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CHLORIDE CONTENT AND ^{36}Cl UPTAKE IN THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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The sodium, potassium and chloride contents of the smooth muscle of the guinea-pig taenia coli have been previously measured (Daniels 1958; Durbin & Monson, 1961; Goodford & Hermansen, 1961) but the observations are not easy to interpret. The kinetics of ^{24}Na and ^{42}K uptake show that these ions are not simply distributed between an intracellular and extracellular space (Goodford & Hermansen, 1961; Goodford, 1962), and the exchange of ^{36}Cl has now been studied in an attempt to throw more light on the distribution of small ions in smooth muscle. A common difficulty has been that measurements of the ionic contents of the taenia coli are not regularly reproducible (Table 3), and the potassium content, sodium content, uptake of ^{42}K and inulin space have now been determined in taeniae from the same animals as were used for the observations of chloride content and ^{36}Cl exchange.

METHODS

The procedures used were essentially those described by Goodford & Hermansen (1961). Pieces of taenia were attached with two loops of cotton to specially constructed 'spring balances' and were immersed in modified Krebs's solution for at least 1 hr, while the tension applied to each was readjusted from time to time until it became steady at 1 g. The muscles were then transferred to the experimental solution, as specified in the description of results, and were finally removed for analysis at intervals.

Solutions. The composition of the modified Krebs's solution was (mM): Na^+ 137·4, K^+ 5·9, Mg^{2+} 1·2, Ca^{2+} 2·5, Cl^- 134, $H_2PO_4^-$ 1·2, HCO_3^- 15·5, D(+)-glucose 11·5; O_2 97 % + CO_2 3 %. It was prepared from isotonic stock solutions as described by Krebs (1950), using Analar grade materials and glass-distilled water. The initial dissection of the taeniae coli was carried out at room temperature in a solution composed of (mM): Na^+ 144, K^+ 5·9, Ca^{2+} 3·7, Cl^- 157. The 'low-chloride' solutions were prepared by using isotonic solutions of sodium and potassium ethanesulphonate (Goodford & Ing, 1959) instead of the corresponding sodium and potassium chloride solutions. The ethanesulphonates were supplied by British Drug Houses, and were purified by passage through a charcoal column which had been previously back-washed to double its volume, with glass-distilled water. The final concentrations of sodium and potassium were checked by flame photometry. Calcium and magnesium ethanesulphonates were not available, so 'low-chloride' solutions still contained the chloride (7·4 mM) corresponding to these cations.

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The Radiochemical Centre, Amersham, supplied a 2·18 M solution of $Na^{36}Cl$ from which a stock solution of isotonic $Na^{36}Cl$ was prepared (specific activity 2·5 μc/m-mole Cl), and the radioactive Krebs's solution was made up from this stock solution in the usual way.

Radioactive chloride determination. Muscle samples were placed on the bottom of cylindrical glass vials 40 mm high and 20 mm diameter, and 1 ml. of 30 % (100 volumes) Analar hydrogen peroxide, containing sodium carbonate 5 mg/ml. was added to each. The samples were slowly warmed and there was a brisk evolution of oxygen, but frothing was prevented by a ring of silicone Antifoam A (Hopkin & Williams) on the inside of the glass just above the solution. The bottom of the vial, when dry, was covered with a uniform crystalline deposit and the radioactive ^{36}Cl in the sample was counted with an end-window G-M tube (window weight 1·7 mg/cm^2) in a lead castle. The crystalline deposit weighed 6–7 mg, and no correction was applied for self-absorption, as this was small and similar for all samples. Count rates were 30 counts/min or more above a background of 7 counts/min (composed of 6·6 counts/min natural background and 0·4 counts/min from the ^{40}K in the glass of the vials), so that counting periods of several hours were necessary to give sample counts with a standard deviation of ± 3 %. Vials which had been previously used were counted before each experiment to make sure that all ^{36}Cl had been washed away. Results have been expressed as the relative activity (R) of the taenia, defined as: (Specific activity of taenia)/(Specific activity of solution).

Chloride determination. The total chloride in each vial was next determined by the method of Cotlove, Trantham & Bowman (1958). 3 ml. acid (0·1 M-HNO_3 in 1·75 M aqueous acetic acid) and 3 drops of gelatine reagent (0·6 % gelatine + 0·01 % thymol blue + 0·01 thymol in water) were added to each vial. The colour of the thymol blue indicator confirmed that the solution was acid, and chloride was determined by coulometric titration. A constant direct current was passed between a pair of silver generator electrodes, causing the release of silver ions into the solution at a constant rate. The end point was indicated, after all chloride had been precipitated, by the increasing concentration of free silver ions which caused a rising current to flow through a pair of silver indicator electrodes. A pre-set increment of indicator current actuated a relay and stopped a timer which ran concurrently with the generation of silver ion, so that the time which had elapsed was proportional to the chloride in the sample. All observations were expressed as mean values, with the estimated standard error of the mean, and the number of observations in brackets, thus: 128 ± 6 (8). The variance of the intercept of the straight line in Fig. 3 was calculated from the formulae used by Goodford & Vaughan Williams (1962).

The chloride content of the taenia coli varied when muscles were dissected from different guinea-pigs, just as the sodium content varied (Goodford, 1962), and close agreement could best be obtained when animals from the same stock were available. Since, in general, guinea-pigs from different sources had to be used, experiments were designed to minimize animal-to-animal variation by using paired muscles.

Control experiments were carried out in order to confirm the accuracy, reproducibility and specificity of the chloride determination, and in order to investigate the possibilities of chemical interference with the method.

The chloridometer itself was very stable, provided the electrodes were carefully maintained, and calibration graphs (which were plotted before and after each experiment) drifted only a few per cent during a period of several months. The addition of Na_2HPO_4, $12H_2O$ 5 mg to the control solution increased the corresponding reading by the equivalent of only 1 μg of sodium chloride, so that phosphate interference was negligible. Sodium ethanesulphonate also had no effect, but reduced glutathione gave a large reading (presumably due to the sulphydryl group) which was completely eliminated when the sample was pre-treated with hydrogen peroxide. It was therefore concluded that sulphydryl groups in the muscle samples would not interfere with the analysis. No chloride could be detected in the cotton used to tie the muscles.

Cotlove (1962) suggested that most reported values of tissue chloride were too high because samples had been inefficiently prepared. Taeniae coli muscles were therefore dissected and the chloride content of alternate pieces was determined by coulometric titration after the alkaline hydrogen peroxide treatment described. The remaining pieces, altogether weighing nearly 100 mg, were analysed for chlorine as one group by Drs Weiler and Strauss, The Microanalytical Laboratory, Oxford. They were dried at 105° C, wrapped in filter paper, and ignited in a flask of oxygen in the presence of aqueous hydrogen peroxide. When the resultant vapour had dissolved, nitric acid was added and after boiling, silver nitrate solution, to yield a precipitate of silver chloride which was weighed, with the usual precautions, to determine chloride. The result by ignition in oxygen was 131·2 m-mole Cl/kg wet wt., and by ashing in alkaline hydrogen peroxide was 128 ± 6 (8) m-mole Cl/kg wet wt.

RESULTS

The chloride content observed immediately after dissection of the taenia coli from a freshly killed guinea-pig varied between 56 and 76 m-mole Cl/kg fresh wt. (Table 1*a*), confirming the results of Daniels (1958), who

TABLE 1. Mean values of various properties of the taenia coli, with S.E. of means and the number of observations in brackets

Condition of muscle	Observations	Result	Units
(*a*) Fresh after killing	Cl content	56–76	m-mole Cl/kg wet wt.
In dissection solution (room temp. = 20° C)	Cl content	128 ± 6 (8)	
In Krebs's solution (35° C)	Cl content	103 ± 2 (52)	
(*b*) In Krebs's solution (35° C) for 1 hr	Cl content	95 ± 1 (5)	m-mole Cl/kg wet wt.
In Krebs's solution (35° C) for 24 hr	Cl content	96 ± 2 (18)	
(*c*) In Krebs's solution (35° C)	Cl content	95 ± 1 (5)	m-mole Cl/kg wet wt.
In Krebs's solution (4° C)	Cl content	95 ± 1 (5)	
(*d*) In Krebs's solution (35° C)	K content	90 ± 1 (49)	m-mole K/kg wet wt.
	Na content	84 ± 2 (30)	m-mole Na/kg wet wt.
	Inulin space	337 ± 9 (24)	ml./kg wet wt.

found 54–65 m-mole Cl/kg fresh wt. During immersion in dissection solution at room temperature it rose to 128 ± 6 (8) m-mole Cl/kg wet wt., and fell again somewhat when the taeniae were suspended in oxygenated Krebs's solution at 35° C (Table 1*a*), reaching 103 ± 2 (52) m-mole Cl/kg wet wt. under normal control conditions. This last fall was probably due to the lower chloride content of Krebs's solution (134 mM) compared with dissection fluid (157 mM). In guinea-pigs from the same stock a similar content was observed after 1 hr at 35° C, and 23 hr later (Table 1*b*), so that the chloride content apparently reached a steady state during the first hour's immersion in Krebs's solution.

Uptake and loss of tracer ^{36}Cl

Figure 1 shows the uptake of ^{36}Cl by the taenia coli at 35° C. The muscles were transferred to radioactive solution after the initial 1 hr

equilibration period, and were subsequently removed for analysis. The relative activity was $0{\cdot}59 \pm 0{\cdot}03$ (8) after the first minute, and this uptake already exceeded the ^{36}Cl which would be dissolved in free solution in the extracellular inulin space (337 ml./kg wet wt.). The rate of entry immediately diminished, however, and after 15 min immersion the relative activity was $0{\cdot}72 \pm 0{\cdot}04$ (7), increasing to $0{\cdot}90 \pm 0{\cdot}03$ (7) after 1 hr. These results show an exponential increase of the relative activity towards 1,

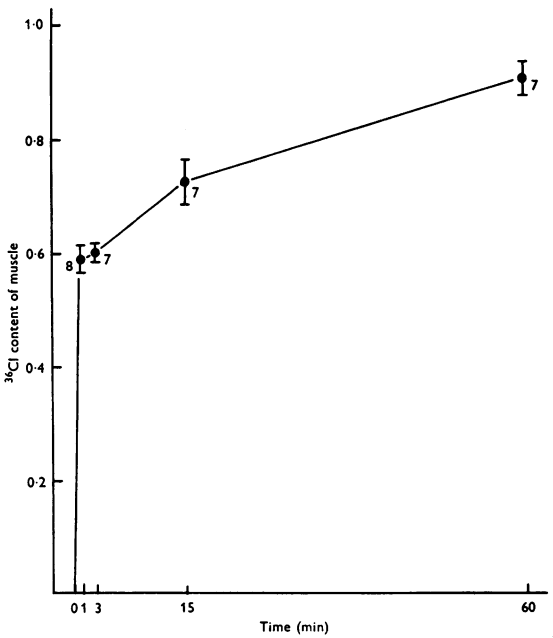

Fig. 1. Observations at 35° C: ^{36}Cl content of the muscle expressed as relative activity (see text) ± S.E., and the number of observations printed next to each point. Abscissa, time after immersion of the taenia in a solution containing tracer ^{36}Cl. The taeniae had previously been allowed 1 hr to equilibrate in inactive solution at 35° C.

the rate constant for this second and slower chloride exchange being $0{\cdot}030 \pm 0{\cdot}004$ (26); or $0{\cdot}032 \pm 0{\cdot}009$ (20) min^{-1} when calculated over the shorter period 1–15 min.

The results showed that there were at least two components of ^{36}Cl uptake, but it was not easy in experiments of this type to eliminate the possibility of a third smaller and still slower fraction. For example, if 5 % of the muscle chloride exchanged very slowly, it would be necessary to differentiate between 95 and 100 % exchange, and even when this small difference had been accurately determined there would still be the risk that it arose from a systematic error rather than a genuine effect.

Pieces of taenia were therefore dissected as usual and suspended in radioactive ^{36}Cl Krebs's solution for 24 hr in an attempt to label all the chloride. The pieces were then transferred to an inactive solution and removed for analysis at intervals. After 90 min the relative activity was only $0{\cdot}012 \pm 0{\cdot}0013$ (6), and after this time it was too small to measure accurately without counting for extremely long periods. One of two conclusions may be reached from this experiment: either the most slowly exchanging chloride was less than about 1 % of the total, or the exchange was so slow that a period of 24 hr immersion in ^{36}Cl solution was not long enough for the tracer to be taken up.

Extracellular space

61 m-mole Cl/kg wet wt. exchanged during the first minute in ^{36}Cl solution, corresponding to a 'chloride space' of 454 ml./kg wet wt., or 447 ml./kg wet wt. if a small conventional correction be applied for the

TABLE 2. 'Extracellular' space measurements corresponding to the rapid uptake or loss of inulin and several small ions by the taenia coli at 35° C. The same method was used to remove the superficial solution from the muscle in every case

Substance	Space (ml./kg wet wt.)	Reference
Inulin	333 ± 8 (40)	Goodford & Hermansen (1961)
Inulin	337 ± 9 (24)	Present work
Li^+	(about 40 %)	Goodford & Hermansen (1961)
$C_2H_5SO_3^-$	409 ± 20 (19)	Goodford & Lüllmann (1962)
Na^+ or Li^+	460	Goodford (1962)
Cl	447	Present work

slow exchange. The 'chloride space', like the 'sodium, lithium, and ethanesulphonate-^{35}S spaces', therefore exceeded the inulin space. Goodford & Hermansen's (1961) inulin space determinations were therefore repeated with the same samples of inulin which had been purified by repeated recrystallization (Willaman, 1922), and an inulin space of 337 ± 9 (24) ml./kg wet wt. was measured. This agreed adequately with the earlier results (333 ± 8 (40) ml./kg wet wt.), and with Born (1962) who found 300 ml./kg wet wt. in the taenia coli.

Calculations based upon the extracellular space and the ionic composition of a tissue can be very misleading, unless identical methods are used to remove the superficial liquid. However, there is still a discrepancy between the 'extracellular' inulin space of the taenia coli and the 'extracellular' chloride space, even when identical drying procedures are used (Table 2).

Low temperature

Pieces of taenia were dissected and left to equilibrate for 2 hr in normal Krebs's solution at either 4 or 35° C, but no difference in chloride content

between the two groups of muscles could be detected (Table 1*c*). By contrast, it has previously been shown (Freeman-Narrod & Goodford, 1962) that the sodium content is high at 4° C, and falls when the temperature is increased, and the potassium content is low at low temperatures but rises on warming.

Low chloride solutions

Pieces of taenia were equilibrated at 35° C in normal solution (134 mM-Cl) and were then transferred to a 'low-chloride' medium (7 mM-Cl) which had been prepared by using sodium and potassium ethanesulphonates instead of the corresponding chlorides. In the first minute this caused

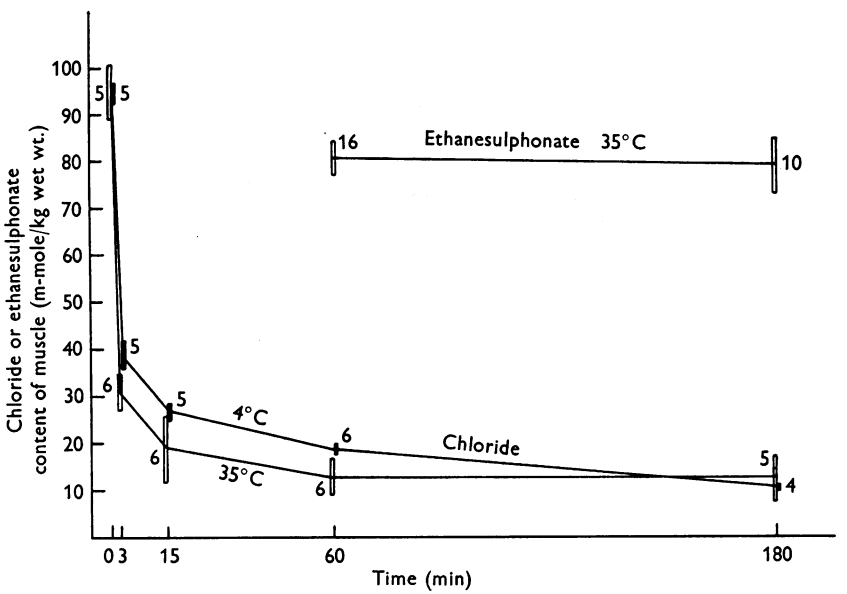

Fig. 2. Observations at 35 and 4° C. Ordinate, the chloride (or ethanesulphonate) content of the muscle in m-mole/kg wet wt. ± S.E., and the number of observations printed next to each point. Abscissa, time after transfer of the taenia to a 'low-chloride' solution which contained 7·4 mM-Cl and 127 mM ethanesulphonate. The taeniae had previously been allowed 2 hr to equilibrate at the respective temperatures.

a rapid loss of 64·2 ± 6·7 (9) m-mole Cl/kg wet wt. from the muscle (Fig. 2) and the 'chloride space' corresponding to the initial loss of chloride again exceeded the extracellular inulin space. During the next hour chloride was lost at a slower rate, until the tissue content reached 13·3 ± 3·6 (6) m-mole Cl/kg wet wt., after which time further observations showed no change. Throughout the whole period the ratio of fresh weight to wet weight remained constant.

One might have expected the extracellular inulin space to contain about 3 m-mole Cl/kg wet wt. during the immersion in 'low-chloride' solution, but the chloride actually measured in the muscle after 3 hr significantly exceeded this estimate. This result may indicate that all the measurements of chloride in the present work were a few m-mole/kg wet wt. too high, as was suggested by Cotlove (1962) for previous investigations of muscle chloride. Alternatively, it may be that some of the tissue chloride was not lost when the muscles were immersed in 'low-chloride' solutions.

The uptake of ethanesulphonate was also measured after 60 and 180 min in these experiments, radioactive ethanesulphonate-^{35}S being used (Goodford & Lüllmann, 1962). It reached a steady state within 60 min (Fig. 2), at which time the total muscle chloride and ethanesulphonate together were 94 ± 5 (10) m-mole/kg wet wt., equalling the chloride originally present at the start of the experiment (95 ± 6 (5) m-mole Cl/kg wet wt.).

Parallel experiments at 4° C gave similar results, showing a fast and slow phase of chloride loss (Fig. 2), with the tissue content falling to $10{\cdot}5 \pm 1{\cdot}3$ (4) m-mole Cl/kg wet wt. after 3 hr. The rapid phase was smaller than at 35° C, and this may be related to the smaller extracellular inulin space which has been observed in the cold (Born, 1962). A similar effect has been observed with the rapid phase of sodium loss into lithium solutions (Goodford, 1962).

Different chloride concentrations

Pieces of taenia were immersed at 35° C in modified Krebs's solutions containing 127, 92, 64 or 34 mM ethanesulphonate instead of the corresponding chloride, and the muscles were analysed after 2 hr equilibration. The relation between the tissue chloride and the chloride concentration in the bathing solution was linear, and this regression was extrapolated in order to assess the tissue chloride in a chloride-free solution (8 ± 1 (22) m-mole Cl/kg wet wt.; Fig. 3).

Cation content

A range of values has been observed for the sodium, potassium, and chloride contents of the taenia coli *in vitro* at 35° C (Table 3). The sodium content ($83{\cdot}6 \pm 1{\cdot}9$ (30) m-mole Na/kg wet wt.) and potassium content ($89{\cdot}6 \pm 1{\cdot}0$ (49) m-mole K/kg wet wt.) were therefore redetermined during the present experiments in order to find the values corresponding to the chloride observations, and it was surprising to find that the muscle contained more chloride than potassium. ^{42}K uptake was measured, and the rate constant, calculated in the same way as for chloride, was $0{\cdot}012 \pm 0{\cdot}0011$ (15) min^{-1}.

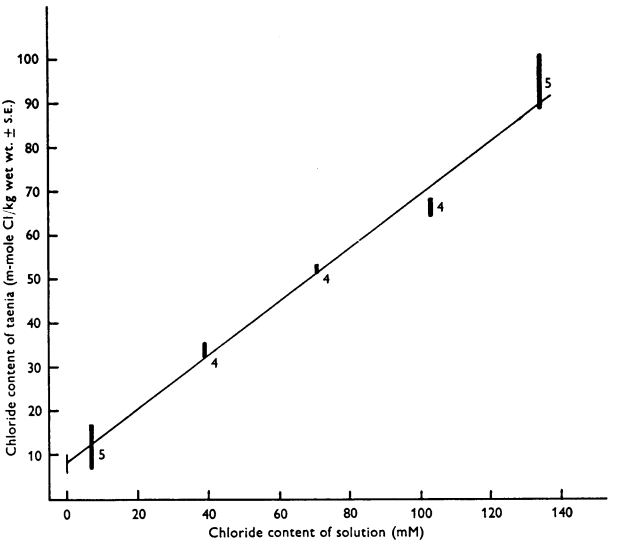

Fig. 3. Observations at 35° C after taeniae had been immersed for 3 hr in solutions of different chloride concentration. The total chloride + ethanesulphonate concentration in the solution was 134 mM. Ordinate, chloride content of the taenia in m-mole Cl/kg wet wt. ± S.E. and the number of observations printed next to each point. The straight line is the best fitting linear regression, and the 5 % limits of the intercept at zero chloride concentration are shown by the thin vertical bar.

TABLE 3. Sodium, potassium and chloride contents (m-mole/kg wet wt.) of the smooth muscle of the guinea-pig taenia coli

	Na	K	Cl	Reference
a Krebs's solution 35–38° C	103·1	51·7	—	Daniels, E. E. (1958)
	100	68	—	Goodford, P. J. & Hermansen, K. (1960)
	97	74	—	Goodford, P. J. & Hermansen, K. (1961)
	91·5	70	—	Freeman-Narrod, M. & Goodford, P. J. (1962)
	80·7	—	—	Freeman-Narrod, M. & Goodford, P. J. (1962)
	70	90	—	Goodford, P. J. (1962)
	83·6	89·6	103	Present work
b In vivo	70·7	89·1	65·0	Daniels, E. E. (1958)
	65	83	—	Goodford, P. J. & Hermansen, K. (1961)

DISCUSSION

Cotlove (1962) showed that inadequate ashing methods and chloride determinations could lead to unreliable estimates of muscle chloride, and for this reason the well established procedures of analytical chemistry were used to check the present observations. The high chloride content of the taenia coli *in vitro* was confirmed not only by this independent analysis, but also by the observation that tracer ^{36}Cl exchanged with at least 90 % of the muscle chloride.

The organic anions normally present in blood were replaced by chloride in the Krebs's solution used for the experiments *in vitro*, and this substitution could account entirely for the chloride content *in vitro*, which was about 40 m-mole Cl/kg wet wt. higher than the content observed immediately after killing a guinea-pig. The results in Fig. 3 show how the chloride content of the muscle depended upon the extracellular chloride concentration so that a blood concentration of about 100 mM-Cl would correspond to the chloride content of 56–76 m-mole Cl/kg fresh wt. actually observed immediately after killing. Chloride is in fact, appreciably bound by serum albumin (Scatchard, Scheinberg & Armstrong, 1950), and this would reduce the activity of chloride ion in blood and lower the amount of intracellular chloride *in vivo* proportionately; moreover, the larger extracellular spaces would contain blood corpuscles immediately after dissection and reduce the total muscle chloride still further. However, both these factors are probably of relatively minor importance in maintaining the lower chloride content observed immediately after killing a guinea-pig.

The inulin space determinations of Goodford & Hermansen (1961) were confirmed by the present observations made under similar conditions with the same sample of inulin, but chloride showed a first rapid phase of uptake in the taenia coli which corresponded to a significantly larger volume. The chloride in this muscle, exactly like the sodium (Goodford, 1962), may therefore be described as exchanging in at least three different ways: (1) a very rapidly exchanging component which is presumably extracellular; (2) another rapidly exchanging component difficult to distinguish from the first, but quantitatively in excess of the chloride in the extracellular inulin space; and (3) a component which exchanges more slowly ($t_{\frac{1}{2}} = 22$ min).

A residual fraction of muscle chloride was also observed after prolonged immersion in a low-chloride medium, although it was not detected in normal solution. Since the control experiments did not rule out a small systematic error, and since, furthermore, the linear extrapolation to zero-chloride concentration in Fig. 3 cannot be justified, the size of this last fraction (8 ± 1 (22) m-mole Cl/kg wet wt.) cannot be stated with certainty.

An ionic distribution was previously calculated for the taenia coli by assuming the two conventional extracellular and intracellular compartments (Goodford & Hermansen, 1961). The traditional inulin space was used in order to estimate the amount of extracellular material, and much of the rapidly exchanging sodium was therefore treated as intracellular. The sodium flux was many times larger than the flux of potassium on these assumptions and at that time 'the essential problem in the interpretation of the sodium movements was to decide how much of the muscle

sodium was truly intracellular and so taking part in transmembrane exchange'. Exactly the same problem now arises in the interpretation of the chloride movements, but it has been shown meanwhile that the first rapid uptake of other small ions, irrespective of their chemical nature or charge, always corresponds to a larger volume than the inulin space (Table 2.). It is difficult to believe that the observed transmembrane potential would be maintained if these ions were all able to cross the cell membrane almost as rapidly as they could travel in the extracellular inulin space, and it therefore seems more likely that the inulin space actually underestimates the proportion of ions which are located outside the cell membrane.

SUMMARY

1. The chloride content of the guinea-pig taenia coli was determined immediately after dissection of the muscle (56–76 m-mole Cl/kg fresh wt.) and *in vitro* (103 m-mole Cl/kg wet wt.). The sodium and potassium contents, the inulin space and the uptake of ^{42}K were measured in taeniae from the same animals.

2. 59% of the muscle chloride exchanged within 1 min of applying ^{36}Cl *in vitro*, and uptake then continued with a half-time of 22 min. The loss of chloride from the taenia into low chloride solution was rapid during the first 3 min and then proceeded slowly.

3. The chloride content *in vitro* was apparently the same at 4 and 35° C and always exceeded the potassium content.

4. The observations indicate that the muscle chloride, like the muscle sodium, is not simply distributed between the intracellular and extracellular space.

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