ION TRANSPORT AND SHORT CIRCUIT CURRENT IN THE RECTUM OF THE DESERT LOCUST, SCHISTOCERCA GREGARIA

by

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We accept this thesis as conforming
to the required standard

The University of British Columbia
September, 1976

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ABSTRACT

An *in vitro* preparation of the rectum of the desert locust *Schistocerca gregaria* is described and characterized. The rectal epithelium was mounted as a flat sheet separating two well stirred chambers and the trans-membrane potential difference (PD), membrane resistance, and short circuit current (SCC) were monitored. The PD and membrane resistance remained relatively constant for at least six hours at 35 mV (lumen positive) and 6000 Ohms . cm^{-2}, respectively. After an initial two hour transient period during which the SCC dropped from 8 uMoles of charge $.cm^{-2}.hr^{-1}$ to 3.5 $uMoles.cm^{-2}.h^{-1}$ an approximate steady-state condition was reached and maintained for at least another four hours. The SCC was consistant with either a net cation transport from the hemolymph to the lumen of the rectum or a net anion transport in the opposite direction.

Since Cl^- was the only major anion in the bathing media some experiments were carried out to evaluate the contribution of active Cl^- transport to the SCC. Substitution of $SO_4^=$ of NO_3^- during the third and fourth hours *in vitro* had no effect on the SCC, although they abolished the initial two hours transient. Similar experiments in which Cl^- was substituted by acetate had a complex stimulatory effect on the SCC. Measurements of $^{36}Cl^-$ fluxes under SCC conditions during the same time

period demonstrated a mean net flux of 1.52 uMoles of $Cl^-.cm^{-2}.hr^{-1}$ from the rectal lumen to the hemolymph. This value is equivalent to one half the simultaneous SCC. These experiments clearly demonstrate that the rectal epithelium actively transports Cl^- in a direction consistent with the observed SCC but that this active transport of Cl^- does not contribute to the SCC during the third and fourth hours in vitro.

Since rectal Na^+ and K^+ transport reported by other workers is in the wrong direction to account for the observed SCC, it seems necessary to propose (1) a Cl^- exchange pump (probably with HCO_3^-) perhaps located on the luminal membrane to account for the fact that active Cl^- transport does not contribute to the SCC and (2) a H^+ and/or HCO_3 pump to account for the SCC. These proposals are incorporated in a model for organization of ion transport processes in the locust rectum.

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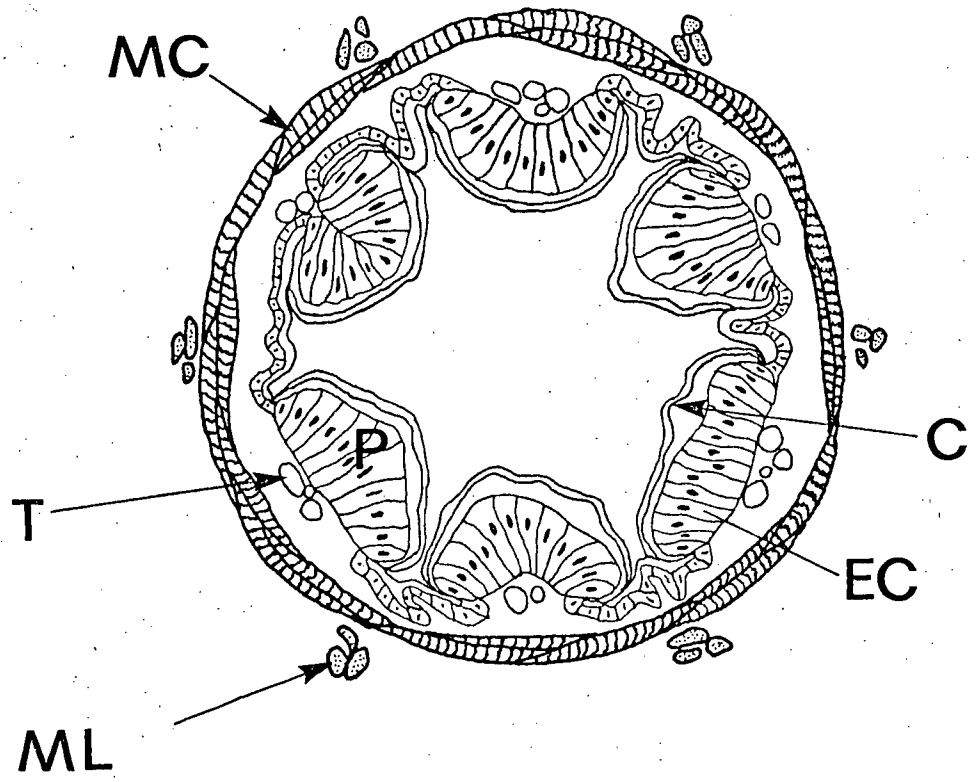
INTRODUCTION

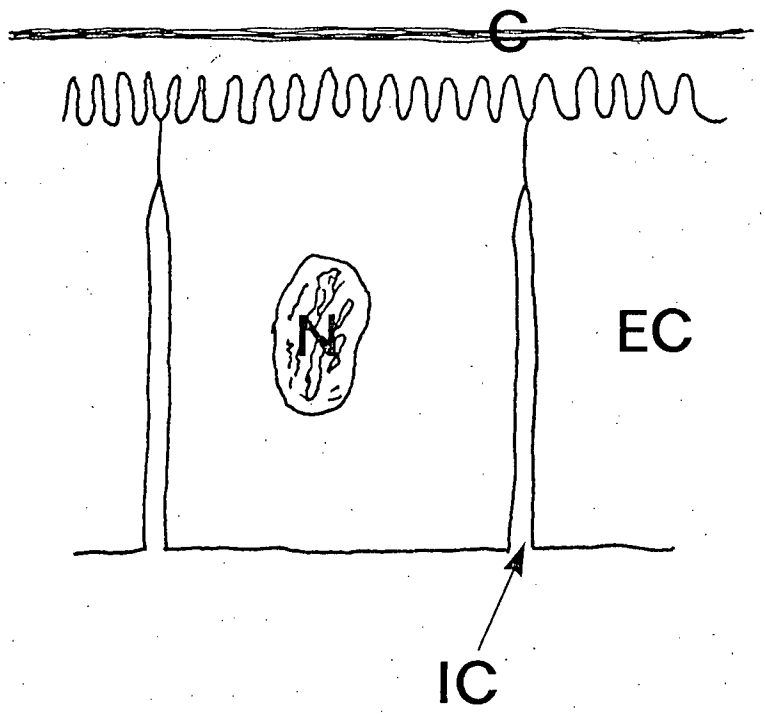
Since its introduction in 1951 by Ussing and Zerahn, the technique of short-circuit current (SCC) has been a powerful tool in the elucidation of ion transport mechanisms in epithelia. First applied to the study of a variety of vertebrate epithelia (Ussing & Zerahn, 1951; Ussing & Anderson, 1955; Hogben, 1955; Leaf & Renshaw, 1958; Cooperstein & Hogben, 1959; Durbin & Heinz, 1959) its use has recently been extended to the study of insect epithelia (O'Riordan, 1969; Wood, 1972; Wood & Harvey, 1975). I wish to report here the application of this technique to the study of ion transport in the rectum of the desert Schistocerca gregaria Forskal.

The morphology and histology of the locust alimentary canal were outlined in detail by Phillips (1964). Briefly, the locust rectum is an extensible tube 5-7mm. long in the contracted state located at the posterior end of the alimentary canal (Figure 1). The lumen of the rectum is lined with a chitinous cuticle or intima which has been shown to act as a molecular sieve with a pore radius of 6.5-8 Å (Phillips & Dockrill, 1968). Beneath the intima the rectal epithelium is thrown up into six longitudinal folds or pads composed of columnar epithelial cells. The epithelial cell layer is surrounded by a continuous sheath of circular muscle outside

Figure 1. Schematized drawing of rectal morphology. (a) Cross section of rectum. (b) Transporting epithelial cell layer. P, rectal pad. C, cuticular intima. EC, transporting epithelial cell layer. MC, circular muscle layer. ML, longitudinal muscle bundles. T, tracheae and tracheoles. N, cell nuclei. IC, intercellular channels.



(a)



(b)

of which are six equidistant longitudinal muscle bands. The rectum is the most heavily tracheated part of the alimentary canal with most of the tracheae entering the rectal tissue along the base of the rectal pads. When the rectum is distended with fecal matter or when it is stretched out as a flat sheet separating two chambers as in most experiments in this study, the rectal epithelium has a uniform thickness of about 1 mm. and the rectal pads appear simply as long silver-white bands beneath the luminal cuticle.

Considerable information on rectal absorption of ions was available for at least one insect, the desert locust, before commencing this study. In the early 1960's, Phillips (1961, 1964) showed that the in vivo locust rectum transports H^+ or HCO_3^-, Na^+, K^+, Cl^+, and H_2O against electrochemical gradients. Transport of the latter four species was later shown to occur in an in vitro preparation by Goh, (1971); Phillips and Goh, in preparation; and Meredith and Phillips, unpublished data. In 1968, Speight showed that the in vivo rectum transports a minimum of 2.2 uMoles cm^{-2} hr^{-1} of H^+ and/or HCO_3^-.

Though the foregoing studies amply demonstrate that ion transport processes occur in the rectal epithelium, for the most part previous studies have focused on water transport. Little was known of the interrelationships bet-

ween the different ion pumps in the rectum nor of their kinetic and other properties. In addition to characterizing a new in vitro preparation, this paper demonstrates that the majority of the electrogenic ion transport (total anions minus total cations; i.e., the SCC) is carried by neither Na^+, K^+, or Cl^- but is probably supported by H^+ or HCO_3^- transport.

While the present paper was in preparation the work of Herrera, Jordana, and Ponz (1976) came to my attention. This paper, which will be considered in the Discussion, also describes the application of the SCC technique to the study of ion transport in the locust rectum. The quite different conclusions which these authors report can be attributed to the use of conditions which failed to sustain transport activity to the same degree as that reported in the present study.

MATERIALS AND METHODS

MATERIAL

Adult female Schistocerca gregaria 1-3 months past their final molt and fed on lettuce or spinach and a mixture of dried grass, bran, yeast and powdered milk were used in all experiments. The animals were maintained in a constant temperature room at 28°C and 50% R.H. in cages each containing a 60 watt incandescent bulb for additional heat and illumination. A photoperiod cycle of 16 hours light and 8 hours dark was used.

METHODS

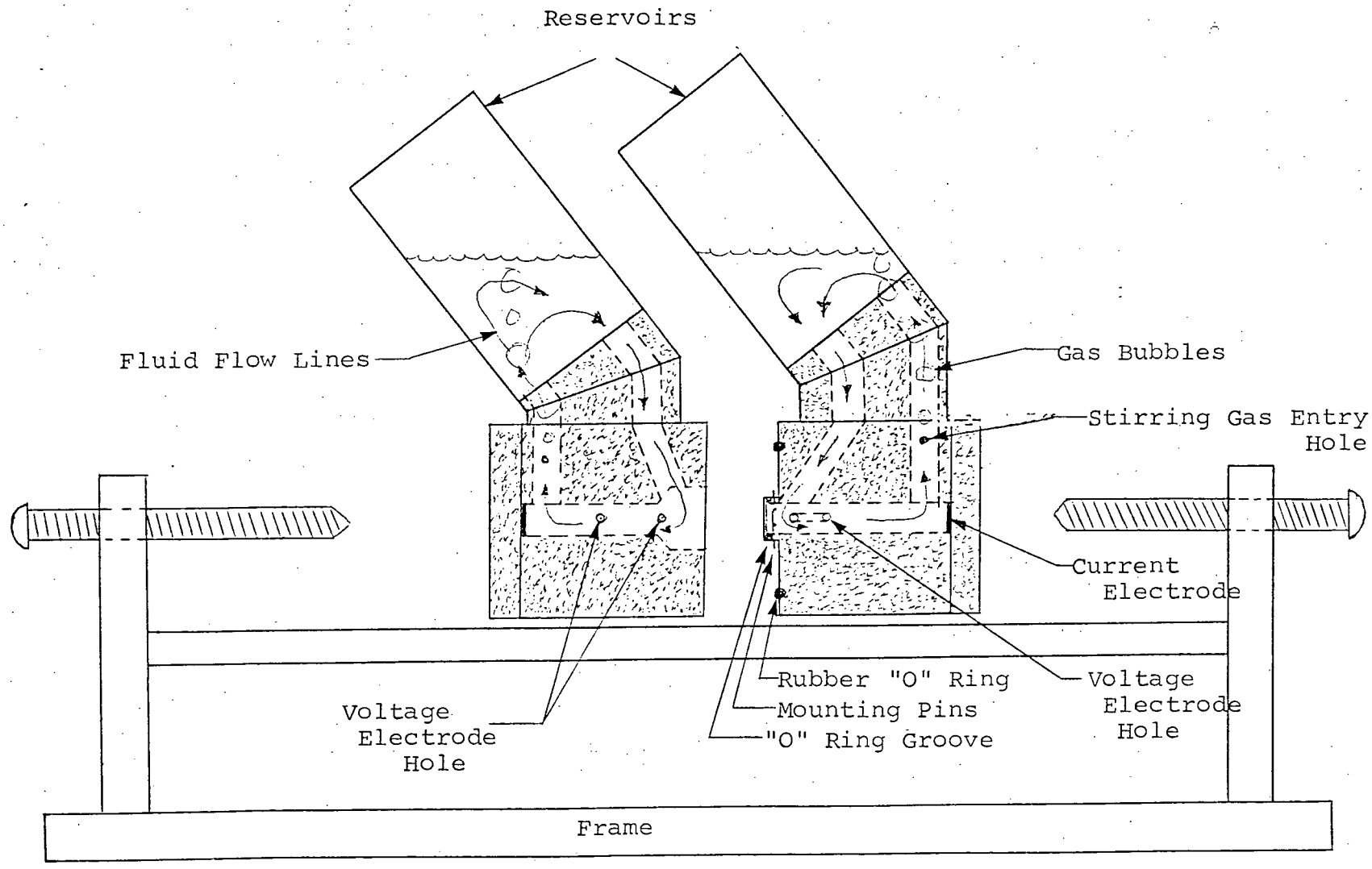
To study ion transport using the SCC method recta were removed from the locust by the following procedure. The head and all the appendages were cut off and the animal was pinned down on a Plasticene block under a dissecting microscope with the left side facing upward. A U-shaped cut was made in the left side of the abdomen starting at the last segment and extending forward 3 segments. The resulting flap of cuticle was folded back dorsally and pinned down to the Plasticene block. Tissues were kept moist during subsequent dissection with the appropriate Ringer's solution. The gut was grasped with a pair of fine watch-makers forceps just

anterior to the rectum, and the fat body, Malpighian tubules and tracheae were all cut and pulled away from the rectum using another pair of forceps. The rectum was then slit lengthwise using iridectomy scissors inserted through the anus. After removing the rectal contents the rectum was severed at its anterior and posterior borders. The resulting square of tissue (about 0.8 cm. on a side) was transferred with the forceps and mounted on an upright half chamber as described below.

The chambers used in these experiments (Figure 2) were modified from those described by Wood, (1972). The fluid reservoirs were inclined at an angle of 45^{o} to the horizontal to permit mounting of the tissue while the right chamber half was filled with Ringer's solution. The chamber halves were held together and supported by a vise-like frame as shown in Figure 2. The diameter of the circular orifice over which the rectum was mounted was 0.5 cm. giving an exposed surface area of 0.196 cm^2. The reservoirs had loose removeable caps to prevent solution loss by spattering and to allow easy access to change solutions and conduct tracer studies. Each chamber half and associated reservoir was filled with equal volumes of Ringer's solution: 20 ml. in most experiments.

Fluid in each chamber half was circulated by a gas-lift pump so as to deliver a flow of saline from the large reservoir against the surface of the rectal wall. A gas

Figure 2. Experimental chambers.


Reservoirs
Fluid Flow Lines
Gas Bubbles
Stirring Gas Entry Hole
Current Electrode
Voltage Electrode Hole
Rubber "O" Ring
Mounting Pins
"O" Ring Groove
Voltage Electrode Hole
Frame

mixture of 95% O_2 and 5% CO_2 was used to provide adequate oxygenation in the absence of intact tracheal connections. Fluid circulation was both rapid and turbulent inside the chambers especially near the membrane as determined by injection of dye at various points in the chamber and reservoirs. Injected dye returned to its starting point in much less than a second and fluid in the reservoirs and chambers became uniformly colored by the dye within a few seconds.

Stirring rate and oxygenation of the solution are intimately related when using gas-lift pumps. Stirring rate was kept constant in these experiments by maintaining the gas flow constant (using a small gas flow regulator) at about twice the rate at which it begins to limit rectal SCC. The stirring gas was raised to 100% R.H. by bubbling through water before being introduced to the chambers in order to minimize evaporation of the Ringer's solution during long experiments.

Most experiments were performed at room temperature. This varied from day to day between 20^{o} - $25^{o}C$ but the change during any one experiment was less than $2^{o}C$. However, to determine the effect of temperature on SCC a coiled glass tube was inserted into each reservoir and a heating or cooling fluid passed through it from a "Haake" model FE constant temperature water circulating pump. Temperatures in the

reservoir were monitored by inserting a thermometer into the top of each reservoir.

When it was desired to change the Ringer's solution during the course of an experiment a large volume of new solution (about 250 ml.) was added through the top of each reservoir while the solution level was maintained by inserting a fixed-level vacuum line into the top of each reservoir. Changing both solutions took about 90 seconds and was 99.9% effective as measured by $^{36}Cl^-$ dilution.

After the rectal epithelium was removed from the locust as described above it was stretched over the mouth of the right chamber half, lumen side up, using two pairs of forceps. The tissue was held down by impaling the peripheral areas of tissue on fine metal pins permanently mounted around the orifice of the chamber half. A rubber O-ring was then fitted over the lip of the chamber above the rows of pins sealing off the orifice of the right chamber. The right and left chamber halves were then fitted together, turned to the horizontal position, mounted in the supporting frame, and clamped firmly together. Then the left chamber half was filled with Ringer's solution and the gas lines for stirring were attached. The voltage and current electrodes were connected last as described below. The initial membrane potential difference (PD) reading was usually taken within 13-15 m. of starting the dissection of the locust.

The basic Ringer's solution used in these experiments (Normal Ringer's) was adapted from Berridge (1966). It contained 24.5 mM NaCl, 10.5 mM $NaHCO_3$, 8.5 mM KCl, 2 mM $CaCl_2$, 13 mM $MgCl_2$, 7.4 mM disodium succinate, 1.87 mM Trisodium citrate, 12.8 mM malic acid, 1.6. mM glucose, 5.56 mM maltose 79.8 mM sucrose, 2.67 mM glycine, 4.61 mM proline, 2.64 mM glutamine, 12.3 mM glutamic acid, 30 mg/l penicillin, and 100 mg/l streptomycin sulphate. The resulting solution was adjusted to pH=7.00 with NaOH. The freezing point of the Ringer's solution was $-0.587^{o}C$ as measured with a "Clifton" Nanoliter Osmometer. In some early experiments Normal Ringer also contained 8.0 g/l of lactalbumin hydrolysate and 4.0 g/l of yeast extract. The freezing point in this case was maintained at $-0.587^{o}C$ by reducing the sucrose concentration to 11.7 mM. Omitting the lactalbumin hydrolysate and yeast extract decreased the SCC by about 20% (see Results) but had no other observable effects on the preparation. Normal Ringer with lactalbumin hydrolysate and yeast extract had a specific resistance of 77.5 Ohm. cm.

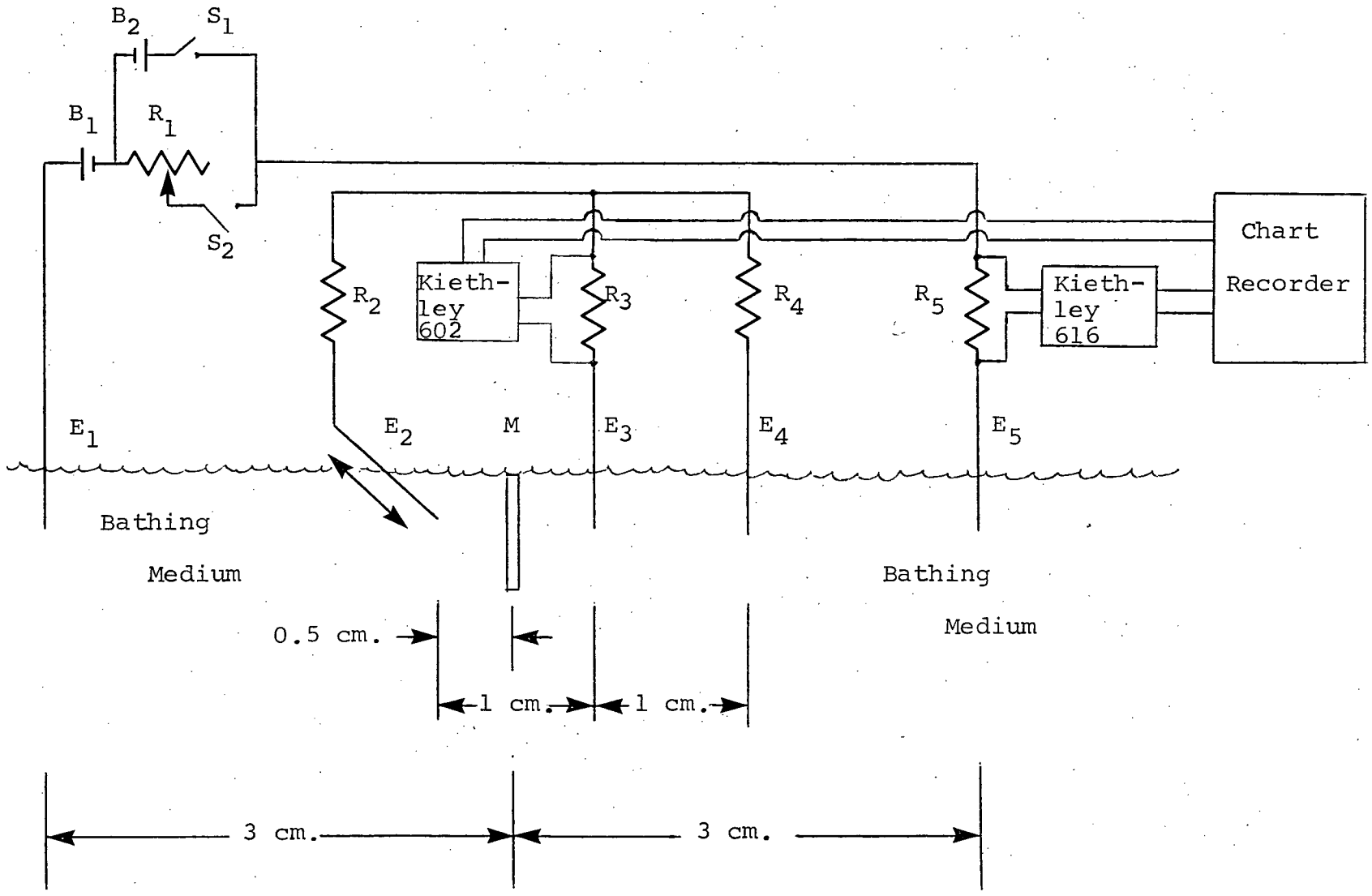
Several other Ringer's solutions were used in this study to investigate the properties of the Cl^- transporting system of the rectum. In these solutions all the Cl^- was replaced with either $SO_4^=$, NO_3^-, or acetate. These Ringer's solutions, which will be subsequently referred to as "$SO_4^=$-Ringer", "NO_3^--Ringer", and "Acetate Ringer" respectively,

were otherwise identical to Normal Ringer, except that $SO_4^=$-Ringer contained 128.3 mM sucrose instead of the normal 79.8 mM in order to keep the freezing point at -0.587°C. Another $SO_4^=$-Ringer's solution contained lactalbumin hydrolysate and yeast extract which introduced small amounts of Cl^- (3 mM) and is referred to as "Low Cl^- $SO_4^=$-Ringer". The Cl^- concentrations of all solutions were measured directly with a "Radiometer" CMT 10 Chloride Titrator.

The Cl^- content of the rectal tissue was measured both immediately after removal from the animal and after varying lengths of time in $SO_4^=$-Ringer. The following procedure was used: rectal preparations were quickly dipped ten times in sucrose solution having a freezing point of -0.587°C to wash of the majority of ions adhering to them, lightly blotted dry on tissue paper and transferred to individual capped vials containing 1 ml. of distilled water. The recta were then stored at room temperature for 24 hours by which time all the cells had lysed releasing their contents into the solution. Then after vigorous shaking to ensure complete mixing, the Cl^- concentration of the solution in each vial was measured as previously described.

The following circuitry (Figure 3) which was modified from Wood (1972) was used to measure the PD and SCC of the <u>in vitro</u> preparation. "M" represents the rectal

Figure 3. Electrical circuitry. See text for explanations of symbols.


B2
S1
B1
R1
S2
R2
Kieth-
ley
602
R3
R4
R5
Kieth-
ley
616
Chart
Recorder
E1
E2
M
E3
E4
E5
Bathing
Medium
Bathing
Medium
0.5 cm.
1 cm.
1 cm.
3 cm.
3 cm.

preparation. R_2, R_3, and R_4 are precision 10^8 ohm ±1% resistors. R_5 is a selected 10^3 ohm carbon resistor. R_1 is a Heathkit decade resistance box, model 1N-17. B_1 and B_2 are 9 volt batteries. The switch S_2 is closed when applying a short circuit current across the rectal wall. The switch S_1 is closed momentarily to pass a current pulse through the preparation to measure transepithelial resistance either under short circuit or open circuit conditions. The voltmeter (a Kiethley 602 electrometer) measures one third of the true membrane PD. The SCC is read as a voltage across R_5 using a Keithley 616 digital electrometer. The membrane PD and SCC are recorded graphically on a 2 pen "Fisher Recordall" strip chart recorder. E_2, E_3, and E_4 are Calomel electrodes connected to the two half-chambers containing the rectal epithelium via salt bridges consisting of polyethlyene tubing (PE-50) with 3 cm. long glass capillary tips and filled with 3M KCl in 3% agar. The glass capillary tubes, which are inserted into the chambers are shown in Figures 2 and 3, were used because they are more rigid than polyethlyene tubing. This rigidity was essential for accurate voltage readings because, when passing current through the chambers, E_2 and E_4 must be equidistant from E_3. Current is applied across the chambers through E_1 and E_5 which are silver-silver chloride electrodes cut from 0.004 inch silver foil. In actual practice three chambers with identical circuits to

that described above were used simultaneously. The two meters and the recorder were switched to each preparation when readings were required.

The three electrode system of voltage measurement was used instead of the usual two electrode system because when E_2 and E_4 are equidistant from E_3 the voltage gradients set up through the Ringer's solution in the two chamber halves by passing a SCC are automatically cancelled out across R_3 and so the only voltage seen across R_3 is one third of the true membrane voltage plus the asymmetry potential. With a two electrode system, the voltage gradients are not cancelled out at the voltmeter. This complicates the measurement of voltage under short-circuit conditions considerably and this, along with technical difficulties related to chamber construction, was considered sufficient reason to use the three electrode system.

The error in voltage measurement introduced by placing E_2 and E_3 such a large distance (i.e., 0.5 cm.) from the membrane can be shown to be negligible. Assuming a membrane PD of 100 mV (over three times the average initial reading in the present study) and considering that the resistance of the voltage measuring circuit $(R_2 + (R_3 \times R_4)/(R_3 + R_4))$ is 1.5×10^8 ohms, the current through the circuit would be 0.66×10^{-9} amperes. The resistance of the Ringer's

solution between E_2 and E_3 is about 60 Ohms. A current of 0.66×10^{-9} amperes through a resistance of 60 Ohms would introduce a voltage drop of only 0.02 microvolts, which is negligible and also well below the internal noise level of the Kiethley 602. For further discussion of electrical measurements using this experimental system see Wood (1972).

Prior to mounting the rectum in the chambers the position of the voltage electrodes was adjusted so that E_2 and E_4 were equidistant from E_3. This was accomplished by first filling the chambers with Ringer's solution, inserting the voltage electrodes, and measuring the asymmetry potential. Then a constant current of 50 microamperes was passed through the solution via the current electrodes. If E_2 and E_4 were equidistant from E_3 the voltage gradients introduced into the solution by this current cancelled across R_3 and only the original asymmetry PD was recorded. If the observed voltage was other than the asymmetry potential, E_2, which intersects the longitudinal chamber axis at about a 35^{o} angle, was moved in or out of its socket as shown in Figure 3 until only the asymmetry potential was observed on the voltmeter. At this point the tip of E_3 was the same distance from E_2 and E_4. The current electrodes were then disconnected and the chambers disassembled without disturbing the position of the voltage electrodes. The rectal epithelium was then mounted in the chambers

as previously described.

Unidirectional flux studies of $^{36}Cl^-$ across the rectal wall were measured to determine the net flux of chloride under short circuit conditions. $^{36}Cl^-$ was obtained as a 2.41 M HCl solution with a specific activity of 4.4 mCi/gm. from New England Nuclear Inc., Boston, Mass., U.S.A. The solution was neutralized to pH=7 by adding the appropriate amount of a NaOH solution of equal molarity and was frozen until ready for use. Approximately 0.31 ml. of the neutralized solution was added to either the lumen or the hemolymph reservoir each of which contained 20 ml. of Normal Ringer at the beginning of the third hour (T = 2:00) after mounting the rectum in the chambers. An equal volume of "cold" NaCl solution of equal molarity was added to the opposite reservoir at the same time. The total amount of chloride added to each side during this procedure was never more than 1 mMole. This increased the Cl^- concentration of the bathing media by 17 mM to give a final Cl^- concentration of 84 mM. Fifteen minutes after adding the isotope four 1 ul samples were withdrawn from the labelled side and each was added to 1 ml. of distilled water in a stainless steel planchette. Specific activity of $^{36}Cl^-$ on the labelled side of the membrane was calculated from these readings. At the same time and every 15 m. thereafter until the end of the fourth hour a 500 ul sample was removed from the initially

unlabelled side and transferred to another planchette. The planchettes were then evaporated to dryness under an infrared lamp and counted to 10,000 counts with a "Nuclear Chicago" Gas Flow Detector, Model 470 coupled to an Automatic Planchet Sample Changer, Model 1042 and a Series 8703 Decade Scaler and Lister. A total of 4 ml. (20%) of the bathing solution was withdrawn during each experiment. Since this solution was not replaced, a gradually increasing hydrostatic pressure gradient from the labelled to the initially unlabelled side developed. This gradient never amounted to more than 0.5 cm. of water and since it was present for flux experiments in both directions its influence on net Cl^- flux was assumed to be negligible. It probably accounts for the small positive slope of the Cl^- influx time course in Figure 16. Unidirectional Cl^- fluxes during each 15 minute interval between samples were computed from the following formula,

$$J_{Cl^-} = \frac{a_2 \cdot V \cdot C}{a_1 \cdot T \cdot A},$$

adapted from Shaw (1955) where

J_{Cl^-} = Flux of Cl^- in uMoles cm^{-2} hr^{-1}

a_1, a_2 = Radioactivity of $^{36}Cl^-$ in counts/min. x ml. on side 1 (initially labelled) and side 2 (initially unlabelled) respectively.

V = Volume of fluid on side 2.

C = Concentration of Cl^- on side 1.

t = Time period over which flux occurred (0.25 hr).

A = Area of membrane (0.196 cm^2).

Due to their extreme variability, flux values for the first 15 minutes after adding the isotope (T = 2:15) were discarded.

RESULTS

ELECTRICAL PARAMETERS AND VIABILITY OF THE *IN VITRO* PREPARATION

Measurements of electrical parameters indicate that the *in vitro* preparation of the locust rectum used in this study survives for more than 6 hours and that a situation approximating a steady state exists during the normal experimental period (third and fourth hour) when flux studies were conducted. The average PD across the rectum (34 mV, lumen positive) remains relatively constant for at least 6 hours (Figure 4) and is in good agreement with the value (15 - 32 mV) observed *in vitro* (Phillips 1964).

The initial trans-epithelial resistance of the open-circuited rectum was 5500 Ohms/cm.^{2}. After 2 hours the resistance had increased to 6700 Ohms/cm^2 where it remained relatively constant for the next three hours; thereafter a slow decline was apparent (Figure 5). The time course of the resistance changes under SCC conditions was quite similar (Figure 6). This suggests that the major resistive elements in the rectal epithelium are not sensitive to membrane PD as are axonal membranes.

The SCC is initially high (7.5 uMoles.cm^{-2}.hr^{-1}),

Figure 4. Time course of trans-rectal PD. ◆, In vitro preparation of Herrera et al. (1976). ●, In vitro preparation described in this study. Mean ± S.E.M. n=14-16 for the first 6 hours, and 2-9 thereafter.

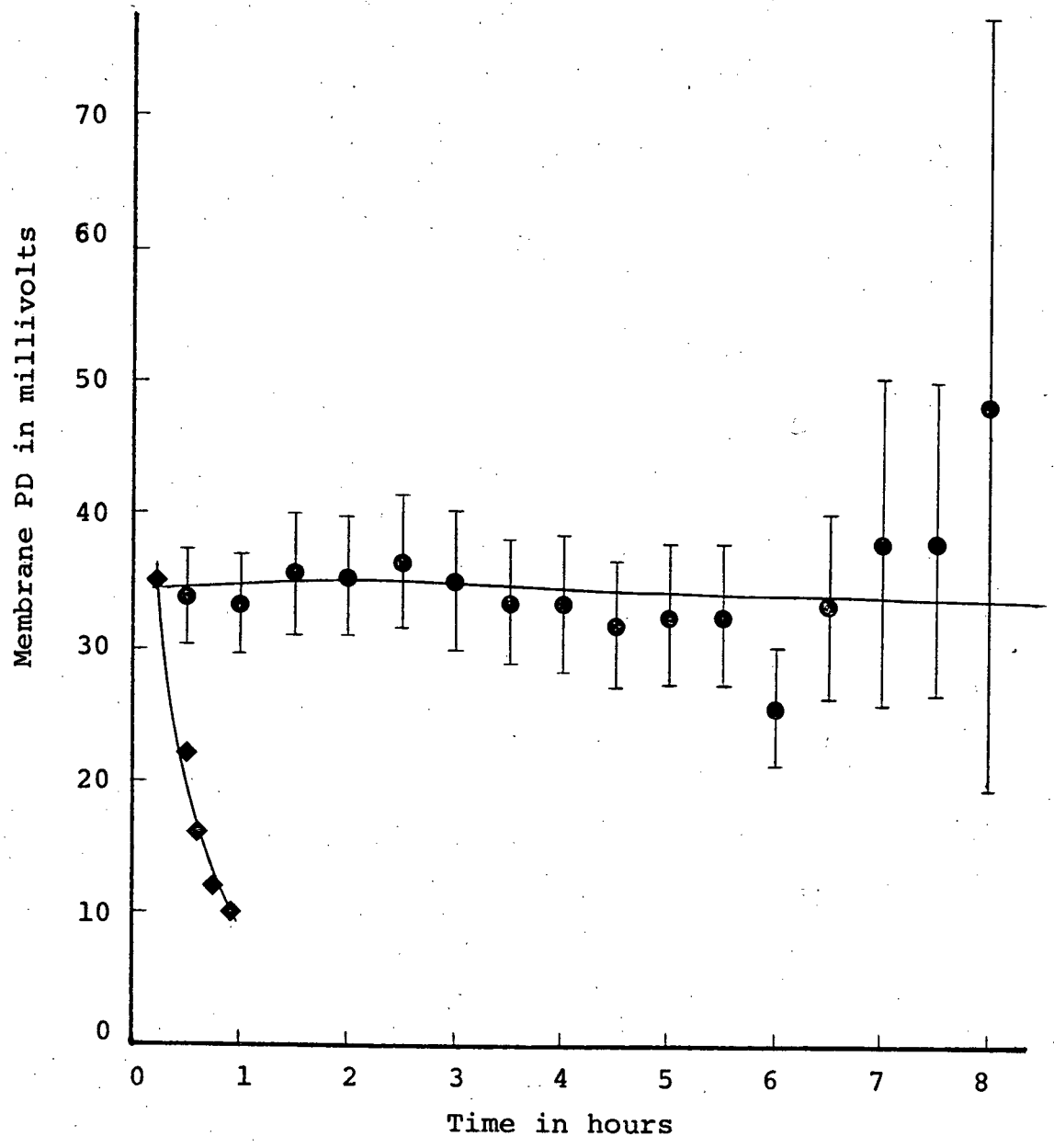

Membrane PD in millivolts
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Time in hours

Figure 5. Time course of trans-rectal resistance under open circuit conditions. Mean ± S.E.M. n=14-16 for all points.

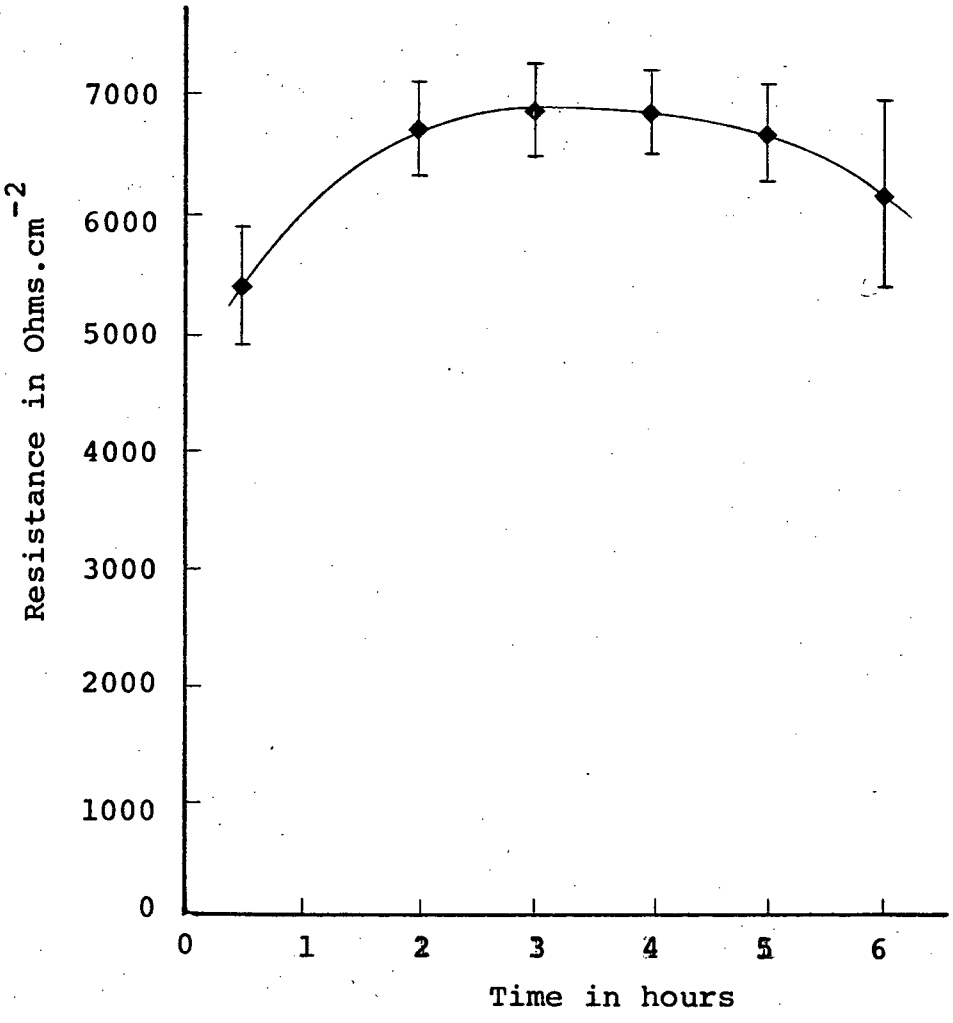
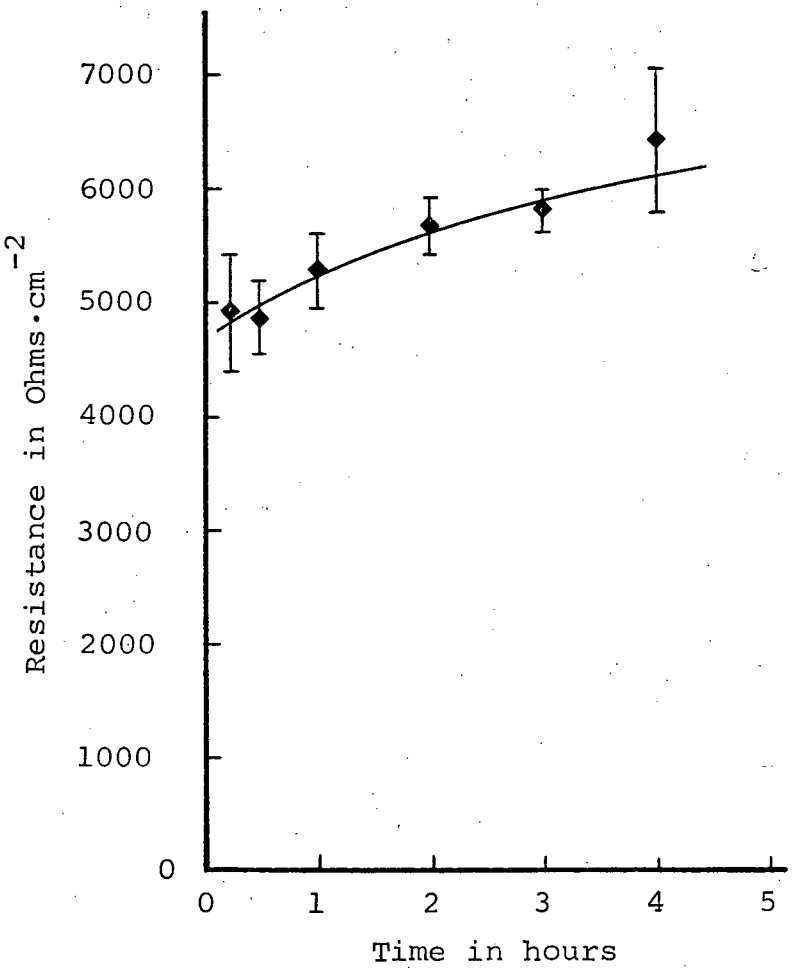

Resistance in Ohms.cm^{-2}
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
5
6
Time in hours

Figure 6. Time course of trans-rectal resistance under SCC conditions. Mean ± S.E.M. n=3.

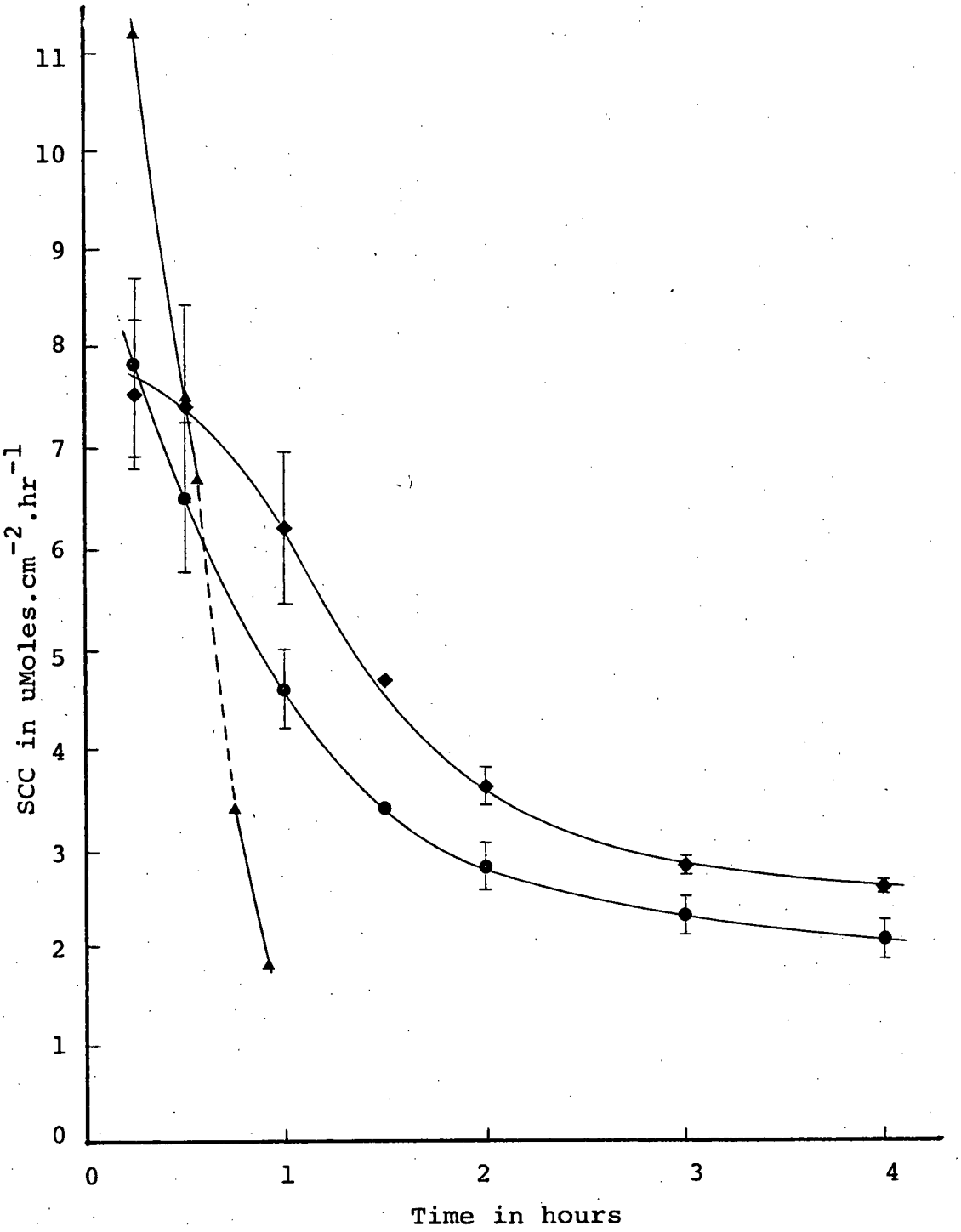

7000
6000
5000
4000
3000
2000
1000
0
Resistance in Ohms·cm^{-2}
0
1
2
3
4
5
Time in hours

drops rapidly to a value of 3 uMoles.cm^{-2}.hr^{-1} within two hours, and thereafter falls slowly over several hours (Figure 7). The latter value for SCC is within the range expected from previous reports of total transport of ions in other *in vitro* preparations of the locust rectum under various experimental conditions (see Discussion).

The Normal Ringer used in some early experiments contained lactalbumin hydrolysate and yeast extract. These were omitted in later experiments where a chloride-free Ringer's solution was desired. Surprisingly, this omission caused a small but significant decrease in the average SCC (Figure 7). However, these two constituents probably contain substantial amounts of organic anions which it will be shown could have marked effect on SCC.

I considered the possibility that the sharp decline in SCC and the concomittant sharp rise in resistance during the initial two hour period were associated with rearrangements of ionic gradients during adjustments of the tissue to SCC conditions, rather than a decline in metabolic activity. A further possibility was that the rate of a voltage-sensitive ion transport process decreased following removal of the normal PD under SCC conditions. However, the fact that open-circuit and short-circuit resistances were of similar magnitude and follow similar time courses argues against the latter

Figure 7. Time course of rectal SCC. ▲, In vitro preparation of Herrera et al. (1976). In vitro preparation described in this study: ◆, rectum bathed in Normal Ringer; ●, rectum bathed in Normal Ringer without lactalbumin hydrolysate and yeast extract. Mean ± S.E.M.; ◆, n=8; ●, n=11.


SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
5
6
7
8
9
10
11
0
1
2
3
4
Time in hours

alternative. If readjustments of ionic gradients in the tissue were the explanation, then the time course of the SCC should remain the same even if the in vitro preparation is left for two hours in the open-circuit state before applying the SCC condition. Figure 8 indicates that this is not the case. The initial SCC observed after two hours in the open-circuit state is almost identical to the SCC at that time in preparations which were in the SCC condition from time zero. Obviously the decline in SCC in the initial two hour period is due to a loss of metabolic or transport activity. This is probably a consequence of eliminating the very heavy tracheal supply to the rectum or possibly it reflects the inadequacy of Normal Ringer as a substitute for hemolymph. It is also possible that removing the rectum from the animal eliminates some natural neural or hormonal stimulation of ion transport.

To demonstrate the metabolic dependence of the SCC generated by the in vitro preparation, I investigated the influence of temperature and metabolic inhibitors on the SCC.

The rectal SCC is markedly dependent on temperature (Figure 9). Decreasing the temperature of the bathing solution from 25°C to 10°C decreases the SCC by more than 66%. The Q_{10} for the temperature range 10°-20°C was estimated to be 2.5 and that for the range 25°-35°C was 2.25. These values of Q_{10} are consistant with a metabolic dependence of the SCC (Prosser, 1973).

Figure 8. Time course of rectal SCC after an initial two hours under open circuit conditions: ◆. Arrow indicates time when short circuiting was initiated. ●, control SCC time course. Mean ± S.E.M. n=2.

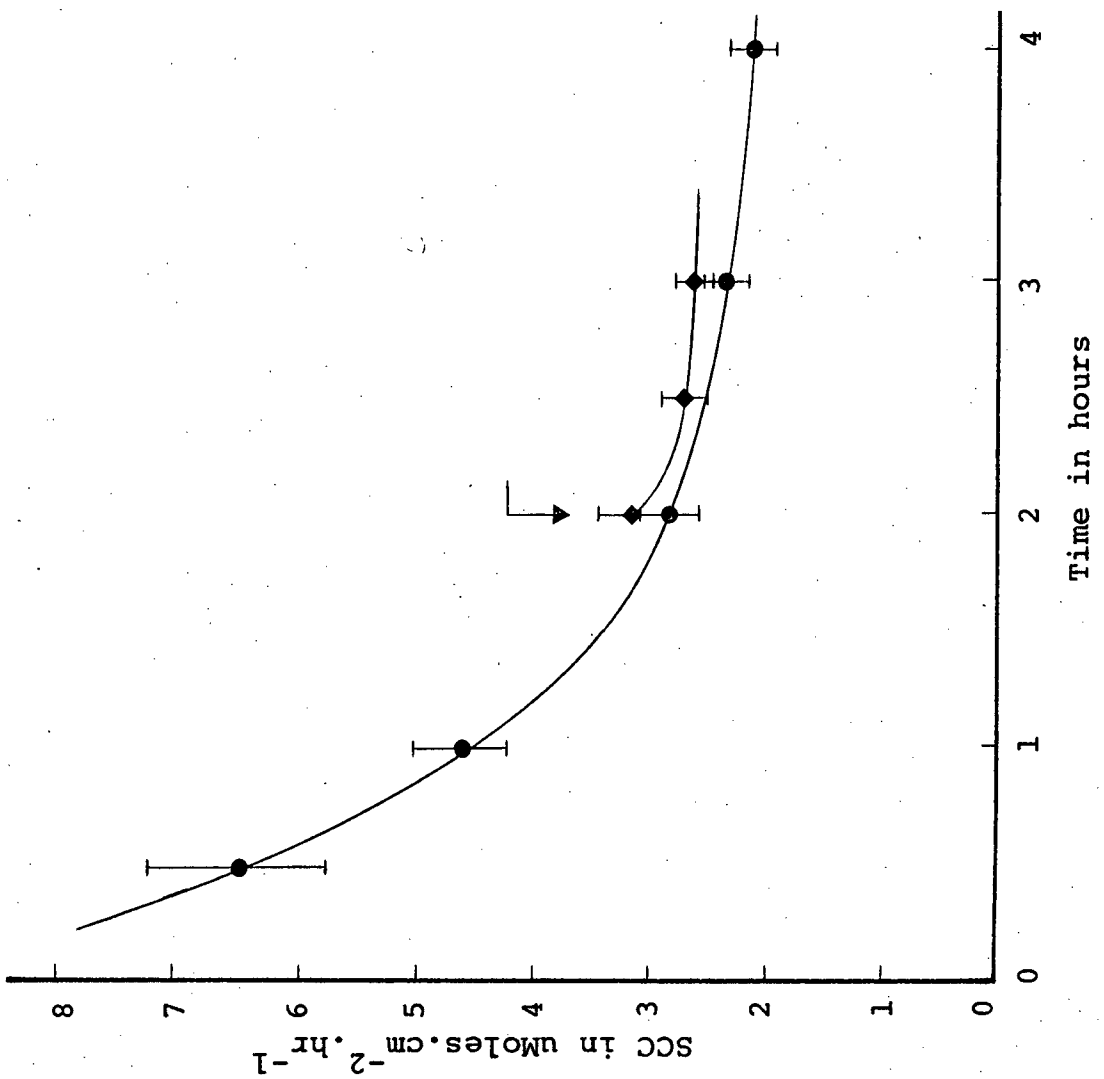
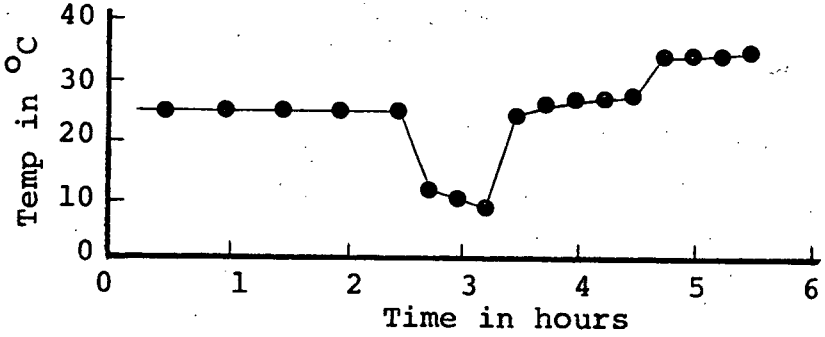
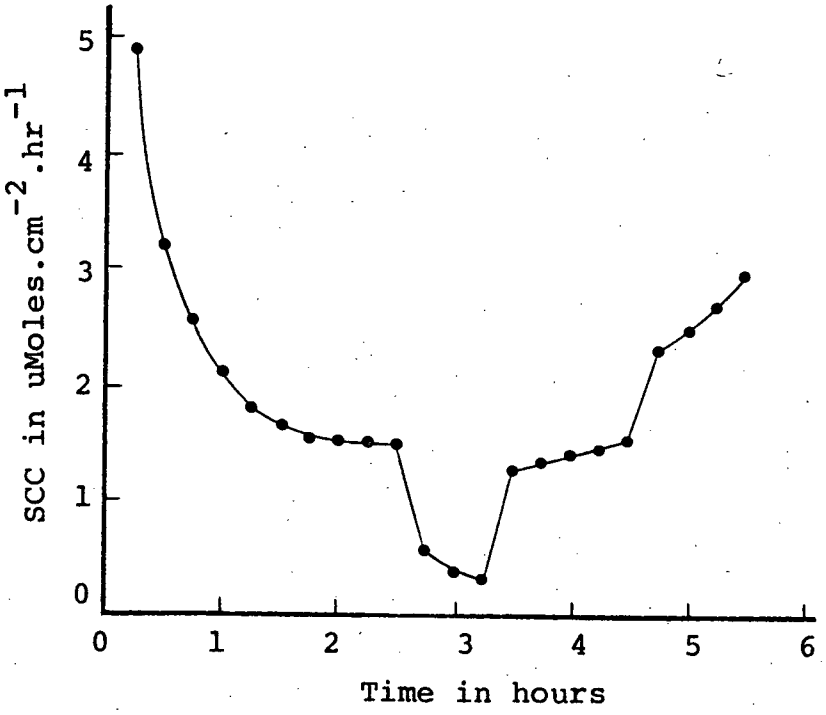

SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
5
6
7
8
0
1
2
3
4
Time in hours

Figure 9. Effect of temperature on rectal SCC in a typical preparation.


Temp in °C
40
30
20
10
0
0
1
2
3
4
5
6
Time in hours


SCC in uMoles.cm^{-2}.hr^{-1}
5
4
3
2
1
0
0
1
2
3
4
5
6
Time in hours

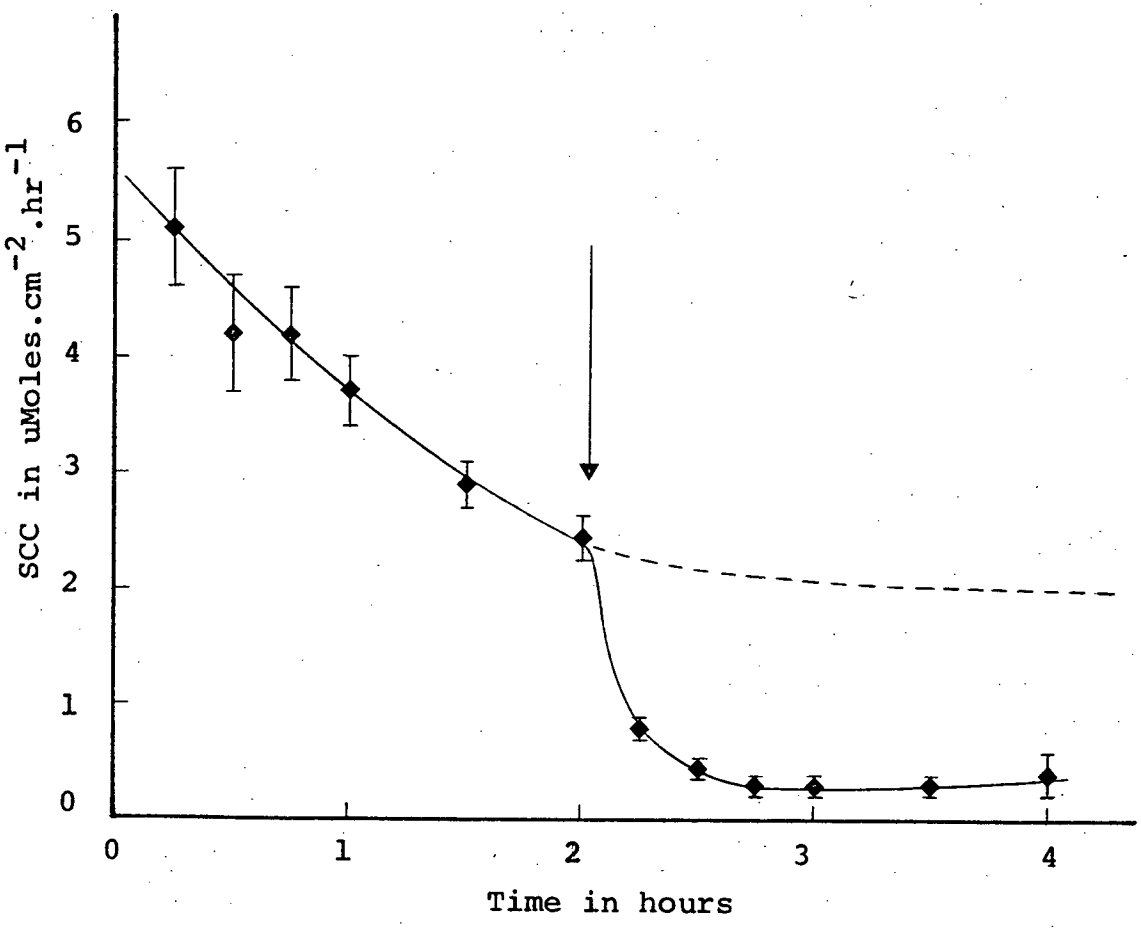
The rectum was also found to be very sensitive to oxygen deprivation. Because the oxygen supply to the bathing solution also functioned as the stirring gas, the effects of oxygen deprivation in this *in vitro* preparation were more easily demonstrated by adding an inhibitor of oxidative metabolism to the bathing solutions. Addition of 1 mM KCN (final concentration) caused a rapid reduction of over 80% in the SCC (Figure 10). Clearly, most of the SCC is fueled by aerobic metabolism though a small anaerobic component cannot be excluded.

CHLORIDE TRANSPORT AND THE SOURCE OF THE SCC

The direction of the observed SCC indicates either a net transport (total cations minus total anions) of negative charges to the hemolymph or positive charges to the lumen side. It is known that the locust rectum *in situ* (Phillips, 1964) and *in vitro* (Goh, 1971; Goh and Phillips,in preparation) absorbs Na^+, K^+, and Cl^- from the lumen against electrochemical gradients. Rapid acidification of the lumen contents indicates either transport of H^+ ions to the lumen or HCO_3^- ions to the hemolymph (Phillips, 1961, 1965; Speight, 1968).

Since Cl^- was the only major inorganic anion in the Ringer's solution used in the present study, transport of this ion seemed a likely source of the observed SCC. If so, the complete replacement of Cl^- ions in the bathing solutions by

Figure 10. Effect of 1 mM KCN on rectal SCC. Arrow indicates time at which KCN was added to the bathing media. Mean ± S.E.M. n=4. Dashed line indicates control.

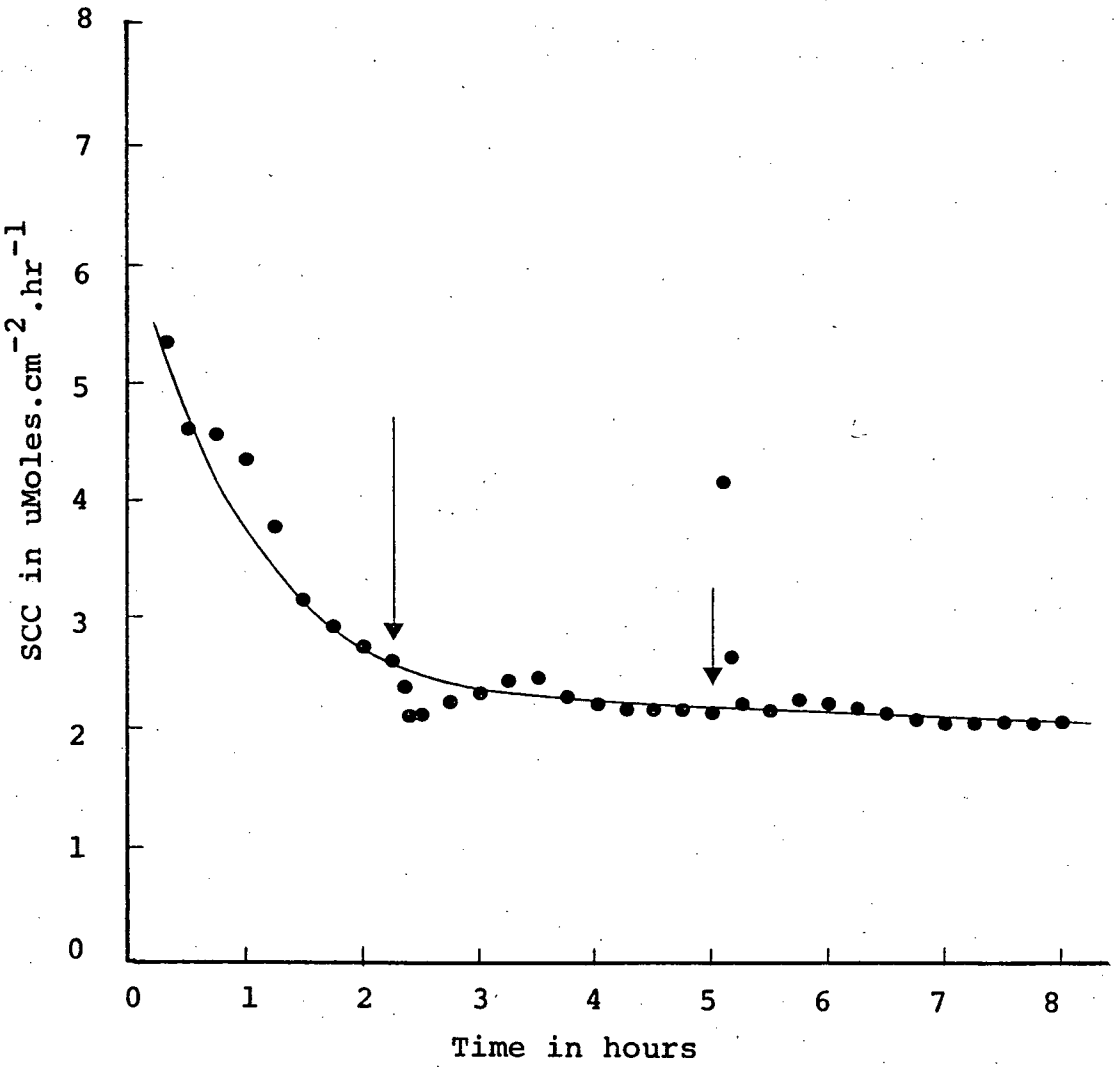

SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
5
6
0
1
2
3
4
Time in hours

other anions might be expected to cause a reduction in, or reversal of the SCC or its complete disappearance if only Cl^- is actively transported.

To this end, a Low chloride $SO_4^=$-Ringer solution was prepared in which nearly all the Cl^- normally present was replaced with $SO_4^=$ to give a final measured Cl^- concentration of less than 3 mM as compared to 67 mM Cl^- in Normal Ringer. Rectal preparations were mounted in the chambers in Normal Ringer solution and a SCC was applied in the usual manner for 2.25 hours. Then the bathing media were washed out and replaced with more than ten times the chamber volume of Low chloride $SO_4^=$-Ringer's solution as described previously. At the start of the fifth hour the recta were again exposed to Normal Ringer's solution. Figure 11 indicates that the SCC was unaffected by substitution of $SO_4^=$ for Cl^- in the Ringer's solution. The results of this experiment were unexpected since the locust rectum has been shown to transport Cl^- *in vivo* and *in vitro* (Phillips, 1961, 1964; Goh, 1971; Phillips and Goh, in preparation; Meredith and Phillips, unpublished data) and since the large SCC which I observed during this time period indicated the viability of the present *in vitro* preparation.

Several possible explanations of these data occured to me: (1) Large stores of tissue Cl^- might maintain the SCC

Figure 11. Time course of SCC during a typical experiment in which recta were exposed to bathing media in which all the Cl^- had been replaced with $SO_4^=$. Arrows indicate time of exposure to $SO_4^=$ media and normal media respectively.

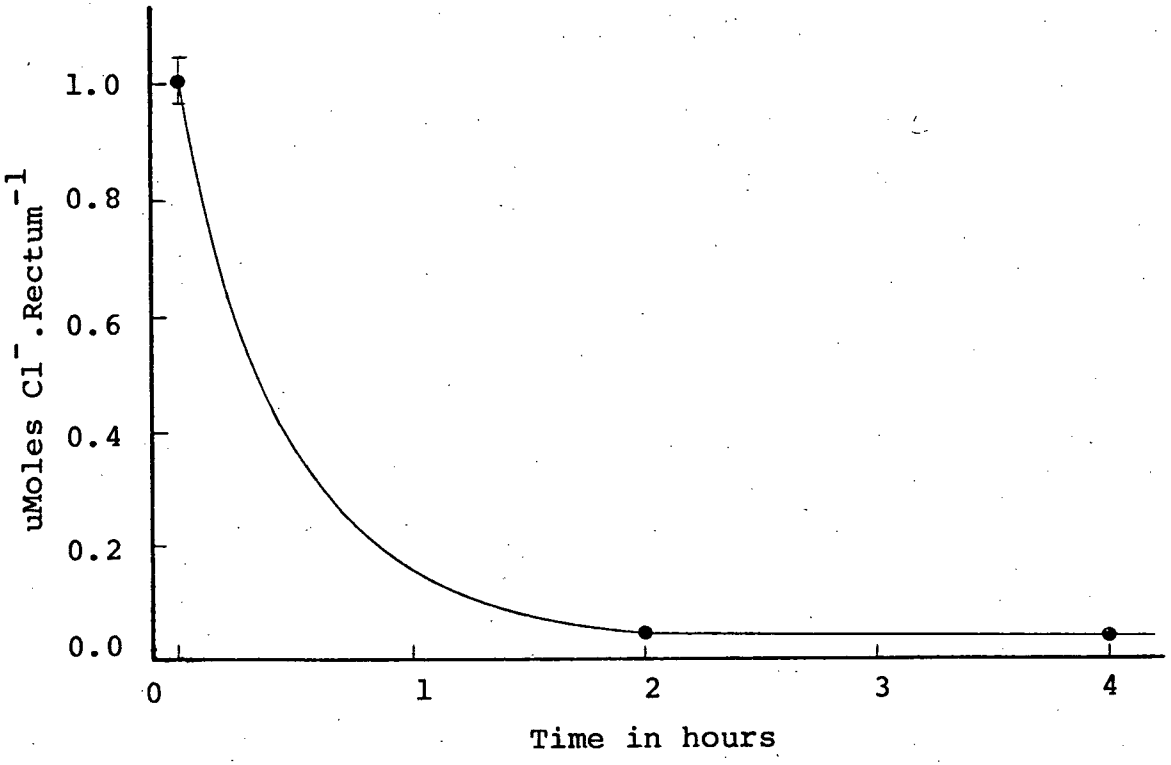

SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
5
6
7
8
Time in hours

for a considerable time after placing the rectum in $SO_4^=$-Ringer's solution. (2) The Cl^- pump, if present, might have a very low affinity constant (K_m); i.e., well below the level of 3 mM Cl^- present in Low-Cl^- $SO_4^=$-Ringer's solution. (3) The Cl^- pump might be a rather non-specific pump which can transfer $SO_4^=$ as well as Cl^-. (4) Possibly this in vitro preparation does not actively transport Cl^-. (5) The Cl^- pump might be tightly coupled to Na^+ and K^+ transport in the same direction such that cation transport stops when Cl^- transport ceases.

To test the first hypothesis, that tissue Cl^- might sustain the SCC, the level of this anion in recta freshly dissected from the locust was measured and found to be 1± 0.04 uMoles rectum. When recta were mounted for various lengths of time in Cl^- free $SO_4^=$-Ringer's solution it was found that more than 90% of the tissue Cl^- was lost within the first two hours (Figure 12). Clearly, tissue Cl^- could not be maintaining the SCC during the 3rd to 5th hour in Figure 11. Moreover the rectum pumps more Cl^- across itself in one hour than is present in the whole of the rectal epithelium (see below).

The second possibility, that a Cl^- pump in the rectal epithelium might have a K_m value well below 3 mM was tested by preparing a "Cl^--free" $SO_4^=$-Ringer's solution from which lactalbumin hydrolysate and yeast extract were omitted

Figure 12. Cl^- efflux from recta incubated for varying times in $SO_4^=$ -Ringer. Mean ± S.E.M. S.E.M. for 2nd and 4th hours was smaller than the point. n=10.


1.0
0.8
0.6
0.4
0.2
0.0
uMoles Cl⁻.Rectum⁻¹
0
1
2
3
4
Time in hours

because they contained some Cl^- ions. Sucrose was added to maintain the same osmotic pressure. The chloride concentration of this $SO_4^=$-Ringer's solution was less than 0.1 mM (i.e., below detectable limits). Recta were bathed in this Cl^--free Ringer's solution both during dissection and subsequently during SCC experiments. The SCC during the third and fourth hours (Figure 13) was indistinguishable from that observed in previous experiments (Figure 11). Clearly, a low K_m value for a chloride pump does not explain the results in Figure 11. However, one surprising difference between the two experiments (Figures 11 and 13) was observed. The initial large decline in SCC during the first two hours in Normal Ringer did not occur when preparations were bathed in $SO_4^=$-Ringer. Rather a value of 2.6 uMoles.cm^{-2}.hr^{-1} was attained within 0.5 hours and was maintained for at least another 3.5 hours.

The third possibility which might explain the results in Figure 11 was that a Cl^- carrier also transported $SO_4^=$. This has been suggested for mammalian red blood cells (Gunn, 1972). The effects of replacing the Cl^- in Normal Ringer with other anions on SCC was therefore tested. The magnitude and direction of the SCC across recta exposed to NO_3^--Ringer (Figure 14) were almost identical to those observed with $SO_4^=$-Ringer (Figure 13). Similar experiments with Acetate Ringer's solution produced very interesting and unexpected results (Figure 15). The initial SCC

Figure 13. Time course of rectal SCC in $SO_4^{=}$ Ringer. Mean ± S.E.M. n=10. Dashed line indicates control.

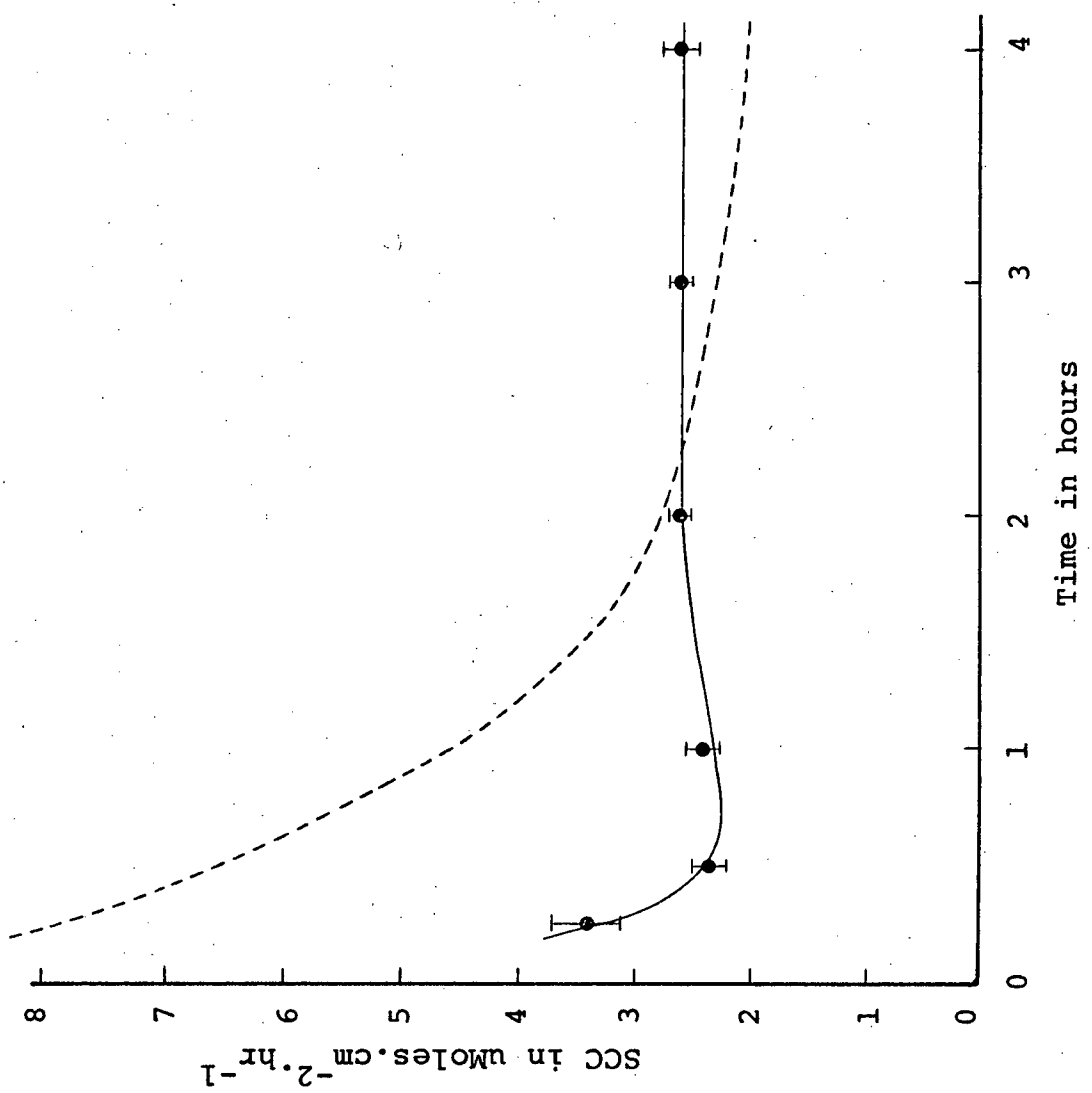

SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
5
6
7
8
0
1
2
3
4
Time in hours

Figure 14. Time course of rectal SCC in NO_3^- Ringer. Mean ± S.E.M., n=6. Dashed line indicates control.

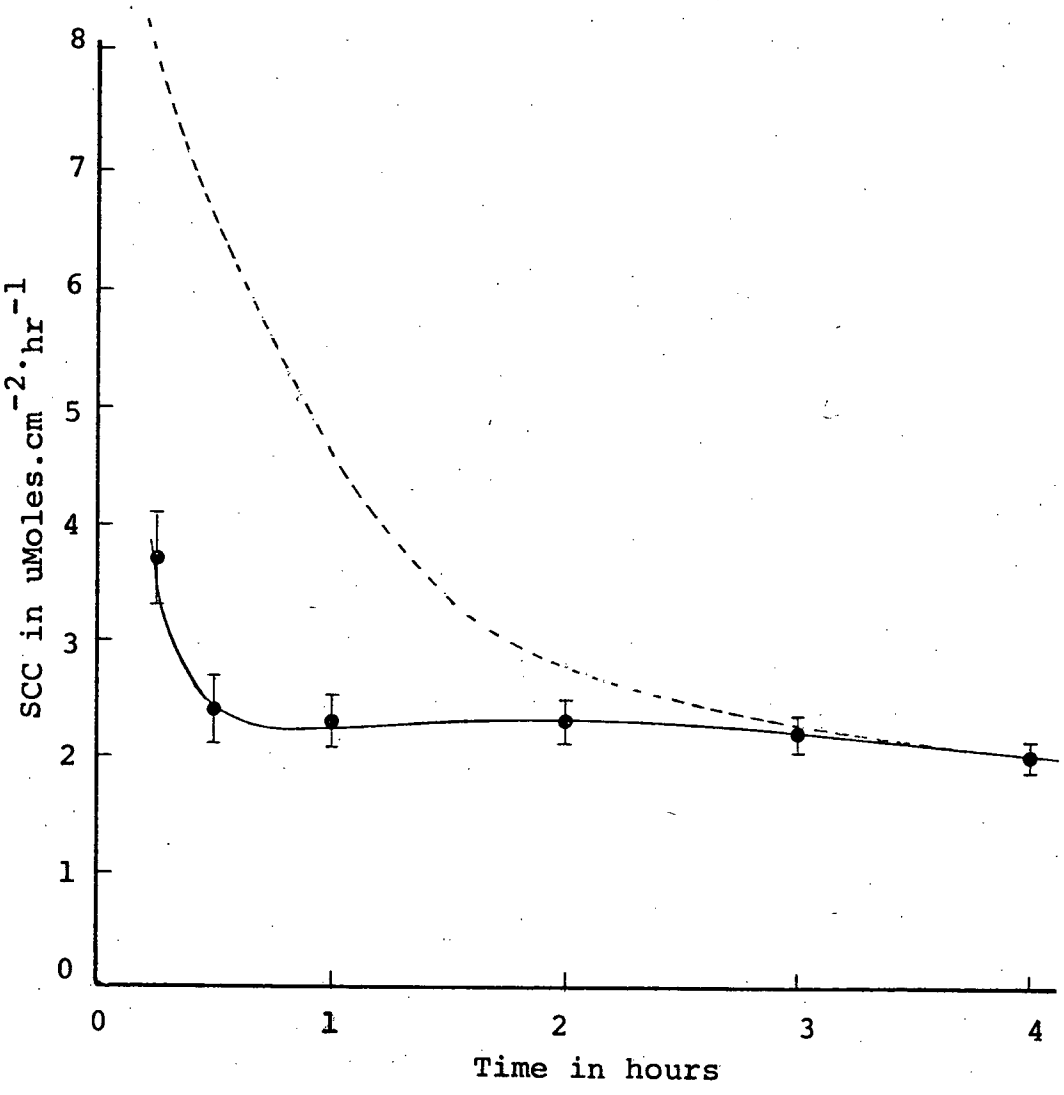
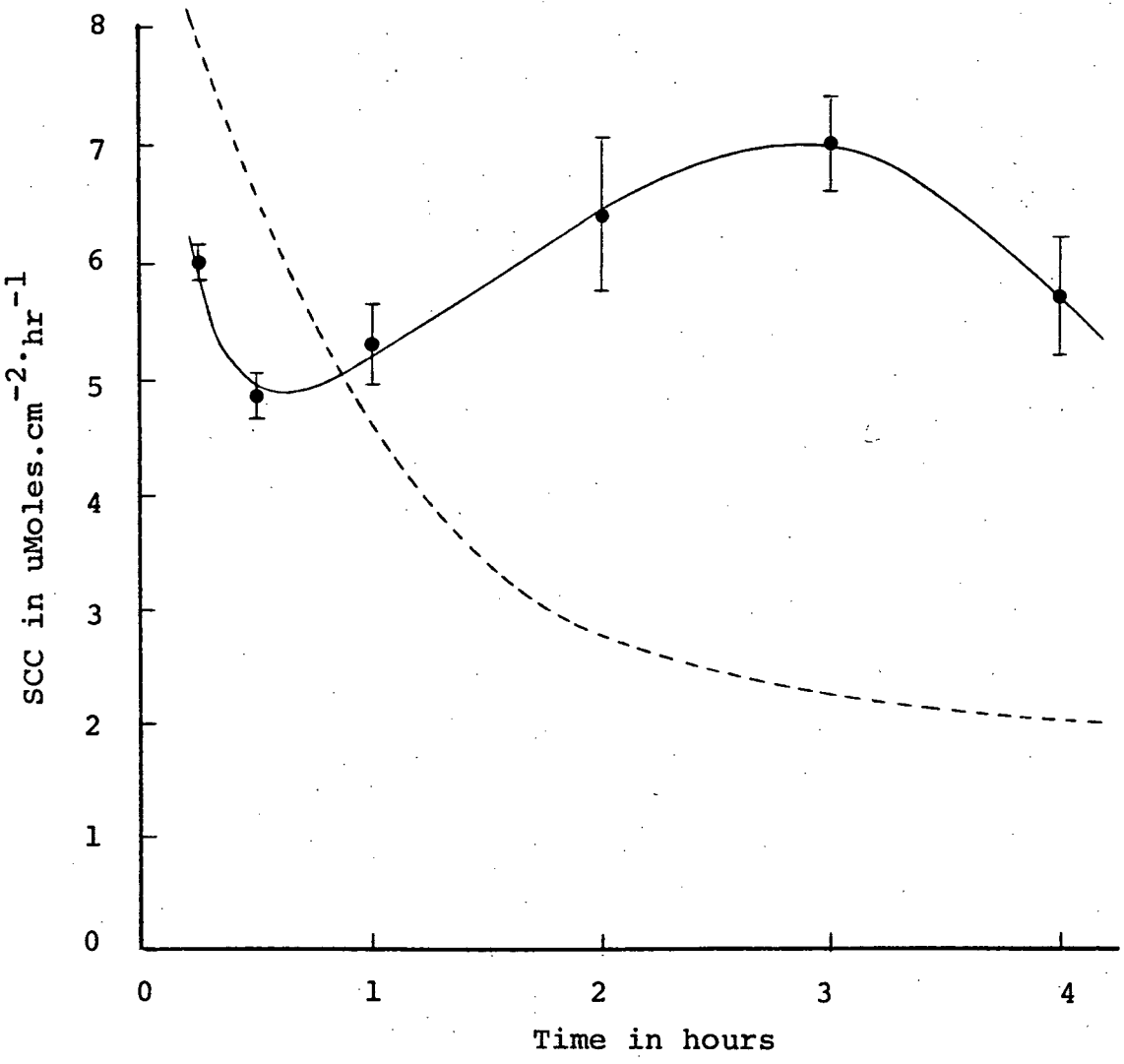

8
7
6
5
4
3
2
1
0
SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
Time in hours

Figure 15. Time course of rectal SCC in Acetate Ringer. Mean ± S.E.M., n=6. Dashed line indicates control.


8
7
6
5
4
3
2
1
0
SCC in uMoles.cm^{-2}.hr^{-1}
0
1
2
3
4
Time in hours

in Acetate Ringer (6 uMoles . cm^{-2} . hr^{-1}) was similar to that initially observed for recta bathed in Normal Ringer (Figure 7). However the subsequent rapid decline which followed in recta bathed in Normal Ringer was not observed. After a small drop within the first hour, the SCC increased gradually to the initial value by the third hour and declined slowly thereafter. Individual preparations all showed this pattern. On the average the SCC during the third and fourth hours was two to three times higher for recta bathed in Acetate Ringer than for those bathed in Normal, NO_3^-, or $SO_4^=$-Ringer's solutions. Possible explanation for this stimulation of SCC by acetate are considered in the Discussion. Clearly none of these experiments in which Cl^- is replaced with acetate, NO_3^-, or $SO_4^=$ fulfill the prediction that removal of Cl^- from the bathing media should lower the SCC during the third and fourth hours. Neither do they exclude the possibility of a general anion pump which can transfer Cl^-, NO_3^-, $SO_4^=$, or acetate. Therefore the net flux of $^{35}SO_4^=$ was measured and found to be very low (Spring, unpublished results). Clearly the SCC across recta during the third and fourth hours in $SO_4^=$-Ringer's solution (Figure 11 and 13) was not maintained by a substitution of $SO_4^=$ for Cl^- transport.

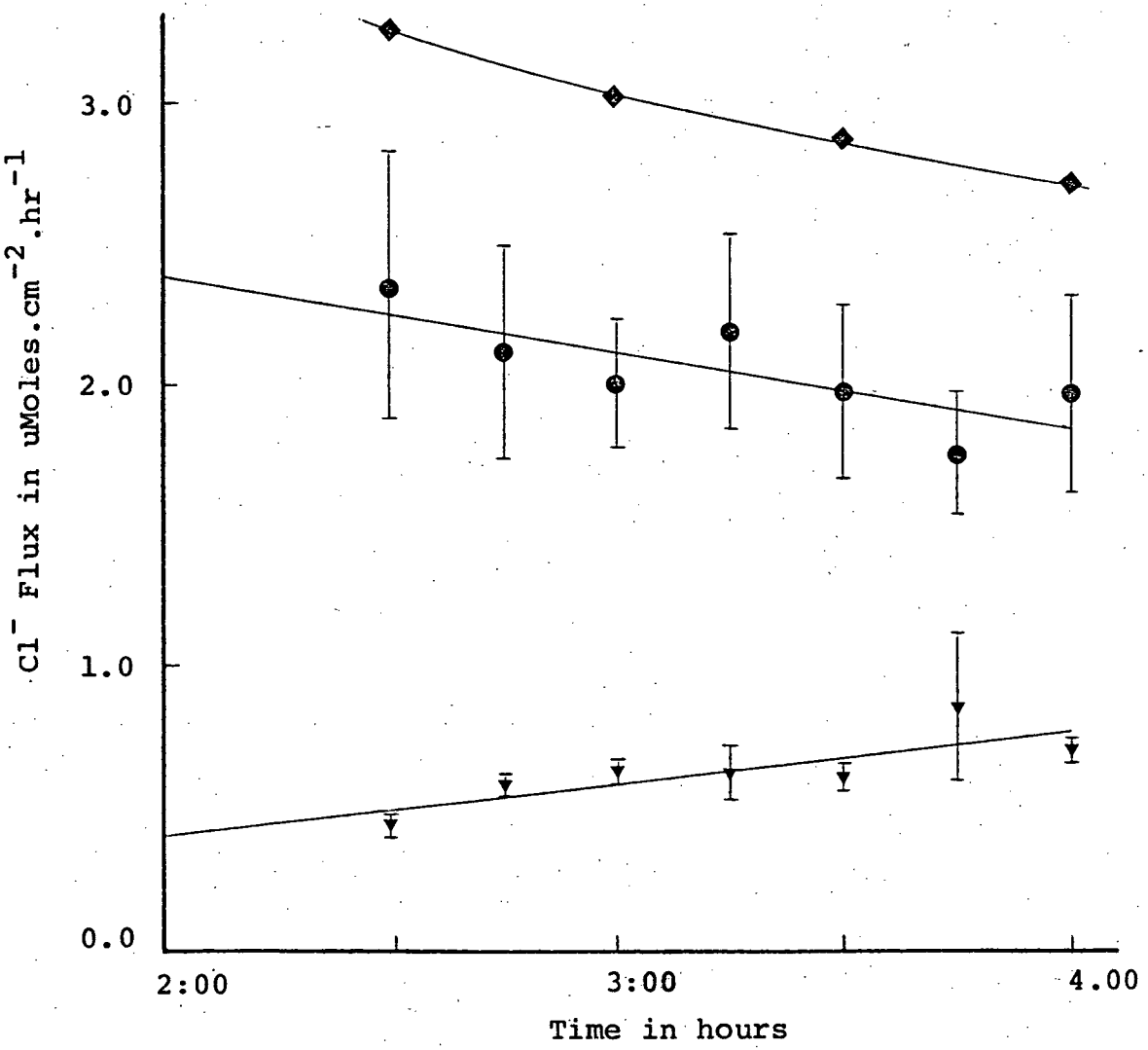
These results led me to question whether significant Cl^- transport occurred in this <u>in vitro</u> preparation (fourth possibility), even though it has been demonstrated both <u>in vivo</u>

(Phillips 1964) and in another type of in vitro preparation (Goh, 1971; Meredith & Phillips, unpublished data). Loss of Cl^- transport in in vitro preparations has been reported for frog skin (Harris, 1960) and in another in vitro preparation of the locust rectum (Irvine & Phillips, 1971). Since further anion substitution studies seemed unlikely to answer this question I turned to flux studies using $^{36}Cl^-$ under SCC conditions to determine if Cl^- is actually transported in this in vitro preparation.

CHLORIDE FLUX STUDIES UNDER SCC CONDITIONS

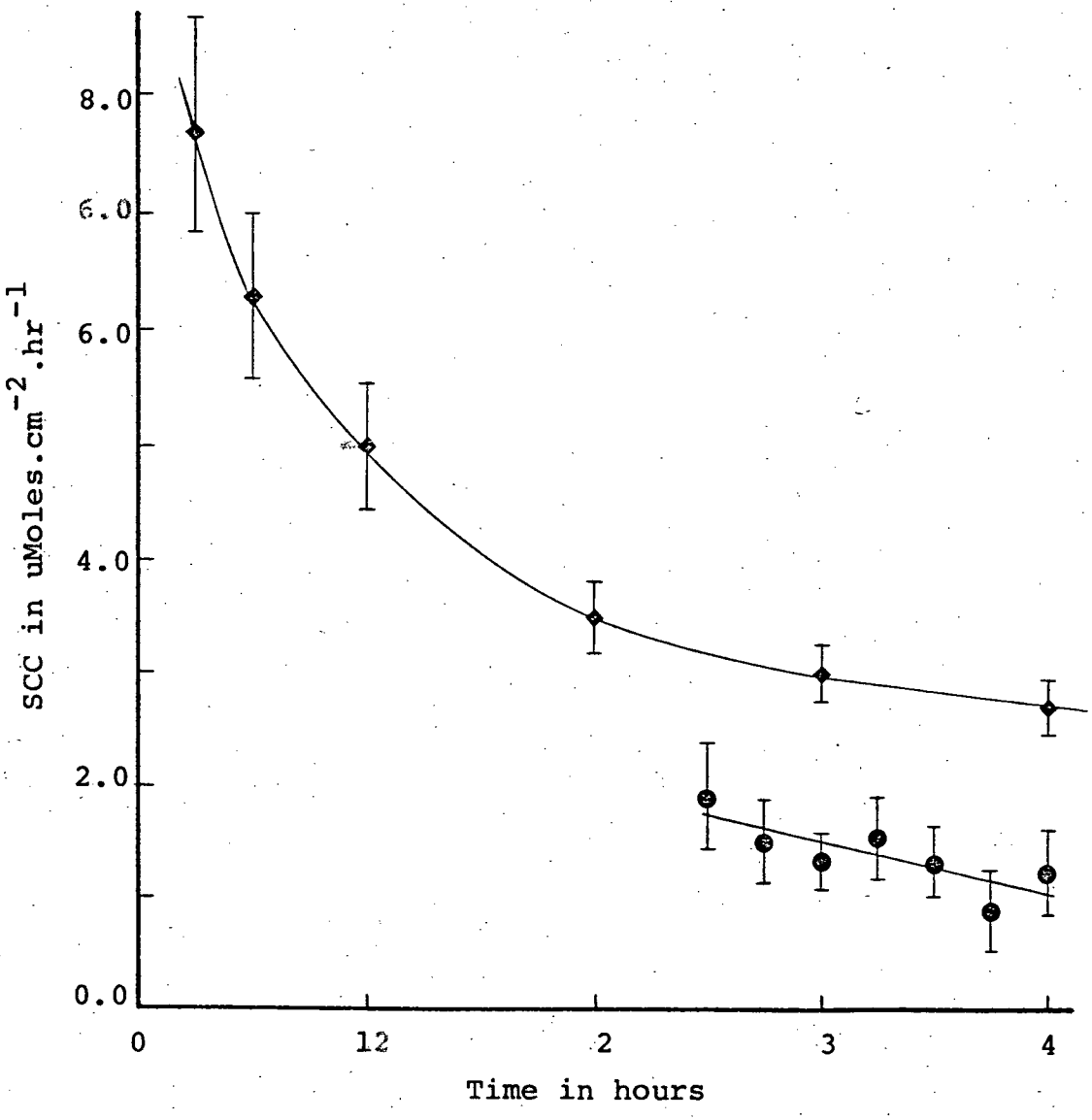
Recta were bathed in Normal Ringer under SCC conditions and unidirectional fluxes of $^{36}Cl^-$ were measured during the third and fourth hours in vitro by adding the isotope to one side at the end of the second hour and observing the subsequent rate at which activity appeared on the opposite side. Mean unidirectional Cl^- fluxes and the mean simultaneous SCC are shown in Figure 16. Under SCC conditions the mean hemocoel to lumen Cl^- flux (influx) remained relatively constant throughout the course of the flux measurement experiments at about 0.6 uMoles. cm^{-2} . hr^{-1}. The mean lumen to hemocoel Cl^- flux (efflux) during the same time period declined during the course of the experiments from an initial value (at T = 2:30) of 2.34 uMoles . cm^{-2}.

Figure 16. Unidirectional Cl^- fluxes measured with $^{36}Cl^-$ and simultaneous mean SCC. Mean ± S.E.M., n=9 for both influx into the rectal lumen and efflux from the lumen. ◆, SCC; ●, efflux; ▼, influx.


3.0
2.0
1.0
0.0
Cl^- Flux in $uMoles.cm^{-2}.hr^{-1}$
2:00
3:00
4.00
Time in hours

hr^{-1} to a final value of 1.95 uMoles.cm^{-2}.hr^{-1} closely paralleling the decline in SCC during the same time period. Subtracting these two uni-directional fluxes (Figure 17), an initial (T = 2:30) net active transport of Cl^- from the rectal lumen to the hemocoel of 1.9 uMoles.cm^{-2}.hr^{-1} is indicated. By the end of the flux experiments (T = 4:00) this active Cl^- transport has decreased to 1.25 uMoles.cm^{-2}.hr^{-1}, closely parallel to the decay in SCC during this time period. The initial value (T = 2:30) for net Cl^- transport is equivalent to about 60% of the simultaneous SCC and is 110-220% higher than previously reported values (Phillips, 1964; Goh, 1971; Meredith and Phillips, unpublished results) for Cl^- transport by the rectal epithelium under open circuit conditions where net absorption is less than true transport due to back diffusion (see Discussion). The only possible conclusion from these data seems to be that Cl^- flux *in vitro* is tightly coupled to the transport of some other ion or ions whose charge is such as to electrically cancel the chloride flux. This might be achieved by anion exchange or co-transport of cations such as Na^+ or K^+. Since previously observed transport of Na^+ or K^+ across the locust rectum is in the wrong direction to explain the observed SCC and since the SCC persists in the absence of Cl^-, the whole of the SCC remains unaccounted for by the transport of these three ions. Amino acids are known to be actively absorbed by the *in vitro* locust

Figure 17. Net Cl^- flux measured with $^{36}Cl^-$ calculated from the date of Figure 16 and simultaneous SCC. Mean ± S.E.M., n=9 for Cl^- flux studies and n=18 for SCC. ♦, SCC; ●, net Cl^- flux.


8.0
6.0
6.0
4.0
2.0
0.0
SCC in uMoles.cm^{-2}.hr^{-1}
0
12
2
3
4
Time in hours

rectum (Balshin, 1973; Balshin and Phillips, 1971) but their total concentration in the bathing media is too low to contribute significantly to the SCC. The only other ion known to be transported in any significant quantity by the rectum is hydrogen and/or bicarbonate. Their transport is in the correct direction to account for the observed SCC.

DISCUSSION

To be useful, the activity of an *in vitro* preparation should approximate a steady-state of sufficient duration to permit the required experimentation and the properties of the preparation should be relatively close to those *in vivo*. The rectal preparation used in this study meets these criteria, as indicated by measurements for PD, SCC and membrane resistance. PD and resistance change very little over a 6 hr. period. The SCC declines only slowly between the 2nd and 6th hr. The size of the current over this period is 4x greater than predicted from estimates of Na^+, K^+, Cl^- and H^+ transport by the locust rectum *in vivo* (Phillips, 1964; i.e. net charge transfer calculated as the difference between total anion and total cation transfer). However, Phillips (1961) pointed out that his value for H^+ transport was an under-estimation since it was made from measurements of large pH change. If the more accurate measurement of the initial rate of pH change by Speight (1968) is used in the calculations, the SCC predicted from *in vivo* measurements (2.16 uMoles.cm^{-2}.hr^{-1}) is almost identical to the value observed *in vitro* in this study during the 2nd to 4th hr. It follows that the initial SCC is 4x greater *in vitro* than predicted from previous *in vivo* studies. However, the *in vivo* values which are available are all for net absorption under open circuit conditions, i.e.

when both electrical potential and concentration difference exist across the rectal wall. Under these conditions net ion absorption is different from true active transport due to diffusion of ions down electrochemical potential gradients. Since the rectal lumen is electrically positive to the hemocoel *in vivo*, which would favour some absorption of Na^+ and K^+ by net diffusion, the true active transport of these cations would be somewhat less than net absorption values. Conversely, the true active transport of chloride, which is normally opposed by an electrical potential difference, would be somewhat greater than net absorption of this anion as measured *in vivo*. The PD under open circuit conditions would also tend to decrease bicarbonate flux from the lumen to the hemocoel and hydrogen flux from the hemocoel to the lumen. In summary, if allowance is made for the PD observed *in vivo*, then the true rate of net charge transfer due to transport of Na^+, K^+, Cl^- and H^+ or HCO_3^- *in vivo* would be somewhat higher than indicated by the data on net absorption by Phillips (1961, 1964) and Speight (1968). In short, the SCC produced by my *in vitro* preparation of the locust rectum is in reasonable agreement with that predicted from studies of ion absorption *in vivo*, suggesting that most of the ion transport activity is retained *in vitro*. Measurements of net flux *in vitro* provide direct evidence that this is indeed the case for chloride (see p. 57).

The time course of the rectal SCC *in vitro* (Figure 8) indicates at least two components; a rapid decline during the first two hours which is followed by the much slower decline thereafter. The second phase might reflect a slow decline in a general viability of the tissue. It is more interesting to speculate on the nature of the first phase. Some evidence exists that this initial rapid decline in SCC is associated with a decrease in chloride transport. As demonstrated by Figures 14 and 15, the absence of chloride from the bathing media during the initial two hour period abolishes the initial rapid decline in SCC. Herrera et al. (1976) also observed that replacement of chloride with sulphate during the first hour *in vitro* causes a large decline in SCC indicating that most of the SCC during this period is carried by chloride transport. This does not necessarily contradict my conclusion that chloride carries little or none of the SCC, since the studies of $^{36}Cl^-$ flux in the present study were performed during the third and fourth hours, by which time the initial rapid decline in SCC had ended. The question of what active transport activities actually decline to produce this rapid initial decrease in SCC remains unresolved. Extension of flux studies to this period would be useful. It is clear however from Figures 14 and 15 that though a large part of the SCC during the first two hours may be carried by chloride transport, a large part (initially 50%) definitely is not.

This observation is contrary to the results of Herrera et al. (1976).

The stimulation of rectal SCC by acetate (Figure 16) is obviously a complex phenomenon. The sharp decline in SCC during the first 15 minutes in vitro is very reminiscent of the similar decline of the current for recta bathed in the $SO_4^{=}$ and NO_3^{-} Ringers (Figures 14 and 15). The obvious explanation is that some ion transport process other than that of chloride decays following removal of recta from the locust. This initial decline appears to be arrested by some process that tends to increase the SCC in the presence of acetate. This may reflect the time required for acetate to enter the tissue. Any one or more of the following hypotheses could account for this subsequent and gradual increase in SCC: (1) Acetate is the anion of a weak acid and its presence in high concentrations in the tissue might provide a source of H^+, resulting in stimulation of H^+ transport into the lumen. (2) Since acetate is readily metabolized in many animal tissues, it might enter the rectal epithelium and provide a favourable energy source to stimulate a more vigorous active transport of other ions. (3) Acetate might itself be transported across the rectal epithelium into the hemocoel. This would increase SCC.

The decline in SCC after the third hour (Figure 16) may reflect a decline of general tissue activity with time. Regardless

of the explanation of the time course, the experiments with acetate Ringer again demonstrate that much or most of the SCC is not necessarily due to transport of Cl^-. Moreover, acetate Ringer sustains SCC current at near the initial value, about 4x higher than observed with other Ringers during the 2nd to the 4th hr. This again refutes the conclusion of Herrera et al. that Cl^- transport accounts for most of the SCC in locust rectum.

These observations might lead one to question the capacity of the present rectal preparation to sustain Cl^- transport. However, net flux of $^{36}Cl^-$ during the 2nd to the 4th hr. (Figure 17) indicates a transport rate of 1.52 uMoles.cm^{-2}.hr^{-1}, a value greater than was previously measured both in vivo and in vitro (Phillips, 1961, 1964; Goh, 1971; Meredith and Phillips, unpublished data; Herrera et al., 1976). This chloride transport (at a rate equivalent to one third of the SCC) is in the right direction to contribute to the SCC, and yet absence of chloride does not change the SCC over the 2nd to the 4th hr. A necessary conclusion from these facts is that at least two other ions are actively transported by my in vitro preparation; one or more ions coupled to the chloride flux to cancel its electrical charge, and one or more ions to account for the SCC. Tight coupling of cation and chloride absorption is one explanation. However, Goh (1971) and Meredith and Phillips (unpublished data)

concluded that chloride transport was independent from sodium and potassium transport under open circuit conditions in another *in vitro* preparation of the rectum, since chloride was still transported against an electrochemical potential difference when sodium and potassium in the bathing Ringer were replaced with choline. Since there are no monovalent cations in the bathing media that could be co-transported with chloride to neutralize its charge (except possibly choline), it seems more reasonable to postulate an anion exchange pump, most likely involving HCO_3^-, to account for the existing data. A second explanation is that Cl^- and HCO_3^- share a common transport process and that in the absence of Cl^-, enhanced movement of HCO_3^- occurs from the lumen to the hemocoel side.

Goh (1971) also concluded that a chloride pump is located on either or both the apical membrane and the membrane facing the intercellular channels of the rectal epithelium. The interior of epithelial cells is negative to the lumen (Phillips, 1961; Vietinghoff *et al.*, 1969) so that active transport of Cl^- may be required across the apical membrane for this anion to enter the cell. It is consistent with the previously mentioned data to propose that a $Cl^- - HCO_3^-$ exchange pump is located at this apical membrane instead of a simple Cl^- pump. Goh (1971) proposed that a chloride pump is located on the membrane facing the intercellular channels as well because when the rectum was incubated

in vitro in choline Ringer (Na^+ and K^+ free), not only did the tissue not swell as it should if a Cl^- pump were located on the apical membrane only, but the final tissue Cl^- concentration was lower than that of the bathing media. It is possible that this pump also transports tissue HCO_3^- into the intercellular channels or else that a separate HCO_3^- pump is located on the same membrane. This hypothesis would explain the anion deficit noted by Meredith and Phillips (unpublished data) in absorbate collected from their in vitro preparations of the locust rectum. They found that the total cation concentration of the absorbate entering the hemocoel was 146 mM while the chloride concentration was only 109 mM. The difference of 37 mM of anion could be accounted for by HCO_3^- transport into the hemocoel. This is in the proper direction to contribute to the SCC. Finally by analogy to the vertebrate kidney and gastric mucosa H^+ secretion into the lumen should yield cellular HCO_3^- which is available for transport to the blood side.

Though the hypothesis of a Cl^--HCO_3^- exchange pump on the apical membrane accounts for the lack of effect of Cl^- removal on the SCC during the third and fourth hours in vitro, it seems inconsistent with the fact that the rectum quickly acidifies any solution injected into its lumen (Phillips, 1961) and also with my earlier conclusion that the majority of the SCC during this period in vitro is carried by H^+ of HCO_3^- transport. However.

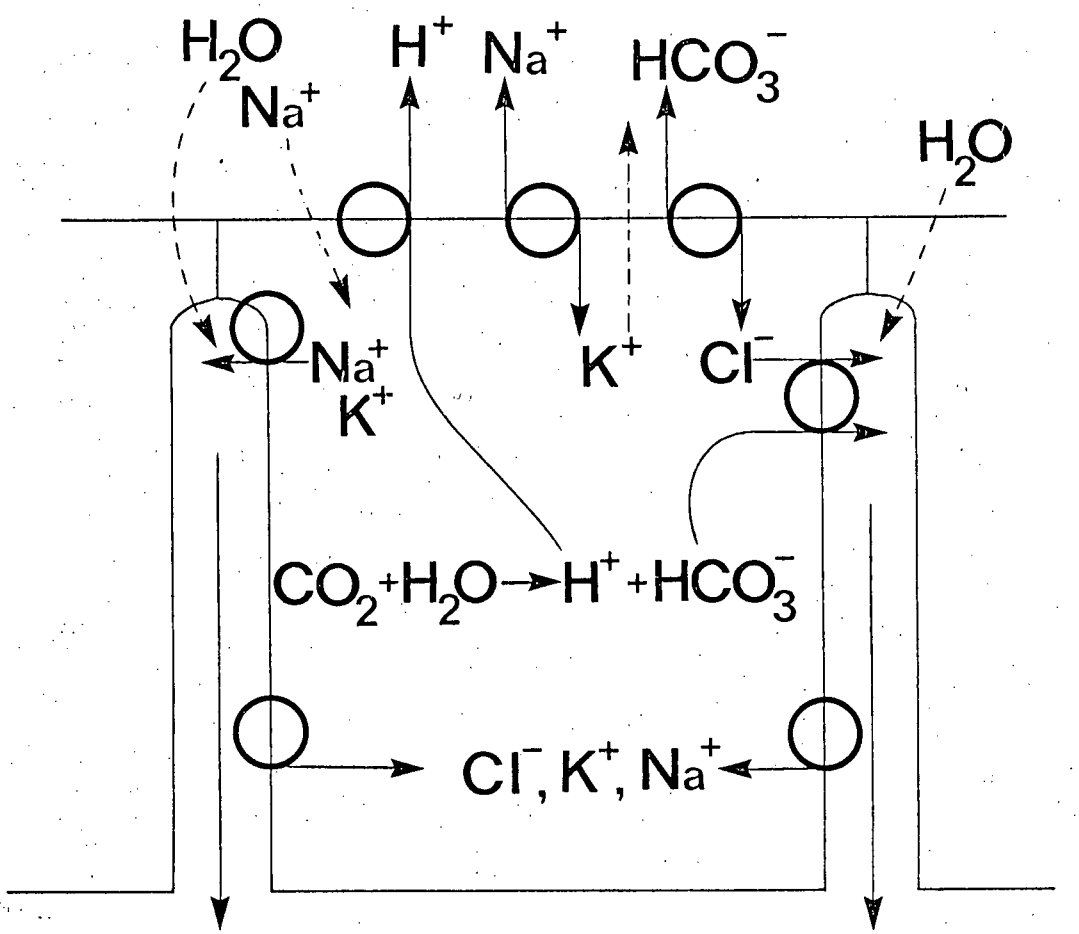
since the rate of chloride transport is probably much lower than the H^+ transport, the required movement of HCO_3^- into the lumen by an exchange pump would only slightly decrease net H^+ secretion, so that the lumen content could still be acidified, as observed by Phillips (1961) and Speight (1968).

These ideas on Cl^-, H^+ and HCO_3^- transport have been incorporated into a model (Figure 18) for organization of ion transport processes in the locust rectum, which is modified from that of Goh (1971). Goh (1971) proposes a cation pump with a much higher affinity for Na^+ than K^+ located on the membrane facing the intercellular channels, transporting cations into these channels. The apical membrane is very permeable to K^+ and it is likely that a typical $Na^+ - K^+$ ATPase maintains the large concentration differences which can exist across this membrane. Furthermore, since the rectum produces hyposmotic absorbate, ion reabsorption is postulated in the membrane facing the intercellular channel.

While this thesis was in preparation, Herrera, Jordana and Ponz (1976) described the effects of various ion substitutions on the PD and SCC in an *in vitro* preparation of the locust rectum. As referred to above, their conclusions do not agree with those of this study. I believe this is because these workers used an inadequate *in vitro* preparation, imprecise electrical methods, and observed only transient events over the first 40 minutes

Figure 18. Model of ion transport processes in the locust rectum. See text for explanation.

LUMEN



HEMOCOEL

in vitro. Over this period the PD and SCC of their control preparation fell to zero (Figures 5 and 8). Toward the end of this period, when tissue activity was reduced to a fraction of the initial value, exposure of rectal preparations to chloride-free Ringer abolished the remaining SCC but other ion substitutions did not. These workers concluded that Cl^- transport accounted for all the SCC and transport of other ion was negligible.

Three major differences in the experimental method used by Herrera *et al*. (1976), as compared to the present study, could explain the failure of their *in vitro* preparation, as indicated by a rapid disappearance of PD and SCC:

(1) These workers bathed their preparations in simple Ringer, whereas I used the complex tissue culture media of Berridge, which may sustain rectal metabolism much better.

(2) Good oxygenation is critical to survival of insect epithelia *in vitro* when tracheal connections are broken (Maddrell, 1971; Bradley, 1976). Herrera *et al*. (1976) used cannulated sacs stirred by bubbling 0_2 on the outside only. I used the recta mounted as a flat sheet, with vigorous stirring and oxygenation on both sides by a method that minimized unstirred layers.

(3) Dissection or mounting of the cannulated recta by ligation may have damaged the tissue (eg. edge damage by ligation).

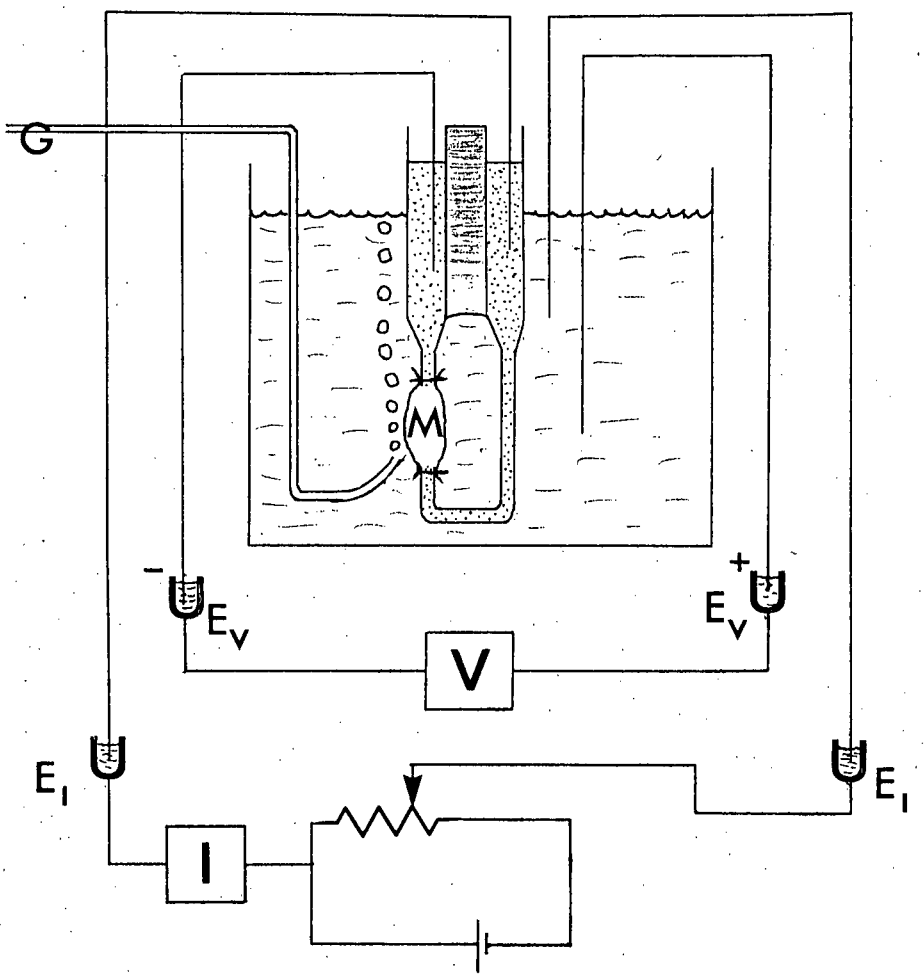
The method used to mount the rectum in the present study avoided application of pressure which is known to cause edge damage in other epithelia. (Dobson and Kidder, 1968).

I also question conclusions based on transient events immediately following dissection of recta from locusts. The tissue is normally exposed to a very hyperosmotic content within the lumen and then abruptly exposed to an isosmotic Ringer of different composition *in vitro*. Higher tissue concentrations of ions and other solutes associated with the previous hyperosmotic state may sustain particular transport activities temporarily at unusual rates. Moreover, the tissue may adjust certain transport processes to achieve some volume regulation following exposure to the more dilute Ringer.

Finally, as Figure 19 indicates, the geometrical arrangement of electrodes used by Herrera *et al*. (1976) to measure voltage and current makes substantial errors in measurement of PD and SCC almost inevitable. In particular, current density is not uniform across the rectal wall and correction for voltage drop through the solution is not indicated. Both factors introduce an error of unknown magnitude into their estimate of SCC.

Furthermore, the last half of their paper cited experiments in which rectal SCC was measured with solutions of radically different ionic composition on the two sides of the

Figure 19. In vitro apparatus of Herrera et al. (1976). V, voltmeter. A, current meter. E_V, voltage electrodes. E_I, current electrodes. M, rectal preparation. G, stirring gas entry tube.


G
M
−
E_v
+
E_v
V
E_I
E_I
I

membrane. These conditions do not satisfy the prerequisites for measurement of SCC and consequently the data are irrelevant. Their conclusion that Cl^- carries all of the SCC then rests entirely on their observation that Cl^- removal reduces the SCC to zero when preparations are clearly near death.

Herrera et al. (1976) also concluded on the basis of pH changes of unbuffered bathing media that the amount of SCC carried by H^+ is negligible (i.e. 0.025 uMoles.cm^{-2}.hr^{-1}). Their value for net H^+ flux is 25 times smaller than the rate observed in vivo by Phillips (1961) and 90 times smaller than that observed by Speight (1968), and is probably not reliable considering that any estimate of net H^+ flux based on pH changes of a solution can only be a minimum value. The only reliable method of measuring net H^+ flux under these conditions is by continuous automatic titration of the luminal bathing medium with a standard base solution. Finally, failure of Herrera et al. (1976) to observe substantial acidification may simply reflect the decreased viability of their preparation (evident from SCC values).

In conclusion then, the development of the in vitro preparation used in the present study should greatly facilitate the study of ion transport in the locust rectum. The most important task is to determine the rate of Na^+, K^+, and H^+ and HCO_3^- transport under SCC conditions. Only after these ques-

tions are answered can the inter-relationships between the various ion transport processes be unravelled and the characteristics of each mechanism studied.

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