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CHLORIDE-INDUCED INCREMENT IN SHORT-CIRCUITING CURRENT OF THE TURTLE BLADDER

EFFECTS OF IN-VIVO ACID-BASE STATE

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Evidence for the participation of conductive and non-conductive (exchange) transmembrane anion pathways in the luminal acidification, alkalinization, and chloride-reabsorptive functions of the turtle bladder is provided from the pattern of Cl^--induced changes in transepithelial electrical parameters of isolated urinary bladders from three groups of donor turtles: control or post-absorptive turtles (those killed 5 days after feeding); acidotic turtles (NH_4Cl-loaded); and alkalotic turtles ($NaHCO_3$-loaded). The predominance of each of the three aforementioned transport functions as well as the response to Cl^--addition is altered by the in-vivo electrolyte balance of the turtle. In post-absorptive bladders, which are poised for acidification and Cl^- reabsorption, the mucosal and serosal addition of Cl^- to Na^+-free, ($HCO_3^- + CO_2$)-containing media increases the negative short-circuiting current (I_{sc}). In acidotic bladders, which are poised for acidification but not Cl^- reabsorption, mucosal Cl^- addition has no effect on this I_{sc} whereas serosal Cl^- addition increases the negative I_{sc} in a manner identical to that observed in the post-absorptive bladders. Alkalotic bladders do not possess an acidification function but instead are poised for Cl^- reabsorption and cAMP-dependent electrogenic alkali secretion (positive I_{sc}). In these bladders, serosal Cl^- addition is without effect while mucosal Cl^- addition produces transient changes in this positive I_{sc}. It is found that these results can be replicated by a model of the turtle bladder in which transmembrane Cl^- and HCO_3^- conductive and exchange paths mediate transepithelial acidification, alkalinization and Cl^- reabsorption.

Introduction

There has been a persistence of two apparently different (yet properly formulated), and heretofore incompatible hypotheses of solely electrogenic or solely electroneutral Cl^- reabsorption in the turtle bladder. This is a result of the prolonged absence of fundamental information about the nature and interaction of Cl^-, and Cl^- transport, with the luminal acidification and alkalinization functions in the turtle bladder. The present investigation provides results, obtained from transepithelial electrophysiological studies, which for the first time allow for the combination of these two hypotheses into a single model which accounts for the present as well as older results on Cl^- transport in the turtle bladder. Previous results which led to the development of this study are the following.

The turtle urinary bladder has been found to actively reabsorb Cl^- by a Na^+-independent pro-

Abbreviations: cp-cAMP, 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate; IBMX, 3-isobutylmethylxanthine; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid.

cess [1,2] which is dependent upon serosal HCO_3^- [3]. An electrogenic Cl^- transport process was invoked by Gonzalez et al. [2] on the following basis. Bladders bathed in Na^+-free, (HCO_3^- + Cl^-)-containing media produce a negative short-circuiting current (negative charges flowing from mucosa to serosa) which was greater in magnitude than the concomitant rate of Cl^- reabsorption. In the absence of mucosal HCO_3^-, the I_{sc} was found to be decreased but equivalent to the rate of Cl^- reabsorption. Gonzalez et al. [2] therefore postulated that the negative I_{sc} observed in Na^+-free (HCO_3^- + Cl^-)-containing media represented the sum of electrogenic HCO_3^- and electrogenic Cl^- reabsorption. Parenthetically, the ionic identity (HCO_3^- reabsorption or H^+ secretion) of this HCO_3^- (or CO_2) dependent current is not currently agreed upon in principle, but the bladder function it represents is agreed to be that of luminal acidification [4–6]. For this reason, the term acidification current will be used to denote the negative I_{sc} observed in the absence of mucosal Cl^-.

An electroneutral $Cl^- : HCO_3^-$ transepithelial exchange process was subsequently invoked by Leslie et al. [3] who found that the presence of serosal HCO_3^- is an absolute requirement for chloride reabsorption and that under pH-statting conditions, the Cl^- reabsorptive rate approximates the rate of luminal titration of alkali (HCO_3^-) without producing net charge transfer.

Recently [7], we tested the validity of the hypothesis for electrogenic Cl^- reabsorption by examining its two main predictions. First, the symmetrical addition (simultaneously to both mucosa and serosa) of Cl^- should increase the negative I_{sc} of bladders incubated in Na^+-free, HCO_3^--containing media. Secondly, the magnitude of this increment in I_{sc} and that of the initiated Cl^- reabsorptive rate should be equivalent. It was found that this type of Cl^- addition did indeed increase the I_{sc} as predicted but that this increment was sometimes but not always equal to the Cl^- reabsorptive rate. It was suggested [7] that this inequality arose from an interaction of Cl^-, or Cl^- transport, with the electrogenic transport of another ion.

In designing the present investigation, two facts were considered. (i) Luminal acidification is increased in the presence of Cl^- [8]; and (ii) the magnitude of the symmetrical Cl^--induced change in I_{sc} is not uniquely attributable to that of the Cl^- reabsorptive rate [7]. If this Cl^- induced increment of I_{sc} represents a function of the Cl^- reabsorptive process, then Cl^- addition to the mucosal solution (the side from which Cl^- is reabsorbed) should uniquely produce this increment in negative I_{sc}. In a preliminary study [9] it was found that in short-circuited bladders from post-absorptive turtles incubated in Na^+- and Cl^--free, HCO_3^--containing media, the addition of Cl^- to either the mucosal or serosal bathing solution produced a similar increment in the negative I_{sc}. The serosal Cl^--induced increase of negative I_{sc} (in direction opposite to that which would be due to Cl^- diffusion) is readily accounted for by postulating the participation of basal-lateral $Cl^- : HCO_3^-$ (OH^-) exchange in the luminal acidification processes. The involvement of such basal-lateral anion exchange in acidification processes has been suggested in preliminary reports for the turtle bladder [9,10] and for the rabbit medullary cortical collecting duct [11].

If, on the other hand, the mucosal Cl^--induced I_{sc} were uniquely due to the Cl^- reabsorptive process, the serosal, but not the mucosal Cl^- response should be evoked in bladders which have a prominent acidification and diminished Cl^- reabsorptive function. Such bladders are obtained from acidotic (NH_4Cl-loaded) turtles [12,13], and therefore it was determined if this mucosal Cl^- effect is absent in this bladder type.

In another approach to determine if the mucosal Cl^--induced change in I_{sc} is characteristic of the Cl^- reabsorptive pathway and not of an interaction with elements of the acidification path, the effects of Cl^- addition were examined on bladders from alkalotic turtles in which the acidification [13,14] but not the Cl^- reabsorptive [12,13] process is physiologically depressed.

It is concluded, upon the basis of the observed Cl^--induced changes in I_{sc} across these bladders from turtles in different physiological states, that the epithelial cells contain transmembrane HCO_3^- and Cl^- selective conductance paths as well as non-conductive $Cl^- : HCO_3^-$ exchange paths. It is found that the interactions of Cl^- and HCO_3^- through these paths at the transmembrane level

can account for the transepithelial $Cl^-:HCO_3^-$ exchange and serosal HCO_3^- dependency of Cl^- reabsorption originally observed by Leslie et al. [3] as well as the association between the negative I_{sc} and Cl^--reabsorption observed by Gonzalez et al. [2].

Methods

In-vivo preparations

Turtles (*Pseudemys scripta*) were kept in a covered stainless steel tank filled with water to a depth of 30 cm. The water was continuously thermostatted at 31–33°C with a submersible heater, and circulated through a floss-charcoal filter. The tank was partitioned into three compartments to allow for separate feedings. The feedings, bovine liver with multivitamin and bone meal supplements, were given once weekly and ingested ad-libitum for 2–4 weeks prior to starting the preparatory treatment on the basis of which the three classes of turtle were defined.

Post-absorptive turtles were defined as those given no feeding for 5–7 days prior to death. At the time of death, the mean values (±S.E.) for the pH (units) and chloride concentration (mM), respectively, in the plasma were: 7.54 ± 0.02 and 85.5 ± 2.2 ($n = 11$); and in the urine, 5.67 ± 0.15 and 0.68 ± 0.16 ($n = 11$).

Acidotic turtles, prepared as recommended by Cohen [12], were defined as those which had been given (by stomach tube) 8 mmol/kg of body weight of NH_4Cl in 7 ml of water twice daily for 4 days prior to death (which also coincided with the 5–7 day fasting period). At the time of death, the mean values (±S.E.) for the pH (units) and chloride concentration (mM), respectively, in the plasma were: 7.24 ± 0.03 ($n = 9$) and 116 ± 6 ($n = 7$); and in the urine, 4.29 ± 0.29 ($n = 9$) and 73.7 ± 9.2 ($n = 8$).

Alkalotic turtles, also prepared as recommended by Cohen [12], were defined as those which had received (by stomach tube) 30 mmol/kg of body weight of $NaHCO_3$ in 7 ml of water twice daily for 4 days prior to death (which coincided with the 5–7 day period of fasting). At the time of death, the mean values (±S.E.) for the pH (units) and chloride concentration (mM), respectively, in the plasma were: 7.84 ± 0.02 ($n = 28$) and 52.8 ± 2.1 ($n = 26$); and in the urine, 7.89 ± 0.05 ($n = 24$) and 0.39 ± 0.13 ($n = 23$).

In vitro procedures

Bladders were removed and prepared for mounting in the form of a sheet as described previously [2], or placed in the form of a sac directly on the pins of the half-chamber and opened into sheet form, and mounted for electrical measurements [2]. Resistance was determined by briefly returning to the open circuit condition and measuring the change in potential difference produced by sending a known current (10 μA) across the tissue as described previously [2].

For Cl^- flux measurements, the bathing chambers were filled with Cl^--Ringer and isotopic Cl^- flux determined as described previously [15]. For Cl^- addition experiments, the bathing chambers were filled with Cl^--free Ringer. These solutions were replaced until Cl^- (in 2 ml samples) was no longer detectable by chloridometry using a Buchler-Cotlove chloridometer. Mucosal solution replacements were made as described previously [7] and serosal solutions were replaced either as described previously [7] or removed by emptying and refilling with Cl^--free Ringer. Cl^- was added by appropriate substitution of the bathing fluid with Cl^--stock Ringer.

For pH-statting experiments, bladders were mounted in HCO_3^--free Ringer on the mucosal side and Cl^--Ringer on the serosal side. The mucosal solution was maintained at pH 4–5 by statting with 0.005 M H_2SO_4. Forward (mucosa to serosa) and backward Cl^- flux determinations in these experiments were determined in bladders from different turtles.

Solutions

Cl^--Ringer, composition in mM: choline HCO_3, 20; choline chloride, 25, choline sulfate, 27.5; K_2SO_4, 2; KH_2PO_4, 0.7; K_2HPO_4, 0.14; $MgSO_4$, 0.8; $CaSO_4$, 2; glucose, 11; and sucrose in amounts to make the final osmolality 220 mosmol/kg. This solution was equilibrated with 5% CO_2 in O_2 and final pH was 7.3.

Cl^--free Ringer: same as above except that choline sulfate (12.5 mM) was substituted for choline chloride.

Cl^--stock Ringer: same as above, except that

choline chloride (80 mM) was substituted for choline sulfate.

HCO_3^--free Ringer: Same as for Cl^--Ringer, except that choline sulfate (10 mM) was substituted for choline HCO_3; K_2HPO_4 was 0.26 mM; and KH_2PO_4, 0.04 mM; and equilibrated with 100% O_2 at pH 4–5.

All serosal solutions contained 10^{-4} M ouabain.

Materials

Turtles were obtained from Lemberger Assoc., Milwaukee, WI. 8-(4-chlorophenylthio) cyclic AMP (cp-cAMP) from Boehringer Mannheim and 3-isobutylmethylxanthine (IBMX) from Sigma.

Results

In the first group of the present experiments, short-circuited bladders from post-absorptive, acidotic or alkalotic turtles were initially bathed on both surfaces by the Cl^--free solution until steady-state levels of short-circuiting current (I_{sc}), potential difference (PD) and resistance (R) were reached. Then changes in these electrical parameters were monitored following the substitution of chloride for sulfate in either the mucosal fluid or the serosal fluid. The directional orientation and the mucosal or serosal sidedness of these electrical changes were found to be characteristic of the prior systemic acid-base state of the intact turtle.

Bladders from post-absorptive turtles

After control levels of I_{sc}, PD (serosa negative) and R were reached in mated pairs of post-absorptive bladders, the mucosal or the serosal addition of chloride (by substitution for sulfate) was followed by rapidly developing and sustained increases in the negative I_{sc} and PD, which were restored to control levels after removal of chloride (by replacement with sulfate) (Fig. 1). The mean values of the mucosal and serosal Cl^--induced changes in these parameters are shown in Table I.

In a separate set of experiments, graded concentrations of chloride (from 0.05 to 25 mM) were added to determine the dependency of I_{sc} on the mucosal or serosal chloride concentration. On the average, the half-maximal increase in I_{sc} was reached at a mucosal Cl^- concentration of 0.2 ± 0.04 mM ($n = 7$) and at a serosal Cl^- concentration of

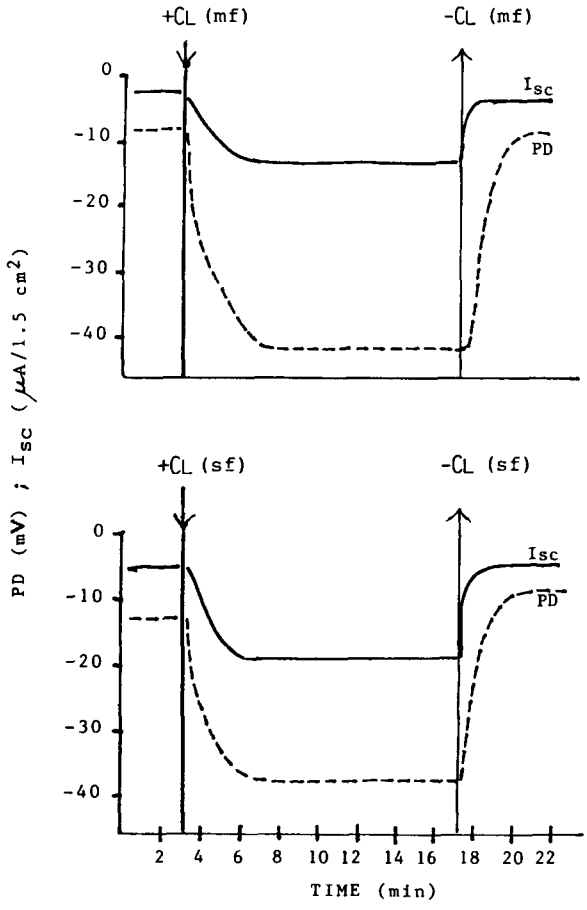

Fig. 1. Effect of the addition of 25 mM Cl^- to the mucosal solution (upper panel) or serosal solution (lower panel) on the short-circuit current (I_{sc}) and potential difference (PD) of two half-bladders from a post-absorptive turtle. Initially the bladder was bathed by Cl^--free Ringer and the PD and I_{sc} were electronegative in direction (serosa electronegative to mucosa, and negative charges flowing from mucosa to serosa). Cl^- addition (downward arrow) increased these parameters which subsequently decreased following Cl^- removal (upward arrow). mf, mucosal fluid; sf, serosal fluid.

0.7 ± 0.2 mM ($n = 7$). The latter value may be higher because of the longer diffusion path from the bulk serosal fluid to the epithelial cell layer.

In other experiments it was found that usually there were no further Cl^--induced changes in I_{sc} or PD, once such a change had been induced from the contralateral fluid compartment (Fig. 2). In a few cases, however (3 out of 25), we did find a second electrical response following contralateral Cl^- addition.

TABLE I

THE EFFECT OF MUCOSAL (*m*) OR SEROSAL (*s*) Cl^- ADDITION (16 OR 25 mM) ON THE MEAN (±S.E.) I_{sc}, PD AND *R* ACROSS BLADDERS FROM POST-ABSORPTIVE AND ACIDOTIC TURTLES

Sign convention: (−) denotes serosal fluid electronegative to the mucosal solution. MID denotes the mean of the individual differences in each parameter.

Post-absorptive	$\overline{I_{sc}}$ (μA)	$\overline{PD}$ (mV)	$\overline{R}$ (kΩ)	*n*
Control	−13.5±2.6	−30.9±5.1	3.0±0.3	18
+Cl^- (m)	−26.9±4.2	−51.5±5.5	2.3±0.2	
Δ (MID)	−13.4±2.1 *	−20.5±2.4 *	−0.7±0.1 *	
Control	−13.2±3.8	−27.3±5.7	2.7±0.3	15
+Cl^- (s)	−26.5±4.4	−47.4±4.7	2.2±0.3	
Δ (MID)	−13.4±1.9 *	−20.5±2.5 *	−0.5±0.1 *	
Acidotic	$\overline{I_{sc}}$ (μA)	$\overline{PD}$ (mV)	$\overline{R}$ (kΩ)	*n*
Control	−4.6±1.4	−12.8±3.1	4.5±0.5	14
+Cl^- (s)	−12.8±2.0	−38.4±4.4	3.5±0.4	
Δ (MID)	−8.1±1.3 *	−22.5±3.3 *	−1.0±0.2 *	

* $p < 0.001$.

Bladders from acidotic turtles

Starting from the standard control conditions under which bladders are initially bathed by Cl^--free Ringer, it was found that the substitution of chloride for sulfate in the mucosal fluid was without effect (Fig. 3). This contrasts with the pronounced effect of mucosal Cl^- addition to bladders from post-absorptive turtles (Fig. 1). The mucosal Cl^--induced electrical responsiveness of the bladder is thus eliminated during the prior in-vivo acidotic state. The subsequent substitution of chloride for sulfate in the serosal fluid was followed by prompt increases in I_{sc} and PD in the negative direction. The mean values of these changes are shown in Table I. The serosally-induced effects in these acidotic bladders were seen in the presence or absence of mucosal Cl^-; and the half-maximal stimulation of I_{sc} occurred at a Cl^- concentration of 0.4 ± 0.06 mM ($n = 12$).

In these bladders from post-absorptive and acidotic turtles, the Cl^--induced increment in

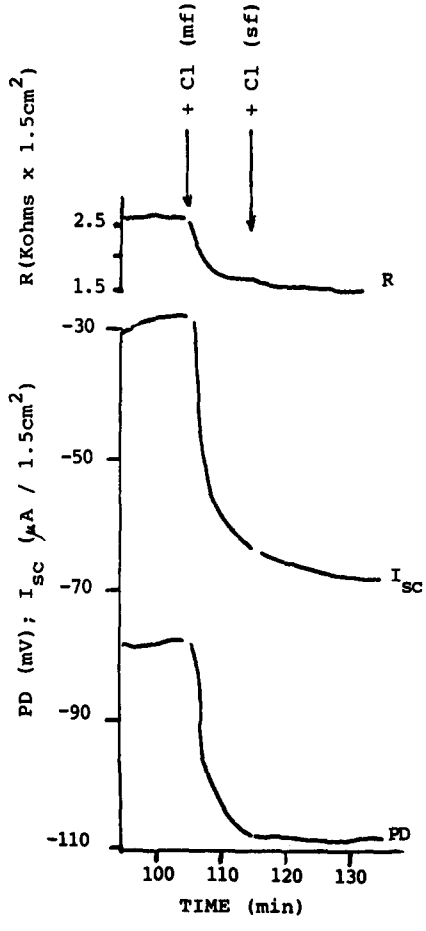

Fig. 2. Effect of Cl^- addition (25 mM) to the mucosal fluid and subsequent Cl^- addition to the serosal fluid on the I_{sc}, PD and *R* in a bladder from a post-absorptive turtle. Initially the bladder was bathed by Cl^--free Ringer. Sign convention as described for Fig. 1.

negative I_{sc} could be due to an increased acidification current. It was therefore of interest to determine whether a similar change could be induced in bladders from alkalotic turtles.

Bladders from alkalotic turtles

Bladders from these turtles have the capability of secreting HCO_3^- by a Cl^--independent electrogenic process which is stimulated by phosphodiesterase inhibitors and cAMP [13,14]. These bladders, as a group, also do not produce an acidification current (or negative I_{sc}) in the presence of HCO_3^-/CO_2 containing bathing fluids as opposed to bladders from other donor types [13].

Fig. 4 depicts the effect of mucosal Cl^- addition to a bladder from an alkalotic turtle. The I_{sc} and PD reached small steady state levels (positively oriented) as has been reported previously [13]. The substitution of chloride for sulfate in the mucosal fluid of these bladders was followed by a rapidly developing biphasic electrical response; initially, I_{sc} and PD increased transiently to a small extent in the negative direction and then increased transiently to a much larger extent in the positive direction with a concomitant transient decrease in R. The addition of Cl^- to the serosal fluid had no effect on the electrical parameters. The addition of Cl^- to the mucosal solution in the presence of serosal Cl^- was followed by the same

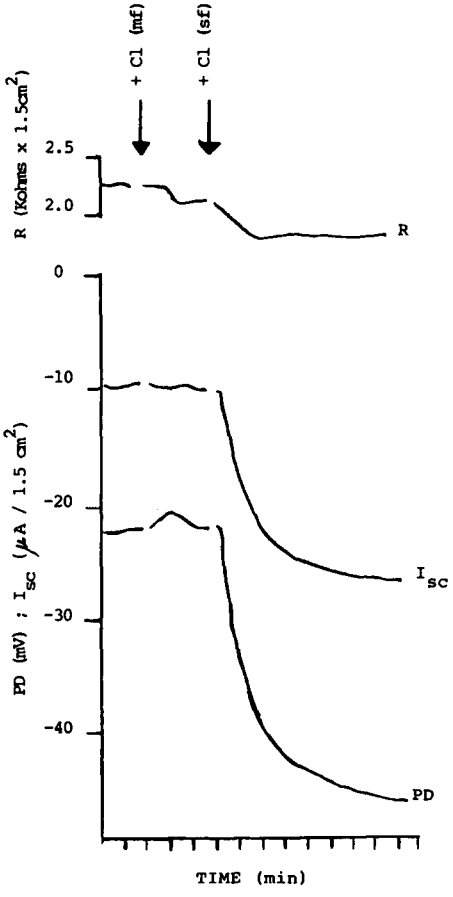

Fig. 3. Effect of Cl^- addition (25 mM) to the serosal solution and lack of effect of mucosal Cl^- addition in a bladder from an acidotic turtle. Initially the bladder was bathed by Cl^--free Ringer. Sign convention as described in Fig. 1.

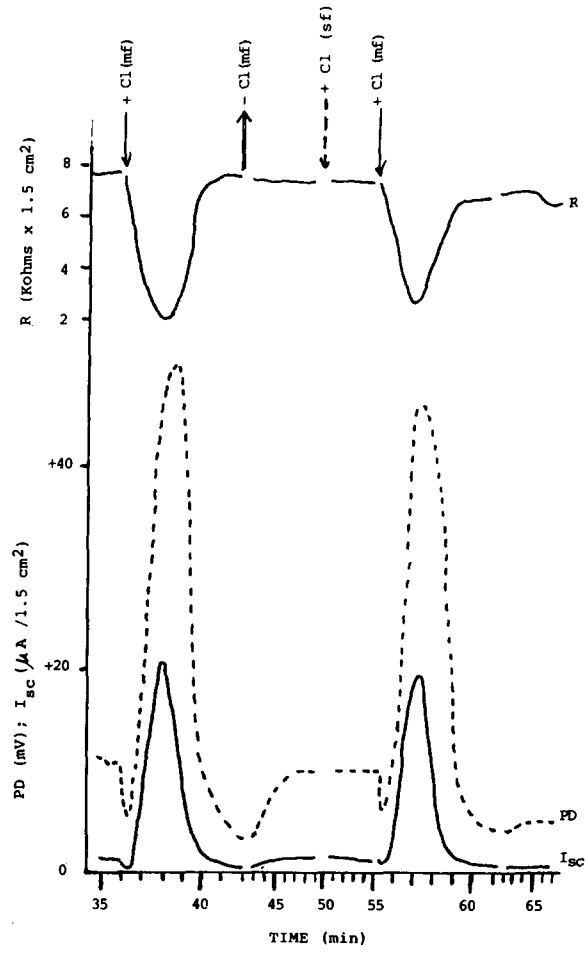

Fig. 4. Effect of Cl^- addition (16 mM) to the mucosal solution and lack of effect of serosal Cl^- addition on the I_{sc}, PD and R of a bladder from an alkalotic turtle. Initially the bladder was bathed by Cl^--free Ringer. PD and I_{sc} initially were positive (sign convention as described for Fig. 1).

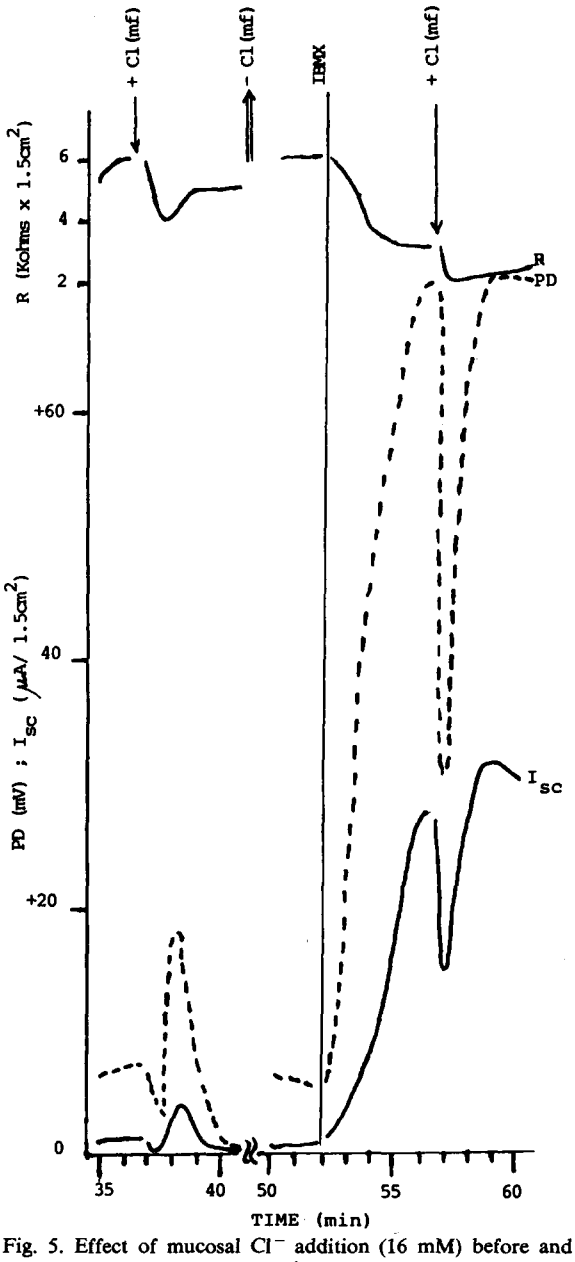

Fig. 5. Effect of mucosal Cl^- addition (16 mM) before and after addition of IBMX (10^{-4} M) to the serosal solution. Initially the bladder was bathed by Cl^--free Ringer and I_{sc} and PD were slightly positive (sign convention as described for Fig. 1). Before IBMX, Cl^- addition elicited the biphasic response as seen in Fig. 4. Following IBMX, Cl^- addition resulted in a change in I_{sc} and PD in the negative direction.

biphasic electrical changes as those found in the absence of serosal Cl^- (Fig. 4). The mean values of the mucosal Cl^--induced changes in the electrical parameters are shown in Table II.

These Cl^--induced changes in positive PD and I_{sc} resemble a transient stimulation of HCO_3^- secretion. If HCO_3^- secretion were first stimulated and maintained by IBMX, then the subsequent addition of Cl^- should fail to induce any transient increase in the positive I_{sc}. This was confirmed in the next set of experiments (Fig. 5). First, Cl^- is added to the mucosal solution, and the I_{sc}, PD and R undergo the transient responses seen above. Next, the mucosal Cl^- was replaced by SO_4^{2-} and then IBMX was added to the serosal fluid. The latter addition was followed by maintained positive increases in I_{sc} and PD. At this point, the mucosal addition of Cl^- was followed by a transient decrease in I_{sc} and PD in the negative direction. Thus, subsequent to the stimulation of HCO_3^- secretion by IBMX, Cl^- addition results in a transient negative deflection in I_{sc} and PD. Table II shows the mean results of experiments of this type. As was observed prior to IBMX addition, serosal Cl^- was without effect. The fact that IBMX changes the pattern of Cl^--induced electrical responses suggests that these agents or the induced alkali secretion interact with Cl^--transport pathways. This issue was addressed in the next set of experiments.

Effect of IBMX + cp-cAMP on Cl^- reabsorption

Post-absorptive turtles. Under pH-stat conditions in Cl^--free media, with the mucosal fluid set at pH 4–5 to inhibit acidification (after the method of Leslie et al. [3]), the addition of IBMX and cp-cAMP has been found to induce a rapidly developing and equivalent increase in the positive I_{sc} and luminal alkali titration rate in post-absorptive and alkalotic bladders but not in acidotic bladders [13]. On this basis, it is concluded that the IBMX and cp-cAMP induced decrease in the negative I_{sc} of post-absorptive turtles is due to stimulation of the alkalinization current and not to inhibition of the acidification current [13].

Since the data of Leslie et al. [3] are consistent with the hypothesis that Cl^- reabsorption occurs by obligatory exchange with HCO_3^- secretion, it would be predicted from that hypothesis that Cl^- reabsorption must be increased after the addition of IBMX and cp-cAMP. However, this was not the case. The serosal addition of (IBMX + cp-cAMP)

TABLE II

THE MEAN (±S.E.) MAXIMUM VALUES OF THE TRANSIENT EFFECT OF MUCOSAL Cl^- ADDITION (16 mM) ON THE I_{sc}, PD AND R ACROSS BLADDERS FROM ALKALOTIC TURTLES, FOR BOTH BEFORE AND AFTER IBMX + cp-cAMP INDUCED STIMULATION OF THE POSITIVE (ALKALINIZATION) I_{sc}

MID and sign convention as described for Table I.

Before stimulation	$\overline{I_{sc}}$ (μA)	$\overline{PD}$ (mV)	$\overline{R}$ (kΩ)	n
Control	0.3±0.6	2.8±3.1	5.1±2.5	19
+Cl^- (m)	11.1±1.8	27.2±3.7	2.6±0.2	
Δ (MID)	10.6±1.6 *	24.3±2.7 *	−2.6±0.3 *	
After Stimulation	$\overline{I_{sc}}$ (μA)	$\overline{PD}$ (mV)	$\overline{R}$ (kΩ)	n
Control	23.3±3.3	51.2±4.9	2.6±0.2	14
+Cl^- (m)	9.5±2.9	17.7±4.6	1.9±0.2	
Δ (MID)	−12.9±2.9 *	−32.3±3.4 *	−0.6±0.1 *	

* $p < 0.001$.

in post-absorptive bladders was followed by decreases in the negative I_{sc}, along with decreases in net Cl^- flux ($J_{net}^{Cl^-}$). The mean values of the individual decrements in these parameters were similar in magnitude (Table III).

Alkalotic turtles. According to the hypothesis of Leslie et al. [3] the mucosal addition of Cl^- initiates an active transepithelial electroneutral exchange of Cl^- for HCO_3^- and thereby increases the luminal alkalinization rate (HCO_3^- secretion). On the other hand, the data shown in Fig. 4 support a hypothesis holding that the mucosal addition of Cl^- transiently increases an active electrogenic HCO_3^- secretion. These hypotheses have as a common base the prediction that Cl^- does interact with HCO_3^- secretion. However, the results on the effect of IBMX on Cl^- reabsorption in post-absorptive turtles (Table III) is explicable by assuming that the stimulation of HCO_3^- secretion can be accompanied by a decrease in Cl^- reabsorption, a finding which is difficult to explain in terms of obligatory transepithelial electroneutral $Cl : HCO_3^-$ exchanging. This assumption was verified by the observed effect of IBMX and cp-cAMP on the simultaneously determined rates of Cl^- reabsorption and luminal alkalinization in alkalotic bladders (Fig. 6). In three of the alkalotic bladders, the change of the mean values of the transport parameters (in $\mu A/9\ cm^2$) before and after IBMX and cp-cAMP addition were the following: (i) I_{sc}, from 16 ± 7.2 to 220 ± 26; (ii) $J_{ms}^{Cl^-}$, from −106 ± 27 to −36.7 ± 5.2; and (iii) $J_{sm}^{HCO_3^-}$, from 118 ± 26 to 208 ± 28. In three other bladders the Cl^- backflux was 22.7 ± 1.8 and 24.0 ± 1.5

TABLE III

THE EFFECT OF IBMX (10^{-4} M) AND cp-cAMP (10^{-3} M) ON THE MEAN (±S.E.) I_{sc}, NET Cl^- REABSORPTION ($J_{net}^{Cl^-}$), Cl^- BACKFLUX ($J_{sm}^{Cl^-}$), AND R IN SIX MATED HALF BLADDERS FROM POST-ABSORPTIVE TURTLES BATHED ON BOTH SURFACES BY Cl^--RINGER

MID and sign convention as described for Table I.

	I_{sc} (μA)	$J_{net}^{Cl^-}$ (μA)	$J_{sm}^{Cl^-}$ (μA)	R (kΩ)
Before	−21.5±8.3	−16.5±5.6	3.2±0.5	1.73±0.17
After	−14.0±6.5	−3.6±2.1	6.0±1.3	1.22±0.17
Δ (MID)	14.2±4.7 *	12.9±3.9 *	2.8±0.9 *	0.49±0.11 *

* $p < 0.05$.

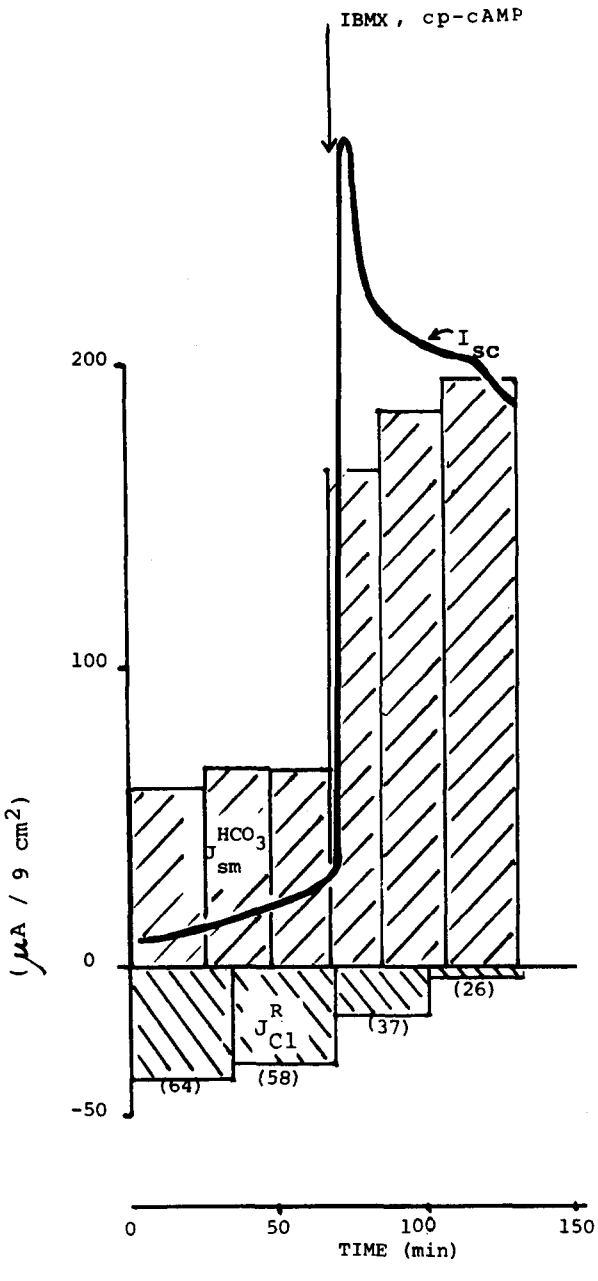

Fig. 6. The effect of the serosal addition of IBMX (10^{-4} M) and cp-cAMP (10^{-3} M) on the Cl^- reabsorption ($J_r^{Cl^-}$), I_{sc} and mucosal pH stat rate ($J_{sm}^{HCO_3^-}$) in a bladder from an alkalotic turtle. $J_r^{Cl^-}$ was estimated from the m to s Cl^- flux (numbers in parentheses) for the bladder depicted minus the average s to m Cl^- flux in bladders from other turtles (see text). Sign convention as described for Fig. 1.

$\mu A/9$ cm^2 before and after IBMX and cp-cAMP, respectively. Although the reduction in Cl^- reabsorption is apparent in these cases, in two other bladders no decrease was observed (45.0 ± 13.0 before and 43.5 ± 13.5 $\mu A/9$ cm^2 after). Such an inconsistency in inhibition of Cl^- reabsorption has been found previously in studies on the effects of disulfonic stilbenes and in those studies it was also found that the insensitivity of Cl^- reabsorption to the agent was found in the group of bladders reabsorbing Cl^- at lower rates [15].

Discussion

The results obtained from this study on the Cl^--induced alterations of transepithelial ion transport as determined by electrophysiological techniques demonstrate that the acid-base states (electrolyte balance) of the donor turtle alter the functional presence of Cl^--selective pathways in the bladder cell membrane. These physiologically induced alterations provide a tool with which to dissect the processes of, and interactions between, Cl^--reabsorption, acidification and alkalinization. In this respect, it is found that the operationally defined placement of conductive and non-conductive Cl^- and HCO_3^- selective transport pathways for each particular bladder type results in the development of a general model which not only resolves a long standing discrepancy about the basic nature of Cl^- reabsorption in the bladder but also provides a foundation upon which further investigations can be logically formulated. It will be shown that when the necessary elements are included to represent the responses of the post-absorptive and acidotic bladders, the derived model cannot account for an electrogenic alkalinization current. However it is found that when the operational placement of transport elements are separately accomplished for the acidotic and then alkalotic bladders, the parallel combination of these two models can successfully account for the conductive characteristics of acidification and alkalinization, and the conductive and non-conductive characteristics of Cl^- reabsorption observed in post-absorptive turtles.

The addition of Cl^- to either the mucosal or serosal fluid of short circuited post-absorptive bladders results in closely similar increments of

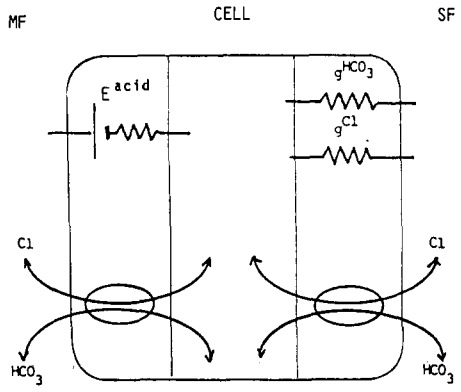

Fig. 7. A model of the turtle bladder epithelial cell containing in the apical membrane an electrogenic acidification pump (E^{acid}) and a $Cl^-:HCO_3^-$ exchanger. The serosal membrane is depicted as containing a conductive HCO_3^- path ($g^{HCO_3^-}$), conductive Cl^- path (g^{Cl^-}) and a $Cl^-:HCO_3^-$ exchanger.

negative I_{sc} (Fig. 1, Table I). On the basis of these results, a preliminary simplifying assumption is made that Cl^- transfer across either cell membrane stimulates luminal acidification, the sustained increment of which results in the steady-state increment in negative I_{sc}. A Cl^--induced activation of the electrogenic acidification pathway is consistent with the fact that the direction of the serosal Cl^--induced increment in I_{sc} is opposite to that of a Cl^--diffusion current. Fig. 7 presents a model which is capable of replicating the observed Cl^--induced changes in I_{sc} in post-absorptive bladders. First, two components are necessary to account for the finite acidification current which has been found in the absence of exogenous Cl^- [7,8]. These are an electrogenic luminal acidification pump * (E^{acid}) in the apical membrane and a HCO_3^--selective conductance path in the basal-lateral membrane. Additional components, an electroneutral $Cl^-:HCO_3^-$ exchanger and a Cl^--selective conductance path (g^{Cl^-}), must be added to the serosal membrane in order to account for the serosal Cl^--induced increment of negative I_{sc}. Thus in this model, the serosal addition of Cl^- initiates a turnover of the $Cl^-:HCO_3^-$ exchanger, reducing the cellular HCO_3^- concentration, and thereby accelerating the luminal acidification rate across the apical membrane producing the observed increment in I_{sc}. In the steady state, this increment in current must be carried by Cl^- diffusion out of the cell through its serosal conductance pathway. An anion exchange-induced increment of acidification has been suggested in brief for the turtle bladder [9,10] and recently for the mammalian collecting duct [11]. To account, in a similar manner, for the presently observed mucosal Cl^--induced increment in I_{sc} it is necessary to place a $Cl^-:HCO_3^-$ exchanger in the apical membrane (Fig. 7). The mucosal addition of Cl^- will then cause HCO_3^- exit from the cell, and thereby increase the steady-state I_{sc} as described above for serosal Cl^- addition. Three characteristics of post-absorptive bladders satisfied by this model are (i) mucosal or serosal Cl^--induced increments in I_{sc} which are similar in magnitude (Table I). (ii) After Cl^- addition to one side increases the I_{sc} the subsequent contra-lateral addition will have little or no effect (Fig. 2). (iii) The symmetrical addition of Cl^- to both media will not necessarily be equal to the rate of Cl^- reabsorption [7]. (iv) Active Cl^- reabsorption [1] effected by secondary coupling to HCO_3^- exit through the luminal exchanger or by assuming that the exchanger per se is active.

An important feature of this model is the prediction of two apparently incompatible results, one of which was explained by invoking electrogenic Cl^- reabsorption [2], and the other, by invoking a transepithelial electroneutral $Cl^-:HCO_3^-$ exchange [3]. In the first of these studies, Gonzalez et al. [2] had shown that the I_{sc} across bladders bathed by $HCO_3^- + Cl^-$ media exceeded the rate of Cl^- reabsorption; and when bathed by HCO_3^--poor mucosal fluids was found to approximate the rate of Cl^- reabsorption. In the model (Fig. 7), a fraction of the luminal acidification current is recycled back to the luminal fluid through the exchange element in the apical membrane. To complete the transepithelial transfer of charge, the electrical equivalent of this current fraction must be carried by the transfer of Cl^- across the serosal

* The identity of the transported ion in either luminal acidification (H secretion [5] or HCO_3^- reabsorption [4]) or luminal alkalinization (H^+ reabsorption or HCO_3^- secretion [13,14]) has not been rigorously established. Thus the phenomenological terms will be used in this discussion. The identity of the transported ion has no effect on the present analysis as both processes are electrogenic.

conductance pathway, or by HCO_3^- recycling between its serosal conductive pathway and the serosal $Cl^- : HCO_3^-$ exchanger. The required consequence is that the I_{sc} exceeds Cl^- reabsorption, which was demonstrated [2]. Furthermore, the model predicts that when the mucosal fluid is diminished in HCO_3^- (and CO_2) the acidification current will decrease, and as the fraction of the recycled current across the apical membrane approaches one, the I_{sc} will approximate the rate of Cl^- reabsorption in accordance with the experimental findings [2]. In the second study, Leslie et al. [3] had found that when the mucosal pH is lowered to the point at which the I_{sc} becomes zero, the subsequent addition of serosal HCO_3^- initiates net Cl^- reabsorption which approximates the rate of luminal alkali titration. In the model, the pH-induced cessation of luminal acidification eliminates current flow across the apical membrane and consequently, the I_{sc} must be zero. Cl^- reabsorption through the luminal and serosal exchangers will then equal HCO_3^- secretion through the same exchangers, resulting in a transepithelial electroneutral exchange of Cl^- for HCO_3^-. This model will therefore satisfy data which indicates that the apparent electrogenic Cl^- reabsorption [2] as well as other data which indicates an apparent transepithelial electroneutral Cl^- reabsorption [3].

With respect to acidotic bladders, the serosal addition of Cl^- increases the I_{sc}, while mucosal Cl^- addition does not (Table I). For the model (Fig. 7) one is forced to invoke a loss of the luminal $Cl^- : HCO_3^-$ exchange process. This decrease of a Cl^- entry path is consistent with the decreased rates of Cl^- reabsorption in the acidiotic bladders, as found by Cohen [12] and our failure to observe ($n = 4$) Cl^- reabsorption in these bladders.

Although the model is consistent with those important characteristics of Cl^- transport and the effects of Cl^- on the I_{sc} in bladders from post-absorptive and acidotic turtles, it has two major short-comings which are sufficient to render it incomplete. First, there are no provisions to account for electrogenic alkalinization after IBMX or cAMP addition [13,14,16] or for the positive levels of I_{sc} observed in Na^+-free media induced by disulfonic stilbenes [15,17]. Consequently, this model cannot account for the presently observed effects of Cl^- addition on the electrical parameters of alkalotic bladders. Secondly, the presence of the conductive Cl^- exit path in the serosal membrane prevents the model from accounting for the critical finding of Leslie et al. [3] that Cl^- reabsorption is dependent upon serosal HCO_3^-. Since the magnitude of the serosal Cl^- conductive path is sufficient to accommodate significant increments in negative I_{sc}, a significant Cl^- reabsorption should be observed in the absence of serosal HCO_3^-. This has as yet not been found to occur [3,15]. In order to develop a model sufficient to account for phenomena observed in all groups of bladders, we will first include the minimal number of elements necessary to account for the characteristics of acidotic

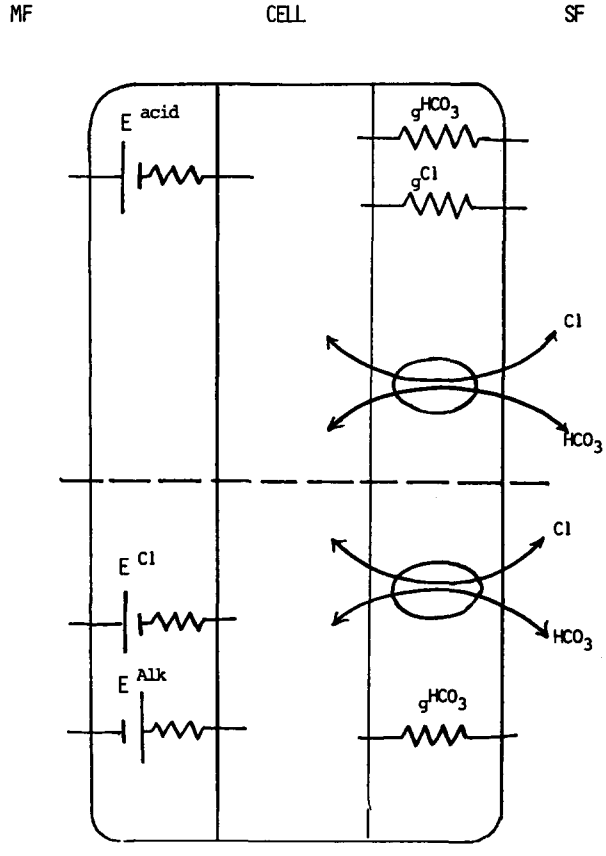

Fig. 8. A model of the turtle bladder epithelial cell with the portion above the dotted line representing the characteristics of an acidotic bladder, and those below the dotted line those of an alkalotic bladder. E^{alk}, electrogenic alkalinization pump; E^{Cl^-}, electrogenic Cl^- reabsorptive pump. Other elements as described for Fig. 7.

bladders and then add those required to account for the distinctly different transport characteristics of alkalotic bladders.

The ideal acidotic bladder is represented with the circuit elements depicted above the dotted line in Fig. 8. The negative I_{sc} will be stimulated by serosal Cl^- through the process described for Fig. 7. Consistent with the data on these bladders, the model predicts; a serosal but not a mucosal Cl^--induced increment in I_{sc} and a finite I_{sc} in the absence of Cl^- (Fig. 3); no conductive alkali secretion [13] and diminished [12] or no Cl^- reabsorption. In general, the ideal acidotic bladder therefore will not be poised to reabsorb Cl^- or to alkalinize the luminal fluid, but will be poised for acidification which is consistent with the low pH and high Cl^- concentrations of blood and urine found in these animals.

The alkalotic bladder lacks the ability to produce a significant negative I_{sc}, and after addition of IBMX, produces a positive I_{sc} which approximates the rate of luminal alkali titration [13]. In order to account for this last phenomenon, it is necessary to include elements which can allow for an electrogenic alkalinization current. This is accomplished by an electrogenic alkalinization pump (E^{alk}) in the luminal membrane and a passive HCO_3^- conductance in the serosal membrane, as depicted below the dotted line in Fig. 8. This is the reverse of, but analogous to, the usual representation of the acidification process.

The explanation of the effect of mucosal Cl^- (and of Cl^- reabsorption) in these bladders is derived on the basis of the following findings. Prior to stimulation of the positive I_{sc}, the mucosal addition of Cl^- results in a transient biphasic response (the second phase of which mimics the effects of IBMX) and subsequent to stimulation of the positive I_{sc} by IBMX, the same mucosal addition of Cl^- is followed by a negative transient in I_{sc}. These responses are represented by a luminal membrane which contains an electrogenic Cl^- pump (E^{Cl^-}) and its series conductance element, in parallel with (but opposite in polarity to) the aforementioned HCO_3^- secretory pump. With respect to Cl^- exit from the cell, the restriction that Cl^- reabsorption must be dependent upon serosal HCO_3^- is satisfied by invoking $Cl^- : HCO_3^-$ exchange mediated Cl^- exit across the serosal membrane. These items are depicted in the lower panel of Fig. 8.

The lower portion of the model allows for the prediction of the following findings. In the ideal alkalotic bladder bathed by Cl^--free, HCO_3^--rich media, the I_{sc} is positive but small (Ref. 13, and Fig. 4), limited by the value of $g^{HCO_3^-}$ and E^{alk}. Subsequent to an IBMX or cAMP induced increment in either E^{alk} or $g^{HCO_3^-}$, the I_{sc} will increase to a higher positive level, R will decrease, and under pH stat conditions, luminal alkali titration will approximate this I_{sc} as is observed [13,14]. Prior to this stimulation, luminal Cl^- addition will first produce a negative current through the activation of E^{Cl^-} and second, through an increase in cell HCO_3^- (due to the exchange facilitated Cl^- exit from the cell) an increase in positive I_{sc}. Since only HCO_3^- can carry current across the serosal membrane, there is a forced electrical coupling between the two apical pumps resulting in a return of the positive I_{sc} toward control levels. This coupling at the transcellular level thus produces the transepithelial finding of similar rates of Cl^- reabsorption and alkali secretion (Ref. 13; and Fig. 6). Subsequent to the IBMX stimulation of the positive I_{sc}, mucosal Cl^- addition will again result in a negative spike of current as described above, but due to the prior stimulation of HCO_3^- secretion, the subsequent balancing between these two ion flows will result in a return to the initial high I_{sc} levels, but not an increase above those levels. These explanations of the Cl^--induced changes in the I_{sc} across the alkalotic bladder indicate that when the transport rate of one path is changed, the rate of the other will also change. The presently reported IBMX and cp-cAMP induced increase in alkalinization current and decrement in Cl^- reabsorption is consistent with such an interaction, predicted in this model as a decrease in the turnover of the serosal $Cl^- : HCO_3^-$ exchange resulting from an IBMX and cp-cAMP induced increase in HCO_3^- flow through the parallel conductance path. With respect to the alkalinization process as depicted in this model, the basal-lateral transfer of HCO_3^- occurs through a conductance path resulting in the observed Cl^--independent secretion of alkali [13].

The ideal alkalotic bladder can thus be represented by the elements depicted in the lower portion of Fig. 8, and this bladder type will be poised

for the secretion of alkali and reabsorption of Cl^- and not for luminal acidification all of which are consistent with the high pH and low Cl^- concentration of the plasma and urine of these turtles.

The post-absorptive bladders contain transport properties characteristic of both acidotic and alkalotic bladders. The characteristics in common with the acidotic bladders are the predominant negative I_{sc} and the serosal Cl^--induced increments in this I_{sc}. The characteristics in common with the alkalotic bladder include active Cl^- reabsorption, an IBMX induced change in I_{sc} in the positive direction; and the fact that under pH stat conditions, the IBMX-induced luminal alkali titration rate approximates the positive I_{sc} [13,14]. Since these bladders have the potential to behave as either type of ideal bladder obtained from the pathological acid-base states, these bladders can be represented with both panels of Fig. 8.

The major predictions from this representation of a post-absorptive bladder are the following. An acidification current which is finite in the absence of Cl^- (Ref. 7, and Table I); and a negative I_{sc} which exceeds Cl^- reabsorption [2] in HCO_3^--rich media due to the parallel addition of the acidification current in the upper panel and Cl^- reabsorption in the lower panel. In the Cl^- reabsorptive path, the current across the serosal membrane is carried by HCO_3^--recycling through its conductive path which is analogous to the aforementioned explanation of Cl^--recycling across the serosal membrane of the acidification pathway. In HCO_3^--poor (and CO_2-poor) mucosal media, the acidification component of the I_{sc} will decrease, and the I_{sc} will approximate the rate of Cl^- reabsorption as was found by Gonzalez et al. [2]. In order to account for the findings of Leslie et al. [3] it is assumed that when the mucosal pH is lowered, current through E^{alk} increases, and electrical coupling between this flow and that of E^{Cl^-} results in the finding that Cl^- reabsorption approximates the luminal alkali titration rate. The effects of IBMX and cp-cAMP on the I_{sc} and Cl^- reabsorption are the same as that given above for the alkalotic bladder. This localization of the effects of these agents on the elements of the lower panel are in harmony with one of the conclusions drawn by Satake et al. [13,14], namely that IBMX and cp-cAMP increase alkalinization but do not inhibit acidification. The mucosal Cl^--induced increment in negative I_{sc} according to this model will be due to the initiation of Cl^--reabsorption across the luminal membrane, while the serosal Cl^--induced increment is due to an increase in the luminal acidification rate, as described above for the acidotic bladder.

In general, the model dictates that bladders from the post absorptive turtles will be poised to reabsorb Cl^-, and reabsorb or secrete HCO_3^- depending upon the particular needs of the animal. Usually, it is found that acidification and Cl^- reabsorption predominate in these bladders, which in the absence of IBMX and cp-cAMP, have a negative I_{sc}. Due to uncontrolled in-vivo requirements, however, some of the bladders may or may not reabsorb Cl^-, which is consistent with the experience (in this laboratory at least) of finding that some bladders reabsorb Cl^- and some do not, despite the lack of obvious dissimilarities between such bladders or between their donor turtles.

One feature of this model is that it provides a systematic basis upon which to analyze the known effects of disulfonic stilbenes on ion transport in the turtle bladder. Although the inhibitory effects of disulfonic stilbenes on net anion (conductive) and exchange transport in the red blood cell has been readily investigated [18,19], the mechanism(s) of the inhibitory effect of these agents on acidification and Cl^- reabsorption in the bladder has remained undefined. This lack of an explanation for these effects was due to the absence of specific information or even a preliminary postulate on the role or type of transmembrane anion pathways involved in these processes. It had been suggested by several investigators that SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic) inhibited acidification and the negative I_{sc} by blocking some undefined basal-lateral anion pathway [15,20,21]. On the basis of the model in Fig. 8, it is now possible to make a somewhat more precise analysis of the site of this SITS blockade. With the assumption that the Cl^- exchange and conductive paths depicted in Fig. 8 are similar to those of the red blood cell, it can be suggested from Knauf et al. [19] that SITS is a more potent inhibitor of the exchangers than of the conductive paths. It would therefore be expected that acidification and Cl^- reabsorption would be more sensitive to SITS than

would alkalinization. Consistent with this expectation, 10^{-4} M SITS in the serosal fluid has been found to decrease luminal acidification and high rates of Cl^- reabsorption [15] but not luminal alkalinization [13]. Preliminary experiments have also been reported in which it was found that in Cl^--free media, SITS retains the capability of inhibiting acidification [13]. In the context of the present model, this effect is envisaged as a SITS-induced decrease in HCO_3^- conductance of the basal-lateral membrane.

It is important to note that the assumed separation or isolation of the transport functions depicted in Fig. 8 has been found necessary in order to produce a model epithelium which can replicate the electrical characteristics of ion transport functions of the three types of bladders. Recent evidence resulting from different experimental approaches has led Husted et al. [22], Schwartz et al. [23] and Gluck et al. [24] to postulate that the non-granular cell of the bladder epithelium is responsible for luminal acidification. With respect to that postulate, the elements depicted in the upper panel of Fig. 8 would reside in that cell type.

There are several concepts arising from the present investigation. The first is that each of the original hypotheses of electrogenic [2] and electroneutral [3] Cl^- reabsorption in the turtle bladder represent the transepithelial manifestation of particular transmembrane characteristics of Cl^- transport. They clearly are not mutually exclusive and both sets of data must be considered in any description of Cl^- transport by the turtle bladder. Another is that serosal transmembrane $Cl^- : HCO_3^-$ exchange participates in the luminal acidification process and remains intact during acidosis even though Cl^- reabsorption is decreased in that condition. Finally, it can be seen that although Cl^- reabsorption interacts with alkalinization (HCO_3^- secretion) these two processes are not obligatorily coupled at both membranes, and thus HCO_3^- secretion can be elicited in the absence of Cl^- reabsorption.

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