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STUDIES ON THE ELECTRICAL CHARACTERISTICS OF CHLORIDE REABSORPTION AND BICARBONATE SECRETION BY THE TURTLE BLADDER

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BY

JOHN HENRY COLLIER DURHAM

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The City University of New York.

1981

This manuscript has been read and accepted for the Graduate Faculty in Biomedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

4 Dec 1981 William A. Brodsky
date Chairman of Examining Committee

8 Dec. 1981
date Executive Officer

Patrick Eggena
Dr. Patrick Eggena

Dr. Rene Gibson

Dr. Peter Reinach

Herm Wyssbrod
Dr. Herman Wyssbrod

Dr. Theodore Schilb

Supervisory Committee

The City University of New York

ABSTRACT

STUDIES ON THE ELECTRICAL CHARACTERISTICS OF CHLORIDE REABSORPTION AND BICARBONATE SECRETION BY THE TURTLE BLADDER

BY

JOHN HENRY COLLIER DURHAM

Advisor: Dr. William A. Brodsky

The turtle bladder (Pseudemys scripta) actively reabsorbs Cl and HCO_3, and secretes HCO_3. The relationship between this Cl reabsorption and electrical characteristics of the short circuited bladder was examined in order to ascertain whether Cl reabsorption occurs via an electrogenic mechanism. It was found that the addition and removal of Cl to and from the bathing media caused decreases and increases in the short-circuiting current (I_{sc}) across bladders bathed by Na-free, HCO_3-containing media. There was a small but significant correlation between the magnitude of this Cl-induced I_{sc} and that of the Cl reabsorption.

In 11 of 27 experiments, the value of the Cl-induced I_{sc} was found to be similar to that of the Cl-reabsorption. In 12 others of the 27 experiments, the value of the Cl-induced I_{sc} was found to be less than that of the Cl-reabsorption. These results can not be accounted for by a hypothesis in which it is assumed that Cl-reabsorption does not affect the simultaneous electrogenic transport of other ions.

In this connection, it was found that the bladder apparently possesses a mechanism for electrogenic HCO_3 secretion. Agents which stimulate this secretion were found to inhibit Cl-reabsorption, indicating that Cl reabsorption and HCO_3 secretion are not obligatorally coupled.

Using the assumption that the electrogenic reabsorption of Cl does affect the electrogenic secretion and reabsorption of HCO_3, an equivalent circuit representing the reabsorption of Cl, HCO_3, and the secretion of HCO_3 as the result of parallel electrogenic flows of these ions was constructed. This circuit model would qualitatively account for the relationship between the Cl-induced I_{sc} and the Cl reabsorption found in the majority of the bladders studied.

ACKNOWLEDGMENTS

First, there is no doubt that I thank Dr. William A. Brodsky, who made my research training both productive and sound. He accomplished this by generously providing not only the necessary physical facilities, but most importantly by producing an environment within which one can experience the principles and practice of empirical investigation.

I would like to thank Dr. Gerhardt Ehrenspeck for taking the time to teach me the fundamentals of research procedures, approaches, and analyses, and for allowing me to work with him.

Furthermore, I thank Ms. Christina Matons for patiently introducing me to the methods and procedures used in this laboratory, and for assistance with this project.

Acknowledgment is also due to Ms. Susan Ehrenspeck for assistance in the early part of this work; Mr. Phillip Cook for suggestions and donation of his expertise in electronics and glassblowing; Ms. Helen Washington for typing and manuscript preparation; and Mr. George Cortes for performing the necessary animal care.

I would also like to mention the value of the many helpful discussions I had with Dr. Peter Reinach and Dr. Herman Wyssbrod concerning this work and general background subjects.

Acknowledgment is also due to Dr. Steven Youmans and Dr. Howard Schoen for many discussions and suggestions.

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INTRODUCTION

1. The general objective of the research findings presented in this thesis has been to determine whether the active reabsorption of chloride by the turtle bladder is an independent electrogenic process or a bicarbonate-dependent electroneutral process by which Cl is reabsorbed only in an exchange with HCO_3 which is secreted.

2. The physiological significance of this problem is its apparently direct relationship to the renal maintenance of the osmolality, and acid-base balance of body fluids.

3. The approach to this problem in the present work is almost entirely mechanistic - focusing more on the intrinsic nature of the ion transport processes (or pump mechanisms) than on the regulation of these processes by hormones, neurotransmitters, or drugs. Nevertheless, possible regulatory aspects are dealt with to a limited extent of the present work insofar as the anion transport rates are influenced by phosphodiesterase inhibitors and cyclic nucleotides. These agents are used in the present work as

convenient tools with which one can not only regulate transport rates but gain additional insight into the intrinsic nature of the anion transport processes.

4. It should be noted that (i) the sodium reabsorptive mechanism in the turtle bladder (like that in the mammalian nephron) is the dominant but not the only active transport mechanism of this epithelium; and that (ii) the chloride reabsorptive mechanism in contrast to that found in gastro-intestinal and other epithelia (Frizzell et al. 1978) is sodium-independent and ouabain-resistant.

Because of these characteristics, it is useful first to provide the reader with a short background of the past and current contributions to our knowledge of the transport functions of the isolated turtle bladder.

STATE OF THE FIELD

1. BASIS FOR THE PRESENT WORK.

(a) It has been established and generally accepted that the isolated urinary bladder of *Pseudemys scripta* turtles (like certain more distal segments of the mammalian nephron) reabsorbs Na, Cl, HCO_3 and water from the lumen and, under certain conditions, secretes HCO_3 into the lumen. Moreover, the active nature of each of the aforementioned ion translocations (with the possible exception of HCO_3 secretion) along with the electrogenicity of the Na and HCO_3 (or H) translocations, has also been established.

(b) There remain gaps however in the present knowledge of how Cl is actively reabsorbed and how HCO_3 is actively secreted. These informational gaps have led more to the development of divergent hypotheses than to the experimental production of new and needed data - a condition which is now being approached and to some extent ameliorated by recent findings in this laboratory.

2. BACKGROUND INFORMATION

a) Sodium reabsorption. When the turtle bladder is isolated in the form of a sheet and interposed between two identical solutions containing Na and Cl, a spontaneous potential difference (PD) is produced such that the serosal solution is electropositive to the mucosal solution. When an electric current from an external source is sent across such a bladder in a quantity sufficient to reduce the PD to zero, the magnitude of this externally-delivered current (in this case, the so-called short-circuiting current, or I_{sc}) is found to be equal to that of the isotopically measured net Na reabsorption. On the basis of such findings, it has been concluded that Na-reabsoption across the bladder is driven by an active electrogenic mechanism (Klahr and Bricker, 1964).

Independent evidence for the active nature of Na reabsorption, along with evidence for Cl and HCO_3 reabsorption has been found when no external electric current is passed across the tissue. Turtle bladders, isolated in the form of a sac bathed on both sides by media rich in Na, Cl and HCO_3 were also found to maintain a PD on the order of 60-120 mV with the serosal solution electropositive to the mucosal solution (Brodsky and Schilb, 1966). Following the substitution of choline for Na in these bathing media, the orientation of the PD becomes reversed and the serosal fluid is maintained electronegative to the mucosal solution by 10-90 mV (Schilb and Brodsky, 1966).

This latter finding indicates that active electrogenic transport mechanisms, other than and independent from that for Na, co-exist with that for Na in the bladder. Further evidence for the presence of independently operating, non-sodium transport mechanisms is provided by experiments in which it was found that the magnitude of the I_{sc} across bladders bathed by media containing HCO_3 as well as Na and Cl was less than that of the concomitantly measured net Na reabsorption (Gonzalez et al. 1967), but equal to that of the net Na reabsorption in bladders bathed on the mucosal surface by media devoid of Cl and HCO_3 (Gonzalez et al. 1967b). Finally, the serosal addition of ouabain (an inhibitor of Na-K-ATPase) reduced the net transport of Na to zero and reversed the orientation of the I_{sc} and PD across bladders bathed by Na, Cl and HCO_3-containing media (Solinger et al. 1968).

Therefore one is forced to conclude that ions other than Na are actively and electrogenically transported by the turtle bladder, and that Cl and/or HCO_3 reabsorption carry part of the transepithelial I_{sc} in the presence or absence of Na or Na transport.

b) $\underline{HCO_3}$ Reabsorption or H Secretion. Isolated bladder sacs have also been shown to acidify the luminal fluid. When sacs are bathed by Na-containing media, the luminal fluid is acidified while the serosal fluid is electropositive to the mucosal solution. When bathed by Na-free solutions, the luminal fluid is acidified while the serosal fluid is electronegative to the mucosal solution (Schilb and Brodsky, 1966) which indicates that the H accumulation in the luminal fluid occurs by an active process (i.e., against an electrochemical gradient).

This accumulation of H could occur by 3 basic mechanisms: (i) H secretion, (ii) HCO_3 reabsorption and (iii) an exchange of H or HCO_3 for another ion (e.g. a K:H exchange or a $Cl:HCO_3$ exchange). There is no evidence which could be accounted for by assuming that either of the exchange mechanisms acidify the luminal fluid in the turtle bladder. In fact, it is generally accepted that the acidification process results from an active electrogenic ion transport mechanism that operates independently of the transport of ions other than H or HCO_3 (Brodsky and Schilb, 1974; Steinmetz, 1974).

What is not agreed upon is the intrinsic nature of the transport mechanism. On the one hand, data from experiments on bladder sacs can be accounted for only by invoking the reabsorption of HCO_3 (Schilb and Brodsky, 1966, 1972; Schilb, 1978); and certain data from experiments on short-circuited bladders can without invoking additional ad-hoc assumptions, be explained solely by HCO_3 reabsorption (Gonzalez and Schilb, 1969). Other data from bladder sacs or from short-circuited preparations can adequately be accounted for by invoking either HCO_3 reabsorption or H secretion (Steinmetz, 1967; Green et al. 1970; Schwartz et al. 1974; Dixon and Al-Awqati, 1979).

In the present context, it should be noted that no attempt is made to identify the intrinsic nature of the acidification mechanism. Since the electrical properities of H secretion are identical to those of HCO_3 reabsorption, the interpretations of any of the presently reported electrical events are not affected by a lack of knowledge about the underlying nature of the acidification transport mechanism. Therefore, the terms HCO_3 reabsorption and H secretion can be considered interchangable for the present purposes.

c) Cl transport. There are at present two different hypotheses on the mode by which Cl is actively reabsorbed by the turtle bladder: one holds that an active transepithelial flow of Cl is mediated by an electroneutral and transepithelial Cl-for-HCO_3 exchange mechanism; and the other holds that the Cl transport is driven by an electrogenic pump mechanism.

Since each hypothesis is consistent with a specific set of data from which it had been derived, both sets will be discussed.

The active nature of a sodium-independent chloride reabsorption by the turtle bladder was first demonstrated unequivocally in the open-circuited sac preparation (Brodsky and Schilb, 1966). In Na-rich media, the bladder sacs transport NaCl into an electropositive serosal fluid; and in Na-free media, the sacs transport choline chloride into an electronegative serosal fluid. Subsequently obtained data from short-circuited turtle bladders in Na-rich media with or without ouabain (or from bladders in Na-free media) showed that the active transport of chloride is independent of the concomitant active transport of Na and independent of the presence of exogenous Na (Gonzalez et al. 1967a,b; Solinger et al. 1968).

Gonzalez et al. (1967a) found in short-circuited bladder preparations bathed on both surfaces by an identical Na-free, (HCO_3 + Cl)-containing solution, that the magnitude of the short-circuiting current (I_{sc}) exceeds that of the net Cl flux (I^{Cl}_{net}):

$$I_{sc} > I^{Cl}_{net} \qquad (1)$$

Postulating that the I_{sc} is equal to the sum of the two parallel electrogenic ion flows of HCO_3 and Cl, Gonzalez et al (1967a) represented the I_{sc} as the sum of both HCO_3 reabsorption and Cl reabsorption.

$$I_{sc} = I_{net}^{HCO_3} + I_{net}^{Cl} \qquad (2)$$

These authors then examined the relationship between the I_{sc} and I_{net}^{Cl} in bladders bathed by HCO_3-poor, CO_2-poor mucosal fluids, but with (HCO_3 + CO_2)-rich serosal fluids. Under this condition, the parallel flow of current due to HCO_3 reabsorption would be decreased, and the I_{sc} should decrease to approximate the value of I_{net}^{Cl}. This was found to be the case; that is, under these conditions, the relationships described in Eq. (2) above reduced to

$$I_{sc} = I_{net}^{Cl} , \qquad (3)$$

and consequently, Gonzalez et al. (1967a) postulated that Cl transport by the bladder occurred via an electrogenic process.

In the presence of a similar transepithelial HCO_3 gradient, Leslie et al. (1973) and subsequently Himmelstein et al. (1974) found that a net reabsorptive increment in the transepithelial Cl flux: (i) occurred only in the presence of serosal HCO_3, (ii) occurred concomitantly with the appearance of a nearly equal amount of alkali in the mucosal fluid, and

(iii) was decreased after the addition of metabolic inhibitors.

These authors therefore concluded that the net (m to s) transepithelial flow Cl occurs via an electroneutral, energy-dependent, Cl-for-HCO_3 exchange mechanism. It should be noted that in order to measure the net secretion of alkali (item ii, above) gradients of HCO_3 from the serosal fluid to a buffer-poor mucosal fluid must exist. When HCO_3 is present in both bathing fluids and when there is no finite HCO_3 gradient across the tissue, alkali secretion, if present, cannot be measured. In order to make the claim that a one-for-one electroneutral exchange of Cl reabsorption for HCO_3 secretion is indeed the mechanism of active Cl reabsorption, one must make the assumption that the exchange process occurs in the absence of a transepithelal gradient when HCO_3 is present in both mucosal and serosal solutions. Because, however, the presence of a transepithelial HCO_3 gradient was required to measure the alkali secretion in the aforementioned experiments, the proposed Cl:HCO_3 exchange has not been shown to be an active process. This is because the measured equality between Cl reabsorption and HCO_3 secretion has not yet been demonstrated in the absence of transepithelial electrochemical gradients of HCO_3, a fundamental requirement for establishing the active nature of any ion transport (Rosenberg, 1948).

On the basis of the aforementioned studies, neither an electrogenic mechanism nor an electroneutral mechanism alone is sufficient to account for all of the characteristics of the demonstrably active reabsorption of Cl in the turtle bladder. This uncertainty led to the present experiments on the response of the short-circuited bladder to symmetrical changes in Cl concentration in the absence of transepithelial electrochemical gradients.

d) $\underline{HCO_3}$ Secretion. That an increase in HCO_3 secretion occurs upon the initiation of Cl reabsorption had been found by Leslie et al. (1973). Recently, however, new evidence on HCO_3 secretion in the bladder indicates that this process is electrogenic and independent of the presence of Cl (Brodsky et al. 1979; Ehrenspeck, 1981; Satake et al. 1981). Since Cl-reabsorption is dependent upon the presence of serosal HCO_3, and since it appears in part to have electroneutral as well as electrogenic characteristics, the existence of a separate pathway for the electrogenic secretion of HCO_3 requires that an alternative hypothesis accounting for both sets of data should be developed. This possibility will be dealt with below.

METHODS

A) MOUNTING AND ELECTRICAL MEASUREMENTS

Turtle (*Pseudemys scripta*) bladders were excised and mounted between two solutions in a modified Rehm-Ussing chamber in the manner described previously (Gonzalez et al. 1967a). For most experiments the chamber was double-barrelled, and each of the mated hemi-bladders were contained within an exposed chamber area of 1.5 cm^2. The volume of the bathing fluids was 10 ml. In the experiments involving pH stat measurements, a single-barrelled chamber was used, having an area of 9 cm^2 and a bathing fluid volume of 15 ml.

The open-circuit transepithelial potential difference (PD) between the mucosal and serosal bathing solutions was measured with calomel electrodes connected to the bathing solution through an asbestos plug as described by Gonzalez et al. (1967a) or through a liquid-liquid (bathing fluid:sat KCl) junction (see below). The open-circuit resistance (R) across the bladder was measured by sending a rapid (1 sec) pulse of known current across the tissue via lead acetate electrodes, and measuring the current induced change in PD (R= PD/ I).

Throughout the experiment (except for brief intervals in which the PD and R were measured) the transepithelial PD was maintained at zero by sending sufficient external current across the tissue (Ussing and Zerahn, 1951) via the lead acetate electrodes. This short-circuiting current (I_{sc}) was measured by the method described by Gonzales et al. (1967a).

B) ISOTOPIC CHLORIDE FLUX MEASUREMENTS

$H^{36}Cl$ was neutralized by titration with an equal amount of choline HCO_3 , and this solution was then added to the mucosal solution of one hemi-bladder (for determination of the forward flux, I_{ms}^{Cl}) and the serosal solution of the other hemi-bladder (for determining the backward flux, I_{sm}^{Cl}). All of the presently reported values of net Cl reabsorption or I_{net}^{Cl} (I_{ms}^{Cl}-I_{sm}^{Cl}) are compared only with the I_{sc} across the same hemi-bladder in which the forward flux was determined.

The final specific activity of ^{36}Cl in the bathing fluid in which it is initially placed ('hot' side) was 33 uCi/mmol. The rate of appearance of isotope in the opposite bathing solution ('cold' side) was calculated from the number of counts per minute (cpm) contained within 0.5 ml samples from that solution, collected at 30 minute intervals. Radioactivity was determined by liquid scintillation counting.

Since the method used for changing bathing solutions in the present study could result in an inexact volume replacement prior to isotopic flux determinations, the final exchangable volume of the 'cold' side was determined at the end of each experiment by determining the degree of dilution of cpm per volume of an added aliquot of solution from the 'hot' side.

C) PH STAT EXPERIMENTS

The apparatus for pH statting was a Metrohm pH meter (Model pH-103) with a combination glass/reference electrode and a microsyringe burette (Model SB2, Micrometric Co., Cleveland, Ohio). The pH-statting was performed at 30 minute intervals by titrating the mucosal fluid to its initial pH with microliter volumes of 0.01 N KOH or 0.01 N H_2SO_4. The change in volume of the bathing fluid as a result of adding titrant was no more than 50 ul/15 ml per period.

D) HOUSING OF TURTLES.

For the bladders used in the experiments depicted in Figures 3, and 6-8, and Tables I and III, the donor turtles were kept at room temperature and had free access to water. For all other experiments, donor turtles were kept in a stainless steel tank containing constantly filtered water at a depth of 25cm, and at a temperature of 30°C. Turtles were fed once weekly with beef liver supplemented with vitamins (Poly-Vi-Sol, Mead-Johnson) and bone meal (Bone-All, Schiff). The turtles were exposed to light on a 16 hour a day cycle.

E) SOLUTIONS

A: Na-free, (HCO_3+Cl)-containing medium (final concentration, mM). Choline HCO_3, 20; choline Cl, 25; K_2SO_4, 2; K_2HPO_4, 0.61; KH_2PO_4, 0.14; $CaSO_4$, 2; $MgSO_4$, 0.8; glucose, 11; and sucrose in amounts to make the final osmolality 220 mOsM/kg; equilibrated with 5% CO_2/95% O_2, gas mixture, pH, 7.3.

B: Na-free, Cl-free, HCO_3-containing medium (final concentraion, mM). As in solution A, except that choline Cl was replaced with choline SO_4, 12.5.

C: Na-free, (HCO_3 + Cl)-containing medium for pH stating experiments (final concentration, mM). As in solution B, except that, K_2HPO_4, 0.26; KH_2PO_4, 0.04; equilibrated with a 2% CO_2/98% O_2 gas mixture pH, 7.6.

D: Na-free, Cl-containing, HCO_3-poor medium, for pH statting experiments (final concentration, mM). As in solution C except that choline HCO_3 replaced by choline SO_4, 10 mM; equilibrated with 100% O_2, pH, 7.6.

E: Na-free, Cl-free, HCO_3 poor medium, for pH-statting experiments (final concenraion, mM); As in soluion D, except that choline Cl was replaced by choline SO_4, 12.5 mM.

F) TECHNIQUES FOR ION SUBSTITUTION IN BATHING FLUIDS

The techniques of changing the ionic composition of bathing solutions during an experiment were of 3 types.

(1) For experiments depicted by data shown in Figs. 3 and Table I, half of the bathing volume (5 ml) was removed and replaced with 5 ml of the desired solution, which formed a new solution. Then half the volume of the new solution was removed and replaced with an equal volume of the desired solution to form a second new solution after which 9 consecutive half-replacements were made until the composition of the final bathing solution was within 1/2000th that of the desired solution. Although satisfactory, this procedure proved tedious in order to avoid causing transient oscillations in the hydrostatic pressure difference across the tissue and interrupting circulation of the bathing solutions.

(2) In the second technique the desired solution was passed from a reservoir into the chamber containing 10 ml of original bathing fluid, the level of which was maintained constant by aspiration. The PD-detecting electrodes were connected to the bathing solution via a readily renewable liquid-to-liquid junction (bathing solution-to-saturated KCl) to avoid the occurrence of diffusion potentials in the PD measur-

ing system occasioned by changes in solution composition (see Figure 1). Note that this method of passing a solution from a reservoir, the level of which is higher than that of the bathing fluid, results not in a geometric dilution of the bathing fluid as does the aforementioned method, but in a much faster replacement of the orginal with the desired bathing solution. Though less definable, the degree of depletion or repletion of Cl in the bathing fluids was determined directly by chloridometry so that the degree of approach toward the desired composition could be verified. The effectiveness of this procedure is ascribed to the fact that the incoming solution travels upward in both connecting tubes, effectively pushing the original solution out of the chamber (see Figure 1). Because the aspiration of the bathing fluid decreases its temperature, the use of aspiration was eliminated and a third technique was developed.

(3) In the third technique, the fluid flowing from the reservoir into the chamber was allowed to flow out of the chamber through a horizontal tube located flush with the upper surface of the bathing fluid in that chamber (Figure 2). Except for this outflow feature, the third technique is the same as the second.

FIGURE 1

One-half of the short-circuiting chamber is depicted. The bathing solution is changed by admitting the desired solution from the appropriate reservoir (not shown) by turning stopcock S_1. Simultaneously aspiration of the bathing fluid is initiated by connecting a vacuum to the glass tube located at the top level of the bathing fluid. The incoming solution travels upward through both tubes, and the bubble lift effected by the small gas line in the upper tubing assures that the incoming solution will pass through the small diameter passages leading to the bladder surface. The bathing solution: SAT KCl junction is continuously renewed during the solution change by opening stopcock S_2.

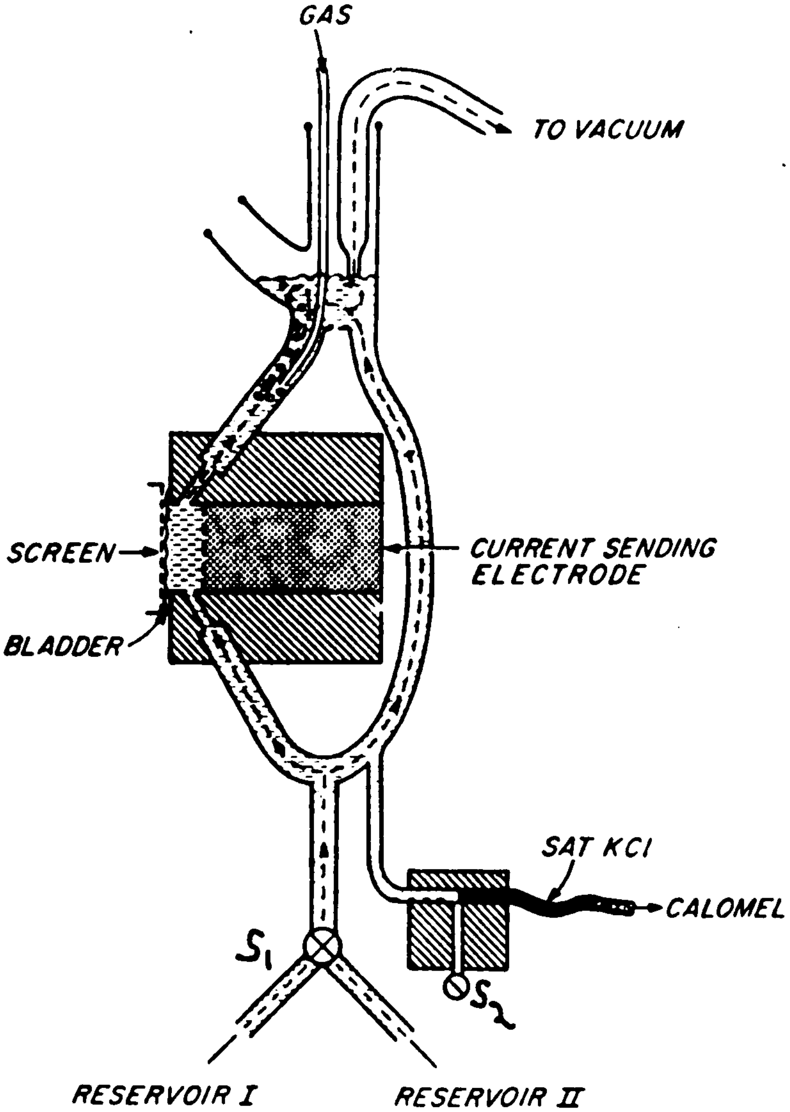
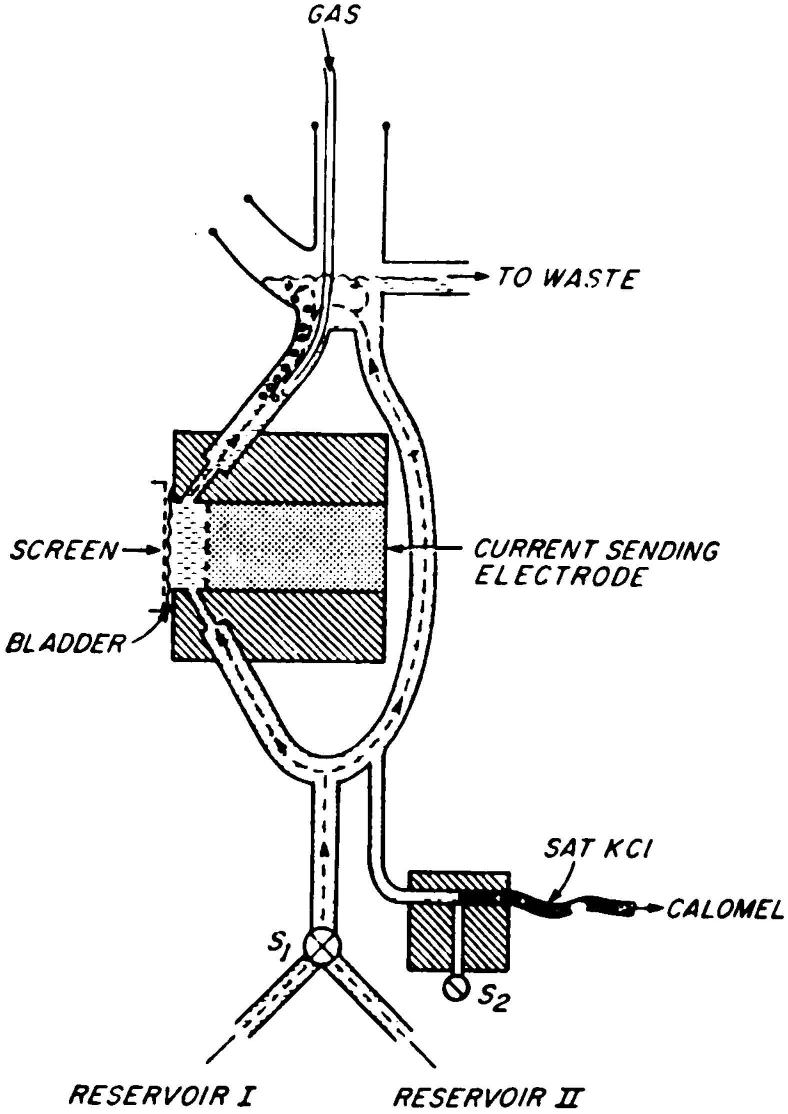

GAS
TO VACUUM
SCREEN
CURRENT SENDING ELECTRODE
BLADDER
SAT KCl
CALOMEL
S1
S2
RESERVOIR I
RESERVOIR II

FIGURE 2

The procedure for changing the bathing solutions is as described for Figure 1. In place of aspiration of the bathing fluid as was used in the method described in Figure 1, the solution was allowed to flow out through the tube located at the surface of the bathing fluid.


GAS
TO WASTE
SCREEN
CURRENT SENDING ELECTRODE
BLADDER
SAT KCl
CALOMEL
S_1
S_2
RESERVOIR I
RESERVOIR II

G) CHLORIDOMETRY

After replacement of the bathing fluids with a solution of different Cl concentration, the chloride concentration of the bathing fluid was determined (Buchler-Cotlove chloridometer). In all experiments Cl was reduced to a level not detectable by the amperometric titration. Thus, in all figures and tables of the present report, '0 mM' Cl concentration signifies that the actual concentration was less than 0.05 mM.

H) SOURCES OF MATERIALS

Turtles were obtained from Lemberger, Milwaukee Wis. The disodium salt of 4-acetamido-4-isothiocyano-2,2-disulphonic stilbene (SITS) was purchased from Pierce Chemical Co., Rockford, Ill., isobutylmethylxanthine (IBMX) from Sigma, St. Louis, Mo., and 8-p-chlorophenyl-cyclic-adenylic monophophoric acid (cp-cAMP) from ICN Radiochemical and Nuclide Division, Irvine, Ca.. Gases were obtained from Union Carbide Corp., Linde Division, Elizabeth, N.J..

RESULTS

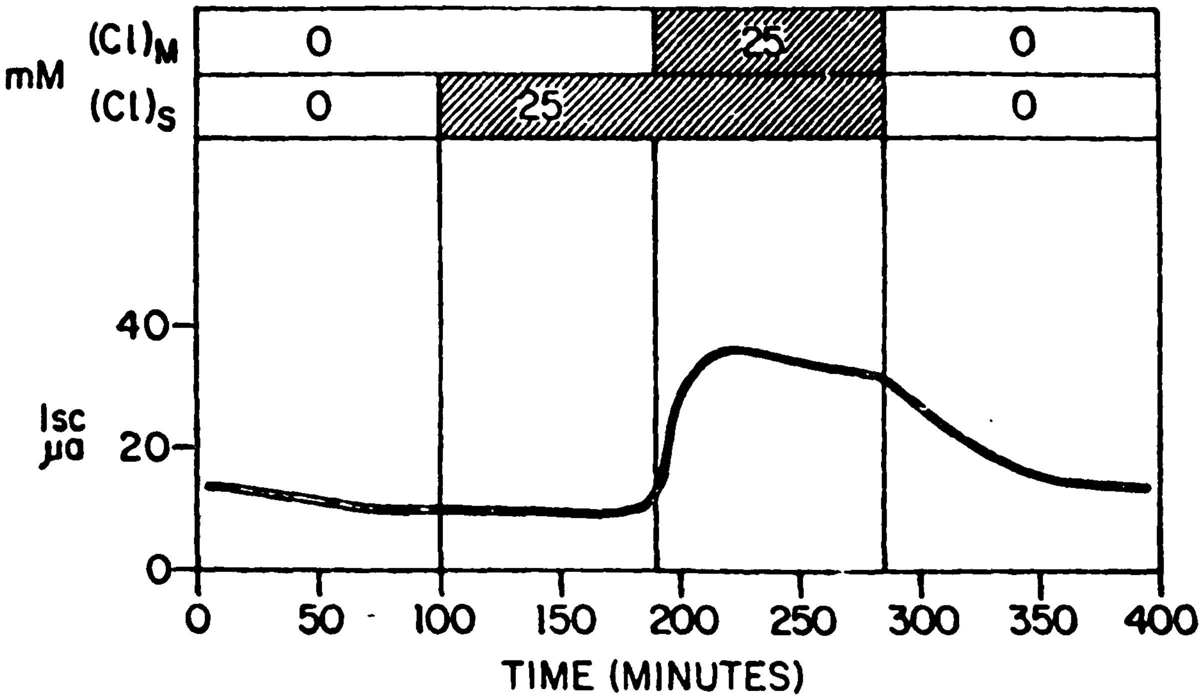
A) CHARACTERISTICS OF CHLORIDE TRANSPORT

If an independently operating electrogenic chloride reabsorption carries an equivalent moiety of the I_{sc} in bladders bathed on both sides by an identical ($Cl+HCO_3$)-rich solution devoid of Na, then the removal and addition of Cl to and from the bathing media should result respectively in a decrease and an increase in the I_{sc}. This prediction was experimentally verified in 7 experiments on bladders initially bathed on both surfaces by Cl-free media (solution B). In one representative experiment (Figure 3), the I_{sc} had reached a steady state in the absence of extracellular Cl and the addition of Cl (via solution A) to the serosal solution caused no change in the I_{sc} for the next 90 min. But the subsequent addition of Cl to the mucosal solution caused an increase in the I_{sc} to a level of about 30 ua. Finally, the removal of Cl from both media resulted in a fall in the I_{sc} to control levels.

FIGURE 3

The effect of the addition of choline-Cl to Cl-free media on the I_{sc} of a turtle bladder.

The bladder was initially bathed on both the mucosal and serosal surfaces by Na-free, HCO_3-rich, Cl-free Ringer's (solution A). The serosal bathing solution was then changed to Cl-containing solution (solution B). Next, Cl was added to the mucosal solution, and the I_{sc} and PD (not shown) increased. Finally, both bathing solutions were changed to Cl-free solution, and the I_{sc} (and PD) fell to control levels. Sign convention is that of positive denoting negative charges flowing from the mucosal to serosal solution.


mM
(Cl)M
(Cl)S
0
25
0
0
25
0
Isc
μa
40
20
0
0
50
100
150
200
250
300
350
400
TIME (MINUTES)

The mean values of I_{sc} and PD for all 7 bladders in Cl-free vs those in Cl-containing media (Table I) show that the Cl-induced increments in these parameters were significant and reproducible. Not shown in the table is the ready reversibility of the chloride-induced changes in I_{sc}. Although these data are predictable from the hypothesis (Gonzalez et al. 1967a) holding that Cl is electrogenically reabsorbed, it remained necessary to demonstrate a one-for-one relationship between the Cl-induced increment in I_{sc} and the net Cl flux. If the hypothesis were correct, then the magnitude of the Cl-induced I_{sc} and the net Cl flux for each bladder should be identical providing that the moiety of I_{sc} carried by HCO_3 (or by any transported ion other than Cl) remains constant during the addition or removal of extracellular Cl. Such an equality was found in 11 out of the next 27 experiments of the aforementioned design. In one of these (Figure 4), the bladder was initially bathed on both surfaces by an identical (HCO_3+Cl)-containing medium devoid of Na (solution A) and under this condition the I_{sc} reached a steady state level of 53 ua. Following the removal of Cl from both the mucosal and serosal solutions (by replacement of solution A with solution B), the I_{sc} began to decrease after a 5 minute interval to reach a steady level of about 43 ua in the next 10-12 minutes. Then Cl was substituted for SO_4 in both the mucosal and sero-

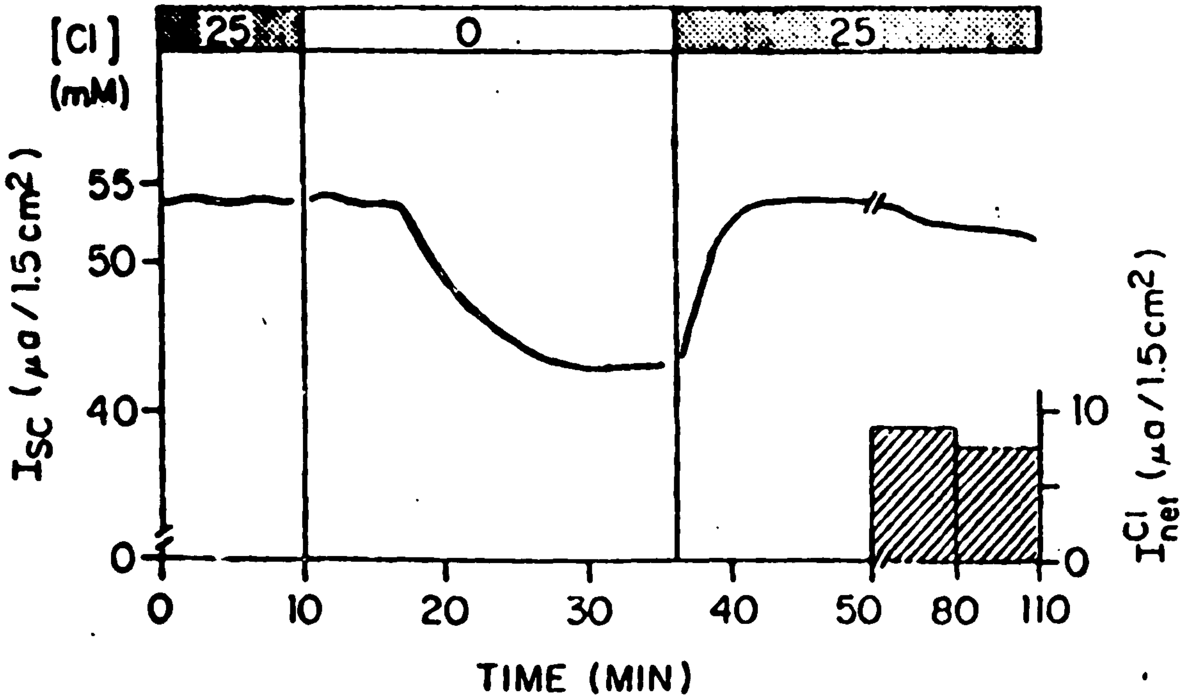
Table I. Effect of addition of Cl to the mucosal solution on the PD and I_{sc} of 7 bladders in HCO_3-rich media.

Cl Conc. (mV)	PD (mV)	I_{sc} (ua)
0	26.8 ± 5.12	15.4 ± 2.24
25	45.9 ± 6.81	40.6 ± 10.2

Bladders were bathed initially on both surfaces by Na-free, HCO_3-rich, Cl-free media (solution B). After a steady state had been attained, Cl was substituted for SO_4 in both solutions (solution B). Isc values are for 1.5 cm^2 of bladder area. Mean value of the 7 increments (not shown) is statistically significant $(P(\Delta = 0) < .01$.

FIGURE 4

The effect of Cl removal from and addition to both the serosal and mucosal bathing media on the Isc of a turtle bladder initially bathed on both surfaces by Na-free, HCO_3-rich, Cl-rich meida (solution A). Open circuit PD (not shown) is that of serosal solution electronegative to the mucosal solution. The I_{sc} and PD decreased (after 5 minute lag period) following the removal of Cl (solution B) from both bathing media, and increased (with little lag time) following the addition of Cl to both bathing media. Net Cl flux was determined after the I_{sc} returned to the control steady state level. Backward flux (s→m) was determined in the mated half bladder. Note that the magnitude of the net Cl flux was similar to that of the increment in I_{sc} following Cl addition to the bathing media.

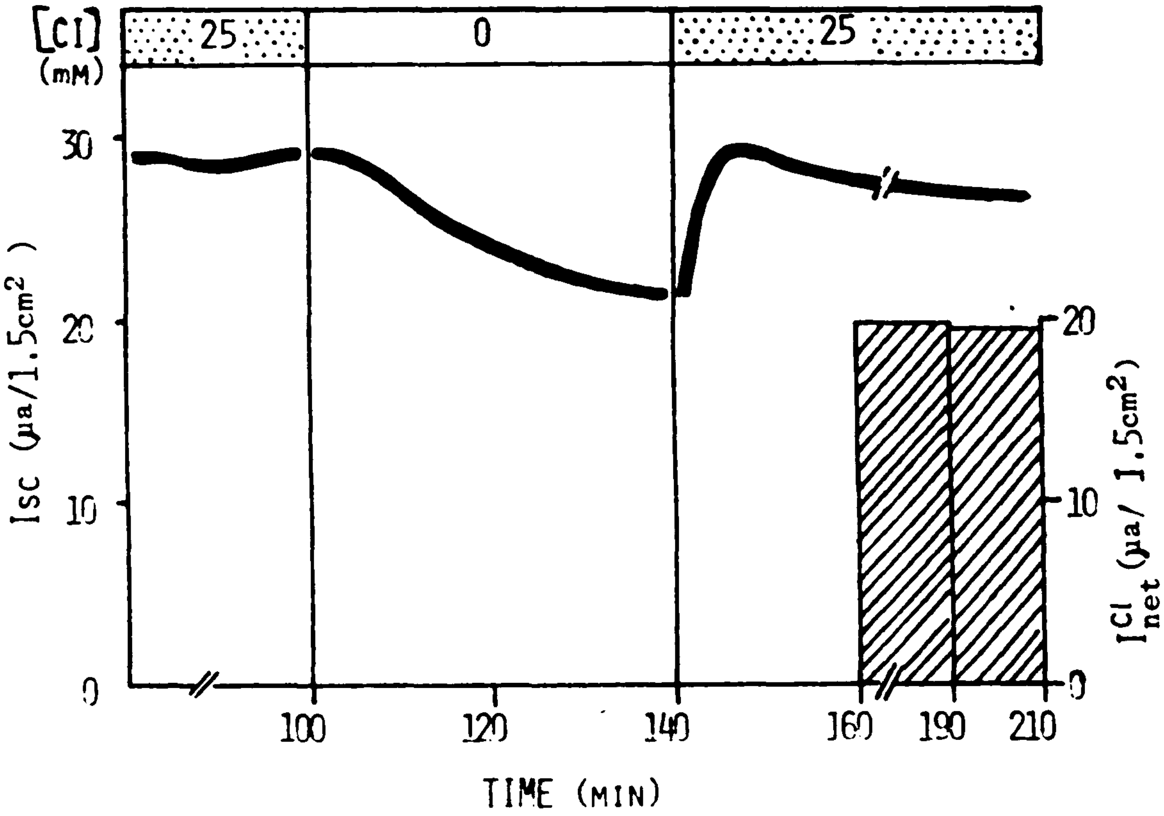

[Cl]
(mM)
25
0
25
I_{SC} (μa/1.5cm^2)
55
50
40
0
I_{net}^{Cl} (μa/1.5cm^2)
10
0
0
10
20
30
40
50
80
110
TIME (MIN)

sal fluids, again keeping the HCO_3 concentration constant. There followed a rapid rise in I_{sc} which returned, after a 10 minute interval, to control levels. Subsequent to this return of I_{sc} to control levels, the forward (m to s) Cl flux was determined in this half-bladder, and the backward (s-to-m) flux was determined in the other (mated) half-bladder. The magnitude of the calculated net reabsorption of Cl (8 ua) was the same as that of the Cl-induced increment in I_{sc} (8 ua) and slightly less than that of the decrement of I_{sc} (10 ua) following Cl removal. So it would appear that the hypothesis of electrogenic Cl transport holds for this experiment.

In other experiments of the aforementioned design, however, inequalities between the Cl-induced changes in I_{sc} and the corresponding Cl reabsorption were found. The most frequently found inequality (12 out of 27 experiments) was that in which the magnitude of the net Cl reabsorption exceeded that of the Cl-induced increment in I_{sc}. In a representative experiment of this kind (Figure 5), the general response of the I_{sc} to the removal and addition of Cl was the same as that described in the previously described experiment (Fig. 4). The value of the net Cl flux (20 ua), however, was over twice that of the Cl-induced change in I_{sc} (8 ua). Clearly the hypothesis holding that Cl transport is a purely electrogenic process does not satisfactorily account for this result.

FIGURE 5

The effect of Cl removal from and addition to both the serosal and mucosal bathing media on the I_{sc} of a turtle bladder. Protocol is the same as described for Figure 4. Note that the magnitude of the net Cl flux was larger than the increment in I_{sc} following Cl-addition to the bathing media.


[Cl] (mM)
25
0
25
Isc (µa/1.5cm²)
0
10
20
30
I^{Cl}_{net} (µa/ 1.5cm²)
0
10
20
100
120
140
160
190
210
TIME (MIN)

Results from all 27 experiments (Table II), indicate that there was a slight but nonetheless significant trend of proportionality between the Cl-induced I_{sc} and the net Cl flux. A more detailed description of the Cl-dependent I_{sc} than that shown in Table II is as follows.

(i) An equality between the Cl-induced I_{sc} and I_{net}^{Cl} occurred in 11 of the 27 experiments;

(ii) The magnitude of the Cl-induced I_{sc} was exceeded by that of the I_{net}^{Cl} in 12 of the 27 experiments

(iii) I_{sc} remained constant ($I_{sc} = 0$) while a significant level of I_{net}^{Cl} was induced in 4 of the 27 experiments.

(iv) A finite Cl-induced increment in I_{sc} occurred when no net flow of Cl was induced by Cl, (i.e. $I_{ms}^{Cl} = I_{sm}^{Cl}$) in 4 of the 27 cases.

Thus neither of the current hypotheses alone (i.e., electrogenic or electroneutral) can account for all of these results.

One of the assumptions concerning the hypothesis of an electrogenic Cl transport is that it is independent of other transport processes. If, however, such an electrogenic translocation of Cl in some way affects the transport of another electrogenically transported ion (or vice versa), then a direct proportionality or even a significant correlation between the Cl-induced I_{sc} and net Cl flux would not be expected. One candidate for such another transport process is that of HCO_3 secretion. Some of the evidence which is needed to support this possibility would be (i) the demonstration of a Cl-induced alkali secretion concomitant with a change in I_{sc}, and (ii) a Cl-independent electrogenic HCO_3 secretion.

B) CL-INDUCED HCO_3 SECRETION WITH ELECTRICAL CHANGES.

In these experiments, Cl was added to a HCO_3-poor mucosal fluid while the bladder was bathed on its serosal surface by a HCO_3-rich serosal fluid. This was followed by a rapidly developing increase in I_{sc} (Figure 6) , similar to that which had been seen in bladders bathed on both surfaces by symmetrical HCO_3 solutions. At the same time, the rate of alkali appearance into the mucosal fluid increased. The direction of the increased I_{sc} was that of increased serosal electronegativity.

Table II. Mean steady state values of Cl-induced increment in short-circuiting current (ΔIsc) vs. those of net chloride flux (I_{net}^{Cl}) in 27 bladders. Protocol was the same as that described under Figure 4.

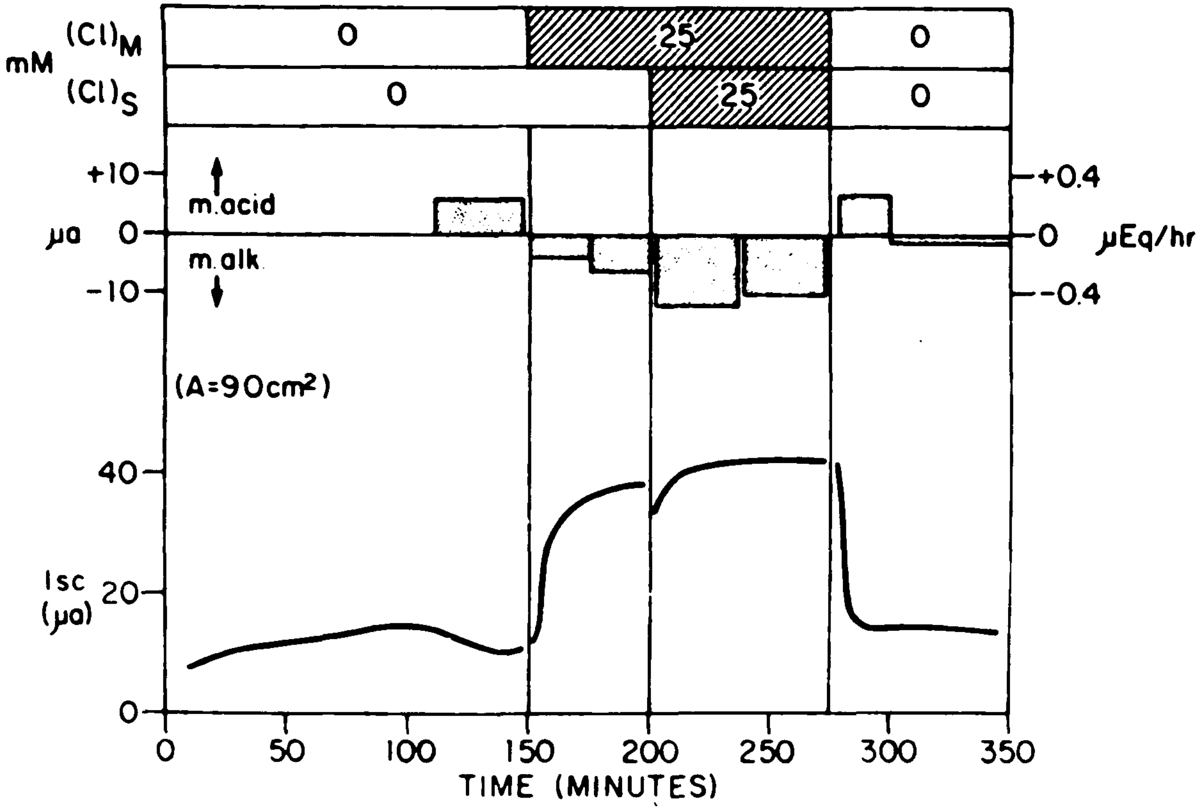
ΔI_{sc} (ua)	I_{net}^{Cl} (ua)	Correlation coefficient[1]
11.2 ± 11.8	16.5 ± 14	0.471 $.05 > P > 0.01$

All values are for 1.5 cm^2 of exposed bladder surface.

[1] Product Moment Correlation Coefficient

FIGURE 6

The effect of the addition and removal of Cl on the I_{sc} and mucosal acidification rate of bladders bathed initially by Na-free, Cl-free media. The mucosal solution was at all times Na-free and HCO_3-poor and gassed with 100% O_2 (solution D). The serosal solution was at all times a Na-free, HCO_3-rich solution and gassed with a 2% CO_2/98% O_2 mixture (solution D). Mucosal acidification (m. acid) and mucosal alkalinization (m. alk.) were measured titrimetrically, maintaining the pH at 7.6. $(cl)_m$- mucosal Cl concentration. $(Cl)_s$-serosal Cl concentration. All values are for 9.0 cm of bladder area. Sign convention is that of positive denoting negative charges moving from the mucosal to serosal solution. Representive of 3 experiments.


mM
(Cl)M
0
25
0
(Cl)S
0
25
0
+10
↑ m.acid
μa 0
m.alk. ↓
-10
+0.4
0 μEq/hr
-0.4
(A=90cm2)
40
Isc (μa)
20
0
0
50
100
150
200
250
300
350
TIME (MINUTES)

On one hand, consistent with the findings of Leslie et al. (1973), the mucosal addition of Cl initiates a flow of alkali into the mucosal fluid in the presence of a HCO_3 gradient. On the other hand, different from the previous findings of Leslie et al. but similar to some of those shown here, the addition of Cl is followed by an increase in I_{sc}. Again, as before, neither of the two hypotheses (electrogenic or electroneutral Cl transport) alone can unequivocally account for both of these results. If, however, there were evidence that the HCO_3 secretion could be stimulated in the absence of Cl and that this secretion carries electrical current, it would be possible to account for both of these effects (Figure 6) and for a major portion of the observed inequalities between the magnitude of the Cl-induced increments of I_{sc} and the net chloride fluxes (Fig. 5).

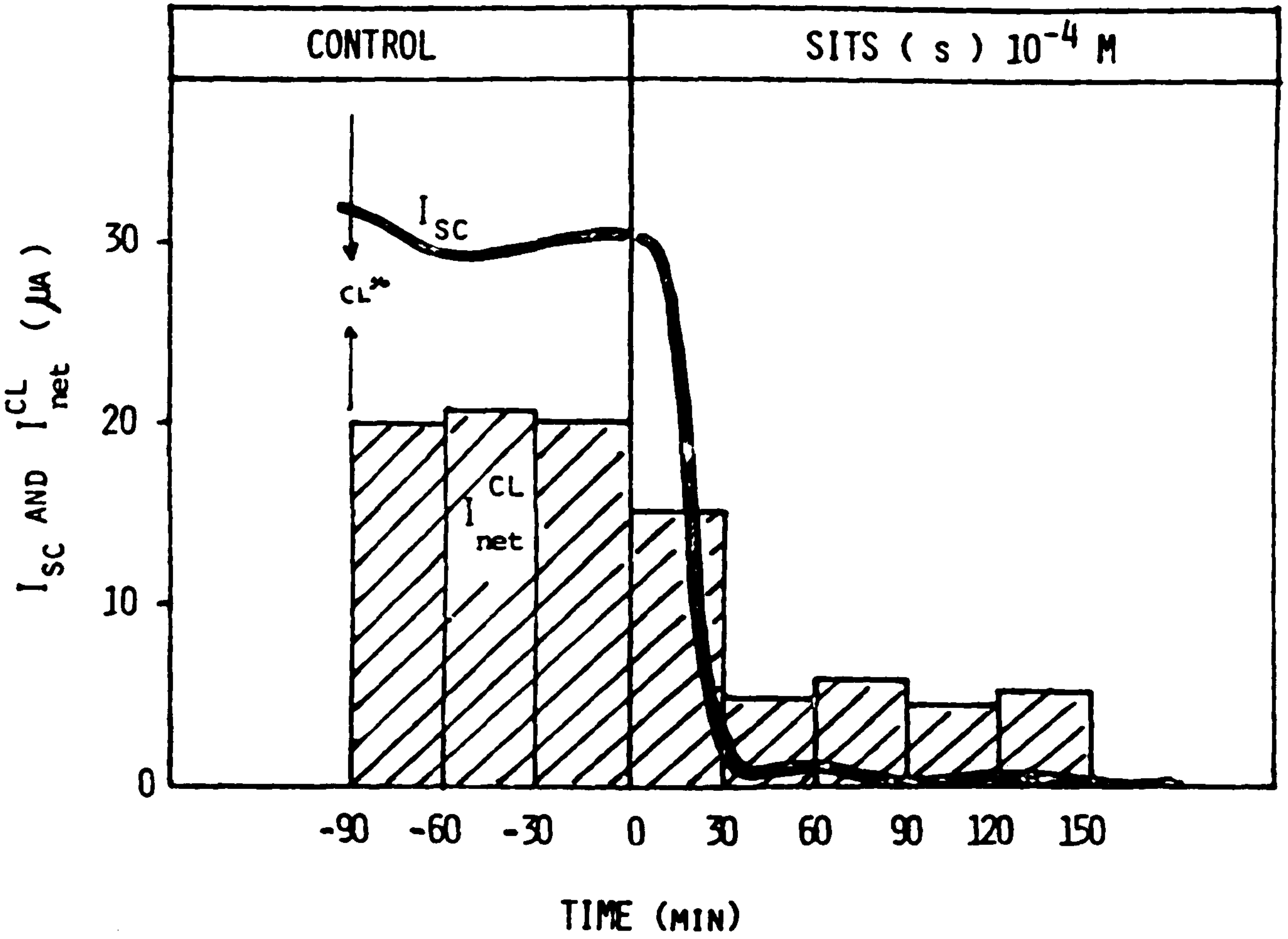
C) AN ELECTROGENIC CL-INDEPENDENT HCO_3 SECRETION.

One line of evidence for an electrogenic Cl-independent secretion of HCO_3 is the effect of SITS on bladders (Brodsky et al. 1979) bathed on both surfaces by identical choline media of two types: (i) HCO_3-rich, Cl-rich (solution A) and (ii) HCO_3-rich, Cl-free (solution B). In medium (i), the I_{sc} and PD are decreased by SITS from the usual orientation under these conditions (mucosal side positive) to zero levels while the chloride reabsorption is decreased but remains finite (Figure 7). In order to account for this electrically-silent post-SITS state, in which a negative charge (Cl) has been translocated, physical law requires that there be a net reabsorption of some cation or a net secretion of some anion other than Cl. A probable candidate for this other translocated charge is the HCO_3 ion, inasmuch as the initiation of a net Cl transport does induce an increase in alkali appearance into the mucosal fluid, at least under certain conditons. This charge-balancing ion flow could be part of an electroneutral exchange with the Cl reabsorption or could be due to an electrogenic HCO_3 secretion. If the latter were the case, then the addition of SITS to the serosal solution of bladders bathed by HCO_3-rich, Cl-free media (solution B) should result in a reversal in the PD and I_{sc}.

Table III presents data which demonstrates that such a reversal in I_{sc} and PD does occur. Although the magnitude of this reversed I_{sc} was small, that of the PD was easily measurable. It was subsequently found (Brodsky et al. 1978) that the addition of theophylline to the mucosal solution increased

FIGURE 7

The effect of the serosal addition of SITS (10^{-4} M) on the I_{sc} and net Cl flux in a bladder bathed by Na-free, HCO_3-rich, Cl-rich bathing media. Note the maintenance of net Cl transport when the I_{sc} (and PD, not shown) is near zero.



CONTROL
SITS (s) 10^{-4} M
I_{SC}
CL^{*}
I_{net}^{CL}
30
20
10
0
I_{SC} AND I_{net}^{CL} (μA)
-90
-60
-30
0
30
60
90
120
150
TIME (MIN)

Table III. The SITS-induced reversal in Isc and PD of bladders (n = 7) bathed on both sides by identical Na-free, Cl-free, HCO_3-rich media. Serosal solutions contained 10^{-4} M ouabain.

Condition	I_{sc} (ua)	PD (mV)
Control	14.6 ± 4.3	26.6 ± 6.7
SITS	-3.5 ± 0.4	-7.3 ± 0.3

Minus signs denote electronegativity of mucosal solution with respect to serosal solution. Isc values are for 1.5 cm^2 of exposed bladder surface.

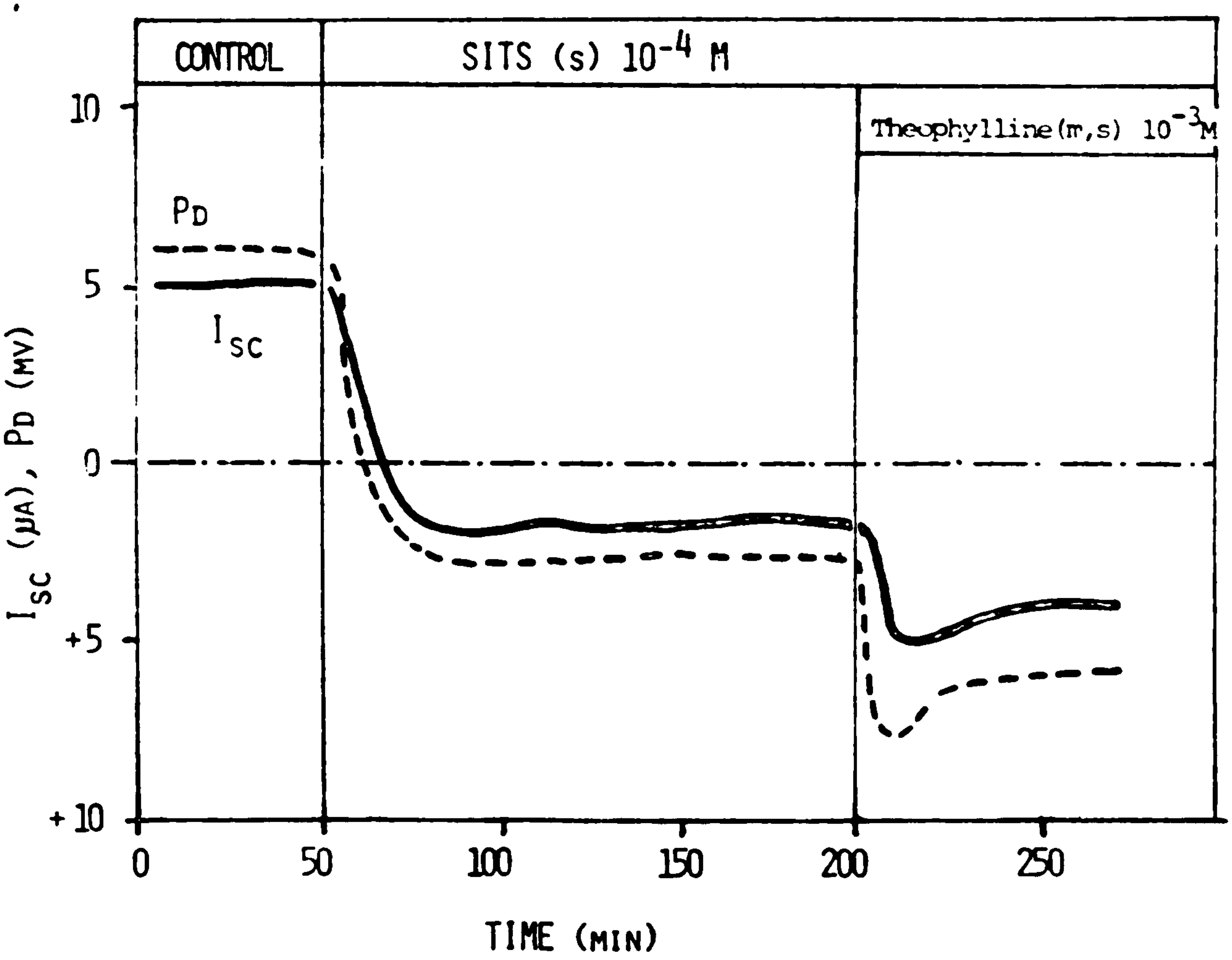
the magnitude of the reversed I_{sc} and PD, indicating that the cellular level of cAMP may be involved in this HCO_3 secretory function of the bladder (Figure 8).

This effect of theophylline has become increasingly significant to us in view of the recent findings of Lief et al. (1980), who showed that theophylline and/or cAMP decreased the rate of luminal acidification (measured by pH statting) by turtle bladders. Although these authors suggested that theophylline and cAMP inhibit the acidification current, it is equally possible that these agents induce an increase in HCO_3 secretion. If so, then a phosphodiesterase inhibitor (e.g., theophylline or IBMX) and/or cAMP might, under the appropriate conditions, induce a reversal in orientation of the I_{sc} and PD across bladders bathed by HCO_3-rich, Cl-free media.

We have recently discovered that the so-called appropriate conditions are those prevailing in bladders obtained from turtles which had been made alkalotic by forced feeding of $NaHCO_3$ (Cohen, 1980). Under these conditions, IBMX and cp-cAMP did increase the apparent HCO_3 secretory current and the measured rate of alkali secretion (Satake et al. 1981). Therefore there is an evident Cl-independent, electrogenic HCO_3 secretion in the turtle bladder.

FIGURE 8

The effect of the serosal addition of SITS (10^{-4} M) on the I_{sc} and PD of a bladder bathed by Na-free, Cl-free HCO_3-rich media (solution B). Following the addition of SITS, the I_{sc} and PD fell to zero and then reversed in orientation, such that the serosal solution was electropositive to the mucosal solution. The subsequent addition of theophylline to both bathing media resulted in an increase in I_{sc} and PD. Sign convention is that of positive depicting the flow of negative charges from the mucosal to the serosal solution.


CONTROL
SITS (s) 10^{-4} M
Theophylline (m,s) 10^{-3}M
10
5
0
+5
+10
PD
I$_{SC}$
I$_{SC}$ (μA), PD (MV)
0
50
100
150
200
250
TIME (MIN)

It was then decided to investigate the effect of IBMX and cp-cAMP on Cl transport during stimulation of the HCO_3 secretory current. On the one hand, the hypothesis holding that Cl reabsorption occurs by an electroneutral transepithelial exchange for HCO_3 secretion would indicate that IBMX and cp-cAMP would either induce an increase or no change (but not a decrease) in Cl transport. On the other hand, a hypothesis holding that both Cl reabsorption and HCO_3 secretion are electrogenic, would not lend itself to any prediction of the response of Cl transport to an IBMX and cp-cAMP induced change in HCO_3 reabsortion or secretion.

We then found that the addition of IBMX and cp-cAMP largely inhibited the net Cl transport while increasing the HCO_3 secretory current in a bladder obtained from an alkalotic turtle (Figure 9). Clearly, the initiation of the 'reversed' I_{sc} occurred concomitantly with an inhibition of Cl transport, which is opposite to the predicted effect of these agents on a HCO_3:Cl exchange operation.

By comparison, the same serosal addition of IBMX and cp-cAMP to bladders from non-alkalotic turtles, also largely inhibited the net Cl reabsorption but decreased the magnitude of the I_{sc} and PD, initially oriented in the direction of active HCO_3 reabsorption (Figure 10). Therefore IBMX and cp-cAMP decrease the net rate of Cl reabsorption during enhancement of a HCO_3 secretion as well as perhaps during inhibition of a HCO_3 reabsorption. <u>Table IVA</u> shows the results of 6 experiments of the type depicted in <u>Figure 10</u>. The addition of IBMX and cp-cAMP did not decrease the I_{sc} and I_{net}^{Cl} to zero levels in every experiment. Of interest, however, is that the decrease in I_{sc} was similar in magnitude and direction to that of the decrease in I_{net}^{Cl} in these experiments and that the decrease in I_{net}^{Cl} was similar to the decrease in the forward flux (<u>Table IVB</u>). It is to be noted that although the similarities in the decrements of I_{sc} and I_{net}^{Cl} are consistent with the hypothesis holding that Cl reabsorption is electrogenic, the IBMX and cp-cAMP induced decrement in I_{sc} does occur in the absence of Cl (Satake et al. 1981).

FIGURE 9

The effect of the addition of IBMX (5 x 10^{-4} M) and cp-cAMP (10^{-3} M) to the serosal solution on the I_{sc}, PD and net (reabsorptive) Cl flux (I_R^{Cl}) of a bladder bathed by Na-free, HCO_3-rich, Cl-rich media (solution A). This bladder was obtained from a turtle which had been given $NaHCO_3$ for four days. Initially, the I_{sc} and PD were slightly negative, while Cl reabsorption was finite (see also Figure 7). The addition of IBMX and cp-cAMP resulted in an increase in I_{sc} and PD, and a concomitant decrease in Cl reabsorption to zero levels. Sign convention is that of positive denoting negative charges flowing from the mucosal to serosal bathing solution. Note that for simplicity, Cl reabsorptive flux is plotted in the negative direction.

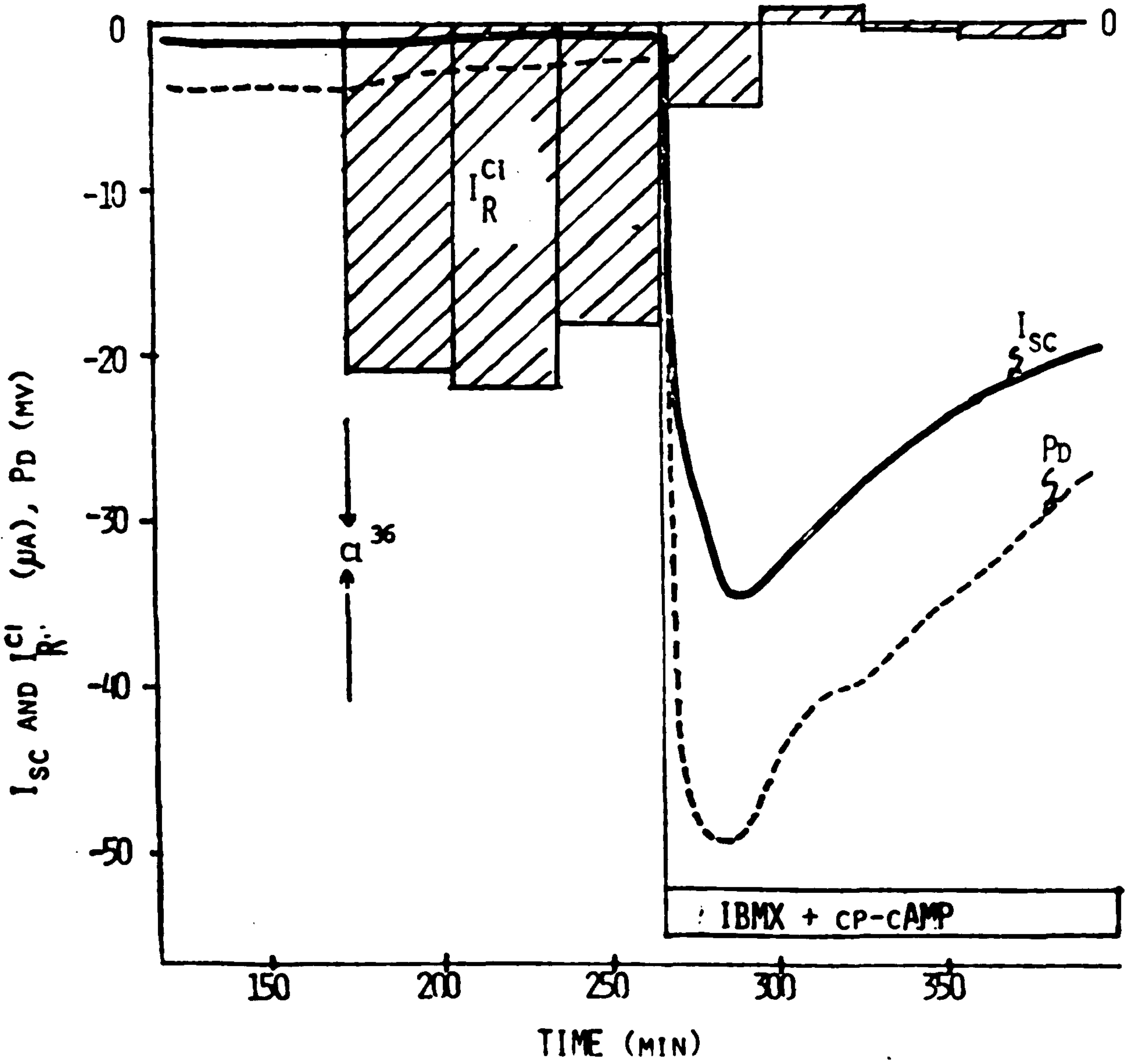

0
-10
-20
-30
-40
-50
0
I_R^{Cl}
I_{SC}
PD
Cl^{36}
IBMX + CP-cAMP
150
200
250
300
350
TIME (MIN)
I_{SC} AND I_R^{Cl} (μA), PD (MV)

FIGURE 10

The effect of the addition of Cl, IBMX (5×10^{-4} M) and cp-cAMP (10^{-3} M) on the I_{sc} and net (reabsorptive) Cl flux (I_R^{Cl}) of a bladder bathed initially by Na-free, HCO_3-rich, Cl-free media (solution B). The addition of Cl was followed by an increase in I_{sc} which was smaller in magnitude than the net Cl flux. The subsequent addition of IBMX and cp-cAMP resulted in transient but rapid decreases in I_{sc} and Cl flux to zero levels.

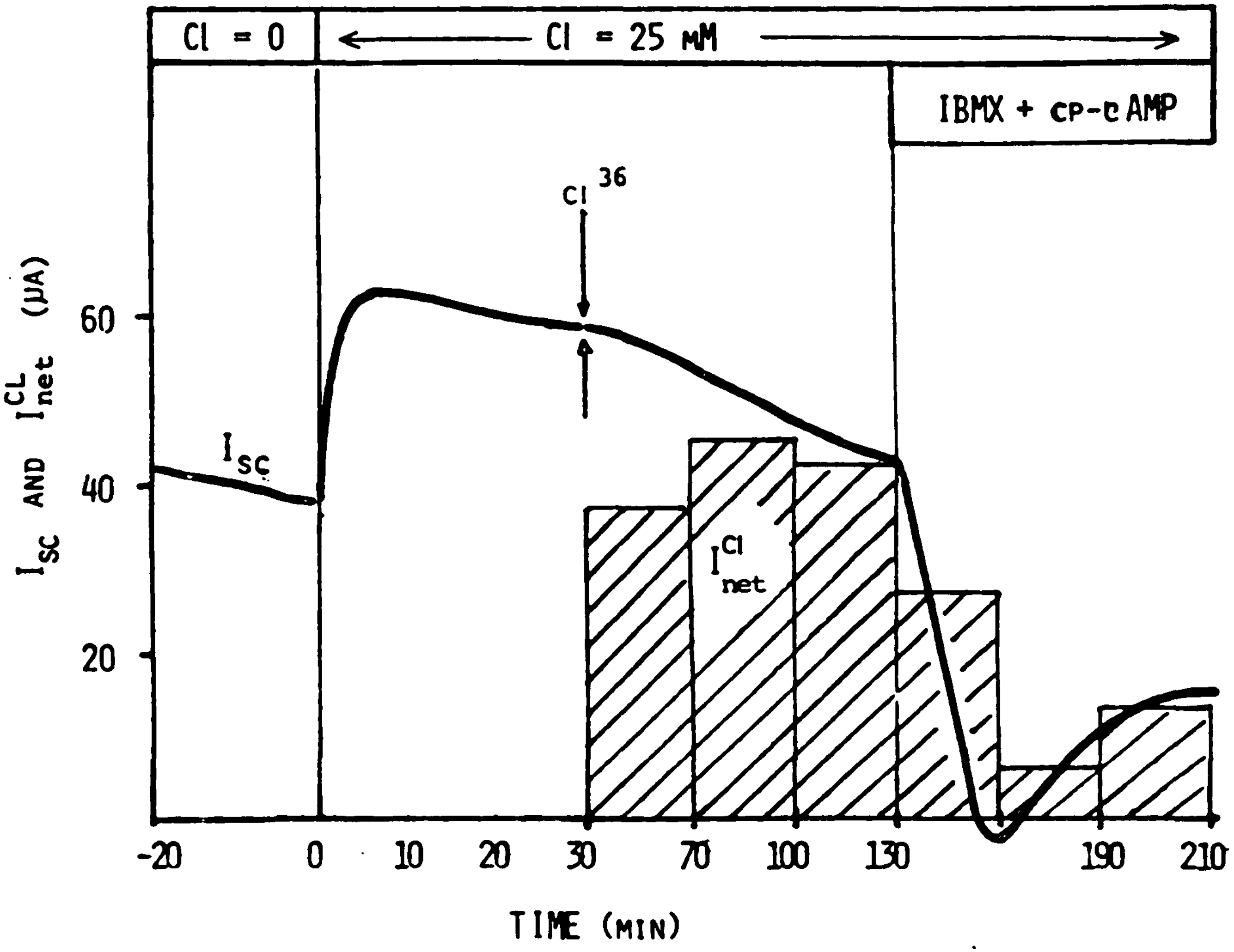

Cl = 0
Cl = 25 mM
IBMX + cp-cAMP
Cl 36
I_{sc}
I_{net}^{Cl}
I_{sc} AND I_{net}^{CL} (μA)
60
40
20
-20
0
10
20
30
70
100
130
190
210
TIME (MIN)

TABLE IV

A. The effect of the serosal addition of IBMX (5×10^{-4} M) and cp-cAMP (1×10^{-3} M) on the Isc and Cl fluxes across 6 bladders bathed by Na-free, (HCO_3 + Cl)-containing media (solution A).

$\Delta\bar{I}_{sc}$ (ua)	$\Delta\bar{I}^{Cl}_{net}$ (ua)	$\Delta\bar{I}^{Cl}_{m\rightarrow s}$ (ua)	$\Delta\bar{I}^{Cl}_{s\rightarrow m}$ (ua)
-16.8 ± 5.4	-15.6 ± 4.7	-11.9 ± 4.1	3.6 ± 0.93

The results (± SEM) are the means of the individual changes (initial - final) in each parameter of each bladder occurring after the addition of IBMX and cAMP. Backfluxes were determined in the mated half-bladder. (-) denotes a decrease. All values are for 1.5 cm^2 of exposed bladder.

B. Significance of the differences between the means of each parameter.

Parameter	Significance (t-test)
$\Delta\bar{I}_{sc}$ vs $\Delta\bar{I}^{Cl}_{net}$	$p > 0.5$
$\Delta\bar{I}^{Cl}_{net}$ vs $\Delta\bar{I}^{Cl}_{m\rightarrow s}$	$p > 0.5$
$\Delta\bar{I}_{net}$ vs $\Delta\bar{I}^{Cl}_{s\rightarrow m}$	$0.05 > p > 0.01$

DISCUSSION

What cannot be denied on the basis of the present results are the following claims:

(i) At least part of the active chloride reabsorption is associated with a transfer of charge across the bladder

(ii) Another part of the chloride reabsorption is associated in some unspecified manner with

  (a) an alteration in the transepithelial flow of another electrogenically transported ion (such as HCO_3) or with

  (b) the interposed action of a Cl:HCO_3 exchange mechanism (antiport) in one or both of the plasma membranes.

(iii) There exists, a Cl-dependent active electrogenic transport of a non-Cl ion; this transport is activated by the presence (not by the net transport) of Cl in the media.

(iv) the bladder posseses a mechanism for a Cl-independent and electrogenic HCO_3 secretion, which is independent of Cl reabsorption

These claims arise from the following experimental observations

(i) the hypothesis holding that Cl transport occurs via an electrogenic process which is independent of all other transport processes would lead to the prediction that there would be a finite Cl-induced increment in I_{sc}, equal in magnitude to the net Cl flux. This equality was found in 11 of the 27 experiments. Therefore without additional assumptions, this hypothesis is capable of accounting for 40% of the present experiments.

(iia) If an electrogenic transport of Cl were in some way coupled to an electrogenic transport of another ion, then the initiation of Cl reabsorption could change the transport rate of the non-Cl ion, which also carries current. In this case, the magnitude of the Cl-induced increment in I_{sc} would not necessarily be equal to or even in direct proportion to the magnitude of the Cl flux. This inequality was indeed found in 12 of the 27 experiments.

(iib) The hypothesis holding that Cl reabsorption occurred via a one-for-one transepithelial exchange for HCO_3 secretion (Leslie et al. 1973) would lead one to predict that the initiation of Cl reabsorption would occur with no change in the I_{sc}. This was found to be the case in 3 of the 27 experiments, and therefore this hypothesis can explain a small number of the present experiments.

(iii) That a Cl-induced increment in I_{sc} can occur in the absence of net Cl reabsorption ($I^{Cl}_{net} \approx 0$) was found in 3 of the 27 experiments.

(iv) IBMX and cp-cAMP inhibit Cl reabsorption while increasing an apparent current-carrying HCO_3 secretion.

Since neither of the two previous hypothesis on Cl transport (i.e., electrogenic or electroneutral) could account for all the data, it became necessary to consider the possibilities that (i) factors other than Cl contribute to the observed changes in I_{sc} subsequent to Cl addition and removal or that (ii) one or both of the hypotheses require additional or modifying assumptions.

It is possible that the effects of Cl removal and addition on the I_{sc} are not due to Cl per se, but to the subsequent effects of respective increases and decreases in the SO4 concentrations of the bathing fluids. In this context, it has been demonstrated that the decreased free Ca levels occasioned by increased SO4 concentration can affect cellular function (Hill and Howarth, 1957). The possibility thus exists that the decrease in free Ca concentration following Cl removal might decrease the basal I_{sc} (HCO_3 reabsorptive or H secretory current), thereby leading to the mistaken conclusion that Cl removal was responsible for the decrement in I_{sc}. Whether this phenomenon is indeed what gives rise to the results seen in the present study requires further investigation. Such an effect due to Ca alone, however, would not be expected to result in a proportionality between the Cl-induced I_{sc} and the net Cl flux (Table II).

According to the hypothesis holding that Cl reabsorption occurs by an electrogenic process (as was stated above) the magnitude of the Cl-induced increment in I_{sc} should equal that of the net Cl reabsorption. Implicit in this prediction is the assumption that the charge-carrying translocation of Cl does not affect the transport of any other ion. If instead, it is assumed that the electrogenic reabsorption of Cl across one or both of the cell membranes does affect other current-carrying ion transport processes, it is possible to account for most of the observed experimental findings.

An Equivalent Circuit Model (Figure 11). In addition to the aforementioned evidence for an active electrogenic transport of Cl, evidence for an active electrogenic secretion of HCO_3 in the turtle bladder has recently been found (Brodsky et al. 1979; Satake et al. 1981), and that for an electrogenic reabsorption of HCO_3 (or secretion of H) has long been established and confirmed. Each of these electrogenic ion transfers can be treated as a flow of electric current through an ion-specific electromotive force (E_R^{Cl}, $E_S^{HCO_3}$, $E_R^{HCO_3}$) in series with a correspondingly specific resistance element. The transepithelial equivalent circuit network of these ion-specific paths represents the apical membrane as a parallel array of the three electrogenic transport mechanism and the serosal membrane as a single anion conductive pathway. The flow of ion-specific currents across this model can be made to replicate the behavior of turtle bladders bathed by Na-free, (HCO_3 + Cl)-rich media with respect to the following phenomena:

(1) a Cl transport-induced increment in I_{sc}

(2) a Cl transport-induced increment in HCO_3 secretion

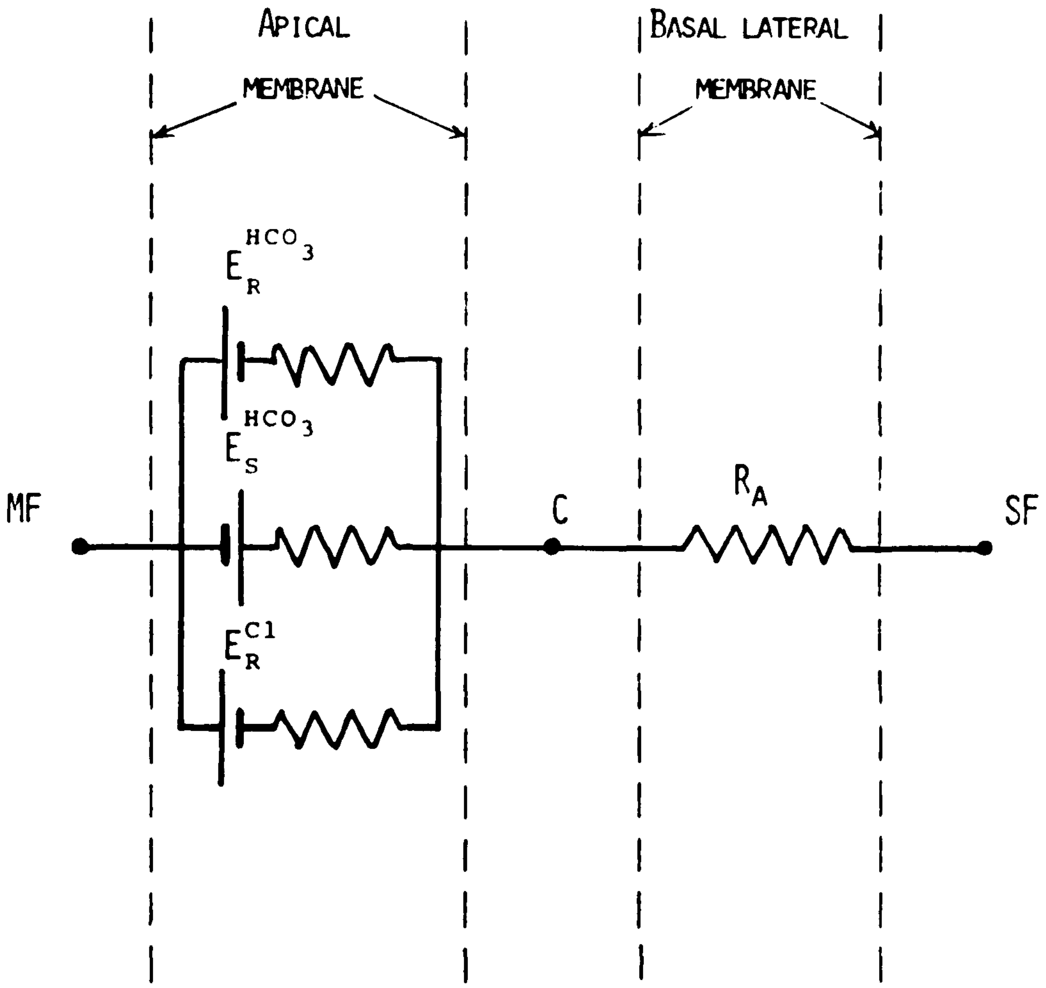
(3) a Cl transport-induced increment in I_{sc} which is equal to or less than the magnitude of the net Cl flux.

(4) a Cl-independent, electrogenic secretion of HCO_3

Assuming that all of the transport elements (conductances) are voltage sensitive one can make the following qualitative predictions. (i) In a steady state (under short-circuiting conditions) with no Cl reabsorption, the cell interior will be electronegative to the mucosal solution, and the I_{sc} across this circuit will be the algebraic sum of the currents through the two HCO_3 transport pathways. (ii) Upon initiation of Cl transport, the addition of the Cl EMF must increase the intracellular electronegativity (with respect to both bathing fluids) in the short-circuited condition. (iii) The increased electric field across the apical membrane will increase the HCO_3 secretion and decrease the HCO_3 reabsorption, the magnitude of these changes depending on the magnitude of each HCO_3 conductance The magnitude of the Cl-induced I_{sc} will thus be a result of the sum of Cl reabsorption and the new rates of HCO_3 reabsorption and HCO_3 secretion. If the latter two transport rates are not changed, then the Cl-induced I_{sc} will be equal

FIGURE 11

Equivalent circuit of the short-circuited turtle bladder bathed by Na-free medium containing HCO_3 and Cl. The apical membrane contains three electrogenic transport elements and the respective series resistances for (i) HCO_3 reabsorption - $E_R^{HCO_3}$, (ii) HCO_3 secretion - $E_S^{HCO_3}$, and (iii) Cl reabsorption - E_R^{Cl}. The basal-lateral membrane is represented as containing a non-specific anion conductive pathway (R_A). MF - mucosal fluid, SF - serosal fluid, CF - cell fluid, See text for discussion of the behavior of this equivalent circuit.


APICAL
MEMBRANE
BASAL LATERAL
MEMBRANE
$E_R^{HCO_3}$
$E_S^{HCO_3}$
E_R^{Cl}
MF
C
R_A
SF

to the rate of Cl reabsorption, the situation found in 11 of the 27 experiments.

On the other hand, if the HCO_3 secretion is increased and/or the HCO_3 reabsorption decreased, the magnitude of the Cl-induced I_{sc} will be less than that of the rate of Cl reabsorption, the situation found in 12 of the 27 experiments.

Since the three transport elements are not obligatorally coupled, they may be varied individually. Thus, the model allows for any agent(s) to stimulate or inhibit one or the other transport process. Such is the case with IBMX and cp-cAMP, where the Cl reabsorption is decreased and the HCO_3 secretion increased in bladders from alkalotic turtles (Fig. 9) and in the case where the I_{sc} is inhibited to the same extent as the I_{net}^{Cl} in bladders from non-alkalotic turtles (Table IV).

This model will account for the majority of the relationships found between the Cl-induced I_{sc} and the Cl reabsorption, and for the IBMX and cp-cAMP-induced decrease in I_{sc} and increase in secretion of HCO_3 and concomitant inhibition of Cl reabsorption. The model, however, has no assigned properties which would predict that (i) a Cl-induced increment in I_{sc} can occur with no induced Cl reabsorption and that (ii) serosal HCO_3 is required for Cl reabsorption. With respect to (i) neither of the current hypotheses on the mode of Cl reabsorption can account for this phenomenon (which was observed in 3 of the 27 experiments). It is possible that these changes in I_{sc} were not due to changes in Cl per se, but rather to the reciprocal SO4 induced changes in free Ca levels (see Hill and Horwath, 1957; and above).

With respect to (ii) above, the dependence of Cl reabsorption on the presence of serosal HCO_3 can be accounted for by an electroneutral Cl for HCO_3 exchange process, such as that proposed by Leslie et al. (1973). But other than the specific dependence of Cl reabsorption on serosal HCO_3, the model of Leslie et al. fails to account for the observed Cl-induced changes in I_{sc}.

Although Cl reabsorption does depend upon the presence of HCO_3 it should be noted that it has been demonstrated that serosal HCO_3 also increases Na-reabsorption (Gonzalez et al. 1969) a process which is without doubt electrogenic. It is thus possible that the serosal HCO_3-dependency of Cl transport is not due to the existence of a Cl:HCO_3 exchanger per se, but to some other effect of serosal HCO_3 on certain transport processes in general.

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