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THE USE OF LANTHANUM TO ESTIMATE THE NUMBERS OF EXTRACELLULAR CATION-EXCHANGING SITES IN THE GUINEA-PIG'S TAENIA COLI, AND ITS EFFECTS ON TRANSMEMBRANE MONOVALENT ION MOVEMENTS

BY A. F. BRADING AND J. H. WIDDICOMBE

From the Department of Pharmacology, University of Oxford, Oxford

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SUMMARY

1. Tissues were allowed to equilibrate in a Tris-buffered Krebs solution and were then exposed to similar solutions containing up to 5 mM-La. La caused shrinkage and significant losses of tissue K, Na, Mg and Ca. The shrinkage was exactly accountable for by a reduction in the extracellular space (e.c.s.) as measured by [^{14}C]sucrose. No significant change was seen in tissue Cl content. Exposure for 1 hr to 5 mM-La led to a total cation loss of 24·3 ±1·6 m-equiv/kg or, correcting for the small change in Cl content, a loss of positive charge of 23·8 ± 2·2 m-equiv/kg fresh wt.

2. Using the radioisotope ^{140}La it was shown that this loss of cation was balanced by an uptake of La^{3+}.

3. Subtraction of the ions in the measured [^{14}C]sucrose space from the total tissue ion contents led to estimates of the 'cellular' ion contents. The effects of 1 hr exposure to 5 mM-La on these were a loss of 12·9 ± 2·4 m-equiv/kg of cation and a gain of 10·0 ± 2·6 m-equiv/kg of Cl.

4. Similar changes in ion content were produced by La on 'Na-loaded' and 'K-loaded' tissues, these being tissues which by exposure to K-free or Na-free (high K) solutions had replaced all their K with Na or vice versa.

5. The uptakes of ^{24}Na and ^{36}Cl by Na-loaded tissues were both describable as the sum of two exponential processes: a fast component ($t_{\frac{1}{2}} \simeq \frac{1}{2}$ min), which was presumed to be extracellular and a slower, presumed transmembrane, component. La reduced the rapid component of uptake of ^{24}Na by an amount greater than that predicted by the reduction in the e.c.s., the extra amount lost being some 10–15 m-equiv/kg. La also reduced the amount of rapidly exchanging ^{36}Cl, but this reduction was entirely accounted for by the change in the e.c.s. La reduced the rate constant of the slow component of ^{24}Na uptake.

6. La reduced the rapidly exchanging component of ^{42}K uptake by normal tissues by an amount equivalent to about 0·5 m-mole/kg fresh wt. of K in excess of the change in the extracellular space.

7. La had little effect on the effluxes of ^{36}Cl and ^{42}K from normal tissues. However, it reduced the size of the fastest component of exchange of ^{42}K efflux from K-loaded tissues by an amount equal to some 10–15 m-equiv/kg in excess of the reduction in the e.c.s. A similar reduction in the rapidly exchanging component of ^{24}Na efflux from normal tissues was also seen. La slowed the efflux of ^{24}Na from Na-loaded tissues at times when the tracer lost could safely be regarded as intracellular.

8. The taenia coli when exposed to K-free solutions gains Na and loses K. In the presence of La the gain in Na was completely blocked. K was still lost, however, being accompanied by Cl and increased shrinkage. La also prevented the uptake of Na from high Na media by ion-depleted tissues (produced by exposure to sucrose media), while having little effect on the uptake of K from high K media by such tissues.

9. The cation displaced by La in excess of that lost due to the reduction of the e.c.s. was shown by tracer studies to be rapidly exchanging. It is concluded that this cation, amounting to some 15–20 m-equiv/kg, is bound to fixed extracellular negative sites.

INTRODUCTION

It has long been known that cell membranes possess superficial fixed negative sites, and that cations are associated with such sites. In the guinea-pig's taenia coli, Goodford (see review, 1970) has presented evidence for the view that Ca, Mg, Na and K all compete for occupancy of these negative sites. In this tissue the surface area:volume ratio is so large that bound ions may contribute a very significant proportion of the total tissue ions, and this must be allowed for when estimates of intracellular ionic concentrations are to be made. Binding of ions may also be an important step in carrier mediated transmembrane ion exchange, and exchange of bound ions with extracellular ions contributes to uptake and efflux curves using radioactive tracers. It is thus important to know the number of negative sites and the amounts of the various cations bound to them.

In the taenia coli there is disagreement as to their numbers; Goodford (1970) believes them to amount to about 4 m-equiv/kg, while Sparrow (1969) estimates them as some 12 m-equiv/kg. In this paper we have attempted to estimate the amount and types of extracellularly bound counter cation in the taenia coli by measuring their displacement by the more strongly binding La^{3+} ion. As long ago as 1949, Bungenberg de Jong

had measured the affinities of some cations for the various types of negative sites found in organic materials, and showed that La^{3+} has an affinity for such sites about 100 times greater than divalent cations, which are in turn some 100 times more potent at binding to these sites than monovalent cations. But the use of La^{3+} in biological sciences only became important after 1964 when Lettvin, Pickard, McCulloch and Pitts predicted that the La^{3+} ion, having a hydrated radius similar to Ca but one extra charge, should bind to and displace Ca from normally Ca binding sites. Since then La has been used as a tool for determining the true intracellular Ca levels in tissues where this is obscured by far larger amounts of extracellularly bound Ca (van Breemen & McNaughton, 1970; van Breemen, Farinas, Gerba & McNaughton, 1972), and since La appears to block Ca influx in all tissues so far investigated, this has led to the use of La to determine whether in smooth muscles a particular drug's action is due to influx of extracellular Ca, or to the release of intracellular Ca stores (for review see Weiss, 1974). However, this emphasis on the effects of La^{3+} on Ca binding and Ca fluxes has led people to overlook the possibility that La^{3+} may affect the fluxes of other ions, and in this paper we have also examined the effects of La^{3+} on monovalent ion movements.

METHODS

Dissection and weighing procedures

White guinea-pigs of either sex, weighing about 400 g, were killed by a blow on the head and bled out. From each guinea-pig, eight to ten pieces of taenia coli, weighing about 15 mg, were dissected and their fresh weight determined, mounted on stainless-steel holders and suspended in warm, oxygenated Krebs solution (pH 7·4, 37° C). At the end of an experiment the muscles were blotted lightly on Whatman no. 1 filter paper and their wet weights determined.

Solutions

As La precipitates out in solutions containing HCO_3^- and $H_2PO_4^-$, Tris Cl replaced the $NaHCO_3$ and NaH_2PO_4 of normal Krebs solution as the buffer in these experiments. The solution was prepared by mixing appropriate amounts of isotonic stock solutions and its composition was 120 mM-Na, 5·9 mM-K, 16·5 mM-Tris, 2·5 mM-Ca, 1·2 mM-Mg, 150 mM-Cl and 11·5 mM glucose. Control experiments showed that tissues remained perfectly healthy in this solution; over an incubation period of from 1 to 9 hr, there were no significant changes in tissue weight, or ion contents. La was added in the form of $LaCl_3$ stock solution in replacement for Tris Cl, so as to keep the Na, Mg, K and Ca concentrations constant. This procedure however led to increases in the Cl content of La-containing media. The La stock solution was markedly acidic due to hydrolysis, and 5 mM-La was the highest concentration that could be buffered effectively. High K (Na-free) solutions and high Na (K-free) solutions were obtained by replacing the NaCl of the normal solution by KCl and vice versa. When La was added to these solutions it again replaced Tris Cl. All solutions were maintained at 37° C, buffered at pH 7·4 and bubbled with 100% O_2.

Determination of ion contents

Following blotting and weighing, tissues were placed in Vitreosil tubes and 1 ml. Aristar grade 30% H_2O_2 was added; they were then digested overnight at 100° C. The digest was dissolved in a known amount of 'diluting fluid' consisting of 0·5 N-HNO_3 containing 9 mM-La_2O_3, this latter being present to prevent calcium precipitation. The ions were then analysed on a Pye Unicam SP 90 atomic absorption spectrophotometer. Blanks were treated in similar ways to allow correction to be made for the small levels of Ca and Na coming from the glassware.

Determination of extracellular space

[^{14}C]sucrose, supplied by the Radiochemical Centre, Amersham, was used throughout. To estimate the e.c.s., for their last 10 min of exposure to an experimental solution, tissues were transferred to an ionically identical solution containing a trace (1 mM) of [^{14}C]sucrose. Following uptake, the tissues were dissolved in 1 ml. NEN protosol (overnight at 50° C), then neutralized with glacial acetic acid and counted in a liquid scintillation counter using 10 ml. Bray's scintillation fluid (Bray, 1960). After counting, small aliquots of the uptake solutions were added to the sample vials, which were recounted and the counts in the uptake media were obtained by subtraction.

Uptake experiments

^{24}Na, ^{42}K and ^{36}Cl were all provided by the Radiochemical Centre, Amersham. ^{24}Na and ^{42}K were supplied as isotonic solutions of the chlorides. In preparing uptake solutions, a given volume of radioactive stock solution replaced the same volume of inactive stock solutions. ^{36}Cl was supplied either as a hypertonic solution of Na^{36}Cl or as H^{36}Cl. In the former case the solution was diluted to isotonicity, while the H^{36}Cl was transformed to a neutral isotonic solution of K^{36}Cl by addition of KOH. Aliquots of these solutions then replaced the same amounts of the inactive stock of NaCl or KCl in the uptake media.

Following uptake of ^{24}Na or ^{42}K, the tissues were placed in Pyrex tubes; 2 ml. distilled water was added and they were then counted on a Packard γ-scintillation spectrophotometer (model 578). Following uptake of ^{36}Cl, the tissues were treated as for the uptake of [^{14}C]sucrose. In all cases, solution samples were taken and treated in similar fashions.

Uptakes were expressed as

$$\text{ml./kg tissue wt.} = \frac{\text{c.p.s./g in tissue}}{\text{c.p.s./}\mu\text{l. in uptake solution}}$$

or as

$$\text{m-mole/kg tissue wt.} = \text{uptake in ml./kg tissue wt.} \times \text{ion content of medium in m-mole/ml.}$$

Effluxes

Muscles, mounted on specially designed holders, were placed in uptake media for sufficiently long periods of time to allow complete equilibration of tracer with inactive ion. They were then placed in the constant flow apparatus described by Brading (1967) and the flow rate of the washout solution adjusted to 2 ml./min for the effluxes of ^{24}Na and ^{42}K and 1 ml./min for the efflux of ^{36}Cl. Samples of the effluent were collected every minute for ^{42}K and ^{24}Na, and every 2 min for ^{36}Cl. At the end of an experiment the muscle piece was removed and counted. The results were then processed by computer (Brading, 1971).

Analysis of uptake data

The uptake of tracer by the taenia coli is describable as the sum of two exponential processes (Goodford, 1966):

$$U_t = A(1-e^{-k_1 t})+B(1-e^{-k_2 t}), \quad (1)$$

where U_t is the uptake of tracer at time $= t$; A is the size of a fast component of exchange, and k_1 its rate constant; B and k_2 are the size and rate constant of the slow component of exchange.

k_1 is very fast and so at times greater than about 2 min, eqn. (1) approximates to

$$U_t = A+B(1-e^{-k_2 t}). \quad (2)$$

If the extracellular and intracellular compartments were in parallel then A and B would correspond to their ion contents. However, they are in series, the uptake of tracer by the extracellular space being modified by its subsequent uptake by the cells. A will thus underestimate the extracellular ion content while B will overestimate the cellular content. Huxley (1960) has analysed this compartmental interaction and the corrected value for $A(A')$ which gives the extracellular ion content is given by

$$A' = \frac{(Ak_1+Bk_2)^2}{Ak_1^2+Bk_2^2}.$$

A' will be little different from A when $k_2 \ll k_1$. However, when $k_1 \simeq k_2$ then the size of the correction becomes large. Values for k_1 for the various monovalent ions range from 1 min^{-1} (Brading, 1971) to 2·8 min^{-1} (Goodford, 1966). Here, consideration of the various factors involved has led to the use of a value for k_1 of 1·5 min^{-1}. With this rate constant the fast component of exchange will be 95 % complete after 2 min.

RESULTS

The effects of lanthanum on ion content

Following an hour's incubation in normal Krebs solution, tissues were placed for a further hour in solutions containing various concentrations of La. Their ion contents and weights were then determined. The results are shown in Fig. 1. The effects of La are shrinkage and a reduction of the tissue Na, Ca and Mg contents in a concentration-dependent fashion, these effects appearing complete at a [La] of 5 mM. La had little effect on either the K or the Cl contents. In Fig. 2 the time course of the ionic changes on exposure to 5 mM-La are shown. Again the tissue Na, Mg, Ca and weight all declined, these changes being essentially complete after 30 min. An additional effect seen in this experiment was a significant fall in the tissue K content. In several experiments with 5 mM-La the effect on tissue K content was variable, ranging from no effect at all to the loss of some 10 m-mole/kg. The slight fall in Cl content seen here was not seen in other similar experiments.

Thus maximal changes in ion content can be produced by treatment with 5 mM-La for 1 hr. Table 1 shows the pooled results from several

experiments in which tissues were treated in this way. Also included are the results from two experiments in which the [^{14}C]sucrose space was measured. The values for cell water were calculated from the wet weight: fresh weight, dry weight: fresh weight ratios and the extracellular spaces listed in Table 1.

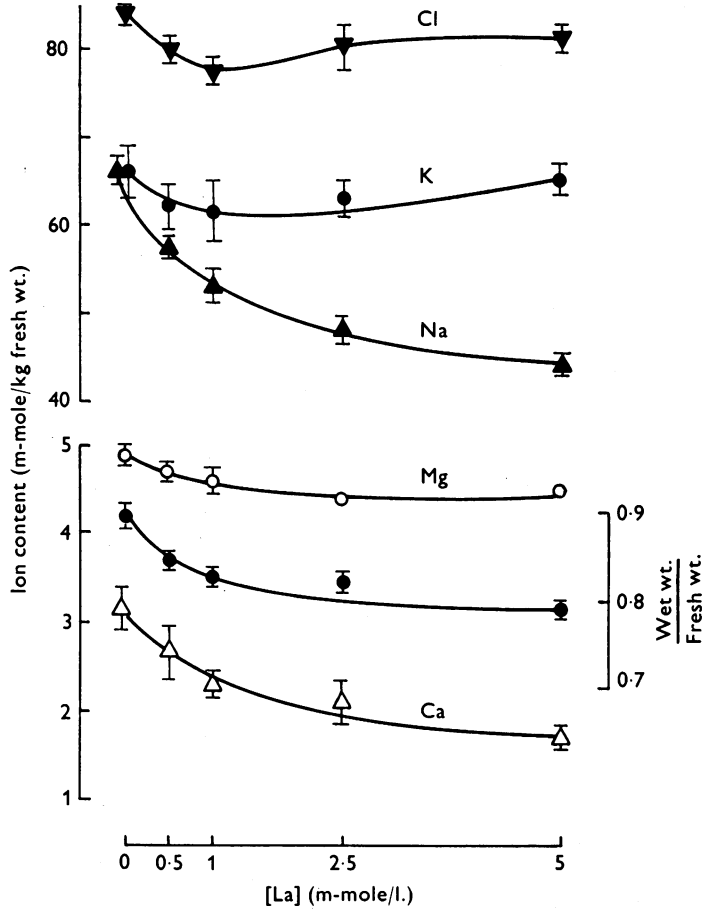

Fig. 1. Tissue ion content and weight following an hour's exposure to Krebs solutions containing various concentrations of La. Mean values (n = 8) ± S.E. of mean are given.

Inspection of Table 1 shows that La produces highly significant decreases in tissue weight, extracellular space and the contents of all cations. No significant changes were detected in the dry weight : fresh weight ratio, the Cl content or the cell water. It can be seen that the shrinkage produced by La is entirely accounted for by a reduction in the size of the e.c.s.

Knowing the size of the e.c.s. and the concentrations of the ions in the bathing solution one can calculate the amount of ions in the e.c.s. and, by subtracting this from the total issue ion content, determine the amount of ions associated with the cells themselves.

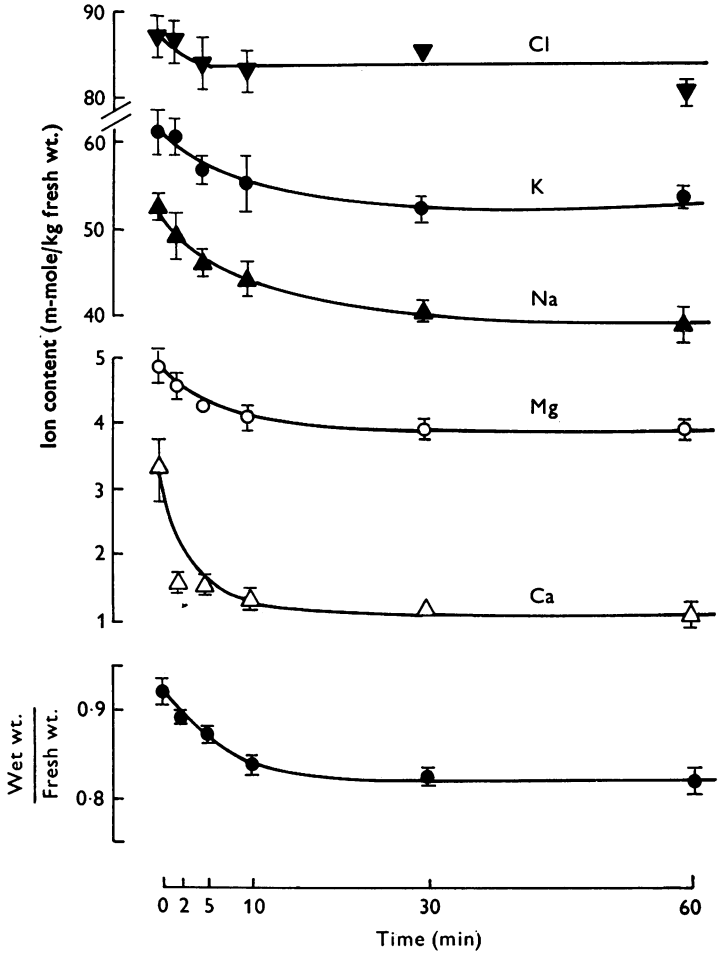

Fig. 2. The time course of ion content and tissue weight during exposure to Krebs solution containing 5 mM-La. The points are the means of six or seven estimates ± S.E. of mean.

This has been done for the results in Table 1 and the values are listed in Table 2. In this Table the changes induced by La are thus the ionic changes over and above those associated with the change in the e.c.s. and they amount to a loss of 12·9 ± 2·4 m-equiv/kg fresh weight of cation and a nearly equal gain of 10·0 ± 2·6 m-equiv/kg fresh weight of Cl.

La thus appears to produce an over-all loss of some 22·9 ± 3·5 m-equiv/kg of positive charge in the tissue. Experiments using the radioactive isotope ^{140}La showed that electroneutrality was maintained by the uptake of La^{3+}. After 1 hr exposure to ^{140}La the uptake as calculated from the specific activity of La in the uptake medium amounted to 17·3 ± 0·4 m-mole/kg fresh wt. However, as in all La-containing solutions, there was some slight precipitation of La it is difficult to know how much of this uptake was ionized La binding to tissue negative sites or previously combined La precipitate. The important thing is that if one assumes all the La taken up to be ionized La, this is more than enough to account for the apparent net loss of positive charge from the tissue.

TABLE 1. The ion contents after 2–3 hr in normal Krebs solution, or after 2 hr in normal and 1 hr in La–Krebs. Means ± S.E., no. of determinations in parentheses. Ion contents in m-mole/kg fresh wt., w.w./f.w., e.c.s., d.w./f.w. and cell H_2O in g/kg fresh wt.

	Krebs soln.	+ 5 mM-La	Δ
Na	54·2 ± 0·9 (64)	39·6 ± 0·6 (63)	− 14·6 ± 1·1
K	65·5 ± 0·6 (63)	60·1 ± 0·9 (61)	− 5·4 ± 1·1
Mg	5·03 ± 0·06 (48)	4·45 ± 0·06 (49)	− 0·58 ± 0·09
Ca	2·73 ± 0·13 (61)	1·15 ± 0·09 (58)	− 1·58 ± 0·16
Cl	84·1 ± 1·0 (57)	83·6 ± 1·1 (50)	− 0·5 ± 1·5
w.w./f.w.	916 ± 5 (78)	829 ± 5 (75)	− 87 ± 7
e.c.s.	371 ± 11 (24)	286 ± 8 (23)	− 85 ± 14
d.w./f.w.	165 ± 1 (25)	168 ± 2 (16)	+ 3 ± 2
Cell H_2O	380 ± 12	375 ± 9	− 5 ± 15

TABLE 2. The effect of La on 'cellular' ion contents. The figures are those of Table 1 with the extracellular ion contents subtracted. Ion contents m-mole/kg fresh wt.; water content in ml./kg fresh wt.

Ion	Krebs solution	+ 5 mM-La	Δ
Na	9·6 ± 1·6	5·2 ± 1·2	− 4·4 ± 2·0
K	63·2 ± 0·9	58·4 ± 0·9	− 4·8 ± 1·3
Mg	4·59 ± 0·06	4·11 ± 0·06	− 0·48 ± 0·08
Ca	1·80 ± 0·13	0·43 ± 0·09	− 1·37 ± 0·16
Cl	28·4 ± 2·0	38·4 ± 1·6	+ 10·0 ± 2·6
Cell H_2O	380 ± 12	375 ± 9	− 5 ± 15

The effects of La on K- and Na-loaded tissues

It was decided worth while to see if La caused changes in the ion contents of Na- or K-loaded tissues similar to the changes seen in normal muscle. Consequently tissues were Na- or K-loaded by incubation in high Na(0K) or high K(0Na) solutions for some 3 hr. At the end of this period Na had replaced almost all the tissue K or K had replaced all the tissue Na. As

Na-loading is slower in tris-buffered solutions than in HCO_3-buffered solutions, Na-loaded tissues were produced by 3 hr incubation in a high Na(HCO_3) medium followed by ½ hr in high Na (Tris) solution. Following Na- or K-loading, tissues were placed in solutions similar to the loading solutions but containing 5 mM-La. As with normal tissues the effects of La consisted of shrinkage and the loss of cations with little change in Cl content. Further, the time courses of these changes in these tissues were similar to that in normal tissues, being essentially complete after 1 hr.

In separate experiments the effects of La on [^{14}C]sucrose spaces of Na- and K-loaded tissues were determined. In K-loaded tissues the e.c.s. amounted to 353 ± 7 ml/kg fresh weight ($n = 8$) and 372 ± 8 ($n = 40$) in Na-loaded tissues, these values agreeing well with the value for normal tissues of 371 ± 11. Five mM-La reduced the extracellular spaces to 273 ± 17 ($n = 8$) ml./kg fresh weight in K-loaded, and to 284 ± 6 ($n = 39$) in Na-loaded tissues, again agreeing well with the value for normal tissues of 286 ± 8. Thus, with K- and Na-loaded tissues, as with normal tissues, the shrinkage caused by La is due largely to a reduction in the extracellular space. In Table 3 the changes produced by 1 hr contact with 5-mM La are compared for the three types of tissue. It can be seen that the changes induced in Na-loaded and normal tissues are very nearly identical. K-loaded tissues, however, show a significant loss of Cl which is accompanied by an increased loss of monovalent cation and greater shrinkage. The changes in e.c.s. and the cation lost in excess of the tissue Cl changes are similar for all three types of muscle.

Correcting for the changes in e.c.s., the 'cellular' ion changes consist of a loss of some 15 m-equiv/kg cation, and a gain of some 12 m-equiv/kg Cl in Na-loaded tissues. In K-loaded tissues the cation loss is 24·5 m-equiv/kg and the Cl gain, 4·5 m-equiv/kg.

^{24}Na uptake

In normal Krebs solution the uptake of ^{24}Na is very rapid, being complete within 5 min, and it is impossible to distinguish a cellular component of uptake (Goodford & Hermansen, 1961). To maximize the cellular exchange, Na-loaded tissues were used.

For each particular tissue, not only the tracer uptake, but also the [^{14}C]sucrose space and total Na content were determined. The results are shown in Fig. 3. The lines fitted through the uptake points are best-fitting solutions to eqn. (2) and represent the slow component of tracer exchange; their intercepts on the ordinates give values for the fast components of exchange. They are described by

$U_t = 45{\cdot}2 \pm 75{\cdot}6\ (1 - e^{-0{\cdot}09t})$ m-mole/kg fresh wt. in the La-free medium

TABLE 3. The changes in tissue weight and ion content produced by 5 mM-La on tissues incubated in high Na, high K or normal Krebs solutions. Differences ± S.E. Ion contents in m-mole/kg fresh wt., e.c.s. and tissue weight in g/kg fresh wt. The values for excess cation lost are in m-equiv/kg fresh weight. The figure in parentheses is an assumed value

	High Na solution	High K solution	Normal Krebs solution
Na, K or Na + K	− 22 ± 3	− 30 ± 4	− 20 ± 1·5
Mg	− 0·4 ± 0·1	− 0·45 ± 0·12	− 0·58 ± 0·09
Ca	(− 1·8)	− 2·00 ± 0·34	− 1·58 ± 0·16
Cl	− 1·5 ± 5·8	− 8·1 ± 3·4	− 0·5 ± 1·5
Weight	− 101 ± 24	− 144 ± 31	− 85 ± 7
e.c.s.	− 88 ± 10	− 80 ± 17	− 85 ± 14
Cation lost in excess of Cl lost	− 24·9	− 26·8	− 23·8

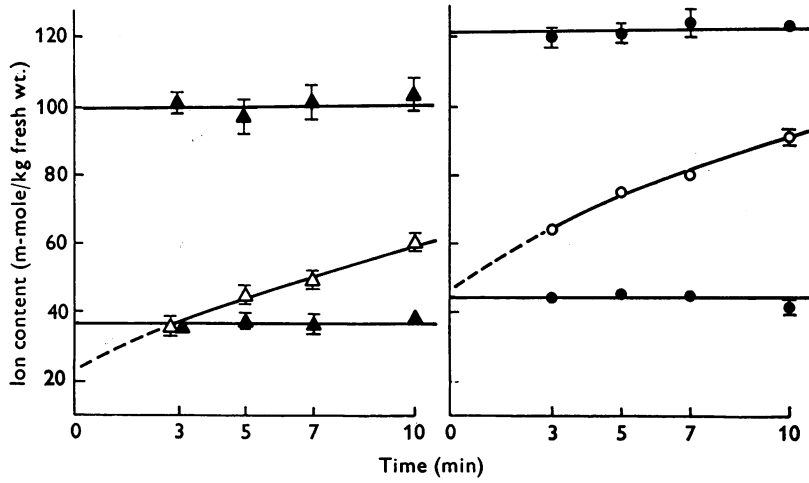

Fig. 3. The time course of the slow component of ^{24}Na uptake by Na-loaded tissues in the presence (triangles) or absence (circles) of 5 mM-La. Open symbols represent the uptake of tracer. The upper filled symbols represent the total tissue Na, and the lower, the extracellular Na contents corresponding to the [^{14}C]sucrose spaces; the horizontal lines drawn through these points are the over-all mean values. Each point is the mean of five determinations. The intercepts of the uptake curves on the ordinates yield estimates of the rapidly exchanging Na.

and by

$$U_t = 23{\cdot}5 \pm 76{\cdot}9\ (1 - e^{-0{\cdot}06t})$$ m-mole/kg fresh wt. in the presence of 5 mM-La.

Thus La can be seen to reduce the rate constant of the slow component of exchange, and to reduce the amount of rapidly exchanging Na, the

reduction in this fraction accounting almost exactly for the decline in total tissue Na content. Applying Huxley's correction the slow components become 66·4 and 70·4 (in La), while the rapid, extracellular components of exchange become 30·0 and 54·4 m-mole/kg fresh wt. in the presence and absence of La respectively. The corresponding Na contents of the extracellular space measured in this particular experiment were 35·8 and 44·1 m-mole/kg fresh wt. Thus of the 24·4 m-mole/kg change in the rapid component of exchange produced by La only some 8·3 m-mole/kg is accounted for by the decrease in the e.c.s., leaving a further loss of 16·1 m-mole/kg.

There seem to be two explanations for the Na lost in excess of the change in the e.c.s. Either La is displacing Na from discrete binding sites on the cell surface, or it is causing a change in the membrane surface potential and excluding Na from the diffuse ionic layer associated with a normally negatively charged surface. If the latter is indeed the case then the change in surface potential should lead to a gain of Cl^- in the diffuse ionic layer equal to the loss of Na and other cations.

To determine which of these two effects was operating experiments were performed in which the uptakes of both ^{24}Na and ^{36}Cl by Na-loaded tissues were followed. The results of one of these experiments are shown in Fig. 4. The lines through the uptake points have been drawn by eye and extrapolated back to zero time to give the rapidly exchanging fractions. The horizontal lines represent the over-all mean values for the extracellular spaces in the presence and absence of 5 mM-La taken from Table 1. La reduces the rapidly exchanging Cl by some 85 ml./kg fresh wt. and this change is entirely accountable for by the change in the e.c.s. The rapidly exchanging fraction of ^{24}Na uptake is reduced, however, by about 180 ml./kg, some 100 ml. in excess of the e.c.s. change. This corresponds to some 12–13 m-mole/kg fresh wt. of Na, in reasonably good agreement with the change of 16·1 seen in the experiment illustrated in Fig. 3.

Thus La causes a decline in the rapidly exchanging Na in excess of the Na lost due to the reduction of the extracellular space, but does not cause corresponding increases in the rapidly exchanging Cl. It seems, then, that the loss of rapidly exchanging sodium in excess of the change in e.c.s. is caused by displacement of this ion from discrete binding sites rather than by an alteration of membrane surface charge and loss of Na from a diffuse ionic layer.

The uptake of ^{42}K

The effects of La on the uptake of ^{42}K from normal Krebs solution are shown in Fig. 5. It is clear that La has little effect on the rate of ^{42}K uptake yet reduces the size of the rapidly exchanging component of exchange.

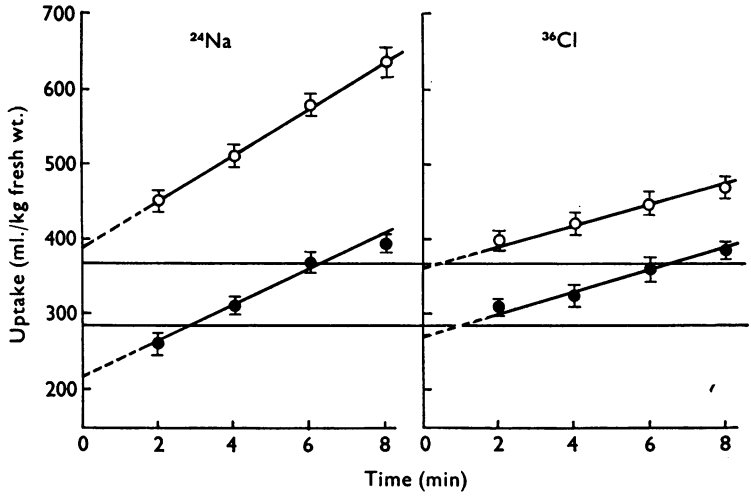

Fig. 4. The slow components of the uptakes of ^{24}Na and ^{36}Cl by Na-loaded tissues in the presence (●) or absence (○) of 5 mM-La. Extrapolation of the uptake curves to zero time yields estimates of the rapidly exchanging tracer. The horizontal lines are the mean values for the [^{14}C]sucrose spaces under these conditions: the lower is that in the presence of La. Symbols are the means of five muscles ± S.E.

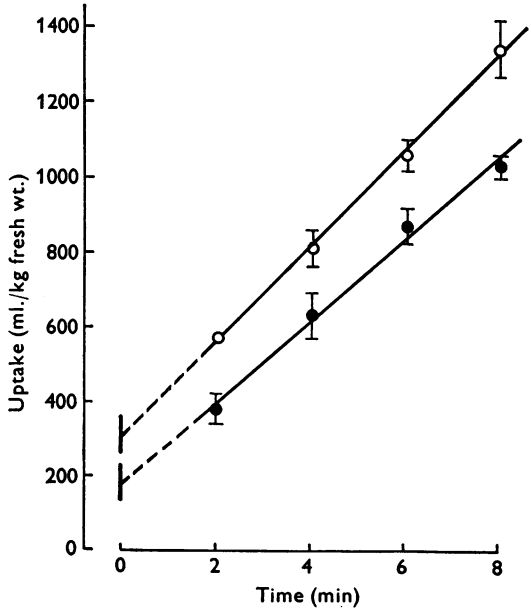

Fig. 5. The slow component of ^{42}K uptake from normal (○) and La-containing Krebs solutions (●). The vertical bars at zero time represent ± S.E. of the intercept. Each symbol shows the mean ± S.E. of five muscles.

The size of this reduction amounts to some 134 ml./kg fresh wt. and is increased to 168 ml./kg fresh wt. if Huxley's correction is applied to the results. This is some 80 ml./kg in excess of the change in the e.c.s. produced by La and is equivalent to a displacement of some 0·5 m-mole/kg of K. However, given the rather large confidence limits of the intercepts not too much reliance should be placed on this figure. In other experiments of this sort, La always reduced the rapidly exchanging K, generally by an amount greater than the change in the [^{14}C]sucrose space.

The effluxes of ^{36}Cl, ^{42}K and ^{24}Na

Normal, K-loaded or Na-loaded tissues were allowed to equilibrate fully with tracer while being incubated in the appropriate medium. The counts were then washed out into media with and without 5 mM-La. Where washouts were performed in the presence of La then for the last ½ hr of tracer loading the tissues had been equilibrated in a La-containing uptake medium.

Brading (1971) describes how the effluxes of tracers from the taenia can be resolved into the sum of three exponential processes. When this was done for the efflux of ^{42}K from high K media it was found that La had little effect on the rates of the slowest components of efflux, yet reduced the size of the fastest component of exchange from 33 to 22 % of the total tissue counts. This reduction is equivalent to some 25 m-mole/kg wet wt. which is a loss of rapidly exchanging K of some 10–15 m-mole/kg in excess of that caused by the reduced extracellular space produced by La.

In other experiments, tissues were loaded with ^{24}Na in, and the counts washed out into, normal Krebs solution. Following washout the same tissues were reloaded in normal solution, transferred for the last half hour of loading to a La-Krebs uptake solution of the same specific activity, and the counts were then washed out into a La-Krebs medium. Control tissues were reloaded in and washed out into normal Krebs solution. Counts leaving in any particular minute of the efflux into La-Krebs solution were subtracted from the counts leaving in the corresponding minute of the efflux into normal Krebs solution. The differences are plotted in Fig. 6 for each minute of the effluxes. The values plotted for the controls are the counts lost during the second efflux subtracted from those lost during the first. This Figure shows that in the control muscles there is little difference in the amount of counts leaving the tissue in any particular minute interval of the two effluxes. However, when the second efflux is into La-Krebs solution, then the amount of counts leaving the tissue during the first few minutes is considerably reduced compared to the efflux into La-free solution; while at longer times there is little difference between the first and second efflux. The mean difference in total tissue counts between

normal and La-treated tissues corresponds to some 13·5 m-mole/kg and, as shown in Fig. 6, this was all rapidly exchanging. The value for the difference in counts lost during the first minute showed great variability. This presumably reflects both differences in the rinsing of the tissues and in the manner in which they were put into the flux apparatus. After the first minute the histogram in Fig. 6 is exponential in nature and one can obtain an extrapolated value for the difference in counts lost in the first minute. Using this value, the change in the rapidly exchanging component of efflux produced by La becomes 17 m-mole/kg in reasonable agreement with the 20–26 m-mole/kg change seen in the uptake experiments. Thus these tracer studies confirm the results on total ion content that La displaces some 20 m-mole/kg of Na, and further show that this Na is rapidly exchanging.

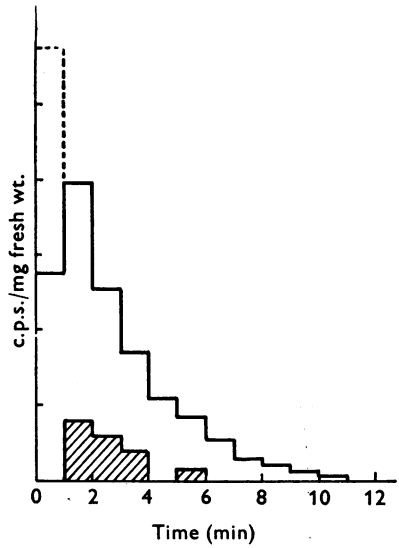

Fig. 6. The excess counts lost during each 1 min interval of the efflux of ^{24}Na into normal compared to the efflux into La-Krebs solution. Each column represents the mean of six estimates. The shaded columns are the values for the controls ($n = 2$), and show the excess counts lost during a first efflux into normal Krebs solution compared to the second efflux. The column marked with dashed lines is an estimated value.

The transmembrane component of Na efflux in normal tissues is small. Na-loaded tissues were therefore used to determine the effects of La on this component. The effect of La was to slow the rate of tracer loss at times when one can safely assume all tracer to be purely intracellular in origin.

In some experiments La was added during the efflux of tracer from

tissues previously unexposed to La into La-free washout solutions. It was without effect on the rates of loss of ^{42}K and ^{36}Cl, but caused a small decrease in the rate of loss of ^{24}Na from Na-loaded tissues.

The effects of K-free solutions

The changes in ion content and tissue weight when tissues were put in tris-buffered K-free solution are shown in Fig. 7. After 3 hr the tissues had lost some 50 m-mole/kg of K and gained about 35 m-mole/kg of Na. The K lost in excess of the Na gained was accompanied by an approximately equal amount of Cl and shrinkage.

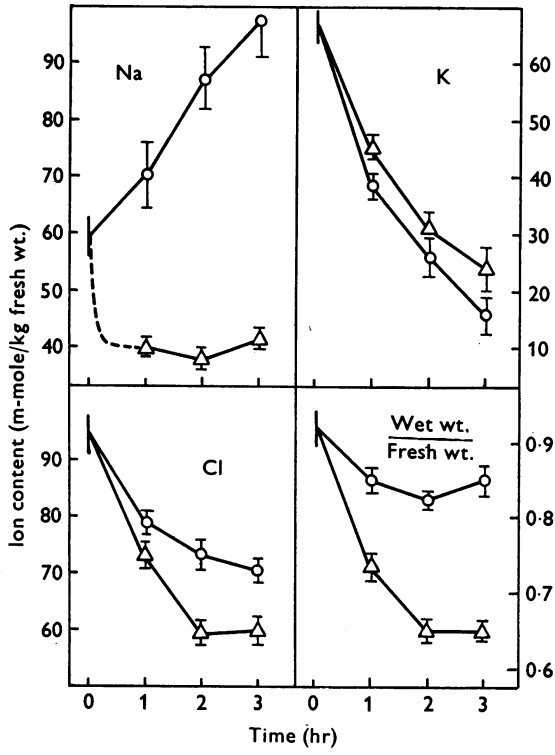

Fig. 7. The changes in ion content and tissue weight with time on exposure to K-free solution (○) or K-free solution + 5 mM-La (△). The points are the means of four to six determinations ± S.E. of mean.

The most marked effect of La on these changes is to completely prevent the uptake of Na. Indeed, not only is Na uptake prevented but there is actually a loss of some 20 m-mole/kg, occurring within the first hour in K-free solution. The loss of K is unhindered by La, but in the absence of a reciprocal gain of Na it is accompanied by an increased loss of Cl and greater shrinkage.

Recovery from ion-depletion

Tissues were placed for 4 hr in a solution in which sucrose replaced both KCl and NaCl. They shrank to 65% of their fresh weight, lost all their Na and their K levels fell to about 20 m-mole/kg fresh wt. These ion-depleted tissues were then placed into either high Na or high K solutions and their Na or K levels determined an hour later. In high Na solution, in the absence of La the tissues took up 83 ± 3 m-mole/kg fresh wt. of Na and swelled to 83 ± 2% of their fresh weight. In La-free high K solution the corresponding values were 89 ± 1 m-mole/kg fresh wt. of K and 79 ± 1% of the fresh weight. When repeated in solutions containing 5 mM-La striking differences were seen in the behaviour of tissues in high-K or high-Na media. In the former the tissues again gained weight and K, the final values being 75 ± 3% of the fresh weight and 75 ± 3 m-mole/kg K. In the high Na+La medium, however, the tissues remained shrunken at 67 ± 2% of the fresh weight and the uptake of Na was only 43 ± 2 m-mole/kg fresh wt.; this latter value is approximately the amount one would expect to be contained in the known e.c.s. of about 300 ml./kg tissue.

DISCUSSION

The results presented in Table 1 show that in normal Krebs solution an hour's exposure to 5 mM-La results in the loss of some $23{\cdot}8 \pm 2{\cdot}2$ m-equiv/kg of cation unaccompanied by Cl. La also caused shrinkage which was due to a reduction in the tissue's e.c.s. Such a change in e.c.s. has also been reported for the longitudinal muscle of the guinea-pig's ileum (Burton & Godfraind, 1974) and the mouse pancreas (Chandler & Williams, 1974). The earlier statement by Widdicombe (1974) that La caused no change in the e.c.s. was based on a single preliminary experiment and was not confirmed in further studies. Correcting for this change, the alterations in cellular ion content consist of a loss of some 12·9 m-equiv/kg of cation and a gain of some 10·0 m-equiv/kg of Cl (see Table 2).

Very similar changes were seen in Na-loaded tissues. In K-loaded tissues, however, the loss of cation was some 24 m-equiv/kg and the gain of Cl only some 5 m-equiv/kg. The shrinkage in the tissues, however, was greater than the change in the e.c.s. Such an effect is explainable by assuming that in these tissues La reduced passive inward K movement leading to a net loss of intracellular K accompanied by Cl^- and water to maintain electroneutrality and isosmolarity. If correction is made for the ions presumed lost with the cell water then the 'cellular' ion changes induced by La in K-loaded tissues are similar to the changes seen in normal or Na-loaded tissues.

The experiments on the uptake of ^{24}Na by Na-loaded tissues, illustrated in Figs. 3 and 4, show that La displaces from 12 to 15 m-equiv of rapidly exchanging Na presumably associated in some way with fixed negative sites in the extracellular space. This could be bound cation displaced by La^{3+} in the fashion

$$3NaR + La^{3+} \rightleftharpoons LaR_3 + 3Na^+$$

as proposed by Goodford (1970) and Sparrow (1969) for the interactions between K, Ca, Mg and Na.

However, La^{3+} is known to reduce the negative surface charge of artificial membranes (van Breemen, 1968) and the membranes of Ehrlich Ascites Tumour cells (Smith, 1976). Thus the Na displaced by La may not be bound but be diffusible cation loosely associated with a negatively charged membrane surface. The reduction in the e.c.s. seen here supports the idea that La is altering surface charge, for water would be held in a Donnan-type equilibrium at a charged surface, and lost when that charge was reduced. However, the cation lost from a diffusible ion layer associated with a change in surface charge should be accompanied by an equal gain of Cl in that layer. The analysis of cellular ion changes shown in Table 2 did reveal a Cl uptake approximately equal to the cation loss. However, the experiment illustrated in Fig. 4 showed that there was no increase in the amount of rapidly exchanging Cl relative to the e.c.s. Thus these experiments leave the exact association of the La-displaceable cation with tissue negative sites uncertain.

The small amount of excess rapidly exchanging K in normal Krebs solution indicates that, at the most, in normal tissues, only some 1 m-mole/kg of K is associated with fixed extracellular negative sites. The 1·37 m-mole/kg of Ca and 0·48 of Mg displaced by La are presumably also counter-cation (see Table 2). The value for rapidly exchanging La-displaceable Na for Na-loaded tissues of 12–15 m-mole/kg is presumably similar to that for normal tissues. Thus adding up we arrive at a figure of from 15 to 20 m-equiv/kg of cation associated with fixed extracellular negative sites. This value is similar to the estimate for fixed negative charge for the taenia by Sparrow (1969) of some 12 m-equiv/kg determined by analysis of the dependence of ^{28}Mg uptake from sucrose solutions on the concentrations of other cations. Using La to displace Ca from Ca-loaded rabbit aorta, van Breemen *et al.* (1972) also arrived at a similar figure of 16·2 m-equiv/kg.

The values of Na and K counter cation of 12–15 and about 1 m-equiv/kg respectively arrived at in the above discussion differ from the total cellular declines in Na and K contents determined by ion analysis of about 4–5 m-mole/kg for both ions (Table 2). However, these figures will reflect

not only the displacement of counter-cation but also changes in the intracellular ion content consequent on La's effects on ion permeabilities and exchanges. The loss of cell K, for instance, could be due to inhibition of Na pumping. This would lead to raised $[Na]_i$ which would tend to mask the displacement of Na counter-cations.

The increased 'cellular' Cl levels produced by La are, at least in Na-loaded tissues, not rapidly exchanging. Hence it seems that these increased Cl levels may be truly intracellular. Though the concentrations of physiological cations were the same in the normal and La-containing solutions, the Cl levels were slightly different. In La-free solutions the [Cl] was 150 mM, while it was 158 mM in 5 mM-La-containing solution. Hence the increased intracellular Cl levels may have been accompanied by increased intracellular levels of monovalent cation on a Donnan redistribution. Alternatively, as suggested by Hodgson, Kidwai & Daniel (1972), La^{3+} may penetrate smooth muscle cells accompanied by Cl^- to maintain electroneutrality.

The most marked effect of La on ion movements is the almost complete suppression of the Na uptake by ion-depleted tissues or by normal tissues placed in K-free solution. This presumably reflects a reduced P_{Na}. The relatively slight effect of La on ^{24}Na movement in Na-loaded tissues may be due to a large contribution to Na movements of Na-exchange diffusion (Brading, 1975). In similar situations where K influx could be considered to be entirely passive La had no such marked effect. Possibly this reflects a fundamental difference in the manner in which K and Na passively cross the cell membrane.

To recapitulate: the important points arising from this study are that La yields an estimate for fixed extracellular negative charge in this tissue, and its bound counter-cation, of some 15–20 mequiv/kg. Most of the counter cation in normal Krebs solution is Na, and this Na exchanges in the rapid phase of the efflux curves. La also greatly reduces bulk passive Na movements, but has less effect on K and Cl movement.

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