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Ion Distribution and Ion Movements in Smooth Muscle

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II. ION DISTRIBUTION AND THE ROLE OF CALCIUM IN CELLULAR FUNCTION

Ion distribution and ion movements in smooth muscle

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The steady-state fluxes of K^+, Cl^- and Na^+ from smooth muscles in normal Krebs solution are described, and some of the problems encountered in the interpretation of such results are discussed. Sodium fluxes are particularly difficult to analyse, and the type of model used to estimate sodium permeability from flux curves, determines the value calculated to a large extent.

In order to simplify the ionic distribution in the tissue, in the hope of obtaining more information about the handling of Na and K by the guinea-pig taenia coli, potassium-free (high Na) tissues and sodium-free (high K) tissues were prepared by soaking for 4 h in the relevant solution. The tissues reach a steady state, and the effluxes of ^{42}K from high K tissues and ^{24}Na from high Na tissues were measured and compared. In these two conditions the electrochemical gradients for Na^+ and for K^+ are probably identical. Nevertheless, the fluxes are markedly different. At 37 °C the Na flux is much faster than the K flux, and a part of the Na exchange is very temperature sensitive. There is also some evidence for a sodium calcium exchange mechanism. It is hoped that further experiments will produce results that will help to understand the processes involved in these exchanges.

Studies on the distribution and exchange of ions in smooth muscle are important on two accounts; first, because theoretically at least, such studies represent one of the few available methods of directly estimating intracellular ion content and membrane permeability, and secondly because they can throw light on the mechanisms by which the smooth muscle cells deal with ions.

Interpretation of steady state flux curves

Normally estimates of cell permeability, which rely on measurements of intracellular ionic content, are carried out under steady-state conditions, that is when the cells are neither gaining nor losing ions, and therefore when the influx and efflux of a particular ionic species are equal. Under these conditions, and if the cell membrane represents the only rate limiting step in the exchange, then measurements of the uptake or washout of a tracer ion should follow a predictable pattern. The flux should show a fast period of exchange that can be described by a diffusion limited process, and will be exchange of extracellular ions, and a slower exponential phase, which will represent exchange across the cell membranes on the condition that the cells are homogeneous with respect to ionic content and membrane permeability. Under such conditions, the extrapolated size of the exponential phase, when corrected for back diffusion of ions (Huxley 1960), should estimate the intracellular ionic content, and should agree with direct measurements. Also the time constant of the exponential phase, if corrections are made to allow for distortion due to diffusional delays in the extracellular space (Harris & Burn 1949; Keynes 1954) should allow an estimate of the rate of exchange across the membrane.

The normal method of plotting an efflux curve is to use semilogarithmic paper, and to plot the total tissue counts against time. On this plot the exponential phase should be linear. This is not a very sensitive method of estimating whether or not a truly exponential phase has been

reached, and it is only too easy to draw a straight line through points that are slightly curved. A more sensitive method of seeing when a true exponential phase had been reached, is to calculate the instantaneous rate constant of the exchange, that is, the counts per minute leaving the tissue over a short interval of time, divided by the average counts in the tissue during that time.

Potassium exchange

Measurements of potassium exchange in smooth muscles have been made by several authors (taenia coli: Goodford & Hermansen (1961), Casteels (1969*a*), Brading (1971); vascular muscle: Jones & Karreman (1969*b*), Wahlström (1972); uterine muscle: Daniel (1963), Jones (1970*b*); vas deferens: Casteels (1969*b*)). In all cases the washout of labelled potassium follows a reasonably predictable course, the majority of exchange being described by a single exponential function, except after long periods of washout where a second slower phase is sometimes apparent (see, for example, Goodford & Hermansen 1961), but this could be associated with tissue deterioration. Although potassium seems to behave in a predictable manner, a more detailed analysis of the potassium efflux curve reveals a few points of interest.

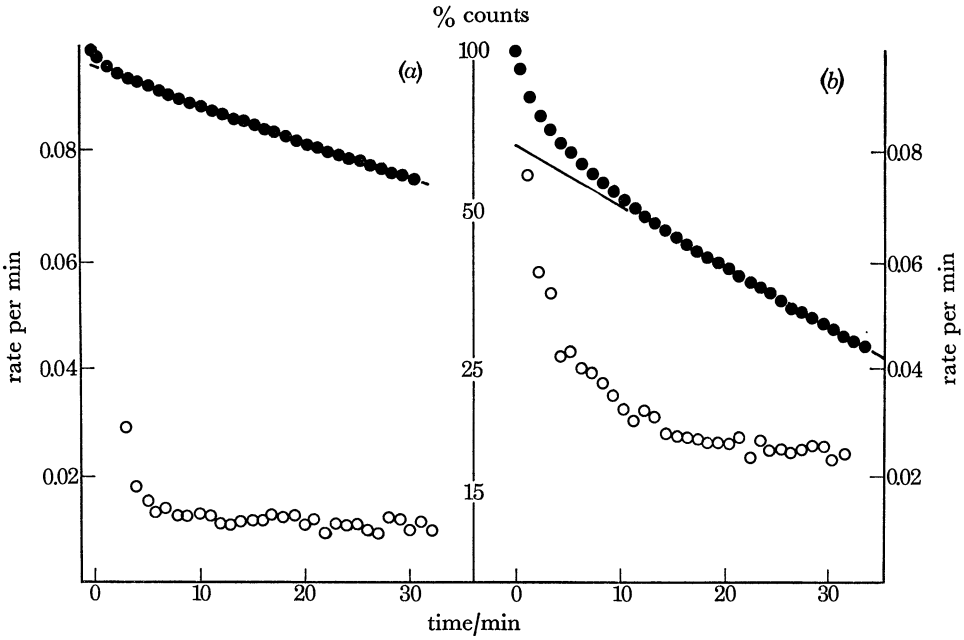

FIGURE 1. Steady-state efflux of ^{42}K from guinea-pig taenia coli. (*a*) Tissue loaded and washed out in normal Krebs. (*b*) High potassium tissue loaded and washed out in high potassium Krebs solution (Na^+ replaced by K^+). ●, counts in the tissues; ○, instantaneous rate constant of the efflux.

Figure 1*a* shows the steady state efflux of ^{42}K from a strip of taenia coli of the guinea-pig, loaded until equilibrium and washed out in normal Krebs solution. Only the early part of the curve is shown, but it can be seen that the rate of loss does quickly reach a constant value, as judged both by the logarithmic decline of the tissue counts, and by the constancy of the instantaneous rate constant. The extrapolation of the linear portion of the curve shows it to contain some 91 % of the tissue potassium, indicating that the major portion of the exchange of this ion is limited by a single step, presumably the membrane. Figure 2 shows that the faster exchanging portion of the potassium is lost in a way that can be described by a diffusion limited process, if reasonable figures are used for the tissue half-thickness (l = 0.045 cm) and the

diffusion coefficient for potassium in the extracellular space ($D = 0.45 \times 10^{-5}$ cm^2 s^{-1}); there is, however, a slight snag, and that is that the amount of fast exchanging material is quite considerably larger than could be accounted for as dissolved in the extracellular fluid. An extracellular space of about 40 % would hold 2.3 mmol/l tissue, whereas the average size of the fast exchanging potassium in this series (Brading 1971) was about 5.8 mmol/l tissue. Similar discrepancies have also been described in the taenia coli by Goodford (1966). Ignoring this point will not introduce much error into estimates of P_K and $[K]_i$, but if one wants to account for the whole exchange, then it is necessary to propose slight modifications to the original model. One could assume that there was some binding of extracellular potassium to anionic sites on the membrane or on extracellular material, as proposed by Goodford (1970), and that the exchange of potassium on these sites with free potassium was rapid, and therefore not rate limiting.

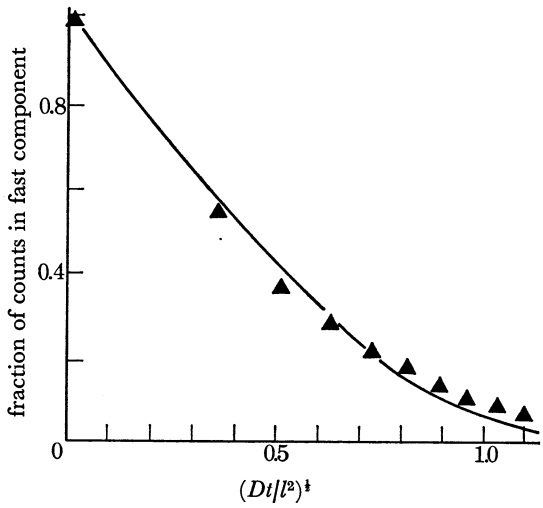

FIGURE 2. The fast components of the efflux of ^{42}K shown in figure 1*a*. The triangles plot the fraction of counts in the fast exchanging material. The abscissa is a non-dimensional time parameter, where D is the diffusion coefficient for potassium in the extracellular space and is taken to be 0.45×10^{-5} cm^2 s^{-1}, and l is the half thickness of the tissue. The curve is a theoretical curve for diffusion from a plane sheet.

A second possibility would be that a compartment of the extracellular space not normally penetrated by the larger molecules of extracellular markers, could hold a significant amount of ions. A possible site would be membrane vesicles. In the case of potassium, however, these ions would have to be concentrated above the extracellular concentration. Another model would be to suppose that the cell population was not homogeneous with respect to the rate of efflux of potassium, and that this function was distributed about a mean value. This means that the transmembrane efflux from the whole tissue would not be described by a single exponential function, but by an aggregate of exponentials. Van Liew (1967) has published curves calculated for aggregates of exponentials, and in fact, if one subtracts the potassium that could be free in the extracellular space, the exchange of the rest of the potassium will fit such a model. Calculations of P_K, using the mean rate constant derived from this model (Brading 1971), give a value of about 7.2×10^{-8} cm s^{-1} as compared with a value of about 6.7×10^{-8} cm s^{-1} derived using the slope of the single exponential phase. Whatever model one in fact uses, rather similar values for P_K and E_K will be estimated.

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Since potassium is the predominant intracellular ion, we might expect that the efflux would be relatively uncomplicated since there is a low extracellular amount of this ion, and we can be reasonably certain that the majority of potassium is intracellular. This is not true for chloride or sodium ions, which are predominantly extracellular. The effluxes of both these ions are more difficult to analyse than that of potassium.

Chloride exchange

Studies of chloride exchange in smooth muscles have shown that it is often difficult to identify a single transmembrane phase of the flux; in several tissues two slow components of chloride exchange can be distinguished (taenia coli: Goodford (1964), Brading (1971); vas deferens: Casteels (1969*b*); portal vein, B. Wahlström (personal communication)). Goodford assumes that the chloride ions are not simply distributed between intracellular and extracellular space, and in 1966, Buck & Goodford, suggested that about 11 mmol/kg fresh tissue are sequestered in some tissue compartment. The chloride efflux from the taenia coli can also be fitted assuming that the transmembrane exchange is an aggregate of exponentials as with the potassium (Brading 1971), but in this case there are quite large differences in the estimates of P_{Cl} and E_{Cl} depending on which model is used. For instance, using the slowest exponential as the transmembrane exchange allows estimates of P_{Cl} of 4.4×10^{-8} cm s^{-1} and E_{Cl} of -45 mV, whereas the model assuming an aggregate of exponentials gives a value for P_{Cl} of 6.7×10^{-8} cm s^{-1} and E_{Cl} of -27 mV.

Sodium exchange

The most difficult of the monovalent ions to analyse is sodium. At normal temperatures sodium exchanges unexpectedly rapidly in smooth muscle, both in cold-blooded vertebrates (frog stomach: Stephenson (1969); toad stomach; Burnstock, Dewhurst & Simon (1963)) and in mammals (taenia coli: Goodford & Hermansen (1961): ileal muscle: Weiss (1969); uterine muscle; Jones (1970*a*); vascular smooth muscle: Keatinge (1968), Jones & Karreman (1969*a*), Wahlström (1972); vas deferens: Casteels (1969*b*); and many others). The rapid exchange of sodium does not often settle down to a clearly measurable exponential phase, and if it does, such a phase usually leaves more sodium exchanging rapidly than can be freely dissolved in the extracellular fluid. Many authors have tried by various techniques to identify a truly transmembrane phase of sodium exchange, for instance by cooling the tissue (Buck & Goodford 1966; Casteels 1969*a*; Burnstock *et al.* 1963) or by identifying a calcium sensitive phase of the exchange as has been done by Keatinge (1968) in vascular smooth muscle. None of these attempts give very reliable estimates of the P_{Na} of the membrane, since it is becoming more and more obvious that there are probably several different mechanisms of sodium exchange between smooth muscle cells and their environment. Anything that alters any of these mechanisms, and puts the cells into a non-steady state, may bring about shifts in the distribution of sodium and may alter the rates of the different mechanisms.

The steady-state efflux of ^{24}Na from the guinea-pig taenia coli muscle in normal Krebs solution is shown in figure 3*a*. The majority of the tissue sodium exchanges extremely rapidly and when the exchange slows down, possibly towards an exponential phase, the counts left in the tissue are so low, that measurements of the counts leaving the tissue and estimates of the instantaneous rate of efflux are very inaccurate. The curve is extremely difficult to analyse. It is not possible to fit the exchange using a model allowing variation in the rate of efflux from cell to cell, i.e. an exponential aggregate system as it is for potassium and chloride.

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It is in the estimates of sodium permeability and intracellular sodium concentration that most discrepancies occur between different workers. If we assume that the slowly exchanging sodium is the transmembrane phase, then calculations of the membrane permeability and intracellular sodium content, taken together with values for chloride and potassium, can be used to predict the membrane potential on the Goldman assumption with reasonable agreements between the predicted and measured values, but one is left with a rather large amount of fast exchanging sodium in excess of that free in the extracellular space. To account for this, one presumably postulates extracellular binding to anionic sites as postulated by Goodford (1970) or some such phenomenon. If, on the other hand, one assumes that more of the sodium is intracellular, and exchanges with a faster rate, then the predicted membrane potential is about 20 mV less than the measured one, and one has to postulate a considerable addition to the membrane potential produced by the activity of a postulated electrogenic pump, as proposed by Casteels (1969*a*).

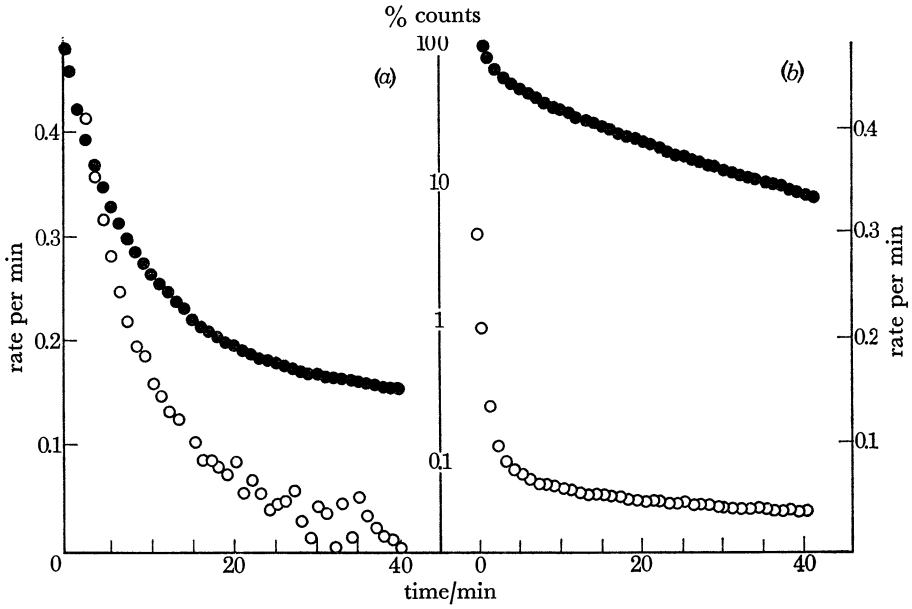

FIGURE 3. Steady-state efflux of ^{24}Na from guinea-pig taenia coli. (*a*) Tissue loaded and washed out in normal Krebs. (*b*) High sodium tissue, loaded and washed out in potassium-free Krebs solution. ●, Counts in the tissue; ○, instantaneous rate constant of the efflux.

The model used causes very large differences in the estimated parameters; for instance Casteels calculates a value for P_{Na} of 1.8×10^{-8} cm s^{-1} and E_{Na} of $+62$ mV, whereas another model (Brading 1971) predicts a value for P_{Na} of about 0.07×10^{-8} cm s^{-1} and E_{Na} of $+100$ mV. The electrophysiological evidence does not support the hypothesis that the membrane permeability to sodium is unusually high in this tissue, and there is general agreement that it must be considerably less than the potassium permeability.

Fluxes from high Na *and high* K *tissues*

Since there is still such a lot unknown about the mechanisms involved in sodium exchange and distribution in smooth muscle, it seems of value to undertake a further investigation into this matter, and the rest of this paper will be spent in describing the approach taken and some results of the investigation.

The approach taken was to simplify the ionic distribution in the tissue by eliminating either

sodium or potassium, so that the fluxes of the two monovalent cations could be studied and compared separately. After 4 h exposure of the tissue to a potassium-free solution (KCl replaced by NaCl) there is virtually complete replacement of K^+ by Na^+ (Axelsson & Holmberg 1970; Casteels, Droogmans & Hendrickx 1971), and similarly when Na^+ is completely replaced by K^+ after 4 h the tissue has lost all of its sodium ions. Effluxes were therefore studied after loading taenia coli with tracer ions during 4 h exposure to potassium-free solution (high sodium tissues) or to sodium-free solution (high potassium tissues). The tissues reach a steady state, and it is probable that there is a relatively low gradient of monovalent cation between the cell and its environment. In fact the intracellular concentration may slightly exceed the extracellular concentration, since it is likely that the intracellular non-diffusible anions may not be as osmotically active as the free extracellular anions. The chloride distribution is not greatly affected, especially when the buffer is HCO_3^-, since in this medium the tissues do not swell. When the buffer used is tris chloride, the tissues do swell in both conditions, as would be predicted by the Gibbs Donnan distribution (Brading & Tomita 1972).

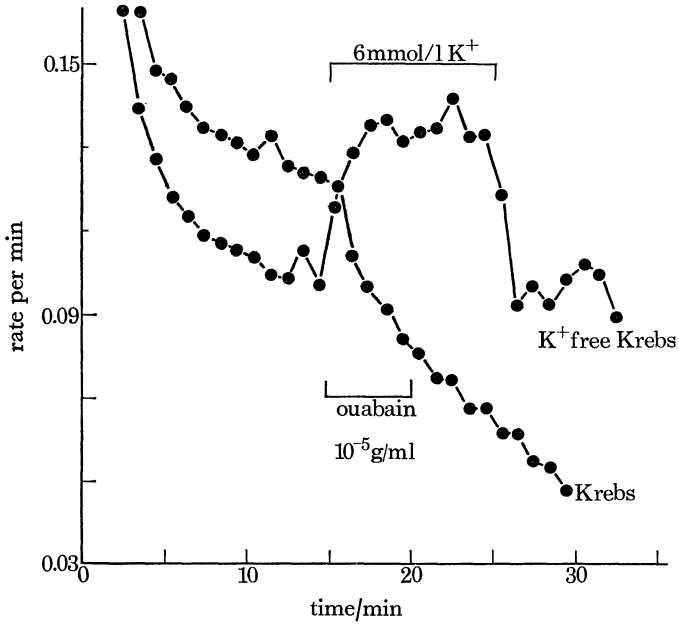

FIGURE 4. Efflux of ^{24}Na from high sodium tissues. The instantaneous rate constant of efflux is plotted for two tissues, one washed out into potassium-free Krebs, and one into normal Krebs. Ouabain, 10^{-5} g/ml was applied for 5 min to the tissue in normal Krebs, and inhibited the efflux. Potassium (6 mmol/l) was added for 10 min to the tissue in potassium-free Krebs and caused a marked increase in the efflux.

Casteels *et al.* (1971) have measured the membrane potential in the potassium-free condition after many hours. They find that it reaches a steady value of about -10 mV. Under these conditions the value they give for E_{Cl} is also about -10 mV. It is quite likely that the ions are all at equilibrium under these two conditions, and therefore the forces acting on the potassium ions and the sodium ions will be virtually identical with respect to chemical and electrical gradients.

It might be argued, particularly in the case of the high sodium tissues, that such a high intracellular sodium content is an extremely abnormal situation, and may cause permanent damage to the tissue. Figure 4 shows that in this condition the tissue is still capable of predictable behaviour, with respect to sodium potassium exchange mechanisms. The figure shows the rate of loss of sodium from high sodium tissues, one being washed out into normal potassium

containing Krebs solution, and showing a faster rate of exchange than the other, which is being washed out into potassium-free Krebs solution. The rate of efflux into Krebs solution is markedly reduced by the addition of ouabain, and the rate of loss into potassium-free solution is markedly increased by addition of potassium, so at least in this respect the tissue is obviously healthy.

All the experiments that I shall describe in the rest of this paper have been carried out on high sodium or high potassium tissues, and in none of them will there be any sodium–potassium exchange mechanism in operation, since only one monovalent cation was present at a time. The experiments are relatively few in number and need confirmation, but they are already beginning to show major differences in the way in which the tissue handles sodium and potassium ions.

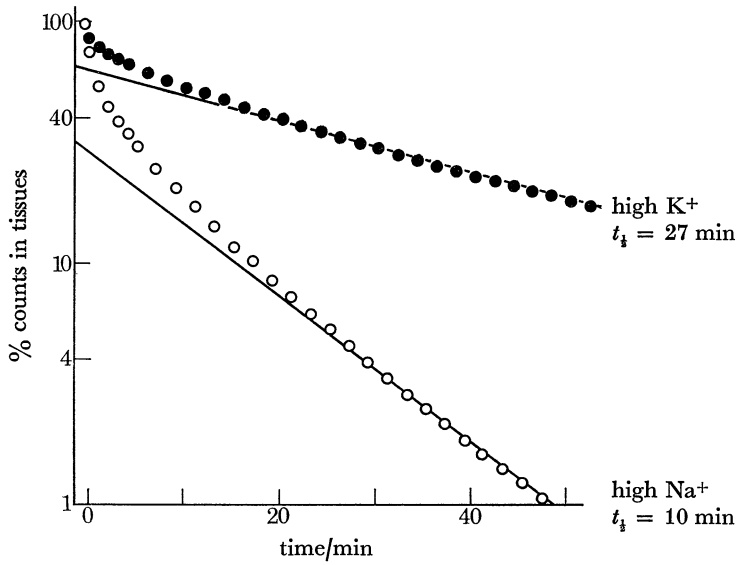

FIGURE 5. Steady-state efflux of ^{42}K from high K tissues and ^{24}Na from high Na tissues. The curves are from figures 1*b* and 3*b*.

Figure 1 compares the steady-state ^{42}K efflux from tissues in normal Krebs solution with that from the high potassium tissues into potassium Krebs solution. The total tissue potassium in the high potassium tissue was the same as in an equivalent weight of the external solution. The flux settles down to a single exponential phase after 15 min, as is judged by the linearity of the tissue counts, and the constancy of the instantaneous rate. The extrapolation of this phase to zero time indicates that about 34 % of the potassium is extracellular, which is a reasonable value for the extracellular space. The faster exchange could be fitted by a diffusion curve. A rough estimate of the P_K for this tissue indicates that it is about 5.4×10^{-8} cm s^{-1}, that is very similar to but a bit less than the permeability in normal solution. In this particular tissue the ions behave as if they were simply distributed between a homogeneous intracellular compartment, and the extracellular space. There is no need to postulate significant amounts of potassium bound to the outside of the cell membrane, or nonhomogeneity of rate of efflux among the cells.

Figure 3 compares the steady-state sodium efflux from normal tissue in Krebs solution with the steady-state flux from high sodium tissue into potassium-free solution. The curve in the latter state is also difficult to interpret. The rate of efflux appears to be approaching a constant value, and after about 35 min, the decline in counts is approximately linear. The highest value for the sodium in this phase that one can get by fitting a line through the tissue counts, is about

40 %, but this line runs through the points when the rate is still obviously declining. A more accurately drawn line drawn when the rate is nearly constant extrapolates to between 25 and 35 % of the total sodium. The rest of the sodium is exchanging faster.

Figure 5 compares the steady state effluxes of ^{42}K from high potassium tissue and of ^{24}Na from high sodium tissue on the same scale. It is obvious that the extrapolation of the linear phases of exchange give quite different intercepts. There is, however, no evidence at all to suggest that there is any less intracellular sodium in the high sodium tissues, than potassium in the high potassium tissues, and it is extremely likely that about 60 to 70 % of the sodium is in fact intracellular. Another obvious difference is the rate of loss of the ions. If one compares the approximately linear parts of the curves, then the efflux of sodium is almost three times faster than the potassium efflux. If this is simply a reflexion of the passive movements of the ions across the membranes, then, since the gradients, both chemical electrical are probably the same in both cases, this should be a comparison of the membrane permeability to the two ions.

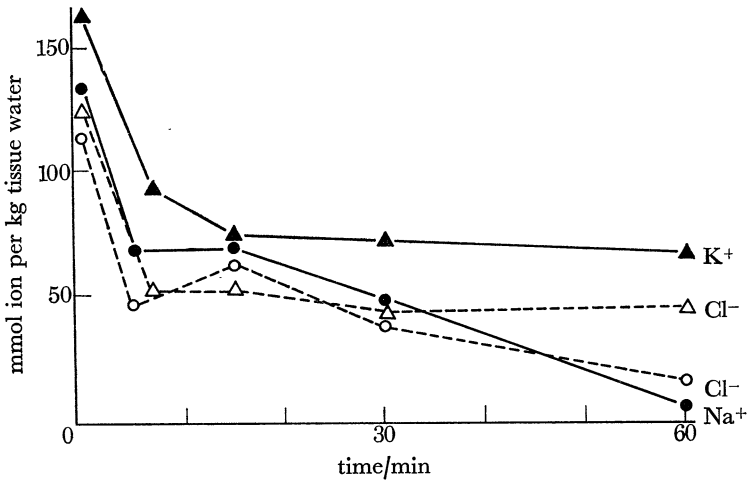

FIGURE 6. Loss of tissue ions into monovalent cation-free sucrose Krebs solution. Ionic content of the tissue expressed as mmol/kg tissue water. ▲,△, Ions during exposure to sucrose Krebs from high potassium tissues; ●, ○, from high sodium tissues.

If one assumes that about 70 % of the sodium in the high sodium tissue is intracellular, then at 36 °C the efflux can be fitted by a model assuming non-homogeneity of rate of loss of sodium from the cells. If this is true then the mean P_{Na} is nearer four times the P_K value in high potassium tissues. However, at lower temperatures such a model cannot be applied. The rate of loss of sodium from sodium-loaded tissues at lower temperatures (11 and 3 °C) again approach a constant value, and extrapolation again shows that some 25 to 35 % of the sodium exchanges with these kinetics. The actual rate at these temperatures is much less, at 11 °C it is about one-tenth that at 36 °C, but does not slow more at 3 °C. The exchange of the faster sodium in each case is very similar, and can be fitted by diffusion limited processes.

It is thus true to say that in high sodium tissues, 25 to 35 % of the tissue sodium exchanges in a manner that is very sensitive to temperature, whereas the remaining sodium, of which a large proportion must be intracellular, exchanges so fast that it appears to be limited rather by diffusion through the tissue than by any rate limiting step such as penetration of the membrane, and this fast phase is relatively insensitive to temperature. Jones (1970*a*) has described very similar behaviour of sodium in rabbit myometrium, and suggests that the sodium distribution

could with advantage be described by some such system as Ling's association induction hypothesis, which gives the intracellular structure a greater role in determining ionic distribution than the selective permeability of the membrane (Ling 1962). However, before abandoning the membrane theory it is worth while to continue with experiments to see whether the behaviour could be accounted for by other membrane phenomena, such as exchange diffusion, etc.

Fluxes into solutions with no monovalent cations

An obvious approach is to study the effect of replacing the monovalent cation in question with foreign cations to see what effect this has on the rate of loss. It is useful for these studies to have the limiting condition, that is to see how the tissue loses monovalent cation into a solution of no monovalent cations. In order to achieve this, the loss of cations was followed into solutions where Na and K had been replaced by sucrose.

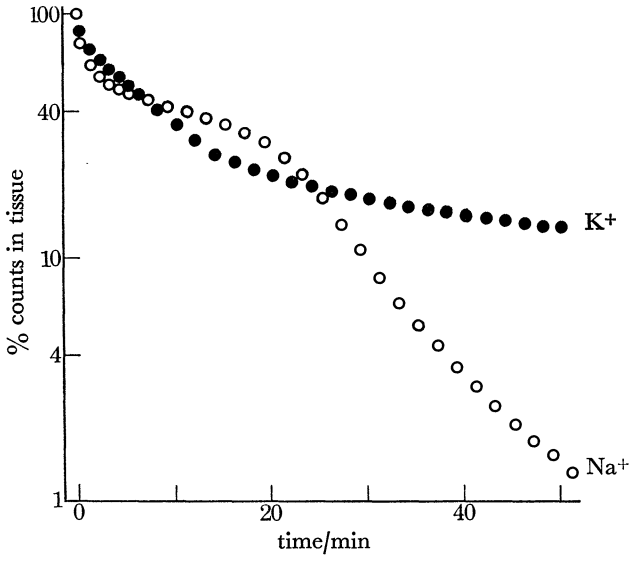

FIGURE 7. ^{24}Na efflux from high Na tissues and ^{42}K efflux from high potassium tissues, both into a Na-free K-free Krebs solution (sucrose replacement). Note the inflexion in the Na curve between 20 and 30 min, indicating an increase in the rate of efflux.

Figure 6 shows the results of an experiment during which tissue ions were determined spectrophotometrically during 1 h exposure to sucrose solution. In this experiment the buffer was Tris chloride, and so in both cases the tissue swelled during the preliminary 4 h exposure to the single monovalent cation Krebs solution, and lost weight continuously during the exposure to the sucrose Krebs solution. The ion contents are expressed as mmol/l tissue water. Taking the high potassium tissue first, there is an initial rapid loss of K and Cl. The lower content of chloride in the tissue is because of the non-permeant anions. After a few minutes, the concentration of the ions in the tissue water reaches a constant value. During this time the tissue lost weight and thus there must be a loss of isotonic KCl.

The loss of Na and Cl from the high Na tissue initially follows a similar time course, but instead of settling down to a steady level, the tissue begins to lose Na and Cl faster than it loses an osmotically equivalent amount of water. It also loses Na faster than it loses Cl, and after 1 h, there is very little sodium left, but a significantly greater amount of chloride.

Figure 7 follows the loss of Na and K using tracer ions. The loss of K starts rapidly, and then

slows down continuously during the exposure. It is interesting to note that the initial loss of tracer K is more rapid in this experiment than when it is being lost in exchange for non-radioactive potassium in the steady state. One must assume that the movement of chloride down its electrochemical gradient which must accompany the potassium into the sucrose solution, in some way accelerates the movement of the potassium ions.

The loss of sodium into the sucrose solution follows an interesting time course. During the initial period the loss proceeds initially fairly rapidly, and then slows down as would be expected, but after 15 to 20 min exposure to the sucrose solution the rate of loss suddenly increases, and the tissue sodium declines rapidly. This sudden inflexion in the efflux curve has been found in all cases (6) so far studied at 35 to 37 °C, although it does not occur at low temperatures (11 °C). Since, as was shown earlier, the sodium is being lost faster than an osmotic equivalent of water, it is probable that it is exchanging with something, and it could be that calcium is involved.

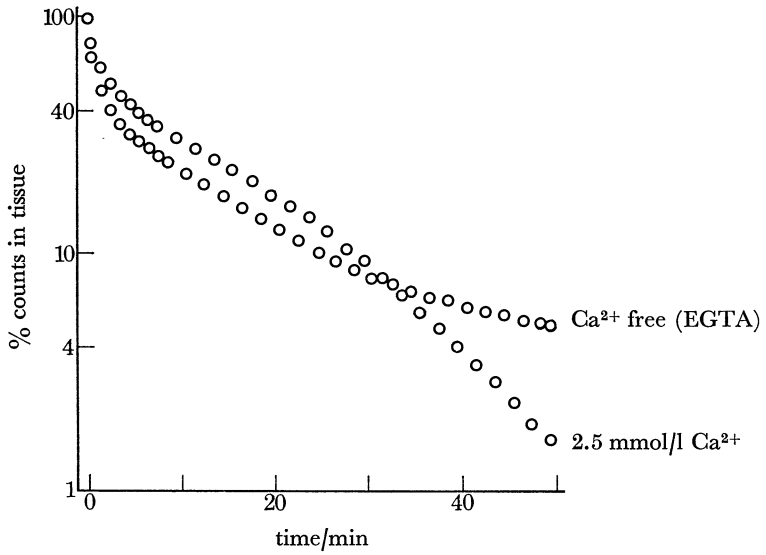

FIGURE 8. ^{24}Na efflux from high Na tissues. One tissue is washed out into a sucrose Krebs solution containing 2.5 mmol/l Ca^{2+}, and the other into a Ca^{2+} free sucrose Krebs solution, where the calcium was replaced by magnesium, and 1 mmol/l EGTA added.

Possible sodium calcium exchange mechanism

Figure 8 shows the results of a similar experiment, where the tissues were loaded for 4 h in potassium-free Krebs and washed out into Na-free K-free Krebs, but in one case the calcium was replaced by magnesium, and 1 mmol/l EGTA was added. As can be seen, the inflexion of the efflux curve did not occur in the Ca-free solution. The inflexion also did not occur in a calcium-containing solution, which had 2.5 mmol/l La^{2+} added to it. In both cases the amount of sodium remaining in the tissue after 50 min was significantly greater than in the control case. As will be shown by Van Breeman in a later paper, the addition of lanthanum to smooth muscle cells appears to prevent movement of calcium across the cell membrane, and also to displace Ca from any extracellular binding sites. Lanthanum has been used by my colleague Jonathan Widdicombe, to see if it is possible to pick up any increase in the intracellular content of calcium, after exposure of high potassium and high sodium tissues to the sucrose solution for 1 h. After this treatment, the tissues were placed in a Na-free K-free Ca-free solution containing 2.5 mmol/l

lanthanum for half an hour before the tissue calcium content was determined. His results show that there is no significant difference in the calcium remaining in the high potassium tissues before or after exposure to the sucrose Krebs solution, whereas there is a highly significant increase in the calcium remaining in the high sodium tissues after exposure to the sucrose Krebs solution. The increase after lanthanum treatment is only small, the calcium content after 4 h exposure to K^+-free solution was 0.51 ± 0.14 (6) and rose to 1.69 ± 0.19 (8) mmol/kg fresh tissue after exposure to sucrose, but the total tissue increase (no exposure to La^{3+}) is larger, 3.9 ± 0.19 (5) to 6.61 ± 0.5 (5) mmol/kg fresh tissue. It is thus probable that there may be a significant exchange of sodium for calcium ions on superficial anionic sites on the membrane. This supposition also fits in with the time course of the sodium loss. If when placed in the low ionic strength sucrose Krebs, there is competition for occupation of superficial anionic sites between sodium ions leaving the tissue, and calcium ions in the external medium, and if transmembrane sodium calcium exchange only can occur when a certain percentage of such sites are occupied by calcium ions, the lag could be explained. The sodium lost in exchange for calcium, as estimated from the flux curves, is between 5 and 7 mmol/kg tissue suggesting an approximately 2:1 Na:Ca exchange. Competition between calcium and sodium has also been shown by Brading, Bülbring & Tomita (1969) in connexion with the role of calcium in the spike mechanism in this tissue.

Summary

In summary, therefore, these experiments have shown that, even under conditions when their distribution in the tissue is virtually identical, the cells handle sodium ions in a manner completely different from potassium ions. Sodium exchanges much more rapidly than potassium at body temperature, and there is a portion of sodium whose exchange is very temperature sensitive. Another portion, which must be intracellular, exchanges so fast that it appears to be limited only by diffusion through the tissue, and is insensitive to temperature. There is also a sodium – calcium exchange mechanism. Experiments have not yet revealed any evidence to suggest how the rapid exchange of sodium comes about, but if one extrapolates from the high sodium tissues to normal tissues, it is likely that some of the diffusion limited sodium exchange in these tissues is intracellular. If this fast sodium exchange is truly representative of a passive movement of sodium ions through the membrane, then P_{Na} is probably higher than P_K, which is contrary to all electrophysiological evidence.

I would like to emphasize in conclusion, the very considerable difficulties in estimating the real transmembrane sodium permeability from flux measurements.

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