IONIC TRANSFER ACROSS THE ISOLATED FROG LARGE INTESTINE*

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ABSTRACT

The unidirectional fluxes of sodium, chloride, and of the bicarbonate and CO_2 pair were determined across the isolated large intestine of the bullfrog, *Rana catesbiana*. The isolated large intestine of the frog is characterized by a mean transmembrane potential of 45 mv., serosal surface positive with respect to mucosal. The unidirectional sodium flux from mucosal to serosal surface was found to be equal to the short-circuit current, thus the net flux was less than the simultaneous short-circuit current. This discrepancy between active sodium transport and short-circuit current can be attributed to the active transport of cation in the same direction as sodium and/or the active transport of anion in the opposite direction. The unidirectional fluxes of chloride and the bicarbonate and CO_2 pair revealed no evidence for active transport of either anion. A quantitative study of chloride fluxes at 45 mv. revealed a flux ratio of 1.8 which is considerably less than a ratio of 6 expected for free passive diffusion. It was concluded that a considerable proportion of the isotopic transfer of chloride could be attributed to "exchange diffusion." Study of the electrical properties of the isolated frog colon reveals that it can be treated as a simple D. C. resistance over the range of -20 to $+95$ mv.

INTRODUCTION

The epithelia of the frog have been demonstrated to be excellent objects for the study of electrolyte and water movement. Studies of ionic movement across the isolated skin and stomach have provided valuable information about the nature of sodium and chloride transport (1, 2). A preliminary report by Ussing and Andersen (3) indicates that the active transport of sodium is essential for the generation of an electrical potential difference across the colon mucosa. Sodium transport alone cannot account for the striking pH gradients which may develop across the large intestinal epithelium of many species (4). The following study confirms and extends the earlier study of the transmucosal

* A portion of these results has been presented previously (*Fed. Proc.*, 1957, **16**, 24).

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potential. In seeking a better explanation of the over-all electrolyte shifts, a detailed study of chloride transfer has disclosed that much of the isotopic transfer of chloride can be attributed to "exchange diffusion" even though chloride moves from a higher to a lower electrochemical potential.

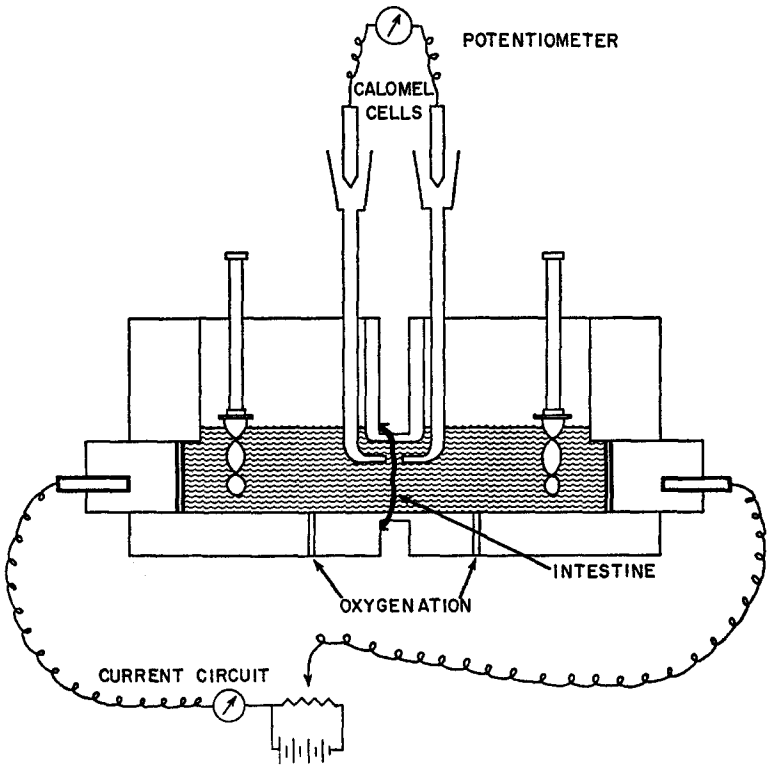

FIG. 1. Intestine flux-chamber assembly.

Methods

Bullfrogs, *Rana catesbiana*, were maintained at 12° for 3 to 6 weeks after receipt, with frequent change of water but no food. This minimized seasonal variations. Within 5 to 10 minutes of pithing, a flat sheet of large intestine was clamped between two lucite chambers (Fig. 1). The solution bathing each surface was aerated and agitated continuously. In most instances, paired experiments were conducted using two portions of the colon from the same frog. One set of flux chambers was used for measuring the flux in one direction and the other set for measuring the opposite flux. The composition of the bathing solution was NaCl 87 mM, $NaHCO_3$ 20 mM, KCl 2.5 mM, NaH_2PO_4 0.3 mM, Na_2HPO_4 1.3 mM, $CaCl_2$ 1.25 mM, $MgSO_4$ 0.5 mM, and glucose 5 mM. Another solution contained NaCl 107 mM and was otherwise the same except for the absence

of HCO_3^-. To minimize bacterial contamination, small amounts of penicillin G (8 mg. per cent) and streptomycin sulfate (16 mg. per cent) were added. 95 per cent O_2 and 5 per cent CO_2 were used to aerate the bicarbonate solution, maintaining the pH at approximately 7.3, and O_2 was used to aerate the bicarbonate-free solution. All experiments were conducted at a room temperature of 24° ± 1.

Electrical.—The fine tips of 3 per cent agar saline bridges were placed within 1 to 3 mm. of the membrane. The electrical potential difference between these bridges was sensed by a pair of calomel cell electrodes leading into a modified Brown-Minneapolis Honeywell continuous recording potentiometer with an input impedance of 1 megohm. Prior to setting up the flux chambers with the large intestine in place, the electrode and bridge junction potentials were determined, and in all cases they were negligible (±1 mv.).

With proper oxygenation the membranes maintained their spontaneous potential difference for periods of up to 12 hours. The average potential was 45 mv., serosal surface positive with respect to the mucosal surface.

By sending a current through an external circuit (Fig. 1), the potential across the mucosa could be adjusted to any desired value. The external circuit electrodes were separated from the solution bathing the membrane by porous discs filled with agar saline. The electrodes were located sufficiently far from the mucosa so that there would be a reasonably uniform potential field across the membrane. Since preliminary study indicated that 45 mv. was a reasonable spontaneous potential, the flux of sodium and chloride was measured when the potential was fixed at this value to ensure consistency. Subsequently, another series of experiments was performed with the potential maintained at zero; this is called the short-circuited state (1). The current necessary to maintain a constant potential difference of 45 mv. or to abolish the potential difference was recorded throughout the experiment. A servo-mechanism was used to maintain the potential at the imposed fixed values.

Radioactivity Assay.—In the experiments in which the fluxes of sodium and chloride were determined, a solution containing both isotopes was placed in contact with the serosal surface. The serosa to mucosa flux was determined from the rate of appearance of the radioisotopes in the solution bathing the mucosal surface. The mucosa to serosa flux was determined separately, by using another portion of large intestine and introducing the radioisotopes into the solution bathing the mucosal surface. Na^{22} was determined without interference in a well-type scintillation counter because the vial containing the analytical aliquot and the aluminum lining of the well effectively absorbed the Cl^{36} beta particles. After counting the Na^{22} disintegrations, duplicate 1 ml. samples were dried on filter paper bonded to copper discs. These samples were then counted with a Geiger-Müller tube. A factor was determined for the relative efficiency of counting Na^{22} by the two methods. Using this factor the total disintegrations detected by the Geiger-Müller tube could be corrected to give the counts attributable to the disintegration of Cl^{36} alone. At least 10,000 disintegrations were counted; the activities were at least several times background.

In order to measure the bicarbonate and CO_2 fluxes, the design of the flux chambers had to be modified to prevent loss of CO_2 to the atmosphere. The bathing solutions were saturated with 95 per cent O_2 and 5 per cent CO_2, and immediately after the addition of the C^{14} bicarbonate, the chambers were sealed. Stirring was maintained with

mercury-sealed stirrers. There was sufficient oxygen to sustain the spontaneous potential and short-circuit current for at least 4 to 6 hours. Analytical samples were immediately placed in alkali. The C^{14} content was determined either by using an infinite thickness of liquid under a mylar windowed proportional counter, or by the liquid scintillation technique of Passmann *et al.* (5).

The flux was calculated from the measured proportion of isotope crossing the epithelium, the known carrier concentration of the ion involved, and the volume of solution. Samples were collected at 1 hour intervals. The area of exposed membrane was 1 cm^2. The flux is expressed in microequivalents $cm.^{-2}$ $hr.^{-1}$. The externally applied current is given in the same units to facilitate comparison.

Chemical Methods.—Chloride was determined by the coulometric-amperometric method of Cotlove *et al.* (6). The bicarbonate was determined after addition of a standard acid, by back-titration with a standard alkali solution (7).

TABLE I

Simultaneous Measurement of Na^+ and Cl^- Fluxes at 45 Mv. in Presence of HCO_3^- + CO_2

	$M \rightarrow S$	$S \rightarrow M$	Net
Na^+ flux	3.39 (±0.3)	1.14 (±0.1)	2.25
Cl^- flux	2.17 (±0.16)	1.17 (±0.1)	1.00
Current	−0.13 (±0.45)	0.12 (±0.43)	
Conductance, *mmhos*	2.57 (±0.15)	2.22 (±0.3)	
No. of animals	10	8	
No. of periods	25	19	

$M \rightarrow S$, mucosal to serosal flux; $S \rightarrow M$, serosal to mucosal flux.

Flux and current expressed as μeq. $cm.^{-2}$ $hr.^{-1}$. Positive current is in the direction $M \rightarrow S$ and negative current $S \rightarrow M$.

(±), standard error of the mean.

RESULTS

The simultaneous fluxes of sodium and chloride were determined at zero and at 45 mv. In the first series of experiments, the membrane was maintained in the short-circuited state (0 mv.). While this represents a more artificial state, it does allow a quantitative analysis of each ion's contribution to the generation of the total short-circuit current. In the second series the membranes were maintained at 45 mv. (serosa positive with respect to mucosa). Since 45 mv. was approximately the average spontaneous potential, the external current required in 44 hourly periods was negligible, averaging 0.01 μeq. $cm.^{-2}$ $hr.^{-1}$ (Table I).

In each instance, at potentials of either zero or 45 mv., the algebraic sum of all the net ionic movements must equal the measured external current. Consequently, we have chosen to stress the comparison of the sum of the net movements of sodium and chloride with the external current. When the sum of net

sodium and chloride fluxes does not equal the external current, there must be net movement of one or more other ions.

Sodium.—The transfer of sodium at both zero and 45 mv. provided evidence that the bullfrog large intestine is capable of transporting sodium actively. At 45 mv. the net sodium flux was 2.3 μeq. cm.$^{-2}$ hr.$^{-1}$; this net movement from mucosa to serosa was from the lower to higher electrical potential. Since the chemical activity of sodium was the same in the two bathing solutions, the driving force for sodium transport must have resided in the membrane.

The quantitative role of active sodium transport is readily ascertained by short-circuiting the mucosa and comparing the net sodium flux with the short-circuit current (Table II). At zero potential there was a net flux of sodium of 3.7 μeq. cm.$^{-2}$ hr.$^{-1}$ when the mean short-circuit current was 4.0 μeq. cm.$^{-2}$ hr.$^{-1}$.

TABLE II

Simultaneous Measurement of Na^+ and Cl^- Fluxes at 0 Mv. in Presence of $HCO_3^- + CO_2$

	$M \rightarrow S$	$S \rightarrow M$	Net
Na^+ flux	4.26 (±0.36)	0.58 (±0.05)	3.68
Cl^- flux	1.45 (±0.10)	1.40 (±0.08)	0.05
Current	4.25 (±0.30)	3.77 (±0.37)	
Conductance, *mmhos*	2.69 (±0.14)	2.28 (±0.26)	
No. of animals	10	8	
No. of periods	26	21	

$M \rightarrow S$, mucosal to serosal flux; $S \rightarrow M$, serosal to mucosal flux.
Flux and current expressed as μeq. cm.$^{-2}$ hr.$^{-1}$. All current is in the direction $M \rightarrow S$.
(±), standard error of the mean.

Thus active sodium transport accounted for all but a small fraction of the short-circuit current. In order to evaluate the significance of this discrepancy, the comparison between the unidirectional fluxes of sodium and the simultaneously measured short-circuit current is given in Table III. In every experiment the serosa to mucosa flux was at least 10 per cent of the simultaneously measured current. In all but one of the ten experiments, the mucosa to serosa flux was approximately equal to or less than the short-circuit current. Even in that one exception (Experiment 1, Table III), the flux was no more than 10 per cent greater than the current. Clearly, the failure of the unidirectional mucosa to serosa flux to equal the short-circuit current in most instances or to exceed it by 10 per cent indicates that net sodium transport cannot account for all of the short-circuit current, and suggests that some other cation is transported in the same direction or an anion in the opposite direction.

It is of interest to compare these results with those previously reported in the toad and guinea pig (3), in which the spontaneous potentials of about 10 to

30 mv. were lower than in the bullfrog, and in which the net sodium flux equalled or exceeded the short-circuit current. In addition to relevant species and seasonal differences, the bathing solutions used in the present study had a higher bicarbonate concentration (20 mM compared with that of ordinary Ringer's solution). In Tables II and IV, a comparison can be made between the presence and absence of the bicarbonate and CO_2 system at a constant pH. In their absence a marked reduction of sodium transport was associated with a marked reduction of short-circuit current. The results of these experiments conducted on the short-circuited membrane, revealed that *in vitro*, the sodium transport mechanism functions better in the presence of bicarbonate and CO_2.

TABLE III

Sodium Fluxes and Simultaneous Short-Circuit Current

Experiment No.	No. of periods	$S \rightarrow M$ Na^+ flux	Current	$M \rightarrow S$ Na^+ flux	Current
1	2	0.56	4.12	5.57	4.95
2	2	0.61	5.72	5.48	5.64
3	2	0.56	3.59	2.88	3.01
4	3	0.80	3.44	3.43	3.44
5	3	0.81	2.44	3.02	2.91
6	3	0.47	4.12	5.06	5.15
7	3	0.52	4.04	4.39	4.33
8	3	0.34	3.35	3.73	4.04
9	3			5.79	5.71
10	2			3.26	3.36

$M \rightarrow S$, mucosal to serosal flux; $S \rightarrow M$, serosal to mucosal flux.
All fluxes and current expressed as μeq. cm^{-2} $hr.^{-1}$.

Their absence produced no significant change in the serosa to mucosa flux of sodium. When bicarbonate and CO_2 were restored to the flux chambers there was a gradual increase of the mucosa to serosa flux of sodium, short-circuit current, and spontaneous potential to their previous higher levels. Although the experiments in Table IV were paired, the fluxes were measured while the short-circuit current was decreasing; this may explain the large difference of 0.5 μeq. between the mean currents.

It is also interesting to compare the musoca to serosa sodium flux with the simultaneously measured short-circuit current when bicarbonate and CO_2 were omitted. The mucosa to serosa flux of 1.5 μeq. $cm.^{-2}$ $hr.^{-1}$ was 0.6 μeq. $cm.^{-2}$ $hr.^{-1}$ greater than the simultaneous short-circuit current of 0.9 μeq. Since the mean opposite flux of sodium was 0.6 μeq. the short-circuit current was in this instance equal to the net sodium flux. The discrepancy between short-circuit current and net sodium flux is eliminated when bicarbonate is absent. This suggests that not only do bicarbonate and CO_2 facilitate the active transport

of sodium but also that they are necessary for the active transport of some other unidentified anion or cation.

Chloride.—At zero potential, the chloride fluxes in the opposite directions were equal, 1.4 μeq. cm.$^{-2}$ hr.$^{-1}$ (Table II). Thus chloride was not actively transported and could not account for the discrepancy between the short-circuit current and net sodium flux in the presence of bicarbonate. Unlike

TABLE IV

Simultaneous Measurement of Na^+ and Cl^- Fluxes at 0 Mv. in Absence of $HCO_3^- + CO_2$

	$M \rightarrow S$	$S \rightarrow M$
Na^+ flux	1.50 (±0.13)	0.60 (±0.08)
Cl^- flux	1.32 (±0.12)	1.39 (±0.10)
Current	0.92 (±0.09)	1.37 (±0.18)
No. of animals	4	4
No. of periods	12	12

$M \rightarrow S$, mucosal to serosal flux; $S \rightarrow M$ serosal to mucosal flux.
Flux and current expressed as μeq. cm.$^{-2}$ hr.$^{-1}$. All current is in the direction $M \rightarrow S$.
(±), standard error of the mean.

TABLE V

Fluxes of Carbon Dioxide-Bicarbonate System Determined with C^{14}

	0 mv.	45 mv.
$M \rightarrow S$	1.96 (±0.13)	1.93 (±0.08)
$S \rightarrow M$	1.86 (±0.20)	1.59 (±0.04)
Net flux	0.10	0.34
No. of animals	6	4
No. of periods	24	18

$M \rightarrow S$, mucosal to serosal flux; $S \rightarrow M$, serosal to mucosal flux.
Flux expressed as μeq. cm^{-2} hr.$^{-1}$.
(±), standard error of the mean.

sodium, neither chloride flux changed when bicarbonate was omitted from the bathing solutions (Table IV).

When the potential difference across the colon was 45 mv. serosa positive with respect to mucosa, there was a net movement of chloride along its electrochemical gradient. The mucosa to serosa flux was 2.2 and the opposing serosa to mucosa flux was 1.2 (Table I).

Bicarbonate-Carbon Dioxide System.—In Table V, the fluxes of the bicarbonate and CO_2 system are given. At zero potential the flux of the C^{14} was 1.9 μeq. cm.$^{-2}$ hr.$^{-1}$ in each direction. At 45 mv., serosa positive with respect to

mucosa, the mucosa to serosa flux was 1.9 and the opposite flux 1.6. Thus the net movement of 0.3 μeq. of bicarbonate was along its electrochemical potential gradient.

An attempt was made to determine whether any H^+ shift occurred when the colon was bathed by bicarbonate saline, and the potential maintained at either zero or 45 mv. No obvious pH changes were detected in the presence of the

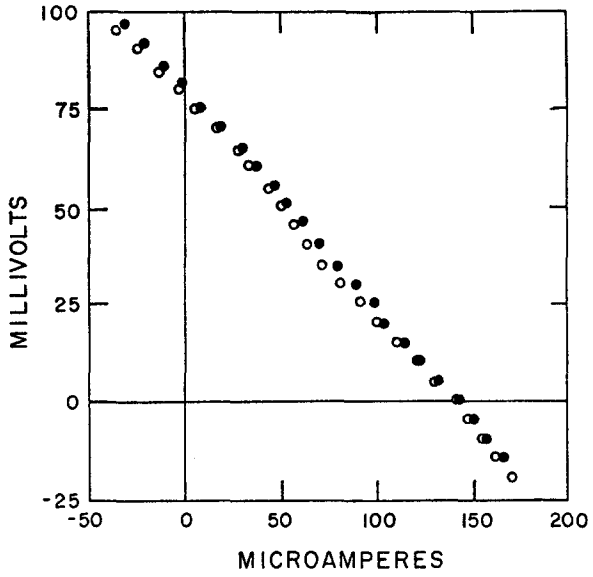

FIG. 2. A plot of the transmucosal potential against the applied external current over the range of −20 to +95 mv. The measurements were made after changing the potential by 5 mv. increments. The open circles represent the sequence from +95 to −20 mv. and the closed circles the opposite sequence.

relatively large amounts of bicarbonate. When the membrane was bathed by bicarbonate-free saline there were no significant pH changes.

Conductance.—In Fig. 2, the transmucosal potential is plotted against the applied external current over a range of −20 to +95 mv. The point at zero applied current is the spontaneous potential. The measurements were made by changing the current to obtain 5 mv. increments of potential. The results when plotted give a straight line. Thus when a small potential change is applied, the membrane can be treated as a simple D. C. resistance and the conductance of the membrane calculated from the ratio of the change of applied current to the change of potential.

When the potential was altered by more than 5 mv. large transients or polarization effects lasting from 2 to 10 minutes were observed (Fig. 3). These

transients indicate that the colon does not behave as a simple D.C. resistance. In spite of this reservation, the reported D. C. conductances of the colon have been calculated from the ratio of steady state short-circuit current to steady state spontaneous potential. The total membrane conductance averaged 2 to 3 millimhos cm.$^{-2}$ (Tables I and II). This estimate of conductance has confidence limits of about ± 15 per cent. The transients noted in the bullfrog colon are much larger than those of the isolated frog stomach (8) but similar to those reported for the frog skin by Linderholm (9).

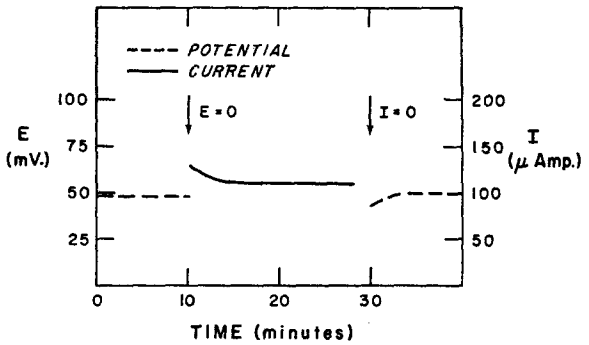

FIG. 3. The transients or polarization effects obtained when potential is altered by greater than 5 mv. At $E = 0$, the membrane was short-circuited and the transient in applied current can be seen. At $I = 0$, the membrane was allowed to return to its spontaneous potential.

DISCUSSION

The frog can live in a hypotonic environment and essential to its survival is its ability to extract salt through its skin, bladder, and large intestine (3, 10). Although the mammal has apparently lost the ability to perform a similar function with its skin or bladder, there have been numerous studies demonstrating the ability of the mammalian colon to reabsorb salt against concentration gradients (11).

The experiments reported here reveal that when the solutions bathing both surfaces of the colonic mucosa are identical, the colon is capable of transporting sodium against its electrochemical gradient. There are no hydrostatic or osmotic pressure differences between the two solutions that would enable an externally induced solvent drag to be responsible for the results obtained. Thus the source of energy for the observed movement of sodium must reside in the membrane.

When the mucosa is short-circuited, the two chloride fluxes are identical. From this, it would appear that chloride is presumably transferred by simple diffusion. However, the flux of chloride at other than zero potential demands a qualification of this conclusion.

Using the Nernst equation, Ussing (12) has reduced the study of ionic flux across a membrane to an equation which had been considered by Behn (13);

$$\frac{M_{12}}{M_{21}} = \frac{a_1}{a_2} \cdot e^{(zF/RT)E}$$

in which M_{12} and M_{21} are the fluxes between solutions 1 and 2 respectively; a_1 and a_2, the chemical activity of the ion in question in solutions 1 and 2 respectively; z, F, R, T have their usual thermodynamic significance, and E is the potential difference between the two solutions.

Under the conditions of the experiment performed here when $a_1 = a_2$, this may be reduced to

$$\log \frac{M_{12}}{M_{21}} = \frac{E(\text{mv.})}{60}$$

At 45 mv. the equation yields a predicted ratio:

$$\frac{M_{12}}{M_{21}} = 6.0$$

Using the flux of chloride from mucosa to serosa of 2.2 and serosa to mucosa of 1.2, the experimental ratio is 1.8. This is significantly lower than the value of 6 predicted for simple passive diffusion. This discrepancy between the observed and expected flux ratio can be attributed to exchange diffusion.

Levi and Ussing first proposed a process of exchange diffusion to explain a discrepancy between energy consumption and the rapid exchange of Na^{24} across the muscle membrane (14). Since the carrier hypothesis is the one most generally accepted, they suggested that the isotope crossed the membrane by combining with some carrier molecule which is part of the membrane or cannot leave the membrane. This carrier-ion complex may alternately come into contact with either solution, where it will exchange the bound ion for an unbound ion of the same species. The portion of flux due to this mechanism would be the same in both directions regardless of the electrical potential across the membrane.

As there is no net transport of chloride at zero potential, we can assume provisionally that the isotopic flux has only two components: passive diffusion and exchange diffusion. If we assume that the partial conductance and the rate of exchange diffusion are independent of the applied potential, we can estimate their magnitudes as follows:—

Let x equal the flux due to exchange diffusion. The passive flux ratio at 45 mv. is given by

$$\frac{M_{12}}{M_{21}} = 6$$

thus if we subtract the exchange diffusion component of each flux, we should have

$$\frac{2.2 - x}{1.2 - x} = 6$$

and $x = 1.0$ μeq. cm.$^{-2}$ hr.$^{-1}$, or 1.0 μeq. of Cl^- is being transported *via* a carrier independent of the electrical gradient. The chloride flux across the short-circuited mucosa could then be partitioned as follows: exchange diffusion 1.0 and passive diffusion 0.4 μeq. cm.$^{-2}$ hr.$^{-1}$. The latter value would constitute a partial conductance for chloride of 0.4 mmho compared to a total membrane conductance of 2 to 3 mmhos.

In Fig. 4 a schematic representation of the movement of chloride by both

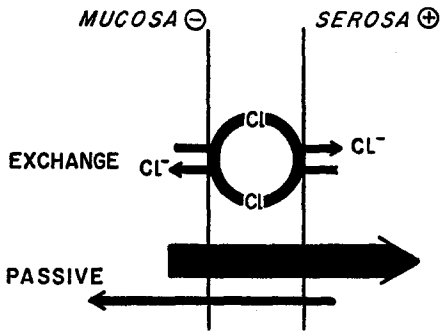

FIG. 4. Chloride diffusion across frog large intestine. See text for description.

exchange diffusion and passive diffusion is given. The carrier is designated as moving to and fro in the membrane. The large passive flux of chloride is in the direction of the electrical gradient.

In contrast to the colon, the flux ratios of chloride across the frog skin have been reported to be within the range expected for passive diffusion (9, 15). Hogben indicated that the chloride movement from the mucosal to the serosal surface of the stomach occurs predominantly by exchange diffusion. This conclusion was based on the observation that the chloride conductance calculated from this flux was greater than the membrane's total conductance. The exchange diffusion of chloride across the short-circuited mucosa of the bullfrog colon, 1.0 μeq. cm.$^{-2}$ hr.$^{-1}$, is less than that across the frog stomach, 3 μeq. cm.$^{-2}$ hr.$^{-1}$ or more (2).

The bicarbonate and CO_2 system is important for normal cellular activity in addition to its more obvious participation in the transepithelial movement of hydrogen ion. The former role is evident in isolated preparations of frog skeletal muscle in which the intracellular composition is maintained more successfully

in the presence of bicarbonate (16). The data presented here (Table IV) indicate that bicarbonate clearly facilitates active transport of sodium by the large intestine of the frog.

The association of carbon dioxide, bicarbonate, and the transepithelial movement of hydrogen ion justifies considering them as a unit. The mammalian colon can render saline alkaline either by the addition of bicarbonate or the reabsorption of hydrogen ion (11, 17). Yet there is considerable variation in the reported values of the pH of the contents within the colon. The guinea pig's cecum has been reported to have acidic contents (4). While it is recognized that bacterial action may render the colonic contents acidic, the extent of this process has not been defined. In the case of the guinea pig cecum, if the low pH were due to active transport of hydrogen ion rather than bacterial activity, it would explain the observation of Ussing and Andersen (3) that the net sodium flux across the cecum is greater than the shortcircuit current.

Isolated sacs of bullfrog large intestine, without modification of the spontaneous potential, reduce the concentration of bicarbonate at the mucosal surface (18) perhaps by passive reabsorption of bicarbonate along its electrochemical potential gradient.

When flat sheets of bullfrog colon have been bathed by highly buffered bicarbonate solutions our only clue to a possible active secretion of bicarbonate or active reabsorption of hydrogen ion is given by the comparison between the short-circuit current and the sum of the observed net ionic movement. At zero potential the net sodium transport is less than the short-circuit current. The difference must be due to net (active) transport of another cation (potassium or hydrogen) from mucosa to serosa or of an anion in the opposite direction. Chloride was excluded as a possibility since no net transport was found at zero potential.

An attempt to assess a possible active transport of bicarbonate by using C^{14}-labelled bicarbonate was inconclusive. At zero potential, the fluxes of C^{14}-bicarbonate and CO_2 were equal in the two directions across the colon, unlike observations on the gastric mucosa (2). When the transmembrane potential was 45 mv., the difference between the two C^{14} fluxes of 1.9 and 1.6 μeq. might be attributed to diffusion of bicarbonate along its electrochemical gradient. This would be consistent with the behavior of isolated sacs of bullfrog colon (18). By applying calculations similar to those used to partition the chloride flux, and assuming the exchange diffusion component to be carbon dioxide, the C^{14} short-circuit flux can be partitioned into a carbon dioxide flux of 1.5 μeq. and a bicarbonate flux of 0.4 μeq.

The mammalian colon has recently been shown to have *in vivo* an electrical potential difference across its epithelial surface which is associated with active reabsorption of sodium (19). While potassium moved into the lumen along its electrochemical potential gradient, there was evidence of an active secretion

of bicarbonate into the lumen which was encountered in some preparations but not in others. This variability may have a neural basis since stimulation of the parasympathetics induces secretion of an alkaline solution by the cat large intestine (17). In view of these observations, it is possible that the discrepancy between net sodium flux and the short-circuit current is a manifestation in the bullfrog of an ability to similarly secrete bicarbonate or reabsorb hydrogen ion.

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