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Conductance, Transport Properties And Mode Of Action Of Barium On The Submucosal Facing Membrane Of The Frog Gastric Mucosa.

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CONDUCTANCE, TRANSPORT PROPERTIES AND MODE OF ACTION OF BARIUM ON THE SUBMUCOSAL FACING MEMBRANE OF THE FROG GASTRIC MUCOSA

by

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A DISSERTATION

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To Uncle Jim

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This is to acknowledge with gratitude the help I have received from my committee chairman and preceptor, Dr. Warren S. Rehm, and from the members of my committee, Drs. Leo Hall, Gordon Schoepfle, Richard Shoemaker, Kenneth Pruitt, and George Hallenbeck.

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INTRODUCTION

In A Nutshell

The major goal of this thesis will be to try to define more clearly the mechanism of ion transport in the gastric mucosa. Specifically this work will provide new evidence for a neutral mechanism of Cl^--bicarbonate exchange in the nutrient facing membrane. A three-pronged attack will be used: first, the establishment of the relationship of PD and resistance to the K^+ concentration of nutrient bathing media - this will be used to establish a minimum resistance of the nutrient membrane which if high enough would assure that the Spangler - Rehm (1968) model did not overlook a proton conductance channel which could otherwise fit nicely with their data; second, the examination of the conductance of the nutrient membrane for HCO_3^-, H^+, OH^- -- if there is none then the mode of bicarbonate exchange must be neutral; third, the examination of the increased resistance that barium causes on the stomach -- if the mechanism of this effect could be elucidated (a major goal in itself) this high resistance depending on its site might reduce to absurdity any conductive mechanism for bicarbonate exchange.

General Definitions

A tremendous amount of work has been done in which the rates of ion movements across membranes and tissues have been determined. With certain tissues it is possible to show that some ionic movements are against their electrochemical gradients. Active transport in the absence of appropriate gradients of other ions refers to the movement of molecules which involves use of metabolic energy of the system in question. Passive transport refers to movement of ions totally accounted for by the forces of the electrochemical and osmotic gradients present in the system.

There are some conditions under which it is possible to determine whether this movement is active or passive. In many cases it has been shown that movement of a particular ion across a membrane is dependent upon metabolic energy. It may be more difficult to determine that an ion is passively transported. A mechanism that is active can involve the transport of ions per se, in which circumstance it is known as an electrogenic mechanism which is a form of conductive mechanism. If the mechanism is passive and the ion is transported per se, this would constitute another form of conductive mechanism. Ions can also move across a membrane in such fashion that there is no net movement of charge involved. Such mechanisms of transport will be referred to henceforth as "neutral". The term "neutral mechanism" carries no implication about the active or passive character of the transport. For instance, a neutral mechanism might involve the transport of

positive and negative ions in the same direction or simultaneous transport of a cation in one direction with that of another cation in the opposite direction (and similarly for anions). The last mechanism has been referred to as "the whirling dervish" type of transport. The essence of the all neutral type of processes is that no net movement of charge occurs.

The Thrust

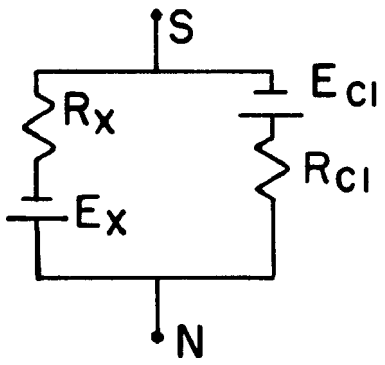
One of the overall goals in this laboratory is to determine whether a given ion is transported via conductive or neutral mechanisms. As stated, one of the primary goals of this dissertation is to present data on the problem of conductive and neutral mechanisms of ion transport in the gastric mucosa. It would therefore be appropriate to present some background information on this tissue. Hydrogen ion and Cl^- transport in this tissue is active. There is a potential difference (PD) across the stomach, both at rest and during the secretion of acid (Rehm, 1964a, b). The PD across the external muscle layer is zero, so that the emf's giving rise to the PD must be in the mucosa. The transport of H^+ and Cl^- constitute about 95% of the total ion transport; small amounts of Na^+ and K^+ are also transported. If an electric current is sent across this tissue from nutrient to secretory side, the rate of secretion of H^+ is increased and vice versa. A theory of HCl secretion has been proposed in which it is postulated that both H^+ and Cl^- are actively transported by separate electrogenic

FIGURE 1.

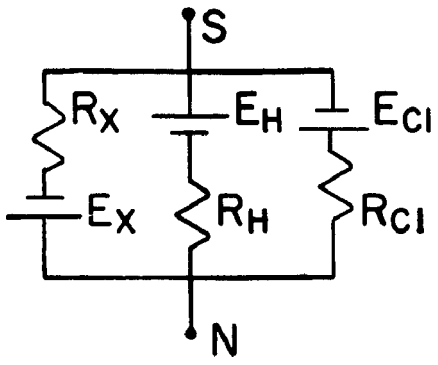
Simplifies equivalent circuits for frog gastric mucosa - at rest, during secretion, and under sulfate conditions.

Represents single equivalent circuit for the secretory mucosa as postulated by the dual site electrogenic theory. The first circuit represents the membrane at rest where the electrogenic Cl^- is the only major ion transport system present and the return limb has a very high resistance so that very little net transport occurs. The second circuit represents the membrane during secretion; an electrogenic H^+ mechanism is present and its orientation would tend to make the nutrient side negative. The nutrient side is, however, experimentally found to be still positive so that the magnitude of the parameters must be such as to account for this orientation. The third circuit represents the secretory membrane under sulfate conditions, i.e., the absence of Cl^-; the H^+ mechanism is the only major ion transport system present under these conditions. In the absence of Cl^- one would predict a reversal of PD and this is what we actually find. R stands for resistance, E for emf, S for the secretory side of the membrane and N for the nutrient. H represents the hydrogen ions; Cl, the chloride ions, and X, all other ions in the system.

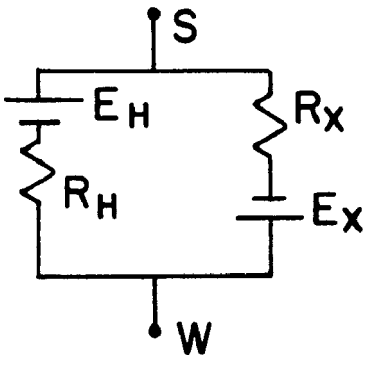
AT "REST"



SECRETING



SULFATE

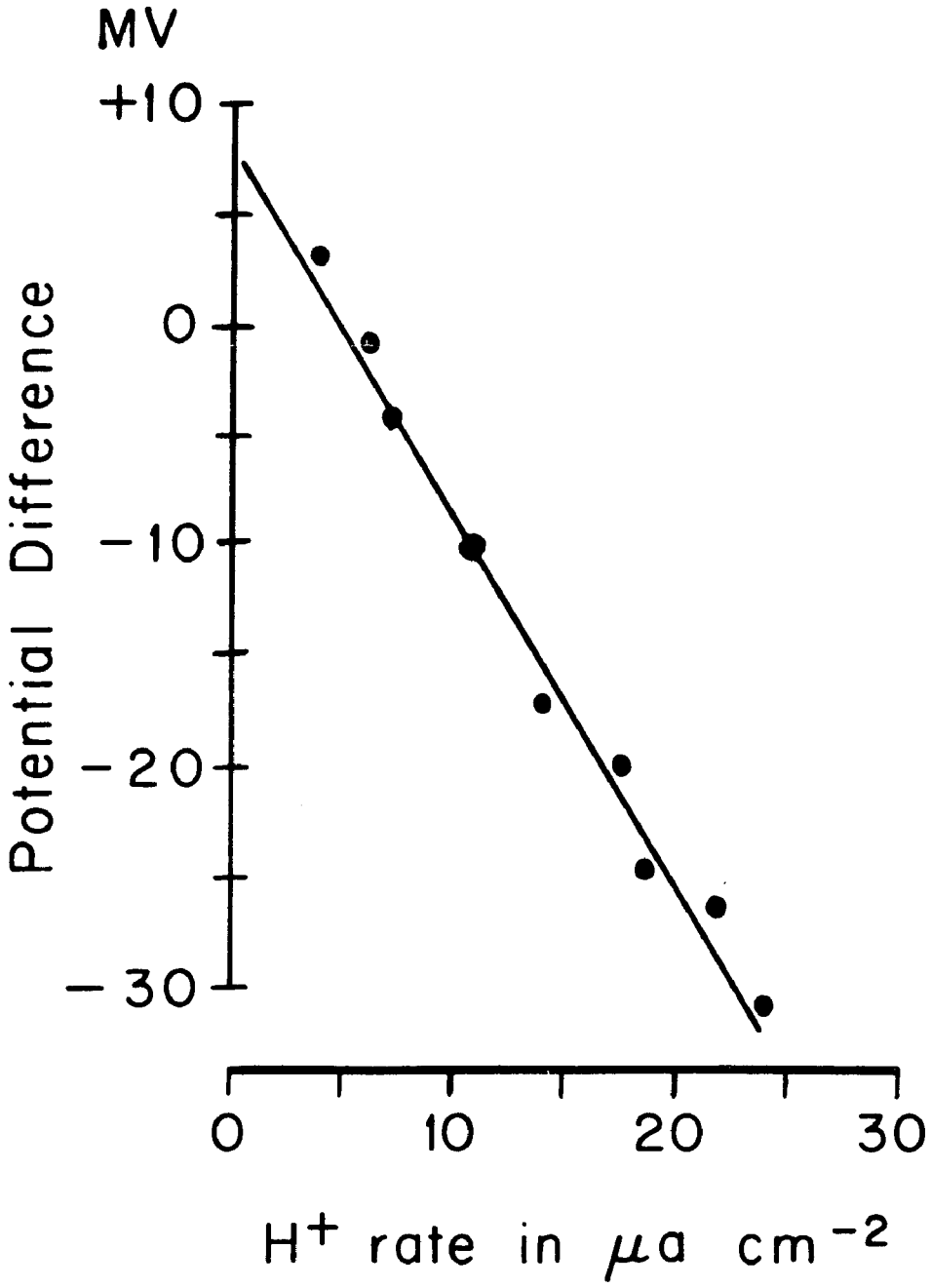


mechanisms (Rehm, 1950). According to this theory, in the stomach at rest (see Figure 1) the electrogenic Cl^- mechanism is the only major ion transport mechanism present and the return limb (R_X and E_X of Figure 1) has a very high resistance so that very little net transport occurs. Under conditions of secretion an electrogenic H^+ mechanism is activated which tends to make the nutrient side negative. Experimentally we know that the nutrient side is still positive so the magnitude of the parameters must be such as to account for this orientation. The circuit under conditions of secretion is represented in Figure 1. In the absence of Cl^- (Figure 1) one would predict an opposite orientation of the observed PD since the H^+ transport mechanism is the only major ion transport system present. When Cl^- was removed from bathing media, this predicted inversion of potential was found (Rehm and LeFevre, 1965; Heinz and Durbin, 1959). In 1965 Rehm and LeFevre looked at the effect of dinitrophenol on the PD, resistance and H^+ rate of the frog stomach. The addition of dinitrophenol to the nutrient side, with Cl^--free bathing media produced a decrease in the H^+ rate and magnitude of the PD with an increase in resistance. A very precise linear relationship was noted between the PD and the H^+ rate. Figure 2 depicts this linear relationship. An analysis of these findings leads to the conclusion that the H^+ mechanism in the absence of Cl^- is clearly electrogenic. These findings have been confirmed with other inhibitors (Schwartz et al., 1968).

FIGURE 2.

PD vs H^+ rate under six sulfate conditions with dinitrophenol inhibition.

Illustrates PD in mv plotted against H^+ rate in μa cm^{-2} when the gastric mucosa is exposed to increasing concentrations of dinitrophenol (DNP). Bathing media in these experiments contained no chloride (sulfate for Cl^-). This represents the first precise linear relationship between PD and rate of ion transport. These experiments were performed by Rehm and LeFevre, 1965. This is the most convincing evidence for an electrogenic H^+ ion pump in biological tissues (Rehm and LeFevre, 1965).


MV
+10
0
-10
-20
-30
Potential Difference
0
10
20
30
H+ rate in μa cm-2

During secretion with regular Cl^- media (Table 1, solution 1, 2) Cl^- enters from the nutrient side to the gastric cells and bicarbonate appears in the nutrient solution (Hanke, Johannesen and Hanke, 1931; Hanke, 1937). There is a one to one relationship between the amount of H^+ secreted into the gastric lumen and the amount of bicarbonate ion excreted in the nutrient bathing media (Davies, 1951; Teorell, 1951). The simplest way to explain how Cl^- and bicarbonate cross the nutrient membrane is to postulate Cl^- and bicarbonate conductance channels in the nutrient membrane (Figure 3). It has been assumed for many years that similar channels were present in the membrane of red blood cells to account for the Cl^- bicarbonate exchange in this tissue. The effect of Ba^{++} vide infra on the frog mucosa threw considerable doubt on the possibility of both Cl^- and bicarbonate conductance channels (Schwartz et al., 1968). This doubt is legitimate only if Ba^{++} can be shown to act on the nutrient membrane (i.e., the membrane facing the submucosal side) -- then the only viable alternative becomes a neutral carrier exchange mechanism for Cl^- and bicarbonate.

This dissertation will consist of experiments dealing with three specific problems: 1) the relationship of the potential difference and resistance of the gastric membrane to various concentrations of K^+ on the nutrient side; 2) the conductance of the nutrient cell membrane for bicarbonate, OH and H^+; and 3) delineation of the site and mechanism of Ba^{++} action with its

implications for the existence of a neutral carrier Cl^- bicarbonate exchange mechanism.

Plugging the Hole in a Model

Spangler and Rehm (1968) looked at the PD response of the nutrient membrane of the frog stomach to step changes in external K^+ and Cl^- concentration of nutrient bathing media. If there were no diffusion barrier one would expect a step change in the PD when the concentration of a conductively permeant ion is changed and replaced by a conductively impermeant ion. However, there is a nutrient diffusion barrier so they set up a mathematical model to take this into account. Changing from 4 mM K^+ to 79 mM K^+ caused a rapid fall in the PD of the system. The reverse change caused a recovery of the PD but with a longer time course as predicted by their model. Similarly, their model predicted the PD changes and time course which occurs when Cl^- concentration is changed. The above experiments and product constant experiments (product of Cl^- and K^+ concentrations kept constant) described in the same paper are consistent with the conclusion that the total conductivity of the nutrient membrane (G_N) can be accounted for by the partial ionic conductances of K^+ (G'_K) and Cl^- (G'_{Cl}).

Other explanations of these data are possible - for instance if the conductance of the nutrient membrane for H^+ were a significant fraction of the total conductance then similar results to those

just described might be anticipated (this will be discussed later). Such high proton conductance in other tissues has been suggested (Campion et al., 1966; Carter et al., 1967a, b).

Some New Data

It is important to establish the relationship of the PD and resistance of the gastric membrane to changes in K^+ concentration in nutrient bathing media. These data are then used to establish the conductance of the nutrient membrane to K^+, to determine the minimum resistance of the nutrient membrane so that the Spangler-Rehm (1968) hypothesis can be more accurately evaluated.

Partial Ionic Conductance to H^+, OH^- and Bicarbonate

More definitive in establishment of a neutral mechanism for bicarbonate exchange at the nutrient membrane than the deductive argument from

$$G_K + G_{Cl} = G_N \tag{1}$$

would be the direct proof that

$$G_H + G_{OH} + G_B = 0 \tag{2}$$

where B stands for bicarbonate. This proposition is the basis for the second set of experiments which work and figures have been previously published (Sanders, O'Callaghan, Butler and Rehm, 1972).

From Barium to Dervish

If the action of Ba^{++} is on the nutrient membrane, this is evidence for a neutral Cl^--bicarbonate exchange mechanism. Let us look at why this should be so. Assume conductive channels for Cl^- and bicarbonate (for equivalent circuit of Figure 3) in the nutrient membrane. If the presence of Ba^{++} could be shown to increase the resistance of this membrane by 500 ohm cm^2 then the resistance of the nutrient membrane must be at least this much. Under steady state conditions I_{Cl} must equal I_B to maintain acid-base balance as can be seen from consideration of the simple circuit of Figure 3. For an H^+ rate of slightly less than 4 microeq. per cm^2 per hour the current in the loop would be about 0.1 ma, so that

$$E_{Cl} + E_B = 0.1\ (R_{Cl} + R_B) \qquad (3)$$

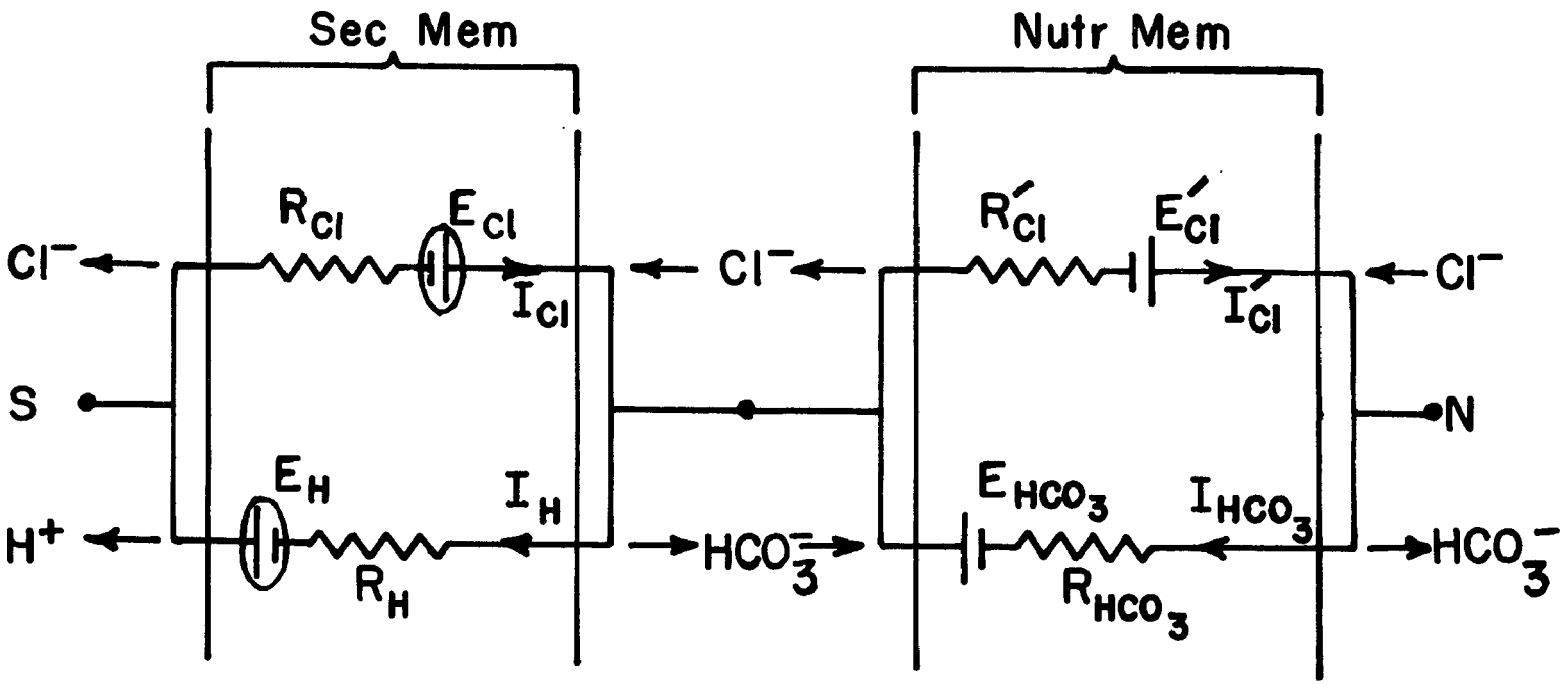
From simple minima considerations if the transmembrane resistance is 500 ohms the minimum loop resistance must be 2000 ohms. This occurs when $R_{Cl} = R_{HCO_3}$. If R_{Cl} does not equal R_B the resistance of the loop must be even greater than 2000 ohms and the sum of E_{Cl} and E_B will be greater than 200 mv.

Setting the extracellular bicarbonate concentration at 25 mM (all nutrient solutions except 9, 12, and 15 of Table 2) as is done here under normal conditions and assuming a maximum intracellular bicarbonate concentration of 100 mM which would be difficult to believe from the point of view of isotonicity, the maximum value for E_B would be 36 mv. This leaves the Cl^- ion to account for

FIGURE 3

Equivalent circuit of the secretory and nutrient membranes of frog stomach with all ion channels being conductive.

Shows one concept of an equivalent circuit for the gastric mucosa. The secretory membrane is depicted having an electrogenic Cl^- and H^+ mechanism (circled) while the nutrient membrane is assumed to have conductance channels for Cl^- and bicarbonate. This mechanism is discussed in the text. R stands for resistance, E for emf. The meaning of the subscripts is self-evident. The prime marking for the Cl^- conductance channel in the nutrient membrane is used to distinguish it from the electrogenic Cl^- mechanism in the secretory membrane.


Sec Mem
Nutr Mem
R_{Cl}
E_{Cl}
I_{Cl}
R'_{Cl}
E'_{Cl}
I'_{Cl}
Cl^-
S
N
E_H
I_H
R_H
H^+
HCO_3^-
E_{HCO_3}
I_{HCO_3}
R_{HCO_3}

164 mv. From a simple Nernst calculation with extracellular Cl^- at 82.6 mM

$$164 = 58 \log \frac{82.6}{C_{Cl}} \quad (4)$$

$$C_{Cl} = 0.123 \text{ mM} \quad (5)$$

where C_{Cl} is concentration of Cl^- in the cell -- an impossible value because it would be difficult to believe it could support the observed gastric acid production. The above considerations would constitute excellent evidence for a neutral exchange mechanism (Figure 4). However, in only a few mucosae does Ba^{++} increase the resistance by 500 to 700 ohm-cm^2. In many mucosae the increase in resistance due to Ba^{++} is only about 200 ohm cm^2.

Using the same circuit (figure 3), the minimum loop resistance, for an increase of mucosal resistance of 200 ohm cm^2, would be 800 ohm cm^2. In this case

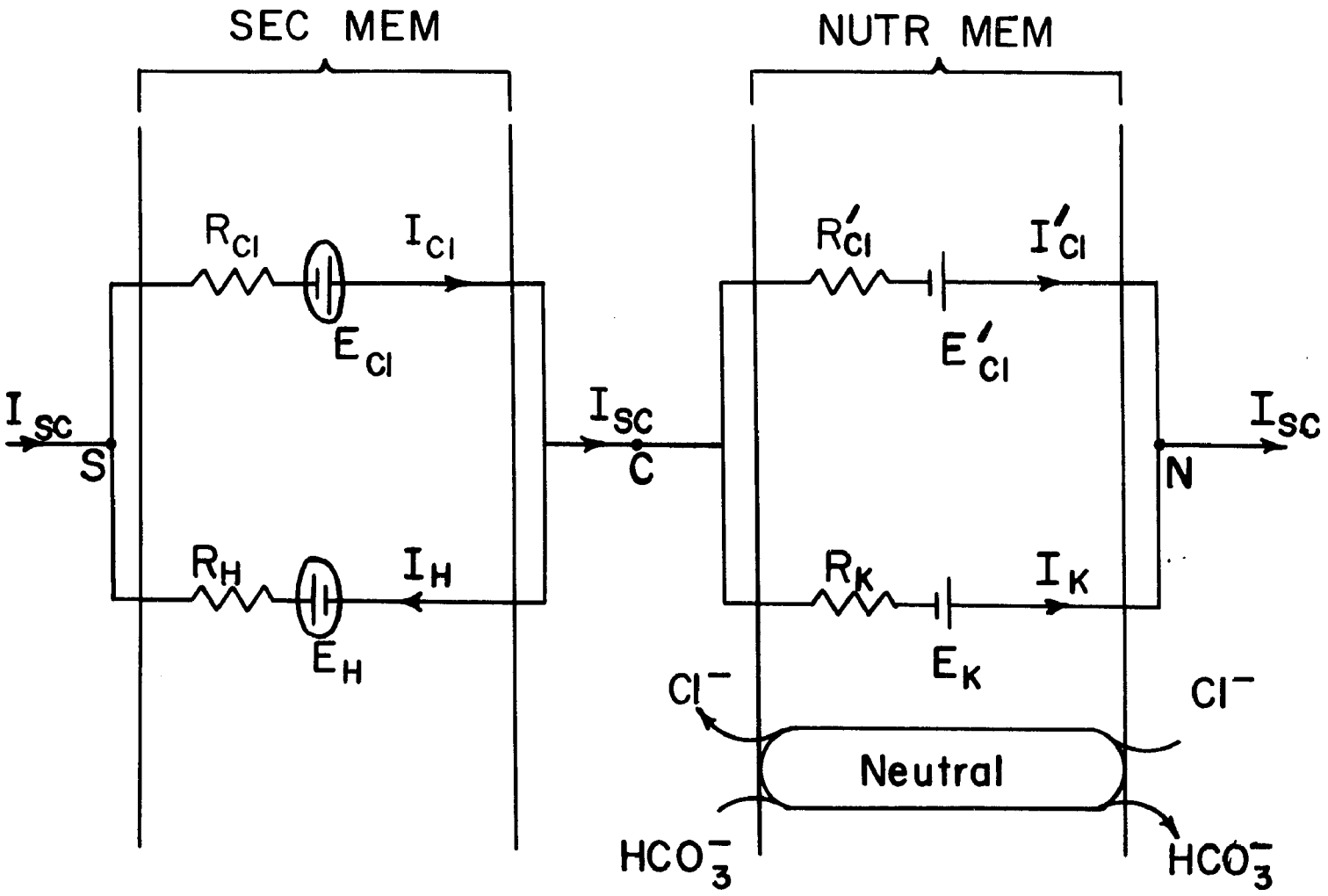
$$E_{Cl} + E_B = 800 \times 0.1 = 80 \text{ mv} \quad (6)$$

Now if $R_B = R_{Cl}$, assuming a 20 mv contribution from the bicarbonate limb and setting our extracellular Cl^- concentration at 82.6 mM (Table 1, solution 2) as is done under standard conditions, then intracellular Cl^- would have to be 7.63 mM or less to account for the necessary emf. This concentration of Cl^- is low but not completely unreasonable for normal HCl rates and is a maximum which would only occur if $R_{Cl} = R_B$. To determine the ratio of R_{Cl} to R_B thus becomes crucial. If it could be demonstrated that the nutrient membrane had no partial ionic conductance to bicarbonate with

FIGURE 4.

Equivalent circuit for new concept of secretory and nutrient membrane of frog stomach.

Depicts what we believe is a realistic scheme for ion transport in the gastric mucosa. The secretory membrane is similar to that shown in Figure 3. The nutrient membrane is pictured with conductance channels for Cl^- and K^+ and a neutral carrier exchange mechanism (a whirling dervish) for Cl^- bicarbonate exchange. It is apparent from consideration of this figure that under steady state short circuit conditions all current must pass through the nutrient membrane via the Cl^- conductance channel, otherwise the intracellular K^+ could not be maintained at a constant level.


SEC MEM
NUTR MEM
R_{Cl}
I_{Cl}
E_{Cl}
R'_{Cl}
I'_{Cl}
E'_{Cl}
I_{SC}
S
I_{SC}
C
I_{SC}
N
R_H
I_H
E_H
R_K
I_K
E_K
Cl^-
Cl^-
Neutral
HCO_3^-
HCO_3^-

fiducial limits such that R_B must be at least twnety times R_{Cl}, then a minimum value for the nutrient loop resistance in Figure 3 becomes $R_B = 20\ R_{Cl}$ and

$$R_N = \frac{20\ R_{Cl}\ R_{Cl}}{21\ R_{Cl}}\ R_{Cl} \qquad (7)$$

$$R_{Cl} = \frac{21}{20}\ R_N \qquad (8)$$

But experimentally R_{Cl} would then be approximately 200 ohm cm^2 so that R_B would have to be 2200 ohm cm^2. Then it is easily shown that

$$R\ (loop) = R_{Cl} + R_B = 2400\ ohms \qquad (9)$$

-- a figure that would make a conductive mechanism for Cl^--bicarbonate exchange prohibitive.

The History of An Ion

Barium historically has been used as a tool to study the electrical properties of membranes. Kruta (1934) claimed that Ba^{++} produced automaticity in the mammalian myocardium. Since the time of Kruta a number of papers (see Sperelakis, Schneider and Harris, 1967, for references) have appeared in which Ba^{++} has been demonstrated to produce spontaneous activity in a variety of tissues including nerves, smooth muscle, crayfish skeletal muscle, frog cardiac (Kleinfeld, Stein and Meyers, 1954) and skeletal muscle and mammalian heart (Dudel and Trautwein, 1958). In 1968 Schwartz et al. demonstrated that Ba^{++} increased the resistance of the frog gastric membrane. In 1967, Sperelakis and co-workers demonstrated

a similar effect on the frog sartorius msucle, while Reid and Hecht (1967) found a rise in resistance of mammalian cardiac muscle when concentrations of greater than 0.75 mM Ba^{++} were added to the bathing media.

In work done on the stomach in the paper by Jacobson, Schwartz and Rehm (1965) these men were originally studying the effects of Ca^{++} depletion on the properties of the gastric mucosae. They found a biphasic effect on the resistance of the frog stomach when all Ca^{++} was removed from the bathing media. Phase I represented a rise in resistance and phase II a fall in resistance, with final total abolition of H^{+} ion secretion (see also Forte and Nauss, 1965). In testing the ability of other divalent cations to substitute for Ca^{++} they found that Ba^{++} would not substitute for Ca^{++}, but that in the presence of the normal concentrations of Ca^{++}, Ba^{++} produced a marked rise in resistance without producing much change in the H^{+} secretory rate. If the site of Ba^{++} action were known, this characteristic (the increase in resistance without inhibition of H^{+} secretion) of Ba^{++} when added to the nutrient bathing media would make it an extremely useful tool in determining the mechanism for transport of other ions. The last set of experiments is designed to elucidate the site and mode of Ba^{++} action and this information is applied to determine the mechanism of nutrient bicarbonate exchange.

Summing It Up

To nail down the hypothesis of a neutral Cl^--bicarbonate exchange mechanism in the nutrient membrane of the frog gastric mucosa represents a substantial advance in our understanding of the mechanisms of ion transport in the gastric mucosa. To provide further evidence for a neutral Cl^--bicarbonate exchange mechanism our approach is to show that the conductance of the nutrient membrane is equal to the sum of the K^+ and Cl^- conductances, to show that the conductance of the nutrient membrane to bicarbonate, H^+ and OH^- is zero, and after establishing the site and mode of Ba^{++} action, to use this to demonstrate that postulation of a conductive mechanism for bicarbonate movement would require the generation of an emf so high as to reduce this proposition to absurdity.

EXPERIMENTAL METHODS AND PROCEDURES

The Preparation of a Stomach

In these experiments frogs of the species Rana pipiens weighing from 60 to 250 grams were used. The frogs were stored in an indoor froggery at room temperature. The frogs were decapitated and the stomach was removed by placing a tie at the second portion of the duodenum then excising the hollow viscus at a point several millimeters above the esophagogastric junction and several millimeters below the distal tie with a "no touch" technique similar to that used in surgery performed for malignant disease. The specimen was immediately placed in an ice cold secretory solution (no. 1 of Table 1) pre-equilibrated with 95% O_2, 5% CO_2. The esophagogastric junction of each stomach was grasped with toothed forceps, the stomach divided along the lesser curvature and stretched by means of three silk ties in triangular fashion across the perfused iced bathing media. Incision would then be made across the external muscle layer approximately to the depth of the submucosa, about 2 mm. proximal to the first portion of the duodenum. By traction blunt dissection the external muscle layer was removed. After removal of this layer, the remaining specimen would be placed under slight tension by means of discrete silk ties placed

circumferentially about the mucosa. At this point the mucosa in the condition just described would be placed between Lucite chambers (Figure 5).

Instrumentation

A four electrode system (Rehm, 1962) was used, two calomel electrodes for measuring the PD and two other electrodes for the purposes of sending current. The calomel electrodes were manufactured by Radiometer of Copenhagen, and for every experiment in this dissertation were demonstrated to have an asymmetry potential much less than one mv, usually in the region of 0.1 mv. The current-sending electrodes were basically of the lead, lead-acetate type. The preparation of these electrodes is as follows: a circumferential lead disk was fitted snugly into the distal end of the cylindrical Lucite casing. A layer of saturated lead-acetate agar heated and in liquid form would then be carefully placed over the lead in such fashion that no bubbles would be present. Lead-acetate was chosen because of its ability to gel in agar; lead nitrate in agar does not gel because of the low pH of this solution. The second layer of melted agar placed in the electrode contains sodium nitrate (0.12 M). Finally the electrode casings are filled with three discrete layers of sodium sulfate (0.1 M) agar. The insolubility of lead sulfate prevents the lead from gaining access in the bathing media used in our preparation. Batteries were used as current sources. Current for all of the experiments

except those involving short-circuiting of the mucosa was 40 microamps for 1.3 cm^2. This current was applied in a square wave pulse of 0.5 second duration. Resistance was taken as the change in PD after 0.5 second divided by the current. Intervals between pulses were varied according to the demands of the experimental protocol. Each pulse would routinely be sent first in one direction then in the other direction.

In all experiments included in this dissertation except those involving short-circuiting of the gastric mucosa, the PD was measured with an Esterline Angus recording potentiometer with a 1/8 second full scale response. The standard paper speed was 0.75 inch per minute except in cases when more rapid changes were expected, at which time the paper speed was increased.

Some experiments were performed with the short-circuiting technique and in these experiments a Honeywell Electronic 194 dual channel recorder was used. This instrument has an accuracy of 0.25% span or one microvolt, whichever is greater, and full scale response time of less than 0.5 second.

A Radiometer (Copenhagen) pH stat, Model TTT-1 with a three decimal precision capability was used in conjunction with a Radiometer titratograph to measure H^+ secretory rates. The secretory solution was maintained at a pH between 4.5 and 5.0. Three different strength titrants were used throughout these experiments. Titrants were made by adding a given amount of a 0.1 N NaOH solution to the appropriate secretory solution. In all the experiments the nutrient fluid contained 10^{-4} M histamine.

The Set Up

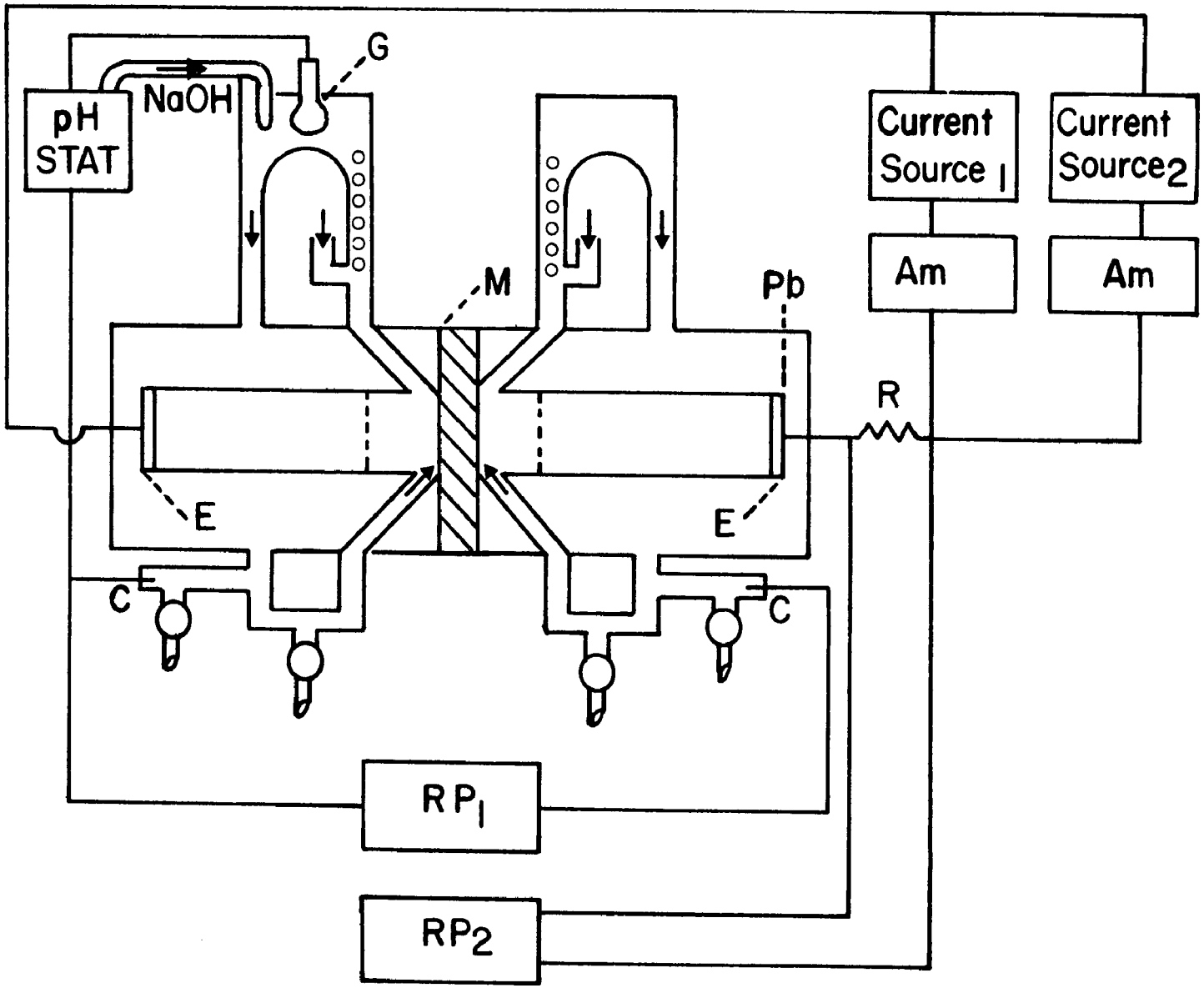
Figure 5 is a diagrammatic sketch of the apparatus except that the second current source and the second recording potentiometer RP_2 were used only in the short circuiting experiments. The chamber had a cross-sectional area of 1.3 cm^2 and circulation was maintained by gas lifts, using 95% oxygen, 5% CO_2 on both sides except where otherwise specified. All solutions used in these experiments were previously equilibrated with the gas mixture used to circulate them. In each case care was taken to maintain equal levels of fluid in the reservoirs on both sides of the chamber so that no hydrostatic pressure gradient would be present. Except at the time of initial mounting, the tubing on the side of the chamber opposite that in which the fluid change was to be made would be clamped both above and below entrances to the chamber. The purpose of this type of clamping was to prevent bulging of the mucosa due to a pressure gradient upon draining. Control experiments showed that with this technique fluid changes can be made with essentially no change in resistance, PD and H^+ rate. Throughout the experiments frequent checks were made to ensure presence of good circulation on both sides of the mucosa.

The actual tissue preparation in our chamber which will be referred to as the gastric mucosa consists of the mucous coat, the invaginated continuous cell layer (one cell thick), the lamina propria, the muscularis mucosa, and part of the submucosa. Some

FIGURE 5.

Schematic representation of experimental system.

This is a schematic representation for the frog chamber and demonstrated used in the described experiments. M shows the position of the gastric mucosa. E represents the agar electrodes used for current sending, C, the calomel non-depolarizable electrodes used in PD measurement, Am, ammeter, arrows indicate the entrance of bubbling gas the direction of flow, pH stat is self-evident and NaOH with its arrow indicates the entrance point of titrant to the secretory side as controlled by our pH stat method. pH further defines our current sending electrodes as being of the lead-lead-acetate type (see description in the text). Current source 2 with its ammeter was present only in the short circuiting experiments where a fifty ohm resistor (R) was inserted to allow direct measurement of the short circuit current by a recording potentiometer (RP_2). Under our short circuit experiments only a single recording potentiometer was present, RP_1.


pH
STAT
NaOH
G
M
Pb
E
E
C
C
R
Current
Source 1
Current
Source2
Am
Am
RP1
RP2

experiments were performed in which other or modified specimens were placed in the chamber but whenever this occurs it will be described in the text.

A Note on Resistance

All resistance measurements were corrected for blank and tissue resistance. Blank resistance is the measured resistance of the solution in the chamber in the absence of tissue. Tissue resistance is the resistance correction for the approximate volume of gastric mucosa when in the chamber. All resistances were corrected to ohm cm^2 unless otherwise specified.

Special Methods

In the K^+ concentration experiments step changes of K^+ were made in the nutrient bathing solution for specific time intervals. From these experiments PD and resistance relationships to K^+ concentration were obtained. Table 1 gives the composition of the solutions used in these experiments.

The method used to determine partial ionic conductance of the nutrient membrane for an ion is to measure the effect on the PD of step changes in the concentration of that ion in the nutrient bathing media by replacing it with an impermeant ion. However, for some of the changes in H^+ and OH^- concentrations no ion substitutions were made. If the H^+ or OH^- conductance of the nutrient membrane were a significant fraction of its total conductance, a step change

in the pH of the nutrient fluid would result in a change in PD. Initial measurements of PD and resistance were obtained within an average of five seconds after solution change, zero time being marked at that point at which the new solution was added. The compositions of the solutions used for this series of experiments are listed in Table 2. Implicit is the assumption that the partial ionic conductance of the nutrient membrane for sulfate is zero so that the bicarbonate is replaced by sulfate and sucrose is used to make up the osmotic loss.

The junction potentials between the various solutions measured by calomel electrodes with readily renewable saturated potassium chloride junctions, were as follows: solution 9-10 (Table 2), +2 mv; solution 13-12, -0.7 mv; solution 14-15, -0.4 mv, (plus sign means first solution positive in an external circuit).

Twelve sets of experiments were designed to help elucidate the site and mode of Ba^{++} action on the gastric mucosa. Solutions for these experiments include some described in Table 1 as well as all those listed in Table 3. These experiments included control protocols to test specific sites for the Ba^{++} effect; examination of the Ba^{++} effect under conditions varying media content, concentration and dwell time. To avoid needless repetition and aid reader orientation these approaches will be discussed under results.

RESULTS

The High Nutrient K^+ Experiments

Changing the K^+

To determine the PD and resistance relationship to K^+ concentration, the time constant for crossing the nutrient diffusion barrier, and the minimum resistance of the nutrient membrane two types of experiments were performed. One involved discrete changes of six minutes duration from a baseline of 4 mM nutrient K^+ to 10, 20, 40, or 80 mM K^+ solutions. At the end of a six minute period the solution with higher concentration of K^+ was thoroughly washed from the nutrient chamber and the stomach again allowed to stabilize with the 4 mM K^+ solution. Figure 6 is a representative experiment from the series. It shows the resistance and PD vs time in minutes. Following the first pulse (80 mM K^+) the resistance does not recover to the original control level. This was sometimes seen after the initial pulsing of the gastric mucosa with higher concentrations of K^+. Usually subsequent elevations to the same high K^+ level were followed by return to pre-pulse resistance levels. The decrease in PD and resistance is greater with increasing concentrations of K^+. Figure 7 is a plot of the average values for resistance vs K^+ concentrations.

FIGURE 6.

Changes in PD and resistance of a representative stomach in response to changing K^+ concentrations on the nutrient side.

Indicates the change in PD and resistance of the gastric mucosa as a function of the concentration of K^+ in the nutrient fluid. At the arrows labeled 80K, 40K, 20K, and 10K the nutrient K^+ was changed from 4 mM to the designated K^+ concentrations. The elevated K^+ concentrations were maintained for periods of 6 minutes. As indicated by the arrows labeled 4 K^+ the standard nutrient fluid replaced the fluid containing elevated K^+ concentrations. After the first high K^+ pulse the resistance settles to a new baseline (cf also Figure 10).

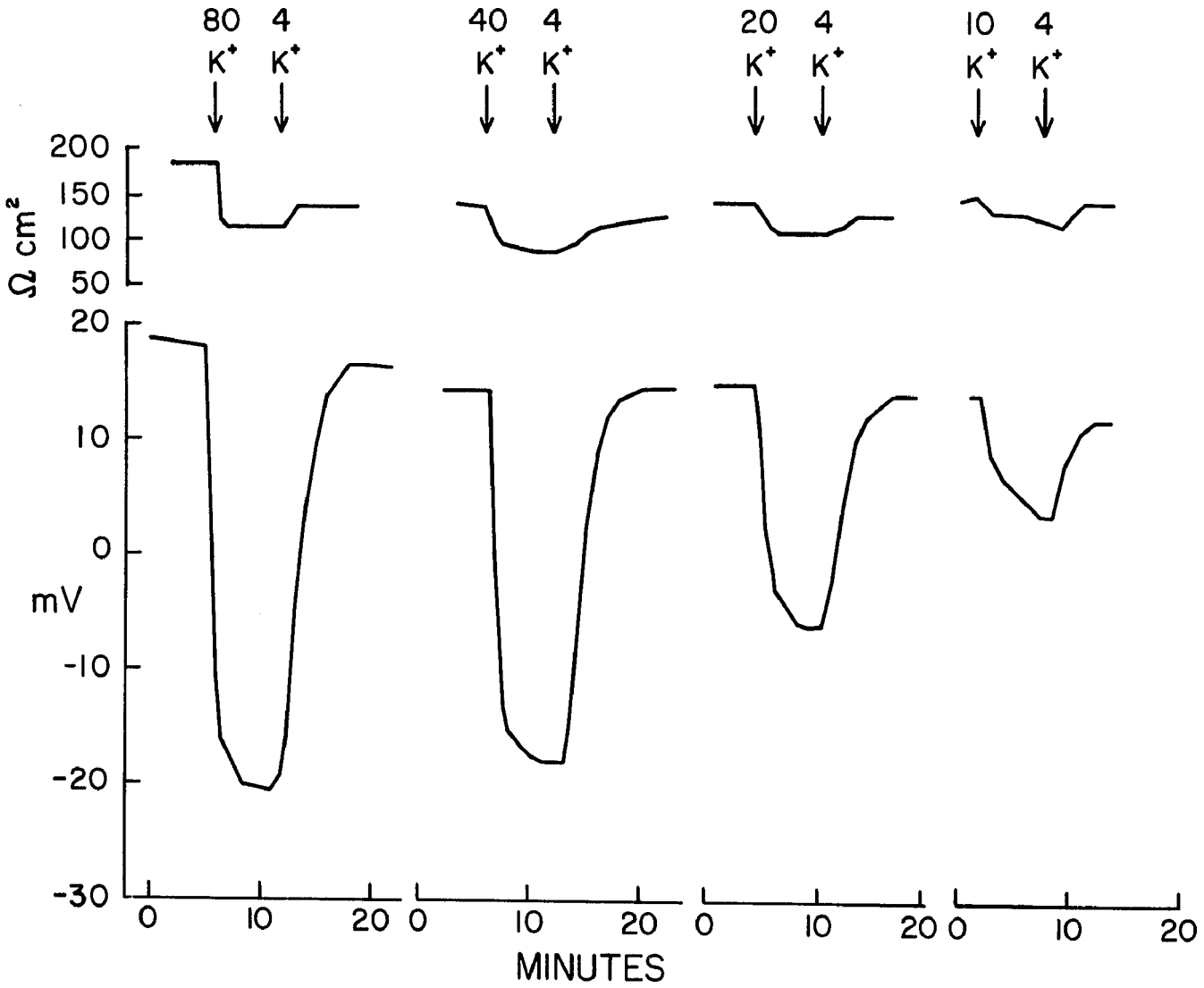
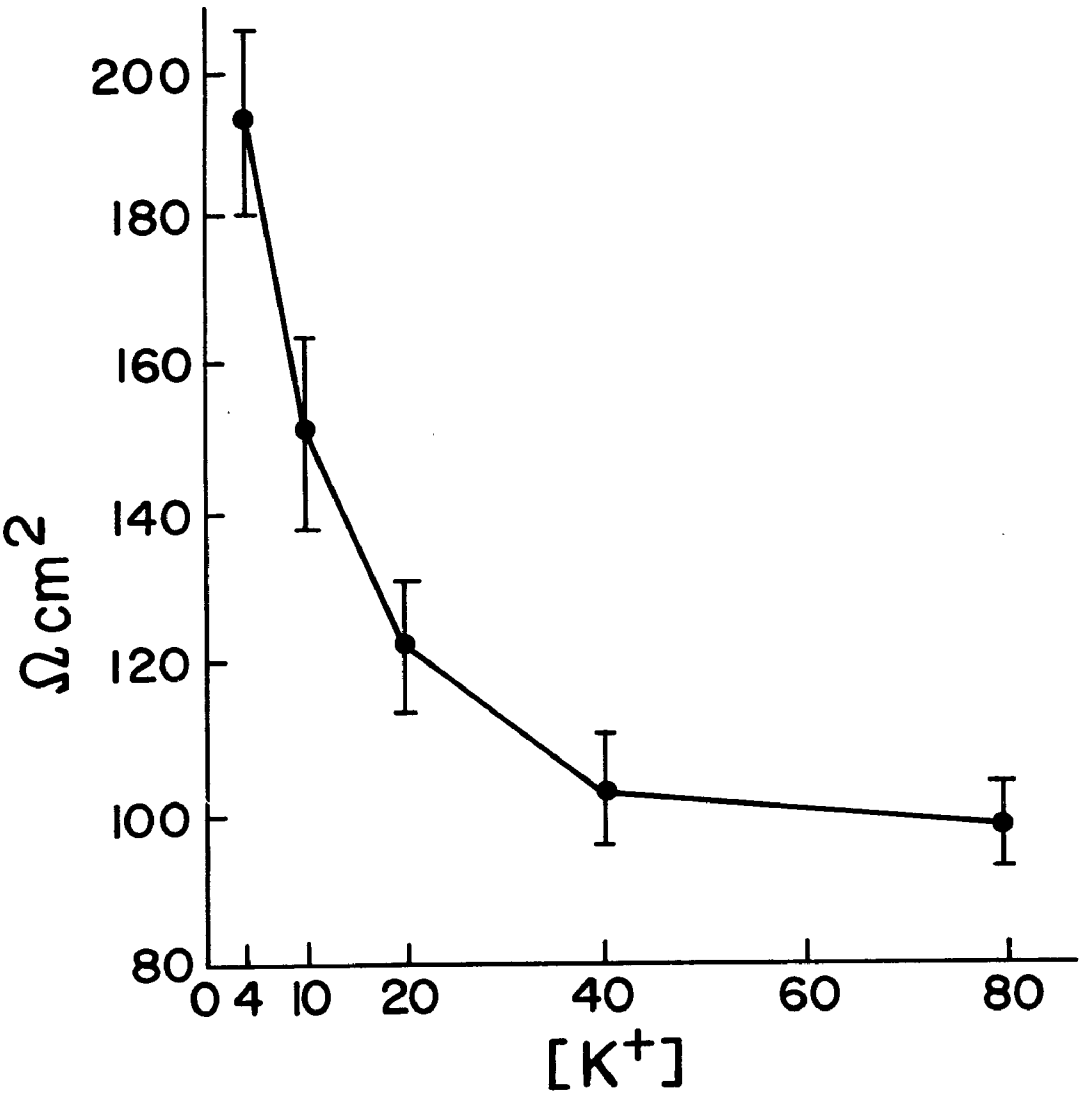

80 4
K+ K+
40 4
K+ K+
20 4
K+ K+
10 4
K+ K+
200
150
100
50
Ω cm²
20
10
0
mV
-10
-20
-30
0 10 20 0 10 20 0 10 20 0 10 20
MINUTES

FIGURE 7.

Resistance vs K^+ concentration for the frog stomach.

This curve represents the average resistance response of six gastric mucosa to increasing nutrient concentrations of K^+. Pulses of 6 minute duration were used and the resistance in ohm cm^2 was taken at the nadir of each pulse. K^+ concentration is in mM, and vertical lines delineate the Standard Error of the Mean for each K^+ concentration.


200
180
160
140
120
100
80
Ω cm²
0 4 10 20 40 60 80
[K⁺]

The resistance was taken at the nadir of the pulses. Each point represents the mean for six experiments with vertical lines depicting their respective ± standard errors of the mean (SEM).

The data showing the PD as a function of the log K^+ concentration for the individual experiments are given in Figure 8. The average PD (± SEM) vs the log K^+ for these experiments is presented in Figure 9. It is apparent from Figures 8 and 9 that the fit to a straight line is excellent.

Changing the Dwell Time

The other type of experiment in this group involved exposing the nutrient membrane to 79 mM K^+ concentrations for discrete 30 second, 60 second, 2 minute and 10 minute pulses. A representative experiment from this group is illustrated in Figure 10. Note the rapidity of change which occurs in both resistance and PD. These experiments are analyzed in terms of resistance (ohm cm^2) change from baseline for each discrete pulse in Table 4. The table includes the mean, standard deviation (SD) and SEM. The figures in parentheses under "average control" are for additional pulses performed on the same membrane of the preceding row. We wanted to show these control values but excluded them from statistical computation to avoid weighting the analysis. The 10 minute pulses were broken into discrete 30 second, 60 second, 2 minute and 10 minute points in Table 5. This effectively allows us to double our observations at 30 seconds, 60 seconds and 2 minutes.

FIGURE 8.

PD vs log K^+ concentration for six individual stomachs.

These curves represent the PD response of each of six gastric mucosae to increasing nutrient concentration of K^+. The ordinate shows PD in mv. The abscissa is the Brigsean log of K^+ concentration. 4 mM, 10 mM, 20 mM, 40 mM and 80 mM K^+ solutions were used. This figure is shown to illustrate the appropriate linearity of each mucosa and as can be seen there is very little spread in the data.

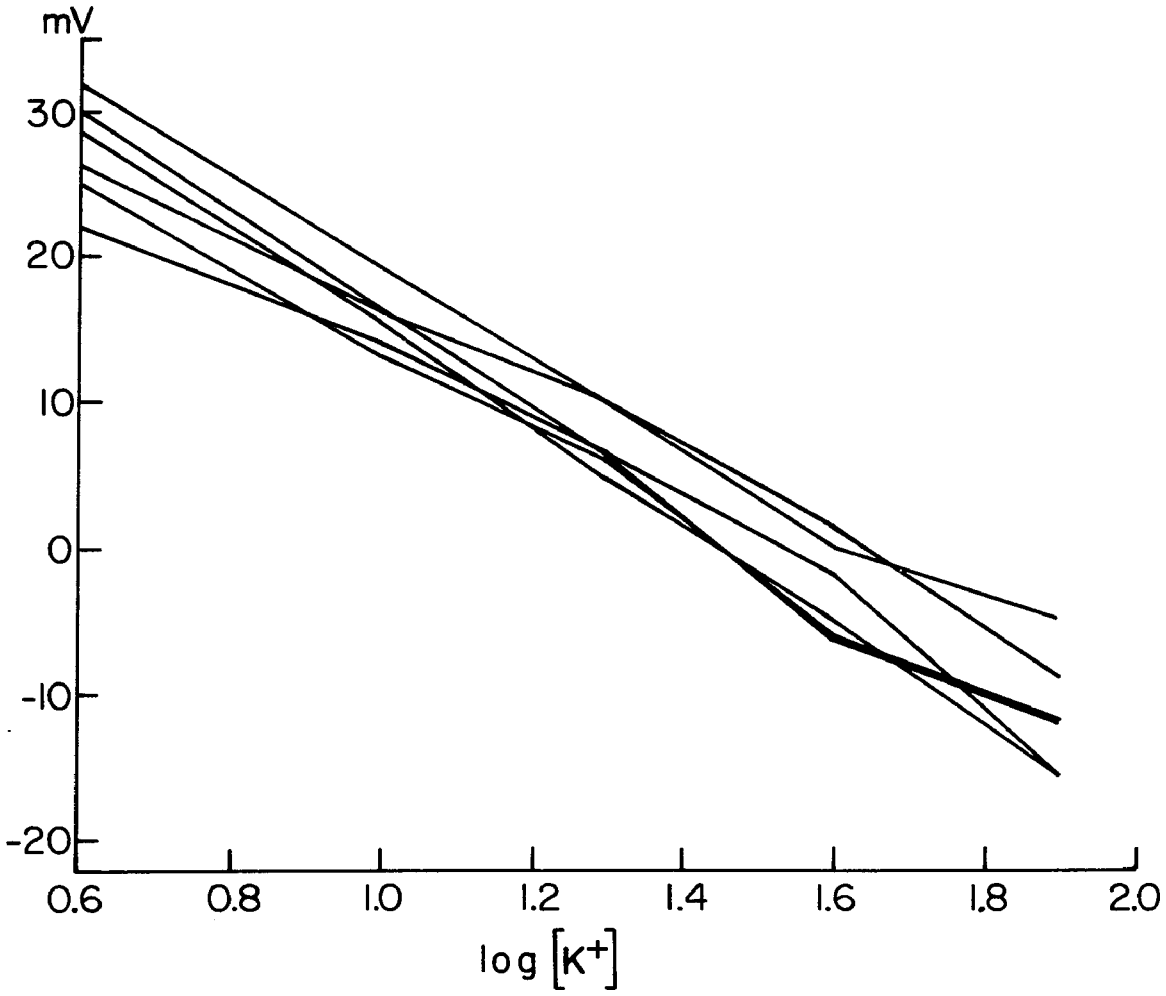

mV
30
20
10
0
-10
-20
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
log [K+]

FIGURE 9.

Average PD vs log K^+ concentration for six stomachs.

This curve shows the average response of the results presented in Figure 8. Vertical lines delineate the Standard Error of the Mean.

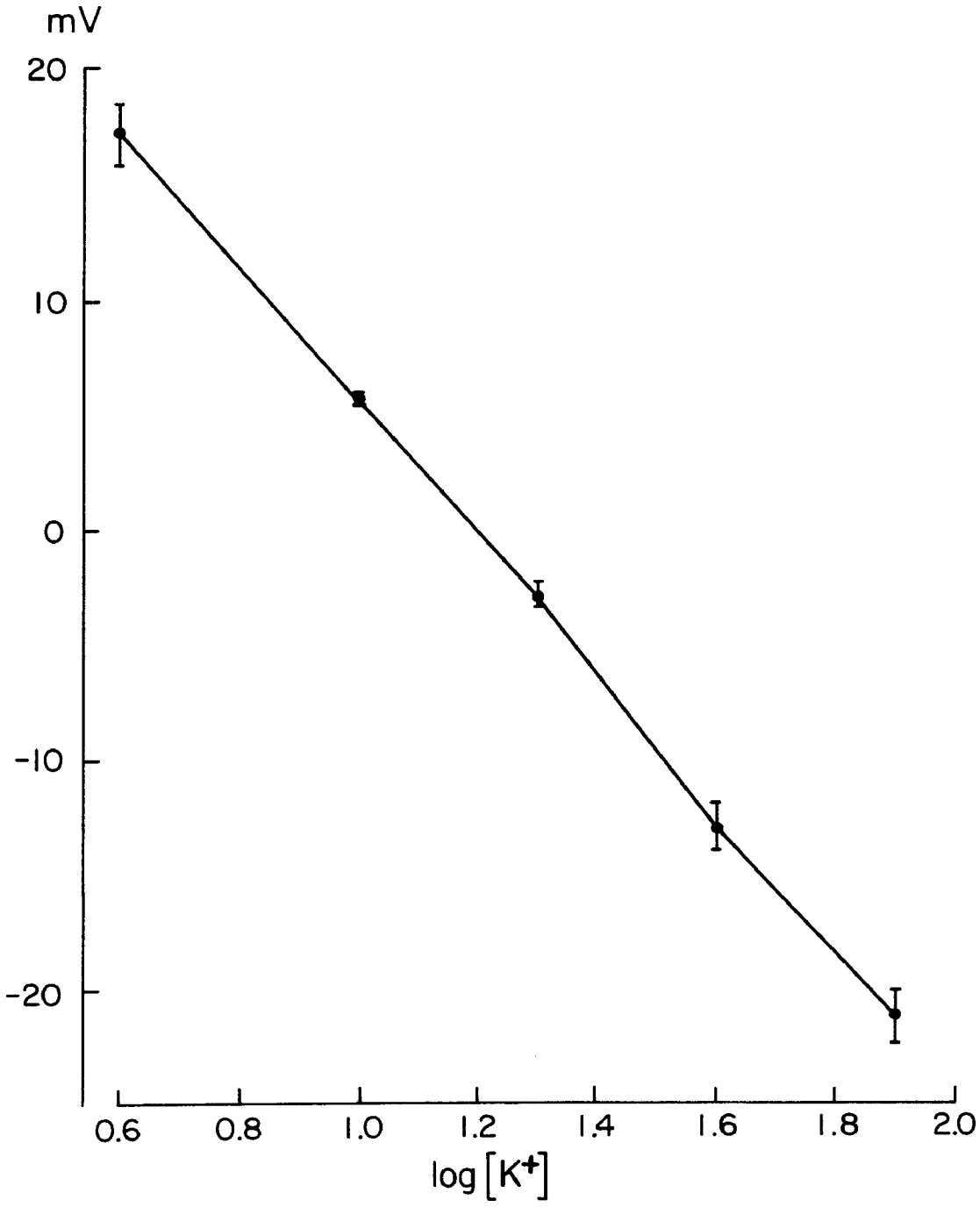
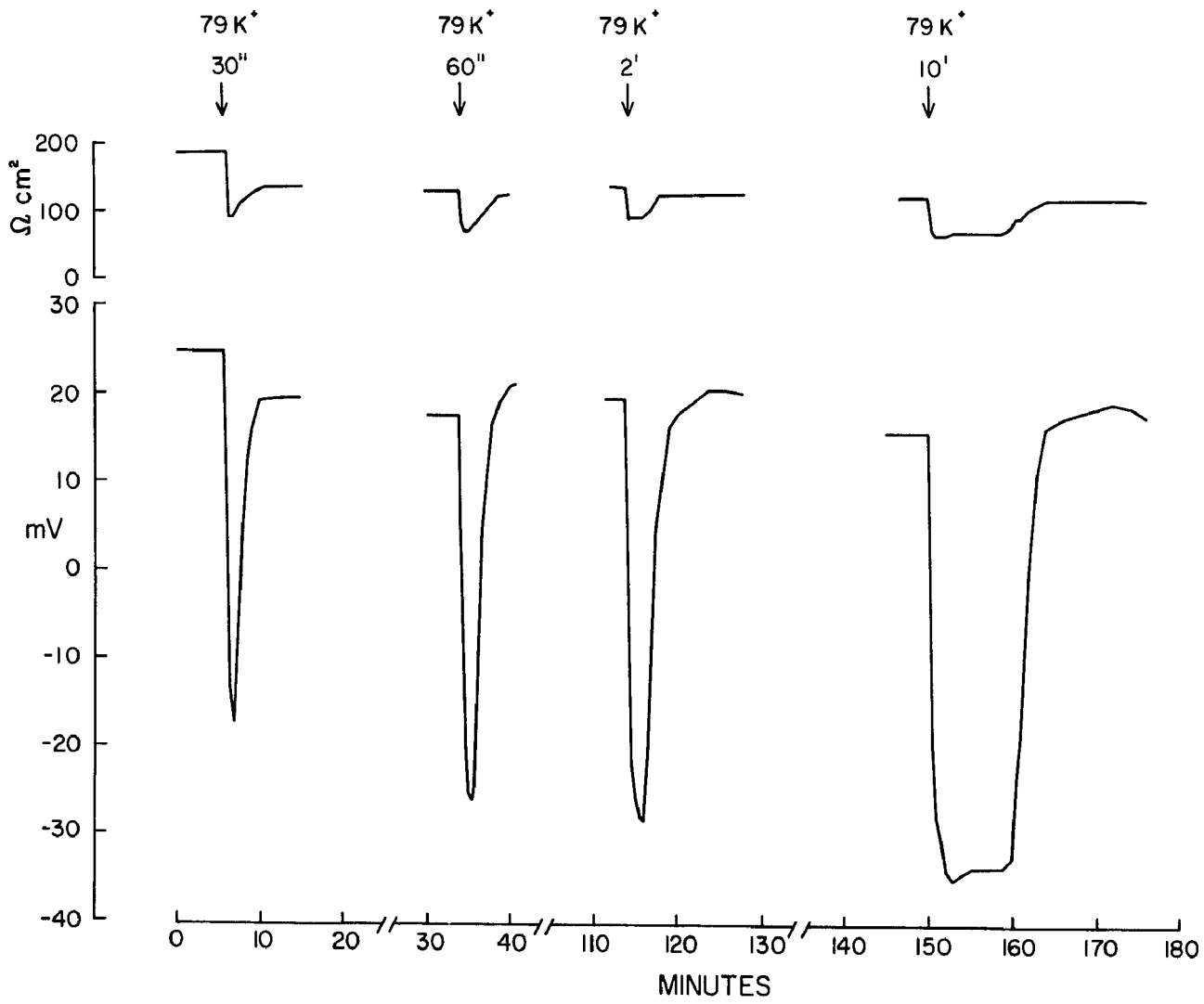

mV
20
10
0
-10
-20
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
log [K+]

FIGURE 10.

PD and resistance changes of a representative stomach for 79 mM K^+ pulses of varying duration.

Represents the change in resistance and PD of the gastric mucosa when step changes of 4 mM to 79 mM K^+ bathing media were made on the nutrient side. The pulses were of 30 second, 60 second, 2 minute and 10 minute duration. Note the failure of resistance to recover to its original baseline after the initial 79 mM K^+ pulse.


79 K+
30"
79 K+
60"
79 K+
2'
79 K+
10'
Ω cm²
200
100
0
30
20
10
mV
0
-10
-20
-30
-40
0
10
20
30
40
110
120
130
140
150
160
170
180
MINUTES

In similar fashion, the PD changes as a function of exposure to 79 mM K^+ for 30 seconds, 60 seconds, 2 minutes and 10 minutes were put in tabular form together with a breakdown for values at the 30 second, 60 second and 2 minute points of the 10 minute curves (Tables 6 and 7).

The Calculation of G_K

It would be nice to gain some approximate idea of the conductance of the nutrient membrane to K^+ and get a handle on the K^+ concentration at this membrane as a function of time when the membrane is exposed to high K^+ solutions. Such data would enable us to calculate the time constant necessary for ions to cross the nutrient diffusion barrier. With this in mind if we make a simplified model for the nutrient facing membrane and assume its resistance is constant we can write

$$V_0 = \frac{G'_K}{G'_K + G'_{Cl}} \; 58 \log \frac{K_i}{4} + \frac{G'_{Cl}}{G'_K + G'_{Cl}} \; E_{Cl} \qquad (10)$$

Where G'_K and G'_{Cl} represent the conductances of the K^+ and Cl^- channels respectively, V_0 is the PD across the nutrient membrane, 58 represents 2.3 (RT/F), K_i the K^+ concentration inside the gastric cell and E_{Cl} the potential of the Cl^-. Equation 11 is exactly like the previous equation:

$$V_1 = \frac{G'_K}{G'_K + G'_6} \; 58 \log \frac{K_i}{C} + \frac{G'_{Cl}}{G'_K + G'_6} \; E_{Cl} \qquad (11)$$

except that C represents a known concentration of K^+ at the surface

of the nutrient facing membrane. By simple subtraction we obtain equation 9:

$$V_0 - V_1 = \Delta V = \frac{G'_K}{G'_K + G'_{Cl}} \; 58 \log \frac{K_i}{4} \; \frac{C}{K_i} \qquad (12)$$

It is obvious (from Tables 6 and 7) that often the maximum change in PD or minimum resistance observed does not occur at the end of the time period during which the membrane is exposed to high K^+. It seems reasonable to assume that the best approximation of a 79 mM K^+ concentration at the nutrient membrane would be obtained by taking the time of greatest change in PD and that any decrease in this magnitude thereafter would be due to changes in the parameters of the system.

Taking this nadir of PD to represent 79 mM K^+ at the nutrient membrane we can now write:

$$\Delta V = G_K \; \log \frac{C}{4} \qquad (13)$$

where G_K represents $\frac{G'_K}{G'_K + G'_{Cl}}$ (58). At this point it becomes a simple matter to plug in the ΔV at shorter time intervals and thus calculate the nutrient membrane K^+ concentration as a function of time by simply rearranging equation 13 to:

$$C = 4 \times 10 \; \Delta V/G_K \qquad (14)$$

Tables 6 and 7 represent the experimental data and the results of these calculations.

Partial Ionic Conductance for H^+, OH^- and Bicarbonate

Nutrient Membrane Conductivity

The purpose of these experiments was to determine directly the presence or absence of nutrient membrane conductance channels for H^+, OH^- and bicarbonate. The method was that of changing concentration of bicarbonate and/or the CO_2 in the nutrient bathing media and observing the change in PD. Figure 11 shows an experiment in which the bicarbonate was changed from 25 mM to 5 mM without changing the gas. It can be seen that there was essentially no change in the PD while the pH shows a substantial change. Simultaneous step changes in bicarbonate and CO_2 had no effect on PD (see Figure 12 and Table 8). Part B of Figure 12 represents the average response of six experiments to the change: 25 mM bicarbonate, 95% oxygen, 5% CO_2 to 5 mM bicarbonate, 99% oxygen, 1% CO_2. Part C shows the response to the reverse change. There is no significant change in PD throughout the first four minutes. Compare with the above a fivefold change in K^+ as shown in part A of Figure 12 where the PD starts changing between 5 and 10 seconds after the new solution is placed in the nutrient chamber. Within two minutes the PD exceeds 50% of its total change. Some experiments were done in which the hydrogen ion concentration was changed by about 0.7 pH unit by changing the gas from 95% oxygen, 5% CO_2 to 99% oxygen, 1% CO_2 with a bicarbonate concentration held constant at 25 mM. As in the experiments described above, there was no

FIGURE 11.

PD changes with time in response to changes in nutrient pH.

Effect of a step change in pH on PD (nutrient positive to secretory side) across in vitro frog gastric mucosa. As time indicated (2 minutes) nutrient chamber was drained and a new solution (previously equilibrated with 95% O_2 - 5% CO_2) placed in this chamber and at 6 minutes new solution was replaced with original one (solutions 3 and 4 of Table 1). Secretory solution was no. 1 of Table 1 and this side was also gassed with 95% O_2 - 5% CO_2. It can be seen that step changes in pH do not result in changes in PD.

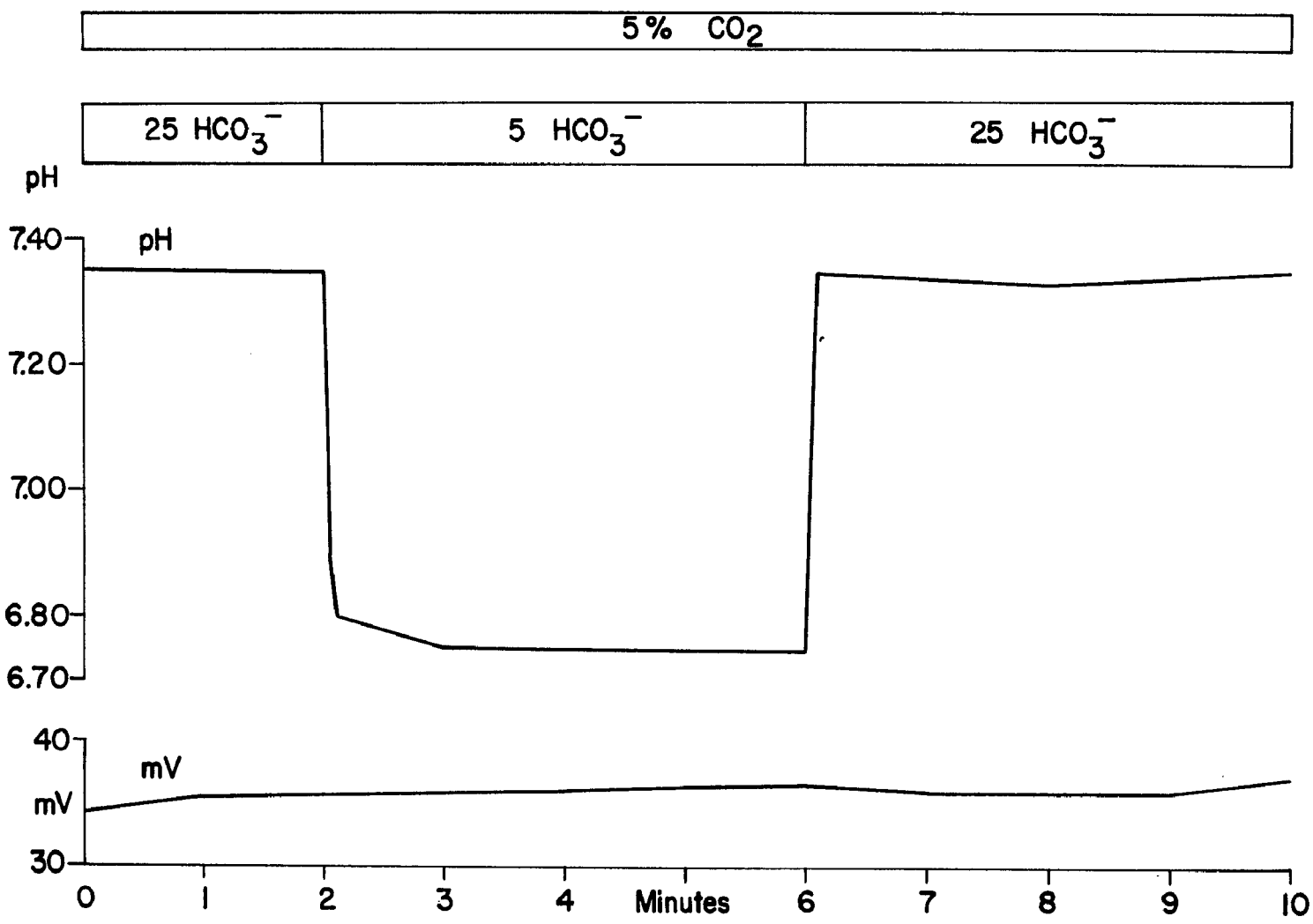
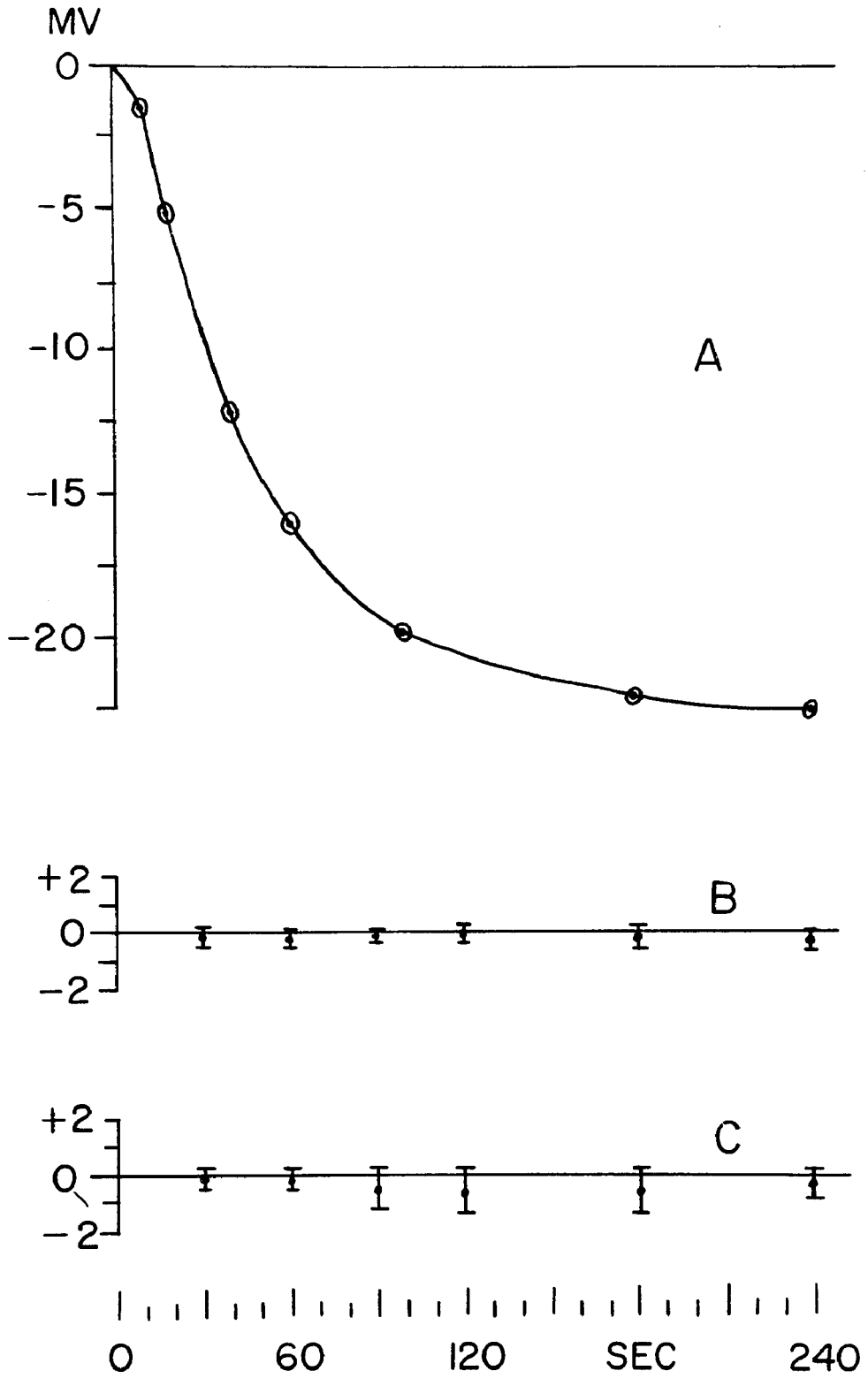

5% CO_2
25 HCO_3^-
5 HCO_3^-
25 HCO_3^-
pH
7.40
pH
7.20
7.00
6.80
6.70
40
mV
mV
30
0
1
2
3
4
Minutes
6
7
8
9
10

FIGURE 12.

PD changes in response to fivefold changes of nutrient media, K^+ or bicarbonate concentrations.

Curve A: effect on PD of a fivefold increase in K^+ of nutrient fluid (4-20 mM, solutions 3-5 of Table 1). Curve B: effect on PD at constant pH of fivefold decrease in HCO_3^- and CO_2 (95% O_2 - 5% CO_2 to 99% O_2 - 1% CO_2) of nutrient (solutions 3 to 4 of Table 1). Curve C: representation of effect on PD of reversed change from that of curve B. For all 3 curves, the ordinates represent the change in PD; a positive ΔPD means nutrient becomes more positive and vice versa. Direction of change in PD for curve A is that predicted for elevation of nutrient K^+.


MV
0
-5
-10
-15
-20
A
+2
0
-2
B
+2
0
-2
C
0
60
120
SEC
240

significant change in PD during the first four minutes after the partial pressures of the gases were changed.

Eliminating a Common Channel

It might be possible that Cl^- and bicarbonate ion share the same conductance channels. We considered this possibility and if we assume for illustrative purposes that the mobilities in these channels for Cl^- and bicarbonate are the same, then it would be the ratio of the sum of chloride and bicarbonate in the external fluid to that in the cell which would determine the emf through these channels. The change in emf, going from 25 to 5 mM bicarbonate would be given by the equation:

$$\Delta E = 58 \log \frac{(81 + 25)}{(81 + 5)} = 5.2 \text{ mv} \qquad (15)$$

where the Cl^- concentration in the nutrient solution is 81 mM and the bicarbonate concentration is either 5 or 25 mM, sulfate is substituted for the bicarbonate ion and sucrose used to make up the osmotic gradient. Using the Spangler-Rehm (1968) value for $G'_{Cl}/(G'_K + G'_{Cl})$ of 0.36 the predicted change in voltage across the nutrient membrane would be only 1.9 mv, that is 5.2 mv times 0.36. With lower Cl^- concentrations the effect of changing the bicarbonate would be expected to produce a greater change in PD. A series of solutions was used with lower Cl^- concentrations (solutions 12-15, Table 2). Again, fivefold changes in bicarbonate did not change the PD. The same was found true in the complete absence of Cl^- (solutions 14, 15, Table 2).

Changing the Cl^- concentration fivefold in solutions resulted in changes essentially the same as those reported in the Spangler-Rehm paper cited above; that is, about 14 mv. It was also found that changing only pH in the absence of Cl^- does not result in a significant change in PD. Finally, the concentration of bicarbonate was elevated from 25 mM to 75 mM in seven experiments and again no change in PD was found.

Table 8 shows the effects on PD of all of these described experiments. The initial PD and resistance measurements with solution changes in these experiments was measured at approximately 5 seconds after the change, zero time being taken as the time at which the previous nutrient facing solution was drained from the chamber. This is significant in view of the Carter et al. hypothesis (1967a, b), to be treated later under "Discussion" and does not allow us to overlook early changes in PD which might be compensated for by transmembrane equilibration of ions (Figure 13).

In Other Words

Changing the bicarbonate ion concentration does not result in a significant change in PD during the first four minutes after the change is made. Since the time constant for the diffusion of ions through the nutrient diffusion barrier is about one minute, one can conclude that the relative bicarbonate conductance is essentially zero. Changing pH produces no significant change in PD. Therefore one can conclude that the relative H^+ and OH^- conductance of the nutrient membrane also is essentially zero.

FIGURE 13.

Model for nutrient membrane with K^+, Cl^-, and H^+ conductance channels.

A: representation of a conceptual model to illustrate consequences of step changes of ionic concentrations of bathing medium on response of transmembrane potential difference (PD). Compartment A represents a small volume comparable to the cell volume and compartment B a large volume comparable to external bathing medium. For purposes of illustration there are 3 arrays of specific conductance channels (for K^+, Cl^-, and H^+) and initially there are 10-fold concentration ratios between compartments A and B so that $E_K = E_{Cl} = E_H = PD_{BA} = 58$ mv. B: illustration of predicted response (15, 16) of PD to a step change in H^+ concentration of compartment B (10^{-7} M to 10^{-6} M). With the H^+ concentrations now same in 2 compartments, E_H will change stepwise to zero and if $R_H << R_K R_{Cl}/(R_K + R_{Cl})$ PD will decrease to almost zero. Current will now flow as to increase H^+ concentration in A (new H^+ concentration in compartment B is fixed) and eventually all 3 emf's will again be equal. Due to large K^+ concentration in compartment A, PD will return to about its original level (high K^+ poises the system). See text for discussion of expected duration of temporary change in PD.

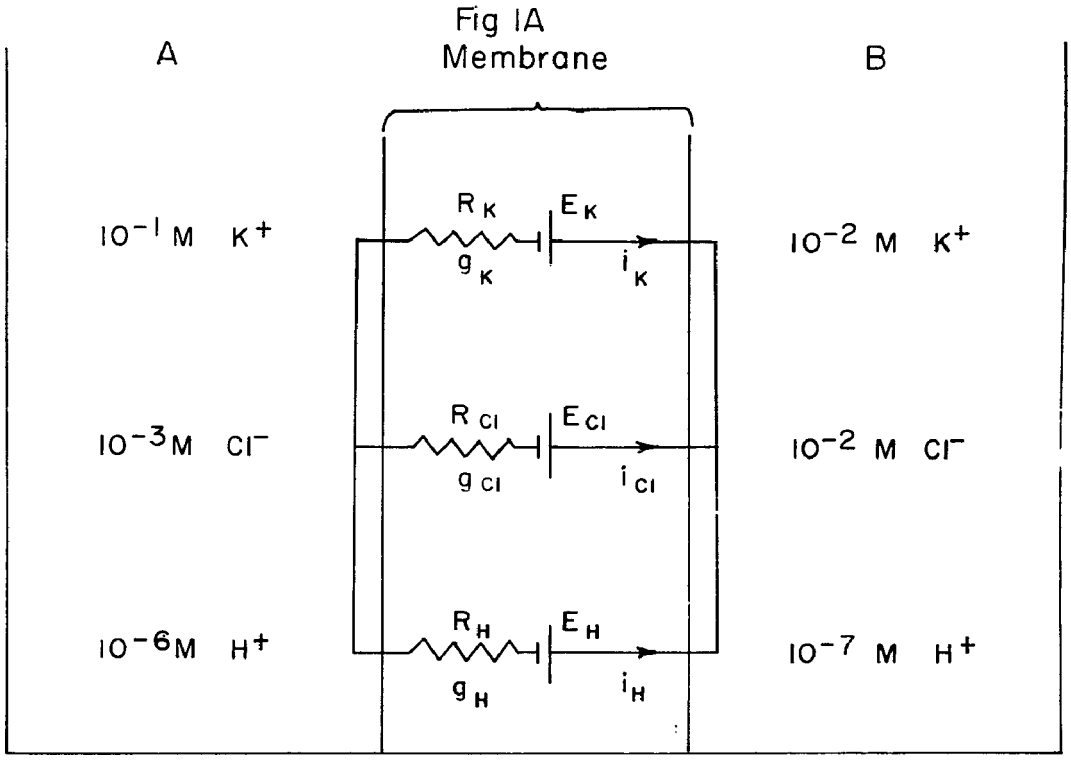


Fig IA
Membrane
A
B
10^{-1} M K^+
10^{-3}M Cl^-
10^{-6}M H^+
10^{-2} M K^+
10^{-2} M Cl^-
10^{-7} M H^+
R_K
E_K
g_K
i_K
R_{Cl}
E_{Cl}
g_{Cl}
i_{Cl}
R_H
E_H
g_H
i_H

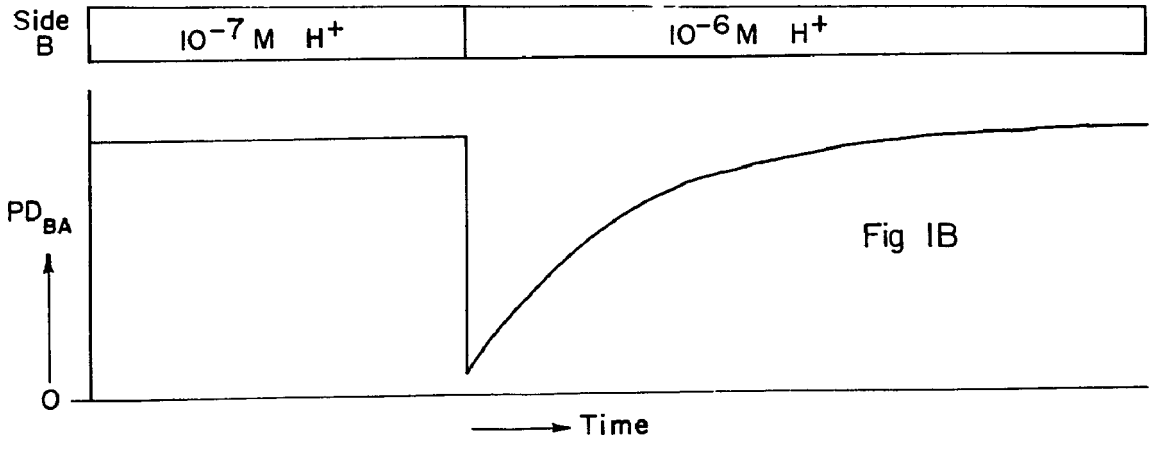

Side B
10^{-7} M H^+
10^{-6}M H^+
PD_{BA}
0
Time

Fig IB

Ba^{++} -- the Site, the Mode, the Tool

This section will describe the results of twelve sets of experiments designed to determine the site and mode of Ba^{++} action on the mucosa. As mentioned earlier, when Ba^{++} is added to the nutrient solution the resistance of the gastric membrane increases. The obvious question arises as to whether the site of Ba^{++} action is on the smooth muscle layers of the stomach, and this will be taken up next.

To Test the Muscle

Experiments were performed in which the frog was dissected, as earlier described, but the gastric mucosa scraped to remove the single layered epithelium as far as practical and allow us to examine the effect, if any, which Ba^{++} might cause on the smooth muscle (muscularis mucosa) of the regular preparation. The external muscle layer (muscularis externa) was also looked at to ascertain any Ba^{++} effects on this portion of the stomach. For these experiments the dissection protocol as described earlier was reversed. Instead of making an incision through the muscle layer just above the pyloric region an incision was made in the mucosa at the pyloric region. The mucosa was then removed and discarded while the external muscle layers and serosa were stretched with silk sutures and placed in the chamber. Although it appeared obvious that smooth muscle should have radial symmetry, nevertheless the orientation of the external muscle layer in our chamber was

always the same and exactly analogous to the orientation of the usual secretory and nutrient sides of the gastric membrane. These scraped mucosae or external muscle layers were placed in our chamber.

As demonstrated in Table 9, scraped mucosae did not show any significant change in resistance or PD in the presence of Ba^{++} in either 1 mM, 5 mM or 10 mM concentrations. When this system was pulsed with high 79 mM K^{+} nutrient solutions, a small change in PD was observed. This change was of the magnitude of and in the direction of the diffusion potential at the junction between the secretory and nutrient solutions. The change in resistance upon the addition of K^{+} always remained negligible. Table 9 presents the change with each individual experiment as well as the mean, SD and SEM.

Six external muscle layers were likewise placed in our chambers. They were pulsed with 1 mM, 5 mM and 10 mM Ba^{++} concentrations as well as 79 mM K^{+} concentrations. The results of these experiments are given in Table 10. There was no response other than that of the expected diffusion potential to either of the various concentrations of Ba^{++} or the high K^{+}.

Resistance and Mass of the External Muscle Layer

This set of experiments provided us with an opportunity to determine the average resistance and the average mass of the external muscle layer for Rana pipiens. Neither of these values

has before appeared in the literature. At the end of each experiment the external muscle layer was carefully excised from our chamber with a #11 blade and weighed. The resistance of each external muscle layer had been measured throughout the course of our normal protocol. The results of these measurements are recorded in Table 10. Keeping in mind that the frogs in this series all weighed from 100 to 200 grams, it was felt that if a fair correlation of resistance in ohms per gram for this tissue could be obtained, this might be accurate across a broader spectrum of specimens. This calculation was in fact carried out and the results included in Table 11. The data show that the resistance of the external muscle layer is about one-third of that for the mucosa in terms of ohms cm^2.

The Anomalous Membrane

One of the "scraped" mucosae deserves special mention. It was the first one we did and in preparing it by scraping off the single cell epithelial layer we did not do as complete a job as we did on all the others in this group. Instead of scraping until all the yellow of the epithelial layer was done and a glistening, white, tough, fibrous membrane was visible, we scraped evenly across the stomach removing some of the characteristic yellow coloring of the intact mucosa, nevertheless leaving a somewhat dull, yellow membrane facing us. When the stomach was placed in the chamber its resistance though lower than that which we usually see for the intact

gastric mucosa (49 ohm cm^2) was nevertheless higher than the other scraped mucosae in this series. We went through our protocol finding the expected negligible changes in PD and resistance.

With time, the resistance of the membrane began to climb. With a keen interest in what was happening we left the membrane in the chamber, continued to perfuse it with standard solutions (solution 1, Table 1). Over the next 12 hours the resistance of this membrane was seen to climb from 49 ohms cm^2 to slightly better than 150 ohms cm^2, which is in the normal range for an intact secreting gastric mucosa. The PD over this time changed but little from minus 1.5 mv to plus 1 mv. However, at the end of this time when the membrane was pulsed with the 79 mM K^+ solution a 6 mv dip in PD was noted. At no time in any of our other experiments with scraped mucosa or external muscle layers did we observe a response of this magnitude. This experiment is singled out at this point because of the implication for in vivo regeneration of the incompletely destroyed gastric cells. The constraints of time prevented us from investigating this phenomenon further but this experiment appeared so unique that we thought it worthy of special note.

High K^+ Pulses in 1 mM Ba^{++}

Next the time course of resistance and PD changes of the nutrient membrane when exposed to 79 mM K^+ nutrient pulses, in the presence of 1 mM Ba^{++} were looked at. The experiment followed exactly the earlier protocol of 79 K^+ pulses (solution 6, Table 1)

of 30 seconds, 60 seconds, 2 minutes, 10 minutes from a baseline 4 mM K^+ solution (solution 2, Table 1), only this time the experiments were performed in the presence of 1 mM Ba^{++}. Figure 14 illustrates a typical experiment from this group. The reversal of the 1 mM Ba^{++} effect by 79 mM K^+ is seen from these figures to be extremely rapid. In shorter time length pulses the effect of 79 mM K^+ on the nutrient membrane is often maximum shortly after removal of high K^+ from the bathing media (Figure 15). This effect is felt to be due to the concentration of K^+ at the nutrient membrane which if it is rapidly increasing at the time of drainage of the chamber will continue to increase for some transient period after it is replaced with a 4 mM K^+ solution until the concentration profile in the diffusion barrier is reversed by the new solution.

Two approaches were used to analyze resistance and PD changes in response to 79 mM K^+ -- both analogous to the 79 mM K^+ pulses in the absence of Ba^{++}. First, tables were made analyzing the discrete 30 second, 60 second, 2 minute and 10 minute pulses, the analogous tables drawn up to analyze the 30 second, 60 second, 2 minute and 10 minute point of the 10 minute pulses. These tables contain the resistance change, the PD change, the calculated G_K and the calculated K^+ concentration at the nutrient membrane in response to these pulses. (See Tables 12, 13, 14 and 15). The implication will be treated in the discussion.

Looking at resistance recovery several phenomena become obvious. First, shorter pulse length tends to result in a more

FIGURE 14.

PD and resistance changes of a representative stomach for 79 mM K^+ pulses of varying duration in the presence of 1 mM Ba^{++}.

Represents the change in resistance and PD of the gastric mucosa when step changes of 4 mM to 79 mM K^+ bathing media were made on the nutrient side. One mM Ba^{++} was present in all nutrient fluids throughout the experiment. The pulses were of 30 second, 60 second, 2 minute and 10 minute duration.

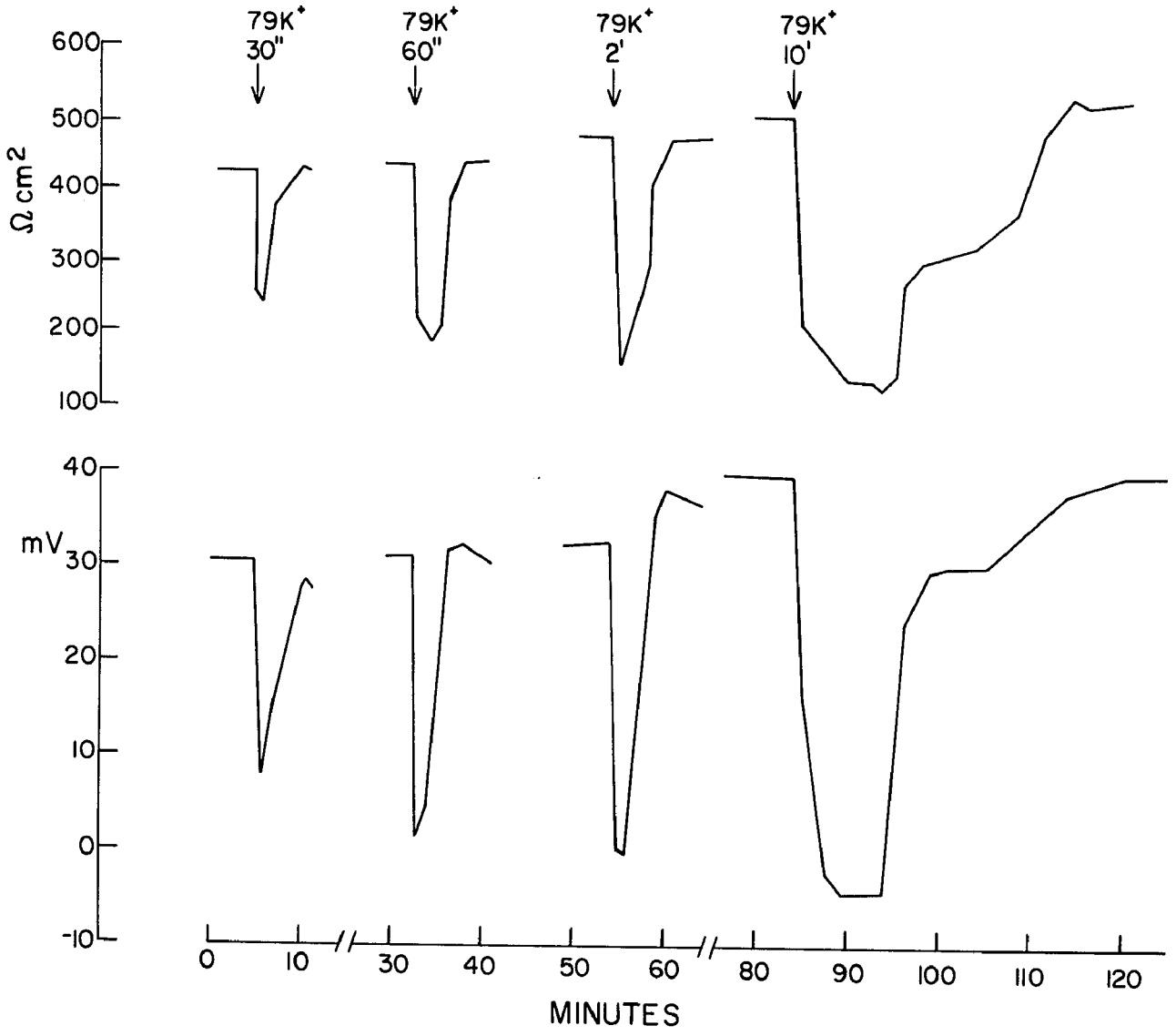
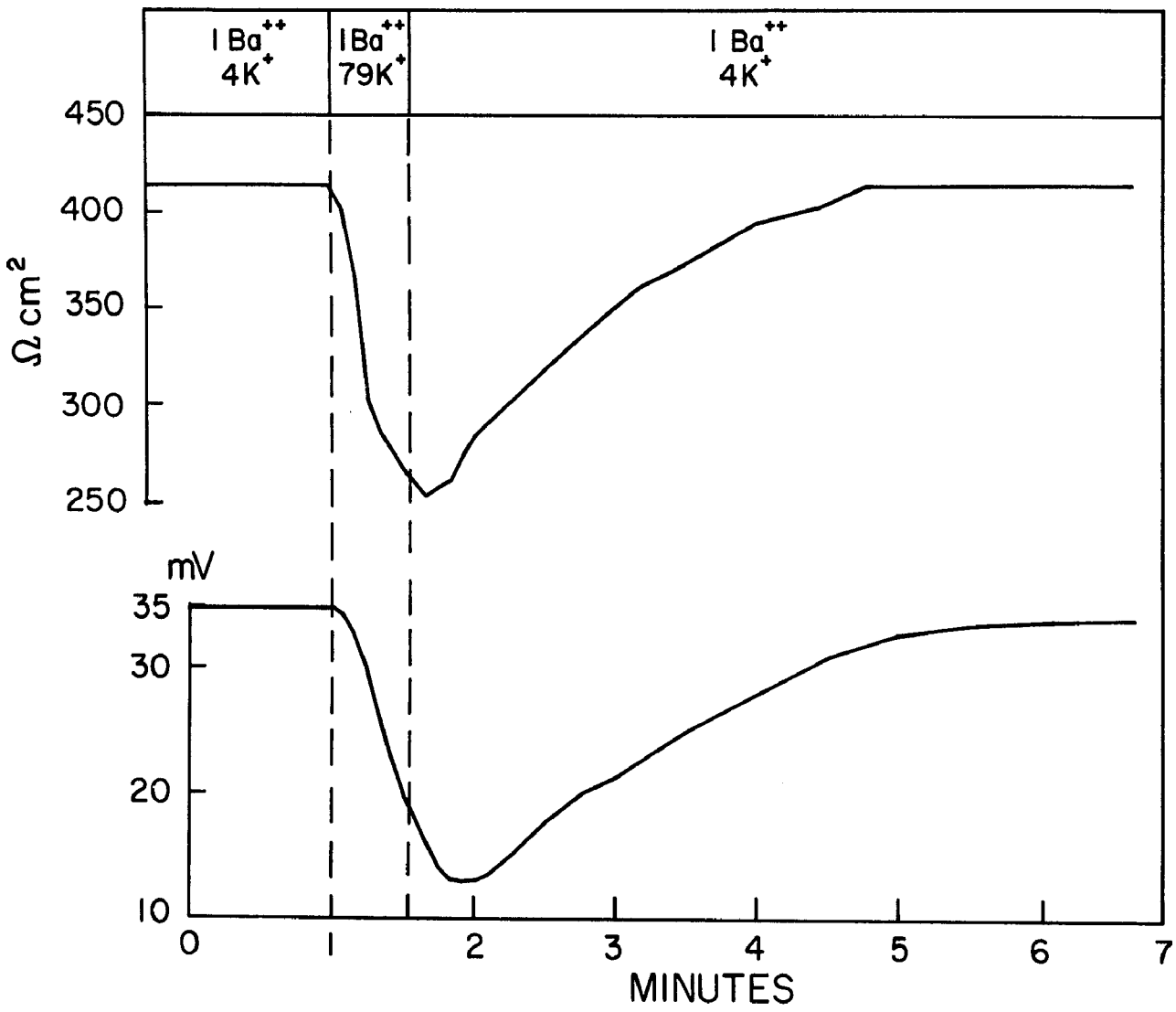

79K+
30"
79K+
60"
79K+
2'
79K+
10'
600
500
400
300
200
100
Ω cm2
40
30
20
10
0
-10
mV
0
10
30
40
50
60
80
90
100
110
120
MINUTES

FIGURE 15.

Time course of PD and resistance changes of a gastric mucosa due to a 79 mM K^+ pulse in the presence of Ba^{++}.

These curves represent the time course of PD and resistance changes of a gastric mucosa when pulsed from the nutrient side with a 79 mM K^+ - 1 mM Ba^{++} solution. Note that the nadir of PD resistance is reached after removal of the high K^+ containing solution from the nutrient chamber. This is shown to illustrate the effects of the K^+ concentration profile in the nutrient diffusion barrier resultant from short time length high K^+ solution in the nutrient chamber as discussed in the text.


1 Ba++
4K+
1Ba++
79K+
1 Ba++
4K+
450
400
350
300
250
Ω cm²
mV
35
30
20
10
0
1
2
3
4
5
6
7
MINUTES

rapid resistance recovery after the removal of 79 mM K^+, while longer pulses appear to have a much slower recovery rate (as illustrated in Figure 14). Second, there is a rapid phase of resistance recovery in Ba^{++} after a 79 high K^+ pulse that is not present in the absence of Ba^{++}. When Ba^{++} is not present the resistance appears to recover at a more even rate and with a much more gradual slope than in the presence of Ba^{++}. It seems reasonable to conclude that the rapid phase of resistance recovery present in Ba^{++} is due to the effect of Ba^{++} on the membrane. For convenience figures are presented comparing resistance recovery from the exactly analogous experiments described earlier (cf. Tables 4 to 7) in the absence of Ba^{++} (Figure 16) with these high K^+ pulses in the presence of Ba^{++} (Figure 17). Figure 16 represents percent resistance recovery of the gastric membrane after a 79 mM K^+ (solution 6, Table 1) pulse in regular chloride Ringer's (solution 2, Table 1) plotted against time; Figure 17 represents percent resistance recovery after a 79 mM K^+ pulse in a 1 mM Ba^{++} Ringer's (solution 18, Table 3) plotted against time. Zero is taken as the resistance at the time the chamber is drained. One hundred percent recovery is taken at the time the chamber is drained. One hundred percent recovery is taken as the time at which the resistance levels off at a new baseline. Each line on the graphs of figures 16 and 17 represents the average recovery of the six experiments. These data demonstrate the more gradual slope in the absence of Ba^{++} as opposed to the tendency towards rapid rise in the presence of Ba^{++}. The

FIGURE 16.

Resistance recovery after 79 mM K^+ nutrient media exposure for varying durations.

Represents the percent recovery of resistance vs time following return to 4 mM K^+ from 79 mM K^+ pulses of 30 seconds, 60 seconds, 2 minutes and 10 minutes. Each line represents the average of six experimental pulses. Zero percent recovery is taken as resistance at the time of 79 mM K^+ removal, 100% recovery is taken as the maximal resistance at which the membrane stabilized after each pulse.

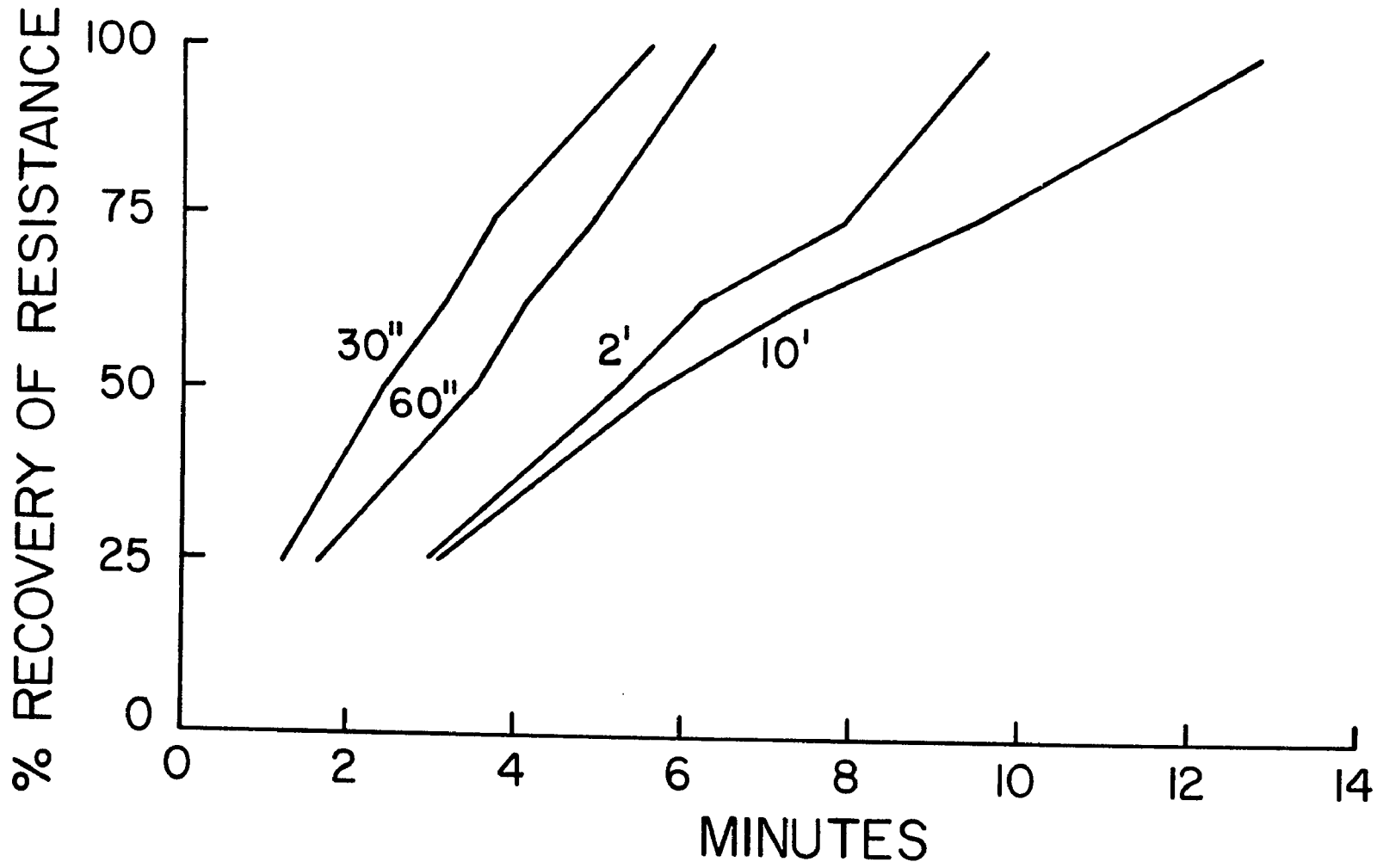
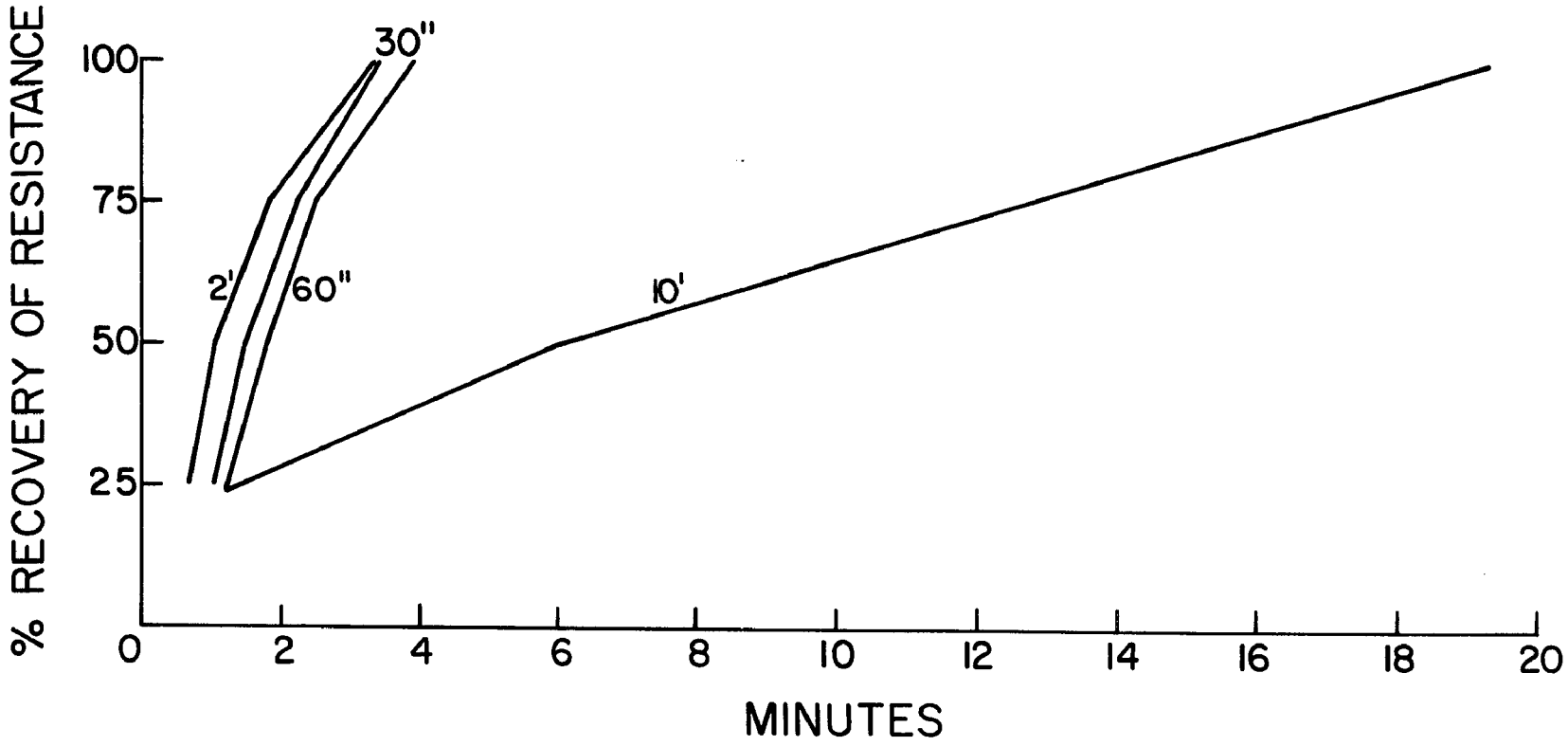

% RECOVERY OF RESISTANCE
100
75
50
25
0
30"
60"
2'
10'
0
2
4
6
8
10
12
14
MINUTES

FIGURE 17.

Resistance recovery after 79 mM K^+ nutrient media exposure for varying durations in the presence of 1 mM Ba^{++}.

This figure is analogous to Figure 16 except that 1 mM Ba^{++} is present in the nutrient solution. The percent recovery of resistance vs time following return to 4 mM K^+ from 79 mM K^+ pulses of 30 seconds, 60 seconds, 2 minutes and 10 minutes. Each line represents the average of six experimental pulses. Zero percent recovery is taken as resistance at the time of 79 mM K^+ removal, 100% recovery is taken as the maximal resistance at which the membrane stabilized after each pulse.


% RECOVERY OF RESISTANCE
100
75
50
25
0
2
4
6
8
10
12
14
16
18
20
MINUTES
30"
2'
60"
10'

10 minute pulse in each series takes longer to reverse than the pulses of shorter duration; while the 30 second, 60 second and 2 minute pulses in the presence of Ba^{++} tend to group and demonstrate a very fast recovery -- faster than in the absence of Ba^{++}, the 10 minute pulse in the presence of Ba^{++} appears to take longer to recover than that under any other conditions measured. The possible significance of these curves will be discussed when we formulate a hypothesis for mechanism of Ba^{++} action.

The Sulfate Wash

The effect of 1 mM Ba^{++} in the nutrient solution may be reversed in other ways than by the administration of high K^{+}. It is possible to drain the nutrient chamber, wash it well with regular 4 mM K^{+} solution (solution 2, Table 1) and thereafter with repeated washings over 15-30 minute time period the Ba^{++} effect may be seen to be reversed. With knowledge of the rapid reversal in 79 mM K^{+} and knowledge of this slow decline with washing in 4 mM K^{+} zero Ba^{++} nutrient (solution 2, Table 1) the question arose: would a wash-out attempt with a solution containing sulfate reverse the effect of Ba^{++} more rapidly? Since sulfate has a high affinity for Ba^{++} and $BaSO_4$ is an extremely insoluble compound, looking at the reversal rate of the Ba^{++} effect by the use of a nutrient fluid containing sulfate (solution 20, Table 3) might give a significant clue to the mode of action of this ion on the nutrient membrane. It seemed reasonable to believe that the Ba^{++} necessary to produce its effect

on resistance might be below threshold concentration once a sulfate-containing solution was used as nutrient bathing media. Five mM sulfate was substituted directly for Cl^- in the nutrient media for these experiments. No attempt was made to compensate for what was felt to be a negligible osmotic difference since in other work from this laboratory it has been found that small changes in osmotic pressure produce essentially no effects on the PD, resistance and H^+ rate.

Figure 18 shows the rate of reversal of the Ba^{++} effect with respect to time in the absence and in the presence of sulfate. Barium was in 1 mM concentration, sulfate was in 5 mM concentration. Considering a residual Ba^{++} solution in the chamber of much less than 1 cc and a new 5 mM sulfate nutrient solution in volume exceeding 10 cc, there should have been adequate sulfate to precipitate all the Ba^{++} present. Figure 18 clearly illustrates that the presence of 5 sulfate does not lead to a quicker reversal of the Ba^{++} effect.

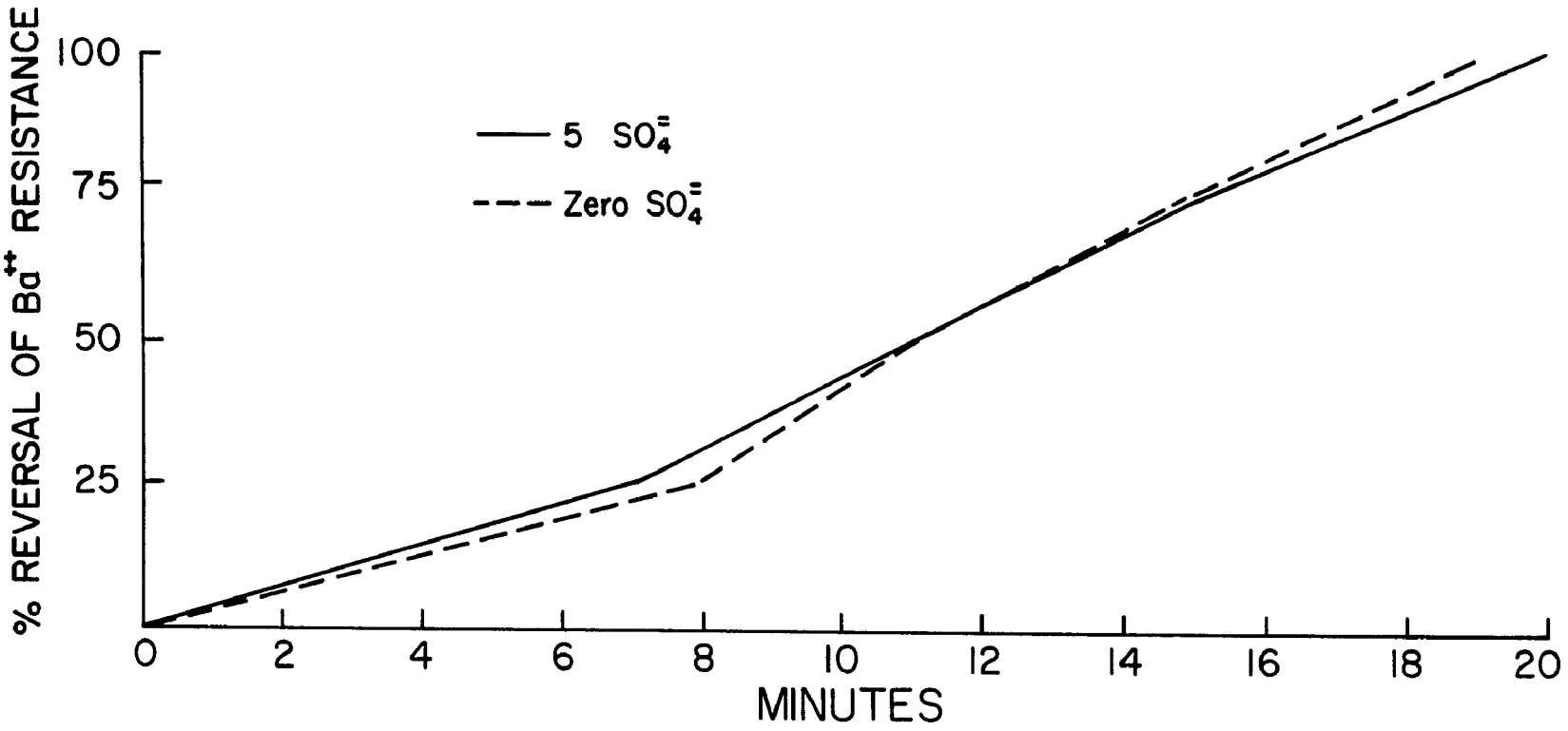
The Pursuit

Pursuing this line of thought further, the question was asked whether there be a Ba^{++} effect if an amount of Ba^{++} sufficient to make a final concentration of 1 mM were precipitated in a 5 mM sulfate nutrient solution (solution 18, Table 3) before addition to the nutrient chamber. Further, it seemed logical to ask whether if there were an effect in the presence of 5 mM sulfate, if such an

FIGURE 18.

Time course of reversal of resistance due to Ba^{++} in absence and presence of sulfate.

These curves represent the time course of percent reversal of elevated gastric resistance after 1 mM Ba^{++} nutrient solution (solution 18, Table 3) was washed from the nutrient chamber. The solid line depicts the reversal as effected by repeated washes with solution 18, Table 3, containing 4 mM K^{+} 5 mM SO_4. The broken line represents the reversal as effected by repeated washes with solution 2, Table 1, containing 4 mM K^{+}, zero SO_4. Each line is an average of six gastric mucosae. The Standard Errors for these curves were small.


% REVERSAL OF Ba^{++} RESISTANCE
100
75
50
25
0
2
4
6
8
10
12
14
16
18
20
MINUTES
5 $SO_4^=$
Zero $SO_4^=$

effect could be reversed by mass action through increasing the sulfate concentration of the nutrient bathing media from 5 mM to 20 mM. In making up a suitable 20 mM solution (solution 22, Table 3) sucrose was used to account for osmotic deficit.

Six experiments were carried out in which a 1 mM Ba^{++}, 5 mM sulfate cloudy precipitate solution was added to the gastric mucosa. In each case a Ba^{++} effect was observed but in each case this effect was of much smaller magnitude than a 1 mM Ba^{++} effect when the Ba^{++} was added to regular nutrient Ringer's (solution 2, Table 1). In each of these six mucosae, the precipitated solution of 1 mM Ba^{++}, 20 mM sulfate was tried in the nutrient chamber. No Ba^{++} effect was observed. Figure 19 is representative of these experiments, demonstrating the effect of 1 mM Ba^{++} in the absence of sulfate, in the presence of 5 mM sulfate, and in the presence of 20 mM sulfate. After each exposure to Ba^{++} its effect on the membrane was completely reversed by washing with regular Cl^{-} Ringer's (solution 2, Table 1) and the membrane allowed to stabilize before being re-exposed.

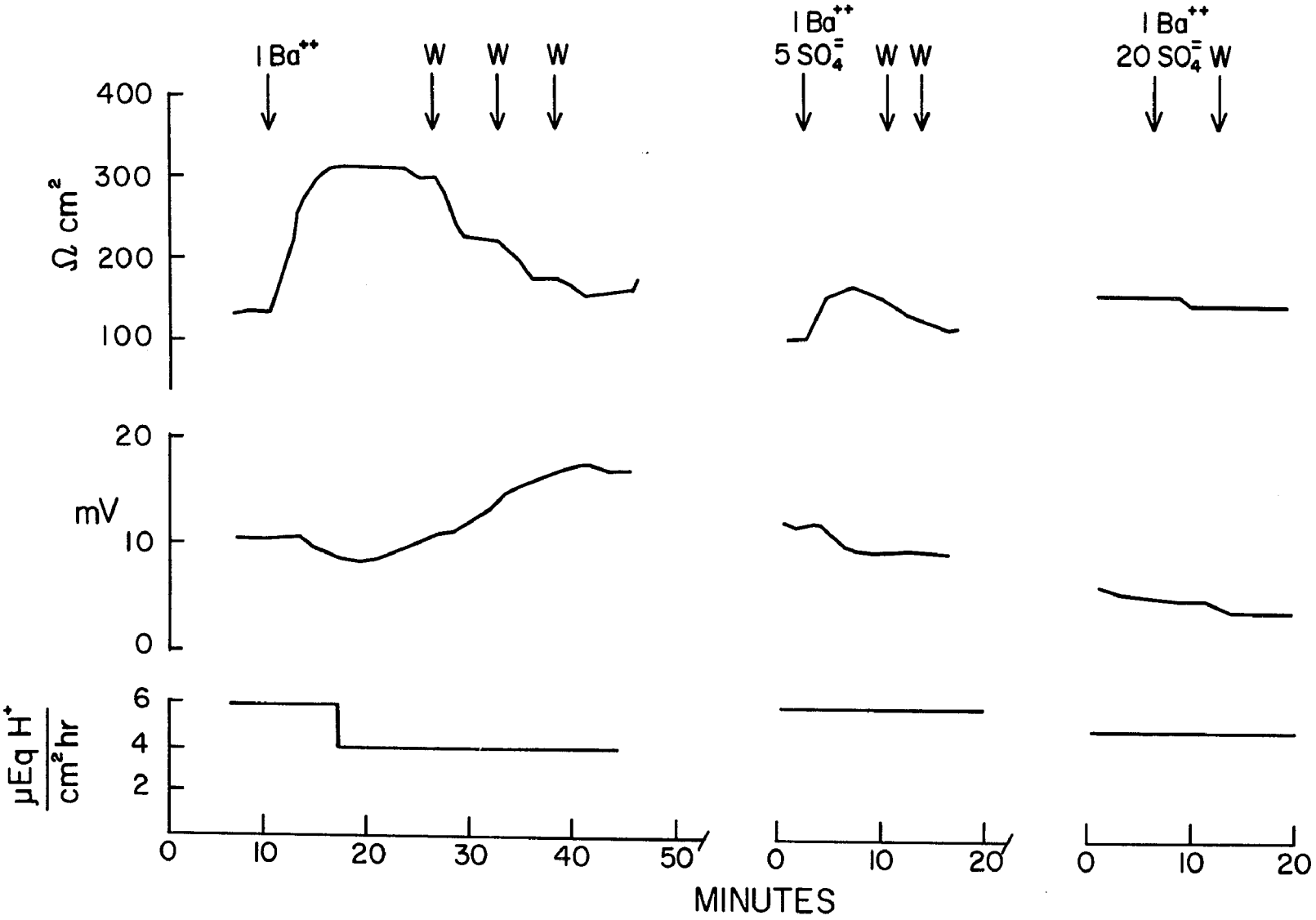
The No-Dwell Experiments

It usually takes from 40 to 100 seconds for the effect of 1 mM Ba^{++} to become obvious in terms of a resistance change, zero time being taken as the point in which 1 mM Ba^{++} nutrient Ringer's was placed on that side. Another series of experiments was performed in which the Ba^{++} solution was in contact with the

FIGURE 19.

The effect of $BaSO_4$ nutrient solutions.

These curves show the effect of adding solutions containing 1 mM Ba Cl_2 to the nutrient chamber on PD and resistance and H^+ rate of the gastric mucosa. The baseline is obtained in the standard solution 2, Table 1. The first curve represents addition of a similar solution with 1 mM Ba^{++} (solution 24, Table 3); the second curve represents a solution containing 5 mM SO_4 plus 1 mM Ba^{++} (solution 18, Table 3); the last curve represents a solution containing 20 mM SO_4 and 1 mM Ba^{++} (solution 21, Table 3). Each represents washing the nutrient side of the membrane three times by draining its solution and refilling the chamber with the appropriate bathing media.


1 Ba++
W
W
W
1 Ba++
5 SO4=
W
W
1 Ba++
20 SO4=
W
400
300
200
100
Ω cm²
20
10
0
mV
6
4
2
μEq H+ / cm²hr
0
10
20
30
40
50
0
10
20
0
10
20
MINUTES

membrane for less than 30 seconds duration. Several experiments of extremely short-dwell times were performed in which the nutrient chamber was drained of its regular nutrient Ringer's (solution 2, Table 1). A few ml of a 1 mM Ba^{++} Ringer's (solution 25, Table 3) was then poured by the mucosa and allowed to drain out the open bottom of the nutrient chamber. The nutrient membrane was immediately and copiously washed with the regular nutrient Ringer's (solution 2, Table 1). These extremely short-dwell times could have represented Ba^{++} contact with the nutrient side of no more than 3 to 5 seconds. The design of these experiments was such that the experimenter was able to look for presence or absence of a Ba^{++} effect after all the Ba^{++} had ostensibly been removed from the chamber. In each case a Ba^{++} effect was found. In the case of the extremely short-dwell times, Ba^{++} effect was much attenuated, resembling that in many ways observed for the precipitated 5 mM sulfate solution.

Instantanicity

To help answer the question as to whether or not the Ba^{++} effect was instantaneous once the Ba^{++} crossed the nutrient diffusion barrier and reached the nutrient membrane, a number of experiments were designed in which the time of onset and Ba^{++} effect as caused by a 1 mM Ba^{++} concentration were compared with time of onset and Ba^{++} effect as caused by 10 mM Ba^{++} concentration. The 10 mM Ba^{++} solution for these experiments was prepared by

simply adding enough $BaCl_2$ to solution 2, Table 1, to bring the Ba^{++} final concentration to 10 mM. No compensation was made for the osmotic change. The 1 mM Ba^{++} solution was solution 25, Table 3.

It can be predicted that if the diffusion time across the nutrient barrier were relatively long with respect to the onset of Ba^{++} effect once it made contact with the membrane (i.e., that the Ba^{++} effect is relatively instantaneous) that increasing the Ba^{++} concentration would appreciably shorten the onset time of Ba^{++} effect. On the other hand, were the time of diffusion across the barrier relatively short compared to the time necessary for initiation of the Ba^{++} effect once the ion reached the membrane, then not much difference in time of onset would be expected. Figure 20 compares the 1 mM and 10 mM Ba^{++} concentrations. Each line represents the average of 10 experiments and it can be seen that the lag time was less for 10 mM Ba^{++} than for 1 mM Ba^{++}.

The Long Time 10 mM Ba^{++} Effect

In performing the last set of experiments a biphasic effect of 10 mM Ba^{++} on the gastric mucosa was observed. The short term effect or phase I effect of 10 mM Ba^{++} resembles in every aspect the effect of a 1 mM Ba^{++} solution. The resistance is raised and the secretory rate remains high. When 10 mM Ba^{++} solution is left in contact with the nutrient membrane for much longer periods of time a second phase of the Ba^{++} effect is seen (see Figure 21). During phase II the PD falls towards zero, the resistance of the

FIGURE 20.

Effect of elevating Ba^{++} concentrations with respect to onset and magnitude of resistance elevation.

These curves show the time course of increasing resistance of the gastric mucosa when the nutrient chamber is filled with 1 mM Ba^{++} vs 10 mM Ba^{++} containing bathing media. Zero time is taken as the time of addition of fluid to the nutrient chamber. Each curve represents the average response in Δ ohms of six membranes to such changes.

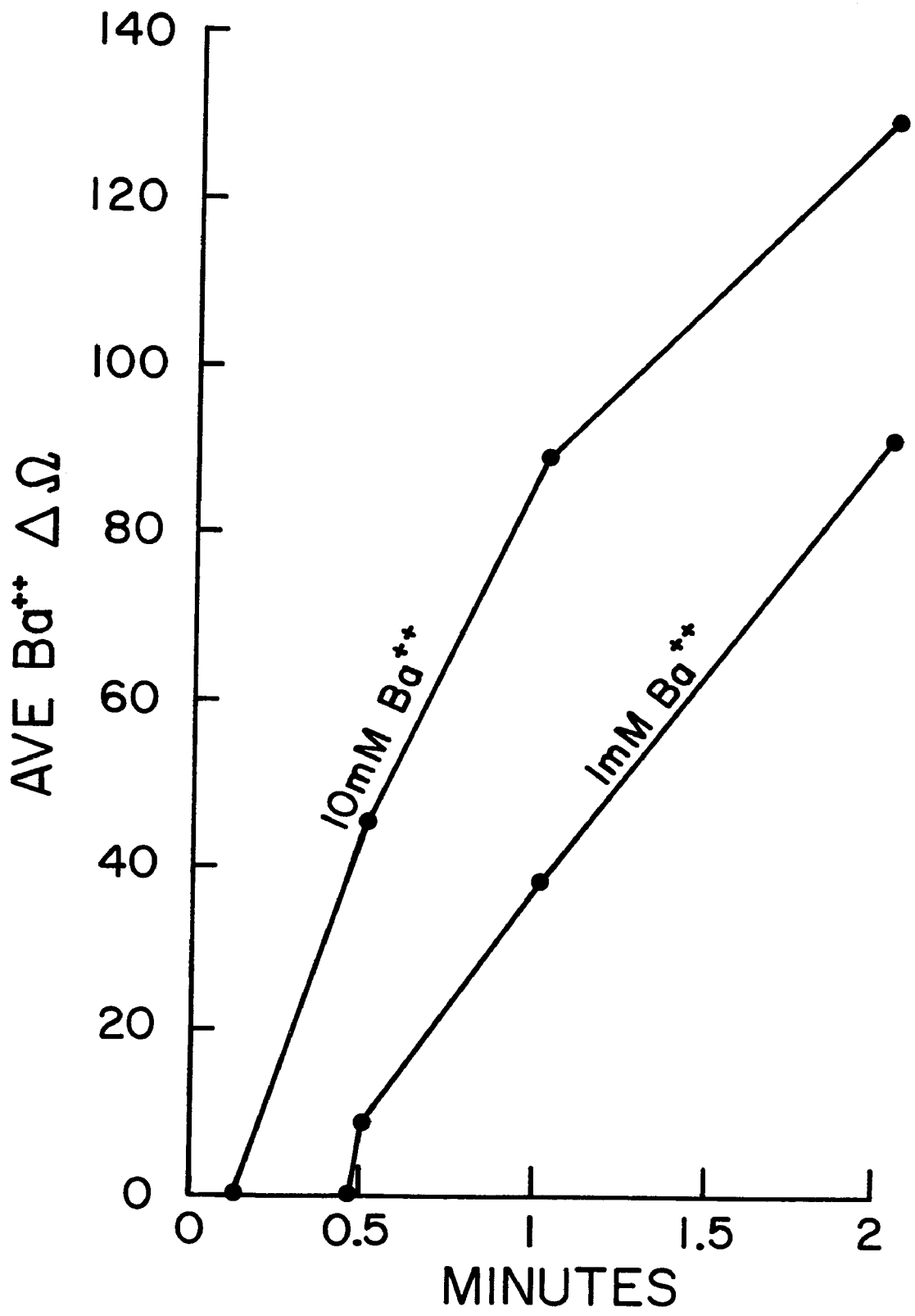
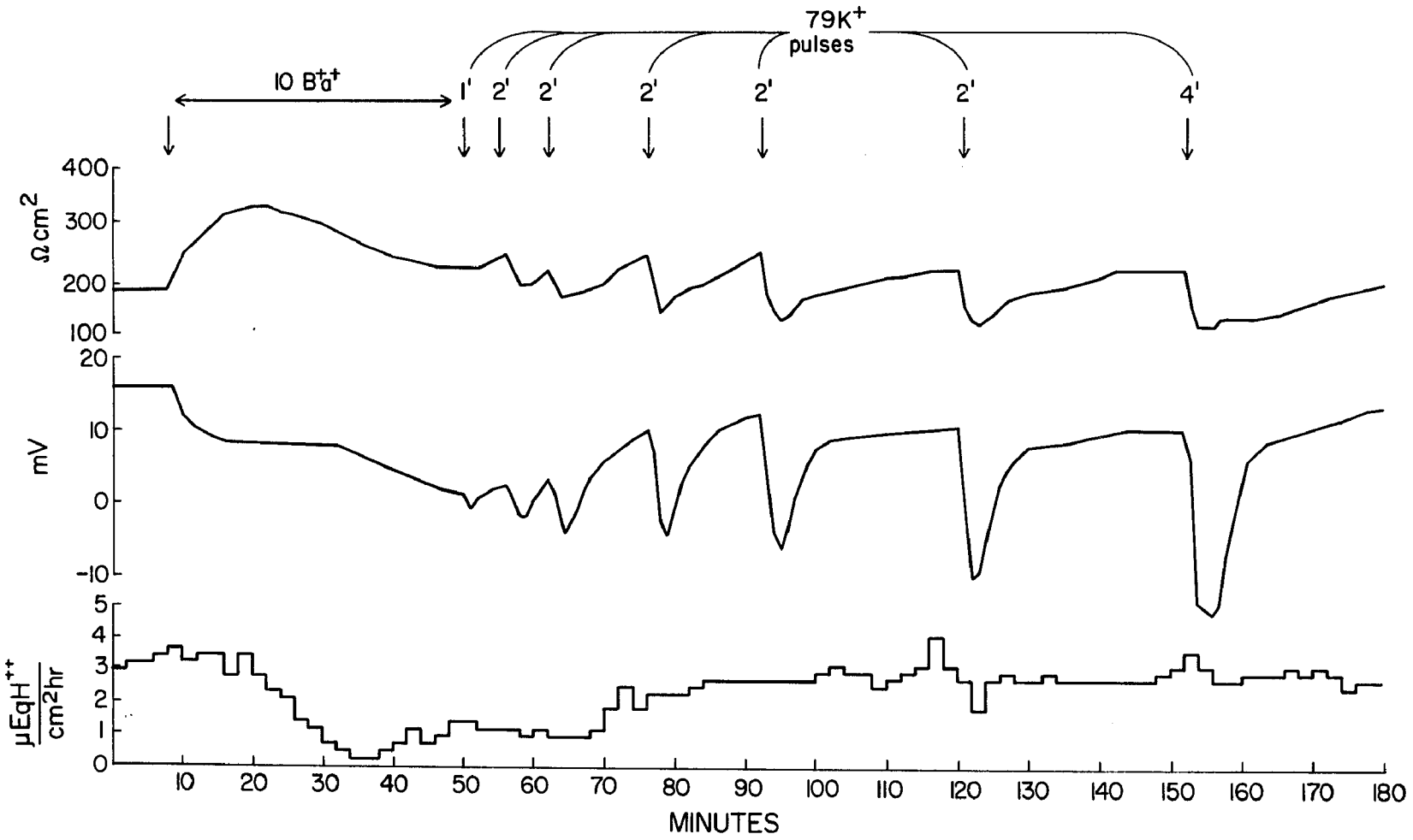

140
120
100
80
60
40
20
0
AVE Ba++ Δ Ω
10mM Ba++
1mM Ba++
0
0.5
1
1.5
2
MINUTES

FIGURE 21.

The effect of 10 mM Ba^{++} on PD, resistance and H^{+} rate.

This is a plot of the resistance, PD and H^{+} rate of a frog gastric mucosa which is exposed to 10 mM Ba^{++} containing nutrient solution (solution 2, Table 1 with 1 cc of 0.1 mM $BaCl_2$ per 10 cc total solution) for 42 minutes. This effect is then reversed by repeated pulses of the nutrient membrane by 79 mM K^{+} (solution 6, Table 1) solutions of the indicated duration. Between pulses the standard solution 2 of Table 1 was maintained in the nutrient chamber. Note the reversibility of this effect of high concentration Ba^{++} as described in the text.


79K+ pulses
10 Ba++
1'
2'
2'
2'
2'
2'
4'
400
300
200
100
Ω cm2
20
10
0
-10
mV
5
4
3
2
1
0
μEqH++/cm2hr
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
MINUTES

membrane decreases markedly and the H^+ rate goes to zero. Startling to us were other characteristics of this 10 mM long term Ba^{++}. The addition of 79 mM K^+ to the nutrient bathing media was very slow in bringing about any change in PD or resistance. The long term high concentration Ba^{++} effect was still reversible by 79 mM K^+ but took a much more extended time period than did the reversal of the 1 mM Ba^{++} effect. Further, if the 79 mM K^+ solution (solution 6, Table 1) were removed from the nutrient chamber during the process of reversal of this phase II effect, one could see evidence that the mucosa was returning to its normal characteristics by passing back through phase I of the Ba^{++} effect; that is, the resistance would recover to a level higher than baseline, the PD would slowly climb back toward its normal value and the H^+ rate would slowly recover.

When the membrane had recovered from the phase II Ba^{++} effect through the phase I effect it could be restored to its pristine state of PD, resistance and H^+ rate simply by drainage of the high K^+ solution and replacement with regular Ringer's (solution 2, Table 1). Figure 21 illustrates the long term 10 mM Ba^{++} effect on the nutrient membrane and its reversal through the phase I effect by 79 mM K^+ pulses (solution 6, Table 1).

The Bounce Experiments

It was now clear that reversal of Ba^{++} by high K^+ solution for the long term phase II effect could lead to a higher than baseline resistance (equivalent to the normal Ba^{++} or phase I

effect) after removal of 79 mM K^+ from the nutrient chamber. This raised the question as to whether the reversal of the usual phase I Ba^{++} effect might not be followed under certain conditions by a return to a high Ba^{++}-like resistance. Accordingly, a number of experiments was designed so that the membrane was exposed to Ba^{++} for one minute. At the end of this one minute period while the Ba^{++} effect was growing in magnitude, the nutrient chamber was drained and filled with 79 mM K^+ nutrient (solution 6, Table 1). As soon as the Ba^{++} effect was seen to be reversed -- reversal being defined as reduction of the membrane resistance to a lower than pre-barium baseline level -- the 79 mM K^+ solution was drained from the chamber and replaced by regular nutrient (solution 2, Table 1). Under these conditions the resistance would climb to greater than baseline values and simulate the recovery of a Ba^{++} effect. If such "bounce" experiments were performed in the face of higher than 1 mM concentrations of Ba^{++}, even more "bounces" might be obtained. Figure 22 depicts such an experiment.

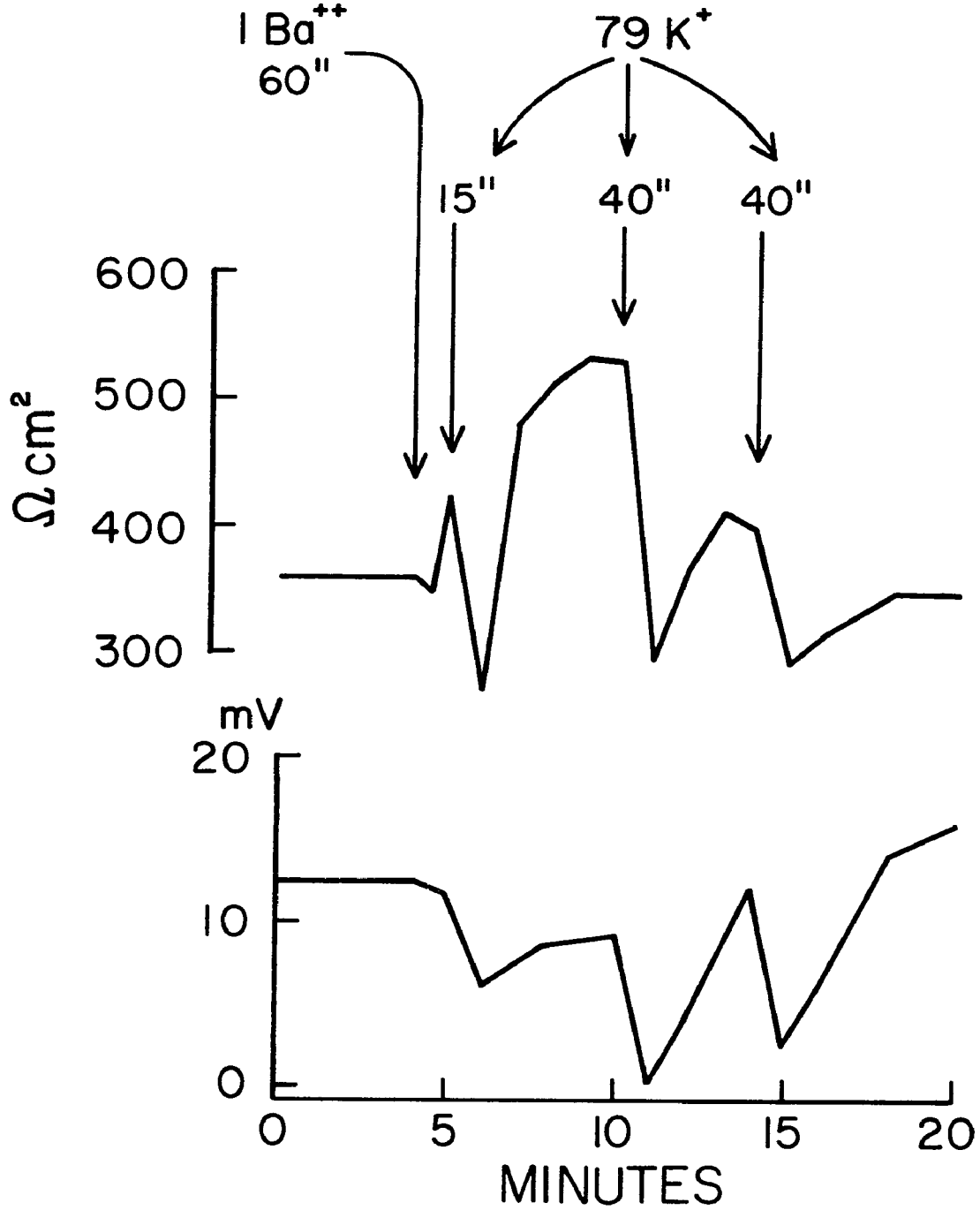
Ba^{++} - No Cl^-

Apropos to the problem at hand is the question of whether or not a Ba^{++} effect might be generated in the absence of Cl^-. To answer this a series of six experiments was performed in which a membrane was set up with our normal solutions (solutions 1 and 2, Table 1), stimulated with histamine and allowed to stabilize. The effect of 1 mM Ba^{++} on the nutrient was then observed. After the

FIGURE 22.

A representative "bounce" experiment.

These curves show the PD and resistance change with time of a gastric mucosa when exposed with a 1 mM Ba^{++} containing nutrient solution for the times indicated. The solution in the chamber between pulses was the standard 4 mM K nutrient (solution 2, Table 1). Note that each 79 M pulse was sufficient in length to reverse the Ba^{++} generated resistance effect below pre-Ba^{++} baseline levels. There is recovery of the Ba^{++} effect after removal of the 79 mM K^{+} solution.


1 Ba++
60"
79 K+
15"
40"
40"
600
500
400
300
Ω cm²
mV
20
10
0
0
5
10
15
20
MINUTES

reversal of this effect by washing (solution 2, Table 1) a change was made to zero Cl^-, Ringer's (solution 23, Table 3) and secretory (solution 16, Table 3). Chloride was replaced by isethionate. The mucosa was repeatedly washed with the isethionate solutions. After stabilization in the absence of Cl^-, with characteristic increased resistance, PD reversal and decreased secretory rate, the nutrient side was pulsed with 1 mM Ba^{++} for 4 minutes. A Ba^{++} effect was observed. A representative effect of Ba^{++} in the presence and absence of Cl^- on the same membrane is shown in Figure 23. Interestingly enough, when the addition of Ba^{++} to the nutrient chamber was made in the form of small aliquots sufficient to raise the final concentration by an increment of less than 0.5 mM almost no Ba^{++} effect would be seen, even though the total Ba^{++} concentration in the nutrient fluid resultant from such aliquots would be 1 mM. This series of experiments was somewhat unhappy in that resistance as measured in the absence of Cl^- would often increase and decrease in wave-like fashion. This may have been accounted for by a change in the peristaltic-like activity of the stomach but, to be truthful, the genesis of this effect is not entirely clear.

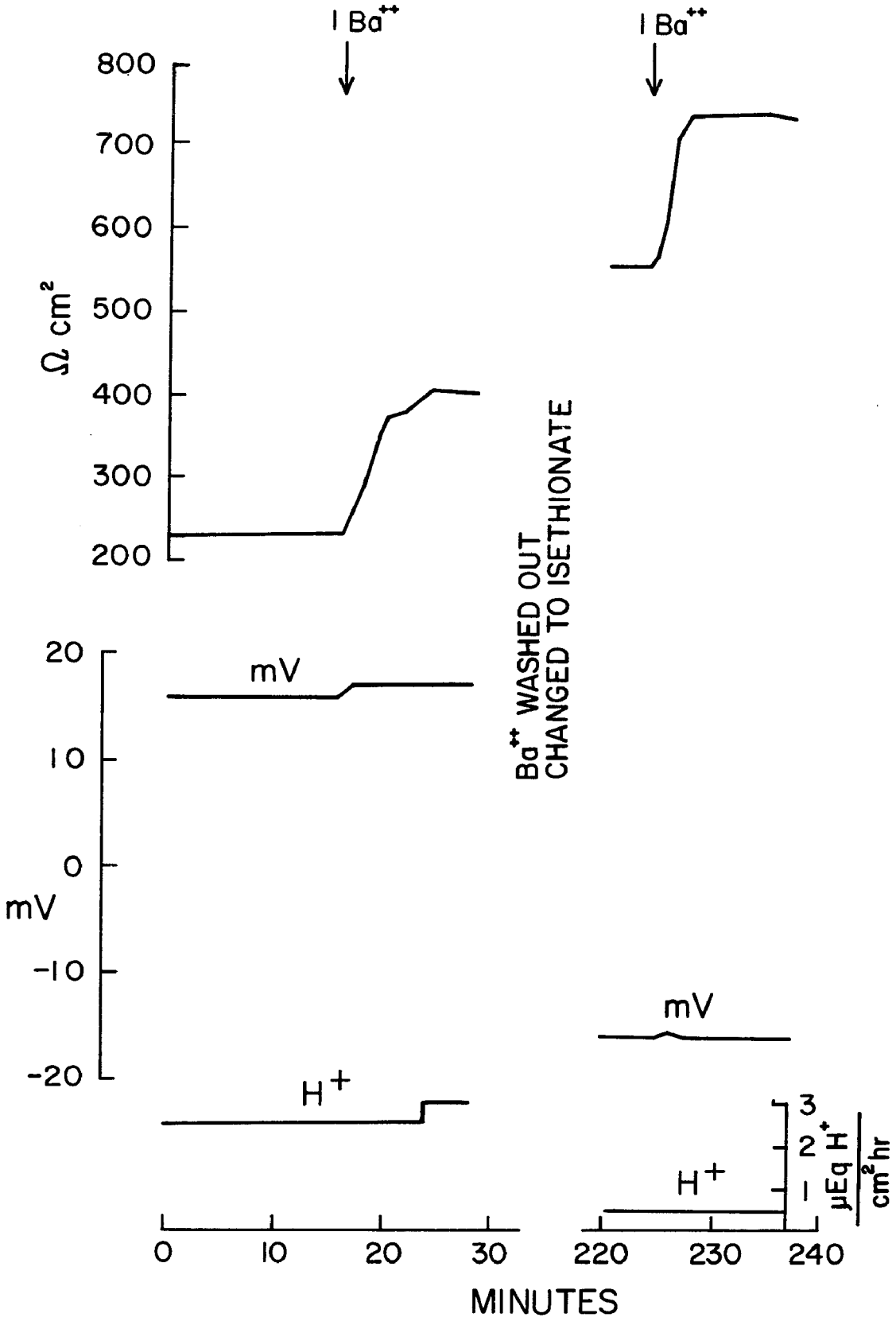
The Ba^{++} Effect Short Circuited

If an equivalent circuit were set up for the secretory and nutrient membranes of the gastric surface epithelium on the basis of what is known from past research (Rehm, 1966) and the work on

FIGURE 23.

Ba^{++} effect in the absence of Cl^-.

These curves are taken from a representative experiment in which a control 1 mM Ba^{++} effect is obtained. All Cl^- and Ba^{++} were then removed from the bathing media of both chambers and replaced with a Cl^--free media (isethionate for Cl^-). After stabilization of the mucosa at the predicted higher resistance, inverted PD and low H^+ rate, 1 mM Ba^{++} was again added to the nutrient chamber. This experiment indicates the presence of a Ba^{++} resistance effect in the absence of a nutrient membrane Cl^- conductance limb (for equivalent circuit see the "sulfate" curve of Figure 1).


1 Ba++
1 Ba++
800
700
600
500
400
300
200
Ω cm²
Ba++ WASHED OUT
CHANGED TO ISETHIONATE
20
10
0
-10
-20
mV
mV
mV
H+
H+
3
2
1
μEq H+
cm² hr
0
10
20
30
220
230
240
MINUTES

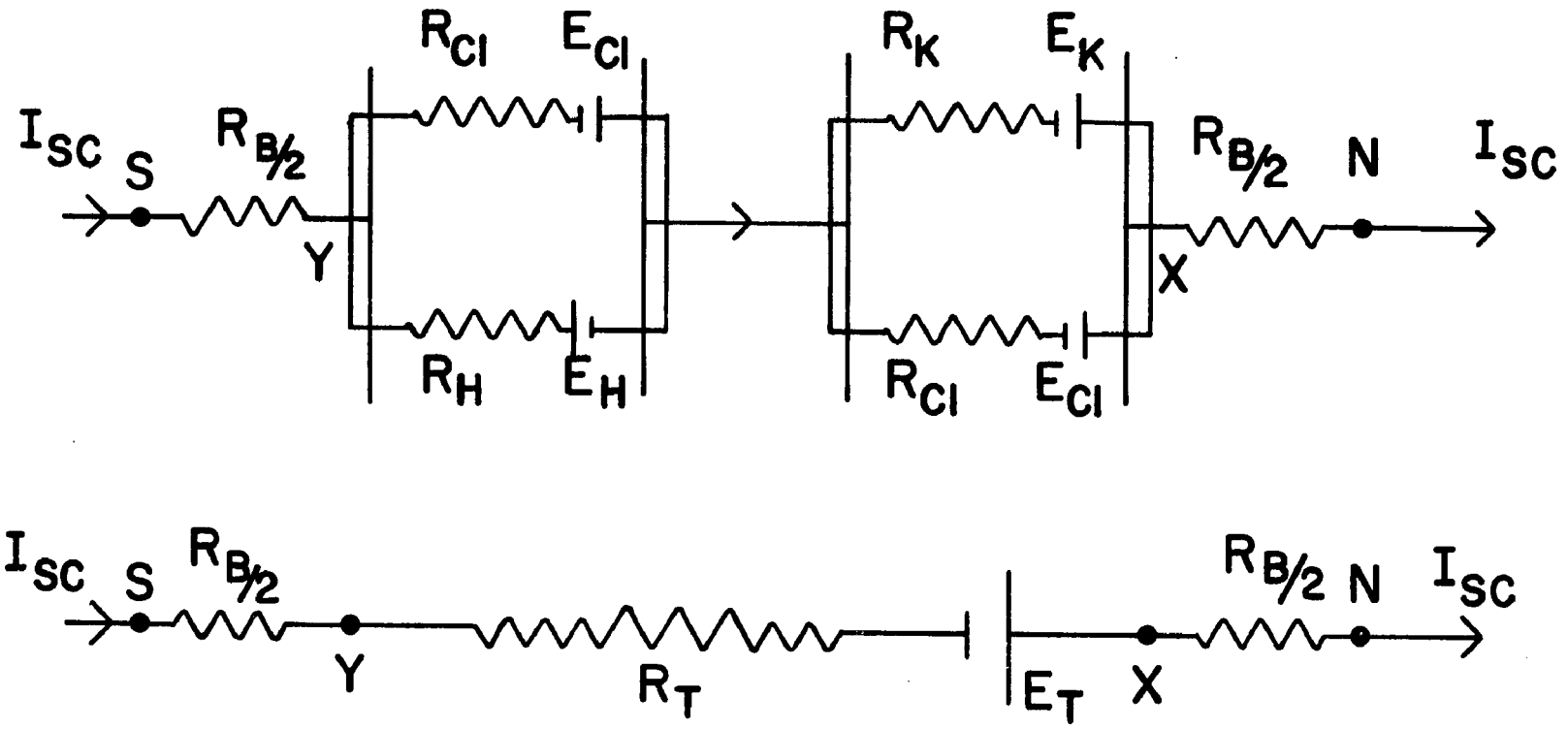
permeability of the nutrient membrane presented earlier in this dissertation, that circuit would be represented by the diagram in Figure 24. Examination of this figure shows an electrogenic Cl^- and H^+ mechanism in the secretory membrane and conductance channels for K^+ and Cl^- in the nutrient membrane. Simple analysis of this circuit shows that under steady state short circuit conditions no current would pass through the K^+ limb of the nutrient membrane; all of the current would pass via the Cl^- limb. If current did pass through this limb, since no new K^+ could get into the cell through the secretory membrane, intracellular K^+ concentration would decrease until there were no more K^+ ions left for possible transport through this limb. If the site and mechanism of Ba^{++} action were limited to the K^+ conductance channel, no decrease in the short circuit current would be predicted. If the Ba^{++} effect involved the Cl^- limb as well, one might predict a decrease in short circuit current under the influence of Ba^{++}. With this in mind another series of six experiments was devised. In this series of experiments the Honeywell Dual Channel Recorder was used which gave the advantage of simultaneous recording of short circuit i_{sc} with PD together with superimposed current for resistance measurement and checks of the open circuit PD. Two battery powered current sources were used in this system. The reader is referred to Figure 5. One of these current sources was used to impose a short circuit current; the other current source was used to change the current by superimposing a square wave of 20 microamps current

FIGURE 24.

Equivalent circuits for short circuiting frog gastric mucosa.

These are equivalent circuits for conductive pathways of the nutrient and secretory membranes of the frog gastric mucosa. I_{SC} represents short circuit current which was sent in the direction of the arrow. The point labelled S represents the secretory probe electrode position and the point labelled N the nutrient probe electrode position. Y and X represent the secretory and nutrient membrane surface. $R_B/2$ measured by each probe electrode E, R, Cl, K and H have their usual significance.

In the lower circuit the emf's (E's) and resistance (R's) of the first circuit are combined for simplicity into an equivalent PD and resistance for the tissue denoted by E_T and R_T, respectively. The other characters have the same meaning as in the circuit above.


R_{Cl}
E_{Cl}
R_K
E_K
I_{SC}
S
$R_{B/2}$
$R_{B/2}$
N
I_{SC}
Y
X
R_H
E_H
R_{Cl}
E_{Cl}
I_{SC}
S
$R_{B/2}$
$R_{B/2}$
N
I_{SC}
Y
R_T
E_T
X

for periods slightly greater than one-half second, first in one direction then in the other, to measure the resistance of the membrane under these conditions.

In the design of these experiments the distinction is made between bringing the PD between S and N and between X and Y of Figure 24 to zero. When a blank resistance of the chamber is a negligible part of the tissue resistance, as in the case of the frog skin where tissue resistance is high, then this distinction does not have much significance. In the frog stomach there is an example of a relatively low resistance tissue. Blank resistance for our chamber is an observed 32 ohms. The chamber takes 1.3 cm^2 membrane area. Thus this figure converts to 41.6 ohm cm^2, a significant portion of the total observed resistance. This cannot be neglected in determining the appropriate short circuit current. An equivalent circuit for our chamber and membrane is represented in Figure 24 where S and N represent the secretory and nutrient probe electrodes. The blank resistance of the chamber (R_B) is divided by two because one-half of the blank resistance will be on each side of the membrane. Y and X are points immediately adjacent to the secretory and nutrient facing membranes respectively. R_T represents the resistance of the tissue and E_T the open circuit PD of the gastric mucosa. From this it can be seen that

$$V_{NS} = V_{NY} + V_{YX} + V_{XS} \tag{16}$$

where the V's represent PD's and the subscripts are defined by

reference to Figure 24. Reducing V_{XY} to 0 through the short circuit technique we can write

$$V_{NS} = -R_B \, i_{sc} = -32 \, i_{sc} \qquad (17)$$

where i_{sc} indicates the short circuit current and 32 represents R_B. On the basis of this:

$$i_{sc} = -32 \, V_{NS} \qquad (18)$$

A table was made to determine the point at which the potentiometer reading should be held for each increment in short circuit current at significant intervals of 0.5 mv. Table 16 represents the values used for these experiments.

Figure 25 is a representative one for these short-circuiting experiments. The acid rate remained fairly stable throughout these experiments, although there is the suggestion that the rate might be slightly decreasing during the time of short-circuiting.

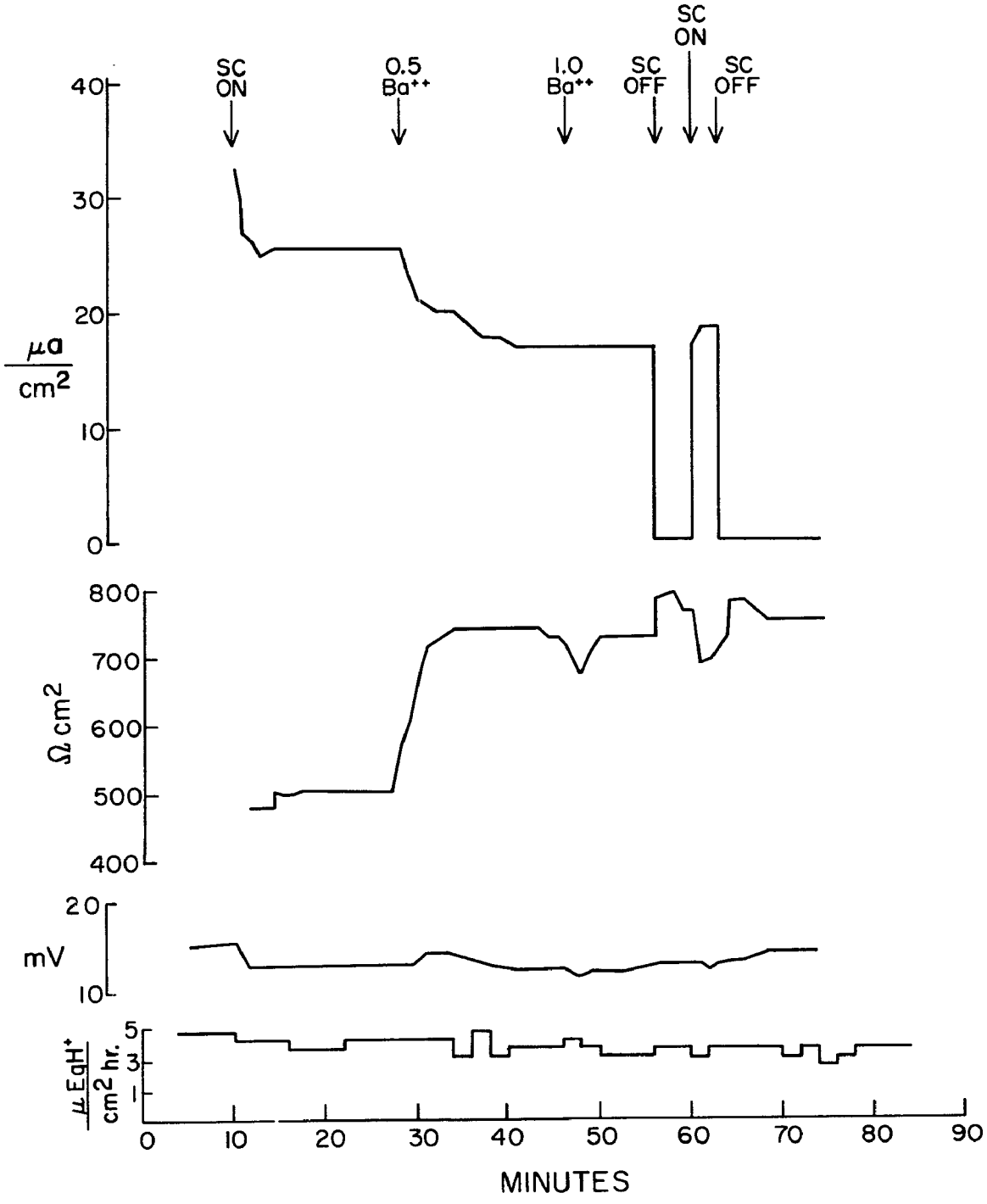
Upon application of the short-circuiting there is a rapid fall in the magnitude of the short-circuit current. This rapid decrease in short-circuit current can be explained on the basis of a model proposed by Kidder and Rehm (1970). After stabilization of the short-circuit current $BaCl_2$ is added to the nutrient solution to bring its final concentration to 0.5 mM Ba^{++}. The resistance of the membrane is seen to rise showing a typical Ba^{++} effect and the short-circuit current decreases. In this particular experiment a full fledged Ba^{++} effect appears to have been obtained with 0.5 mM Ba^{++} and no further increase in resistance or decrease in short-circuit current is obtained upon increasing the concentration

FIGURE 25.

Ba^{++} effect under short circuit conditions.

This is a plot of an experiment in which after baseline stabilization a frog gastric mucosa was short circuited. The short circuit current, resistance, PD and H^+ rate are shown. The initial drop in short circuit current is typical for this type of experiment. Significant is the decrease in short circuit current precipitated by addition of Ba^{++} after a steady state has been reached. Of significance also the square wave-like rise of the short circuit current in the presence of Ba^{++}. Since under steady state short circuit conditions all current must pass through the Cl limb of the nutrient conductance pathways as discussed in the text and illustrated in Figure 4. This is a clear demonstration of a Ba^{++} effect on the Cl^- conductance mechanism of the nutrient membrane.

(SC in this figure stands for short circuit current).


SC ON
0.5 Ba++
1.0 Ba++
SC OFF
SC ON
SC OFF
40
30
20
10
0
μa/cm2
800
700
600
500
400
Ω cm2
20
10
mV
5
3
1
μEqH+/cm2 hr.
0
10
20
30
40
50
60
70
80
90
MINUTES

Ba^{++} in the nutrient fluid to 1 mM. In other experiments of this series, the short circuit was seen to fall further and the resistance increase when the final concentration of the nutrient solution was raised to 1 mM Ba^{++}.

To summarize the effects of Ba^{++} under short circuit conditions, for six experiments the mean initial short circuit current (I_{sc}) was 46.2 μa (± SD 8.9), the mean steady state I_{sc} was 38.6 μa (± SD 10.4) the mean I_{sc} with 0.5 mM Ba^{++} was 32.0 μa (± SD 11.9) and the mean I_{sc} with 1 mM Ba^{++} was found to be 24.5 μa (± SD 7.3). This evidence makes it difficult to explain the Ba^{++} effect entirely on the basis that the only action of Ba^{++} is to increase the resistance of the K^{+} conductive pathway of the nutrient membrane. It appears that the resistance of the Cl^{-} limb of the nutrient membrane is also increased.

DISCUSSION

Traditionally workers in biophysics have been interested in the passage of ions across biological membranes. The rate of passage, and the mechanism of transport are important goals in the field. The present work is set out to define more clearly the mode of ion passage across the nutrient membrane of the gastric mucosa. Since the movement of Cl^- across this membrane and the appearance of bicarbonate in the nutrient bathing media are part of the total process of gastric secretion, to discover the exact mechanism by which this movement occurs becomes an important question. This dissertation attempts to show that the preceding experimental results clearly point to a neutral Cl^--bicarbonate -- "whirling dervish" type of exchange. In an attack on this problem the partial ionic conductances of the nutrient membrane to H^+, OH^- and bicarbonate were determined. Experiments have been performed which enable one to determine the minimum resistance of the nutrient membrane and calculations show that the sum of the absolute conductances of the nutrient membrane to H^+, OH^- and bicarbonate is essentially zero. Finally, attempt will be made to define the mechanism of Ba^{++} action more precisely and then use the Ba^{++} effect to establish a neutral mechanism (or mechanisms) the end result of which is a Cl^--bicarbonate exchange.

Partial Ionic Conductance for H^+, OH^- and Bicarbonate

The Need to Know

Earlier it was noted that the work of Spangler and Rehm (1968) is evidence that

$$G_N = G'_K + G'_{Cl} \tag{18}$$

where it be recalled G_N is the total conductance of the nutrient membrane and G'_K and G'_{Cl} are the conductance of the K^+ and Cl^- limbs. They found that a step change in K^+ and Cl^- concentrations with the product of their concentrations held constant resulted in a change in the magnitude of the PD per unit log of either the K^+ or Cl^- of about 56 (SD ± 6.3) mv. This is not significantly different from the theoretical value of 58 mv. for a membrane with conductive mechanisms for K^+ and Cl^- alone. Although the above findings clearly establish that there are conductive pathways for K^+ and Cl^-, these data as will be seen in the following analysis are insufficient to eliminate the possibility of conductive channels for other ions. The data presented under Results indicate that the sum of the partial ionic conductance of the nutrient membrane for H^+, OH^- and bicarbonate is essentially zero. In spite of this appearance, it is clear that only a small amount of H^+ ion would actually have to cross the nutrient membrane at the physiologic pH before a new electrochemical equilibrium is reached for the system. The above allegation will become clearer upon examination of a model which we will now consider (Figure 13). The essential problem is whether the change in PD occurring after altering H^+ concentration

in the bathing media might occur so fast that it might be completely overlooked giving the false impression of a zero conductance for this ion.

The Scrutiny

Figure 13 depicts the model in which a membrane has conductive channels for K^+, Cl^-, and H^+ and is impermeable to all other ions. With such a model a limiting membrane could have a much higher H^+ conductance relative to the K^+ and Cl^- conductances. To illustrate this latter point one can assume that side A of Figure 13 represents a small volume of fluid comparable to the intracellular volume. Side B represents a large volume comparable to the extracellular fluid. It is easily shown that the PD between A and B (V_{BA}) is given by:

$$V_{BA} = \frac{G_H E_H + G'_K E_K + G'_{Cl} E_{Cl}}{G_H + G'_K + G'_{Cl}} \qquad (19)$$

where the G's and E's refer to conductances and emf's and the meaning of the subscripts is self evident. With initial ten-fold concentration ratios of the ions in our model, all emf's would initially equal 58 mv (2.3 RT/F = 58 mv). Since a change in H^+ concentration must always be associated with a simultaneous change in OH^- concentration, a channel for OH^- could have been used in place of the H^+ channel and a high OH^- conductance and zero H^+ conductance could have been assumed, or it could have been assumed that the conductances for both the H^+ and OH^- were high. However, for simplicity one can let the membrane have a zero OH^- conductance.

In developing equation 19, it was assumed that the E's are determined by the Nernst equation

$$E_i = \frac{RT}{F} \ln \frac{(C_A)_i}{(C_B)_i} \qquad (20)$$

where the symbols have been previously defined. For the purpose of illustration it is assumed that the (G')'s of equation 19 are independent of concentrations. One may assume that G_H is much greater than G'_K or G'_{Cl}, and for purpose of illustration, that $G_H = 20\ G'_K$ and $G'_K = G'_{Cl}$. Then equation 19 becomes

$$V_{BA} = \frac{20\ E_H + E_K + E_{Cl}}{22} \qquad (21)$$

Now changing the H^+ concentration on side B ten-fold (i.e. from 10^{-7} M to 10^{-6} M) would result in a reduction of E_H to zero and V_{BA} from equation 21 would then change from 58 mv to 5.5 mv. E_K and E_{Cl} would still be equal to 58 mv and with E_H now zero, K^+ would move from A to B, while Cl^- would move from B to A, as H^+ move from B to A. These movements would continue until all the emf's were again equal. Following the step change it is assumed that compartment B is large enough compared to compartment A so that concentrations in compartment B remain essentially constant. The H^+ concentration in compartment A would increase to approximately ten times its old concentration, i.e., from 10^{-6} M to 10^{-5} M. The large concentration of K^+ in compartment A would poise the system so that the initial step change in PD would be followed by an exponential-like recovery as illustrated in Figure 13 to a level very close to the original one. If one were not aware of this

possibility the temporary change in PD might easily be overlooked and one might conclude that the relative H^+ conductance is essentially zero. Changes in the K^+ and Cl^- concentrations (with their product held constant) would result in a change in PD (ignoring the time course of the response with a slope of 58 mv per ten-fold change in the concentrations of K^+ or Cl^- so that one might conclude that the total conductance of the membrane is equal to the sum of the K^+ and Cl^- conductances. Obviously then the duration of the temporary change in PD across this membrane is of importance in assessing the relative ionic conductances. Without a buffer in the system very little H^+ would have to be transported to change the concentration in compartment A from 10^{-6} M to 10^{-5} M. A step change in compartment B to a fluid with the same H^+, but different Cl^- and K^+ concentrations (but the product of K^+ and Cl^- held constant) would result in a small initial change in PD followed by an exponential-like change to a new level determined largely by the K^+ gradient. Without buffer the PD could rapidly reach its new study state and on a condensed time scale a plot of the PD response would appear almost like a step change.

The Rebuttal

In view of the model just discussed it is obviously important to determine the time course of the PD response to changes in nutrient fluid.

The duration of the PD response to changes in nutrient pH would be a function of the volume of compartment A, the amount of

buffer in compartment A and the magnitude of the partial ionic conductances of the nutrient membrane.

For purposes of illustration one can assume that the H^+ ion conductance is substantially greater than that of the Cl^- and K^+ conductances. Specifically assumption can be made that the H^+ conductance is twenty times the sum of Cl^- and K^+ conductances. Then the resistance of the loop linking the H^+ limb with the K^+ and Cl^- limbs would be about twenty-one times the resistance across the nutrient membrane. The resistance across the nutrient membrane as will be discussed in detail in the next section is at least 85 ohm cm^2, hence the loop resistance is at least 21 x 85 or 1785 ohms for a square centimeter of tissue. Calculations with this loop resistance and a cell volume of 4×10^{-2} cm^3 per cm^2 show that with a bicarbonate-CO_2 buffer system it would take well over a minute for the H^+ ion concentration in compartment A to increase to a new electrochemical equilibrium. Therefore we should early observe a change in PD if there is a H^+ conductance channel. In fact detailed calculations (Rehm et al., 1973) reveal that if the H^+ conductance were as low as 5% of the total conductance of the nutrient membrane an easily observable PD change should be seen in response to the H^+ change in nutrient bathing media.

A similar situation would be found for a model in which the membrane had a high OH^- conductance. These considerations are not necessary in discussing bicarbonate because it is present in much higher concentrations.

pH vs PD

It might appear that simultaneous changes in pH and ion concentrations could generate canceling emf's. Consideration of Figure 13 and the changes made as illustrated in Table 8 would orient all H^+, OH^- and bicarbonate emf's in the direction in these experiments so that they would be additive.

The K^+ Relationships

Perigee Nutrient Resistance

If it could be demonstrated that the action of a high concentration of K^+ is upon the nutrient membrane, then it follows that the amount of the decrease in resistance of the nutrient membrane represents a minimum value for the resistance of this membrane by the principle of NEMO DAT QUOD NON HABET. It has been established (Rehm, 1972) that the effect of high K^+ in lowering the transmembrane mucosal PD and resistance could not be from action at the intracellular tight junction.

One must now face the problem of whether the rapidity of the change in resistance and PD when the nutrient chamber is filled with high K^+ solution establishes the nutrient membrane as its site of action or whether sufficient concentration of K^+ could build up in the continuous phase of the cell to cause changes at the secretory membrane.

Assuming what might be considered realistic ion concentrations for this system, intracellular Cl^- concentration of 40 mEq/liter

(Davenport and Alzamora, 1962; Hogben, 1955; Villegas, 1962) and intracellular K^+ concentration of 8 mEq/liter (Davis et al., 1965; Harris and Edelman, 1964) the maximum emf of the K^+ limb with 79 mM K^+ in the nutrient fluid would be from the Nernst equation:

$$E_K = 58 \log \frac{79}{8} = 58 \text{ mv} \tag{22}$$

while that of the Cl^- limb would be

$$E_{Cl} = 58 \log \frac{80}{40} = 18 \text{ mv} \tag{23}$$

This gives a loop emf of 76 mv or an initial loop current of

$$I = \frac{76 \text{ mv}}{340 \text{ ohm cm}^2} = \frac{0.22 \text{ ma}}{\text{cm}^2} \tag{24}$$

where the 340 ohm cm^2 loop resistance will be established later. Using a reasonable intracellular volume of 4×10^{-2} cm^3 per cm^2 of mucosa, the rate of buildup of K^+ would be approximately .06 mM/second. Looking at Tables 6, 7, 14 and 15 it becomes apparent that most of the PD change and practically all the resistance change seen in response to a 79 mM K^+ occurs in the first 30 seconds. This would allow an intracellular buildup of K^+ of no more than 1.7 mEq/liter, hardly enough to justify the postulate that the effect is on the secretory membrane. Although this does not eliminate the possibility of high concentrations of K^+ triggering a messenger mechanism located in the nutrient membrane which would cause an effect at the secretory membrane, one would be forced to postulate a messenger system with a short latent period which could explain the time course of the change in PD and resistance. It should be

recalled the detailed time course for changes of K^+, Cl^- and product constant experiments are predicted on the basis of the simple hypothesis of Spangler and Rehm (1968) and to postulate a messenger system which could duplicate these quantitative effects becomes almost ludicrous. On the basis of the above arguments, we conclude that the nutrient membrane is the site at which high K^+ lowers the resistance and PD of the gastric mucosa.

The K^+ Time Constant

In the last section the expected diffusion time of K^+ in the nutrient diffusion barrier was referred to as part of the evidence against a messenger mediated K^+ effect on the secretory membrane. Throughout this dissertation a time constant of one minute has been used for diffusion of ions across the nutrient diffusion barrier. This figure has been assumed as one previously established (Spangler and Rehm, 1968). Now it would seem appropriate to check the accuracy of our tables against each other and use these data as an independent check on the accuracy of this one minute figure. To say that the time constant for the diffusion of an ion to the nutrient membrane is one minute means that if we place a 79 mM K^+ solution in the nutrient bathing media then at the end of one minute the concentration at the nutrient membrane would be i.e. 63% of 79 mM. The simple calculations as carried out in Table 10 the calculated average concentration of K^+ at the end of one minute's time is 46 mEq/liter and in Table 11 - 50 mEq/liter. Furthermore, to cross check in the other direction, the average change in PD as recorded

in Table 6 is 34.60 mv at the end of a minute, as opposed to 33.67 mv at the end of a minute in Table 7. If these calculations are interpolated to Figure 11, Table 6 would lead one to predict the PD of -17 mv, while Table 7 would lead one to predict the PD of -16 mv at the end of a one minute 79 mM K^+ pulse if the estimate of the K^+ diffusion time constant is correct. Looking at the graph one can see that at the exact concentration of one time constant we find a PD of -16 mv. One should also be able to double check this independently through measurements of resistance at the end of one minute's time. Figure 7 shows that a nutrient concentration of 49 mM K^+ should be reflected in an average gastric membrane resistance of 103 ohm cm^2. Tables 4 and 5 show actual 60 second pulse average resistance of 105 and 103 ohm cm^2 respectively. This close agreement supports both the accuracy of the one minute time constant and the internal consistency of the experiments.

The Promised Determination

Having argued that the immediate effect of high K^+ on PD and resistance is on the nutrient membrane, it is obvious that the decrease in resistance produced in this membrane by high K^+ pulses represents the minimum resistance value that this membrane could have. Simple subtraction of values from Figure 7 put the perigee at approximately 90 ohm cm^2 while consideration of Tables 4 and 5 indicate it could be as low as 85 ohm cm^2. Looking back at the schema in Figure 4 it is obvious that maximum current flow across this membrane will occur when $R_K = R'C_1$ -- i.e. when loop resistance

is at a minimum. Assuming the lower 85 ohm cm^2 figure for nutrient membrane resistance (R_N) according to the relation

$$\frac{1}{R_N} = \frac{1}{R_K} + \frac{1}{R_{Cl}} \qquad (25)$$

the minimum loop resistance must be 340 ohm cm^2 (the value used in equation 24).

The Denouement

With the calculated one minute time constant for the nutrient diffusion barrier, the response of the PD resulting from a change in the concentration of an ion with significant conductance at the membrane would be about 4 minutes. As illustrated in Figure 12, curve A, more than one-half of the PD response to a five-fold K^+ change would occur within the first minute. From this one might infer that if there were no response in the first minute, any subsequent change could be attributed to changes in the parameters of the system. Looking at curve B of Figure 12, at constant pH a five-fold decrease in bicarbonate ion concentration of nutrient solution results in no such change. Similarly, neither does the reverse change represented in curve C of Figure 12. From the constancy of the PD it would appear that our conclusion of zero conductance for H^+, OH^- and bicarbonate is well documented.

The Audit

The mechanism of acid base balance of the gastric cells deserves attention. On basis of the work in this dissertation it is clear that acid base balance is maintained by a neutral

mechanism (or nutrient mechanism). Bicarbonate has been shown to appear in the nutrient medium in a one to one ratio for each H^+ secreted into the gastric lumen (Teorell, 1951; Davies, 1951). This does not in itself establish a neutral carrier exchange for Cl^--bicarbonate across the nutrient membrane. The cell could maintain its acid base balance by: 1) entrance of H^+ via a neutral mechanism together with Cl^- into the cell; 2) a neutral exchange between OH^- and Cl^-; or 3) a neutral exchange of bicarbonate with Cl^-. Intracellular production of lactate or pyruvate could neutralize the OH^- provided the lactate or pyruvate ion moved across the nutrient membrane per se. However, it is experimentally known that the rate of appearance of these ions in the bathing media is only a small fraction of the rate of hydrogen ion secretion (Davenport, 1943; Davies, 1951; Hogben, 1955).

The simplest hypothesis of the three mentioned would be neutral carrier exchange mechanism for bicarbonate and Cl^-. The relatively small concentrations of OH^- and H^+ in the cell and nutrient solutions make these latter two options less likely. It is of interest in this connection that subsequent studies of Harris and Pressman, 1967, Hoffman, 1962, and Hunter, 1967 demonstrate a neutral mechanism for Cl^--bicarbonate shifts in the red cells.

Ba^{++} -- the Site, the Mode, the Tool

The Site

There is now at hand good evidence that Ba^{++} acts on the

nutrient facing membrane of the epithelial layer of frog gastric mucosa. First, looking at the histology of the stomach, the smooth muscle and fibrous layers beneath the nutrient facing membrane are loosely knit. It would be hard to imagine from a histologic viewpoint this diffusion barrier being responsible for such high resistance. But even more so, our experiments with scraped mucosae which we feel are fairly representative of diffusion barrier and our experiments with external muscle layers, which again contain much smooth muscle, demonstrate very clearly that there is no Ba^{++} effect on the resistance of these layers. Neither is there seen to be any effect of high K^{+} on the resistance of these layers. If one places Ba^{++} on the secretory side of the membrane there is no rise in resistance (Sanders et al., 1973) which decreases the probability of Ba^{++} acting on the secretory membrane.

Occam's Razor

It could be argued that though a Ba^{++} effect occurs only when Ba^{++} is placed on the nutrient side of the membrane, yet the change in resistance seen could represent a change in the secretory membrane much in the fashion of an ADH (antidiuretic hormone) effect on tissues susceptible to this hormone, e.g., toad bladder and frog skin (Fuhrman and Ussing, 1951; Koeroed-Johnsen and Ussing, 1952). Like Ba^{++}, ADH acts only when placed on the nutrient side of the membrane. There does not appear to be much data in the literature in the way of experiments to define the minimum latent period for an ADH effect. However, from what is in the literature, this

period appears to be from 2 to 4 minutes as a minimum (Capraro and Bernini, 1952). The rapidity of onset of Ba^{++} effect when placed on the nutrient side make a messenger-mediated effect on the secretory membrane seem unlikely. In other words the possibility of the increase in resistance due to Ba^{++} being an increase in the resistance of the secretory membrane seems unlikely. Further, the effects of Ba^{++} are rapidly reversed by 79 mM K^{+} with the fall in resistance being seen often within seconds. We know from the calculation presented earlier in this paper that K^{+} concentration would not be expected to build up in the cell over this short time period.

Postulation of a messenger-mediated Ba^{++} effect on the secretory membrane with this speed and the almost instantaneous reversal by high K^{+} practically eliminates a messenger hypothesis from serious consideration.

The Antagonists

Over and over the reversal of Ba^{++} effect by high nutrient K^{+}, even high K^{+} on the secretory side, when left for a time, has been seen to cause a two-thirds decrease in Ba^{++} resistance (Sanders and Rehm, 1970). Thus K^{+} appears to reverse the effects of Ba^{++} from inside as well as outside the cell. From experiments performed and described earlier in this dissertation, high concentrations of Ba^{++} are less readily reversed by K^{+}. Ten mM Ba^{++} is much more slowly reversed than 1 mM Ba^{++} concentrations. The shorter the exposure time of the nutrient membrane to Ba^{++} the

more quickly its effect appears to be reversed. When 10 mM Ba^{++} is left for a long time at the nutrient surface, reversal with 79 mM K^+ may take 30 minutes. From Tables 4, 5, 12 and 13 resistances of stomachs with Ba^{++} do not appear to reach quite the nadir that stomachs without Ba^{++} reached for the same K^+ concentration. Changes in potential difference and calculated G_K's in Tables 6, 7, 14 and 15 for experiments with Ba^{++} are also lower than for membranes with no Ba^{++} exposure. In most cases it will be noted that the resistances, PD's and calculated G_K's differ by 3 to 6 standard errors of the mean in each respective case. These data are further evidence in support of a Ba^{++}-K^+ antagonism.

Gastric Permeability to K^+

The cells constituting the gastric epithelium appear permeable to K^+ when this epithelium is exposed to bathing media containing K^+ in relatively high concentrations. As evidence for this the reader is reminded of the permeability calculations earlier in this paper, the K^+ conductance channels established by Spangler and Rehm (1968) as well as the aforementioned ability of high concentrations of K^+ on the secretory side to at least partially reverse the effect of Ba^{++} though taking a longer time than high nutrient K^+.

Looking at the experiments described earlier, specifically Figures 16 and 17, nutrient pulses of high K^+ concentration of longer duration require a much longer time for membrane resistance recovery to normal levels after restoration of usual 4 mM K^+ nutrient Ringer's

(solution 2, Table 1). Since on washing the nutrient chamber most of the outside K^+ is carried off, we could argue that if no K^+ got into the gastric membrane itself the resistance recovery curves should always take the same amount of time. Slower recovery times after high K^+ pulses of longer duration is taken to represent the outward diffusion of high K^+ concentrations built up in the cell. Implicit in this statement is the concept that cytoplasmic K^+ concentration is not much higher than that of the nutrient bathing media and that traditional higher measurements of intracellular K^+ are the result of compartmentalization of this ion within discrete portions of the cell (Davis et al., 1965). If no K^+ got into the cell we would expect the same recovery time no matter what the duration of exposure. The slower recovery rate after long time exposure appears compatible with slow outward leakage of increased cytoplasmic concentrations of K^+. In other words, it is postulated that K^+ can oppose the Ba^{++} effect when elevated on either side of the nutrient membrane. Ten minute pulses of 79 mM K^+ are felt to increase intracellular K^+ concentrations. Restoration of 4 mM K^+ to nutrient bathing media rapidly reverses the high concentration of K^+ outside the nutrient membrane and a rapidly rising Ba^{++} effect on resistance is seen. This effect is then arrested by the higher than usual intracellular K^+ concentration and can only climb to the normal resistance of the Ba^{++} effect as this extra K^+ diffuses from the cell bringing the cytoplasmic K^+ concentration back down to normal levels.

The Sulfate Effect on Ba^{++}

In an attempt to elucidate further the site of Ba^{++} action on the nutrient membrane, experiments were designed in which paired Ba^{++} pulses of either 8 or 10 minutes were used and were washed out either with regular Ringer's or with 5 mM sulfate Ringer's (solution 18, Table 3). Figure 18 shows the results of these experiments. The presence of 5 mM sulfate does not significantly shorten the reversal of the Ba^{++} effect. One possible explanation is that the membrane surface has such an incredible affinity for Ba^{++} and such a low dissociation constant that the well known high affinity of sulfate for Ba^{++} has no effect in speeding the Ba^{++} reversal. Another more plausible explanation would be that the Ba^{++} is not readily available to the sulfate for precipitation -- that is, one can consider the Ba^{++} ions responsible for the Ba^{++} effect as being within the nutrient membrane. With this concept in mind if one looks at the wash-out curves of the zero sulfate (solution 2, Table 1) as compared to the wash-out curves of 5 mM sulfate (solution 20, Table 3), the surprise (i.e., that they are extremely similar) is explained by the fact that for all practical purposes when a Ba^{++} ion leaves the membrane it rapidly diffuses (time constant 1 to 2 minutes) into an "infinite" reservoir. Therefore for all practical purposes it may as well be precipitated and we can see that under such conditions precipitation with sulfate would not be expected to alter the time course of reversal of the Ba^{++} effect.

The Ba^{++} Threshold

Another question that arose was whether or not a precipitated solution of 1 mM Ba^{++} final concentration in 5 mM sulfate (solution 18, Table 3) would be sufficient to cause a Ba^{++} effect. When such a solution was placed in the nutrient chamber an attenuated Ba^{++} effect was noted (Figure 19). At first glance this might be surprising but it would seem worthwhile to point out that this case is not at all analogous to our last case in which we described an attempt to reverse the effect of 1 mM Ba^{++} with a 5 mM sulfate solution. In this case a solution was made up and precipitated so that we had 1 mM Ba^{++} for 5 mM sulfate final concentration. This gives us a 1 to 5 ratio of these ions. In the previous case when the 1 mM solution was drained from the chamber it was immediately washed and drained 3 times with a 5 mM sulfate solution. With this procedure if there were an equivalent of .1 cc residual in our chamber after washings the ratio of sulfate ion to Ba^{++} would be 1,000 to 1. Since after several minutes' equilibration this whole mixture was again drained and washed 3 times with sulfate then refilled, on the basis of the same calculations the ratio of sulfate to Ba^{++} ion would at this point be a million to one, quite a different case from the 1 mM final concentration solution described. With this distinction, it was surprising with the known low solubility product of $BaSO_4 \simeq 10^{-10}$ (Chemical Handbook) to find even an attenuated Ba^{++} effect with this solution. Schwartz et al. (1968) demonstrated a threshold effect for Ba^{++} at about .005 mM

concentration. The effect they noted at this concentration was small and this appears to be the lowest concentration of Ba^{++} that they attempted to use. The presence of a Ba^{++} effect with 5 mM sulfate (solution 18, Table 3) would tend to indicate a much lower threshold for this phenomenon.

If the explanation used here of the 1 mM Ba^{++} 5 mM sulfate experiments is correct and the presence of a Ba^{++} effect simply represents low membrane threshold, then logically one might expect to find no effect of Ba^{++} with a higher sulfate concentration. This led to the experiments in which 1 mM Ba^{++} was precipitated in a solution of 20 mM sulfate as described under Results (Figure 19). The above expectation was reinforced by finding that the addition of this solution produced no Ba^{++} effect.

Experiments were performed in which the Ba^{++} dwell time at the nutrient border of the mucosa was exceedingly small. In some of these experiments several ml of solution 24, Table 3, containing 1 mM Ba^{++} were simply poured through the nutrient chamber. This resulted in no more than a few seconds contact with the mucosa which was then copiously and repeatedly washed with a solution containing no Ba^{++} (solution 2, Table 1). Obviously enough Ba^{++} got into the diffusion barrier and reached the nutrient membrane to produce a Ba^{++} effect. Although determination of final concentration of Ba^{++} at the surface of the nutrient membrane would be mathematically complex and well nigh impossible considering the geometrical complexity of the diffusion barrier one might logically

expect this concentration under such circumstances to be very small indeed. This set of experiments is further evidence for an extremely low threshold of the nutrient membrane for Ba^{++} effect.

The 10 mM Enigma

Interestingly, when testing the effects of increasing the concentration of Ba^{++} on the nutrient membrane up to 10 mM final concentration, the usual Ba^{++} effects were always obtained. The higher Ba^{++} concentrations were less readily reversed by 79 mM K^{+} solutions in that the resistance and PD decreases were slower after membrane exposure to higher concentrations of Ba^{++}. Ten mM Ba^{++} was discovered to have a biphasic effect on the membrane, as discussed under the section on Results and was reversed by 79 mM K^{+} nutrient solution.

One possible explanation for this long term effect of high Ba^{++} concentrations is that the low resistance represents Ba^{++} moving across the nutrient membrane into the gastric cells. However, the longer time for reversal by 79 mM K^{+} could be explained by higher concentration of Ba^{++} in the membrane rather than a higher concentration of Ba^{++} in the cell.

Evidence for Ba^{++} in the Nutrient Membrane

If the effect of Ba^{++} were a surface phenomenon at the nutrient membrane it would be difficult to conceive of its reversal by high K^{+} solutions as anything more than a simple dislodgement of Ba^{++} by a barrage of K^{+}. Once the dispersal of Ba^{++} was

accomplished into the quasi-infinite reservoir of bathing medium, one would not predict a recovery of Ba^{++} effect without the addition of more Ba^{++} to the nutrient medium. On the other hand, were the Ba^{++} site of action within the membrane itself and its reversal by high K^{+} due to fierce competition for conductive loci, then one might predict a recovery of the Ba^{++} effect if the competing high concentration of K^{+} ions was lowered before Ba^{++} were dislodged from within the membrane.

The "bounce" experiments described earlier in which the Ba^{++} effect reappeared several times after reversal by K^{+} and in the absence of new Ba^{++} is strong evidence that the Ba^{++} effect takes place within the nutrient membrane. The phenomenon of obtaining more "bounces" after a one minute exposure to a higher concentration of Ba^{++} could be logically explained by a larger amount of Ba^{++} being present in the nutrient membrane.

Ba^{++} Without a Cl^{-} Limb

The Ba^{++} effect demonstrated in the isethionate experiments where no Cl^{-} was present tends to substantiate our concept of the association of Ba^{++} with K^{+} in the nutrient membrane. These experiments clearly demonstrate the ability of Ba^{++} to raise the resistance of the nutrient membrane in the absence of the Cl^{-} limb of the nutrient conductance channels.

The Short Circuit Implication

The decrease in short circuit current due to Ba^{++} is of

importance in determining its site and mode of action since it indicates that Ba^{++} has an effect on the Cl^- conductance of the nutrient membrane. On the basis of our present picture of the characteristics of the nutrient membrane (Figure 24) short circuit current can only travel via the Cl^- limb, i.e. the short circuit current must equal the Cl^- current through the nutrient membrane. Since the short circuit current is decreased by Ba^{++}, the implication of these experiments is that any explanation for the effect of Ba^{++} must include a decrease of the conductance of the Cl^- limb of the nutrient membrane.

THE EXEGESIS

The purpose of this dissertation has been to define the mechanism of ion transport in the submucosal facing membrane of the frog gastric mucosa. Since Ba^{++} has the unique characteristic of elevating the gastric resistance without inhibiting its secretory rate, it can be considered the Rosetta Stone for understanding nutrient membrane transport. Therefore, considerable attention was given to its mode of action.

In approaching this problem many characteristics of the nutrient membrane have been determined including

1. definition of the site of action of high nutrient K^+ as the submucosal facing membrane
2. determination of the relationship of PD and resistance of this membrane to nutrient K^+ concentration as plotted in Figures 7 and 9
3. establishment of a minimum value for the resistance of this membrane at 85 ohm cm^2
4. demonstration that the conductance of this membrane for H^+, OH^-, and bicarbonate is zero
5. further evidence that the time constant for passage of ions across the nutrient diffusion barrier is one minute

6. demonstration that Ba^{++} does not act on the secretory membrane through a messenger mediated influence
7. further evidence that Ba^{++} acts on the nutrient membrane
8. determination that Ba^{++} raises membrane resistance in the absence of Cl^{-}; this effect is further evidence that Ba^{++} acts to decrease the K^{+} conductance limb of the nutrient membrane
9. establishment that Ba^{++} decreases the short circuit current which is evidence to support the concept that Ba^{++} decreases the Cl^{-} conductance of the nutrient membrane
10. evidence that the Ba^{++} effect is not likely a surface phenomenon at the nutrient membrane but probably acts within the membrane.

These data establish a neutral mechanism or mechanisms for the entrance of Cl^{-} into the gastric cell and appearance of bicarbonate ions in the nutrient bathing media. This concept when applied to the Cl^{-}-bicarbonate shift in the red cell was called by Passow "the revolution in red cell physiology". It represents a substantial advance in our understanding of the mechanism of gastric secretion.

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APPENDIX

TABLE 1

Composition of solutions (mM) used in experiments to determine the effect of high nutrient K^+ on PD and resistance. All nutrient solutions contain 10^{-4} M histamine. Solutions 3 through 6 are referred to in the text as containing 10-20-40-80 mM K^+ for convenience.

Solution No.	Na	K	Ca	Mg	HCO_3	Cl	SO_4	P_i	Ba^{++}	Suc.*	Glu.*
Secretory											
1	100	4	0	0	0	104	0	0	0	0	0
Nutrient											
2	102	4	1	0.8	25	82.6	0	1	0	0	10
3	96.13	9.88	1	0.8	25	82.6	0	1	0	0	10
4	86.25	19.75	1	0.8	25	82.6	0	1	0	0	10
5	66.50	39.5	1	0.8	25	82.6	0	1	0	0	10
6	27	79	1	0.8	25	82.6	0	1	0	0	10

* Suc. = sucrose; Glu. = glucose.

TABLE 2

Composition of solutions in mM/l. Secretory refers to solutions bathing lumen or mucosal side, and nutrient to solution bathing the submucosal side.†

Solution No.	Na	K	Ca	Mg	HCO_3	Cl	SO_4	P_i	Suc.
Secretory									
7	100	4	0	0	0	104	0	0	0
8	100	4	0	0	0	0	52	0	80
Nutrient									
9	102	4	1.0	0.8	25	81	0.8	1.0	0
10	102	4	1.0	0.8	5	81	10.8	1.0	10
11	86	20	1.0	0.8	25	81	0.8	1.0	0
12	102	4	1.0	0.8	25	31	25.8	1.0	25
13	102	4	1.0	0.8	75	31	0.8	1.0	0
14	102	4	1.0	0.8	25	0	41.3	1.0	40
15	102	4	1.0	0.8	5	0	51.3	1.0	50

† All nutrient solutions contained 10 mM glucose and 10^{-4} M histamine.

TABLE 3

Composition of solutions (mM) used in experiments to determine site and mode of Ba^{++} action. Some solutions for this series of experiments appear in Table 2. All nutrient solutions contain 10^{-4} M histamine.

Solution No.	Na	K	Ca	Mg	HCO_3	Cl	SO_4	P_i	Ba^{++}	Iseth.*	Ace.*	Suc.*	Glu.*
Secretory													
16	100	4	0	0	0	0	0	0	0	104	0	0	0
17	100	4	0	0	0	106	0	0	1	0	0	0	0
Nutrient													
18	102	4	1	0.8	25	74.6	5	1	1	0	0	0	10
19	106	0	1	0.8	25	84.6	0	1	1	0	0	0	10
20	102	4	1	0.8	25	72.6	5	1	0	0	0	0	10
21	102	4	1	0.8	25	44.6	20	1	1	0	0	20	10
22	98	4	1	0.8	25	0	0	1	0	75	3.6	0	10
23	23	79	1	0.8	25	0	0	1	0	75	3.6	0	10
24	102	4	1	0.8	25	84.6	0	1	1	0	0	0	0

* Iseth. = isethionate; Ace. = acetate; Suc. = sucrose; Glu. = glucose.

TABLE 4

Resistance (ohm cm^2) changes of mucosa resulting from 79 mM K^+ pulses of 30 second, 60 second, 2 minute and 10 minute duration in absence of Ba^{++}.

Exp. No.	Av. Control	30 sec.	60 sec.	2 min.	10 min.
1	160	62	94	88	101
2	149	88	88	69	75
3	285	184	155	129	192
	(285)	--	--	--	192
4	126	87	71	79	80
	(126)	--	--	72	--
5	147	72	103	100	100
	(147)	--	120	--	--
MEAN	173.40	98.60	105.1	89.50	123.33
S.D.	63.59	48.96	29.3	22.40	54.20
S.E.M.	28.44	21.89	12.0	9.15	22.13

TABLE 5

Resistance (ohm cm^2) changes of mucosa at 30 second, 60 second, 2 minute and 10 minute points resulting from 79 mM K^+ pulses of 10 minutes' duration in the presence of 1 mM Ba^{++}.

Exp. No.	Control	30 sec.	60 sec.	2 min.	10 min.
1	166	120	120	114	101
2	123	75	62	62	75
3	283	172	153	192	192
4	283	--	--	--	192
5	120	120	79	80	80
6	155	88	101	104	104
MEAN	188.33	115.00	103.0	110.40	124
S.D.	75.46	37.51	35.33	49.95	53.88
S.E.M.	30.81	16.75	15.89	22.34	22.00

TABLE 6

PD (mv) changes and calculated K^+ concentration (mM) at nutrient membrane resulting from 79 mM K^+ pulses 30 second, 60 second, 2 minute and 10 minute duration in the absence of Ba^{++}.

Exp. No.	30 sec. ΔPD	30 sec. K^+	60 sec. ΔPD	60 sec. K^+	2 min. ΔPD	2 min. K^+	10 min. ΔPD	10 min. K^+	G_K*
1	33	62	36	79	33	62	31	52	27.79
2	18	12	30	25	49	79	43	55	37.82
3	19	13	29	23	49	79	41	49	37.82
4	--	--	--	--	--	--	40	79	30.87
5	43	55	43	55	49	79	49	79	37.82
6	33	42	35	48	42	79	40	69	32.42
MEAN	29.20	36.80	34.60	46.00	44.40	75.60	40.67	63.83	34.09
S.D.	10.59	23.32	5.59	23.15	7.06	7.60	5.82	13.60	4.35
S.E.M.	4.74	10.43	2.50	10.35	3.16	3.40	2.38	5.55	1.78

* $G_K = \frac{G'_K}{G'_K + G'_{Cl}}$ where G'_K and G'_{Cl} are the K^+ and Cl^- conductance of the nutrient membrane, respectively.

TABLE 7

PD (mv) changes and calculated K^+ concentration (mM) at 30 second, 60 second, 2 minute and 10 minute points resulting from 79 mM K^+ pulses of 10 minutes' duration in the absence of Ba^{++}.

Exp. No.	30 sec. ΔPD	30 sec. K^+	60 sec. ΔPD	60 sec. K^+	2 min. ΔPD	2 min. K^+	10 min. ΔPD	10 min. K^+	G_K
1	28	54	32	79	32	79	31	72	24.70
2	20	16	32	37	38	56	43	79	33.19
3	16	13	28	31	34	47	41	79	31.65
4	18	15	28	32	34	51	40	79	30.87
5	40	46	44	58	49	79	49	79	37.82
6	23	21	38	63	41	79	40	73	31.65
MEAN	24.17	27.00	33.67	50.00	38.00	65.17	40.67	76.83	31.65
S.D.	8.82	17.81	6.25	19.64	6.29	15.42	5.82	3.37	4.23
S.E.M.	3.60	7.27	2.55	8.02	2.57	6.30	2.38	1.38	1.73

TABLE 8

Effect on potential difference across in vitro frog gastric mucosae of changing HCO_3^- and/or CO_2 of fluid bathing submucosal (nutrient) side. A plus value for ΔPD means nutrient side becomes more positive and vice versa. The averages (± one standard deviation) are given for the values one minute after the change to the new solution and the corresponding values for the maximum ΔPD during the first 4 minutes.

ΔHCO_3 / ΔCO_2	Sol. Δ see Table 2	ΔPD at 1st min.	Max. ΔPD during 1st 4 min.	No. of Exps.
Chloride Solutions				
25 HCO_3 / 5% CO_2 → 5 HCO_3 / 5% CO_2	3→4	0.08 (±0.89)	-0.44 (±2.45)	23
5 HCO_3 / 5% CO_2 → 25 HCO_3 / 5% CO_2	4→3	0.02 (±0.91)	1.09 (±2.05)	23
25 HCO_3 / 5% CO_2 → 5 HCO_3 / 1% CO_2	3→4	0.82 (±0.80)	1.26 (±1.23)	47
5 HCO_3 / 1% CO_2 → 25 HCO_3 / 5% CO_2	4→3	-0.20 (±0.40)	-0.57 (±0.82)	6
25 HCO_3 / 5% CO_2 → 25 HCO_3 / 1% CO_2	3→3	-0.54 (±0.85)	-0.54 (±0.85)	10
25 HCO_3 / 1% CO_2 → 25 HCO_3 / 5% CO_2	3→3	-0.18 (±0.53)	0.72 (±1.13)	10
25 HCO_3 / 5% CO_2 → 75 HCO_3 / 5% CO_2	6→7	-1.1 (±0.89)	-1.1 (±0.89)	4
75 HCO_3 / 5% CO_2 → 25 HCO_3 / 5% CO_2	7→6	0.0 (±1.4)	-0.12 (±2.7)	4
25 HCO_3 / 5% CO_2 → 75 HCO_3 / 1% CO_2	6→7	-0.66 (±1.1)	-1.0 (±1.5)	3
75 HCO_3 / 1% CO_2 → 25 HCO_3 / 5% CO_2	7→6	0.0 (±0.5)	0.16 (±0.8)	3

TABLE 8 (continued)

ΔHCO_3 ΔCO_2	Sol. Δ see Table 2	ΔPD at 1st min.	Max. ΔPD during 1st 4 min.	No. of Exps.
Sulfate Solutions				
25 HCO_3 5% CO_2 → 5 HCO_3 5% CO_2	8→9	0.36 (±1.39)	-1.0 (±1.9)	15
5 HCO_3 5% CO_2 → 25 HCO_3 5% CO_2	9→8	0.50 (±0.90)	1.13 (±1.3)	15
25 HCO_3 5% CO_2 → 5 HCO_3 1% CO_2	8→9	-0.16 (±1.2)	-0.16 (±1.2)	3
5 HCO_3 1% CO_2 → 25 HCO_3 5% CO_2	9→8	-0.66 (±1.3)	-1.6 (±1.83)	3
25 HCO_3 5% CO_2 → 25 HCO_3 1% CO_2	8→8	0.16 (±0.41)	1.16 (±1.71)	6
25 HCO_3 1% CO_2 → 25 HCO_3 5% CO_2	8→8	0.2 (±0.91)	-0.63 (±1.05)	6

TABLE 9

PD and resistance changes of scraped mucosa resulting from 1-5-10 mM Ba^{++} and 79 mM K^{+} solutions. All resistances in these and following tables are corrected for tissue and blank resistance and given in ohm cm^2.

Exp. No.	Control PD	Control R	1 mM Ba^{++} ΔPD	1 mM Ba^{++} ΔR	5 mM Ba^{++} ΔPD	5 mM Ba^{++} ΔR	10 mM Ba^{++} ΔPD	10 mM Ba^{++} ΔR	79 mM K^{+} ΔPD	79 mM K^{+} ΔR
1	0	49.1	0	0	0	0	0.3	0	2.8	6.5
	-0.5	49.1	0.4	0	--	--	0.2	2.6	2.6	6.5
	-1.0	58.2	0.1	0	--	--	0.3	0	4.0	13.0
	-1.5	62.1	0.3	0	--	--	--	--	3.0	0
2	-1.0	6.0	0.1	0.81	0.8	0	0.02	0	0	0
	-3.0	8.8	0.05	0.5	0.1	0.98	0.2	0.9	0.2	0.9
3	-0.3	6.8	-0.5	0	--	--	0.2	0	2.7	0
	-1.2	8.8	0	0	--	--	--	--	1.4	0
	-0.3	5.9	--	--	--	--	--	0	2.4	3.0

TABLE 9 (continued)

Exp. No.	Control PD	Control R	1 mM Ba^{++} ΔPD	1 mM Ba^{++} ΔR	5 mM Ba^{++} ΔPD	5 mM Ba^{++} ΔR	10 mM Ba^{++} ΔPD	10 mM Ba^{++} ΔR	79 mM K^{+} ΔPD	79 mM K^{+} ΔR
4	-1.7	7.5	0	0	0	0	0.5	0	3.8	4.0
	-1.4	6.2	0	0	0	2.60	0.2	0	2.0	0
	0	10.1	--	--	--	--	--	--	2.3	0
MEAN			0.15	0.13	0.18	0.72	0.21	0.36	2.27	2.79
S.D.			0.19	0.28	0.35	0.14	0.15	0.85	1.23	4.08
S.E.M.			0.06	0.09	0.16	0.51	0.05	0.77	0.36	1.38

TABLE 10

PD and resistance (ohm cm^2) changes of external muscle layers resulting from 1-5-10 mM Ba^{++} and 79 mM K^+ solutions.

Exp. No.	Control		1 mM Ba^{++}		5 mM Ba^{++}		10 mM Ba^{++}		79 mM K^+	
	PD	R	ΔPD	ΔR	ΔPD	ΔR	ΔPD	ΔR	ΔPD	ΔR
1	0	50.0	0	0	--	--	0	0	--	
	0	50.0	0	0	--	--	0	0	--	
2	-1.2	77.3	0	0.9	0.0	0.3	0	2.6	0.5	2.6
	-2.8	86.8	0.1	0.3	0.2	1.3	0.2	3.3	0.6	4.0
3	1.3	29.6	0.1	0	0.1	0	0	0	0.4	0
4		81.6	0	0	0	0	0	0	4.7	0
5	-2.0	55.6	0.1	0	0	0	0.1	0	2.4	0
6	-2.4	46.5	+.1	0	+.1	0	+.1	0	1.8	1.8

TABLE 10 (continued)

Exp. No.	Control PD	Control R	1 mM Ba^{++} ΔPD	1 mM Ba^{++} ΔR	5 mM Ba^{++} ΔPD	5 mM Ba^{++} ΔR	10 mM Ba^{++} ΔPD	10 mM Ba^{++} ΔR	79 mM K^{+} ΔPD	79 mM K^{+} ΔR
MEAN	-1.1	59.5	0.03	0.2	0.07	0.3	0.04	0.7	1.6	1.4
S.D.	1.4	20.1	0.07	0.3	0.08	0.5	0.06	1.4	1.5	1.7
S.E.M.	0.5	7.1	0.03	0.1	0.03	0.21	0.02	0.5	0.6	0.7

TABLE 11

Resistance of external muscle layer as a function of area and weight of tissue.

Exp. #	Av. ohm-cm^2	Wt. gm	Wt. gm/cm^2	Resistance ohm/gm
1	82.1	.26437	.203362	403.46771
2	29.6	.091670	.070515	419.48552
3	81.6	.25900	.199231	409.47443
4	55.6	.13190	.10146	547.75281
5	46.5	.12138	.09337	497.75088
MEAN	59.1	.17361	.13359	455.58627
S.D.	22.8	.08172	.06286	64.06722
S.E.M.	10.2	.03654	.02811	28.65173

TABLE 12

Resistance (ohm cm^2) changes of mucosa resulting from 79 mM K^+ pulses of 30 second, 60 second, 2 minute and 10 minute duration in the presence of 1 mM Ba^{++}.

Exp. No.	Pre-Ba^{++} Resistance	Average Control with 1 mM Ba^{++}	Length of 79 K^+ Pulse			
			30 sec.	60 sec.	2 min.	10 min.
1	218	497	315	--	--	196
2	244	439	246	155	151	143
3	218	449	198	172	153	156
4	153	301.93	166	133	133	130
5	153	448.4	250	192	192	155
MEAN	197.20	426.80	235	163.00	157	156
S.D.	41.72	73.90	56.74	25.07	24.85	24.73
S.E.M.	18.66	33.05	25.37	12.54	12.43	11.06

TABLE 13

Resistance (ohm cm^2) changes of mucosa at 30 second, 60 second, 2 minute and 10 minute points resulting from 79 mM K^+ pulses of 10 minutes' duration in the presence of 1 mM Ba^{++}.

Exp. No.	Pre-Ba Baseline	Control	30 sec.	60 sec.	2 min.	10 min.
1	218	546	351	204	136	196
2	244	416	188	179	169	143
3	218	493	188	169	156	156
4	153	324	130	130	136	130
5	153	519	223	191	191	155
MEAN	197.20	459.60	216.00	174.60	157.60	156.00
S.D.	41.72	90.00	82.52	28.17	23.35	24.73
S.E.M.	18.66	40.75	36.90	12.60	10.44	11.06

TABLE 14

PD (mv) changes and calculated K^+ concentration (mM) at nutrient membrane resulting from 79 mM K^+ pulses of 30 second, 60 second, 2 minute and 10 minute duration in the presence of 1 mM Ba^{++}.

Exp. No.	30 sec. ΔPD	30 sec. K^+	60 sec. ΔPD	60 sec. K^+	2 min. ΔPD	2 min. K^+	10 min. ΔPD	10 min. K^+	G_K
1	19	15	--	--	--	--	33	79	25.47
2	26	37	33	67	35	79	32	61	27.02
3	22	25	30	48	36	79	32	57	27.79
4	15	26	23	70	16	29	24	79	18.52
5	22	18	30	31	32	35	44	79	33.96
MEAN	20.80	24.20	29.00	54	29.75	55.5	33.0	71.0	26.55
S.D.	4.09	8.53	4.24	18.17	9.32	27.25	7.14	11.05	5.53
S.E.M.	1.83	3.81	2.12	9.08	4.66	13.63	3.19	4.94	2.47

TABLE 15

PD (mv) changes and calculated K^+ concentration (mM) at 30 second, 60 second, 2 minute and 10 minute points resulting from 79 mM K^+ pulses of 10 minutes' duration in the presence of 1 mM Ba^{++}.

Exp. No.	30 sec. ΔPD	30 sec. K^+	60 sec. ΔPD	60 sec. K^+	2 min. ΔPD	2 min. K^+	10 min. ΔPD	10 min. K^+	G_K
1	17	18.6	--	--	--	--	33	79	25.47
2	26	43	31	66	33	79	32	72	25.47
3	23	23	36	63	39	79	32	46	30.10
4	17	33	21	54	24	79	24	79	18.52
5	19	15	25	22	34	40	44	79	33.96
MEAN	20.40	26.32	28.25	51.25	32.50	69.25	33.00	71.00	26.50
S.D.	3.97	11.05	6.60	20.16	6.24	19.50	7.14	14.30	5.86
S.E.M.	1.78	4.95	3.30	10.08	3.12	9.75	3.19	6.38	2.62

TABLE 16

Actual magnitude of V_{NS} during short circuiting for each current range. Current is in microamps per 1.3 cm^2.

I_Y Microamps	V_{NS} mv	I_Y Microamps	V_{NS} mv
0 to 15	-0.5	108.5 to 124	-4.0
15 to 31	-1.0	124 to 139.5	-4.5
31 to 46.5	-1.5	139.5 to 155	-5.0
46.5 to 62	-2.0	155 to 170.5	-5.5
62 to 77.5	-2.5	170.5 to 186	-6.0
77.5 to 93	-3.0	186 to 217	-7.0
93 to 108.5	-3.5	217 to 248	-8.0

GRADUATE SCHOOL
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Major Subject Physiology and Biophysics

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