part of the curve, representing the membrane conductance, represents a period during which the electrotonic potential could not be measured.

Under steady-state conditions the pump intensity becomes lower because of the low $[Na]_i$, but the high value of the membrane resistance makes the electrogenic component of the membrane potential remain large.

Some characteristics of the Na-K *exchange in taenia coli cells*

From the characteristics of the ion exchange in other tissues it may be expected that the ion pump will be activated by the intracellular Na and by the external K and might be inhibited by extracellular Na. Experiments designed to study the active sodium extrusion as a function of $[Na]_i$ values between the steady-state value of 13 mmol/l cell water and the maximal value after complete K depletion have not been successful. The main reasons for this failure are the rapid changes in intracellular ion concentrations, in membrane potential and membrane resistance which make it very difficult to calculate the active fraction of the ion fluxes.

The effect of different concentrations of external K on the changes of the intracellular ionic concentrations of K-depleted cells are represented in figure 6. The quadratic regression lines fitting the experimental points for the different external K concentrations are given in table 3. The initial decrease of $[Na]_i$ after adding K to the external medium may be considered as

Downloaded from rstb.royalsocietypublishing.org

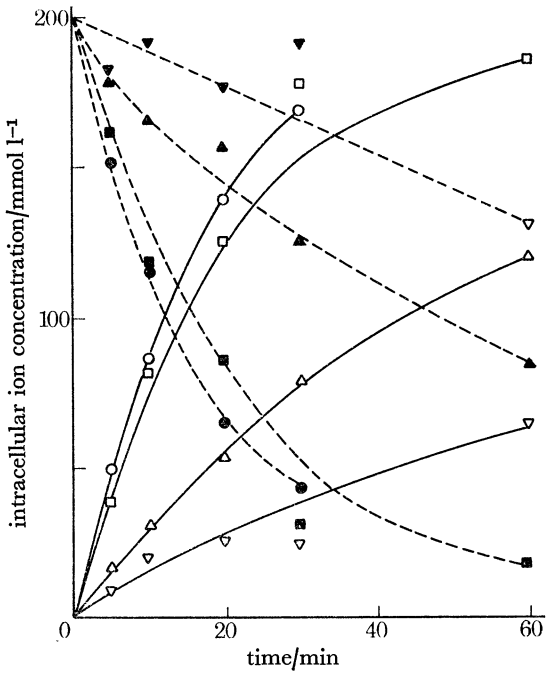

FIGURE 6. The intracellular Na (filled symbols) and K (open symbols) concentrations during reaccumulation of K by K-depleted cells as a function of time of exposure to solutions with different external K concentrations. The following external K-concentrations have been used: 23.6 mmol/l is represented by circles, 11.8 mmol/l by squares, 2.95 mmol/l by triangles and 1.2 mmol/l by inverted triangles. The lines drawn through the experimental points represent the quadratic regression lines given in table 3. The full lines represent the regression lines for $[K]_i$, the pecked lines the regression lines for $[Na]_i$. (From Casteels *et al.* 1971*b*.)

TABLE 3. QUADRATIC REGRESSION LINES CALCULATED TO FIT THE EXPERIMENTAL POINTS REPRESENTED IN FIGURE 6

external K concentration / mmol l^{-1}	functions describing the changes of $[Na]_i$ and $[K]_i$
23.6	$[Na]_i = 195 - 9.39t + 0.145t^2$ $[K]_i = 1.6 + 9.88t - 0.144t^2$
11.8	$[Na]_i = 196 - 7.60t + 0.077t^2$ $[K]_i = 0.4 + 8.45t - 0.089t^2$
2.95	$[Na]_i = 194 - 2.48t + 0.011t^2$ $[K]_i = 0.8 + 3.17t - 0.019t^2$
1.2	$[Na]_i = 201 - 1.23t + 0.003t^2$ $[K]_i = 3.4 + 1.07t - 0.002t^2$

depending largely on active transport because at zero time E_{Na} and the membrane potential have the same value (Casteels, Droogmans & Hendrickx 1971*b*). Consequently the initial active Na efflux ($J^{act}_{Na,\ t=0}$) at different $[K]_o$ can be calculated by the equation

$$J^{act}_{Na,\ t=0} = \left(\frac{d[Na]_i}{dt}\right)_{t=0} \frac{V}{A}\frac{1}{60}$$

in which V/A represents the volume/surface ratio of the cells. These calculated values are represented in figure 7*a* as a function of $[K]_o$. The estimated maximal value for the Na efflux is 22.2 pmol cm^{-2} s^{-1} and the $[K]_o$ value which causes half maximal activation amounts to 4.7 mmol/l. The $[K]_o^{0.5}$ value is higher than the value observed for the isolated ATP-ase (Skou 1965). This is probably due to a shift of the activation curve to the right by the competitive

Downloaded from rstb.royalsocietypublishing.org

inhibition of external Na as observed in other tissues by Baker & Connelly (1966) and by Sjodin (1971). Experimental evidence in favour of this action of external Na will be presented in the next section.

The fractional values of the Na efflux are plotted against the logarithm of the normalized values of $(K)_o$, i.e. $[K]_o/[K]_o^{0.5}$ in figure 7*b*. The experimental points are best fitted by a second-order system. This type of activation curve can be explained by the existence of a carrier system in which the carrier has to be occupied by 2 K to activate the ion transport.

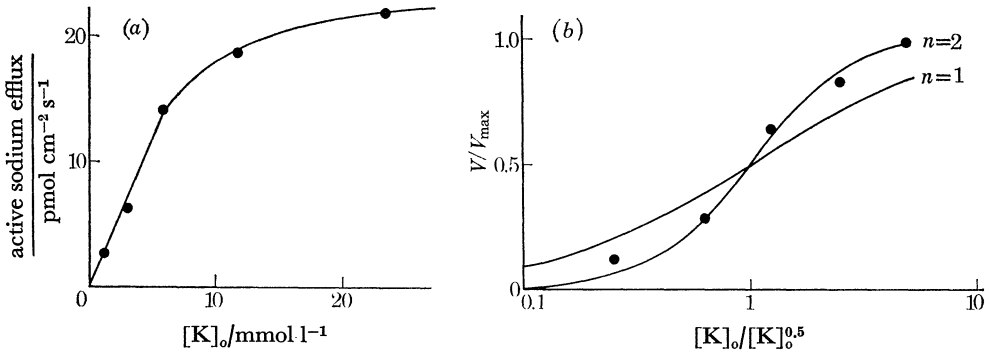

FIGURE 7. (*a*) The initial active sodium efflux at different external K-concentrations. The active Na fluxes have been obtained by differentiation at zero time of the quadratic functions which fit the experimental points represented in figure 6 and which are given in table 3. (*b*) The fractional active Na efflux (V/V_{max}) plotted as a function of $[K]_o/[K]_o^{0.5}$. The experimental points are compared with the normalized dose-response curves of a first- and second-order system.

From the net passive fluxes under steady-state conditions we may deduce a ratio of $-4/6$ for the active K influx/active Na efflux (see table 1), suggesting that also the K/Na flux ratio in taenia coli has an approximate value of 2/3 as in other tissues. This deduction implies that the K ions which have to occupy the carrier to activate the transport are also transferred across the membrane. The ratio active K influx/active Na efflux calculated for K-depleted cells during K reaccumulation equals $-9.2/23$ or -0.4 (Droogmans 1972). This value is lower than the ratio found in steady-state conditions, probably because of the occurrence of Na-exchange diffusion in Na-loaded tissues.

Effect of external Na *on the* Na–K *exchange pump*

External Na ions inhibit the Na–K exchange system in several tissues (Baker & Connelly 1966; Priestland & Whittam 1968; Sjodin 1971). Such an effect has also been observed in taenia coli cells. Replacing half of the extracellular Na by Li or choline causes a much faster uptake of K ions than in solutions containing the same K concentration and 142 mmol/l Na. Moreover, a 50 % replacement of Na by Li causes a larger uptake of K than a 50 % replacement with choline as shown in figure 8. This is probably due to a supplementary activation of the ion pump by Li as described previously in other cells (Whittam 1964; Beaugé & Sjodin 1968). The active uptake of Li in taenia coli cells has been demonstrated by following the changes of intracellular Li and Na during an exposure of K-depleted cells to a 71 mmol/l Na–71 mmol/l Li solution. The uptake of Li against its electrochemical potential can be inhibited by 10^{-5} mol/l ouabain (Casteels, Droogmans & Hendrickx 1972).

The active Na–Li exchange can be further demonstrated by following the efflux of ^{22}Na from K-depleted cells in a 142 mmol/l Li solution. Such a modification of the washing medium does

Downloaded from rstb.royalsocietypublishing.org

not appreciably change the rate of Na efflux, but if a similar experiment is performed in the presence of 2×10^{-5} mol/l ouabain, the ^{22}Na efflux decreases to about 47 % of its control value (figure 9). This result can be interpreted by assuming that in the absence of ouabain the active Na–Li exchange is balanced by a reduction of the Na exchange diffusion. This reduction of the Na exchange is readily observed in the presence of ouabain and could explain the discrepancy between the ratio of active K/Na fluxes in steady-state conditions and in K-depleted cells which are extruding Na.

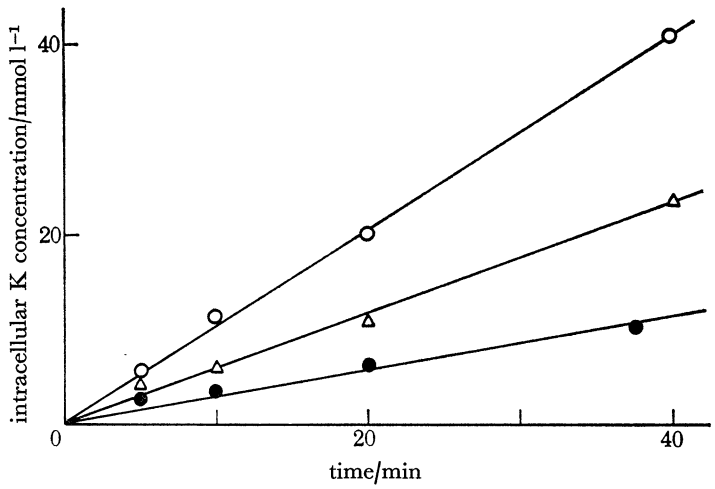

FIGURE 8. Intracellular K concentration during K accumulation by K-depleted cells as a function of time of exposure to a solution containing 0.59 mmol/l K. The filled circles represent the $[K]_i$ values in a solution containing 142 mmol/l Na, the open circles the values in a solution containing 71 mmol/l Na–71 mmol/l Li, and the triangles the values in a 71 mmol/l Na–71 mmol/i choline solution.

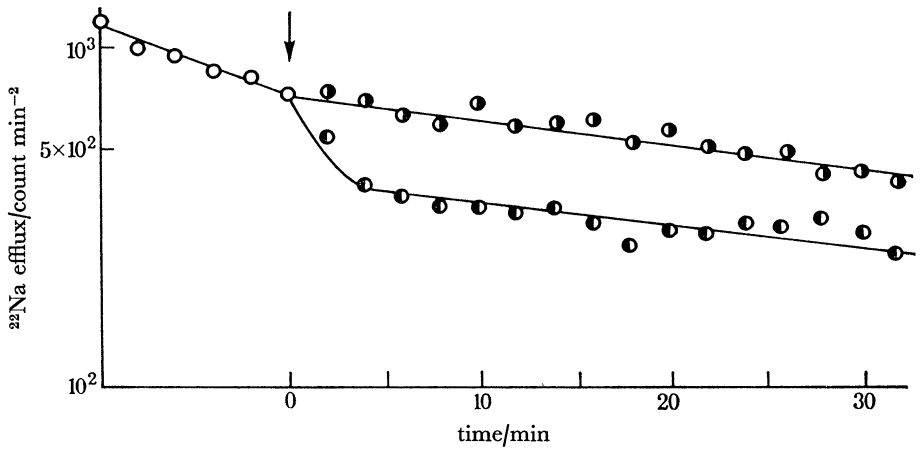

FIGURE 9. ^{22}Na-efflux (count min^{-2}) from K-depleted cells in a K-free solution. The efflux is started in a solution containing 142 mmol/l Na (○). At the arrow the tissues are exposed either to a 142 mmol/l Li solution (◑) or to a 142 mmol/l Li solution containing 2×10^{-5} mol/l ouabain (◐).

Conclusion

In taenia coli cells the Na–K exchange pump determines the resting potential by maintaining a diffusion potential and by producing an electrogenic potential. The important contribution of the electrogenic pump to the membrane potential under steady-state conditions is due to the

Downloaded from rstb.royalsocietypublishing.org

high membrane resistance of the cells. The characteristics of the Na–K exchange are similar to the ones observed in many other cells. This Na–K exchange is activated by $[Na]_i$ and $[K]_o$ and is inhibited by $[Na]_o$. The ratio of active K influx to Na efflux is about 2/3. In K-depleted cells Na-exchange diffusion is responsible for 47 % of the Na-exchange.

The main problems in the study of this tissue are the rapid changes of the intracellular ion content and the changes in membrane permeabilities caused by some experimental conditions.

References (Casteels *et al.*)

Abe, A. L. & Tomita, T. 1968 *J. Physiol., Lond.* **196**, 87–100.
Adrian, R. H. & Slayman, C. L. 1966 *J. Physiol., Lond.* **184**, 970–1014.
Baker, P. F. & Connelly, C. M. 1966 *J. Physiol., Lond.* **185**, 270–297.
Beaugé, L. A. & Sjodin, R. A. 1968 *J. gen. Physiol.* **52**, 408–423.
Bülbring, E. 1962 *Physiol. Rev.* **42**, suppl. **5**, 160–178.
Burnstock, G. 1958 *J. Physiol., Lond.* **143**, 183–194.
Casteels, R. 1966 *J. Physiol., Lond.* **184**, 131–142.
Casteels, R. 1969 *J. Physiol., Lond.* **205**, 193–208.
Casteels, R. 1971 *J. Physiol., Lond.* **214**, 225–243.
Casteels, R., Droogmans, G. & Hendrickx, H. 1971*a* *J. Physiol., Lond.* **217**, 281–295.
Casteels, R., Droogmans, G. & Hendrickx, H. 1971*b* *J. Physiol., Lond.* **217**, 297–313.
Casteels, R., Droogmans, G. & Hendrickx, H. 1972 *J. Physiol., Lond.* **224**, 61–62*P*.
Casteels, R., van Breemen, C. & Waytack, F. 1972 *Nature, Lond.* (in the Press).
Droogmans, G. 1972 Ph.D. thesis, Universiteit Leuven, Belgium.
Finkelstein, A. & Mauro, A. 1963 *Biophys. J.* **3**, 215–237.
Frumento, A. S. 1965 *Science, N.Y.* **147**, 1442–1443.
Hodgkin, A. L. & Katz, B. 1949 *J. Physiol., Lond.* **108**, 37–77.
Hodgkin, A. L. & Rushton, W. A. H. 1946 *Proc. R. Soc. Lond.* B **133**, 444–479.
Kernan, R. P. 1962 *Nature, Lond.* **193**, 968–987.
Kumamoto, M. & Horn, L. 1970 *Microvasc. Res.* **2**, 181–201.
Priestland, R. N. & Whittam, R. 1968 *Biochem. J.* **109**, 369–374.
Sjodin, R. A. 1971 *J. gen. Physiol.* **57**, 164–187.
Skou, J. C. 1965 *Physiol. Rev.* **45**, 596–617.
Tomita, T. 1966 *J. theor. Biol.* **12**, 216–229.
Ussing, H. H. 1949 *Physiol. Rev.* **29**, 127–155.
Whittam, R. 1964 *The cellular functions of membrane transport* (ed. B. Hoffman). New Jersey: Prentice Hall.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES