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SODIUM/SODIUM EXCHANGE IN THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. External Na has been shown to initiate a loss of ^{24}Na from high-Na smooth muscle of the guinea-pig taenia coli. This is an ouabain-insensitive effect, and there appears to be a 1:1 exchange of Na ions, suggesting a classical Na/Na exchange mechanism.

2. The Na/Na exchange has many properties in common with a similar exchange studied in high-Na beef erythrocytes by Motais (1973), and Motais & Sola (1973). It is very temperature-sensitive, it is partially inhibited by the sulphydryl reagents studied and it has a fairly low external affinity for Na.

3. The affinity of the external site for alkali metal cations is Na > Li > K > Rb > Cs.

4. It has proved impossible to estimate the affinity of the intracellular sites for Na. The curve relating intracellular Na content with the stimulated efflux reaches a maximum and then declines slightly.

5. Another unexpected finding was that after the rate of loss of Na has been reduced in a Na-free medium, reintroducing Na causes an overshoot in the rate, it increases to a value beyond the original one, and then slowly declines to it.

6. Unlike the classical Na/Na mechanism, the process is reduced, but not abolished by metabolic inhibition that depletes the tissue of ATP.

7. The results are interpreted to suggest that the Na/Na exchange is occurring from a cellular compartment of limited volume, which is itself exchanging with the main cell compartment. It is suggested that this small compartment is the sarcoplasmic reticulum, and the effect of metabolic inhibition is to interfere in some way with the relationship between this compartment and the cell membrane.

INTRODUCTION

It has long been realized that in smooth muscle Na exchange between the bathing solution and the tissue is extremely rapid, and follows a complex time course (for references, see Brading, 1973). It appears probable that a considerable fraction of the cell Na is held in association with negative sites on the cell membrane (Goodford, 1970; Widdicombe, 1974), and in tracer studies this rapidly exchanging fraction masks the slower and smaller transmembrane fluxes of the ion, making these processes difficult to study.

In the taenia coli of the guinea-pig it is fortunate that Na can be induced to replace all the tissue K, simply by exposing the muscle to K-free solution at body temperature for a few hours (Axelsson & Holmberg, 1971; Casteels, Droogmans & Hendrickx, 1971; Brading, 1973). The tissues reach a steady state, and will regain their K rapidly when this ion is introduced into the bathing medium. These high-Na tissues are very suitable for studying the transmembrane fluxes of Na.

The steady-state effluxes of Na from such high-Na tissues in the absence of K also follow a complex time course (Brading, 1973), even though there will be no Na/K exchange operating. After the 10 min necessary to remove the fast exchanging fraction of labelled Na (Brading & Jones, 1969) the rate constant of the efflux still slowly declines, and usually has not reached a steady value after 50–60 min. This suggests either that the population of cells is heterogeneous as regards Na permeability, or that the efflux is from a rather complex arrangement of cell compartments.

Recently Casteels, Droogmans & Hendrickx (1973) have suggested that half of the Na efflux from Na-rich tissues is due to a ouabain insensitive Na-exchange diffusion. A similar but very small ouabain insensitive fraction of Na exchange has been shown in red blood cells by Garrahan & Glynn (1967) and has recently been studied in some detail in high-Na beef erythrocytes (where it contributes the major fraction of the efflux) by Motais (1973) and Motais & Sola (1973). Keynes & Steinhardt (1968) have also demonstrated this phenomenon in striated muscle, and have proposed that the sodium is distributed in a multi-compartment arrangement in this tissue. They suggest that the Na/Na exchange takes place between the sarcoplasmic reticulum and the extracellular fluid via the 'T' tubules. Rogus & Zierler (1973) propose a similar compartmental arrangement to account for their studies on Na fluxes in striated muscle.

The aim of the present investigation was to make a detailed study of the properties of the Na/Na exchange in the taenia coli. No ouabain-sensitive Na/Na exchange has been detected, but the ouabain-insensitive exchange is shown to have properties very similar to those determined by Motais &

Sola in the beef erythrocyte, with a few interesting exceptions, and the results of the investigation are consistent with the hypothesis that the Na/Na exchange occurs from an intracellular compartment of limited volume, which might tentatively be identified with the sarcoplasmic reticulum recently described in taenia by Gabella (1971, 1973).

A preliminary account of the work was given in a communication to the Physiological Society (Brading, 1974).

METHODS

Tissue preparation

Male white guinea-pigs were stunned and bled, and thin pieces of taenia were dissected from the caecum. The pieces were weighed immediately to determine the fresh weight (F. wt.), and mounted at approximately *in situ* length on appropriate stainless-steel holders. They were then left to equilibrate in Krebs solution at 36–37·5° C for at least 1 hr before the experimental procedure was begun. After the experiment tissues were blotted and reweighed to determine the wet weight (W. wt.) before analysis.

Solutions

See Table 1. All solutions were made by adding appropriate amounts of isotonic stock solutions of the following concentrations (mM) NaCl 154·98, KCl 154·26. $NaHCO_3$ 154·76, $CaCl_2$ 108·11, $MgCl_2$ 103·28, NaH_2PO_4 153·84, glucose 299·73. Stock solutions of LiCl, RbCl and CsCl were all made up to 154 mM, and Tris Cl at approximately 154 mM, adjusted to pH 7·4 at 36° C. Except where specified, tissues were loaded in HCO_3/PO_4 buffered solutions, and washed out in Tris buffered solutions.

Chemical analysis

Solutions and muscle pieces were analysed for Na and K content by atomic absorption flame photometry. Aliquots of the solutions and tissues were extracted in Vitreosil tubes containing a 'diluting fluid' consisting of 1 N-HNO_3 containing La^{3+} 18 mM (La_2O_3) and Li^+ 5·5 mM ($LiCO_3$) to minimize ionic interaction in the flame and Ca precipitation. Standard solutions were prepared in the same diluting fluid. Glass distilled and then deionized water was used.

Preparation of high-Na ^{24}Na-loaded tissues

Tissues were exposed to non-radioactive K^+-free Krebs solution for 1½ hr then transferred to a small (13 ml.) loading bath containing ^{24}Na K^+-free Krebs for 2½ hr and then for a final 1 hr to a second loading bath with an identical solution. All solutions were kept at 36–37·5° C. Analysis of Na and K content of the second loading bath was routinely performed at the end of each experiment, and the K^+ content was always negligible.

Efflux measurements

Effluxes were measured using the continuous flow apparatus and method described by Brading (1971). The flow rate was a little over 2 ml./min. The samples were counted in a Packard gamma scintillation counter, and the results computed.

Table 1

Solutions	Concentration (mM)									
	Na^+	K^+	Ca^{2+}	Mg^{2+}	$Tris^+$	Cl^-	$H_2PO_4^-$	HCO_3^-	Glucose	Gas
Krebs	136·9	5·9	2·5	1·2	—	133·5	1·2	15·5	11·5	97% O_2 3% CO_2
K^+ free Krebs	142·8	—	2·5	1·2	—	133·5	1·2	15·5	11·5	97% O_2 3% CO_2
Tris-buffered Krebs	120·3	5·9	2·5	1·2	16·6	150·2	—	—	11·5	100% O_2
$MgCl_2$ washout solution	—	—	—	88·2	16·6	193	—	—	11·5	100% O_2
NaCl washout solution	126·2	—	—	3·7	16·6	150·2	—	—	11·5	100% O_2

Uptake measurements

Tissues were mounted on small stainless-steel holders, and after various pre-treatments were transferred into the radioactive solution for the desired time, and then exposed to ice-cold $MgCl_2$ washout solution for 5 or 10 min to remove extracellular Na, blotted, weighed and placed in vitreosil tubes. Diluting fluid was added and the tubes were capped and counted, and later analysed for Na and K content. Aliquots of loading solution were similarly treated.

Instantaneous rate constant of efflux

This value is the fraction of counts leaving the tissue at any instant. It is determined by dividing the counts leaving the tissue during a 1 min interval by the average counts in the tissue during that interval. If there is only a single rate limiting step in the exchange, the instantaneous rate constant should reach a steady value.

RESULTS

Some observations on steady-state high-Na tissues

When studying Na/Na exchange it is necessary to be able to remove Na completely from the external medium, and since it is not always possible to get the bicarbonate of the replacing cation, Tris Cl has been used as a buffer in all the washout solutions of experiments described in this paper unless otherwise stated. There is, however, evidence that the behaviour of the tissue is altered when HCO_3 ions are omitted from the medium (Brading & Tomita, 1972), and experience in our laboratory has also shown that after several hours in a Tris buffered medium, the Na pump is reduced in efficiency. Good *et al.* (1966) have also shown that Tris is often inhibitory to metabolic processes, even at buffering concentrations. For these reasons the loading and K-depleting of tissue was always carried out in the HCO_3/PO_4 buffered solutions, and exposure to Tris was kept only for the duration of the washout.

The exception to this was in an experiment where the effluxes of tissues loaded, K-depleted and washed-out in Tris buffered solutions were compared with those from tissues from the same animals loaded, depleted and washed-out in HCO_3/PO_4 buffered solutions. The effluxes at 35° C in both cases were very similar. This experiment was also designed to investigate the effect of temperature on these fluxes. Tissues were loaded and K-depleted at 35° C, and the washout was carried out at the experimental temperature indicated. Table 2 includes the results of an experiment on ion content, that shows that on transferring tissues from solutions at 35 to 10° C no change in the size or ionic content of the tissue occurs, and the tissues are thus in a steady state. Nevertheless it was apparent that the effluxes were greatly slowed as the temperature was reduced. Fig. 1 shows the instantaneous rate constants of the effluxes plotted using a

semilogarithmic plot. This plot not only emphasizes that the rate constant is continuously declining, even at the lowest temperatures, but also enables the temperature sensitivity of the flux to be seen. The bar at the left of the figures shows a multiplication factor of $\times 3$. The Q_{10} varies with the stage of the efflux curve, being greater at longer intervals than early intervals. Between 5° and 15° C and 15° and 25° C in Tris buffered solutions the Q_{10} reaches a value of 3·6, whereas at early times it is not much greater than 1. However, in both solutions the Q_{10} of the flux between 25° and

TABLE 2. The effect of exposure to a temperature of 10° C on tissues K-depleted in Tris buffered K^+-free solution

	Time of exposure to solution at 10° C				
	0 min	15 min	30 min	60 min	120 min
W. wt. % F. wt.	105·7 (8) ± 3·9	105·6 (8) ± 2·1	109·6 (8) ± 2·2	107·0 (8) ± 1·8	109·9 (8) ± 2·1
Cl m-mole/kg F. wt.	128·8 (8) ± 4·3	125·0 (8) ± 4·8	125·3 (8) ± 4·4	121·7 (8) ± 2·1	128·4 (8) ± 5·2
Na m-mole/kg F. wt.	125·5 (8) ± 3·4	124·8 (8) ± 3·8	127·7 (8) ± 2·9	128·6 (8) ± 1·6	135·2 (8) ± 4·0

35° C does not seem to change markedly with time. These experiments do not reveal anything about the mechanisms of efflux in these tissues, but do strongly suggest that several processes are involved which have different Q_{10}'s, and also, since the tissues remain in a steady state so far as ionic content goes, the exchange processes are probably exchanging Na ions in a ratio of 1:1.

Differences are apparent in this experiment between the behaviour of tissues in solutions with HCO_3/PO_4 and Tris buffers, but they may have been due to the fact that the same Tris buffer was used at all temperatures, and thus the pH of the solutions varied from 7·6 at 35° C to 8·2 at 5° C, whereas in the Krebs solution the pH was 7·5 at 35° C and 7·2 at 5° C. Later experiments in Tris-buffered solutions showed no obvious effect on the ^{24}Na efflux at 36° C of changes in the pH from 7·5 to 8·4.

The effects of replacing external Na

A suitable Na replacement had to be found to enable investigations of the Na/Na exchange system to be made. Casteels *et al.* (1973) have shown in these high-Na tissues, that replacing external sodium with choline causes the membrane to become leaky after about 20 min. I have shown a similar phenomenon (Brading, 1973) when sucrose was used to replace NaCl, and this is also shown in Fig. 2. In Fig. 2 the instantaneous rate

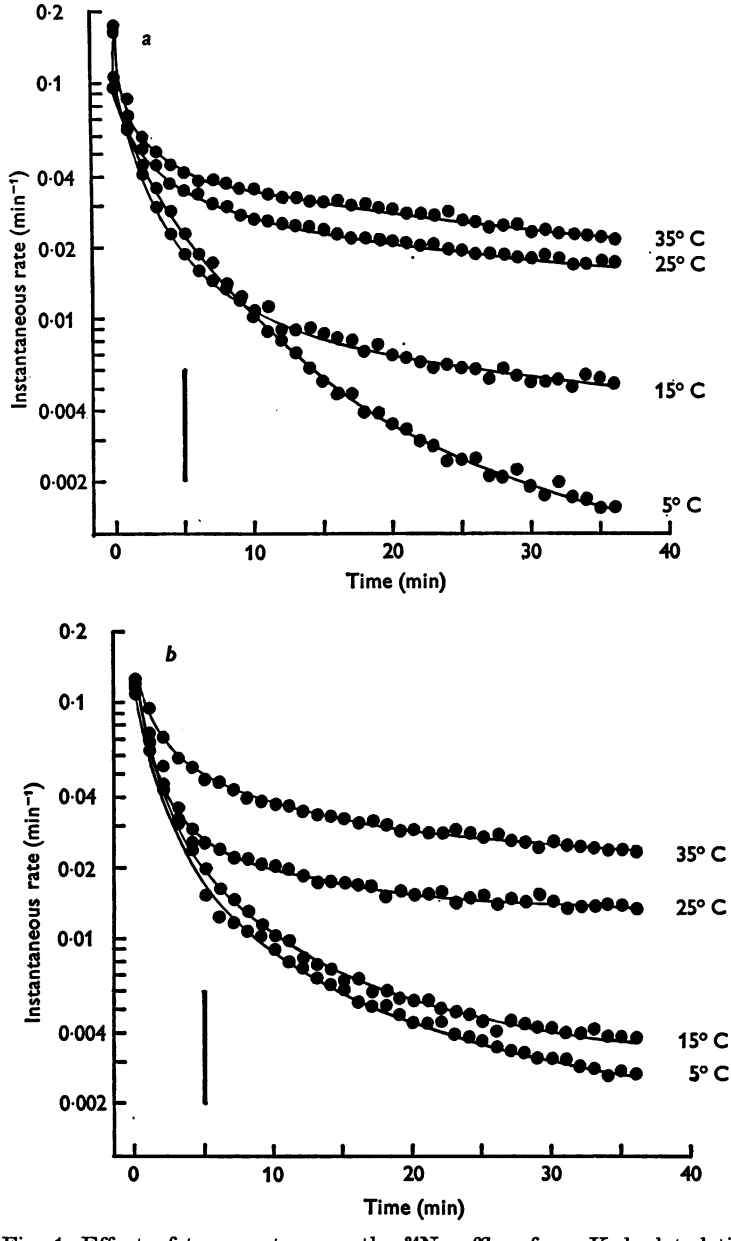

Fig. 1. Effect of temperature on the ^{24}Na efflux from K-depleted tissues. *a*, tissues loaded and washed in Tris-buffered K-free solution. *b*, tissues loaded and washed in HCO_3/PO_4 buffered K-free Krebs solution. The graphs show semilogarithmic plots of the instantaneous rate constant of the efflux against time. The bars show a multiplication factor of $\times 3$. Note that the Q_{10} of the rate constant varies with the stage of the efflux, becoming greater with time.

constant of the ^{24}Na efflux has been plotted for high-Na tissues washed out in Tris buffered solutions with several different Na replacements.

All these solutions also contained the normal concentrations of Ca^{2+} and Mg^{2+}. It can be seen that when sucrose replaces NaCl there is a sudden increase in the rate of loss of sodium that occurs after 15 min. A similar phenomenon was apparent when DDA (dimethyl-diethanolammonium

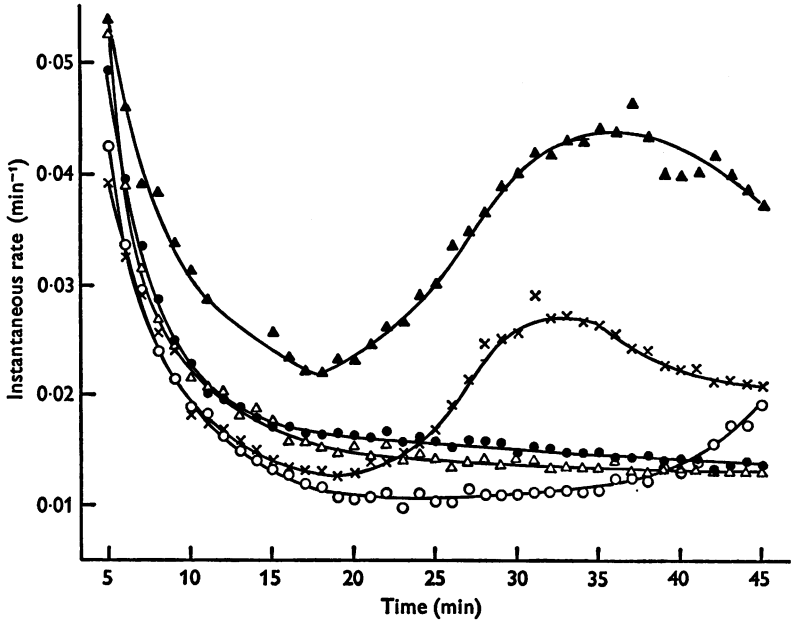

Fig. 2. Rate of loss of ^{24}Na from high-Na tissues into Na-free K-free solutions containing Tris buffer and the normal Mg^{2+} and Ca^{2+} content. The following sodium substitutes were used: ▲, sucrose; ×, DDA; ○, Tris; △, Mg^{2+}; ●, Li^{+}.

chloride – see Lorente de Nó, 1949; Kleinhaus & Kao, 1969; Anderson, Ramon & Snyder, 1971) was used as a sodium replacement, but the increase occurred later, and later still when Tris was used to replace Na. When the Mg was omitted from the washout solutions the increase in the rate constant with Tris and DDA as Na replacements began earlier (after 15 min) and was considerably greater. Only when lithium or magnesium were used to replace Na did the efflux rate constant remain low and continue to decline. As later experiments showed clearly that Li could substitute for Na to some extent in the Na/Na exchange mechanism, and also as Casteels *et al.* (1973) have shown that external lithium can activate the ouabain-sensitive Na pump, for the rest of these experiments Mg^{2+} was

used routinely as a Na substitute. Ca ions were omitted from the washing solutions since they are implicated in the increase in membrane permeability described above (Brading, 1973; and Discussion).

Demonstration of Na/Na exchange

Fig. 3 shows the effect on the ^{24}Na efflux from high-Na tissues of removing and replacing Na in the washout solution, using Mg^{2+} as a Na replacement. This is compared with a similar set of experiments showing the effect on the $^{42}K^{+}$ efflux from Na-free high-K tissues of removing and replacing K^{+} in the washout solution. The graphs represent the mean of

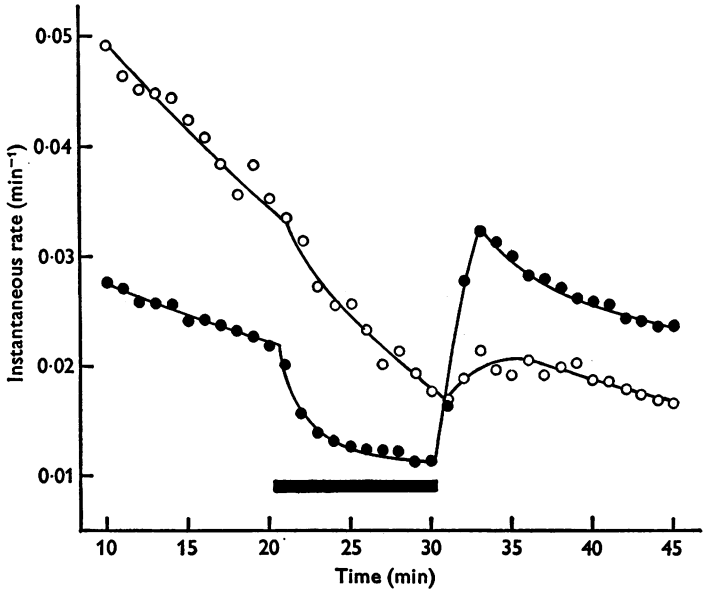

Fig. 3. The rate of loss of ^{42}K (○) from Na-free high-K tissues into Na-free high-K medium, and the rate of loss of ^{24}Na (●) from K-free high-Na tissues, into K-free medium. For the duration of the bar, both tissues were exposed to the Na-free K-free $MgCl_2$ washout solution. Each curve is the mean of three experiments.

three experiments in both cases. Ouabain had no effect on these results. It is interesting to note that the effect of replacing Na is much greater than the effect of replacing K, and on re-admitting Na after 10 min the rate constant of the efflux overshoots its previous value, an effect that is not observed on re-admitting K. This experiment was performed to reduce the possibility that the changes in efflux are due solely to membrane potential changes. It is probable that in both high-K tissues in Na-free medium and high-Na tissues in K-free medium, that the membrane potential is very

low (about −10 mV) and determined by the chloride equilibrium potential (Casteels, 1971). If the passive Na permeability of the membrane is less than the passive K permeability in the two conditions, as seems likely, the removal and replacement of K^+ would be expected to cause much larger changes in potential than the removal and replacement of Na, and the fact that the rate is actually more affected by Na suggests that the majority of this effect is due to an efflux mechanism that is directly activated by external Na ions.

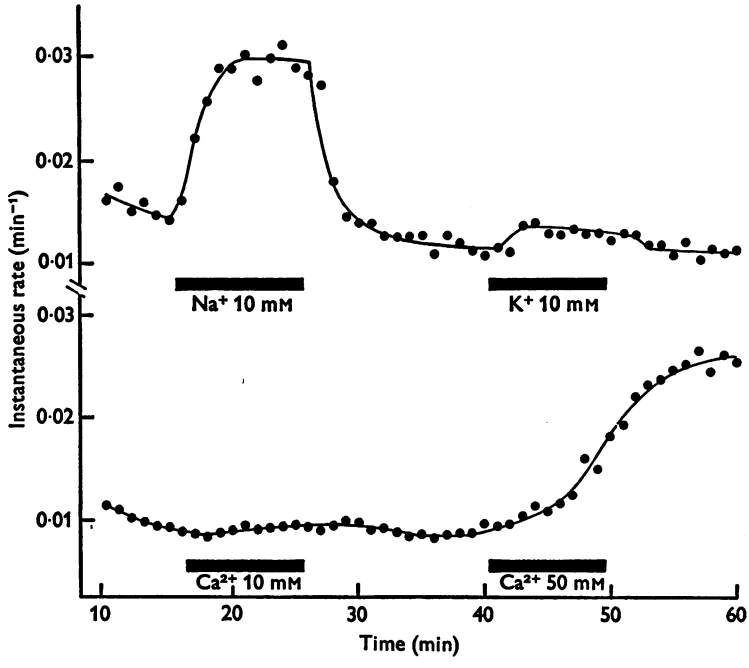

Fig. 4. The effect of introduction of various cations on the rate of loss of ^{24}Na into $MgCl_2$ washout solution. The upper trace is from an experiment where 10^{-5} M ouabain was used throughout.

The reduction of the rate of loss of ^{24}Na on removal of external Na is not simply a non-specific effect of the high external Mg concentration, since very similar results are obtained when Li, DDA or Tris are used for the Na substitute. But as these ions may either cause non-specific changes in permeability, or substitute for Na in several exchange mechanisms, they have not been used further as Na replacements.

Fig. 4 is a plot of the rate of loss of ^{24}Na into $MgCl_2$ washout solution, and the effects of introducing various cations. As can be seen, in the presence of 10^{-5} M ouabain, a Na concentration as low as 10 mM causes a

marked increase in the efflux of Na, while 10 mM-K^+ has only a small effect. In the absence of ouabain this concentration of K would cause a marked increase in efflux, presumably due to the activation of the Na/K pump. Addition of 10 mM-Ca^{2+} in the absence of ouabain had no appreciable effect, but 50 mM-Ca^{2+} induced a slowly rising irreversible increase in Na efflux, in time course and reversibility markedly different from the addition of Na, but similar to the increased efflux that develops when other Na substitutes are used (see Fig. 2).

To compare the ability of alkali metal cations to induce an increase in Na efflux

Fig. 5 compares the effect of reintroducing 46 mM monovalent cation during an efflux in $MgCl_2$ washout solution. Ouabain 2×10^{-5} M was present throughout, except during exposure to K^+ and Rb^+ containing solutions, where the concentration was increased to 10^{-3} M ouabain. It is clear from this experiment that each of these metal cations can stimulate an increase in the efflux of Na, and that their order of potency is Na > Li > K > Rb > Cs. This is the Eisenman series X (Eisenman 1962). It is interesting

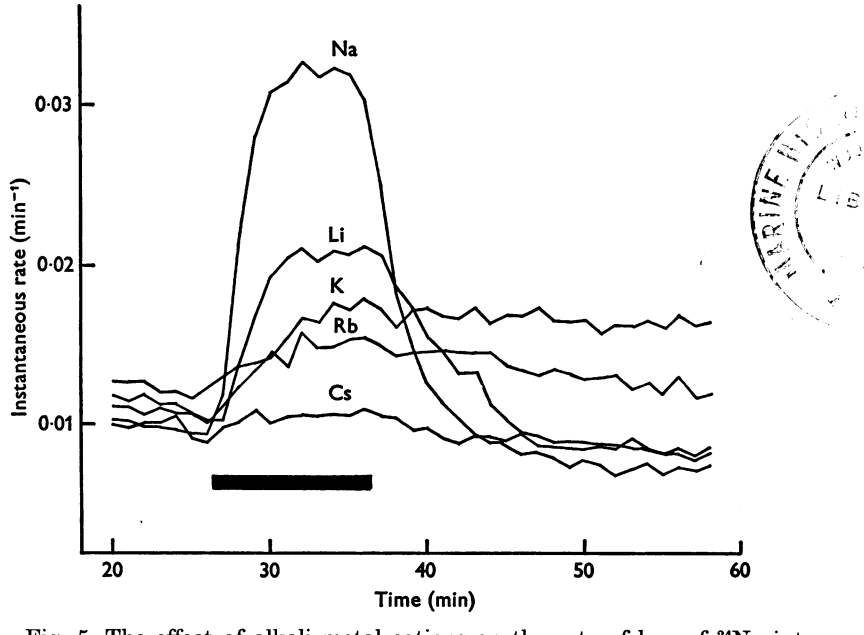

Fig. 5. The effect of alkali metal cations on the rate of loss of ^{24}Na into $MgCl_2$ washout solution. During the time indicated by the bar, the washout solution contained 46 mM alkali metal cation replacing some of the Mg. All solutions contained Ouabain 2×10^{-5} M, except the K^+ and Rb^+ containing solutions which contained 10^{-3} M ouabain. The effectiveness in stimulating the ^{24}Na efflux was of the order Na > Li > K > Rb > Cs.

to note that K and Rb, both ions known to be very active in stimulating Na/K pumping in many tissues, and also in the taenia (where the affinity of alkali metal cations for the external pump site follows Eisenman series IV, K ≏ Rb > Cs > Na > Li, J. H. Widdicombe, personal communication), induce a small increase in Na efflux, but on returning to $MgCl_2$ washout solution, the rate of efflux continues to be elevated. It is possible that K and Rb ions enter the cell during exposure to these ions, and later when they leave the cell down their concentration gradients, can occupy enough pump sites even in the presence of ouabain, to activate the pump slightly. For all the other ions, the flux returns to its previous value in an exponential manner.

Effect of changes of external Na concentration

The effectiveness of different concentrations of external sodium in stimulating Na efflux was studied on pieces of taenia from the same guinea-pig, and the results are plotted in Fig. 6.

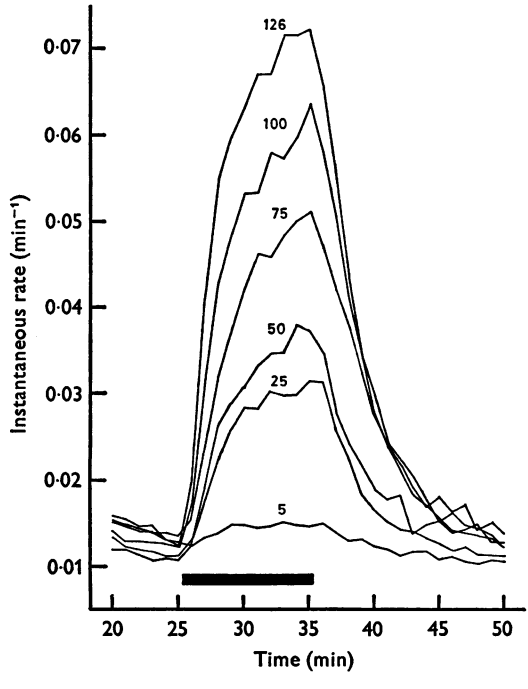

Fig. 6. The effect of various concentrations of extracellular Na on the rate of loss of ^{24}Na into $MgCl_2$ washout solution. The Na was introduced for 10 min (indicated by the bar) at the concentration shown on the graph (mM).

Fig. 7 includes the results from this experiment and two others. The Figure shows that there may be considerable variation in the magnitude of the Na efflux that can be stimulated, but in both cases the points can be fitted by a Langmuir plot with a K_e of 60 mM. This would only approach saturation at very high levels of external Na, and it is apparent that any binding site involved has a rather low affinity for Na. The points are the apparent amount of Na leaving the tissue by the Na stimulated efflux. This apparent amount was calculated by estimation of the c.p.s./mg W. wt.

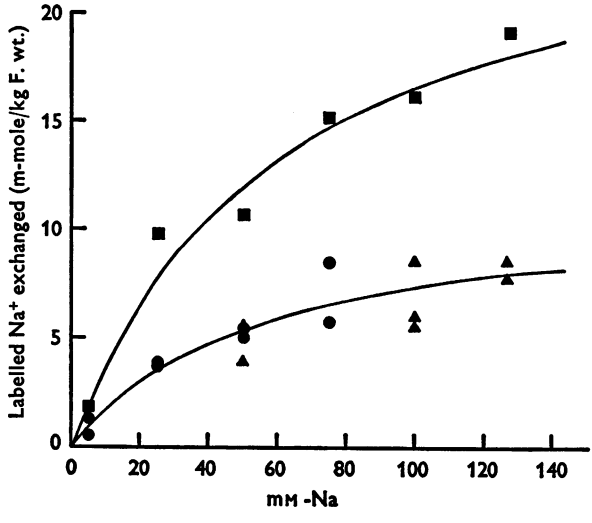

Fig. 7. A graph of the labelled Na lost during 10 min exposures to various concentrations of extracellular Na. Each symbol indicates tissues from the same guinea-pig. The upper points are from an experiment using a different batch of guinea-pigs from the lower points. The curves are both Langmuir plots with a K_e of 60 mM.

above the basal level for each point, summing them and estimating the amount of Na by reference to the specific activity of the loading medium. The actual amount of Na leaving will be underestimated, particularly at the higher levels, since the specific activity of the internal Na will be declining during exposure to external Na.

Effect of altering cellular Na content

The intracellular Na content of tissues was altered, keeping the specific activity of the Na constant, by incubating tissues first with the normal K-depleting ^{24}Na-loading schedule, and then transferring the tissues for different times to a loading solution of identical ^{24}Na specific activity, but containing 6 mM-K^+. In this solution Na is lost from the tissue and

replaced by K. The washout curves were again followed in $MgCl_2$ washout solution for 25 min before transferring to NaCl washout solution (Na 126 mM) for 10 min. Ouabain 10^{-4} M was present in both washout solutions. The apparent amount of Na leaving the tissue was clearly reduced as the total tissue Na became low, but it was also found that the peak amount of Na lost occurred not at the highest tissue Na levels (with zero K) but at slightly lower levels where some K was present in the cells. This same

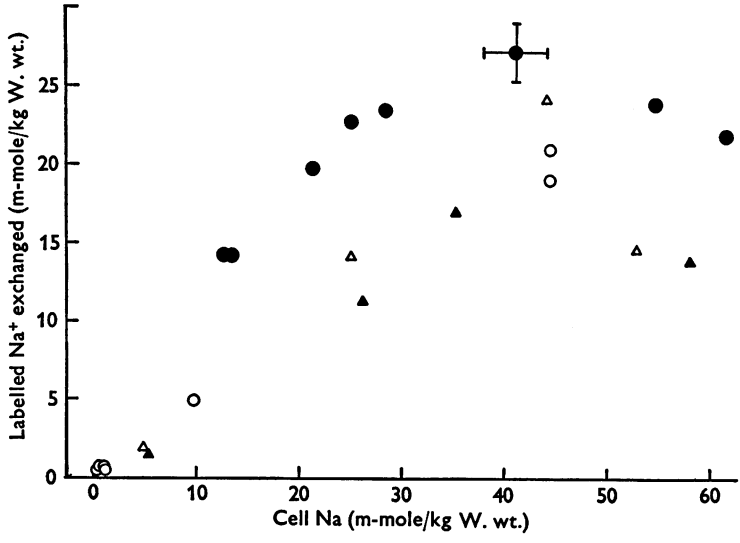

Fig. 8. A graph to show the relationship between the Na flux stimulated by 10 min exposure to 126 mM-Na and the cellular content of Na. The filled circles are the stimulated uptake of Na, and each point is the mean of six tissues, in which the ^{24}Na uptake and total Na content were estimated. The standard errors of one point are indicated by the bars. The other symbols are all from efflux experiments, each symbol indicating tissues from one guinea-pig. The efflux points will underestimate the real stimulated efflux because of declining specific activity in the tissues (see text).

phenomenon was apparent when the uptake of ^{24}Na by tissues with different total Na content during a 10 min exposure to ^{24}Na K-free Krebs was measured. In this experiment the buffer was HCO_3/PO_4 and therefore the extracellular Na was 143 mM. In this case the real and not apparent Na uptake will be measured, but this will include any Na entering through the passive permeability of the membrane. The results are shown in Fig. 8. As it is not possible to estimate the Na concentration adjacent to the site involved in the Na stimulated Na loss (see Discussion), no estimate of the affinity of the binding sites to internal Na can be made.

Stoichiometry of the exchange process

High-Na K-depleted tissues were used to measure simultaneous ^{22}Na efflux and ^{24}Na uptake during a 10 min exposure to various concentrations of extracellular Na (125, 82 and 47 mM). Tissues were first K-depleted and loaded with ^{22}Na-labelled solution, then washed for 5 min in $MgCl_2$ washout solution at 2° C (the aim being to remove extracellular Na with the minimum loss of intracellular ^{22}Na); they were then placed for 10 min into ^{24}Na-containing K-free Krebs solution at 36·5° C, washed again at 2° C, blotted, weighed and analysed for ^{22}Na, ^{24}Na and total tissue Na and K content. Controls were treated as above, omitting the exposure to ^{24}Na and the second rinse. Table 3 contains the uncorrected means of the uptake and efflux, and the mean ratio of the two. However, these fluxes need to be corrected for passive movements through the membrane. To do this it has been assumed that the passive movements are proportional to the concentrations from which they occur. From other experiments on the loss of Na into Na-free solutions, an approximate estimate of the passive loss was made, and taken to be 6·5 m-mole/kg. wt. for the three periods (2 m-mole for the two rinses, and 4·5 m-mole for the 10 min exposure). The passive uptake at 125 mM-$[Na]_o$ is assumed to be 4·5 m-mole, 2·95 at 82 mM-$[Na]_o$ and 1·69 at 47 mM-$[Na]_o$. These corrections are clearly only approximate, and make no allowance for changes in potential that might occur. The means of the individually corrected values are also presented in Table 3. The means of the corrected ratios of uptake and influx in the different solutions are 0·9, 1·02, and 0·89. The figures are not significantly different, and are not inconsistent with there being a 1:1 exchange of Na stimulated by the extracellular Na.

The effect of temperature

Fig. 9 illustrates the effect of temperature on the Na-stimulated Na efflux. The process shows a marked temperature sensitivity. In the experiment illustrated the areas under the curves representing the efflux stimulated by the external Na are in the ratio of 1:0·247:0·043:0·012 for the four temperatures, which gives a Q_{10} from 25 to 35° C of 3·9, from 15 to 25° C of 6·0, and from 5 to 15° C of 3·8.

The effect of metabolic inhibitors

Metabolic inhibition was achieved by including 2×10^{-4} M dinitrophenol (DNP) and 10^{-3} M iodoacetic acid (IAA) in the washout solutions, and using N_2 instead of O_2 to bubble the solution. This is sufficient to inhibit completely the Na/K pump and to reduce ATP levels in the tissue to

TABLE 3. Uptake of ^{24}Na and efflux of ^{22}Na from tissues exposed to extracellular Na for 10 min (m-mole/kg F. wt.)

	$[Na]_o$ 125 mM			$[Na]_o$ 82 mM			$[Na]_o$ 47 mM		
	Na uptake	Na loss	Ratio	Na uptake	Na loss	Ratio	Na uptake	Na loss	Ratio
Uncorrected means	30·6 (8) ± 1·0	36·6 (8) ± 1·8	0·85 (8) ± 0·06	25·1 (7) ± 1·8	25·5 (7) ± 1·8	0·862 (7) ± 0·09	19·2 (9) ± 0·75	27·2 (9) ± 1·76	0·727 (9) ± 0·05
Corrected means	26·1 (8) ± 1·03	30·1 (8) ± 1·8	0·90 (8) ± 0·08	22·1 (7) ± 1·8	23·9 (7) ± 2·8	1·02 (7) ± 0·1	17·5 (9) ± 0·75	20·7 (9) ± 1·8	0·89 (9) ± 0·08

zero (Van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth 1973). The Na stimulated efflux is markedly reduced, but not abolished by this treatment as shown in Fig. 10. Another interesting observation was that the efflux into $MgCl_2$ washout solution was also reduced by this procedure. Fig. 11 shows the results of an experiment where the total washout curves in Na solution and $MgCl_2$ have been followed in the presence and absence of metabolic inhibitors.

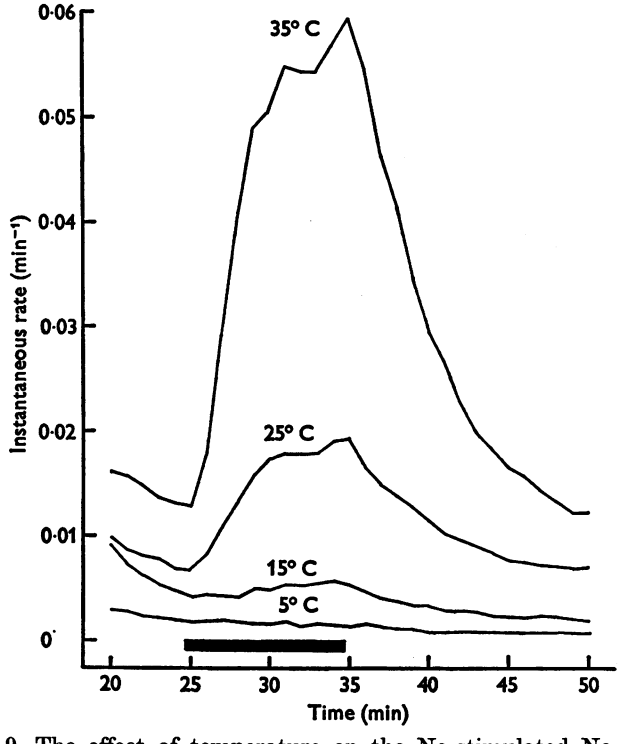

Fig. 9. The effect of temperature on the Na-stimulated Na efflux. The tissues were washed out in $MgCl_2$ solution except for a 10 min exposure to 126 mM external Na as indicated by the bar.

The effects of sulphydryl reagents

Motais & Sola (1973) describe the effects of sulphydryl reagents on the Na/Na exchange in beef erythrocytes. Amongst other agents, they describe a marked inhibition (to 36% of the control value) by 5×10^{-6} M *p*-chloromercuribenzene sulphonate (PCMBS) whereas ethacrynic acid (10^{-3} M) only reduced the exchange to 86% of the control. At a higher concentration (10^{-4} M) PCMBS they found a non-specific increase in membrane permeability. The effects of these concentrations of the two drugs was also

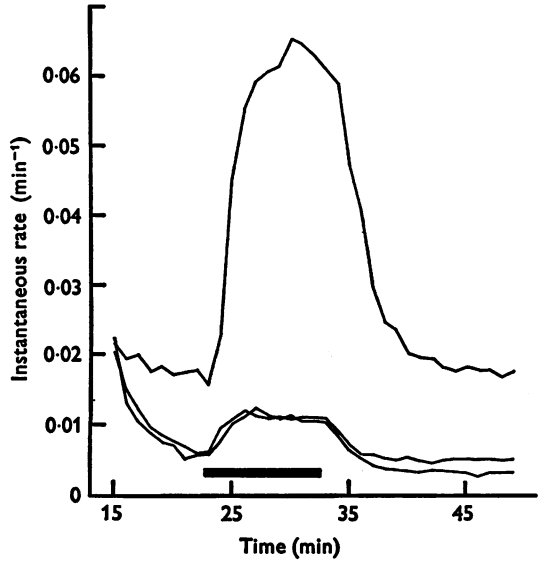

Fig. 10. The effect of metabolic inhibition on the Na stimulated Na efflux. The upper curve is a control showing the increased rate of efflux that occurs on exposure of the tissue for 10 min to 126 mM-Na in the washout solution. The lower curves are from tissues from the same guinea-pig, but both the $MgCl_2$ washout solution and the Na-containing solution included DNP 2×10^{-4} M and IAA 10^{-3} M, and were bubbled with N_2 instead of O_2.

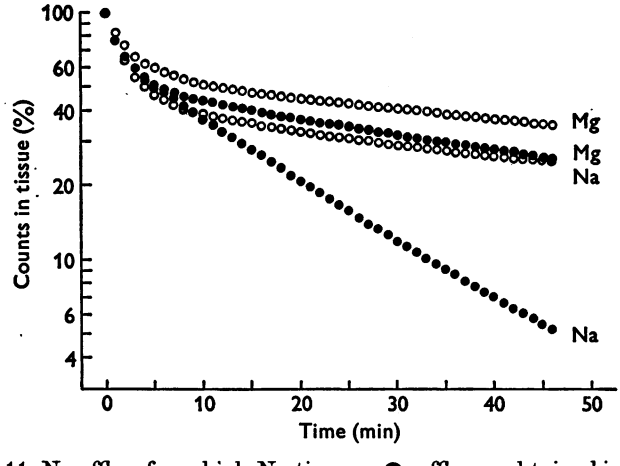

Fig. 11. Na efflux from high-Na tissues. ●, effluxes obtained in the absence of metabolic inhibition, into $MgCl_2$ washout solution or K-free Na-containing washout solution. ○, effluxes into the same solutions, but containing DNP 2×10^{-4} M and IAA 10^{-3} M, bubbled with N_2.

investigated on the taenia for comparison. It was found that ethacrynic acid reduced the Na/Na exchange to 41 and 45% of the control in two experiments, and the lower concentration of PCMBS reduced the Na stimulated efflux to 81% of the control. At higher concentrations of PCMBS the reduction of the Na stimulated Na efflux was to 73% of the

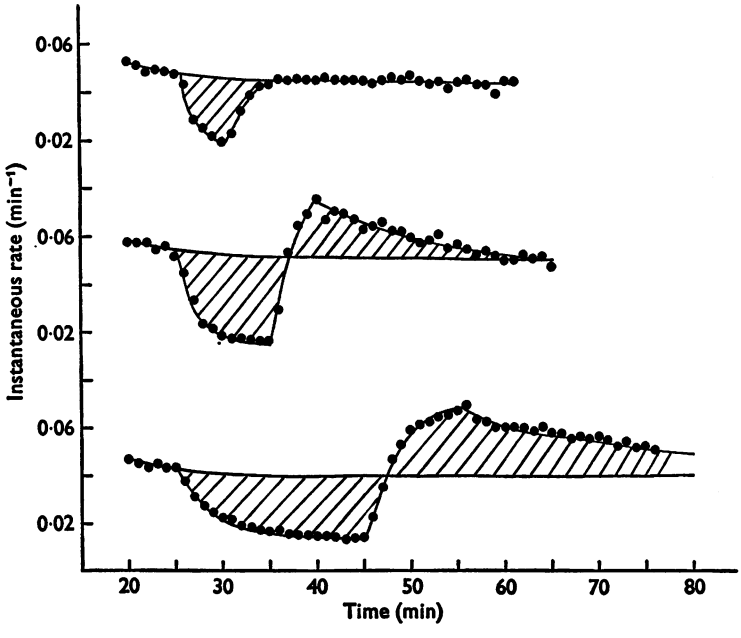

Fig. 12. The effect of removing Na on the rate of loss of ^{24}Na from high-Na tissues. The tissues were exposed to $MgCl_2$ washout solution for 5, 10 and 20 min. Note the effect on the overshoot.

control, but a marked increase in the rate of loss of Na into the $MgCl_2$ washout solution also occurred, indicating that a non-specific increase in membrane permeability also occurs in this tissue.

Experiments on the increased Na efflux following Na-free conditions

As shown in Fig. 3 when Na is removed from the washout solution for 10 min, the rate of loss of Na is reduced, and on reintroducing Na the rate rises to a level higher than it was previously. It was thought interesting to see what effect varying the length of exposure to the Na-free solution had on this subsequent increase. Fig. 12 shows the results from three tissues from one guinea-pig. To get some idea of the effect, the shaded areas on the curves have been compared. If A is the area enclosed by the reduction in rate constant, and B the area enclosed by the overshoot, then for 5 min

exposure to Na free solution, $A:B$ is 1:0, for 10 min exposure it is 1:0·7, and for 20 min, by extrapolation of the curves, $A:B$ is 1:1·24. For the two increased Na effluxes the declining rate constant after the overshoot was approximately exponential.

DISCUSSION

Sodium substitutes

The search for a suitable Na substitute to enable the experiments to be carried out, of itself led to some interesting observations. The sudden increase in rate of loss of ^{24}Na that occurs after exposure of the tissue to several of the Na substitutes has also been observed by Casteels *et al.* (1973) when choline was used as a Na substitute. It has been shown (Brading, 1973) that a corresponding increase in K permeability does not occur during the washout of ^{42}K from high-K tissues in the absence of Na. The increase in loss of Na when it occurs is associated with a gain in total tissue Ca^{2+}, at least when sucrose is used as a Na substitute, and if the external Ca is chelated with EGTA, or if La^{3+} is added to the sucrose, the increase in Na loss does not occur. It appears that to some extent Mg^{2+} can antagonize this increase of permeability, since if the normal amount of Mg is omitted from the bathing solution containing the Na substitute, the increase in rate of loss of Na occurs more rapidly, and more markedly. These findings all suggest that Ca ions are in some way responsible for this effect. The increase in rate of ^{24}Na loss always occurs under conditions which in other tissues are known to lead to an inwardly directed movement of Ca on a Na/Ca exchange mechanism (e.g. Baker, Blaustein, Hodgkin & Steinhardt, 1969; Reuter, Blaustein & Haeusler, 1973). Van Breeman *et al.* (1973) have shown that the presence of metabolic inhibitors leads to a gain in Na and Ca in the taenia, and an increase in membrane permeability also occurs, as judged by an increase in the [^{14}C]sorbitol space. This leakiness is abolished by La^{3+}, which has been shown to prevent Ca^{2+} entry into the cells. In the present experiments metabolic inhibition did not cause an increase in membrane permeability (see Fig. 11), but Ca ions had been omitted from the medium. I should like to suggest that the increase in loss of Na observed when sucrose, DDA and Tris were used as Na substitutes, could be explained by assuming that the large outwardly directed Na gradient so established is favourable for Ca^{2+} entry on a Na/Ca exchange mechanism. This Ca^{2+} entering the cell interacts in some way with the inner surface of the membrane, and causes a non-specific increase in membrane permeability. Li does not always cause this effect, and this may be due to its ability to replace Na on the Na/Ca exchange mechanism (Baker *et al.* 1969). Mg^{2+} also prevents this increase in permeability by competing with Ca^{2+} for sites on the carrier. It is possible that this mechanism can exchange Mg with Na, because the Na loss into $MgCl_2$ washout solution is reduced by metabolic inhibitors (see Fig. 11), and partial dependence of the Na/Ca mechanism on metabolic energy has been postulated by Baker & Glitsch (1973). If some Mg entry does occur through this mechanism, then it must be assumed that its effect on the inner membrane of the cell is different to the effect of calcium. A consiserable proportion of the Na loss into $MgCl_2$ solution must, however, be due to loss of NaCl and water, as the tissues shrink progressively during exposure to this solution. Nevertheless, $MgCl_2$ appears to be the best substitute for NaCl, allowing continued survival of the cells in a relatively normal state. It is reassuring that the Na/K exchange pump works well when small amounts of K^+ are introduced into the $MgCl_2$ solution.

Evidence for Na/Na exchange

The results described in this paper provide clear evidence that a Na/Na exchange occurs under the conditions of the experiments, that is from high-Na K-free tissues. Removing Na from the washout solution during an efflux rapidly reduces the rate of loss of Na and reintroduction of Na during a washout into $MgCl_2$ washout solution markedly stimulates the rate of loss of Na. The experiments on the stoichiometry of the exchange using simultaneous uptake and efflux measurements, although difficult to analyse accurately, suggest that a 1:1 Na exchange is taking place, and the steady-state fluxes performed at different temperatures, in which the tissues undergo no change in their Na content, even though the Na/Na exchange is markedly affected, are good evidence for the exchange being a 1:1 process. Experiments with high-K tissues give evidence that these effects are unlikely to be secondary to changes in potential.

Properties of the exchange process

If it is assumed that the exchange process is mediated by some carrier, then it is clear that the sites have an affinity for all the alkali metal cations, although the affinity for Na ions is greatest. The order of effectiveness of these cations for stimulating the process is Na > Li > K > Rb > Cs, which is series X of the Eisenman series (Eisenman, 1962). This might suggest that the binding energy of the sites is rather high, since the sequence, except for the order of Na and Li, is that of the unhydrated ion size. Another biological membrane that may show this selectivity is the outer border of the frog skin (Lindley & Hoshiko, 1964), although they found it difficult to establish the order of Rb, K and Cs.

The actual affinity of the sites for Na is, however, rather low, since there is no sign of saturation of the stimulated efflux even when the external Na is increased to the normal concentration. It is difficult to be certain that interaction at only one site is occurring, although in Fig. 7 points relating the size of the stimulated efflux to the extracellular Na can be fitted reasonably well by a Langmuir plot with a K_e of 60 mM. The figure is, however, a little misleading in this respect, as the points at increasing extracellular Na concentrations progressively underestimate the actual stimulated efflux, because the specific activity of the intracellular Na will be declining. If, as is suggested later, the Na/Na exchange is occurring from a limited intracellular compartment, there seems at present no justifiable way of correcting for this effect.

It seems probable that the Na/Na exchange is occurring by an ion exchange mechanism that is separate from either the Na/K pump or any Na/Ca exchange. It is unlikely to be operating via the Na/K pump since

it is not abolished or reduced by ouabain even at concentrations as high as 10^{-3} M, and the affinity of the alkali metal cations for the external exchange sites is different to that of the Na/K pump in this tissue (Eisenman series X for Na/Na exchange, series IV for Na/K exchange). It also seems unlikely that the exchange is using a mechanism normally involved in Na/Ca exchange, since the presence or absence of extracellular Ca (2·5 mM) did not affect the Na stimulated Na efflux. As discussed earlier there may be a Na/Ca exchange operating in the taenia (although this is not yet proved, and its properties not established.) At higher levels external Ca is able to stimulate an increase in Na efflux (see Fig. 4) in a Na-free solution, even in the presence of high $[Mg^{2+}]_o$, but in contrast to Na efflux stimulated by the monovalent cations, the latency of the effect is long, it is not immediately reversible, and the increased Na efflux brought about is probably largely due to an increase in membrane permeability, since the chloride efflux is also increased (Brading, 1974).

It is interesting to compare the Na/Na mechanism described here with that occurring in high-Na beef red cells, the properties of which have been elegantly characterized by Motais (1973) and Motais & Sola (1973). In these cells there is good evidence that the Na/Na exchange, which accounts for 90 % of the total Na efflux, may involve only a single site reacting with Na at each side of the membrane. The sizes of the stimulated efflux related to both extracellular and intracellular Na can be fitted by a rectangular hyperbola, but the affinity of the outer site is lower than that of the inner site. The K_e at the outer site was 40–50 mM and for the inner site 5–10 mM. In the taenia it has proved impossible to estimate the K_e for the inner sites, because of the strange shape of the curve, and the possibility of intracellular compartmentalization.

The Na/Na exchange from both beef red cells and taenia is markedly temperature sensitive, and both processes are reduced by sulphydryl reagents, although ethacrynic acid is more effective in reducing the exchange in smooth muscle than it is in the red cells, and PCMBS is less effective. The non-specific increase in membrane permeability induced in the red blood cells by higher concentrations of PCMBS is also observed in smooth muscle.

The two most obvious differences between the Na/Na exchange of beef red blood cells and taenia are first, that the exchange of these cells is not effected by metabolic inhibitors, whereas in metabolically inhibited smooth muscle the Na/Na exchange is greatly reduced. This is inconsistent with a classic exchange system (Ussing, 1947). Secondly, the relationship between intracellular sodium and the Na stimulated efflux in RBC's is described by a rectangular hyperbola, whereas the curve for smooth muscle reaches a maximum and then declines.

There are two other interesting features of the Na fluxes in Na loaded taenia coli; one is the constantly declining rate constant, even when the tissues are in a steady state, and the other is the overshoot of the rate constant that occurs on readmitting Na after exposure to Na-free solution for a short time.

Compartmentalization of intracellular Na

The discrepancies between the Na/Na exchange in the beef high-Na red blood cells and in the high-Na taenia coli smooth muscle, and also the unexplained features of the Na efflux from the taenia referred to above,

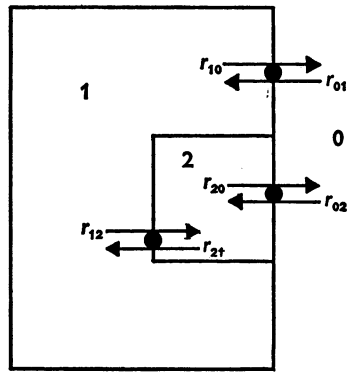

Fig. 13. Suggested compartmental arrangement of cells. 0 is the extracellular compartment, and 1 and 2 intracellular compartments.

could all be accounted for if it is assumed that the Na/Na exchange occurs across the membrane from a limited intracellular compartment. The arrangement suggested is shown in the diagram (Fig. 13).

Compartment 0 is the extracellular compartment, and 1 and 2 are cell compartments. r_{12} is the rate of Na transfer, or amount of Na moving in unit time from compartment 1 to compartment 2 (this will depend on the concentration of Na in compartment 1, or its specific activity when the movement of radioactive Na is considered, the surface area of the membrane between 1 and 2, and a constant depending on the mechanisms involved in the transfer). The rate constant λ_{12} is the proportion of the Na content of compartment 1 moving into 2 in unit time, which for radioactive Na would be the cps/min leaving compartment 1, divided by the c.p.s. present in compartment 1 during that minute. In the steady state it is suggested that $r_{20} = r_{02} > r_{12} = r_{21} > r_{10} = r_{01}$. When the tissue is loaded to equilibrium with ^{24}Na, and if the rates of transfer are considered for the radioactive ions (r^*), then on washout, the extracellular space and

any bound ions will exchange rapidly, and the specific activity in the extracellular compartment will approach zero, and so will r^*_{02} and r^*_{01}. As the exchange between compartments 2 and 0 is the most rapid, the specific activity in compartment 2 will initially decline faster than the specific activity in compartment 1, until a steady state is reached when r^*_{20} will depend on r^*_{12}, the rate at which Na enters compartment 2. The total rate constant for the whole tissue would thus be expected to decline with time (after the initial fast extracellular exchange), and eventually reach a steady value, which should depend on r^*_{12} and r^*_{10}. This is not inconsistent with the results as the rate constant in the steady-state efflux does decline, and although it has not reached a constant value after 40–50 min, it appears to be approaching a steady level.

If the Na movement between compartment 2 and 0 is mediated entirely by the Na/Na exchange mechanism described in this paper, then removal of extracellular Na should immediately reduce the rate constant to a value depending on r_{10}, as the only route for Na exchange between compartment 2 and the extracellular space is through compartment 1. However, when one switches off r_{20}, the specific activity in 2 is less than in 1, and r_{21} will be less than r_{12}, which will result in the specific activity in 2 progressively increasing until it is the same as in 1. When the Na/Na exchange mechanism is switched on again, r_{20} which depends on the specific activity in 2, will be greater than it was previous to removing Na, and so the total rate constant of the whole tissue will overshoot the original level, and then slowly decline to the old level.

It is thus apparent that such a model could account for the declining rate constant of the efflux from high-Na tissues, and the overshoot phenomenon observed when Na is removed and reintroduced in the external medium. It could also account for the relationship between total cellular Na and the Na stimulated efflux, if it is assumed that one of the processes that could be involved in the movements r_{12} and r_{21} is the normal Na pump. This of course would not be operating in K-free tissues, but changing $[Na]_i$ in these experiments involved increasing the $[K]_i$, and it is possible that in the presence of the right amount of intracellular Na and K the pump could result in a higher Na concentration in compartment 2 than could be achieved in the absence of K. The highest values of stimulated efflux occurred when intracellular Na was high, but there was also a small amount of cell K.

The anatomical correlate of compartment 2 in this model would be the sarcoplasmic reticulum that has been shown to occur in the taenia, under the plasma membrane, and around the membrane caveolae by Gabella (1971, 1973), and estimated to account for about 2% of the cell volume (Devine, Somlyo & Somlyo, 1973). The model requires that the compart-

ment exchanges directly with the extracellular space, and thus would have to contact the plasma membrane. It is interesting to note that Gabella (1973) reports that 'all the superficial sarcoplasmic reticulum appears to contact the plasma membrane at some point' and he also says of the sarcoplasmic reticulum that 'the relationship with the caveolae seems too extensive to the fortuitous'. Thus it is possible that where the sarcoplasmic reticulum contacts the membrane, either at the surface or on the caveolae, a pathway occurs for the Na/Na exchange described in this paper. If the relationship of the sarcoplasmic reticulum with the plasma membrane is modified or abolished in the presence of metabolic inhibitors, then the reduction of the Na/Na exchange could be explained, without having to postulate a requirement for metabolic energy for the process. The remaining Na/Na exchange could be between the sarcoplasm and the extracellular solution.

This type of arrangement would then be analogous with the sarcoplasmic reticulum and 'T' tubular system in striated muscle. It has been suggested (Keynes & Steinhardt, 1968; Rogus & Zierler, 1973) that exchange of Na between the sarcoplasmic reticulum and external fluid does occur via the 'T' tubules, the membrane of the triad being permeable. Keynes & Steinhardt have also suggested that this is the site of the ouabain insensitive Na/Na exchange seen in this tissue. Compartmentalization of intracellular Na has often been postulated in striated muscle and is supported by such evidence as the unexpectedly low Na activity measured with ion-sensitive microelectrodes; see, for example, Lea & Armstrong (1974).

In conclusion, therefore, it is suggested that the Na/Na exchange observed in the high-Na taenia coli is exchanging Na predominantly between the sarcoplasmic reticulum and the external medium, through pathways where reticulum contacts the plasma membrane or caveolae. It is interesting to speculate on the relevance of this arrangement in normal tissue. If the sarcoplasmic reticulum membrane does contain a Na/K pump as is suggested, then it is possible that the Na content in the reticulum is normally much higher than in the sarcoplasm. In this case the $[Na]_i$ of the sarcoplasm is probably very much lower than is normally calculated. The gradient of Na between the two compartments would then be favourable for uptake of Ca by a Na/Ca exchange mechanism, and such a mechanism might assist in the ability of the sarcoplasmic reticulum to accumulate Ca. One might also speculate that the Na/Na exchange studied in this paper, in the absence of K^+ and Ca^{2+}, is taking place on a mechanism capable under normal circumstances of carrying several ionic species depending on their relative concentrations and the affinity of the carrier for the species in question. The mechanism then may be partly responsible for regulating the concentrations of ions in the sarcoplasmic reticulum.

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