POTENTIAL RESPONSES OF NUTRIENT MEMBRANE OF FROG'S STOMACH TO STEP CHANGES IN EXTERNAL K^+ AND Cl^- CONCENTRATIONS

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ABSTRACT With an in vitro chamber method experiments were performed to determine the relative ionic conductances of the nutrient membrane (membrane facing muscularis mucosa). The concentration of a given ion in the nutrient bathing solution was changed, and the ensuing time course of the change in transmucosal potential difference (PD) was recorded. Changing K^+ from 4 to 79 mM produced a response in PD which occurred markedly faster than the response for the reverse change, and similar results were obtained by changing the Cl^- concentration. It was found that these differences were predicted by the analysis of an idealized model consisting of a membrane in series with a diffusion barrier. When both the K^+ and Cl^- were changed, such that the product of their concentrations remained constant, the time courses of the responses were again similiar to those predicted on the basis of the model. From the magnitudes of the total PD responses it is shown that in the presence of a 4 mM K^+ nutrient solution, the conductivity of the nutrient membrane appears to be entirely due to the K^+ and Cl^- conductances, the K^+ conductance being about twice that of the Cl^-. It is also shown that with a 79 mM K^+ nutrient solution the parameters of the membrane were changed such that the conductances of the two ions were approximately equal. The time constant for diffusion of KCl or NaCl across the barrier consisting of the submucosa, muscularis mucosa, and lamina propria is about 1 min.

INTRODUCTION

The work on the in vitro frog gastric mucosa presented in this paper is primarily concerned with the determination of the relative ionic conductances of the limiting membrane of the mucosal cell layer facing the submucosa and certain characteristics of the adjacent diffusion barrier. The barrier consists of the lamina propria, the muscularis mucosa, and part of the submucosa (the external muscle layers together with a portion of the submucosa are removed and discarded). The above membrane will be referred to as the nutrient membrane, and the solution bathing the submucosal side as the nutrient solution; the solution bathing the opposite side of the tissue will be referred to as the secretory solution.

The relative ionic conductances were assessed by determining the effect on the transmucosal potential difference (PD) of changes in the ionic composition of the nutrient fluid. It was found that the time course of the absolute value of the change in PD following a change from a low to a high concentration of a permeant ion was quite different from that following the reverse change. This finding raised the possibility that changes in ionic composition of the nutrient solution produce changes in the values of the parameters of the mucosal cell layer such that the response of the PD cannot be used to accurately assess the relative ionic conductances of this membrane. It will be shown that the time course for the rapid change in PD is essentially that predicted on the basis of an idealized model consisting of a membrane with fixed permeability properties in series with a diffusion barrier. The analysis is similar to that used by Kidder et al. (1) for the frog skin. Harris and Edelman (2) also studied the relative ionic conductances of this membrane by determining the effect of changes in ionic composition on the PD. However, they used values of the PD 20–40 min after the change in composition. Hogben (3) has criticized their approach on the basis that the values of the parameters of the system may change so that the changes in PD may not be a simple direct function of change in ion gradients across the nutrient membrane. Comment on both the work of Harris and Edelman and the criticisms of Hogben will be given in the discussion.

METHODS

A previously described technique (4) was used on stomachs of *Rana pipiens*. In most experiments the stomach minus the external muscle layer (referred to as the gastric mucosa) was mounted between two Lucite chambers. In some experiments either the external muscle layer itself or the scraped gastric mucosa was mounted between chambers. The scraped mucosa consists of portions of the submucosa and muscularis mucosa after the mucosal cell layer has been removed by scraping. Two pairs of electrodes were used, one for sending current and the other for measuring the PD. The PD was recorded by means of an Esterline-Angus Recording Potentiometer (Esterline-Angus Instrument Company, Inc., Indianapolis, Ind.) and the resistance was determined as previously described (5) by sending pulses of direct current of about 0.5 sec duration across the preparation (resistance = $\Delta PD/I$). The secretory rate was measured with a recording pH stat technique. Both nutrient and secretory solutions were gassed with 95% O_2 and 5% CO_2. The regular nutrient solution had the following composition (in mM): Na^+ 102; K^+ 4; Ca^{++} 1.0; Mg^{++} 0.8; Cl^- 81.0; HCO_3^- 25; phosphate 1; $SO_4^=$ 0.8; glucose 10; and histamine 10^{-4}. In modified nutrient solutions Na^+ was replaced by K^+ and/or Cl^- by $SO_4^=$ plus sucrose (to maintain isotonicity). In all experiments on the gastric mucosa the solution bathing the secretory side contained (in mM) Na^+ 100, K^+ 4, and Cl^- 104.

In some experiments reservoirs containing the regular nutrient solution and the experimental nutrient solution were arranged so that flow through the nutrient chamber could be switched from one to the other in a few seconds. In other experiments the nutrient chamber was drained and washed once with the new solution, zero time being taken at the time of addition of the aliquot of the new solution used for washing; the first PD reading was obtained in about 10–15 sec. During the change in nutrient solution the inflow and outflow tubes to the secretory chamber were clamped to prevent bulging of the mucosa. There were no apparent

differences in the results regardless of the technique used, and neither the techniques nor the results are separately designated.

In the experimental arrangement, saturated KCl calomel electrodes made contact with the circulating fluid via tubing about 4 cm in length. In most experiments the liquid junction potential was renewed at the time of the change in the new solution while in other experiments the old solution remained in the 4 cm length of tubing, thus establishing a liquid junction potential between the new and old fluids. After the PD reached a steady state, the electrode junction was flushed and the ΔPD was noted. This method for measuring the magnitude of the liquid junction potentials gave the same values as did a more elaborate method described previously (6). Except for a 9.3 mv potential between the 4 K^+, 81 Cl^-, and the 81 K^+, 4 Cl^- solutions, the liquid junction potentials between solutions used in these experiments were 4 mv or less.

RESULTS

Fig. 1 shows the effects on the PD and H^+ secretory rate of changing the K^+ concentration from 4 to 79 mM and back again. A positive PD means that the nutrient side is positive. At the first arrow, the regular nutrient fluid was replaced with one containing 79 mM K^+ (K^+ replacing Na^+). The PD decreased, reached a negative peak within a few minutes, and then started increasing. At the second arrow, the 79 mM K^+ solution was replaced with the regular nutrient solution containing 4 mM K^+ and the PD returned towards its original control level.

Following the change to 79 mM K^+, the H^+ secretory rate started decreasing after the negative peak of the PD was reached. The average time for the H^+ rate to start decreasing was significantly greater than the average time for the PD to reach its negative peak. It has been previously shown that our method would detect a de-

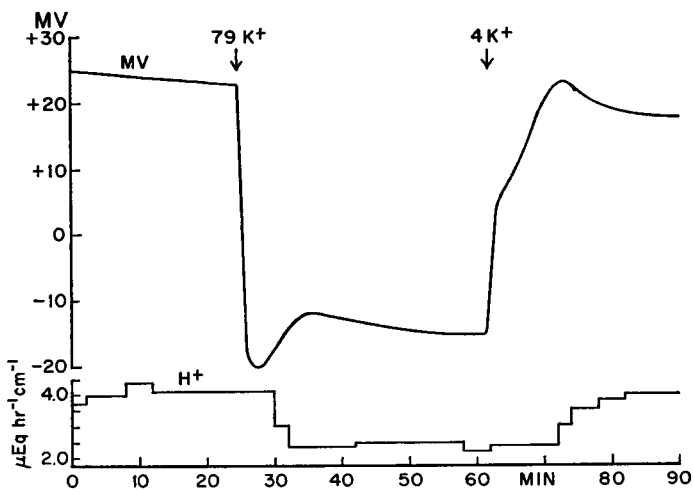

FIGURE 1 Effects on PD and H^+ secretory rate of changing K^+ in the nutrient solution from 4 to 79 mM (at arrow labelled 79 K^+) and back to 4 mM (at arrow labelled 4 K^+). A positive PD means that the nutrient side is positive to the secretory side.

crease in the H^+ rate within one minute (inhibition of H^+ secretion by adding thiocyanate to the nutrient fluid (4)). In 79 mM K^+, the steady-state H^+ secretory rate was significantly lower ($P < 0.01$) than in 4 mM K^+; the average in 79 mM K^+ was 73% (SD ± 9.2) of the previous and 69% (SD ± 11.8) of the subsequent level in 4 mM K^+ (No. of experiments = 5). In returning from 79 mM K^+ to 4 mM K^+ the H^+ secretory rate did not start to increase until the PD had almost returned to its control level.

As shown in Fig. 2 the resistance with 79 mM K^+ was less than with 4 mM K^+, and following a change from 4 to 79 mM K^+ the decrease in resistance occurred

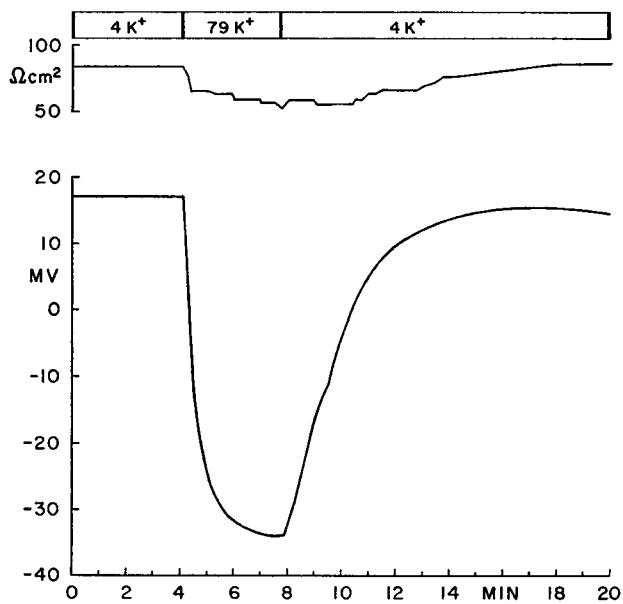

FIGURE 2 Effect on PD and resistance of changing K^+ in the nutrient solution from 4 to 79 mM and back to 4 mM.

during the change in PD. This was true for all of the experiments, and the average steady-state level of the resistance in 79 mM K^+ was 68.6% (SD ± 12.8, 15 experiments) of the average of the control levels (difference significant $P < 0.01$). Upon returning to 4 mM K^+ the resistance remained depressed and only gradually returned towards its original control level.

Comparison of the time courses of the response of the PD shows that there is a distinct time lag in the change from 79 to 4 mM K^+ as compared to the response resulting from a 4 to 79 mM K^+ change. This difference can be seen better in Fig. 3 in which the absolute values of the change in PD are plotted. The solid curves represent the experimental data. The dotted lines indicate the steady-state PD predicted on the basis of a model to be described below. It can be seen that the

ΔPD after 4 or 5 min was less in going from 79 to 4 mM K^+ than it was for the opposite change.

On the basis of the data presented so far one might be skeptical that the ΔPD method gives an accurate measure of the relative ionic conductances of the nutrient membrane. It might appear that the elevation of K^+ alters the characteristics of the mucosal cells and that the change in PD is a complex function of a number of factors. However, if it could be shown that the time lag in the response is predicted on the basis of a model consisting of a membrane and a diffusion barrier, and that in the steady state the parameters of the nutrient membrane are different with a nutrient fluid containing 4 mM K^+ than they are with a nutrient fluid containing 79 mM K^+, then one would have renewed confidence in this method. In the next section it will be shown that the time lag is predicted on the basis of a model, and in a

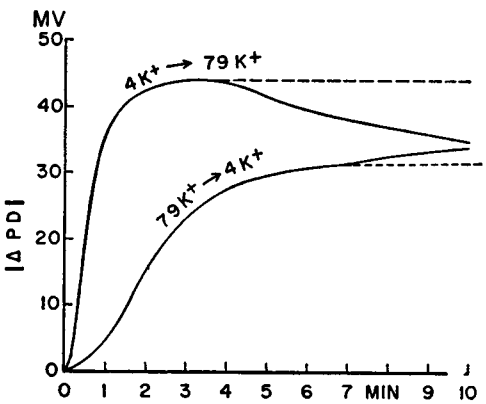

FIGURE 3 Absolute values of PD responses when nutrient solution K^+ is changed from 4 to 79 mM and vice versa. Broken lines indicate the total response expected if the parameters of the tissue had not changed.

subsequent section it will be shown that the steady-state characteristics of the gastric mucosa are different in 79 mM K^+ than they are in 4 mM K^+.

Calculations of the PD Response on the Basis of a Model

The model is represented in Fig. 4 and consists of a homogeneous diffusion barrier (from $x = 0$ to $x = a$) separating a bathing solution (in the region $x > a$) from membrane at $x = 0$. It is assumed that the rate of movement of ions through the barrier is much greater than the rate of movement across the membrane and for purposes of calculation the rate of movement of ions across the membrane is taken to be zero. It is also assumed that the concentration of ions to the left of the membrane ($x < 0$) is constant, that the bulk flow through the barrier is zero, that there is no liquid junction potential in the barrier, and that the PD across the membrane is a linear function of the log of the concentration of a permeant ion on the diffusion barrier side of the membrane.

On the basis of the above assumptions the boundary value problem of the model

for the concentration function $C(x, t)$ is then

$$C_t(x, t) = D\, C_{xx}(x, t) \qquad (0 < x < a, t > 0) \tag{1}$$

$$C(x, 0) = C_i \qquad (0 < x < a) \tag{2}$$

$$C_x(0, t) = 0,\ C(a, t) = C_f \qquad (t > 0) \tag{3}$$

where the subscripts t, x, and xx indicate partial derivatives, t the time in seconds, and D the diffusion coefficient for the appropriate ion. Subscripts i and f denote

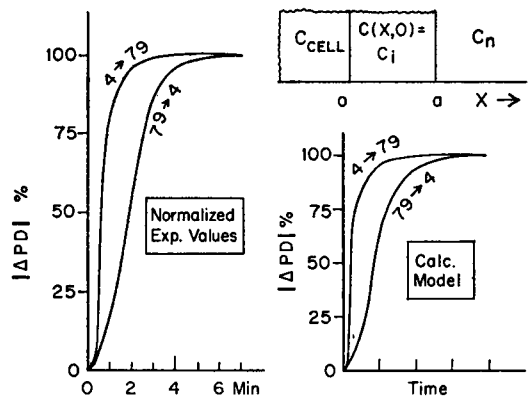

FIGURE 4 The left graph represents normalized experimental curves of the type shown in Fig. 3. The right graph represents responses calculated on the basis of the model (upper right). The membrane of the model (corresponding to the nutrient membrane) is at $x = 0$, the diffusion barrier is between $x = 0$ and $x = a$. C_{cell} corresponds to the K^+ concentration inside the cell, C_i is the initial K^+ concentration in the bathing fluid (at all $x > 0$ when $t = 0$), and C_n is the K^+ concentration to the right of $x - a$ when $t > 0$.

initial and final concentration. According to Churchill (7) a solution of the equation is

$$C(x, t) = C_f + \frac{4(C_i - C_f)}{\pi} \sum_{n=1}^{\infty} \frac{(-1)^{n-1}}{2n - 1} \cdot \left[\cos \frac{(2n - 1)\pi x}{2a}\right] \exp\left[-\frac{(2n - 1)^2 \pi^2 D t}{4a^2}\right]. \tag{4}$$

When $x = 0$, equation 4 reduces to

$$C(0, t) = C_f + \frac{4(C_i - C_f)}{\pi} \sum_{n=1}^{\infty} \frac{(-1)^{n-1}}{2n - 1} \exp\left[-(2n - 1)^2 \alpha t\right] \tag{5}$$

where

$$\alpha = \frac{\pi^2 D}{4a^2}. \tag{6}$$

The equation (13) at which we shall finally arrive is applicable to any model where there is a linear relationship between ΔPD and $\log_{10}C$ of a given cation or anion and where the PD effects of the two ions are additive. However, it will be derived on the assumption that there are two permeant ions, a cation and an anion, and that there are separate channels (parallel limbs) for each ion. The PD (t) across the membrane would then be given by

$$\text{PD}\ (t) = \frac{g_+}{g_+ + g_-} E^+(t) + \frac{g_-}{g_+ + g_-} E^-(t) \tag{7}$$

where g's are conductivities and E's are emf's due to the cations (+) and anions (−). E^+ and E^- are given by

$$E^+(t) = 2.303 \frac{RT}{F} \log_{10} \frac{C^+_{\text{cell}}}{C^+(0, t)} \tag{8}$$

and

$$E^-(t) = 2.303 \frac{RT}{F} \log_{10} \frac{C^-(0, t)}{C^-_{\text{cell}}} \tag{9}$$

where R, T, and F have their usual meaning and C^+_{cell} is the concentration of the given ion to the left of the membrane ($x < 0$). The initial potential is given by

$$\text{PD}\ (0) = G^+ \log_{10} \frac{C^+_{\text{cell}}}{C_i^+} + G^- \log_{10} \frac{C_i^-}{C^-_{\text{cell}}} \tag{10}$$

where

$$G^+ = \frac{g_+}{g_+ + g_-} 2.303 \frac{RT}{F} \tag{11}$$

and

$$G^- = \frac{g_-}{g_+ + g_-} 2.303 \frac{RT}{F}. \tag{12}$$

Subtraction of equation 10 from the combination of equations 8 and 9 with 7 and

rearrangement yields

$$\Delta PD\ (t) = PD\ (t) - PD\ (0) = G^+ \log_{10} \frac{C_i^+}{C^+(0, t)} + G^- \log_{10} \frac{C^-(0, t)}{C_i^-} \quad (13)$$

where $C^+(0, t)$ is given by equation 5.

Equation 13, then, is the basis for predicting the time course of the response of the PD when the external concentration of either or both of the permeant ions is changed. If only a single permeant ion is changed, the term involving the other ion on the right side of equation 13 is zero.

The per cent of the total ΔPD vs. time is plotted in Fig. 4 (right graph) for a change in a single ionic species of C_i = 4 mM to C_f = 79 mM and C_i = 79 mM to C_f = 4 mM. This graph, valid for either anion or cation concentration changes of 4 to 79 and 79 to 4 mM, is independent of the magnitude of G^+ since it is a per cent plot. No values have been placed on the time scale since the same curves (for these values of C_i and C_f) can represent the relationship for any value α of equation 6. By a linear adjustment of the concentration scale, a single curve can represent a plot of the concentration at $x = 0$ vs. time for changes in either direction. This is not true for a plot of ΔPD vs. time because of the logarithmic function. It can be seen that for calculations made on the basis of the model there is a decided time lag in the 79 to 4 response as compared to the 4 to 79 response.

Inspection of equation 5 reveals that when $t = 0$, $C(0, t) = C_i$ since the sum of the infinite series equals $\pi/4$. The value of α (see below) ranges from 0.0091 to 0.032 sec^{-1}, and when t = 20 sec the error in using only the first term of the infinite series is about 5% and rapidly diminishes with time since this is a monotonically decreasing alternate sign series.

Comparison of Experimental Results with Results Predicted by the Model

In the K^+ experiments there was a peak in the response for the 4 to 79 mM change. For the 79 to 4 mM change inflection points similar to that seen in Fig. 3 where the dotted line begins were quite distinct in all but a relatively few experiments; in some experiments the PD actually levelled off for a short while before continuing its rise. The peak ΔPD of the 4 to 79 mM change and the ΔPD at the inflection point for the reverse change were taken as the best estimates of total transient response. The subsequent slow changes in PD are apparently due to changes in the characteristics of the mucosa since, as will be shown below, in the presence of 79 mM K^+ the nutrient membrane becomes relatively less permeable to K^+ and more permeable to Cl^-.

A normalized plot of a typical experiment is shown in the left-hand graph of Fig. 4. It is clear that the response found experimentally is quite similar to that predicted by the model. In every experiment the 79 to 4 mM K^+ response lagged the response for the reverse change. The average time for half maximal response of the

PD calculated from the experimental data (13 experiments) was 26.9 sec (SD ± 9.2) for the 4 to 79 change while that for the 79 to 4 response was 62 sec (SD ± 25.1). On the basis of these two concentrations (4 and 79 mM K^+) the values of G^+, $|\Delta PD/\log_{10}(C_i/C_f)|$, are 37.9 mv (SD ± 4.3) for the 4 to 79 change and 24.3 mv (SD ± 5.6) for the 79 to 4 change (difference significant, $P < 0.01$).

Comparison of PD vs. $\log_{10}$ K^+ Plots for 4 mM K^+ and 79 mM K^+ as Reference Solutions

A possible explanation accounting for the difference in the G^+'s in the foregoing analysis is that in the presence of the 79 mM K^+ nutrient solution, the relative K^+ conductance of the nutrient membrane is less than in the presence of the 4 mM K^+ nutrient solution. If this be the case, the absolute value of the slope of a plot of

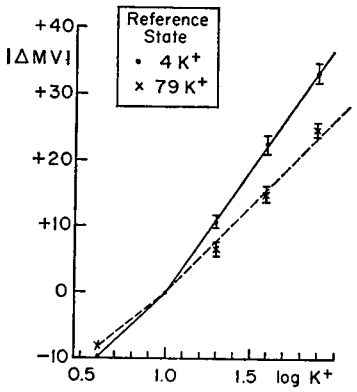

FIGURE 5 Absolute values of PD response vs. log of nutrient K^+ concentration. The solid curve is for the data when 4 mM K^+ was the reference concentration, the broken curve when 79 mM K^+ was the reference concentration. For convenience of comparison the curves were plotted so that they intersected at 10 mM K^+.

ΔPD vs. $\log_{10}$ K^+ should be less when the reference solution is 79 mM in K^+ than when the reference solution is 4 mM in K^+. In an experiment a steady state was obtained in the presence of the 4 mM K^+ nutrient solution. A change was then made to a 10, 20, 40, or 79 mM K^+ nutrient solution, and the ΔPD was recorded until a negative peak was reached. A change was then made back to the 4 K^+ reference, and the above steps were repeated until changes had been made to each of the higher K^+ concentrations. A similar procedure was used with 79 mM K^+ nutrient solution as the reference. In the latter case changes were made to solutions of lower K^+ concentrations; instead of peak ΔPD's, inflection points (similar to that seen in Fig. 3 for the 79 to 4 K^+ response) were obtained. The best estimate of a total response was again taken as the ΔPD occurring at a peak or an inflection point.

The results are plotted in Fig. 5 (5 experiments); the length of the vertical lines represents the standard error of the mean. It can be seen that with 4 mM K^+ as the reference solution the value of G^+, the absolute value of the slope, was greater than it was with 79 mM K^+ as the reference. The average value for G^+ in the 10 to 79 mM range was 36.3 mv (SD ± 4.0) with 4 mM K^+ while with 79 mM K^+ it was 27.4 mv

(SD ± 2.1, difference significant, $P < 0.01$). In other words, these results are consistent with the hypothesis that in the presence of a high K^+ concentration, the relative K^+ conductance of the nutrient membrane decreases.

Effect of Changes in Cl^- Concentration in the Nutrient Fluid

The above analysis is applicable to any ion that contributes substantially to the conductance of the nutrient membrane. Fig. 6 shows an experiment in which the Cl^- concentration was changed from 81 to 4 mM and back again. It can be seen

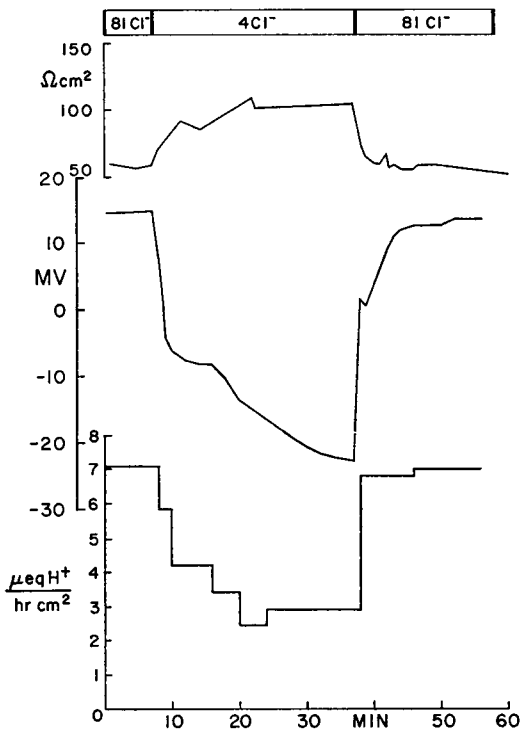

FIGURE 6 Effect on the PD, resistance and H^+ secretory rate of changing Cl^- in the nutrient solution from 81 to 4 mM and back to 81 mM.

that in going from 81 to 4 mM Cl^- the PD decreased and reached a relatively constant level in about 6 min. and then started decreasing again at about 9 min. after the change. The delayed change in PD occurred in four experiments; in two experiments the PD following the initial change remained relatively constant over the subsequent 10–20 min. period, and in one experiment it reached a negative peak and began to ascend. The increase in resistance shown here was atypical: in this experiment the control resistance was lower and the H^+ rate higher than average. An analysis by the method of paired variates indicates no significant change in the resistance (average $\Delta R = -2.1$ ohm cm², SD ± 21.6, $P < 0.5$, 7 experiments).

The H^+ rate in these experiments decreased and reached a steady state that was 60.5% (SD ± 14.2) of its value before the change and 65.9% (SD ± 19.5) of its subsequent value in regular nutrient solution (the H^+ secretory rate was significantly lower in 4 mM Cl^- than in 81 mM Cl^-, $P < 0.01$). Following the change back to 81 mM Cl^-, the PD rapidly increased to about two-thirds of the control level, paused, and gradually increased to within a few millivolts of the original level. The pause was seen in two experiments, a return to a constant level in one, and a peak response in four. By the end of the phase of the rapid change in PD, the H^+ rate started to increase and reached within a few minutes a new steady-state level not significantly different from the original level.

Plots of the normalized ΔPD vs. time for the Cl^- experiments again showed a time lag similar to that predicted on the basis of the model. In seven experiments the average time for one-half of the total PD response in going from 81 to 4 mM chloride was 165 sec (SD ± 82.7) and for the 4 to 81 mM changes, 21.1 sec (SD ± 9.1). The average G^-, $|\Delta PD/\log_{10}(Cl_i^-/Cl_f^-)|$, for the 81 to 4 mM Cl^- change was 18.7 mv (SD ± 1.6) and for the reverse change, 16.2 mv (SD ± 4.0 mv). Since the relative K^+ conductance of the nutrient membrane with 4 mM K^+ is greater than with 79 mM K^+ as the nutrient fluid, one might expect the relative Cl^- conductivity to be increased in the presence of high K^+. Three experiments were performed in which the nutrient Cl^- was changed from 81 to 4 mM, first in the presence of a nutrient fluid with 81 mM K^+ and then in the presence of the regular nutrient fluid (4 mM K^+). The average G^- for the first change was 27 mv while the average for the controls with 4 mM K^+ was 20.5 mv. The differences in G^- in the individual experiments were 6.4, 7.3, and 5.7 mv. These results are further evidence showing that the characteristics of the nutrient membrane change when the nutrient K^+ concentration is increased.

Effect of Simultaneous Changes of K^+ and Cl^- such that the Product of their Concentrations Remains Constant

From the data presented above, the values of G^+ and G^- are about 38 and 19 mv, respectively, when the regular 4 K^+ nutrient solution is the reference solution. The fact that the sum of G^+ and G^- is 57 mv indicates that the relative conductivity of the nutrient membrane to other ions is essentially zero. The question naturally arises as to whether the use of the "product constant" technique of Hodgkin and Horowitz (8) would yield a similar value. With this technique the Cl^- and K^+ concentrations are simultaneously changed, but the product of their concentrations is held constant. Experiments were performed in which the nutrient solution was changed from 4 mM K^+, 81 mM Cl^- to 81 mM K^+, 4 mM Cl^- and then back again, and a typical experiment is shown in Fig. 7. In 15 experiments, the average value for the sum of G^+ and G^- was 56.3 mv (SD ± 7.8) for the change from 4 K^+, 81 Cl^- to 81 K^+, 4 Cl^-, and 50 mv (SD ± 5.8) for the reverse change. There was a decrease

in H^+ secretory rate during the period of high K^+ and low Cl^-; the average H^+ rate during this period was 34.6% (SD ± 7.9) of the previous control level and 40.8% (SD ± 10.9) of the subsequent control level.

A normalized plot of a typical "product constant" experiment is shown in the right graph of Fig. 8. It can be seen that there is only a small time lag between the

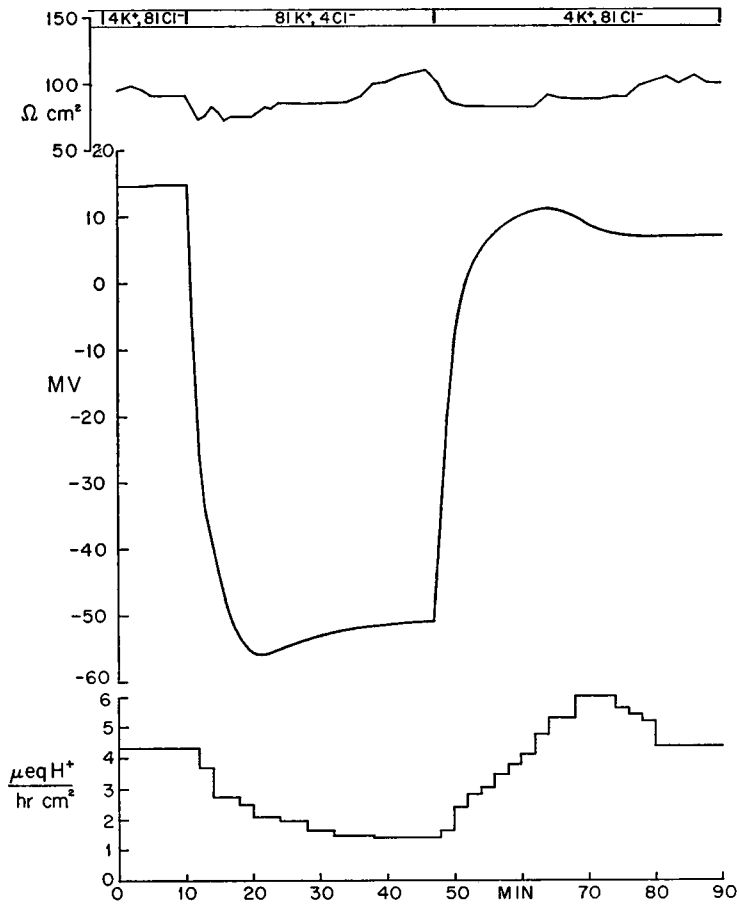

FIGURE 7 Effect on the PD, resistance, and H^+ secretory rate of changing the nutrient solution from 4 mM K^+, 81 mM Cl^- to 81 mM K^+, 4 mM Cl^- and back to 4 mM K^+, 81 mM Cl^-.

PD response to the changes in one direction as compared to those in the opposite direction. The average time for one-half of the total ΔPD for the change from 4 K^+, 81 Cl^- to 81 K^+, 4 Cl^- was 78.3 sec (SD ± 10.4) and for the opposite change 83.5 sec (SD ± 12.3).

It is clear from equation 13 that if G^+ and G^- are equal, the PD response for a change in one direction would be exactly the same as for the reverse change. Calculations on the basis of the model were made with the assumptions that $G^+ = 38$

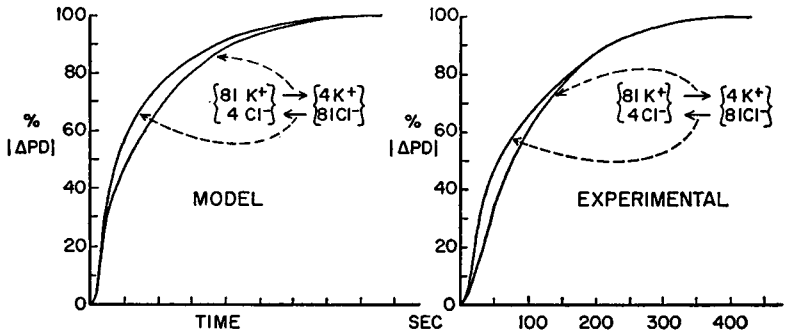

FIGURE 8 The right graph represents normalized experimental plots of the PD response when the nutrient solution is changed from 4 mM K^+, 81 mM Cl^- to 81 mM K^+, 4 mM Cl^- and for the reverse change. The left graph represents normalized responses calculated on the basis of the model.

and $G^- = 19$ for the change from 4 K^+, 81 Cl^- to 81 K^+, 4 Cl^- and $G^+ = 25$ and $G^- = 25$ for the reverse change. The responses are plotted in the left graph of Fig. 8. Calculations with other assumptions regarding the values of G^+ and G^- illustrate the prediction that the time course of the response to changes in one direction is very similar to that resulting from changes in the opposite direction when G^+ and G^- are not markedly different.

Calculations of the Time Constant for Diffusion Across the Nutrient Barrier

Table I shows values of α calculated from the time for half of the total change in PD following a change in the ionic composition of the nutrient fluid. In the case of

TABLE I

VALUES OF α, D, AND THE DIFFUSION TIME CONSTANT CALCULATED FOR EACH CHANGE FROM THE AVERAGE TIME REQUIRED FOR HALF OF THE TOTAL PD RESPONSE

Change in nutrient solution	Time for one-half total ΔPD	α	Time constant $T = 1/\alpha$	No. of experiments	$D \times 10^5$ cm^2 sec^{-1}
mM	*sec*	sec^{-1}	*sec*		
4 → 79 K^+	26.9 SD ± 9.2	0.017	59	13	0.43
79 → 4 K^+	62 SD ± 25.1	0.032	31	13	0.81
81 → 4 Cl^-	165 SD ± 82.7	0.021	48	7	0.53
4 → 81 Cl^-	21.1 SD ± 9.1	0.012	83	7	0.30
4 K^+, 81 Cl^- → 81 K^+, 4 Cl^-	73.8 SD ± 10.4	0.0091	110	10	0.23
81 K^+, 4 Cl^- → 4 K^+, 81 Cl^-	83.5 SD ± 9.9	0.011	93	10	0.28

calculations involving changes between the 4 mM K^+, 81 mM Cl^- and 81 mM K^+, 4 mM Cl^- solutions it was necessary to use values for G^+ and G^- obtained in the experiments on single ion changes. It can be seen that the values for the time constant (the inverse of α) for the concentration changes (not the time constant for the PD changes) at the nutrient membrane range from 32 to 110 sec.

Calculations of D (in this case the empirical diffusion coefficient) were made by substituting the values of α in equation 6 on the assumption that the thickness of the diffusion barrier is 2.5×10^{-2} cm (on the basis of unpublished observations). The values of D ranged from 0.23×10^{-5} to 0.81×10^{-5} cm^2 sec^{-1}, the maximum value being about one-half of the value of D for KCl and NaCl in free solution.

Effect of Changes in Composition of Bathing Media on the PD Across Scraped Mucosae and External Muscle Layers

It is possible that the response of the PD to changes in the ionic environment might be due in part to changes in the PD across the muscularis mucosa. In order to test

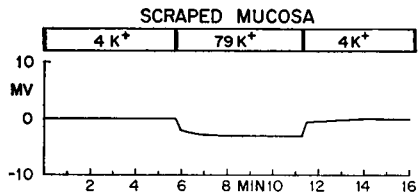

FIGURE 9 Effect on the PD across a scraped mucosa with nutrient solution on both sides when the K^+ on the anatomical nutrient side is changed from 4 to 79 mM and back to 4 mM.

this possibility, scraped mucosae were mounted between the chambers and bathed on both sides by the 4 K^+ nutrient solution. Both the gastric mucosa and the scraped gastric mucosa possess spontaneous motility, and the scraped mucosa can develop about as much tension as the intact mucosa (unpublished observations), indicating that there is a substantial amount of viable smooth muscle tissue remaining after scraping. The effect on the PD of changing the K^+ on the anatomical nutrient side was determined, and a typical experiment is shown in Fig. 9. The PD rapidly changed following both the change from 4 to 79 mM K^+ and the reverse change. The magnitude and orientation of these changes are those predicted on the basis of measurements of the liquid junction potential between the two nutrient solutions. Similar experiments were performed with the external muscle layers mounted between the chambers, and the results were the same as with the scraped mucosae. It is concluded that, apart from transient liquid junction potentials, the changes in PD of the gastric mucosa resulting from changes in the ionic composition of the nutrient fluid occur at the mucosal cell layer and not in either the submucosa or muscularis mucosa.

DISCUSSION

The situation in the in vitro frog gastric mucosa is considerably more complex than that in the idealized model. The following is a list (together with appropriate com-

ments) of the main factors that might alter the magnitude and the time course of the ΔPD from that predicted on the basis of the model:

(*a*) The muscularis mucosa possesses a spontaneous motility which could result in a "to and fro" type of bulk movement and influence the movement of ions across this barrier (9, 10, 11).

(*b*) A small elevation in K^+ concentration of the nutrient fluid increases the tone of the muscularis mucosa while a large increase produces a marked decrease in tone, i.e., the geometry of the barrier would not necessarily remain constant in the face of changing K^+ concentrations (unpublished work of White, Sanders, and Rehm).

(*c*) Examinations of frozen sections of mucosae revealed that the thickness of the muscularis mucosa was relatively constant, but that of the submucosa varied from region to region (unpublished observations).

(*d*) The nutrient fluid is rapidly recirculated and the flow of fluid past the submucosal surface might cause a rhythmic deformation of the barrier which would result in bulk flow within the barrier.

(*e*) Following a change in ionic composition, the PD does not approach a limiting value in the exponential fashion predicted on the basis of the model, but may peak or pass through an inflection point. Changes occurring before a peak or an inflection are associated with changes predicted by the model, and changes occurring after a peak or an inflection point are interpreted as due to changes in the parameters of the system, e.g., changes in membrane conductance or emf's associated with HCl secretion. When these effects overlap each other, the choice of the total ΔPD resulting from a change in ionic gradients across the nutrient membrane becomes somewhat arbitrary. In the experiment in Fig. 6 the ΔPD occurring during the first 9 min. after a change of Cl^- from 81 to 4 mM is that predicted on the basis of the model, whereas the subsequent change in PD is undoubtedly due to changes of the parameters of the system.

(*f*) We made the assumption that the net movement of an ion across the nutrient membrane is negligible during the transient change in PD. Calculations which will not be given here reveal that this assumption is not completely justified. However, in the experiment shown in Fig. 6 it is unlikely that during the first 4 or 5 min. after the change to 4 Cl^-, an appreciable Cl^- moved from the cell across the nutrient membrane into the diffusion barrier.

(*g*) The liquid junction potential between the old and new solutions in the barrier would vary with time and it would be necessary to correct for it in order to accurately determine the time for the transmembrane ΔPD to reach one-half its total value. This is a complicated calculation; since the error involved would be small and the main point of interest in this analysis is the difference in PD between the control value and the value after the concentration at the nutrient membrane approximates that of the new solution, we have not attempted to make the correction.

(*h*) The smooth muscle cells in the barrier could act as a "source" or "sink" for ions during the transient.

(*i*) In the model of Fig. 4 the assumption that the mucosal cell layer is a flat sheet of cells is an oversimplification.

(*j*) It is implicitly assumed that the nutrient membrane is homogeneous. There are two cell types in the mucosal cell layer: the surface epithelial cells and the tubular cells, so two different nutrient membranes are involved (12).

On the basis of the above factors it is not surprising that the values of α of equations 5 and 6 vary from one type of ion change to the next (Table I). In spite of the variation, the facts are that the time courses of the changes in PD are very similar to those predicted on the basis of the model and that $G(K^+) + G(Cl^-)$ equals approximately 57 mv for both the single ion and "product constant" experiments. Furthermore, it has been shown that changes in the concentrations of HCO_3^-, OH^-, H^+, Na^+, and Ca^{++} in the 4 mM K^+ nutrient fluid produce essentially no change in PD (5, 13, and unpublished work of Spangler, Sanders, and Rehm). These findings are compatible with the conclusion that the sum of the K^+ and Cl^- conductances represents the total conductance of the nutrient membrane.

Harris and Edelman (2) studied the effects of changes in ionic composition of the nutrient media on the PD and used values of the ΔPD 20–40 min. following the change in ionic composition. Hogben (3) has contended that the changes in PD observed by Harris and Edelman could be due to changes in the parameters of the system and may not yield reliable information concerning the relative ion conductances of the nutrient membrane. On the basis of the evidence presented in this paper, the smaller values of the ΔPD's used by Harris and Edelman (20–40 min. after the change in concentration) are interpreted as due in part to changes in the parameters of the system. Our work is an extension of the work of Harris and Edelman and provides substantial support for their conclusion that the conductance of the nutrient membrane is primarily due to the sum of the conductances of the potassium and chloride. On the basis of their work alone, however, one might conclude that in the presence of 4 mM K^+ the conductance of the nutrient membrane to other ions is appreciable.

One may still contend that our explanation is not unique and that the observed transient changes in PD are caused by a mechanism other than that described in this paper. We readily agree that any additional model capable of accurately predicting the experimental results presented above deserves equally serious consideration.

One of the purposes of this work was to determine the time constant for diffusion of KCl into and out of the nutrient diffusion barrier in order to lay a foundation for a more rational approach for the determination of the extracellular fluid volume on the nutrient side of the mucosa. In contrast to other tissues, such as striated muscle, determinations of the extracellular fluid volume for the frog's gastric mucosa are

unsatisfactory. Inulin, radioiodinated serum albumin, sucrose, and other solutes have "spaces" that show wide variation (14, 15). In most of the work it is assumed that equilibration of the tag with the extracellular space would only be complete over a period of hours. On the basis of the values of α given in Table I it would appear that K^+ and Cl^- would equilibrate with the nutrient extracellular space in about 5 min. (a period equal to about five time constants). Furthermore, sucrose would be expected to have a time constant of about 4 min.; thus, experiments could be designed on a more meaningful basis than heretofore.

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