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CALCULATION OF THE MEMBRANE POTENTIAL IN SMOOTH MUSCLE CELLS OF THE GUINEA-PIG'S TAENIA COLI BY THE GOLDMAN EQUATION

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SUMMARY

1. The intracellular K^+, Cl^- and Na^+ concentrations in the taenia coli cells of the guinea-pig have been estimated from the total ion content or the extrapolated intracellular tracer content, the sorbitol space and the dry wt./wet wt. ratio.

2. The exchange of K^+, Cl^- and Na^+ was studied by following the uptake and the efflux of these ions with radioactive isotopes. The following efflux values have been calculated: m_K, 4 p-mole.cm^{-2}.sec^{-1}; m_{Cl}, 8·4 p-mole.cm^{-2}.sec^{-1} and m_{Na}, 7·2 p-mole.cm^{-2}.sec^{-1}. These flux values agree well with the influx values, obtained under the same experimental conditions.

3. The slowness of diffusion in the extracellular space reduces the Na flux by about 2·5 % and the K flux by about 30 %. A correction factor of 1·3 has to be introduced to obtain the true K flux.

4. The values for the permeability constants calculated by the constant field assumptions are for P_K, 11×10^{-8} cm/sec; P_{Cl}, $6{\cdot}7 \times 10^{-8}$ cm/sec and for P_{Na}, $1{\cdot}8 \times 10^{-8}$ cm/sec. The introduction of these values and of the ion concentrations in the Goldman equation gives a resting potential of -37 mV.

5. One of the possible explanations for the discrepancy between the measured resting potential and the calculated one, is that the resting potential of these smooth muscle cells is partly a diffusion potential and partly due to the operation of an electrogenic Na pump.

INTRODUCTION

The resting potential in taenia coli cells, as measured by several authors, has a value between -50 and -56 mV (Holman, 1958; Bülbring & Kuriyama, 1963). This 'resting membrane potential' varies with the frequency of the spontaneous spike discharge and is determined by most authors as the maximum polarization registered by the micro-electrode.

However, there is no agreement on the mechanism by which this resting potential is generated in smooth muscle cells. Different values for intracellular ion concentration and fluxes have been calculated and several hypotheses have been proposed (Bozler, 1964; Bennett, 1966; Buck & Goodford, 1966).

This paper is concerned chiefly with attempts to measure the absolute values of the intracellular ion concentration and the absolute sizes of ion fluxes in taenia coli cells. We can confirm Schatzmann's (1964) conclusion that the measured value of the resting potential does not agree with the potential calculated by the constant field equation from experimental values for the ion content and ion permeabilities. Several mechanisms which could explain this discrepancy will be discussed. A preliminary account of this work was presented at the XXIV International Congress of Physiological Sciences (Casteels, 1968).

METHODS

Taenia coli strips, weighing between 10 and 14 mg were cut from the caecum of male guinea-pigs. The tissues were mounted isometrically on Perspex or Teflon rods and transferred to Krebs solution at 35° C. This solution contained (mM): Na^+ 137; K^+ 5·9; Mg^{2+} 1·2; Ca^{2+} 2·5; Cl^- 134; $H_2PO_4^-$ 1·2; HCO_3^- 15·5 and glucose 11·5; and was aerated with 97 % O_2 and 3 % CO_2. The pH at 35° C was 7·35. The tissues were always left at least for 1 hr in this solution.

Analytical methods. For all determinations the tissues were blotted according to a standard procedure with filter paper Whatman no. 54. To determine the ion content, the tissues were ashed chemically in Teflon tubes, with H_2O_2 containing the appropriate amount of $AgNO_3$. The Na, K and Cl contents were determined by flame photometry as described by Casteels & Kuriyama (1965). The extracellular space was measured by exposing the tissues for 10 min to a Krebs solution, containing [^{14}C]sorbitol (Goodford & Leach, 1966). The water content of the tissues was determined from the wet weight and the dry weight. The latter value was obtained by drying the tissue for 20 hr at 95° C. The intracellular ion concentration was calculated per litre fibre water according to the method of Boyle, Conway, Kane & O'Reilly (1941).

Measurement of fluxes. After equilibration in Krebs solution, the tissues were transferred to a solution containing ^{22}Na (supplied by NEN Chemicals), ^{42}K (supplied by Studiecentrum voor Kernenergie, Mol, Belgium) or ^{36}Cl (supplied by NEN Chemicals). In efflux experiments the exposure time to the radioactive solutions was 20–30 min for ^{22}Na, 40 min for ^{36}Cl and 2–4 hr for ^{42}K. Only in the ^{42}K experiments

was the exposure time too short to obtain complete equilibration of the intracellular ions with the isotope.

After loading, the tissues were transferred through a succession of test-tubes, containing inactive Krebs solution, bubbled with the O_2–CO_2 mixture, at 1 min intervals for ^{22}Na and for ^{36}Cl and at 5 min intervals for ^{42}K. The amount of radioactivity in the tissue at the end of the efflux was added to the activity in the successive samples, in reverse order, giving the amount of radioactivity in the fibres as a function of time (counts.min^{-1}). The radioactivity leaving the muscle per unit time was expressed in counts.min^{-2}. Both values (total activity and radioactivity leaving

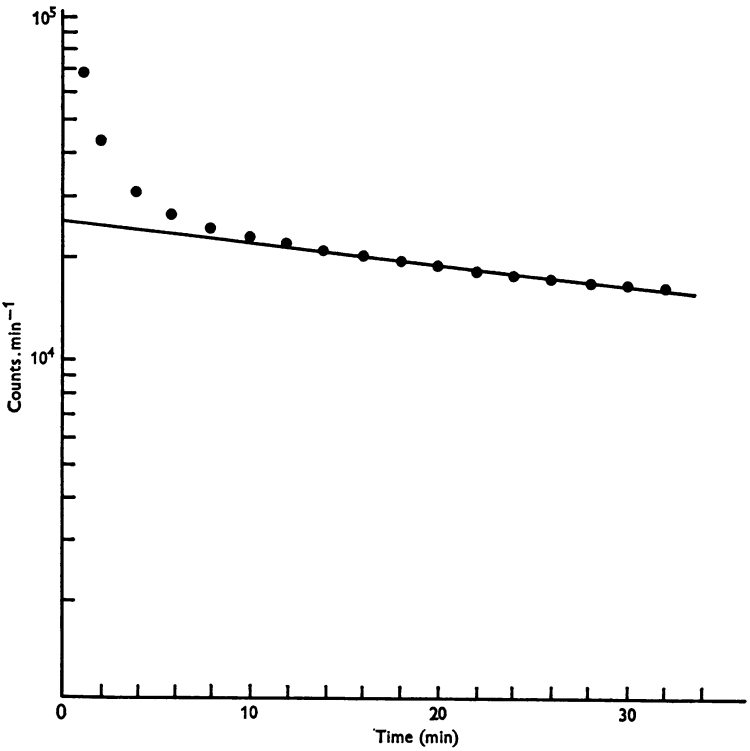

Fig. 1. ^{22}Na efflux from taenia coli at 0° C after loading in radioactive Krebs solution at 35° C. By extrapolating the linear part of the curve, one can estimate the amount of tracer, which has penetrated into the intracellular compartment during the exposure to the radioactive solution.

the muscle) were plotted logarithmically against time. A comparison of the curves can give information on the homogeneity of the later phase of the ion exchange (Persoff, 1960).

The influxes of ^{22}Na and ^{36}Cl were determined by exposing the tissues for 1 and 2 min to the active solution at 35° C. However, it is difficult to distinguish the intracellular tracer from the extracellular one under physiological conditions, because the rate of movement of Na and Cl in smooth muscle tissue at 35° C is rather fast and because the amount of Na and Cl in the cells is rather low compared to the amount of these ions in the extracellular space. A clearer separation between these fractions can be obtained by performing an efflux at low temperature, after loading the tissue

at 35° C, because by this procedure the permeation of ions through the membrane is slowed down. By extrapolation to zero time of the late linear part of the curve representing the logarithmic decrease of activity in the tissue as a function of time, it was possible to estimate the amount of Na or Cl that has penetrated into the cells during the period of exposure to the active solution (Fig. 1). Using the same procedure but with exposure times to the radioactive solution until there was a complete exchange of isotope in the cells, we had an independent method of calculating the intracellular Na and Cl content. The amount of Na and Cl, penetrating into the intracellular compartment of the tissue has been plotted in Fig. 2 as a linear function of

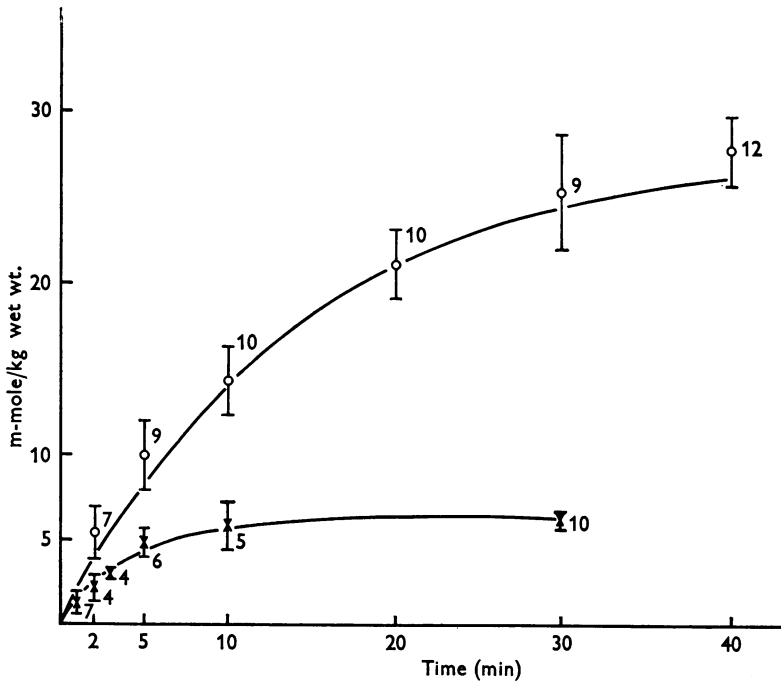

Fig. 2. The points represent the uptake (mean ± 1 S.D.) of Na (×) and Cl (○), with the number of observations printed beside the point. The ordinate gives the amount of ions which penetrated into the cells in m-mole/kg wet weight and the abscissa the time in min of exposure to the radioactive solutions at 35° C. The curves, drawn through the experimental points, were worked out from the equation $Y_{it} = Y_{i\infty}\{1-\exp(-kt)\}$, by introducing for the Na curve $Y_{i\infty} = 6{\cdot}2$ m-mole/kg wet wt. and $k = 0{\cdot}239\ \text{min}^{-1}$. For the Cl curve $Y_{i\infty} = 27{\cdot}8$ m-mole/kg wet wt. and $k = 0{\cdot}0714\ \text{min}^{-1}$.

the time of exposure to the radioactive solution. The initial part of these uptake curves can be considered as linear and this justifies the use of an exposure time of 1 min for calculating the Na uptake and of a 2 min exposure time for calculating the Cl uptake. Moreover, in these tissues having a diameter of less than 1 mm, the effect of diffusion delay on these uptakes (see p. 203) cannot be very important.

The uptake of ^{42}K was determined by measuring the amount of tracer in the tissue after 10 min exposure to the active solution and after a wash of 1 min in inactive

Krebs solution. This procedure is justified by the low exchange rate for K and by the high value of the ratio of intracellular over extracellular K.

The activity of the samples containing ^{36}Cl or ^{14}C was measured in a Packard liquid scintillation spectrometer 3003 using a scintillation mixture described by Patterson & Greene (1965). The activity of samples containing ^{42}K or ^{22}Na was measured in a Packard autogamma spectrometer 5019.

RESULTS

Intracellular ion concentration. The total content of K, Na and Cl ions of taenia coli tissues, expressed in m-mole/kg wet wt., the extracellular sorbitol space expressed in ml./kg wet wt. and the dry wt./wet wt. ratio percentage are given in Table 1.

TABLE 1. Total ion content (m-mole/kg wet wt.), sorbitol space (ml./kg wet wt.) and dry wt./wet wt. ratio (percentage) of strips of the guinea-pig's taenia coli, given as mean values with S.E. of mean. The number of observations is given in brackets

K	80·7 ± 0·6 (80) m-mole/kg wet wt.
Na	56·3 ± 0·8 (80) m-mole/kg wet wt.
Cl	72·3 ± 0·6 (80) m-mole/kg wet wt.
Sorbitol space	343 ± 19 (12) ml./kg wet wt.
dry wt./wet wt.	18 ± 0·4 (22) g/100 g wet wt.

TABLE 2. Calculated intracellular ion concentration in m-mole/l. cell water, and equilibrium potentials in mV, calculated by the Nernst equation. The values in brackets have been obtained by the extrapolation procedure (see text)

	m-mole/l. cell water		mV
$[K]_i$	164	E_K	−89
$[Na]_i$	19 (13)	E_{Na}	+52 (+62)
$[Cl]_i$	55 (58)	E_{Cl}	−24 (−22)

The discrepancy of these values with the ones of Goodford & Hermansen (1961) and Goodford (1964) is caused by the use of a different blotting procedure, resulting in a difference between the extracellular spaces in the two sets of data.

From these experimental values the mean intracellular ion concentrations expressed in m-mole/l. cell water have been calculated (Table 2). Also the equilibrium potentials for K, Na and Cl, calculated by the Nernst equation are given in Table 2. The intracellular ion concentrations of Table 2 are not significantly different from the values given by Casteels & Kuriyama (1966) and Casteels (1966).

The second method for estimating the intracellular ion content by means of radioisotopes gave a mean value for the intracellular chloride of 27·8 ± 0·2 m-mole/kg wet wt. (n = 12). The intracellular ion concentration cal-

culated from this value and the intracellular water gives 58 m-mole/l. For Na we find by the same method an intracellular content of $6{\cdot}2 \pm 0{\cdot}3$ m-mole/kg wet wt. ($n = 10$) and an intracellular concentration of 13 m-mole/l. This latter value is appreciably lower than the $[Na]_i$ value obtained by the analytical procedure. This discrepancy could be due to the fact that a small fraction of the total Na does not belong to the free intracellular or extracellular Na, but is somewhere bound in the tissue.

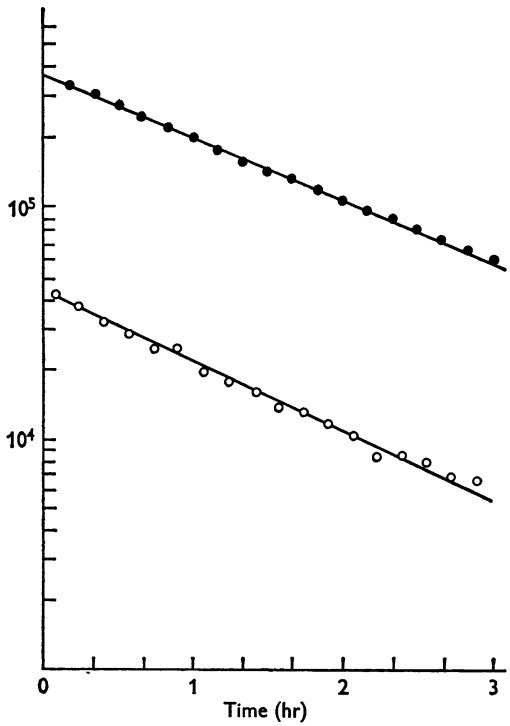

Fig. 3. ^{42}K efflux from taenia coli at 35° C. The open circles represent the decrease of activity in the effluent and the filled circles the decrease of activity left in the tissue. Both efflux values are given as logarithmic functions of time in hr.

Potassium fluxes. There is not much controversy about the value of the K efflux because its rate constant is small and because the intracellular K concentration is much higher than the extracellular concentration. After the first 10 min the decrease of the radioactivity left in the tissue proceeds along a simple exponential with a mean rate constant of 0·61 hr^{-1}. For the decrease of the activity in the effluent the mean rate constant is 0·646 hr^{-1}. This means that the curves representing the decrease of tracer in the tissue and its differential (Fig. 3) are nearly parallel. This observation supports the hypothesis that the potassium is lost from a single uniform intra-

cellular compartment. The small discrepancy between the two values could be due to the presence of a small intra- or extracellular fraction of K with a slower exchange. We assumed that this latter compartment has probably no relation to the generation of the membrane potential and therefore we use the rate constant for the decrease of activity in the effluent to calculate the K flux.

The efflux of K ions was calculated by the equation of Keynes & Lewis (1951), $m_o = k.C_i.V/A$, where k is the rate constant, C_i the intracellular ion concentration and V/A the mean volume/surface ratio of the cells. This ratio V/A was calculated from transverse histological sections of taenia coli cells, in which the shrinkage was negligible. Using the method of point-counting, as described by Sitte (1967), a mean value of $1{\cdot}4\,\mu$ was found (unpublished observations). The K efflux, obtained from the above values, amounted to $4{\cdot}1 \times 10^{-12}$ mole.cm^{-2}.sec^{-1}.

The influx of K under the same experimental conditions at 35° C can be calculated from the amount of K taken up by 1 mg of tissue and from the surface to weight ratio for taenia coli. This value, calculated from the tissue density 1·05 (Goodford & Hermansen, 1961), the extracellular space (34·3 %) and from the volume/surface ratio of $1{\cdot}4\,\mu$ amounts to 4·35 cm^2/mg. If we assume that the K uptake during the first 10 min of exposure to radioactive solution proceeds in a linear fashion, we find a value of $18{\cdot}1 \times 10^{-12}$ mole.mg^{-1}.sec^{-1}. From this value and the surface over weight ratio, a K uptake of $4{\cdot}16 \times 10^{-12}$ mole.cm^{-2}.sec^{-1} has been calculated.

Chloride fluxes. Also the Cl efflux of taenia coli cells at 35° C expressed as the decrease of the activity left in the tissue or the decrease of activity in the effluent, proceeds after about 10 min as a single exponential function (Fig. 4). The rate constant, calculated from the experimental values, has a mean value of 0·0654 min^{-1} for the decrease of activity in the tissue and of 0·0714 min^{-1} for the decrease of activity in the effluent. The efflux, calculated from the higher rate constant, the intracellular ion concentration and the mean cell diameter, is $8{\cdot}4 \times 10^{-12}$ mole.cm^{-2}.sec^{-1}. The influx of Cl in steady-state condition at 35° C is 45×10^{-12} mole.mg^{-1}.sec^{-1} corresponding to $10{\cdot}3 \times 10^{-12}$mole.cm^{-2}.sec^{-1} for a surface/weight ratio of 4·35 cm^2/mg.

The reliability of the methods used for calculating the influx and efflux is somehow demonstrated in Fig. 2. The experimental points, obtained in uptake experiments of ^{36}Cl, nearly fit the curve representing the equation given by Hodgkin (1951) for calculating the influx of tracers in cells

$$Y_{it} = Y_{i\infty}\{1 - \exp(-kt)\},$$

where Y_{it} represents the amount of radioactive ions in the cells after an exposure of time t to the radioactive solution and $Y_{i\infty}$ the amount of radioactive ions after complete exchange with the external solution, i.e. for

chloride 27·8 m-mole/kg wet wt.; k is the rate constant, which in steady-state conditions should have the same value for influx and efflux (0·0714 min^{-1}).

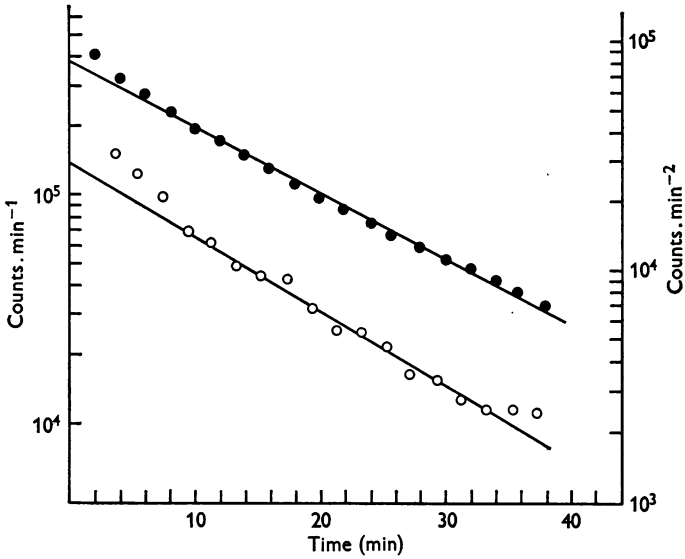

Fig. 4. ^{36}Cl efflux from taenia coli at 35° C. The open circles represent the decrease of activity in the effluent and the filled circles the decrease of activity left in the tissue. Both efflux values are given as logarithmic functions of time in min.

Sodium fluxes. The transmembrane Na flux is the most controversial factor in the study of membrane permeabilities of smooth muscle cells. This is due to the fact that the Na efflux (decrease of activity left in the tissue), proceeds rather quickly along a curve which cannot be fitted by a single exponential. Moreover, the intracellular Na concentration (or activity) also remains a largely unknown factor of which the value varies according to the experimental method used for its determination. Therefore calculating a Na flux always implies some assumptions, which cannot yet be fully justified. Some justification of the present calculation procedure used for the sodium efflux was provided by the fact that this efflux was very similar to the influx of Na calculated for the same experimental conditions.

Figure 5 shows the efflux of ^{22}Na from taenia coli at 35° C. The points representing the decrease of radioactivity left in the tissue as a function of time do not correspond after the first 10 min to a single exponential. However, the points representing the decrease of activity in the effluent can be fitted by a straight line between 10 and 25 min. The initial deviation from linearity is due to the loss of extracellular Na, while the later slow

phase of the curve could be explained by the presence in the tissue of some slowly exchanging Na. The mean rate constant for the Na efflux, calculated from the linear part of seven curves, representing the decrease of activity in the effluent, is 0·239 min^{-1}. Although this procedure is rather arbitrary, this value of 0·239 min^{-1} is justified to some extent by the rate constant observed in Fig. 6. In this experiment the tissue has been loaded

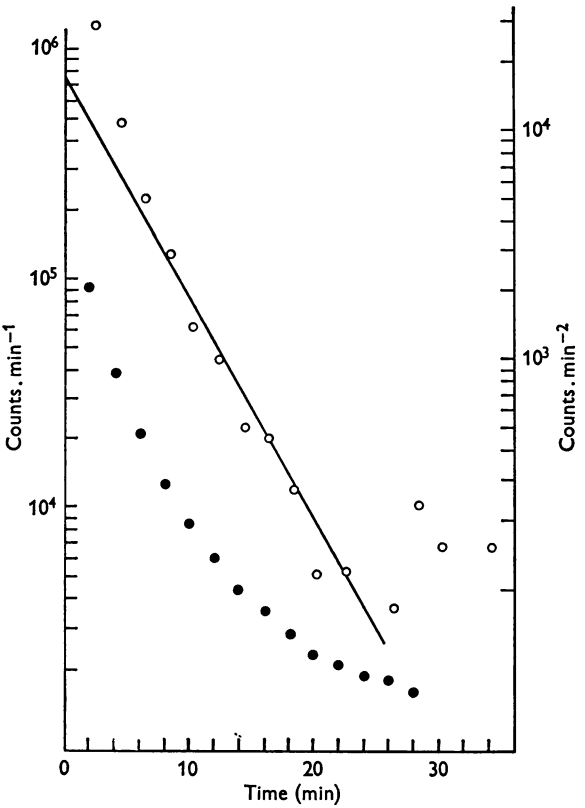

Fig. 5. ^{22}Na efflux from taenia coli at 35° C. The open circles represent the decrease of activity in the effluent and the filled circles the decrease of activity left in the tissues. Both efflux values are given as logarithmic functions of time in min.

at 35° C in Krebs solution, containing ^{22}Na and the efflux was measured for the first 15 min at 4° C, in order to wash away all the extracellular tracer Na and maintaining most of the intracellular tracer. The temperature was then suddenly raised to 35° C and thereupon the Na efflux increased to a rate, which was similar to the one estimated under physiological conditions from the decrease of activity in the effluent.

From the rate constant 0·239 min^{-1}, the lower value for the intracellular

Na concentration (Table 2) and the mean cell diameter, a Na efflux of $7{\cdot}2 \times 10^{-12}$ mole.cm^{-2}.sec^{-1} is calculated. The Na influx under the same experimental conditions is $21{\cdot}7 \times 10^{-12}$ mole.mg^{-1}.sec^{-1} or $5{\cdot}0 \times 10^{-12}$ mole.cm^{-2}.sec^{-1}. The rather arbitrary assumptions made in the calculation of the efflux are somewhat justified by the agreement between the calculated influx and efflux values and by the fact that the experimental values obtained in uptake experiments (Fig. 2) fit the curve, representing

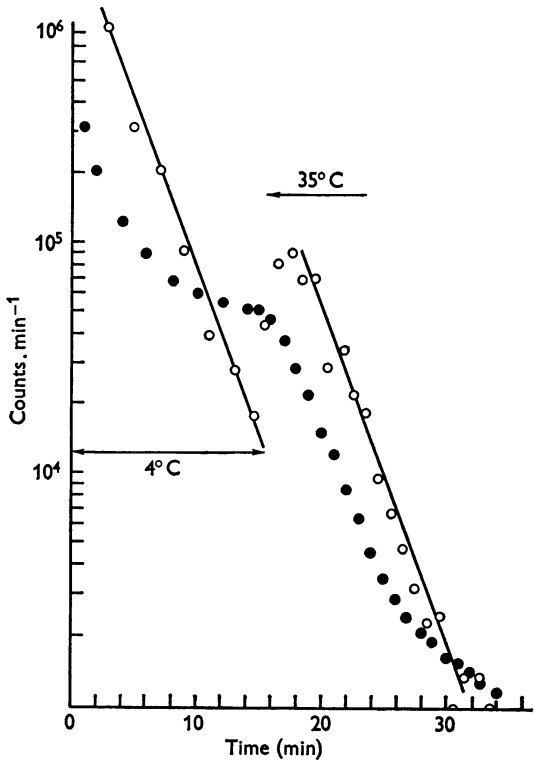

Fig. 6. Efflux of ^{22}Na, after loading the tissue in a radioactive solution at 35° C. During the first 15 min of this efflux the temperature was kept at 4° C, to reduce the loss of intracellular Na. Thereafter the temperature was increased to 35° C. The open circles represent the activity in the effluent, and the filled circles the activity left in the tissue. Time in min.

the equation of Hodgkin (1951) using a value for $Y_{1\infty}$ of 6·2 m-mole/kg wet wt. and a value for k of 0·239 min^{-1}. Therefore a steady-state transmembrane Na flux at 35° C between 5 to 7×10^{-12} mole.cm^{-2}.sec^{-1} might be accepted.

Rate of diffusion in taenia coli. Using the procedure described by Keynes (1954), a rough estimate was made of the diffusion constant of Na^+ ions in the extracellular space of taenia coli. Because it was difficult to

separate at 35° C the intracellular from the extracellular tracer, the exchange from the intracellular compartment was slowed down by performing the experiment at 25° C. The counting rate for the extracellular radioactivity was obtained by subtracting the extrapolated intracellular counts from the total counts (Fig. 7). The resulting figures were plotted logarithmically against time and a straight line was drawn through the middle part of the curve. From the half-time of this exponential function

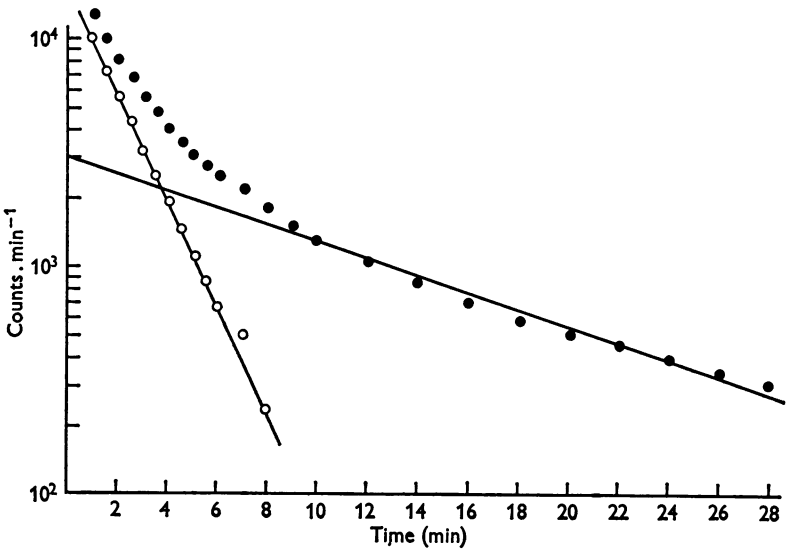

Fig. 7. Efflux of ^{22}Na at 25° C. The filled circles represent the logarithmic decrease of activity as a function of time. The linear part of this curve is extrapolated to zero time and subtracted from the total counts, giving the counting rate for the extracellular radioactivity. Time in min.

($t_{\frac{1}{2}}$), and the radii of the total muscle (r), the diffusion coefficient for Na ions in the extracellular space (D') was calculated by the equation of Hill (1928)

$$t_{\frac{1}{2}} = 0{\cdot}118r^2/D'.$$

The half-times for eight tissues varied between 45 and 35 sec, and the tissue radii between 341 and 445 μ. The mean value of D' was $5{\cdot}55 \times 10^{-6}$ cm^2/sec. This is slightly larger than the third of the diffusion coefficient for NaCl at 25° C (Robinson & Stokes, 1959). From the values of the diffusion constants, one can calculate, by the equation of Keynes (1954) how much the slowness of diffusion reduces the apparent Na and K fluxes below the true ones. It was found that the Na flux is not appreciably hindered by diffusion and is only 2·5 % higher than the observed flux. The true K flux was calculated using a diffusion coefficient for K ions in the extracellular

space of $7{\cdot}24 \times 10^{-6}$ cm^2/sec. This value was obtained from the diffusion coefficient of K ions in free solution, assuming a similar reduction factor in the extracellular space as calculated for Na ions. This true K flux is about 30 % higher than the observed flux. We will therefore use in the following calculations the apparent value of the steady-state Na flux and a value for the K flux of $5{\cdot}4 \times 10^{-12}$ mole.cm^{-2}.sec^{-1}. The correction factor for the K flux in taenia coli is thus higher than the value given by Keynes (1954) for frog toe muscle.

Calculation of the membrane permeabilities. Having estimated the intracellular ion concentrations (neglecting the activity coefficients), the flux values and the membrane potential, we can calculate the permeability coefficients P_K, P_{Na} and P_{Cl}, by the constant field assumptions (Goldman, 1943; Hodgkin & Katz, 1949). We have also to assume that the inward and outward movements of an ion species are independent of each other and that effluxes of K and Cl ions and the influx of Na ions are due only to their own electrochemical forces. The Cl influx cannot be considered as being passive, because the electrochemical potential of Cl ions is higher in the cytoplasm than in the extracellular fluid (Casteels, 1965). Because we are dealing with ions, which are also affected by the electric field, their permeabilities are determined by the ratio of flux-concentration, multiplied by a factor that represents the effect of the electric field on the movement of ions across the membrane. This factor is according to Goldman (1943)

$$\frac{EF/RT}{1-\exp(-EF/RT)}$$

and amounts to 0·3 for the outward movement of K and to 2·46 for the inward movement of Na and the outward movement of Cl at a membrane potential of -55 mV and a temperature of 35° C.

From the value of the corrected K flux, from $[K]_i$ and the factor representing the influence of the potential on the ion movement, we obtain for P_K a value of 11×10^{-8} cm/sec. The calculated value of P_{Cl} amounts to $6{\cdot}7 \times 10^{-8}$ cm/sec and of P_{Na} to $1{\cdot}8 \times 10^{-8}$ cm/sec. Several simplifying assumptions have been made in these calculations. One of these assumptions, the volume/surface ratio, can be cancelled by determining the ratio of P_{Na}/P_K and P_{Cl}/P_K. The ratio P_{Na}/P_K is 0·16 and P_{Cl}/P_K is 0·61. Introducing these ratios and the calculated intracellular ion concentrations (Table 2) in the Goldman equation, gives a value for the membrane potential of -37 mV, which is about 20 mV less negative than the mean value of the measured membrane potential.

DISCUSSION

The values for the total ion content of taenia coli are not very different from the data of other authors (Goodford & Hermansen, 1961; Goodford, 1964), but the calculated intracellular ion concentrations vary according to the assumptions about the extracellular space and about the distribution of the ions over various compartments in the tissue.

In this paper it has been assumed that practically all the K and Cl is free and located in an intracellular and an extracellular compartment. The present experiments do not suggest a sequestration of K or of Cl. The similarity in slope between the linear parts of the logarithmic plot of the tissue activity and of the activity of the effluent is in agreement with a uniform intracellular compartment. The slightly slower exchange, obtained from the tissue plot as compared with the effluent plot, could be due to the presence of some cells or of some compartments with a slow exchange rate. The deviation from linearity in the later part of some efflux curves could be explained by a similar mechanism, consisting of a distribution about a mean value of the rate constants of the K exchange of individual cells (Van Liew, 1967).

The calculated values for the K and Cl fluxes are not very different from the values obtained from the data of Born & Bülbring (1956) or the values published by Durbin & Monson (1961), Goodford & Hermansen (1961) and Goodford (1964).

The Na distribution in taenia coli cells remains a controversial subject. This could be due to the existence of several compartments in which Na might be bound or sequestered. Sequestration of a small amount of Na (6 m-mole) has also been considered by Goodford (1962) and Buck & Goodford (1966). This uncertainty not only makes it difficult to estimate the real intracellular Na concentration, but also raises some doubt concerning the significance of the exchange constants, derived from efflux experiments. A comparison, however, between the exchange constant obtained from Fig. 4 and from Fig. 5, suggests that we might have used the right fraction of the curve in Fig. 4. Moreover measuring the Na influx gives an independent and direct method to check the steady-state Na flux. The rather good agreement between these two estimates justifies the use of a steady-state value of 6×10^{-12} mole.cm^{-2}.sec^{-1}.

A comparison of the permeability constants calculated for smooth muscle and of the value given by Hodgkin & Horowicz (1959) and Adrian (1960) for frog striated muscle, points to some interesting aspects of the membrane permeability in smooth muscle. The Na permeability is not significantly different from the value in striated muscle, but the K and Cl permeability of the membrane are much lower in smooth muscle than in

striated muscle. It is therefore tempting to conclude that the low membrane potential in smooth muscle cells is caused by the low value of P_K and not by a high value of P_{Na}. Moreover, this conclusion regarding the potassium permeability explains the fact that the maximal slope of the curve, representing the change of the resting potential as a function of the logarithm of the external concentration is only 35 mV for a tenfold change of $[K]_o$ (Casteels & Kuriyama, 1966). The low value of the Cl permeability may be essential for the maintenance of a non-passive Cl distribution in smooth muscle cells. Only under these conditions can an inward movement of Cl produce such a non-passive distribution.

The K conductance (G_K) and Cl conductance (G_{Cl}) of the smooth muscle membrane can be calculated from the P_K and P_{Cl} values by the equations given by Hodgkin & Katz (1949). Because the potassium (E_K) and the Cl equilibrium potential (E_{Cl}) are different from the resting potential (E) we have to use the two following equations

$$G_K = P_K \frac{F^2}{RT} \frac{E}{E_K - E} \frac{[K]_o - [K]_i \exp(-EF/RT)}{1 - \exp(-EF/RT)},$$

and

$$G_{Cl} = P_{Cl} \frac{F^2}{RT} \frac{E}{E - E_{Cl}} \frac{[Cl]_i - [Cl]_o \exp(-EF/RT)}{1 - \exp(-EF/RT)}.$$

The simplified versions of these equations $G = F^2.M/RT$ can only be used if the equilibrium potential is similar to the membrane potential.

The K conductance (G_K) calculated from the experimental values is $0{\cdot}9 \times 10^{-5}$ mho.cm^{-2} and the Cl conductance (G_{Cl}), $1{\cdot}9 \times 10^{-5}$ mho.cm^{-2}. Assuming that G_K and G_{Cl} are largely responsible for the membrane conductance (G) we find that this membrane conductance is $2{\cdot}8 \times 10^{-5}$ mho.cm^{-2} and that the membrane resistance is $36 \times 10^3\ \Omega$ cm^2. This value agrees well with the value, obtained by electrophysiological methods (Tomita, 1966).

The fact that the resting potential calculated, by introducing our experimental values in the Goldman equation, is appreciably lower than the measured potential, can be explained by several hypotheses. First, one has to consider the fact, that the Goldman equation can only give an estimate of the diffusion potential across a complex biological membrane. However, the large discrepancy between the measured and the calculated value points to a more fundamental problem. A first possibility is that we have overestimated the P_{Na}/P_K value. This could be due to experimental errors, but also to the existence of single file diffusion for K^+ (Hodgkin & Keynes, 1955). No experimental data, suggesting a single file diffusion of these ions in taenia coli, are as yet available. The possibility of experimental error or misinterpretation is rather unlikely, because of the agree-

ment between influx and efflux values. A last possibility, which can be considered, is that the resting potential in taenia coli cells is not only due to a diffusion potential, as expressed by the Goldman equation, but also, partly to a continuous electrogenic extrusion of Na ions. Such an electrogenic process has already been proposed by Burnstock (1958) and Bülbring (1962) to explain the hyperpolarizing action of adrenaline in taenia coli cells. An argument in favour of the existence of an electrogenic Na pump is the fact that taenia coli cells which have been depleted of K by prolonged exposure to K-free solution, suddenly repolarize by readmitting K in the external solution. The membrane potential during this phase is 15–20 mV more negative than the K equilibrium potential (Casteels & Hendrickx, 1969).

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