Sorenson

Electrophysiological Properties of Resting Secretory Membranes of Lamellibranch Mantles

Interaction between Calcium and Potassium

A. L. SORENSON, DONALD S. WOOD, and LEONARD B. KIRSCHNER

From the Department of Pharmacology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil and the Department of Zoology, Washington State University, Pullman, Washington 99163. Dr. Sorenson's present address is Department of Physiology, Albert Einstein College of Medicine, Bronx, New York 10461; Dr. Wood's present address is Laboratory of Muscle Physiology, Department of Neurology, College of Physicians & Surgeons of Columbia University, New York 10032.

ABSTRACT This study concerns the effects of ions on the shell-secreting membrane of clam mantles. The average resting potentials were −47 mV for freshwater mantles and −60 mV for marine mantles. Elevation of potassium in the absence of chloride gave a maximal slope of depolarization equivalent to 59 mV for a 10-fold change in the marine form but much less in the freshwater form. In normal potassium, a 10-fold reduction in calcium produced a hyperpolarization of 6 mV for the freshwater mantle. Neither reduction nor elevation of calcium affected the potential of marine mantles in the presence of normal potassium, but a hyperpolarization of 8 mV occurred when calcium was deleted in a low-potassium medium. Elevated calcium reduced the depolarization induced by raised potassium in both species and resulted in an increased effective membrane resistance in marine mantles. Lowered calcium enhanced the hyperpolarization caused by reduction in potassium in freshwater mantles but not in the marine species. Replacement of chloride by large anions produced transient depolarization in both freshwater and marine mantles and resulted in a maintained increased effective membrane resistance in marine mantles. The effects of sodium and magnesium on the membrane potential were not significant in normal potassium. We conclude that the secretory membrane of freshwater and marine clam mantles is permeable mainly to potassium and chloride, and that responses of the membrane potential to calcium are mediated through its effect on the permeability to potassium.

INTRODUCTION

In spite of the large number of studies on exocrine glands, much more information is available for basilar receptive membranes than for their apical

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/80/01/0021/17 $1.00 21
Volume 75 January 1980 21-37

counterparts, the secretory membranes (Petersen, 1976). There is good reason for this disparity, because in most preparations the apical membrane is inaccessible. Thus it is difficult or impossible during electrophysiological studies either to control the ionic composition of the fluid bathing the apical membrane or to study directly its membrane characteristics. In recent studies of sweat and salivary glands (Imai, 1974; Berridge et al., 1975; Sato, 1977), attempts were made to estimate electrical characteristics of the apical membrane. In these cases it proved necessary either to calculate the apical membrane potential from the difference between the basilar and the transepithelial potentials or, more directly, to measure the apical membrane potential between an intracellular electrode and a reference electrode placed close to the duct orifice. In order to elucidate the events of stimulus- or excitation-secretion coupling, and especially the role of calcium, direct measurements from the secretory membrane in both resting and active states are essential. Our solution has been to use a morphologically simpler exocrine gland.

In lamellibranch mantles, the cells responsible for secretion of the proteins of the shell lie in an epithelial sheet with the secretory membranes exposed (Wilbur, 1972). In the first electrophysiological studies to take advantage of this tissue, the trans-mantle potential was found to be shell-side positive and calcium-dependent (Lund, 1947; Kirschner et al., 1960; Istin and Maetz, 1964). On the basis of intracellular recordings, Istin and Kirschner (1968) later reported that the effect of calcium was restricted to the apical secretory membrane. They also suggested that the secretory membrane was permselective for calcium, and that a calcium diffusion potential was responsible for the potential across the apical membrane surface.

The study of Istin and Kirschner (1968), however, was restricted to the effect of calcium on the resting potential. In a variety of tissues, calcium has been shown to affect the membrane potential indirectly by acting on the permeability to other ions (Koketsu, 1969). In the present study we have examined the effects of other ions in the presence of different calcium concentrations in order to define the electrophysiological role of calcium in the mantle. Our study deals primarily with the ionic dependence of the electrical potential across the apical membrane of mantles from marine and freshwater clams, supplemented with studies of the ionic dependence of the apical input resistance. We find that calcium is best described as a modulator of the resting permeability to potassium. Chloride is permeant and appears to be distributed passively across the apical membrane. The permeability to sodium is finite, but small, relative to that of potassium or chloride. Some of our results have been presented in preliminary form (Wood et al., 1972).

MATERIALS AND METHODS

Animals

Freshwater clams (*Anodonta* sp.) were bought from a Midwest supplier (Steinhilber, Oshkosh, Wis.) and stored at 12°C in dechlorinated tap water for 2 wk before use. Marine clams (*Anomalocardia brasiliana*) were collected from Guanabara Bay near the

anes (Petersen, 1976). There is good reason
ost preparations the apical membrane is
or impossible during electrophysiological
omposition of the fluid bathing the apical
nembrane characteristics. In recent studies
., 1974; Berridge et al., 1975; Sato, 1977),
ectrical characteristics of the apical mem-
necessary either to calculate the apical
rence between the basilar and the trans-
ectly, to measure the apical membrane
electrode and a reference electrode placed
to elucidate the events of stimulus- or
specially the role of calcium, direct mea-
brane in both resting and active states are
use a morphologically simpler exocrine

ls responsible for secretion of the proteins
t with the secretory membranes exposed
hysiological studies to take advantage of
al was found to be shell-side positive and
Kirschner et al., 1960; Istin and Maetz,
r recordings, Istin and Kirschner (1968)
ium was restricted to the apical secretory
t the secretory membrane was permselec-
diffusion potential was responsible for the
e surface.
r (1968), however, was restricted to the
ntial. In a variety of tissues, calcium has
e potential indirectly by acting on the
1, 1969). In the present study we have
s in the presence of different calcium
e electrophysiological role of calcium in
arily with the ionic dependence of the
membrane of mantles from marine and
n studies of the ionic dependence of the
calcium is best described as a modulator
ım. Chloride is permeant and appears to
apical membrane. The permeability to
o that of potassium or chloride. Some of
eliminary form (Wood et al., 1972).

s

ught from a Midwest supplier (Steinhilber,
echlorinated tap water for 2 wk before use.
ere collected from Guanabara Bay near the

Federal University of Rio de Janeiro (Ilha do Fundão) and were used fresh or within a few days of storage in seawater. Experiments were performed at room temperature, 20–23°C for *Anodonta* and 24–28°C for *Anomalocardia*.

Clams selected for experiments were judged to be healthy if they pumped water actively while undisturbed and if they closed their valves quickly when handled. Mantles were obtained from clams that had a smooth and glistening inner shell surface. Mantles with a brittle plaque covering all or part of the epithelium and with a shell that was rough and chalky in appearance were not used since the animals were probably anaerobic and resorbing shell (Dugal, 1939).

Morphology and Histology

Lamellibranch mantles are double epithelia derived from lateral extensions of the body. The two epithelia are separated by an interstitial space containing hemolymph. One epithelium faces the mantle cavity and is bathed by the environmental medium. The other faces the shell and secretes the shell components into a thin layer of "extrapallial" fluid similar in composition to the hemolymph in the interstitial space (de Waele, 1930; Crenshaw, 1972).

The shell-facing epithelium is composed of two major portions: a folded peripheral strip responsible for increment in shell area and a large central region responsible for increasing the shell thickness. Our study was confined to the central mantle region. Histological (Beedham, 1958) and ultrastructural studies (Neff, 1972) of the central portion show a single layer of columnar cells as tall as 30 μm and as wide as 10 μm. These cells comprise the bulk of the central epithelium although a few mucous cells are present. The cells rest on a basal lamella and there are conspicuous convolutions of the basilar plasma membrane. *Zonulae occludentes* and *zonulae adhaerentes* appear on the lateral surfaces near the apical end and the apical membranes are comprised of microvilli. (Neff, 1972; Wood, 1973).

Solutions

The *Anodonta* saline was based on analyses of clam blood reported by Potts (1954). It contained (in millimolar): NaCl, 10.0; KCl, 0.5; $CaCl_2$, 6.0; and HEPES-Tris buffer (pH 7.2), 7.0. In experiments involving ion substitutions, Na was replaced with HEPES-Tris, Ca and K were replaced by equiosmolar Na, and Cl was replaced by propionate. The *Anomalocardia* saline was based on analyses of extrapallial fluid reported by Crenshaw (1972). It contained (in millimolar): NaCl, 410; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 25; $MgSO_4$, 25; and Tris maleate buffer (pH 7.2), 5.0. Tris was used as a substitute for Na; Ca, K and Mg were replaced by equiosmolar Na. Cl was replaced by gluconate or propionate. Osmolarity was constant at the expense of ionic strength.

Dissection

Marine mantles ~2 cm in diameter were excised and placed shell-side up in a small plastic chamber on the stage of a compound microscope. The mantles were secured by a stainless steel spring clip on a small piece of peripheral mantle. In experiments that required intracellular recordings to be made during solution changes, the mantle was placed over a small plastic pedestal to reduce movement. The basilar membranes of marine mantles were shielded from the solutions by the second epithelium (see *Morphology and Histology*) and from diffusion from the edges by using cells in the center.

For freshwater mantles, the cavity-facing epithelium was removed by dissection and the shell-facing epithelium was clamped horizontally between two cylindrical plastic chambers with an exposed surface area of 2 cm^2.

Electrical Recording Apparatus

Micropipettes were pulled from acid-cleaned 0.8-mm (o.d.) pyrex capillary tubing. Microelectrodes for measuring voltage had between 8 and 20-MΩ resistance and were filled with 3 M KCl. For passing current, 5-MΩ resistance electrodes filled with 2.5 M K citrate were used. The measured tip potentials (Adrian, 1956) were <5 mV.

Micropipettes were connected by chlorided Ag wires to electrometer amplifiers for voltage measurements, or to a radio frequency isolation unit and a large resistor in series with a Tektronix 161 pulse generator (Tektronix, Inc., Beaverton, Ore.) for constant current stimulation. Membrane potentials were displayed on a Tektronix 564B oscilloscope for photography or on a Beckman RS chart recorder (Beckman Instruments, Inc., Fullerton, Calif.). Ground electrodes were Ag:AgCl wires connected to the bath directly or through 3 M KCl-agar bridges.

Membrane Potential Measurements

The micropipettes were held at an angle to the mantle surface, advanced by micromanipulators until a small voltage deflection appeared, and inserted by vibration. Successful electrode placements gave a rapid negative deflection to a steady level with complete reversibility when the electrode was withdrawn. After insertion of the electrode, an increase in the initially recorded potential sometimes occurred. If this later increase due to sealing was 5 mV or less and completed within 60 s, the final value was accepted. Recordings which showed either loss of potential or increments larger than 5 mV were rejected. For marine mantles, stable resting potentials could be recorded for at least 30 min after successful penetrations of the apical membrane. Penetrations of freshwater mantle cells were more difficult and less stable; we accepted recordings which were stable for 5 s or more. We also experienced difficulty in obtaining stable voltage measurements in solutions with lowered calcium or after exposure to increased K concentrations for both marine and freshwater mantles.

When the electrode was advanced further after a successful penetration, there was a rapid decrease in the recorded potential to about −5 mV. This indicated that the basilar membrane had been passed; it was rarely possible to regain the original resting potential during withdrawal of the electrode from a transepithelial position.

In experiments involving ion substitution, five or more penetrations of different cells were made in the control solution; the procedure was then repeated in the experimental solution and then for a second time in the original. The average results for both controls were taken for comparison with the experimental series. Measurements of membrane potential were started 3–5 min after solution changes and the series was usually completed within 10 min. Solution changes, as measured by dyes, were complete in 15 s.

Membrane Resistance Measurements

Current from one side of the isolation unit was injected from a second micropipette inserted within 100 μm of the voltage electrode. The second lead from the isolation unit was connected to the oscilloscope for current measurement as an IR drop across the 1-MΩ input resistance. Because, in both marine and freshwater mantles, voltage deflections in response to the current were recorded at electrode separations, which exceeded the cell dimensions, the cells are probably electrically connected (Lowenstein et al., 1965; Bennett and Trinkaus, 1970).

The decay of potential with distance in cells connected in two dimensions is nonlinear (Noble, 1962; Jack et al., 1975), requiring multiple penetrations at different distances to determine specific membrane resistance (Shiba, 1971), and secondary ion

shifts might then occur. Howe
the electrodes in place and th
membrane resistance for a g
control solutions. Owing to
freshwater mantle, our exper
marine species.

Statistical Procedures

To determine the significanc
determination of both experi
paired *t* test. Unless noted
criterion for statistical signif
standard deviation (SD) (Sne

RESULTS

The first part of our report
ions, and to some interact
mantles. In the second p
investigation of the electr
mantles.

Experiments on Membr

NORMAL RESTING PO
potential for mantles fron
negative relative to the ex
38 marine clams was signi
typical penetrations are sl

Our value for the apic
significantly greater than
(1968). In our hands, the
was measured after retra
variable membrane poter
the result of lack of a sati

As seen in Fig. 1, there
cells in either freshwater
potentials would be exp
which indicate uniformit
(Beedham, 1958; Neff, 1

EFFECT OF POTASSIU
and magnitude to that
consistent with a K diffu

Fig. 2 shows the result
of Cl. Elevated K depol
marine mantles, but in
produced only a 26 m
maximum depolarizatio
change. Reduction of K

leaned 0.8-mm (o.d.) pyrex capillary tubing
had between 8 and 20-MΩ resistance and were
it, 5-MΩ resistance electrodes filled with 2.5 M
potentials (Adrian, 1956) were <5 mV.
lorided Ag wires to electrometer amplifiers for
equency isolation unit and a large resistor in
erator (Tektronix, Inc., Beaverton, Ore.) for
ne potentials were displayed on a Tektronix
on a Beckman RS chart recorder (Beckman
)und electrodes were Ag:AgCl wires connected
Cl-agar bridges.

angle to the mantle surface, advanced by
e deflection appeared, and inserted by vibra-
ve a rapid negative deflection to a steady level
ectrode was withdrawn. After insertion of the
corded potential sometimes occurred. If this
or less and completed within 60 s, the final
showed either loss of potential or increments
arine mantles, stable resting potentials could
cessful penetrations of the apical membrane
ere more difficult and less stable; we accepted
or more. We also experienced difficulty in
in solutions with lowered calcium or after
for both marine and freshwater mantles.
ther after a successful penetration, there was
ial to about −5 mV. This indicated that the
as rarely possible to regain the original resting
rode from a transepithelial position.
ution, five or more penetrations of different
n; the procedure was then repeated in the
ond time in the original. The average results
ison with the experimental series. Measure-
ted 3–5 min after solution changes and the
nin. Solution changes, as measured by dyes,

nit was injected from a second micropipette
ectrode. The second lead from the isolation
r current measurement as an IR drop across
oth marine and freshwater mantles, voltage
re recorded at electrode separations, which
probably electrically connected (Lowenstein

e in cells connected in two dimensions is
requiring multiple penetrations at different
resistance (Shiba, 1971), and secondary ion

shifts might then occur. However, we found it possible to change solutions slowly with the electrodes in place and thereby were able to obtain an estimate of the effective membrane resistance for a given electrode separation in both experimental and control solutions. Owing to the difficulty in obtaining stable penetrations in the freshwater mantle, our experiments on membrane resistance were confined to the marine species.

Statistical Procedures

To determine the significance of the experimental results, we used each mantle for determination of both experimental and control measurements and used a two-tailed paired *t* test. Unless noted otherwise, a probability level of 0.05 was used as the criterion for statistical significance. Experimental variability was recorded as the standard deviation (SD) (Snedecor, 1956).

RESULTS

The first part of our report deals with the membrane potential, its response to ions, and to some interactions between those ions in marine and freshwater mantles. In the second part we report corroborative evidence obtained by investigation of the electrical resistance of the apical membrane of marine mantles.

Experiments on Membrane Potentials

NORMAL RESTING POTENTIALS The average (± SD) apical membrane potential for mantles from 14 freshwater clams was −47 ± 3 mV, cytoplasm negative relative to the extrapallial fluid. The apical membrane potential for 38 marine clams was significantly greater, averaging −60 ± 5 mV. Records of typical penetrations are shown in Fig. 1.

Our value for the apical membrane potential in the freshwater mantle is significantly greater than the value of −15 mV reported by Istin and Kirshner (1968). In our hands, the method used in that study, in which the potential was measured after retracting the electrode back into the cell, gave low and variable membrane potentials (−7 to −42 mV, see Methods). These could be the result of lack of a satisfactory seal around the micropipette tip.

As seen in Fig. 1, there does not appear to be more than one population of cells in either freshwater or marine mantles. A single mode of apical membrane potentials would be expected from histological and ultrastructural studies which indicate uniformity of cell structure in the central portion of the mantle (Beedham, 1958; Neff, 1972).

EFFECT OF POTASSIUM The apical membrane potential is similar in sign and magnitude to that recorded for numerous animal cells and would be consistent with a K diffusion potential (Grundfest, 1966).

Fig. 2 shows the results of our experiments on the effect of K in the absence of Cl. Elevated K depolarized the apical membrane in both freshwater and marine mantles, but in the former, an increase in K from 0.5 to 10 mM produced only a 26 mV depolarization, whereas in marine mantles the maximum depolarization was equivalent to a 59 mV change for a 10-fold change. Reduction of K was less effective: a reduction in K from 0.5 to 0.1

mM hyperpolarized the apical membrane by only 7.5 mV in freshwater mantles, and in marine mantles a reduction of K from 10 to 1.0 mM produced a hyperpolarization of only 3.5 mV.

The response to K in the physiological range cannot be described as that of a "good" K electrode for either marine or freshwater mantles, although the response of marine mantles approaches the theoretical in the case of very elevated K. Departure of the apical membrane from that expected for a K diffusion potential could be due to permeability to other cations.

EFFECT OF CALCIUM In five freshwater mantles, reduction of Ca from 6.0 to 0.6 mM resulted in a significant hyperpolarization from -46 ± 1.2 mV to -52 ± 3.3 mV. This was a small effect in spite of the increased activity of calcium expected from the 20% reduction in ionic strength, but qualitatively the change was in the same direction as that found by Istin and Kirschner

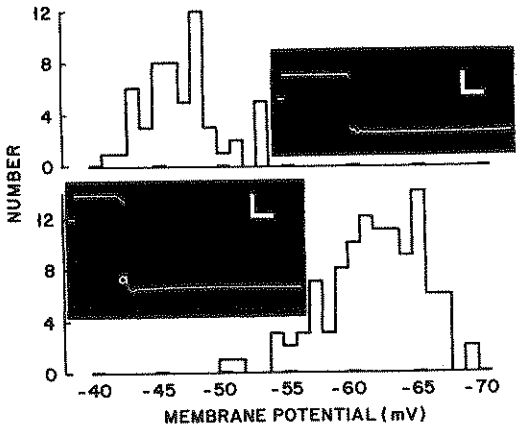


FIGURE 1. Records of impalements and histograms of membrane potentials of freshwater (above) and marine (below) mantle cells. For the histograms, six freshwater (47 penetrations) and four marine (106 penetrations) mantles were used. Calibrations for the impalement records, 20 mV and 10 s.

(1968), and in judging only by its effect on the membrane potential, we observe that a small resting permeability to Ca might be present. In three marine mantles, however, even complete deletion of Ca resulted only in a minor depolarization, from -65 ± 7.9 mV to -62 ± 10 mV. Three additional mantles gave no response to elevation of external Ca to 100 mM (-56 ± 2.5 mV in normal Ca and -55 ± 1.7 mV in elevated Ca). Finally, two more mantles tested at 0.1, 1.0, 10, and 100 mM Ca also showed only minor changes in the resting potential. Inasmuch as it was unlikely that a different mechanism of action of Ca existed in the two types of mantles, the effect of Ca was investigated further in the marine form.

EFFECT OF CALCIUM IN LOWERED POTASSIUM An important difference between the saline solutions for the two types of mantles lies in the Ca:K ratio, and because examples of interaction between the two ions are well known (Höber, 1945), we undertook a series of experiments on marine mantles

with 1 mM K. Five man
a significant hyperpolar
similar to that found for
of hyperpolarization was
be indirect.

EFFECT OF CALCIUM
altering the permeabilit
Fig. 2). Ca significantly
mantles to K (Fig. 3).

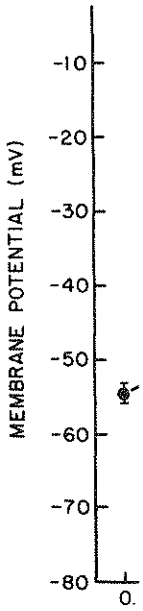


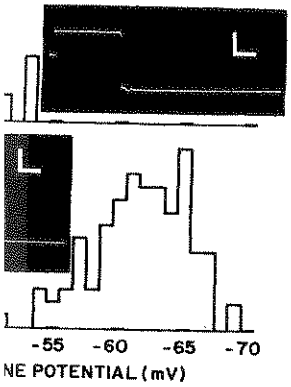
FIGURE 2. Relatio
external potassium.
(●) mantles. These
bar represents 1 SD

Experiments on fresh
reduced (0.6 mM) Ca
reduced Ca. The char
elevated K were not st
those found for marine

Larger changes in Ca
was placed in saline w
with 100 mM Ca in t
results from three ma
marked: an increase in
a further increase in K

embrane by only 7.5 mV in freshwate
duction of K from 10 to 1.0 mM produce

gical range cannot be described as that o
rine or freshwater mantles, although th
aches the theoretical in the case of very
l membrane from that expected for a K
permeability to other cations.
reshwater mantles, reduction of Ca from
nt hyperpolarization from −46 ± 1.2 mV
effect in spite of the increased activity o
action in ionic strength, but qualitatively
on as that found by Istin and Kirschner



ts and histograms of membrane potentials of
elow) mantle cells. For the histograms, six
our marine (106 penetrations) mantles were
ent records, 20 mV and 10 s.

effect on the membrane potential, we
bility to Ca might be present. In three
plete deletion of Ca resulted only in a
) mV to −62 ± 10 mV. Three additional
n of external Ca to 100 mM (−56 ± 2.5
nV in elevated Ca). Finally, two more
mM Ca also showed only minor changes
was unlikely that a different mechanism
types of mantles, the effect of Ca was
m.

D POTASSIUM An important difference
two types of mantles lies in the Ca:K
raction between the two ions are well
series of experiments on marine mantles

with 1 mM K. Five mantles examined for the effect of Ca withdrawal showed a significant hyperpolarization of 7.8 ± 2.7 mV. This result is qualitatively similar to that found for freshwater mantles, but since in both cases the degree of hyperpolarization was small, it became possible that the action of Ca might be indirect.

EFFECT OF CALCIUM ON THE RESPONSE TO POTASSIUM Were Ca to act by altering the permeability to another ion, a likely candidate would be K (see Fig. 2). Ca significantly altered the response of both marine and freshwater mantles to K (Fig. 3).

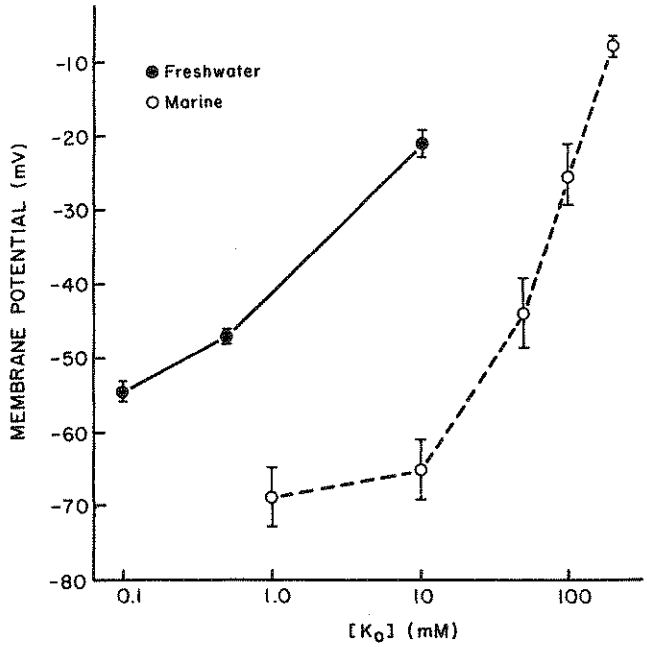


FIGURE 2. Relation between apical membrane potential and concentration of external potassium. Average results for three marine (○) and five freshwater (●) mantles. These experiments were done in the absence of chloride. Length of bar represents 1 SD unit.

Experiments on freshwater mantles were performed in normal (6 mM) and reduced (0.6 mM) Ca. Hyperpolarization in lowered K was enhanced by reduced Ca. The changes effected by Ca in the depolarization caused by elevated K were not statistically significant but were in the same direction as those found for marine mantles.

Larger changes in Ca were used for the experiment on marine mantles: one was placed in saline without added Ca and the other was placed in saline with 100 mM Ca in the absence of Na. Included in Fig. 3 are the average results from three mantles in normal Ca. The effect of elevated Ca was marked: an increase in K to 100 mM produced only a 6.5 mV depolarization; a further increase in K to 210 mM was necessary to effect a slope approaching

that of the mantles in normal or lowered Ca. The response to reduced K wa also modified by elevated Ca: instead of hyperpolarization, depolarizatio occurred. This latter finding was confirmed in three other mantles, also in th absence of Na, where in lowered K (1 mM), depolarization from −65 ± 8. mV to −56 ± 4.5 mV was produced by raising Ca to 100 mM.

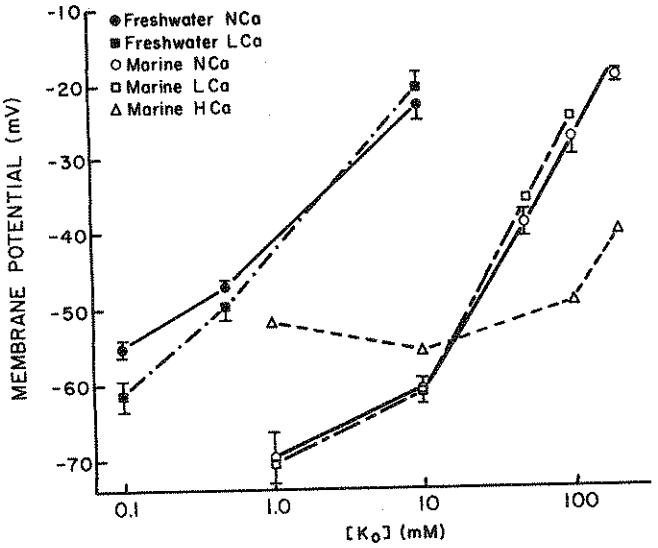


FIGURE 3. Effect of calcium on the relation of apical membrane potential to the concentration of potassium. Results are shown from five freshwater mantle and five marine mantles. For the marine mantles, empty circles (○) represen average results from three mantles with normal calcium (N Ca, 10 mM). Th results from two additional mantles in low calcium (L Ca, no Ca added, □) an high Ca (H Ca, 100 mM, △) are presented. For the experiments on freshwat mantles each of the five mantles was subjected to both normal calcium (6 mM ●) and lowered calcium (0.6 mM, ■) for three levels of potassium. The experiments were done in the presence of normal constant chloride concentra tions for marine mantles and 0 chloride for the freshwater mantles. Th maximum slope (normal Ca) in high potassium was 34 mV for marine and 18. mV for freshwater mantles for a 10-fold change. Length of bar represents 1 SI unit.

The effect of elevated Ca in lowered K could be interpreted either a reflecting a small permeability to Ca, as a depression of the permeability t K, or both. The effect of high Ca in elevated K, however, is not consisten with the first possibility and is most simply explained as an inhibition of th resting K permeability.

EFFECT OF CHLORIDE Cl withdrawal resulted in transient depolarizatio in both freshwater and marine mantles (Fig. 4). In these experiments th marine mantles were placed in saline solutions with 1 mM K. Results simila to those in Fig. 4 were obtained for two additional freshwater and thre

marine mantles. Two of t in normal K. The depola it is transient indicates (Hodgkin and Horowic the marine mantle was mantles in normal K (3 showed 2-mV depolariz determine whether the c

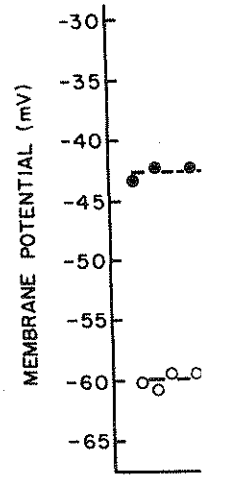
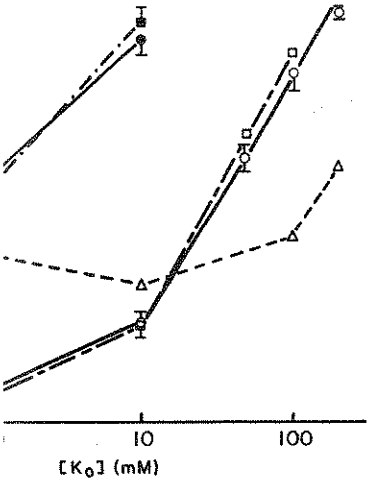


FIGURE 4. Effect sults for one fresh freshwater experim experiment was pe

EFFECT OF SODIU effect of Na replacem saline solutions. Three of −46 ± 0.5 mV with gave −59 ± 7.3 mV difference, although n small permeability to in marine mantles bat studied. An average significant ($P = 0.10$).

EFFECT OF MAGN much as we were not in the marine form ir mV without Mg; 13 with Mg, −78 ± 5.5

wered Ca. The response to reduced K wa
tead of hyperpolarization, depolarizatio
nfirmed in three other mantles, also in th
(1 mM), depolarization from −65 ± 8.
d by raising Ca to 100 mM.



the relation of apical membrane potential t
Results are shown from five freshwater mantle
marine mantles, empty circles (○) represen
es with normal calcium (N Ca, 10 mM). Th
les in low calcium (L Ca, no Ca added, □) an
presented. For the experiments on freshwate
was subjected to both normal calcium (6 mM
M, ■) for three levels of potassium. Thes
esence of normal constant chloride concentra
0 chloride for the freshwater mantles. Th
igh potassium was 34 mV for marine and 18.
10-fold change. Length of bar represents 1 SD

wered K could be interpreted either a
Ca, as a depression of the permeability t
in elevated K, however, is not consisten
t simply explained as an inhibition of th

drawal resulted in transient depolarizatio
antles (Fig. 4). In these experiments th
ne solutions with 1 mM K. Results similar
for two additional freshwater and three

marine mantles. Two of the experiments with marine mantles were performed in normal K. The depolarization recorded indicates that Cl is permeant; that it is transient indicates that Cl is redistributed across the cell membrane (Hodgkin and Horowicz, 1959). The apparent steady hyperpolarization for the marine mantle was also found in the second mantle (2 mV) and in two mantles in normal K (3 and 5.5 mV), but two other mantles in normal K showed 2-mV depolarizations, so further experiments would be necessary to determine whether the distribution of Cl is not completely passive.

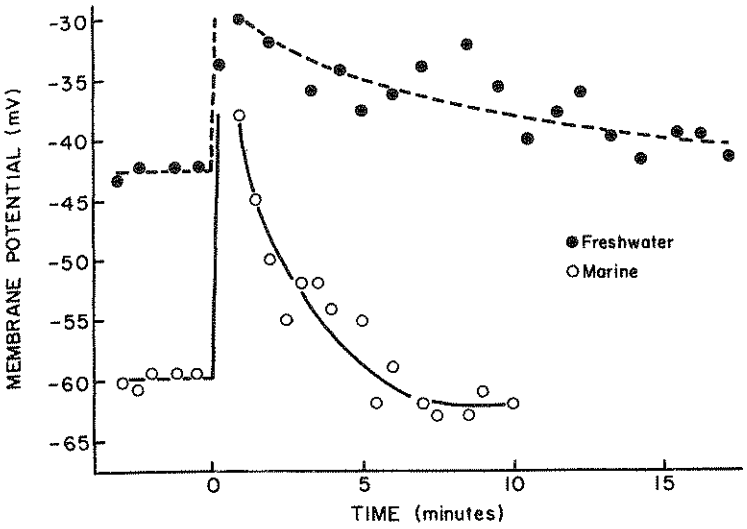


FIGURE 4. Effect of chloride withdrawal on apical membrane potential. Results for one freshwater (●) and one marine (○) mantle are shown. The freshwater experiment was done in normal potassium (0.5 mM); the marine experiment was performed in lowered potassium (1.0 mM).

EFFECT OF SODIUM We were unable to demonstrate any significant effect of Na replacement on the membrane potential in otherwise normal saline solutions. Three freshwater mantles gave apical membrane potentials of −46 ± 0.5 mV with and −46 ± 0.3 mV without Na. Two marine mantles gave −59 ± 7.3 mV with Na and −62 ± 6.4 mV without Na. This latter difference, although not significant, was in the proper direction to indicate a small permeability to Na, so we performed Na withdrawal experiments also in marine mantles bathed in solutions containing 1 mM K. Five mantles were studied. An average hyperpolarization of 8.8 ± 6.2 mV was found to be significant ($P = 0.10$).

EFFECT OF MAGNESIUM Mg was not tested in freshwater mantles inasmuch as we were not able to demonstrate significant effects of Mg withdrawal in the marine form in either normal K (−61 ± 3.9 mV with Mg, −61 ± 4.8 mV without Mg; 13 mantles) or in the presence of 1 mM K (−77 ± 5.9 mV with Mg, −78 ± 5.5 mV without Mg; 3 mantles).

Studies on Apical Input Resistance

These experiments were undertaken to provide added evidence concernin the permeability of the apical membranes of marine mantles to Ca and to Cl

EXPERIMENTS IN NORMAL SALINE In our first experiments we found tha currents injected at a distance from the voltage electrode caused a smoothl rising change in membrane potential to a steady final level. This is evidenc that the cells are electrically connected. The relation between current an voltage was linear for small potential changes in either the hyperpolarizing o the depolarizing direction (Fig. 5). Thus the membrane behaves in an ohmi manner and small perturbations can safely be used to estimate membran resistance.

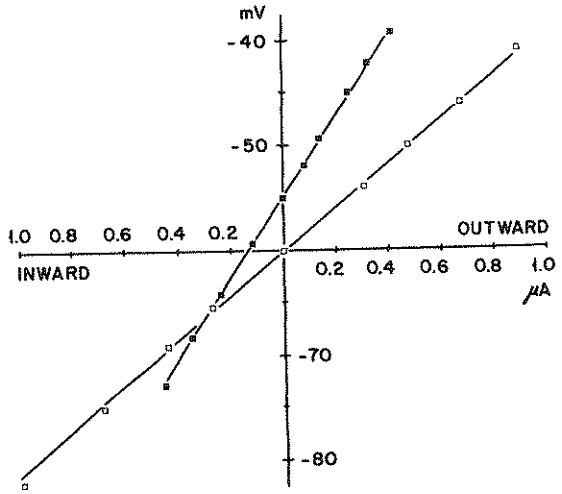


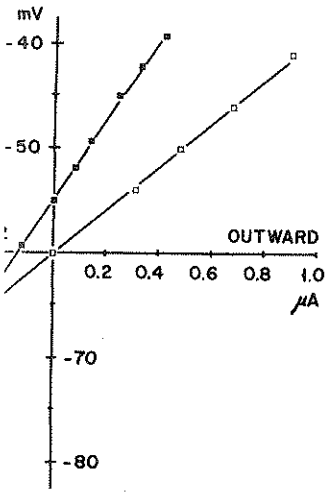
FIGURE 5. Current-voltage relationship in two marine mantles. Electrode sep aration, 22 μm for one mantle (■) and 62 μm (□) for the second mantle. Th responses of the two mantles can not be compared directly because of th different electrode separations.

Larger hyperpolarizing currents elicited no marked deviations from thi linearity but larger depolarizing currents elicited time-variant potentia changes in response to constant currents (Fig. 6). In the marine mantle, thes responses were found to be all-or-none, propagated, and not dependent on th presence of Na or Cl (Sorenson, 1978). They are also cardiac-like in appearanc in that there is an initial upstroke and overshoot followed by a rapid repolarization to a plateau which is terminated by a second rapid repolari zation (Cranefield, 1975). Preliminary experiments on freshwater mantle showed them to be electrically excitable in the sense that time-variant potentia changes were present (Grundfest, 1966) but no action potentials per se wer found.

EFFECT OF CHLORIDE ON MEMBRANE INPUT RESISTANCE If Cl is permeant as suggested by the results in Fig. 4, its replacement by an impermeant anion

ce

ten to provide added evidence concernin
nbranes of marine mantles to Ca and to C
NE In our first experiments we found tha
m the voltage electrode caused a smoothl
tial to a steady final level. This is evidenc
nected. The relation between current an
ial changes in either the hyperpolarizing o
Thus the membrane behaves in an ohmi
can safely be used to estimate membran



ionship in two marine mantles. Electrode sep
■) and 62 μm (□) for the second mantle. Th
an not be compared directly because of th

elicited no marked deviations from this
currents elicited time-variant potential
rents (Fig. 6). In the marine mantle, these
ne, propagated, and not dependent on the
3). They are also cardiac-like in appearance
ike and overshoot followed by a rapid
s terminated by a second rapid repolari-
nary experiments on freshwater mantles
ble in the sense that time-variant potential
966) but no action potentials per se were

ANE INPUT RESISTANCE If Cl is permeant,
its replacement by an impermeant anion

should result in an increased membrane input resistance. Fig. 7 shows one such experiment in which the solution was changed with the electrodes in place while recording the response to repeated hyperpolarizing currents. The resistance rapidly increased and then remained steady as expected. Five determinations on three marine mantles gave an average increase in membrane input resistance to 176 ± 58%.

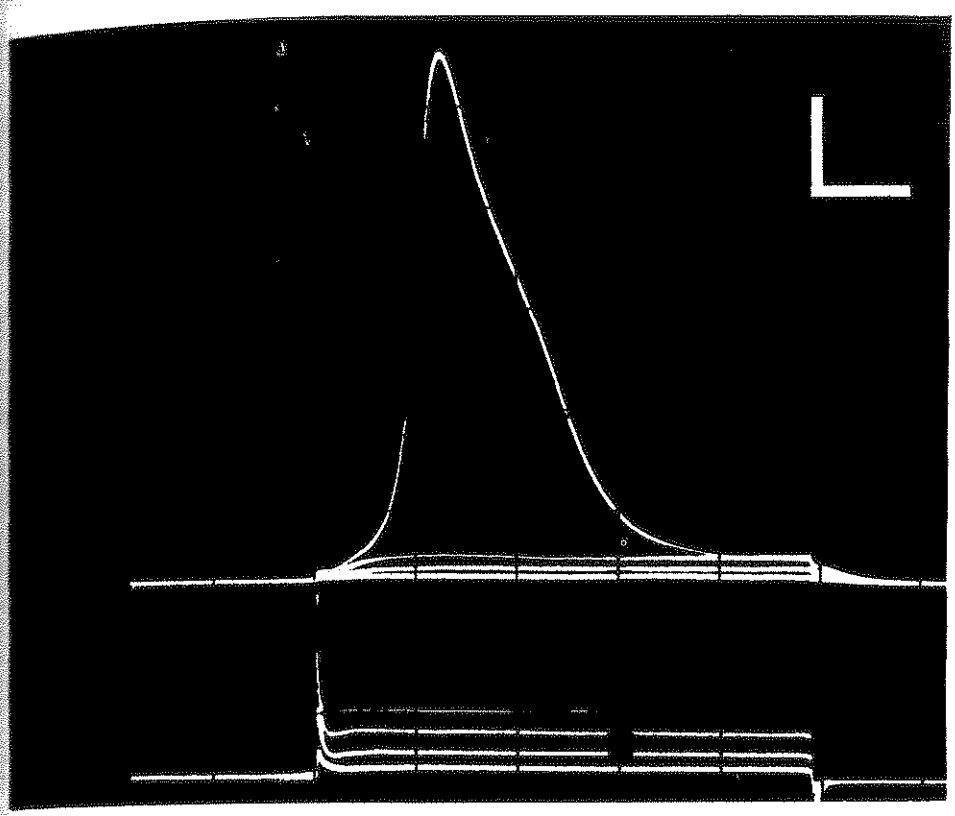


FIGURE 6. Action potential in apical membrane of a marine mantle. Electrotonic responses (upper traces) to three subthreshold currents (lower traces), followed by the active response. Calibrations: 20 mV/cm, 10 μA/cm, and 10 ms/cm. Resting potential 60 mV.

EFFECT OF CALCIUM ON MEMBRANE INPUT RESISTANCE The possibility that Ca acts primarily by modification of the permeability to K rather than by directly contributing to the resting potential was tested by measuring its effect on membrane resistance. Neglecting possible voltage-dependent changes in conductance (see Fig. 5), added Ca should cause an increased membrane resistance if it modifies the permeability to K. If Ca itself is significantly permeant, added Ca should result in a decreased membrane resistance as it increases the concentration of ions that can carry current. Elevated, rather than lowered, Ca was used because decreased input resistance in low Ca could

result from either an increased shunt around the electrode because of po sealing or from a true decrease in input resistance. That elevated Ca mig also increase sealing resulting in a spurious increase in input resistance is l likely, for this effect would be reflected in greater recorded membrane pote tials as well and this was not found (see EFFECT OF CALCIUM). Fig. 8 shows t results from one such trial in a marine mantle. The effective membra resistance clearly increased in response to added Ca. Three determinations o this mantle showed that Ca caused an average increase in input resistance

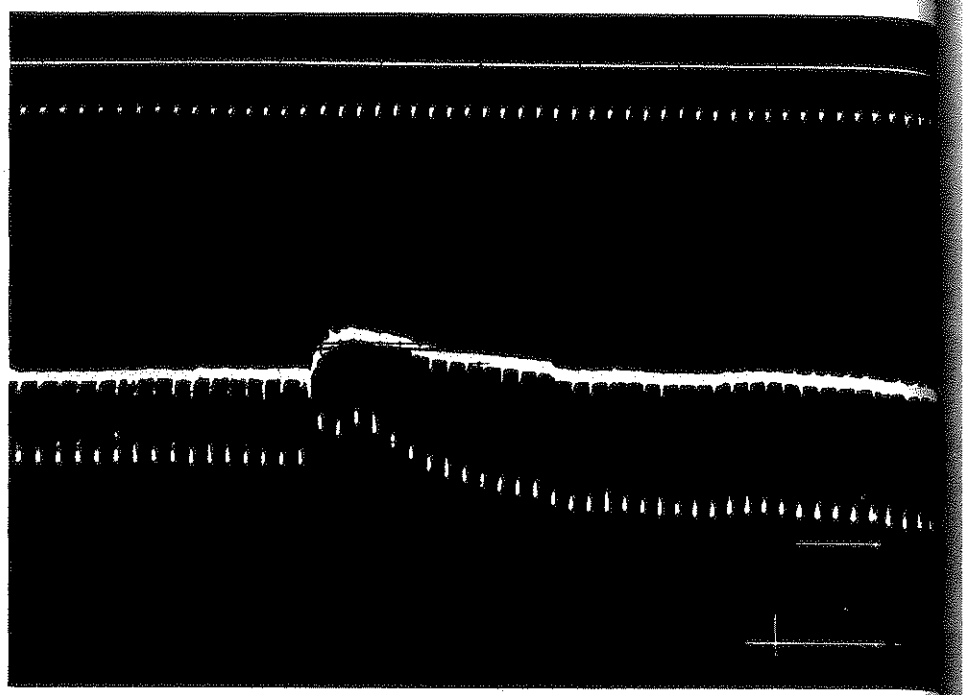


FIGURE 7. Effect of chloride withdrawal on membrane input resistance. Co tinuous recording of current (upper trace) and voltage responses (lower trac Pulse frequency 2/s, voltage calibration 10 mV/cm, current 2.0 μA/cm. Reco from marine mantle.

159 ± 9%. Further experiments would be necessary to show that Ca is acti on the permeability to K alone, but our results are clearly counter to t proposition that Ca is relatively highly permeant.

DISCUSSION

Our main conclusion is that the secretory membrane of mantle cells permeable mainly to K and to Cl and less so to Na. Judging by o electrophysiological findings, Ca is relatively impermeant but exerts a stron influence on the permeability to K.

Permeability to Chloride

Depolarization following Cl withdrawal (Fig. 4) and an associated increase in input resistance (Fig. 7) constitute good evidence that the apical membranes of the mantle are permeable to Cl (Hodgkin and Horowicz, 1959). The decreased slope of K depolarization in constant Cl concentration (compare Figs. 2 and 3) can also be explained by the presence of an emf for Cl acting to offset the effect of elevated K if the redistribution of KCl in these experiments is slower than in our experiments involving Cl withdrawal. This appears likely, for the electrochemical gradient for KCl entry is much less in the experiments at constant Cl than for KCl loss after Cl replacement. This repolarization following Cl withdrawal indicates that Cl is lost from the cell across the apical membrane (Hodgkin and Horowicz, 1959), and to the extent

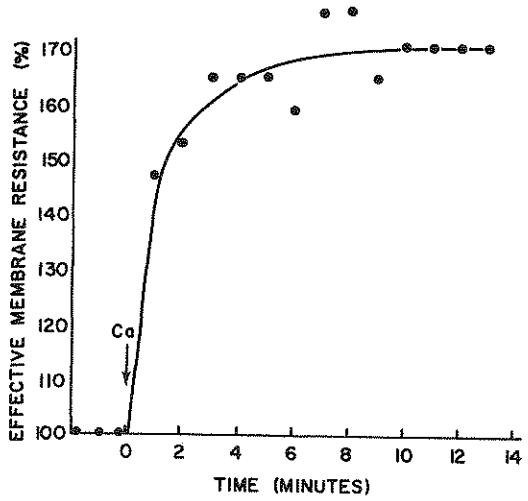


FIGURE 8. Effect of calcium on membrane input resistance. Experiment done in the same manner as for Fig. 7, but presented directly in terms of percent change in membrane resistance. Data from a marine mantle. At the arrow, the concentration of calcium was raised from 10 to 100 mM.

that the original membrane potential is recovered, that the distribution of Cl can be considered to be in accordance with the membrane potential.

Permeability to Sodium

Our observation that the marine mantle cells hyperpolarize upon removal of Na (in low K salines) is consistent with a finite resting permeability to that ion. Williams (1970) has ascribed the departure of the membrane potential from that predicted by the Nernst relation in secretory tissues to an increased permeability of Na relative to that of K.

Permeability to Potassium

Our evidence suggests that the resting potential across marine and freshwater apical membranes is established by a diffusion potential for K (Fig. 2). Marine

mantles, however, are more responsive to K than those of the freshwa species; a possible explanation for this will be discussed in a later section the effect of Ca on the permeability to K.

Relative Impermeability to Calcium

The results of three types of experiments led us to conclude that Ca is relativ impermeant. First, the response of the membrane potential to Ca withdraw in marine mantles was minor depolarization when the bathing solutio contained normal levels of K, and even in low K solutions (c.f. Fig. 3), r response to lowered Ca (freshwater mantles) and to Ca deletion (mar mantles) was small. Second, the effect of Ca on the membrane potential solutions of elevated K (Fig. 3) gave results of a sign inappropriate fo permeant Ca in both freshwater and marine mantles. Finally, in solutio with normal levels of K, elevating Ca had no effect on the membrane potenti in marine mantles, while with elevated Ca the input resistance was signi cantly increased (Fig. 8).

Hyperpolarization caused by lowered Ca is unusual but not unprecedente Hoffman and Suckling (1955) observed the same effect in dog ventricular a Purkinje tissue, and Cuthbert (1966) reported such an effect in smooth mus of mammalian mesenteric vein. The former authors interpreted their resu as representing an influence of Ca on the permeability to K; we believe th Ca has a similar influence in mantle apical membranes.

Effect of Calcium on the Permeability to Potassium

In mantle secretory membranes, Ca seems to act through an increase membrane resistance. Two kinds of evidence suggest that Ca acts prima on the permeability to K. First, added Ca increased the membrane inp resistance to current flow and the ion with the greatest resting permeabil appears to be K (Fig. 2). Second, added Ca decreased the response of apical membrane potential to K (Fig. 3).

Ca could act by screening or binding fixed negative surface charges (Fra kenhaeuser and Hodgkin, 1957; Huxley, 1959; McLaughlin et al., 197 Since Ca acted even in the presence of high Mg and since Mg itself w without significant effect, some specificity for Ca appears to exist. T differences we observed between freshwater and marine mantles could related to this action. Freshwater mantles had lower membrane potenti smaller responses to K, and greater responses to Ca than marine mantles. T lower ionic strength of the freshwater saline, and the further reduction wh Ca was replaced, would result in greater effective surface charge seen by and thus a lower permeability to K. However, Ca may also influence t permeability to other ions.

Effect of Calcium on the Permeability to Sodium and Chloride

In other tissues, Ca appears to act primarily by controlling the permeabil to Na (Koketsu, 1969); lowered Ca could not produce hyperpolarization increasing the conductance to Na, but it is possible that alterations in might influence an electrogenic extrusion of Na. This would be consist

with Ca-induced depolarization and with hyperpolarization induced by Na withdrawal in low K. Without experiments using pump inhibitors, it is difficult to predict whether it is also consistent with results on input resistance (Fig. 8).

Hyperpolarization in lowered Ca also seems unlikely to be caused by an increase in the conductance to Cl, for our results suggest that Cl distribution is largely determined by the membrane potential (Fig. 4).

Electrically Excitable Responses

Direct evidence for electrical excitability of apical membranes was shown in Fig. 6. Indirect evidence is suggested by the shift produced by Ca of the relation between external K and the membrane potential (Fig. 3). A propagated spike constitutes strong evidence that the muscle cells are electrically coupled (Bennett, 1966). A study of its physiological role is in progress. Electrically excitable responses in tadpole skin have been regarded as sensory (Roberts, 1971). On the other hand, the responses reported by Mackie (1976) for a coelenterate gland were considered by him to be involved in secretion. The ionic basis of the action potential in clam mantle secretory membranes may bear on this issue (Hagiwara, 1974).

Transport of Calcium to the Shell

From the first studies of the mantle transepithelial potential, it was inferred that Ca played a direct role in the genesis of the potential and that this potential thus reflected the active transport of Ca to the shell (Kirschner et al., 1960). Neither inference has been borne out. The transepithelial potential is in fact sensitive to Ca, but our data indicate that it acts indirectly by influencing the permeability of the apical membrane to K.

Two studies using radioactive Ca indicated that the movement of Ca across the mantle toward the shell was passive (Kirschner and Sorenson, 1964; Istin and Maetz, 1964), but in these studies intact mantles with both epithelial cell layers were used. Istin and Kirschner (1968) suggested that movement of Ca from the shell-facing cells to the extrapallial fluid could be passive and provide a basis for the membrane potential, if the mantle cells exhibited a high resting permeability to Ca. Our present results do not support this suggestion.

If these mantle cells share with numerous other cells the property of a low internal Ca, the electrochemical gradient strongly opposes Ca extrusion from the cell, and active transport thus appears necessary if the route of flux is transcellular. Measurements of Ca efflux from the cell to the extrapallial fluid are now needed.

We wish to thank Dr. John P. Reuben for a valuable critical review of an earlier version of this article.

This work was supported in part by grants GM-04254 from the National Institutes of Health, TC 16-841 and TC 17-456 from the Conselho Nacional de Pesquisas in Brazil, and 7447 and 7454 from the Westchester Heart Association.

Received for publication 16 February 1979.

REFERENCES

ADRIAN, R. H. 1956. The effect of internal and external potassium concentrations on membrane potential of frog muscle. *J. Physiol. (Lond.)*. **133**:631–658.

BEEDHAM, G. E. 1958. Observations on the mantle of the lamellibranchia. *Q. J. Microsc. Sci.* 181–197.

BENNETT, M. V. L. 1966. Physiology of electronic junctions. *Ann. N.Y. Acad. Sci.* **137**:509–539.

BENNETT, M. V. L., and J. P. TRINKAUS. 1970. Electrical coupling between embryonic cells by way of extracellular space and specialized junctions. *J. Cell Biol.* **44**:592–610.

BERRIDGE, M. J., B. D. LINDLEY, AND W. T. PRINCE. 1975. Membrane permeability changes during stimulation of isolated salivary glands of *Calliphora* by 5-hydroxytryptamine. *J. Physiol. (Lond.)*. **244**:549–567.

CRANEFIELD, P. F. 1975. The Conduction of the Cardiac Impulse. Futura Publishing Co., Inc. Mount Kisco, N.Y. 404 pp.

CRENSHAW, M. A. 1972. The inorganic composition of molluscan extrapallial fluid. *Biol. Bull. (Woods Hole)*. **143**:506–512.

CUTHBERT, A. W. 1966. Electrical activity in mammalian veins. *Bibl. Anat.* **8**:11–15.

DE WAELE, A. 1930. Le sang d'*Anodonta cygnea* et la formation de la coquille. *Mem. Acad. R. Belg. Classe Sci.* **10**:1–51.

DUGAL, L. P. 1939. The use of calcareous shell to buffer the product of anaerobic glycolysis in *Venus mercenaria*. *J. Cell. Comp. Physiol.* **13**:235–251.

FRANKENHAEUSER, B. and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol. (Lond.)*. **137**:218–244.

GRUNDFEST, H. 1966. Comparative electrobiology of excitable membranes. *Adv. Comp. Physiol. Biochem.* **2**:1–116.

HAGIWARA, S. 1974. Ca spike. *Adv. Biophys.* **4**:71–102.

HODGKIN, A. L. 1958. Ionic movements and electrical activity in giant nerve fibres. *Proc. R. Soc. Lond. B. Biol. Sci.* **148B**:1–37.

HODGKIN, A. L., and P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (Lond.)*. **148**:127–160.

HÖBER, R. 1945. Physical Chemistry of Cells and Tissues. Blakiston, Philadelphia. 676 pp.

HOFFMAN, B. F., and E. E. SUCKLING. 1956. Effect of several cations on transmembrane potentials of cardiac muscle. *Am. J. Physiol.* **186**:317–324.

HUXLEY, A. F. 1959. Ionic movements during nerve activity. *Ann. N.Y. Acad. Sci.* **81**:221–246.

IMAI, Y. 1974. On secretory processes and membrane potential of dog submaxillary gland. In Secretory Mechanisms of Exocrine Glands. N. A. Thorn and O. H. Petersen, editors. Academic Press, Inc., New York. 199–215.

ISTIN, M., and L. B. KIRSCHNER. 1968. On the origin of the bioelectrical potential generated by the freshwater clam mantle. *J. Gen. Physiol.* **51**:478–496.

ISTIN, M., and J. MAETZ. 1964. Perméabilité au calcium du manteau de lamellibranches d'eau douce étudiée à l'aide des isotopes ^{45}Ca et ^{47}Ca. *Biochim. Biophys. Acta.* **88**:225–227.

JACK, J. J. B., D. NOBLE, and R. W. TSIEN. 1975. Electric Current Flow in Excitable Cells. Clarendon Press, Oxford. 502 pp.

KIRSCHNER, L. B., and A. L. SORENSON. 1964. Calcium movement across the isolated clam mantle. *Fed. Proc.* **23**:115. (Abstr.)

KIRSCHNER, L. B., A. L. SORENSON, and M. KRIEBEL. 1960. Calcium and electric potential across the clam mantle. *Science (Wash. D.C.)*. **131**:735.

KOKETSU, K. 1969. Calcium and the excitable cell membrane. *Neurosci. Res.* **2**:1–39.

LOWENSTEIN, W. R., S. J. SOCOLAR, S. HIGASHINO, Y. KANNO, and N. DAVIDSON. 1965. Intercellular communication: renal, urinary bladder, sensory, and salivary gland cells. *Science (Wash. D.C.).* **149:**1248–1249.

LUND, E. J. 1947. Bioelectric Fields and Growth. University Cooperative Society, Austin, Tex. 391 pp.

MACKIE, G. O. 1976. Propagated spikes and secretion in a coelenterate glandular epithelium. *J. Gen. Physiol.* **68:**313–325.

MCLAUGHLIN, S. G. A., G. SZABO, and G. EISENMAN. 1971. Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.* **58:**667–687.

NEFF, J. M. 1972. Ultrastructure of the outer epithelium of the mantle in the clam *Mercenaria mercenaria* in relation to calcification of the shell. *Tissue & Cell.* **4:**591–600.

NOBLE, D. 1962. The voltage dependence of the cardiac membrane conductance. *Biophys. J.* **2:** 381–393.

PETERSEN, O. H. 1976. Electrophysiology of mammalian gland cells. *Physiol. Rev.* **56:**535–577.

POTTS, W. T. W. 1954. The inorganic composition of the blood of *Mytilus edulis* and *Anodonta cygnea*. *J. Exp. Biol.* **31:**376–385.

ROBERTS, A. 1971. The role of propagated impulses in the skin of young tadpoles. *Z. Vgl. Physiol.* **75:**388–401.

SATO, K. 1977. The physiology, pharmacology, and biochemistry of the eccrine sweat gland. *Rev. Physiol. Biochem. Pharmacol.* **79:**51–131.

SHIBA, H. 1971. Heaviside's "bessel cable" as an electric model for flat simple epithelial cells with low resistive junctional membranes. *J. Theor. Biol.* **30:**59–68.

SNEDECOR, G. W. 1956. Statistical Methods. Iowa State University Press, Ames, Iowa. 534 pp.

SORENSON, A. L. 1978. Action potential in a secretory epithelium. Proceedings 6th International Biophysics Congress. 375.

WILBUR, K. M. 1972. Shell formation in molluscs. *Chem. Zool.* **7:**103–143.

WILLIAMS, J. A. 1970. Origin of transmembrane potentials in non-excitable cells. *J. Theor. Biol.* **28:**287–296.

WOOD, D. S. 1973. Calcium Movement and Electrogenesis Across the Isolated Clam Mantle. Ph.D. Thesis. Washington State University, Pullman, Wash. 112 pp.

WOOD, D. S., A. L. SORENSON, and L. B. KIRSCHNER. 1972. Analysis of the bioelectric potential across a freshwater clam mantle. *Physiologist.* **15:**304. (Abstr.)