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# ELECTROLYTE TRANSPORT BY BULLFROG COLON *IN VITRO*

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SUMMARY

1. Studies were done on the transport function of the large intestine of *Rana catesbeiana* by measurement of the electrical potential difference (PD) and solute concentration gradients developed by the everted sac preparation during incubation in different media *in vitro*, and the effect of acetazolamide on these gradients.

2. A high PD, serosa positive to mucosa, is maintained by this preparation. The PD was dependent on the ambient $Na^+$ concentration and dropped when this was lowered to 15 mM or less. A decrease in PD associated with an increase in net serosal (s) to mucosal (m) $K^+$ movement was effected by elevating ambient $K^+$ concentration.

3. $Na^+$ was transported from the m to the s fluid against this PD, resulting in a high concentration gradient. This concentration difference was independent of the presence of $K^+$ and $HCO_3^-$ in the medium.

4. A high osmolal gradient was developed along with the $Na^+$ gradient since there was little net transmural movement of water.

5. $Cl^-$ and $HCO_3^-$ moved in roughly equal amounts along with $Na^+$. This anion movement occurred down an electrochemical gradient. Marked qualitative differences were demonstrated in the behaviour of these two anions, however. The net movement of $HCO_3^-$ was positively correlated with that of $Na^+$, whereas this was true for $Cl^-$ only in $HCO_3^-$-free medium or in the presence of acetazolamide. Further, $HCO_3^-$ movement varied with $K^+$ transport in contrast to $Cl^-$. Reversed net s to m movement of $HCO_3^-$ was observed in $Na^+$-free media and in the presence of acetazolamide. Reduction of m to s $HCO_3^-$ movement was compensated for by an equal increase in that of $Cl^-$, when caused by substitution of *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid for ambient $HCO^-_3$ but only partially when caused by acetazolamide inhibition of $HCO_3^-$ transport.

6. Net s to m movement of $K^+$ was demonstrated to take place even against an electrochemical gradient. This movement increased in the absence of ambient $HCO_3^-$. $K^+$ movement was increased by acetazolamide in absence of ambient $HCO_3^-$ but not in its presence. On prolonged incubation of the preparation, net movements of $K^+$ and $HCO_3^-$ decreased together relative to the transport of other ions.

Abbreviations: s, serosal; m, mucosal; PD, transmural (s *minus* m) electrical potential difference; $\Delta S$, s *minus* m concentration difference of a solute found at end of incubation; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

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7. It is concluded that the everted colon preparation shows evidence of (1) an electrogenic mechanism actively transporting $Na^+$ from m to s fluid independently of $K^+$, (2) another electrogenic mechanism actively transporting both $K^+$ and $H^+$ into the m fluid. The $HCO_3^-$ generated by the process which supplies $H^+$ to this pump moves passively in the s direction along its electrochemical gradient, as does $Cl^-$. (3) A minor mechanism transporting $HCO_3^-$ in the s to m direction may also exist.

## INTRODUCTION

*In vitro* studies of amphibian epithelial membranes have contributed a great deal to our present knowledge of electrolyte transport. They survive for prolonged periods under conditions in which environmental and regulatory factors can be altered. As compared to the skin and urinary bladder, the colon has been the object of little study although it is an important and fascinating transport organ, and in addition has a striking functional analogy to the distal tubule of the kidney.

Since USSING AND ANDERSEN[1] in 1955 first demonstrated active $Na^+$ transport across the colon wall of the toad, *Bufo bufo*, CHALFIN *et al.*[2] in 1958, and COOPERSTEIN AND HOGBEN[3] in 1959 reported studies on the colon of *Rana catesbeiana in vitro*, concluding that there was no proof of active transport of ions other than $Na^+$. COFRÉ AND CRABBÉ[4,5] in 1965 and 1967 published data from studies with the colon of the toad *Bufo marinus*, which demonstrated that aldosterone and antidiuretic hormone both stimulated $Na^+$ transport probably through increasing the permeability of the mucosal diffusion barrier to this ion.

We report here a more general exploration of the transport activity of the colon of *R. catesbeiana* as studied *in vitro* using the everted sac incubation technique. Our findings widen the functional analogy of this preparation with distal kidney tubule: it is poorly permeable to water and builds up a marked osmolal gradient in addition to maintaining an electric potential gradient due to active $Na^+$ transport. It acidifies the luminal fluid, and secretes $K^+$ into it, even against an electrochemical gradient.

## MATERIALS AND METHODS

### *Experimental*

Bullfrogs, *R. catesbeiana*, 250–600 g body wt., were obtained from Lemberger Co., Oshkosh, Wisc., U.S.A., during the period October–June, and kept unfed at 4° for 2–7 days until taken to room temperature the night before use. After pithing, the whole large intestine was dissected free, emptied, washed with 0.64 % NaCl solution and rinsed in the buffer to be used. It was then everted and each end tied over short pieces of polyethylene tubing to make a loop[6] (for sequential incubations), or only at the distal end and closed at the other to make a sac. 1.00 ml of buffer (s solution), was then instilled from a syringe into the loop or sac and the colon was placed in a beaker containing 10.0 ml (m solution) of the same buffer, where not otherwise stated. The preparation was then incubated at 22° with constant shaking in a Dubnoff metabolic shaking incubator in appropriate atmosphere ($O_2$–$CO_2$ (95:5, v/v) for

$HCO_3^-$ buffers, and 100 % $O_2$ for the $HCO_3^-$-free* buffer) for 4 h or, in case of sequential incubations, for 3 h. At the end of the incubation the s solution was harvested by draining with air washes into a tube. In sequential incubations, the loop was then rinsed with the buffer, emptied by air washes, the new s and m solutions added and the loop returned to the incubator.

Two general types of experiment were used: (1) single incubation experiment in which preparations from different animals were compared and (2) sequential incubation experiment in which each preparation served as its own control. When the effect of changing the electrolyte composition of medium from A to B was studied in the sequential type experiment half of the loops were incubated with change of the medium in sequence A–B–A; and sequence B–A–B for the other half. The mean of the transport indices of the 1st and 3rd period was compared with the index of the 2nd period. For the study of drug effect the single incubation experiment was used.

*Buffer solutions*

The basic buffer used, a modified high-$K^+$ reduced osmolal Krebs–Henseleit buffer, had the following composition (in mM): $Na^+$, 104; $K^+$, 16; $Ca^{2+}$, 1.3; $Mg^{2+}$, 1.0; $Cl^-$, 103; $HCO_3^-$, 20; $SO_4^{2-}$, 1.0; phosphate, 1.0; glucose, 17.5; total osmolality, 245 mosM/kg. It was aerated with $O_2$–$CO_2$ (95:5, v/v). This buffer was further modified when indicated by substitution of an osmotically equivalent amount of either mannitol, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), $K^+$, choline$^+$, $Li^+$ or $NO_3^-$ for one or several of the following: $Na^+$, $K^+$, $HCO_3^-$ and $Cl^-$; and aeration with 100 % $O_2$ when $HCO_3^-$ free buffer was used. The pH of all buffers was between 7.10 and 7.20. The different buffers are identified by giving the concentrations of $Na^+/K^+/Cl^-/HCO_3^-$ followed by substituent, if other than mannitol (*e.g.* $Na^+/K^+/Cl^-/HCO_3^-$=96/16/103/0/TES). Acetazolamide (Diamox) was obtained from Lederle Laboratories.

*Measurements*

For determination of the PD the s and m solutions of the preparation were connected to matched calomel electrodes through 0.64 % NaCl solution in 4 % agar-gel bridges of fine polyethylene tubing and voltage measured with a Keithley 602 solid state electrometer. The PD varied, as a rule, less than 5 % when the serosal agar bridge was moved to different locations inside the loop. The highest consistent reading was recorded. The volume of the s fluid recovered was measured with a pipette calibrated with 0.01-ml divisions to the tip.

The final s and m solutions and the original buffer solutions were analyzed for osmolality using an Advanced freezing point osmometer, for $Na^+$ and $K^+$ with an internal standard flame photometer, for $Cl^-$ with a Cotlove chloridometer, for total $CO_2$ content with a Natelson manometric microgasometer, and for lactate by measuring the NADH generated from $NAD^+$ in the presence of lactate dehydrogenase.

*Presentation of results*

As essentially no transmural water movement took place, the concentration difference of a solute, s *minus* m, at the end of the incubation (designated as $\Delta S$)

* The $CO_2/HCO_3^-$ system is referred to as $HCO_3^-$ and the term $HCO_3^-$ transport is used understanding that the moving moiety could be $CO_2$.

is used as index of transport of the solute. For the purpose of assessing the electrochemical gradient, the ratio of the s and m concentrations of the solute is used.

To determine the rapidity with which the concentration gradients were developed, the fluids were sampled at 3, 4, and 5 h in three experiments. The mean of the differences of the individual preparations were for PD 79, 83 and 85 mV, for $\Delta$ $Na^+$ 71, 78 and 84 mM, for $\Delta$ $K^+$ −13.0, −13.8 and −14.2 mM and for $\Delta$ $Cl^-$ 15.0, 15.3 and 14.7 mM at 3, 4 and 5 h, respectively.

## RESULTS

### *Transmural electrical potential difference*

There was a PD between the two fluids, s positive to m, with highest recorded readings above 100 mV (Fig. 1). This was dependent on the presence of some ambient $Na^+$ and fell only when this was reduced to 15 mM or less (Fig. 2). This was true irrespective of whether $Na^+$ was replaced by mannitol, $K^+$, or choline. At ambient $Na^+ = 0$, a reversed PD (−4 and −2 mV) was observed in two of four experiments with $K^+ = 55$ mM, but in none of six with $K^+ = 42$ or 20 mM. In a sequential incubation experiment, increase of the ambient $K^+$ from 5 to 24 mM was shown to depress the PD at ambient $Na^+$ levels of 97 and 16 mM (Fig. 3).

The correlation PD/$\Delta Na^+$ was fair ($r = 0.46$ for medium $Na^+/K^+/Cl^-/HCO_3^- = 104/16/103/20$), see Fig. 4.

During sequential 3 h incubations with the same initial medium the PD fell

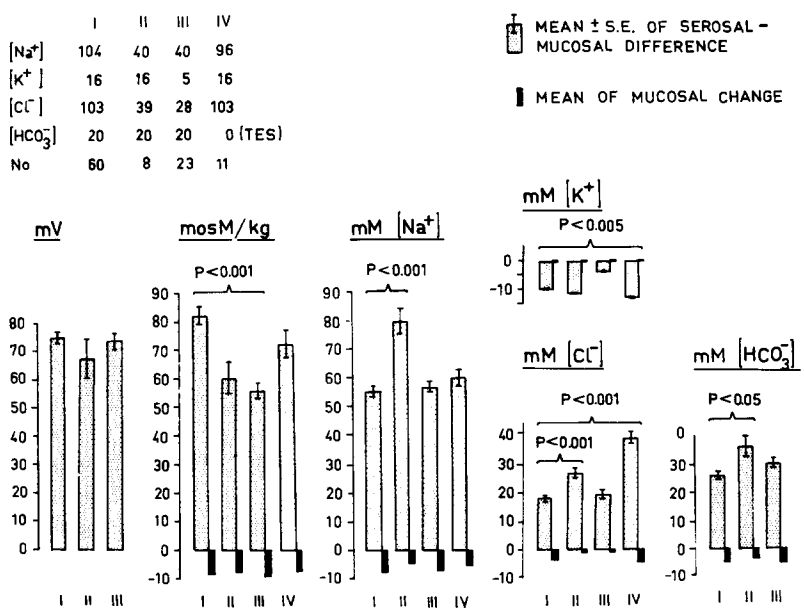


Fig. 1. s–m differences developed by everted frog colon sacs, incubated in four different media. The incubations were started with 1.00 ml of the medium on the s, and 10.00 ml on the m side. The varied part of the composition of the Krebs–Henseleit-type media (I–IV) used is given in the figure. The measurements were done after 4 h incubation at 22°. PD was not measured in experiments with Medium IV.

significantly only in the third 3-h period (Table I). Acetazolamide had no effect on PD (Table II).

*$Na^+$ transport*

$Na^+$ moved against an electrochemical gradient in all the solutions studied (Fig. 1). Change in ambient $K^+$ from 5 to 24 mM brought about a slight decrease in $\Delta Na^+$ at ambient $Na^+$ = 16 mM but no change at 97 mM, in sequential incubation experiments (Fig. 3). Reduction of $K^+$ from 5 to 0 mM at $Na^+$ = 16 mM had no effect on $\Delta Na^+$.

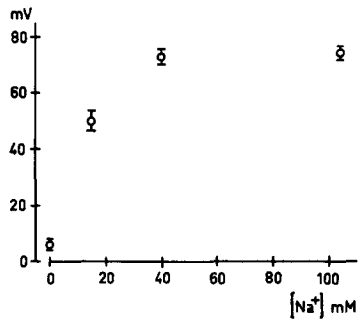


Fig. 2. Transmural electrical potential difference of everted frog colon sacs incubated at different $Na^+$ concentrations of bathing medium. The measurements were done after 4 h incubation. s side was positive to the m side. Mean ± S.E. is given for groups of a minimum of 10 experiments.

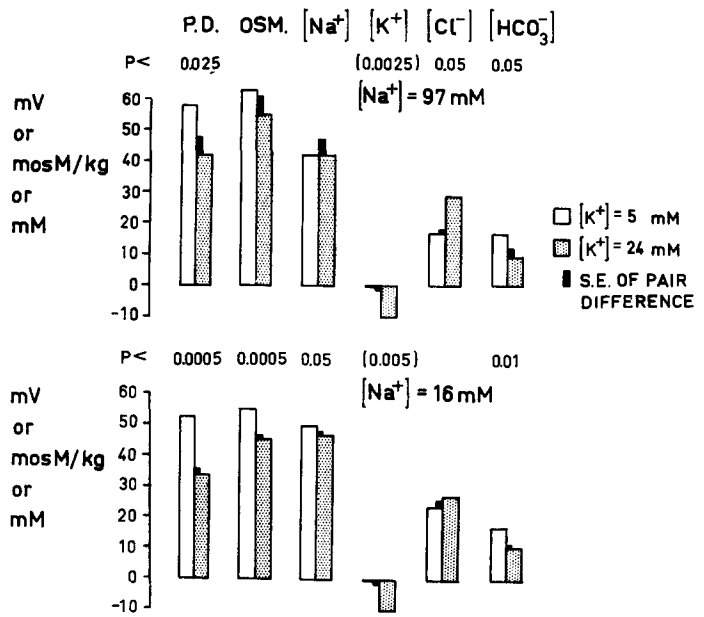


Fig. 3. Effect at two different ambient $Na^+$ concentrations of change in $K^+$ concentration on s–m differences developed by everted frog colon loops. Eight loops were incubated for 3 consecutive 3-h periods alternating the two media in sequences A–B–A and B–A–B. 1.00 ml of the medium was placed on s side, 10.00 ml on m side. The two buffers of each pair were identical except for the indicated reciprocal difference in the concentration of $K^+$ and choline. Means of the differences developed in the 1st and 3rd period were compared with the differences found after the 2nd period. The general means for each medium are shown by the columns.

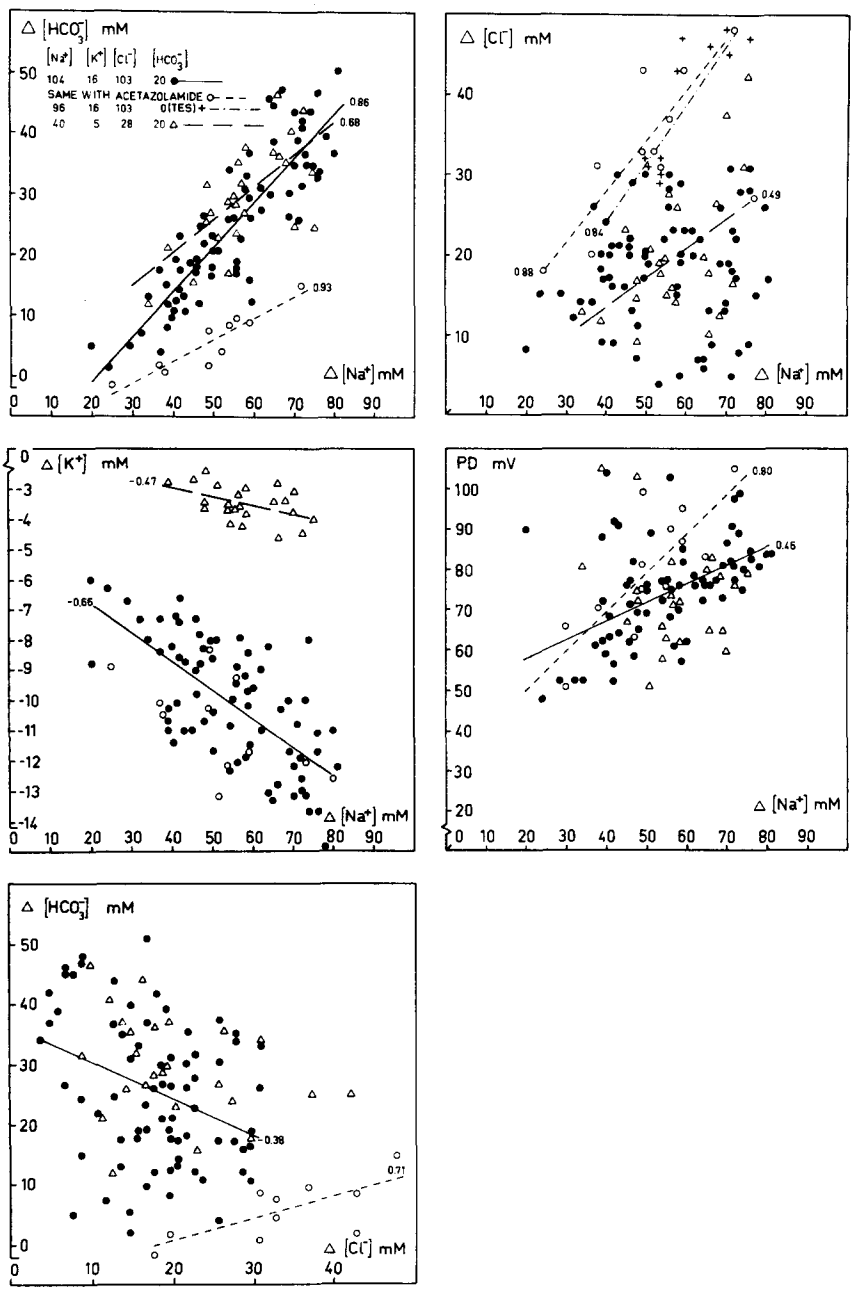


Fig. 4. Some correlations of the s–m differences developed by everted frog colon sacs incubated in different media. 1.00 ml of the medium was placed on the s side, 10.00 ml on the m side and the differences were measured after 4 h incubation. The varied part of the Krebs–Henseleit-type media used is given in the figure. For significant correlations, the regression lines have been drawn and the corresponding correlation coefficients (Pearson) are given.

TABLE I

COMPARISON OF CONSECUTIVE INCUBATION PERIODS—EVERTED FROG COLON SACS

10 sacs were incubated for 3 consecutive 3-h periods, and 10 others for 2 periods, in $Na^+/K^+/Cl^-/HCO_3^-$ = 104/16/103/20 mM medium. Values are mean ± S.E. for volume recovered (1.00 ml being introduced), and s–m difference developed during incubation. *P* values are for difference from paired analysis.

| *Period* | *Serosal volume (ml)* | *PD (mV)* | *Osmolality (mosM/kg)* | $[Na^+]$ *(mM)* | $[K^+]$ *(mM)* | $[Cl^-]$ *(mM)* | $[HCO_3^-]$ *(mM)* |
|---|---|---|---|---|---|---|---|
| A | 1.05 ± 0.04 | 74 ± 3 | 79 ± 6 | 54 ± 4 | −10.4 ± 0.5 | 17.8 ± 1.7 | 24.4 ± 2.9 |
| B | 1.11 ± 0.05 | 73 ± 3 | 83 ± 6 | 54 ± 3 | − 6.8 ± 0.4 | 21.7 ± 1.7 | 24.3 ± 2.9 |
| $P_{A-B}$ | <0.05 | | <0.025 | | <0.001 | <0.05 | |
| C | 1.08 ± 0.07 | 62 ± 6 | 71 ± 9 | 46 ± 5 | −4.1 ± 0.7 | 20.2 ± 1.7 | 18.7 ± 4.2 |
| $P_{A-C}$ | | <0.05 | | | <0.001 | | |
| $P_{B-C}$ | | | | | <0.01 | | <0.01 |
| $P_{A-B,\ A-C}$* | <0.05 | | | | <0.001 | <0.001 | <0.1 |

* Pooled comparison between 1st and subsequent periods.

On sequential incubation with same initial medium, $\Delta$ $Na^+$ showed a slight and statistically insignificant decrease in the third 3-h period only, paralleled by a decrease in PD (Table I). Acetazolamide decreased $\Delta$ $Na^+$ significantly in the presence of $HCO_3^-$ but not in its absence (Table II).

*Water and total solute transport*

The colon wall was obviously poorly permeable to water as transmural osmotic gradients of up to 130 mosM/kg were developed (Fig. 1). The mean volume of s fluid recovered from loops after (first) 4 h incubation period with the basic buffer was 1.07 ± 0.06 (S. E.) ml, 1.00 ml having been introduced. There was a slight but statistically significant increase in the water movement in sequential incubation (Table I).

There was an apparent discrepancy between the $\Delta$ of ions and total solute in the mannitol-containing media (Fig. 1, Buffers II and III). The highest osmolal gradients were seen in loops with the basic medium (I) whereas loops with the mannitol-containing media had the highest $Na^+$ gradients. It is obvious by calculation, that up to more than half of the mannitol originally present in the s fluid had entered the tissue. The apparent hindrance to further $Na^+$ movement was the total osmolal gradient in the mannitol-free media, but the $Na^+$ gradient in the mannitol-containing media.

*Anion transport and changes in acidity*

In the $HCO_3^-$ containing media, both $Cl^-$ and $HCO_3^-$ accumulated in the s fluid, the sum of their net movements being equal to the measured net cation movement (Fig. 1). There was marked individual variation among the loops with respect to the proportions of $HCO_3^-$ and $Cl^-$ transported .Qualitative differences were evident in the behavior of these two anions. $\Delta$ $HCO_3^-$ showed a high correlation with $\Delta$ $Na^+$ ($r$ = 0.86 for the basic medium) whereas this was not true for $\Delta$ $Cl^-$ ($r$ = 0.16) (Fig. 4). Increase of ambient $K^+$ from 5 to 24 mM brought about a marked decrease

TABLE II

EFFECT OF ACETAZOLAMIDE ON TRANSFER OF IONS AND WATER BY EVERTED FROG COLON SACS INCUBATED IN $HCO_3^-$ AND $HCO_3^-$-FREE MEDIUM

Values are mean ± S.E., for volume recovered (1.00 ml being introduced) and for s–m difference developed during 4 h incubation. Acetazolamide concentration was 0.25–1.0 mM. PD was not measured in the experiments with $HCO_3^-$-free media.

| *Medium* ($Na^+/K^+/Cl^-/HCO_3^-$ (*mM*)) | *Number of experiments* | *Serosal volume* (*ml*) | *PD* (*mV*) | *Osmolality* (*mosM/kg*) | $[Na^+]$ (*mM*) | $[K^+]$ (*mM*) | $[Cl^-]$ (*mM*) | $[HCO_3^-]$ (*mM*) | $[H^+]$ (*nM*) |
|---|---|---|---|---|---|---|---|---|---|
| Without acetazolamide (104/16/103/20) | 10 | 1.02 ± 0.04 | 77 ± 4 | 82 ± 5 | 60 ± 4 | −11.4 ± 0.5 | 16.7 ± 2.6 | 28.7 ± 3.8 | |
| *P* | | | | | <0.05 | | <0.001 | <0.001 | |
| With acetazolamide | 13 | 0.96 ± 0.03 | 80 ± 4 | 74 ± 6 | 49 ± 4 | −10.6 ± 0.5 | 33.7 ± 3.1 | 5.7 ± 1.6 | |
| Without acetazolamide (96/16/103/—/TES) | 11 | 0.83 ± 0.06 | | 72 ± 5 | 60 ± 3 | −12.5 ± 0.5 | 39.1 ± 2.4 | | −43 ± 9 |
| *P* | | | | | | <0.0125 | | | <0.025* |
| With acetazolamide | 4 | 0.93 ± 0.03 | | 71 ± 10 | 61 ± 6 | −14.5 ± 0.4 | 36.8 ± 7.7 | | −19 ± 6 |

* Test for paired difference.

in the net m to s movement of $HCO_3^-$ relative to that of $Cl^-$ (Fig. 3). Decrease of $K^+$ from 5 mM to zero did not cause a further effect. In $Na^+$ free media ($Na^+/K^+/Cl^-/HCO_3^-$ = 0/55/38/20 and 0/20/28/20 choline 25), the net $HCO_3^-$ movement was reversed, the mean $\Delta$ $HCO_3^-$ for all experiments ($n$ = 10) being —4.9 ± 0.8 (S.E.) mM in contrast to mean $\Delta$ $Cl^-$ of 9.3 ± 2.9 mM. As the PD was positive in all but two of these loops (*vide supra*) the net $HCO_3^-$ movement was against an electrochemical gradient. It may be significant that the regression $\Delta HCO_3^-/\Delta Na^+$ in the basic medium gives $\Delta HCO_3^-$ = —15.8 mM at $\Delta Na^+$ = 0 mM (Fig. 4). In no system was there any indication of transport of $Cl^-$ against an electrochemical gradient.

A significant decrease of $\Delta HCO_3^-$ along with similar decrease of $\Delta K^+$ relative to other gradients and especially to $\Delta Cl^-$ occurred on sequential incubation of the loops with same initial medium (Table I). There was an actual increase in $\Delta Cl^-$.

A fall in the ratio of $\Delta Cl^-/\Delta HCO_3^-$ with decreasing ambient $Cl^-$ as seen with similar preparations of rat colon[7] could not be demonstrated with frog colon.

Substitution of TES for $HCO_3^-$ in the basic medium did not reduce $\Delta Na^+$; although TES moved poorly (mean $\Delta$TES by calculation = 8.5 mM) there was a compensatory increase of $\Delta Cl^-$ and a significant increase in $\Delta K^+$ (Fig. 1). In this $HCO_3^-$ free system, a high correlation appeared between $\Delta Cl^-$ and $\Delta Na^+$ (Fig. 4).

Almost the same regression of $\Delta Cl^-/\Delta Na^+$ was found in the basic medium in the presence of acetazolamide as in the $HCO_3^-$ free TES medium (Fig. 4). Here also a marked increase in $\Delta Cl^-$ was found (Table II) which almost compensated for the decrease of $HCO_3^-$ transport caused by acetazolamide. Inhibition of net m to s $HCO_3^-$ movement was the only unequivocal primary effect of the carbonic anhydrase inhibitor in this system, as the decrease in $\Delta Na^+$ could be secondary to inhibition of anion transport (Table II).

To explore further the acetazolamide effect we incubated four loops sequentially for 90-min periods increasing acetazolamide concentration in the sequence 0, 0.1, 1.0 and 10 mM. There was no suppression of $\Delta Cl^-$ below the control level even at 10 mM acetazolamide, but $\Delta HCO_3^-$ became negative in every preparation (range —1.6 to —6.4 mM, with actual rise in m $HCO_3^-$ at the highest drug concentration.

Presumably, there was acidification on the side opposite to that of $HCO_3^-$ accumulation. No actual pH determination was done, except in the experiments with $HCO_3^-$ free media in which acetazolamide was found to decrease the $\Delta H^+$ (Table II).

In contrast to rat colon preparation[7], the frog colon was found to generate only negligible amounts of lactic acid. The highest measured s lactate concentration was 1.3 mM; it was nondetectable in the m fluid.

*$K^+$ transport*

The net transport of $K^+$ was consistently from s to m fluid. This was true even at ambient $K^+$ = 40 mM, which was the highest concentration tested. Substituting TES for $HCO_3^-$ in the basic medium significantly increased $\Delta K^+$ which suggests inhibition of $K^+$ movement by the $CO_2/HCO_3^-$ system (Fig. 1). The largest net $K^+$ movement recorded in the regular type incubations were in the TES medium in the presence of acetazolamide (Table II). The lowest s/m ratio of $K^+$ recorded was 0.12, the mean for this system being 0.24 ± 0.015 (S.E.). This ratio was also

significantly lower ($P < 0.005$) in the $K^+ = 5$ mM than in the 20 mM medium (0.34 ± 0.02 and 0.41 ± 0.01, respectively.). These ratios could be the result of transport down the electrochemical gradient, since according to the Nernst equation a PD of only 43 mM is necessary to maintain a ratio of 0.20. The only observations of $K^+$ apparently moving against the electrochemical gradient in the standard experiments were in the $Na^+ = 0$ media where ratios below 0.70 were measured at PD = 3 mV or less.

$\Delta K^+$ showed a fair negative correlation with $\Delta Na^+$ ($r = -0.66$), PD ($r = -0.53$) and $\Delta HCO_3^-$ ($r = -0.68$) but less with $\Delta Cl^-$ ($r = -0.33$); all these $r$ values are for the basic medium. The regression $\Delta K^+/\Delta Na^+$ gives $\Delta K^+ = -4.9$ at $\Delta Na^+ = 0$, which suggests that part of the s to m movement of $K^+$ is by a system independent of the reverse transport of $Na^+$ (Fig. 4).

The findings on sequential incubation with the same initial medium suggest progressive impairment of the movement of $K^+$ and $HCO_3^-$ with prolonged survival of the tissue (Table I).

Acetazolamide had no effect on s to m $K^+$ movement in the basic medium but increased it significantly in the $HCO_3^-$ free medium (Table II).

*Demonstration of $K^+$ movement against electrochemical gradient*

A different experimental design was selected to obtain more definite proof of $K^+$ transport against an electrochemical gradient and for possible evidence for similar $Cl^-$ movement. The incubations were started with a transmural gradient for $Na^+$ as high as had been found to develop during the regular incubations, and for $K^+$ and $Cl^-$ of normal direction but higher than that which the loop could be expected to maintain by the predicted PD calculated from the Nernst equation. To reduce anion movement in this system, $SO_4^{2-}$ was used as mucosal anion, and TES substituted for $HCO_3^-$ in both s and m solutions. Three different mucosal buffers were used to give different gradients of $K^+$ and $Cl^-$ and these are labelled below by the PD to which they corresponded. The mM composition was: $Ca^{2+}$ 1.3, $Mg^{2+}$ 1.0, phosphate 1.0, TES 22 and glucose 17.5, *plus* the ions which were varied as given in Table III.

The measured osmolality was 330 mosM/kg for the s and 244 mosM/kg for the m buffers. Obviously, the activity coefficients of univalent ions were markedly different on the two sides, especially those of cations, because of the high m sulphate concentration. Activity coefficients 0.86 and 0.70 for the cations, and 0.80 and 0.90 for the anions, for s and m fluid, respectively, were calculated from the composition

TABLE III

THE VARIABLE PART OF COMPOSITION OF MEDIA DESIGNED FOR DEMONSTRATION OF $K^+$ TRANSPORT AGAINST ELECTROCHEMICAL GRADIENT

| *Medium* | $[Na^+]$ (*mM*) | $[K^+]$ (*mM*) | $[Cl^-]$ (*mM*) | $[SO_4^{2-}]$ (*mM*) | *Mannitol* (*mM*) |
|---|---|---|---|---|---|
| Serosal | 150 | 4.2 | 150 | 1.0 | — |
| Mucosal "57 mV" | 24 | 43 | 21 | 20 | 111 |
| Mucosal "67 mV" | 25 | 66 | 14 | 36 | 85 |
| Mucosal "77 mV" | 26 | 92 | 10 | 51 | 47 |

and the measured osmolalities, assuming a coefficient of 1.00 for non-electrolytes. As this latter is too high, the estimated coefficient for m cations is too low and the estimates for m/s activity ratio of $K^+$ are minimum estimates. Loops were incubated with the different m media in sequence, and the final activity ratios were compared to the actual PD .

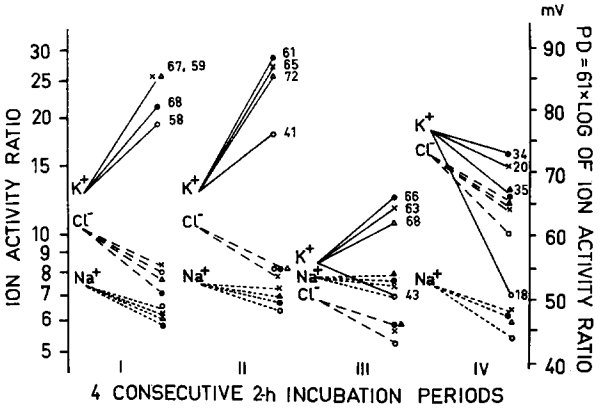


Fig. 5. Demonstration of transport of $K^+$ against electrochemical gradient by everted frog colon loops. Four sequential experiments are illustrated. The incubations were started with m medium different from the s to give the activity ratio indicated on the scale on the left in the picture (m/s for $K^+$ and s/m for $Na^+$ and $Cl^-$), as explained in the text. The ratios found at the end of the incubation periods are shown with a symbol (×, △, ●, ○) for each individual loop, with figure giving the PD actually measured at the symbol giving the $K^+$ ratio. This PD should be compared with the theoretical PD necessary to maintain such ratio (of $K^+$ and $Cl^-$) according to the Nernst equation, as indicated on the scale on the right.

The results of four such experiments are shown in Fig. 5. The high initial m to s gradient of $K^+$ was increased further in all loops during the first two incubation periods. The actual PD measured at the end of the periods was from 16 to 46 (mean 28) % lower than would have been needed to maintain the final gradient. PD was also measured at the midpoint of the periods: none was higher than at the end but all were from 0 to 13 mV lower. In the 3rd period the ratios changed (increased in three, decreased in one) to approximately the levels predicted by the actual PD. In the 4th period a fall was seen in the m/s ratio of all loops which shows that the loops were permeable to $K^+$ in the m to s direction. No decrease in ionic activity ratio was seen below that predicted for $K^+$ from the actual PD but such a decrease did occur with respect to $Cl^-$.

*Effect of unphysiological ions*

In the sequential incubation type experiment 20 mM of either LiCl or choline chloride was substituted for an osmotically equivalent amount of mannitol in $Na^+/K^+/Cl^-/HCO_3^-$ = 40/5/28/20 buffer. $Li^+$ caused a significant ($P < 0.05$) reduction in PD from a mean of 67 mV to a mean of 56 mV, and in $\Delta K^+$ from a mean of —3.4 mM to a mean of —2.5 mM ($P < 0.05$). No such effects were evident when choline was substituted. Neither altered the $\Delta$ of osmolality, $HCO_3^-$, or $Na^+$. Both accumulated slightly in the mucosal fluid, as assessed indirectly from measurement of total osmolality and concentration of other ions. Substitution of $NO_3^-$ for 20 mM

of $Cl^-$ similarly in medium $Na^+/K^+/Cl^-/HCO_3^-$ = 60/5/48/20 did not bring about any apparent alteration in the transport indices.

## DISCUSSION

Study of the transport function of frog colon by the simple everted sac preparation makes possible the determination of concentration gradients between the two surfaces of the intestinal wall. It is evident, though, that we were not observing steady-state gradients in our standard experiments starting with identical medium on both sides of the membrane. Thus the transport indices determined were only approximate measures of the capacity for maintaining concentration differences between the two phases. The approach used to demonstrate movement of $K^+$ against electrochemical gradient measured the capacity more closely. The standard type experiment was clearly useful, nevertheless, in providing a general picture of the transport possibilities of the colon *in vitro*.

Investigations in a number of animal species have established the colon as a membrane furnished with an effective mucosal to serosal $Na^+$-pump mechanism associated with high transmembrane PD (for review see SCHULTZ AND CURRAN[8]). The highest PDs reported in mammalian colon, 50–60 mV, were observed by EDMONDS AND GODFREY[9], and by DALMARK[10] in human colon. By aldosterone treatment or $Na^+$ depletion colonic PDs up to 100 mV have been obtained both in man[9] and rat[11]. Our present measurements on frog colon *in vitro* gave consistently higher PDs than the mean of 45 mV found in the same preparation by CHALFIN *et al.*[2] and markedly higher than the mean of 4.5 mV found in sacs of the colon of *Bufo marinus* by COFRÉ AND CRABBÉ[5]. The PD maintained by our preparation was of the same magnitude as found for turtle bladder *in vitro*[12].

COFRÉ AND CRABBÉ[5] reported that the short-circuit current across the toad colon depended on ambient $Na^+$ and fell when the mucosal $Na^+$ was decreased to about 25 mM. This is in agreement with our finding in the frog that the PD was unchanged when ambient $Na^+$ was decreased to 40 mM, but fell on further decrease to 15 mM (Fig. 2). Marked depression of $Na^+$ movement in absence of ambient $HCO_3^-$ was demonstrated by COOPERSTEIN AND HOGBEN[3] in the colon of *R. catesbeiana*, and LEW[13] reported that the removal of $HCO_3^-$ in the serosal fluid abolished the short-circuit current by the colon of *B. arenarium*. The same effect has been reported from experiments with turtle bladder[14] and frog skin[15]. In marked contrast to these observations, replacing the ambient $HCO_3^-$ by TES in our experiments caused no apparent change in $Na^+$ transport. The reason for this discrepancy is unknown but it could be related to two differences between the systems studied. Either TES could be a more physiological substitute for $HCO_3^-$ or the presence of $CO_2/HCO_3^-$ is not critical at the high ambient $K^+$ in our experiments.

GOLDSCHMID AND DAYTON[16] first observed absorption of hypertonic fluid from isotonic colon contents in the dog. POWELL AND MALAWER[17] perfusing segments of rat jejunum, ileum and colon *in situ* with isotonic salt solutions, found that the perfusate osmolality fell progressively, and thus probably the permeability to water decreased, in the aboral direction of the intestine. Everted sacs of rat colon build up transmural osmolal gradients as high as 50 mosM/kg (refs. 7 and 18). The same value has been reported for sacs of turtle bladder[12]. Our preparation developed

osmolal gradients up to 130 mosM/kg, which is still less than the frog skin is able to maintain[19]. CHALFIN *et al.*[2] observed considerable movement of water out of everted sacs of *R. catesbeiana* colon. As this was more than from noneverted sacs it was probably due to damage on handling.

Active colonic transport of $Cl^-$ has not been conclusively demonstrated. It has been implied in human colon by DEVROEDE AND PHILLIPS[20] who found that more $Cl^-$ than $Na^+$ was absorbed from NaCl perfusion solution. We found the same in *in vitro* experiments with rat colon[7]. In the present experiments $Cl^-$ moved down the electrochemical gradient in all situations.

Evidence has been presented for active secretion of $HCO_3^-$ into the colonic lumen in dog[21,22], with equilibrium concentration as high as 75 mM (ref. 22) and coupling of this secretion with the absorption of $Cl^-$ has been suggested[20]. In the two reports which give data on net bicarbonate movement in frog colon[2,3], studied *in vitro*, this was always in the m to s direction. The same was true in our present experiments, except in $Na^+$-free ambient medium and in the presence of high concentrations of acetazolamide, under which conditions the net movement was into the m fluid. This was transport against the electrochemical gradient and suggests that the frog colon also has a minor active mechanism pumping $HCO_3^-$ from s to m fluid which does not require carbonic anhydrase activity.

An unequivocal effect of acetazolamide found in these experiments was inhibition of both the s accumulation of $HCO_3^-$ and the corresponding acidification of the m fluid. That this was evident in the $CO_2/HCO_3^-$ rich ambient medium suggests that carbonic anhydrase was necessary for the generation of $H^+$ even in this medium, contrary to views presented by BRODSKY AND SCHILB[23]. Actually, GONZALEZ AND SCHILB[24] have observed the acetazolamide effect on turtle bladder in similar medium. It may mean that the $P_{CO_2}$ is much lower inside the tissue than in the medium. Maintenance of transmembrane $CO_2$ gradients has been observed[23].

Acetazolamide has been found to inhibit $Cl^-$ transport in dog ileum[25], frog gastric mucosa[26] and cornea[27], foot pad of the cat[28] and turtle bladder[14]. We have shown inhibition by acetazolamide of both $Cl^-$ and $NO_3^-$, or of $Na^+$ movement in everted preparations of rat colon[7]. In the present acetazolamide experiments there was a depression of $Na^+$ transport along with suppression of $HCO_3^-$ movement, despite compensatory increase in $Cl^-$ translocation, which was evident still at 10 mM drug concentration. In experiments in which $HCO_3^-$ was replaced by TES the increase in $Cl^-$ movement was enough to fully compensate for the lack of $HCO_3^-$ movement and $Na^+$ transport was not reduced. Inhibition of either $Na^+$ or $Cl^-$ movement by acetazolamide can only be slight.

We have demonstrated s to m movement of $K^+$ against an electrochemical gradient. GIEBISCH[29] does not believe that such movement occurs in the distal tubule of mammalian kidney but it has been demonstrated in the colon of the rat[7,11,30]. Evidence has also been presented for this phenomenon in the dog colon[31], but the authors were unsure about their results and the mean colonic PD observed by them was much lower than reported in the dog by others[21].

Our observations (Table IV) suggest an interrelation between the s to m movement of $K^+$ and the m to s movement of bicarbonate. It is of interest that amiloride has been found[32] to depress the movement of these two ions distinctly more than that of other ions. The data are compatible with competition between

TABLE IV

FACTORS FOUND TO AFFECT BOTH THE NET s TO m $K^+$ MOVEMENT AND THE NET m TO s $HCO_3^-$ MOVEMENT

| *Factor* | *Effect on $\Delta K^+$* | *Effect on $\Delta HCO_3^-$* |
|---|---|---|
| Presence of ambient $CO_2/HCO_3^-$ | Decrease | Increase |
| Increase in ambient $[K^+]$ | Increase | Decrease |
| Acetazolamide in $CO_2/HCO_3^-$-free TES medium | Increase | Decrease |
| Prolonged survival of tissue | Decrease | Decrease |
| Amiloride in mucosal medium[32] | Decrease | Decrease |

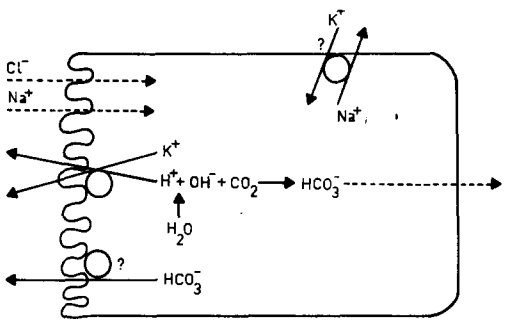


Fig. 6. Model suggested for the ion transport by the m cells of frog colon. The continuous lines indicate suggested active transport, the discontinuous ones suggested passive movement.

$K^+$ and $H^+$ transport and with inhibition of both functions by the same agents. The finding that acetazolamide did not increase $K^+$ transport in the basic medium does not fit this concept, however, and remains unexplained.

Since m accumulation of $H^+$ is associated with s accumulation of $HCO_3^-$ we suggest the following mechanism which could explain the interrelations listed above. $K^+$ and $H^+$ are transported from within the cell into the m medium by a common system for which they compete. That this pump generates a PD, mucosa positive to serosa, is suggested by the observation that increasing ambient $K^+$ reduced the reverse PD maintained by the $Na^+$ movement. $OH^-$ generated with $H^+$ react with $CO_2$ within the cell, catalyzed by carbonic anhydrase, to form $HCO_3^-$. This ion either moves passively along the electrochemical gradient to the s medium, or part of it may be pumped into the m medium.

Fig. 6 depicts a tentative model to account for some of the transport phenomena observed. The $K^+/H^+$ pump has been tentatively located on the luminal border, as STEINMETZ[33,34] has produced evidence for this location of the $H^+$ pump in turtle bladder and also because of the action of amiloride on m side only[32]. STEINMETZ *et al.*[35] did not observe any effect on m acidification of varying the $K^+$ of m medium. This might be expected as the $K^+$ to be pumped originates mainly from the s fluid. Secretion of $K^+$ into m fluid has been observed in the turtle bladder, but not proven to occur against an electrochemical gradient[36].

Whether the s or intercellular pumping of $K^+$ into the cell is coupled with extrusion of $Na^+$ is unknown. Our observation that $Na^+$ movement was unaffected in absence of ambient $K^+$ does not rule out such coupling as there is ample $K^+$ in the tissue. In the thin membrane of turtle bladder, $Na^+$ transport ceases in absence

of ambient $K^+$ (ref. 37). HERZER *et al.*[38] have demonstrated histochemically the presence of ATPase at the intercellular membrane in rat colon. It is likely that the uphill movement of $K^+$ takes place at the basal or lateral cell border to maintain high intracellular $K^+$. Thus what is required at the luminal membrane may only need to be some kind of "controlled leak". However, locating a $K^+/H^+$ pump to the basal or lateral membranes would imply intracellular accumulation of $H^+$ whereas if it is at the luminal border, $CO_2$ will buffer the $OH^-$ generated.

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