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ANALYSIS OF THE EFFLUXES OF SODIUM, POTASSIUM AND CHLORIDE IONS FROM SMOOTH MUSCLE IN NORMAL AND HYPERTONIC SOLUTIONS

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SUMMARY

1. Efflux curves of ^{24}Na, ^{42}K and ^{36}Cl from the guinea-pig taenia coli were obtained in normal Krebs solution, and in hypertonic Krebs solution in which the osmolarity had been doubled by the addition of sucrose.

2. The efflux curves were complex, and in order to get average curves each was analysed as the sum of three exponential terms, and average curves were constructed from the means of the constants found.

3. In order to estimate the membrane permeability to the ions, it was necessary to make assumptions as to the distribution of ions in the tissue. Several models have been examined and the predictions of the models with respect to the membrane properties were compared with the data obtained by using electrophysiological methods by other workers.

4. It was found that reasonable predictions of membrane properties could only be made using models in which the majority of the rapidly exchanging sodium is considered to be extracellular. This amount of sodium is more than can be accounted for as freely dissolved in the extracellular water.

5. A possible interpretation of the ion exchange and distribution would be to suppose that some proportion of the three ions is contained in an extracellular compartment not available to the normal extracellular markers, and limited in its exchange by the rate of diffusion in the extracellular phase, and that the truly intracellular fractions of the tissue ions do not exchange with the external solution in a simple exponential manner, but in a manner described by an aggregate of exponential terms due to inherent variation in the permeabilities of the individual cell membranes.

6. There is no evidence for any change in the membrane permeabilities to sodium and potassium in hypertonic solution, but there is evidence for a decrease in chloride permeability in this solution.

INTRODUCTION

This paper is an attempt to correlate ion flux data with some of the known electrophysiological properties of smooth muscle and to study the effects of hypertonic solution on the membrane parameters.

The taenia coli reaches a steady state in Krebs solution of twice the normal osmolarity made by addition of sucrose. In this solution, the electrical and mechanical behaviour of the tissue is modified. Tomita (1966*a*) has shown that the membrane of the muscle cell undergoes hyperpolarization, accompanied by a cessation of spontaneous activity. The tissue is, however, still able to conduct action potentials, although the mechanical response is reduced or abolished. Under this condition the tissue shrinks to about 75% of its fresh weight (Brading & Setekleiv, 1968). The cells retain most of their sodium and potassium content, although they lose a certain amount of chloride (in excess of the sodium + potassium lost). The concentration of sodium and potassium ions in the cell, however, must have increased, and the hyperpolarization observed could be accounted for by an increased potassium equilibrium potential.

It is also possible that treatment with hypertonic solution may alter the membrane permeability to the ions, and this may contribute to changes in the membrane potential. It is important to establish whether such changes in permeability occur. The quiescent tissue in hypertonic solution has been used extensively for studying the electrophysiological properties of the tissue, and may provide a stable preparation for the study of the factors affecting ion fluxes. The use of hypertonic solution reduces artifacts due to tissue movement, and eliminates the contribution by ion movements during spontaneous spike activity.

The most difficult problem encountered, when trying to gauge the effect of hypertonic solution on the permeability of the cell membrane to ions, is the interpretation of the steady-state efflux curves in both normal and hypertonic solutions. In this paper the approach taken will be to calculate the membrane permeabilities using various assumptions as to the ionic distribution in the tissue, and the factors limiting the exchange of ions, in the hope of finding a model which will predict the membrane parameters that have been established by electrophysiological techniques in the two solutions used.

METHODS

Apparatus. The apparatus is illustrated in Fig. 1. It consisted of a narrow channel in which the tissue was inserted on a special holder and washed by a continuous flow of solution. Solutions were saturated with the gas mixture, and were kept at constant level in storage baths above the apparatus, with which they were connected through jacketed tubes. The chamber and storage baths, and also the organ baths

used for loading and keeping the mounted tissues were all kept at the same constant temperature with circulating water. The washing solution was collected in a series of tubes mounted on a sliding rack beneath the apparatus.

Solutions. A modified Krebs saline was used of the following composition (mM): Na^+ 136·9 K^+ 5·9 Ca^{2+} 2·5 Mg^{2+} 1·2 Cl^- 133·5 HCO_3^- 15·5 $H_2PO_4^-$ 1·2 and glucose 11·5, equilibrated with a gas mixture of 3 % CO_2 and 97 % O_2 at 37° C. The solution was made hypertonic by addition of 10 g sucrose to every 100 ml. saline. This increased the volume by about 6 %, which was taken into account in the calculation of the results.

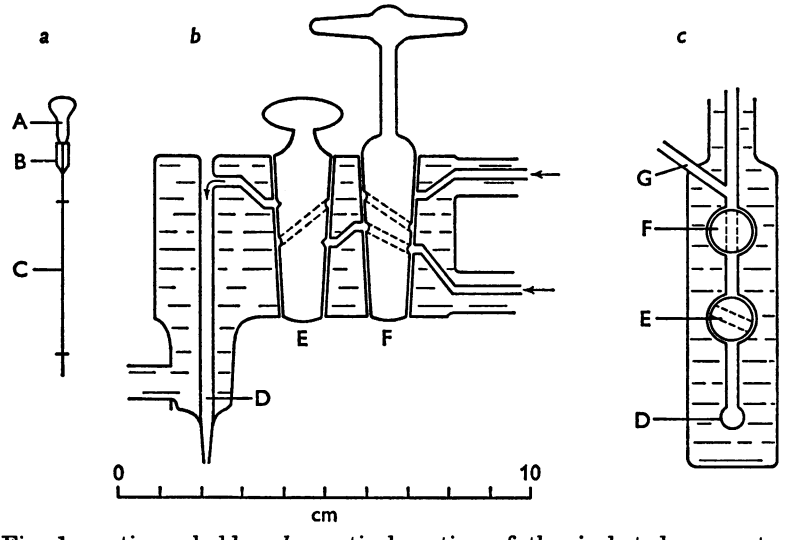

Fig. 1. *a*, tissue holder; *b*, vertical section of the jacketed apparatus; *c*, diagrammatic horizontal section. A, glass stopper; B, polyethylene sleeve; C, stainless-steel wire with cross bars; D, channel for tissue and holder; E, tap controlling rate of flow of solution; F, two way tap allowing change of solution; G, bleed out tube (one for each solution) to allow removal of stagnant fluid. Arrows show direction of flow of solution (from Brading, 1967).

Dissection. Male white guinea-pigs were stunned and bled, and thin pieces of taenia coli were dissected from the caecum. The *in situ* length of each piece was measured, and the pieces were weighed after removal, on a torsion balance, to determine the fresh weight (F. wt.). The pieces were then mounted on special holders (Fig. 1). The pieces were then placed in gassed Krebs solution, at 37° C, for at least 1 hr to reach a steady state.

Loading. The tissue was loaded with either ^{24}Na, or ^{42}K or ^{36}Cl obtained from the Radiochemical Centre, Amersham. Sodium and potassium isotopes were supplied as isotonic solution of the chloride, and the chloride isotope as a solution of sodium chloride, which was diluted to isotonicity before use. To make the loading solutions, the relevant radioactive isotonic solution was used to replace some of the isotonic stock solution used. Small heated water-baths, capacity 15 ml., were used to take the radioactive solutions. The tissues were loaded in the normal or hypertonic active solutions for sufficient time for equilibration of the radioactive ions with the tissue ions, and for a steady state to be reached in the hypertonic solution. Normally, a

minimum of 1 hr was used in each experiment before the first washout, but most tissues were loaded for several hours.

Washout. When a washout was started, a holder with the muscle was removed from the loading bath, washed in a large bath of heated non-active solution for about one second to remove adhering surface activity, and plugged into the dry channel of the apparatus. The flow rate was then turned up to maximum, and the solution run through until no air bubbles remained in the channel. The flow was then quickly adjusted to the desired rate. The liquid run through the channel during this procedure (usually about 1 ml.) was collected in the first tube in the rack, and was later counted and used in the estimation of the efflux curves. When the correct rate of flow had been established, the second tube was placed to collect the efflux. The time elapsing from the removal of the holder from the loading bath until the correct flow had been established was usually about 25 sec. Subsequently, the washing solution was collected for 1 min in each tube, with the same number of drops in each sample.

Counting procedure. For sodium and potassium, the washing solution was collected as 2 ml. samples in glass tubes, and these were counted in a sodium iodide well crystal. After the end of the washout the radioactivity of the tissue was counted whilst it was still on the holder, in two mls of solution. Tissues could then be reloaded and used for a second time. For chloride, the efflux was collected in 1 ml. samples in polyethylene tubes which could be used for counting in an automatic liquid scintillation counter (IDL Tritomat). Five ml of Bray's scintillation fluid (Bray, 1960) was added directly to the washout sample. To count muscle ^{36}Cl activity, the tissue was removed from the holder. The muscle was extracted in distilled water for 12 hr, and an aliquot was taken and counted. Thus, in the chloride experiments the tissue could only be used once. On two occasions, to check for reproducibility of results, tissues were used twice and, to work out the results, a reasonable figure for the tissues' radioactivity was assumed. Standards were diluted from the loading solutions, and these and blanks were treated identically to the efflux samples.

Analysis of results. The counts per second in the muscle at each minute interval were calculated from the count rate of the samples by summing the counts in reverse order. These figures were normalized by taking the total counts per second in the muscle at the beginning of the experiment as unity. A computer programme was written to calculate this result for ^{24}Na, ^{42}K and ^{36}Cl. The programme also corrected the sodium and potassium results for decay of the isotopes. The normalized figures were plotted against time on semi-logarithmic graph paper, and further analysis was done fitting lines by eye, as described by Solomon (1960). The counts in the tissue at zero time were converted into m-mole/kg F.wt.:

$$\text{Total ions (m-mole/kg F.wt.)} = \frac{\text{c.p.s./mg in tissue at zero time}}{\text{c.p.s./mg in loading solution}} \times \text{ion content of Krebs solution.}$$

Chemical analysis. In the sodium and potassium efflux experiments, after counting the radioactivity of the tissue, it was returned to a non-active medium to allow for recovery from effects of exposure to room temperature. Then the pieces were removed, blotted and weighed, and treated for determination of ion content, by the methods described by Casteels & Kuriyama (1965). In the chloride efflux experiments, an aliquot of the solution in which the muscle had been extracted in distilled water for 12 hr was analysed.

Analysis of efflux curves. The efflux curves calculated from tissue washouts using the apparatus described were very similar to those obtained by the other method of transferring the tissue through a series of tubes containing non-radioactive solution. The curves were smooth and fluctuations cut down to a low level. Due to variations

in the amount of surface radioactivity carried over from the loading solution, the washout curves were shifted up and down the y axis, and it was difficult to construct average curves for washouts of the same ion, and thus difficult to compare the effluxes in normal and hypertonic solution. In order to construct a meaningful average curve, individual results were subjected to exponential analysis, as described by Solomon (1960).

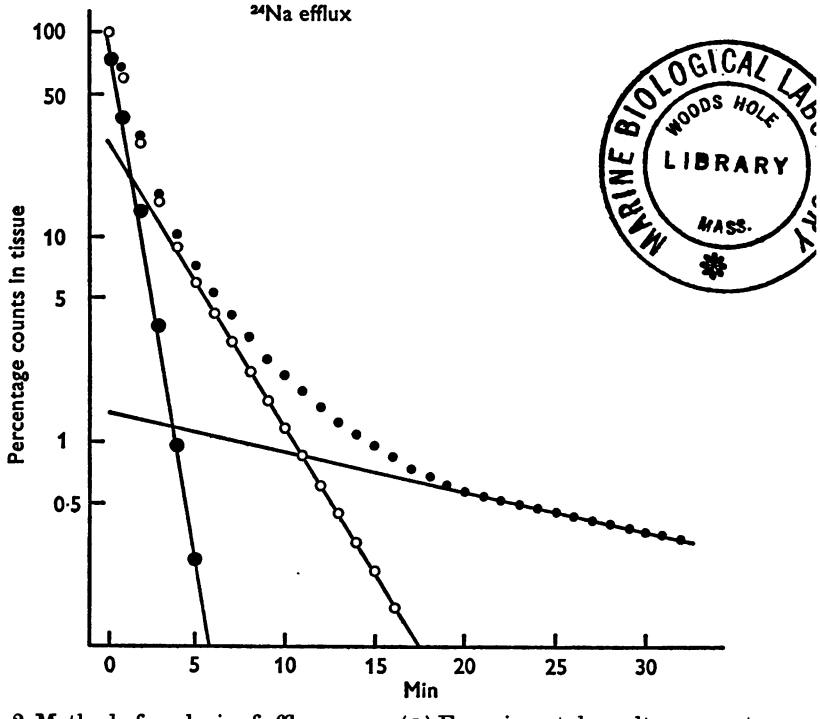

Fig. 2. Method of analysis of efflux curve. (●) Experimental results, percentage radioactivity in the tissue on a logarithmic scale plotted against time after beginning washout of the activity. After 20 min the points can be considered to fall on a straight line. The middle component of the curve is obtained by subtracting the line from the experimentally determined points. The values of this second component (○) also can be considered to fall on a straight line after about 5 min, and subtraction of this line from the earlier values gives the third component, the values for which (●) can all be considered to fall on a straight line. The slopes of the three lines represent the rate constants for the three terms, and the extrapolation of the lines to zero time give the values A, B and C (Table 1).

It was found that it was impossible to get a good fit for any of the effluxes by using two exponential terms, but in every case the curves could be satisfactorily described as the sum of three exponentials, so that the counts in the tissue at the time (t) after the beginning of the washout (P_t) could be calculated from the equation:

$$P_t = A\exp(-\lambda_1 t) + B\exp(-\lambda_2 t) + C\exp(-\lambda_3 t),$$

where λ_1, λ_2, λ_3 are the rate constants of the components (min $^{-1}$), and A, B, and C are the extrapolations of the components to zero time (% total counts). Fig. 2 illustrates

this method of analysis applied to a sodium efflux curve in normal solution. The lines have been fitted by eye.

It was thus possible to evaluate the constant terms in the equation for each result, and to calculate means and standard errors for each constant in the two conditions, and to construct average curves, with some estimate as to the significance of any differences between the effluxes. It should be pointed out that no physiological significance need be placed on the fact that the curves can be described in this manner.

RESULTS

The washouts of sodium, potassium and chloride were carried out on many tissues in normal and hypertonic solutions. Each efflux curve was analysed by the method described. The mean and S.E. of the six variables were obtained for the three ions in both solutions, and these are given in Table 1. This table also shows the half-time of exchange for each component:

$$\text{half-time } (t_{\frac{1}{2}}) = \frac{0{\cdot}693}{\text{rate constant}}.$$

Equilibration of the radioactive ions

The total sodium, potassium and chloride in the tissue determined by flame photometry, was not significantly different from the values estimated from the effluxes using the specific activity of the bathing medium, indicating that the radioactive ions were probably completely equilibrated with the non-radioactive species. This was true for all three ions, although the conditions in the ionic estimations were not exactly parallel. The efflux curves yield estimates of the concentration at the beginning of the washout curve, after an initial rinse in non-radioactive solution, whereas the ionic content determined by flame photometry represents the value at the end of the washout, and after the surface solution had been removed by blotting.

Sodium efflux

The total amount of sodium in the tissue at zero time calculated from the efflux curves was equivalent to 65·8 m-mole/kg F.wt. in the normal solution, and 64·3 m-mole/kg F.wt. in hypertonic solution. The difference was not significant, and it appears that the tissue loses no sodium under these conditions during exposure to hypertonic solution. This contrasts with the findings of Brading & Setekleiv (1968) who reported a loss of about 10–20% sodium in the hypertonic solutions. The tissues were mounted in the previous work at considerably less than their *in vivo* length, and some of the loss could be explained by a reduction in the extracellular space. In the present experiments the tissues were stretched approximately

TABLE 1. Average values from three compartment analysis. N: normal solution, H: hypertonic solution; the values are given as mean (n) ± S.E.

	K efflux		Na efflux		Cl efflux	
	N	H	N	H	N	H
A Extrapolated fast component (m-mole/kg F.wt.)	3·0 ± 0·5 (9)	4·3 ± 1·3 (7)	45·1 ± 2·9 (15)	35·5 ± 3·2 (12)	35·2 ± 2·0 (11)	38·1 ± 1·7 (8)
	$P > 0·5$		$P = 0·025$		$P = 0·3$	
B Extrapolated middle component (m-mole/kg F.wt.)	2·8 ± 0·3 (12)	3·1 ± 0·5 (10)	18·9 ± 1·8 (15)	27·1 ± 2·7 (12)	13·7 ± 1·7 (11)	15·5 ± 1·1 (9)
	$P > 0·5$		$P = 0·01$		$P = 0·4$	
C. Extrapolated slow component (m-mole/kg F.wt.)	58·9 ± 3·2 (12)	61·9 ± 2·9 (10)	1·77 ± 0·47 (15)	1·78 ± 0·52 (12)	15·0 ± 2·0 (11)	6·0 ± 1·2 (9)
	$P > 0·4$		$P > 0·5$		$P = 0·001$	
Total $A+B+C$	64·7 ± 4·2 (9)	67·3 ± 3·1 (7)	65·8 ± 3·0 (15)	64·3 ± 3·1 (12)	64·0 ± 3·4 (11)	59·1 ± 1·8 (8)
	$P > 0·6$		$P > 0·7$		$P > 0·2$	
Fast rate constant (min^{-1})	1·211 ± 0·076 (9)	0·980 ± 0·175 (7)	0·958 ± 0·035 (15)	1·014 ± 0·039 (12)	1·090 ± 0·05 (11)	0·952 ± 0·10 (9)
λ_1	$P > 0·2$		$P = 0·3$		$P = 0·2$	
$t_{\frac{1}{2}}$ (min)	0·57	0·71	0·72	0·68	0·64	0·73
Middle rate constant (min^{-1})	0·207 ± 0·015 (12)	0·203 ± 0·022 (10)	0·291 ± 0·009 (15)	0·346 ± 0·018 (12)	0·191 ± 0·014 (11)	0·210 ± 0·014(9)
λ_2	$P > 0·8$		$P < 0·01$		$P = 0·3$	
$t_{\frac{1}{2}}$ (min)	3·34	3·42	2·38	2·00	3·62	3·29
Slow rate constant (min^{-1})	0·011 ± 0·001 (12)	0·015 ± 0·001 (10)	0·035 ± 0·004 (15)	0·031 ± 0·005 (12)	0·055 ± 0·004 (11)	0·055 ± 0·008 (8)
λ_3	$P = 0·001$		$P = 0·5$		$P = 0·6$	
$t_{\frac{1}{2}}$ (min)	64·74	46·59	19·54	22·46	12·70	12·58

to their *in vivo* length, and there is some evidence that there was little change in extracellular space under these conditions.

Fig. 3*a* was obtained by computing the curves from the figures for the variables given in Table 1. The graph shows the average curves for sodium efflux in normal and hypertonic solution. Typical curves from one experiment are shown in Fig. 3*b*. The curves are very similar, and it would be

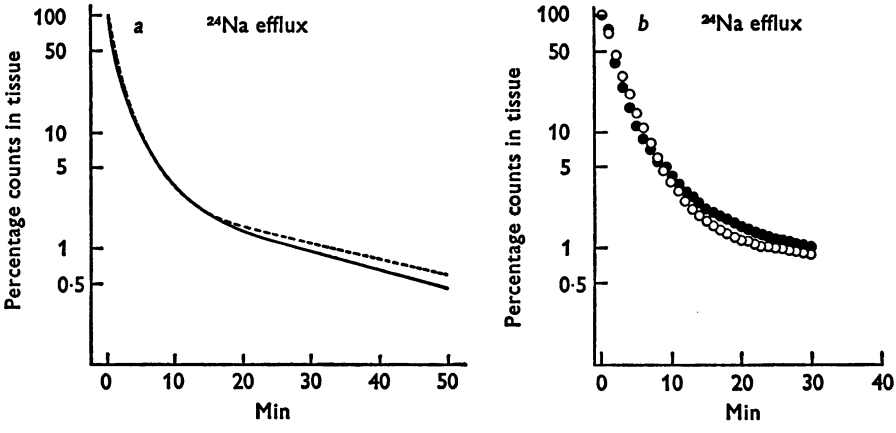

Fig. 3. Efflux curves of ^{24}Na in normal (continuous line) and hypertonic (dashed line) solution.

a. These curves are average curves and have been computed from the equation

$$P = A\exp(-\lambda_1 t) + B\exp(-\lambda_2 t) + C\exp(-\lambda_3 t),$$

where P is the percentage of the counts in the tissue at time t, and the values for the six variables are the average values shown in Table 1.

b. The results from a typical experiment. The tissue was first loaded with ^{24}Na and a washout curve obtained, using normal Krebs solution (filled circles), and then the tissue was reloaded and equilibrated in hypertonic solution (open circles), and a second washout curve obtained.

impossible to detect significant differences by observation only. The exponential analysis does, however, indicate that there may be some significant difference between the curves. The rate constants and extrapolated size for the fastest and for the slowest components in normal and hypertonic solutions were not significantly different from each other (see Table 1). The rate constant of the middle component was, however, significantly higher in hypertonic solution, and the extrapolation to zero time significantly larger than in normal solution.

Potassium efflux

The total amount of potassium calculated from the efflux curve was 64·7 m-mole/kg F.wt. in normal solution and 67·2 m-mole/kg F.wt. in hypertonic solution. Again these figures are not significantly different,

indicating no loss of potassium from the tissue. This also is in contrast to a slight loss noted by Brading & Setekleiv (1968).

In the potassium efflux, there is no significant difference between the behaviour of the tissue in normal and hypertonic solutions with respect to the rate and extrapolated size of the fast and middle components (see Table 1). There is, however, a significant difference between the slow rate constants in the two solutions, the exchange being faster in hypertonic solution than in normal solution, although the extrapolated sizes of these components are not significantly different.

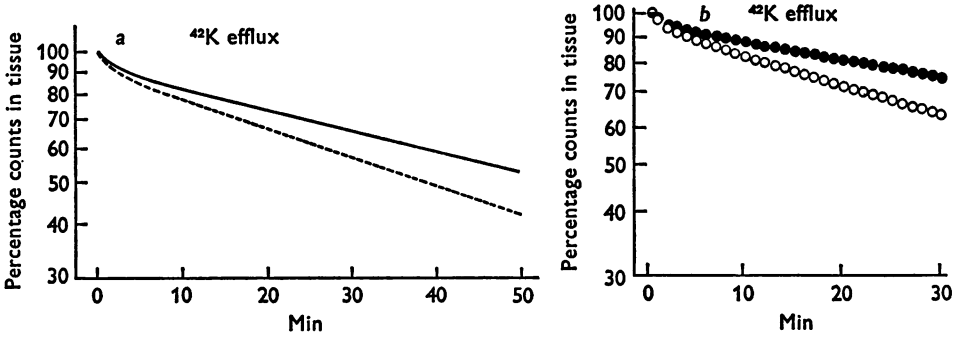

Fig. 4. Efflux curves of ^{42}K in normal (continuous line) and hypertonic (dashed line) solution.

a. These curves are average curves and have been computed from the equation

$$P = A\exp(-\lambda_1 t) + B\exp(-\lambda_2 t) + C\exp(-\lambda_3 t),$$

where P is the percentage of the counts in the tissue at time t, and the values for the six variables are the average values shown in Table 1.

b. The results from a typical experiment. The tissues was first loaded with ^{42}K and a washout curve obtained, using hypertonic Krebs solution (open circles), and then the tissue was reloaded and equilibrated in normal (filled circles) Krebs solution and a second washout curve obtained.

The average curves are shown in Fig. 4*a*, and curves from a single experiment in Fig. 4*b*. The values for the size and rate of the two faster components of the potassium efflux are probably the most inaccurate of the whole series, since the majority of the tissue potassium exchanged relatively slowly. Very small errors in measuring the slope of the slow component can result in large differences in the faster two components, and therefore not much weight should be placed on these figures.

Chloride efflux

The total amount of chloride calculated from the efflux curves was 64·0 m-mole/kg F.wt. in normal solution, and 59·1 m-mole/kg F.wt. in hypertonic solution. These figures are not significantly different, although

the extrapolated size of the slow component is very significantly larger in normal solution than in hypertonic solution (normal 15·0 m-mole/kg F.wt. hypertonic 6·0 m-mole/kg. F.wt.). This reduction may reflect the loss of chloride shown by Brading & Setekleiv (1968). The rate constants and extrapolated sizes of the other components are not significantly different in the two solutions (see Table 1). The average curves are shown in Fig. 5*a*, and curves from single experiments in Fig. 5*b*.

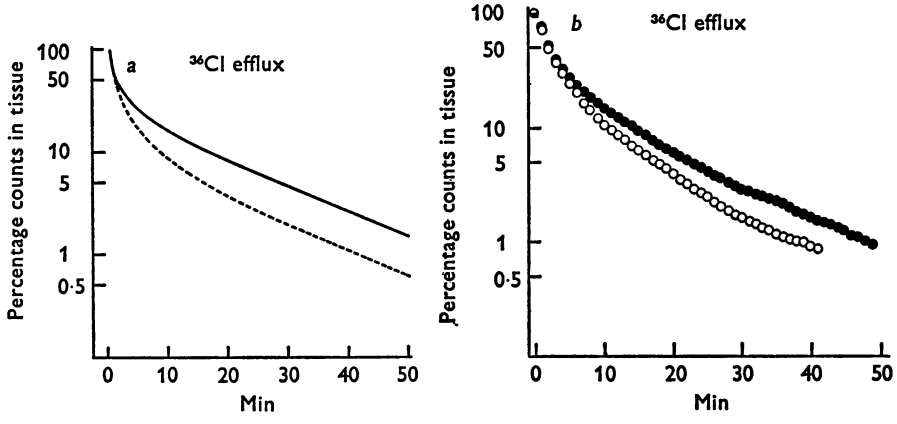

Fig. 5. Efflux curves of ^{36}Cl in normal (continuous line) and hypertonic (dashed line) solution.

a. These curves are average curves and have been computed from the equation

$$P = A\exp(-\lambda_1 t) + B\exp(-\lambda_2 t) + C\exp(-\lambda_3 t),$$

where P is the percentage of the counts in the tissue at time t, and the values for the six variables are the average values shown in Table 1.

b. The results from a typical experiment. One tissue was loaded with ^{36}Cl and washed out using normal Krebs solution (filled circles), and a second tissue from the same animal was loaded and washed out using hypertonic Krebs solution (open circles).

Interpretation of efflux curves

In order to make estimates of the membrane permeability to an ion species from flux data, it is necessary to know the concentration of the species on either side of the membrane, and the rate of exchange of the ion across the membrane.

If a tissue could be considered simply as a two-compartment system, consisting of an extracellular and a uniform intracellular phase, and the tissue ions taken to be freely dissolved in the two phases, then theoretically the washout of tracer ions should be described by the sum of two exponential terms, or by a diffusional and an exponential term if the exchange of the extracellular material is limited by diffusion. In such a tissue, the

extrapolation of the slower exponential phase of the efflux curve to zero time, when corrected for possible back diffusion of ions (Huxley, 1960) should give an estimate of the amount of ion in the intracellular compartment, and the slope of this line can be used to give an estimate of the rate of exchange between the compartments (if suitable corrections are made to allow for distortion due to diffusional delays in the extracellular space; Harris & Burn, 1949; Keynes, 1954). From the efflux curve, knowing the extracellular concentration of the ion in question, one should also be able to calculate the size of the extracellular compartment. Ideally those values should agree with estimates of extracellular space and ion distribution calculated directly from a knowledge of the total tissue water and ions.

In smooth muscle, the efflux curves are more complex than this ideal, and it is difficult to identify with certainty one phase of exchange as being from a homogeneous intracellular compartment, especially in the case of Na^+ and Cl^-. Estimates of internal ionic concentration and membrane permeability become very uncertain and depend to a large extent on the assumptions made as to the distribution of ions in the tissue. These difficulties in interpretation are commonly met in other tissues, as, for example, kidney (Kleinzeller, Janácek & Knotkova, 1962) and intercostal muscle (Lipicky & Bryant, 1966); also a similar complexity is seen in more detailed analysis of the efflux from the frog skeletal muscle (Keynes & Steinhardt, 1968).

Casteels (1969) has attempted to analyse the membrane permeabilities of the guinea-pig taenia coli in Krebs solution, using the simple two-compartment model. He has some difficulty in determining the rate constant of exchange, particularly for sodium ions, and he uses information from total tissue analysis and from uptake measurements to estimate intracellular ion concentrations. His estimates, when inserted into the Goldman equation, predict a membrane potential of -37 mV, and using his values of G_K and G_{Cl}, and a value for G_{Na} calculated from his figures, the predicted total membrane resistance can be shown to be about 30 kΩ cm^2. Using a similar model with the results presented here (giving a higher value of $[Na]_i$ and thus P_{Na} than described by Casteels) the predicted membrane potential in normal Krebs solution was -21 mV, and in hypertonic Krebs solution -24 mV, and the predicted membrane resistance was 24 kΩ cm^2 in normal and 21 kΩ cm^2 in hypertonic solution.

This model is therefore inadequate to describe the tissue, which has been shown (Tomita, 1966*a*) to have a membrane potential of about -51 mV in normal solution and -63 mV in hypertonic solution, and a membrane resistance in hypertonic solution of 30–50 kΩ cm^2 (Abe & Tomita, 1968). The fact that this model is inadequate means that it is necessary to consider other models. The most immediately obvious

possibility is the presence of an extra tissue compartment, exchanging with kinetics different from the exchange of ions across the cell membrane. Before any such explanation is assumed, however, it is necessary to examine other possible sources which could account for the complex curvature of the efflux analysis. If it is assumed that in this tissue there are basically two compartments, and that the cell membrane is the only rate-limiting step in the exchange of intracellular electrolytes with those in the extracellular space, then two factors can distort the efflux from the tissue, and lead to deviations from a single exponential relationship. These are inhomogeneity of cell sizes, and variations in the individual membrane permeabilities of the cells.

Zierler (1966) has considered the interpretation of tracer washout curves from a population of muscle fibres of different sizes, and comes to the following conclusion: If the radii of the majority of the cells differ by less than 50 %, and if the washout from one fibre follows a single exponential, then the washout from the whole population will mimic a single exponential for several time constants, whether the distribution of radii is normal or skewed. In the taenia coli, the muscle is reasonably homogeneous with respect to cell size (Dr H. Nishihara, personal communication), and, as the variation is probably well within the limits specified by Zierler, it seems justifiable to ignore this factor in the present interpretations.

Van Liew (1967) has investigated the other situation, where the morphology of the tissue is homogeneous, but the rate processes vary from cell to cell within the tissue. Such variations can result in curvature, instead of a straight line in a semilogarithmic plot. This author gives details of analysis which allow estimations to be made as to whether or not a particular flux curve could describe an aggregate of simple processes. Analysis of the present results by this method will be considered in the next section.

Another possibility that will be examined is that the slowly exchanging fraction of the ions (represented by the third exponential term of the original analysis) is the transmembrane phase. This leaves the faster exchanging fractions to be accounted for, and the possibility that these can be described by diffusion curves will be examined.

Models

In this section, the aim is to examine some possible interpretations of the efflux curves, and use them to predict the electrical properties of the cell membrane. The usefulness of a model can be gauged by comparisons of the predicted properties with results obtained with micro-electrode studies by other workers. In the next few paragraphs the numerical values and equations used will be described, and then these will be fitted to two basic models. These two models were the only useful ones out of a number tried.

The size of the extracellular compartment will be taken from [^{60}Co] EDTA spaces obtained from washout experiments as described by Brading & Jones (1969). This

method will be used because the extracellular space from tissues mounted at their *in vivo* length (as in washout measurements) is smaller than the space in tissues mounted for tissue analysis (Brading & Setekleiv, 1968) at less than their *in vivo* length. The average value for nine washouts of [^{60}Co]EDTA was $33{\cdot}06 \pm 1{\cdot}5$ % F.wt. In the following calculations, a value of 33 % F.wt. will be taken for the extracellular space in normal and hypertonic Krebs solution. A value of 17 % F.wt. will be taken for the dry weight in both cases (unpublished observations). From these values the cell water is 50 % F.wt. in normal solution and 25 % F.wt. in hypertonic solution (tissue behaving as a perfect osmometer, Brading & Setekleiv, 1968). No corrections have been made for tissue density.

A value for the volume/surface area ratio is needed for estimations of the transmembrane fluxes. The value of $1{\cdot}5\ \mu$ has been used in normal solution for the whole cell volume (Freeman-Narrod & Goodford, 1962). If it is assumed that the intracellular ions are dissolved in the cell water, the volume of the cell water has to be used instead, and the ratio becomes $1{\cdot}07\ \mu$. The calculated transmembrane fluxes are the same whichever concept is used (Casteels (1969) determined the V/A ratio from the whole cell volume, but assumes the ions to be dissolved in a volume equivalent to the volume of cell water, and thus his values for the fluxes are higher than they would have been if the same concept had been used throughout). In calculating the membrane permeabilities, the assumption used will alter the values of P_K and P_{Cl}, since the efflux of these two ions is passive, and the calculation requires a value for the intracellular concentrations without V/A. In hypertonic solution, the volume of the cell water is half that in normal solution, and, on the assumption that the surface area of the membrane does not change, the ratio V/A becomes $0{\cdot}963\ \mu$ assuming that the ions are dissolved in the whole cell volume, and $0{\cdot}573\ \mu$ if they are dissolved in the cell water.

The calculations of the membrane properties will be worked out on the assumption that the intracellular ions are dissolved in the volume occupied by the cell water. If the other assumption is used, the calculated membrane permeabilities for potassium and chloride ions are 30–40 % greater in normal solution, and 60–80 % greater in hypertonic solutions, depending on the model used. If the surface area of the membrane decreases in hypertonic solution, then the fluxes per unit area will be greater, and this will be reflected in an increased value of the permeabilities of the membrane to the three ions in hypertonic solution. The ratio of the permeabilities should, however, remain unchanged.

Ion fluxes (M) can be calculated from the equation of Keynes & Lewis (1951)

$$M = K(V/A)C_i,$$

where K is the rate constant (sec^{-1}), V/A the volume/surface area ratio, and C_i the intracellular ion concentration. Harris & Burn (1949) and Keynes (1954) have pointed out that this estimate of the apparent flux will in fact be incorrect if there is an appreciable diffusion delay in the exchange of ions between the centre of the muscle and the surrounding medium. The flux values will thus be corrected using Keynes correction. The diffusion coefficient (D) for sodium is taken as $3{\cdot}1 \times 10^{-6}$ cm^2 sec^{-1} (see model 1) and for potassium and chloride $4{\cdot}5 \times 10^{-6}$ cm^2 sec^{-1}. The radius of the tissue pieces is taken to be $300\ \mu$ in normal solution as calculated approximately from the length and weight of the tissues, and $260\ \mu$ in hypertonic solution. The same value for D will be used under both normal and hypertonic conditions. In the two models described below, only the corrected value of the potassium fluxes were significantly different from the apparent fluxes, being 12–33 % higher, depending on the conditions.

The permeability of the membrane to the ion species will be calculated using the

method described by Katz (1966). This method holds good only for passive fluxes of ions. For effluxes, the permeability (P) can be calculated from the equation

$$P = \frac{M}{C_i \times \frac{VF/RT}{1-\exp(-VF/RT)}},$$

where V is the membrane potential, and is taken to be negative when the ion movement is opposed by the electric field, and positive when the movement is assisted. In normal solution the value for the membrane potential is taken to be -50 mV, and in hypertonic solution -62 mV (Tomita, 1966*a*). For influxes, the extracellular concentration is used. Under steady-state conditions, it may be assumed that the efflux and influx are equal. If it is assumed that there is a sodium–potassium exchange pump in the taenia coli, then the passive fluxes of these two ions will be the potassium efflux and the sodium influx, and these fluxes have been used to estimate the membrane permeabilities to these ions. There is some evidence (Casteels, 1965) that there is an active component of chloride influx, and so the membrane permeability to this will be calculated from the efflux.

Membrane potentials can be predicted from the constant field equation, and at 37° C can be estimated from the equation:

$$E = 61{\cdot}5 \log_{10} \frac{P_K[K_i] + P_{Na}[Na_i] + P_{Cl}[Cl_o]}{P_K[K_o] + P_{Na}[Na_o] + P_{Cl}[Cl_i]}.$$

The ionic conductances will be calculated from the following equations from Hodgkin & Katz (1949)

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

$$G_{Cl} = P_{Cl} \frac{F^2}{RT} \frac{E}{E-E_{Cl}} \times \frac{[Cl_i] - [Cl_o]\exp(-EF/RT)}{1-\exp(-EF/RT)},$$

$$G_{Na} = P_{Na} \frac{F^2}{RT} \frac{E}{E-E_{Na}} \times \frac{[Na_o] - [Na_i]\exp(-EF/RT)}{1-\exp(-EF/RT)}.$$

Model 1

The model will use data from the analysis of the efflux curves. It will be assumed that the slow components of the efflux curves represent the transmembrane fluxes of the ions. The middle component of exchange together with the fastest phase are both considered to be extracellular. This may seem an unlikely situation because the amount in these two components will be greater than that which could be present dissolved in the extracellular medium. There is, nevertheless, some justification in this approach. Brading & Jones (1969) observed that the kinetics of exchange of the extracellular markers, [^{60}Co]EDTA and [^{14}C]sorbitol, were remarkably similar to the two fast phases of the sodium exchange in smooth muscle, and that these exchanges could all be fitted by a bulk diffusion curve, although the 'sodium space' was larger than the [^{60}Co]EDTA and [^{14}C]sorbitol spaces. Fig. 6 in the present paper shows the two fast phases from the

average curves of the three ions sodium, potassium and chloride in normal and hypertonic solutions, in relation to bulk diffusion curves calculated for diffusion through a plain sheet (Crank, 1956). The value D/l^2 for each of the curves shown is 0·00344 which for sodium represents a diffusion coefficient of $0{\cdot}31 \times 10^{-5}$ cm^2 sec^{-1}, and a half thickness of 0·03 cm. The

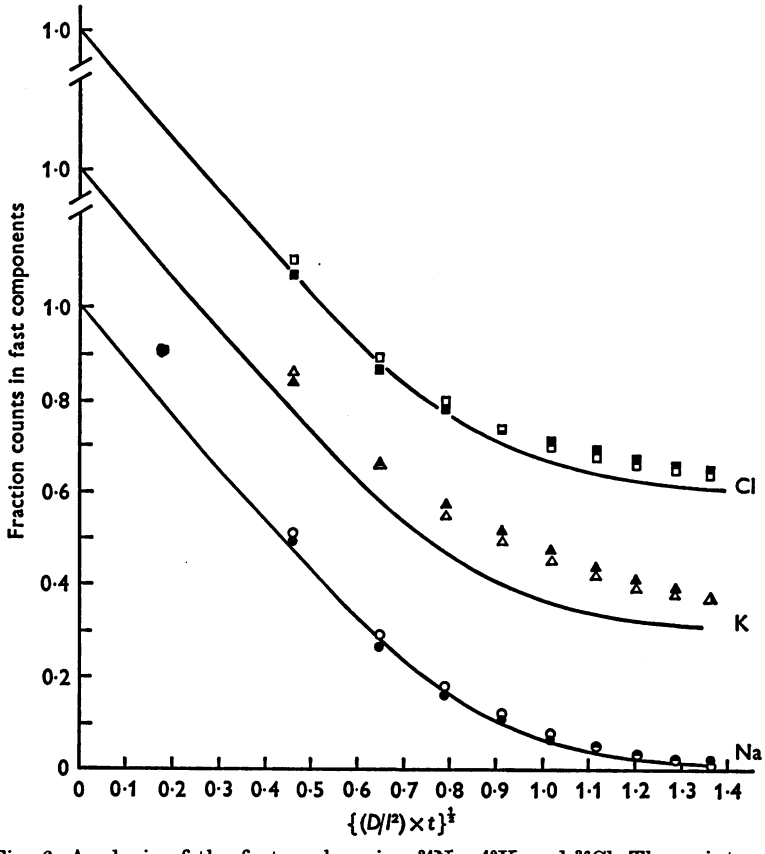

Fig. 6. Analysis of the fast exchanging ^{24}Na, ^{42}K and ^{36}Cl. The points are from the sum of the two fastest exponential terms from the efflux curve analysis. The filled symbols are from effluxes in normal solution, and the open symbols from effluxes in hypertonic solution. Ordinate: fraction of counts in the fast components. Abscissa: non-dimensional time parameter (Crank, 1956; see text). The curves represent the theoretical relationship based on bulk diffusion through a plain sheet.

value D for the best fit is identical with the value obtained by Keynes (1954) for diffusion in the extracellular space of frog toe muscle. If the diffusion constant for potassium and chloride ions is taken to be $0{\cdot}45 \times 10^{-5}$ cm^2 sec^{-1} (see Keynes, 1954) then the half thickness of the pieces would be 0·036 cm. In fact, the piece of taenia dissected for the sodium

flux experiments were normally cut thinner than those for potassium and chloride to reduce time taken for diffusion of sodium since Goodford & Hermansen (1961) had shown that sodium entry was limited by diffusion in the extracellular space. The potassium points can be fitted better by using a larger value for l or a smaller value for D, but since these points are likely to be the most inaccurate, no great significance should be attached to them. It can be seen that it is possible to get a reasonable fit for all three ions, although the fit for potassium and chloride ions is never as good as that for sodium ions. It follows that the two fast phases of ion exchange could be described as a single component whose exchange is limited by diffusion in the extracellular space, although this may be an over-simplification, especially for potassium and chloride ions.

If then, the slowly exchanging components are considered to represent the transmembrane components, the next problem is to estimate the size of these components. The information available is the size of the extrapolated components, and their rate constants. However, as has been pointed out previously, the size of the extrapolated components does not correspond exactly with the actual size. If the system is considered to be a two-compartment one, then Huxley's correction factor (Huxley, 1960) may give an estimation of the actual size. This correction factor has been derived from a consideration of efflux curves that can be described as a sum of two exponential terms, which is not the case here, but in the absence of any other simple method it may give a reasonable estimate of the size of error involved.

The correction factor predicts that the size of the slowly exchanging component will be

$$\frac{XY(\lambda_x-\lambda_y)^2}{X\lambda_x^2-Y\lambda_y^2},$$

where Y is the extrapolation to zero of the slowly exchanging compartment, and λ_y its rate constant. X is the extrapolation of the extracellular component to zero time and λ_x its rate constant. Calculations show that with the values of Y and λ_y in these experiments, the size of X makes relatively little difference to the corrected size of the slowly exchanging compartment, and the magnitude of the correction is determined by the rate λ_x; the smaller the value of λ_x the larger the correction. The value taken when applying the correction was an intermediate value, obtained by averaging the two values λ_1 and λ_2 (see Table 1). Table 2 gives the parameters worked out assuming a size for the intracellular compartment calculated using the above correction.

The ratio of the permeabilities are $P_{Na}/P_K = 0{\cdot}0098$ in normal solution and 0·0082 in hypertonic solution. $P_{Cl}/P_K = 0{\cdot}66$ in normal and 0·58 in

hypertonic solution. The predicted membrane potentials are −57·2 mV in normal and −76·0 mV in hypertonic solution, and the total membrane resistance calculated from the sum of the ionic conductances is 72·2 kΩ cm² in normal and 74 kΩ cm² in hypertonic solution.

TABLE 2. Properties of Model 1

	Normal			Hypertonic		
	Na	K	Cl	Na	K	Cl
Extracellular ionic concentration (mM)	136·9	5·9	133·6	129·2	5·56	126
Total intracellular ions (m-mole)/kg F. wt.	1·57	56·99	12·51	1·65	58·67	4·87
Concentration in cell water (mM)	3·14	113·98	25·02	6·6	234·28	19·48
Transmembrane rate constant (min^{-1})	0·0355	0·0107	0·0546	0·0309	0·0149	0·0551
Apparent fluxes (p-mole cm^{-2} sec^{-1})	0·20	2·17	2·44	0·18	3·12	0·96
Corrected fluxes (p-mole cm^{-2} sec^{-1})	0·20	2·6	2·44	0·18	4·03	0·96
Permeabilities (cm $sec^{-1} \times 10^{-8}$)	0·066	6·71	4·4	0·056	6·84	3·94
Equilibrium potentials (mV)	+100·8	−79·1	−44·7	+79·4	−99·9	−49·9
Ionic conductances G(mho $cm^{-2} \times 10^{-6}$)	0·13	5·73	7·99	0·12	7·64	5·75
Predicted membrane potential (mV)		−57·23			−75·99	
Predicted membrane resistance (kΩ cm²)		72			74	

Model 2

This model will be used to consider the possibility that the slow and middle components of potassium and chloride fluxes represent efflux from a population of fibres with rates of efflux distributed about a mean value. Van Liew (1967) has published populations of curves that are aggregates of exponential processes, and has described methods of estimating the mean and standard deviation of the rate constants from the efflux curves. These methods have been applied to the last two phases of efflux of the three ions. This curve for sodium ions will not fit any of Van Liew's curves,

and is, in fact, so different as to render negligible the chances that this method can be applied to sodium ions. On the other hand, the curves for potassium and chloride do appear to be similar to Van Liew's curves. Fig. 7 shows points from the last two components of the chloride curve plotted using Van Liew's technique. The lines are the predicted efflux from an exponential aggregate system of components with a truncated normal distribution. The ratio of the standard deviation to mean halftime of the rates are given. The experimental points follow the same shape, and have a ratio of S.D./$\bar{X}_{\frac{1}{2}}$ similar to the value for Van Liew's curve, although the

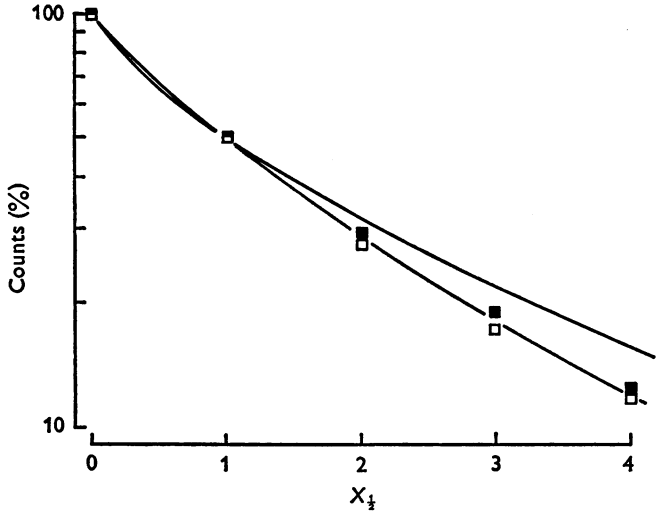

Fig. 7. Analysis of the slowly exchanging ^{36}Cl. The points are from the sum of the middle and slow components of the efflux curve analysis for chloride in normal (filled squares) and hypertonic (open squares) solution. Ordinate: percentage counts in the slowly exchanging components. Abscissa: time expressed in terms of the half-time ($X_{\frac{1}{2}}$) of the curves. The curves taken from Van Liew (1967) are of exponential aggregate systems with truncated normal distribution, the upper curve having the ratio S.D./$\bar{X}_{\frac{1}{2}}$ of 0·76, and the lower curve 0·56. The S.D./$\bar{X}_{\frac{1}{2}}$ of the chloride points in normal solution is 0·54, and in hypertonic solution 0·66.

agreement is better in normal solution than in hypertonic solution. The deviation of the S.D./$\bar{X}_{\frac{1}{2}}$ from Van Liew's curve may indicate that some other process is occurring, or that the distribution of the individual values is asymmetric. The calculated mean and S.D. of the chloride flux was mean $t_{\frac{1}{2}}$ 8·3 min S.D. ± 4·5 (S.D./$\bar{X}_{\frac{1}{2}}$ = 0·54) in normal solution, and mean $t_{\frac{1}{2}}$ 6·1 min S.D. ± 4 (S.D./$\bar{X}_{\frac{1}{2}}$ = 0·66) in hypertonic solution. The potassium effluxes were not carried out for long enough to compare them with Van Liew's but the general shape seems to be similar. The calculation of the

mean and S.D. of the potassium fluxes are, in normal solution: mean $t_{\frac{1}{2}}$ 62·0 min S.D. ± 11 (S.D./$\overline{X}_{\frac{1}{2}}$ = 0·18) and in hypertonic solution: mean $t_{\frac{1}{2}}$ = 44·5 min S.D. ± 9·2 (S.D./$\overline{X}_{\frac{1}{2}}$ = 0·21).

In this model the parameters for the sodium exchange and distribution will be the same as in model 1. In order to make an estimate of the intracellular amount of potassium and chloride ions, the Huxley correction

TABLE 3. Properties of Model 2

	Normal			Hypertonic		
	Na	K	Cl	Na	K	Cl
Extracellular ionic concentration (mM)	136·9	5·9	133·6	129·2	5·56	126
Total intracellular ions (m-mole/kg F. wt.)	1·57	60·67	24·6	1·65	63·19	16·81
Concentration in cell water (mM)	3·14	121·34	49·2	6·6	252·76	67·24
Transmembrane rate constant (min^{-1})	0·0355	0·0112	0·0829	0·0309	0·0156	0·1136
Apparent fluxes (p-mole cm^{-2} sec^{-1})	0·20	2·42	7·28	0·18	3·52	6·84
Corrected fluxes (p-mole cm^{-2} sec^{-1})	0·20	2·96	7·28	0·18	4·70	6·84
Permeabilities (cm $sec^{-1} \times 10^{-8}$)	0·066	7·17	6·69	0·056	7·4	3·95
Equilibrium potentials (mV)	+100·8	−80·8	−26·7	+79·4	−101·9	−16·8
Ionic conductances G (mho $cm^{-2} \times 10^{-6}$)	0·13	6·35	17·5	0·12	8·84	11·9
Predicted membrane potential (mV)		−40·9			−54·0	
Predicted membrane resistance (kΩ cm^2)		41·6			47·9	

will be used, although it is realized that it will not strictly apply; nevertheless, it can be used to correct in some measure for back diffusion from the extracellular space. The values X and λ_x will be taken from the fast component of exchange, and Y will be the sum of the extrapolation to zero time of the slow and middle components, and λ_y from the mean $t_{\frac{1}{2}}$ values given above. From the corrected values, some idea of the amount of potassium and chloride ions in the fastest component can be gained. If all

the fast exchanging chloride is free in the extracellular space, the size of the space would be 29·5 % F.wt. in normal solution and 33·6 % F.wt in hypertonic solution. These values are within the range of extracellular spaces calculated from the [^{60}Co]EDTA space quoted above. There is more potassium ion content in the corrected fast exchanging fraction than could be freely dissolved in an extracellular space of this size. Assuming a space of 33 %, the extra potassium is 2·08 m-mole/kg F.wt. in normal and 2·28 m-mole in hypertonic solution.

Table 3 lists the values for the ion fluxes and membrane permeabilities of this model. The ratios of the permeabilities are: P_{Na}/P_K is 0·0092 in normal and 0·0074 in hypertonic solution; P_{Cl}/P_K is 0·962 in normal and 0·534 in hypertonic solution. The predicted membrane potential is $-41·0$ mV in normal solution and $-54·0$ mV in hypertonic solution, and the total membrane resistance 41·6 kΩ cm^2 in normal and 47·9 kΩ cm^2 in hypertonic solution.

DISCUSSION

The original aim of the experiments described in this paper was to evaluate the effect of solutions made hypertonic with sucrose, on the permeability of the membranes of the smooth muscle of the guinea-pig taenia coli. It soon became obvious that although it was comparatively simple to get good efflux curves for the various ions studied, the interpretation of these curves and the estimation of the membrane permeability were extremely difficult. The approach taken was to consider possible ion distributions, and various factors that might contribute to the shape of the curves, and to construct models of the tissue with which to evaluate the various membrane parameters. Each model was then tested to see how far the parameters it predicted in both normal and hypertonic solutions agreed with the values established under these conditions by other workers using electrophysiological techniques.

Many more models were tested than the two described in detail in this paper. The main conclusion that was reached was that any model that considered to be intracellular all the sodium that could not be contained dissolved in the extracellular water, gave very unsatisfactory predictions of the membrane parameters in both solutions. Models were similarly unsatisfactory if the rate of exchange of sodium was taken to be relatively fast, with a half-time similar to that of the middle phase of the sodium exchange.

Casteels (1969) has described a model with relatively fast exchanging transmembrane sodium, which predicts properties not in agreement with the established parameters, and he postulates some other factors such as an electrogenic pump to explain the discrepancy. When the values from

the efflux curves in the present experiment were used in a similar model to Casteels, the predicted parameters were even less satisfactory, and also failed to predict the hyperpolarization observed in hypertonic solutions. The results in this paper suggest that it is not necessary to involve some unknown factor in order to account for the membrane properties, if it is assumed that the intracellular sodium concentration is low, and the rate of exchange is derived from the slowly exchanging fraction of this ion.

There are two justifications for using this distribution and exchange of sodium. Electrophysiological evidence clearly suggests that the membrane is much more permeable to potassium that it is to sodium, since the effects of sodium on the membrane potential (Kuriyama, 1963) and on the membrane resistance (Tomita, 1966*b*; H. Ohashi, personal communication) are very much smaller than those of potassium which both indicate a low membrane permeability to sodium. Also the experiments of Brading & Jones (1969) indicate that the majority of sodium is only limited by diffusion in its exchange with the external medium. It is unlikely that an exchange diffusion process is occurring in the tissue, since Goodford (1966) showed that there was little change in the efflux of sodium in a sodium-free medium.

The two models described in detail in this paper both assume that the slowly exchanging part of the efflux curve for sodium represents the transmembrane exchange. It must be pointed out that the exact values for the parameters predicted by both models depend to a quite considerable extent on the sizes taken for the extracellular, and thus intracellular spaces. The variable that this effects most markedly is the intracellular chloride concentration, and hence the values of E_{Cl} chloride flux and membrane permeability to chloride ions.

In formulating the first model, the slow phases of the exchange of the three ions only were considered to be the transmembrane fluxes. This assumes that there is a proportion of all three ions over and above those dissolved in the extracellular water, that is only limited by diffusion in its exchange with the bathing medium. In justification of this it was shown that the faster exchanges of the three ions could be reasonably well (but not perfectly) described by diffusion curves from a plane sheet. This model predicts an increase in the membrane potential in hypertonic solution, but the potentials and the resistances are rather higher than those obtained by more direct measurements. It would seem probable that this model underestimates both the amount of ions in the intracellular water, and also their rates of exchange.

The second model uses the same assumptions for sodium distribution, but for potassium and chloride the fastest phase of exchange for each ion is considered extracellular exchange, and the rest to be transmembrane. The curvature of this latter phase of the efflux curve is considered to be due

to cell to cell variations in the permeability of the membrane to ions, and in justification it is shown that this phase of efflux of potassium and chloride follow roughly the shape calculated by Van Liew (1967) for efflux from a system consisting of an aggregate of exponential processes with a truncated normal distribution. This model also predicts the increase in membrane potential in hypertonic solution, and predicts reasonable figures for the membrane resistance, but the predicted values for the potentials are a little lower than those measured. It seems probable that this model over-estimates the amounts of intracellular potassium and chloride.

The fact that neither model exactly predicts the membrane parameters might be expected from the fact that experimental results cannot be precisely described using either of the assumptions. It seems that there is no easy way to get a direct estimate of the membrane permeability from the efflux curves. The models do, however, suggest that a likely interpretation would be that there is a certain amount of extracellular material not freely dissolved in the extracellular water, but nevertheless exchanging sufficiently fast to be limited in its exchange by diffusion, and that the true transmembrane exchange will be curved on a semi-logarithmic plot due to a variation of individual cells in their ionic permeabilities. Such a combination of features might predict membrane parameters near to the means of model 1 and 2 estimates, so that the predicted resting potential in normal solution would be about 49 mV, and 65 mV in hypertonic solution, with membrane resistances of 57 kΩ in normal and 52 kΩ in hypertonic solution, which values are reasonably close to the experimentally observed ones.

Goodford (1970) has calculated that there may be about 4 m-equiv of fixed anionic sites on the cell membranes, and he has derived equations which suggest that 5·2 m-mole/kg wet wt. of sodium ions, 0·45 of potassium and 6 of chloride might be held in association with these sites. (Also 1·45 m-mole/kg wet wt. of Ca^{2+} and 0·72 of Mg^{2+}.) These would be rapidly exchanging, and might well explain the additional amounts of the ions in the extracellular phase proposed in this paper.

The evidence suggests that solutions made hypertonic by the addition of sucrose do not markedly affect the membrane permeability, although it is possible that the chloride permeability may decrease slightly under these conditions. None of the models examined suggested any significant change in potassium permeability.

In conclusion, it seems that although neither of the models described in this paper precisely predicts the membrane parameters derived from electrophysiological experiments, nevertheless they suggest that these properties could be predicted from the passive membrane properties and

distribution of ions, without having to postulate any special mechanisms such as electrogenic pumping to account for the experimental results.

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