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Cl^- Diffusion Potential of Nutrient Membrane of Frog Gastric Mucosa (40698)

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Forte *et al.* (1) were the first to show that isethionate ion like SO_4^{2-} replacing Cl^- in the bathing media of the *in vitro* frog gastric mucosa reversed the transmembrane potential difference (PD) across the mucosa. In another aspect of their investigation, changing from 15 to 60 m*M* Cl^- in the nutrient solution with isethionate as the replacement ion for Cl^-, they obtained a Cl^- diffusion potential of 54 mV per 10-fold change in Cl^- concentration. The latter approached the value of 58 mV predicted by the Nernst equation. In a model involving conductance pathways on the nutrient membrane (2), their results would suggest that the Cl^- conductance pathway was the only high conductance pathway across the nutrient membrane. On the other hand, Spangler and Rehm (2) obtained a diffusion potential of 19 mV using SO_4^{2-} as the replacement ion for Cl^-. Their results suggest that Cl^- contributes about one-third to the total conductance of the nutrient membrane. In the work of Spangler and Rehm (2), the relative conductance of an ion was assessed by determining the effect on the PD resulting from rapid changes of the ionic composition of the nutrient fluid. According to their considerations, if readings of the PD were taken during the first few minutes, there would be no marked changes in the characteristics of the mucosa arising from ionic changes. If, then, this method were applied to isethionate as well as SO_4^{2-} as the replacement ion for Cl^-, would the Cl^- diffusion potential give essentially the same results for both replacement ions or might differences still arise due to differences in permeability between isethionate and SO_4^{2-}? The results of this investigation are described herein.

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described in detail elsewhere (3). Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was obtained as the change in PD per unit of applied current. The H^+ secretory rate was determined by the pH stat method introduced by Durbin and Heinz (4). The pH of the secretory side was maintained at 4.90. The regular nutrient bathing solution contained (in m*M*): Na^+, 102; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81; HCO_3^-, 25; phosphate, 1.0; and glucose, 10; and the secretory bathing solution contained: Na^+, 102; K^+, 4; and Cl^-, 106. Both sides of the mucosa were gassed with 95% O_2 and 5% CO_2. Histamine was added to the nutrient solution to a concentration of 10^{-4} *M*. Isethionate or SO_4^{2-} replaced Cl^-. For experiments with divalent ions, the sucrose concentration was altered to insure isosmolality. Generally the nutrient solution was drained and washed once with the new solution. The first PD reading could be taken about 15 sec after the change. Further details are provided elsewhere (2).

The PD of the nutrient side is considered relative to the secretory side.

Results. Figure 1 represents the results of a typical experiment in which the nutrient solution containing 6 m*M* Cl^- was replaced with one containing 81 m*M* Cl^-. In the first part of the experiment, SO_4^{2-} replaced Cl^- in the nutrient solution and, in the second part, isethionate replaced Cl^-. The change to 81 m*M* Cl^- caused the PD to attain a maximum value within 2–3 min. The maxima were, respectively, about 26 and 22 mV above the control value. During this period, the resistance showed a small decrease. The H^+ secretory rate increased to a small extent during the first part and showed no change in the second part. Generally, the H^+ secretory rate showed a small but significant increase. Reversal of ion replacement (that is, isethionate first and SO_4^{2-} second) gave essentially the same results.

Table I summarizes the results of three kinds of concentration changes in the nutrient solution: (i) from 6 m*M* Cl^- to 81 m*M* Cl^-, (ii) from 81 m*M* Cl^- to 6 m*M* Cl^-, and (iii)

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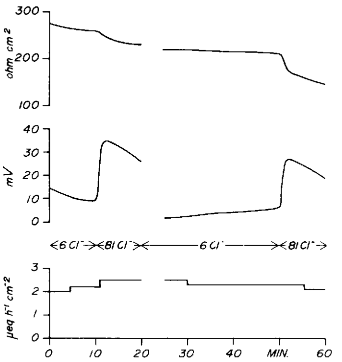

FIG. 1. Effect of changing 6 m*M* Cl^- in nutrient solution to 81 m*M* Cl^- on resistance, PD, and H^+ secretory rate versus time. In the first part (0 to 10 min) SO_4^{2-} replaced Cl^-. The break in the curve represents several washings of the nutrient membrane with 6 m*M* Cl^- and isethionate ion in place of Cl^-.

from 15 m*M* Cl^- to 60 m*M* Cl^- for both isethionate and SO_4^{2-} in place of Cl^-. The results were averaged irrespective of the order of ion replacement. We note further that, for changes from low to high concentrations of Cl^-, the maximum change in PD was recorded as well as the corresponding changes in resistance and H^+ secretory rate. For changes from high to low concentrations of Cl^-, the data at inflection points were recorded as explained in the discussion.

We first note that the change in PD never exceeded 20 mV per 10-fold change in Cl^- concentration for either SO_4^{2-} or isethionate ion. The resistance either did not change or showed a small significant change. The H^+ secretory rates showed similar results. In experiments of Spangler and Rehm in which Cl^- was changed in the nutrient solution from 81 m*M* Cl^- to 4 m*M* Cl^- using SO_4^{2-} as the replacement ion, they obtained a decrease in PD of 18.7 mV per 10-fold change and no significant change in resistance. For similar changes in concentration, our results gave similar changes in PD and resistance. From Table I, line 2, we determined that, for a 10-fold change, the PD decreased by 19.3 mV compared to 18.7 mV and that the change in resistance was not significant. Their data suggest that a decrease in H^+ secretory rate is expected, as indicated in our Table I. For reverse changes in concentration, they obtained an increase in PD of 16.2 mV compared to our increase of 17.9 mV per 10-fold change. Both groups found that |ΔPD| for changes from low to high concentrations of Cl^- was less than |ΔPD| for the reverse changes. Moreover, isethionate gave values of |ΔPD| somewhat less than those obtained with SO_4^{2-}.

Lastly, in changes in Cl^- concentration from 15 to 60 mV, we obtained, according to the data of Table I, ΔPD = 20 mV for SO_4^{2-} replacing Cl^- and ΔPD = 18 mV for isethionate replacing Cl^- per 10-fold change in con-

TABLE I. EFFECTS OF THE CHANGE IN Cl^- CONCENTRATION IN THE NUTRIENT SOLUTION ON RESISTANCE, PD, AND H^+ SECRETORY RATE OF THE FROG GASTRIC MUCOSA[a]

Original (m*M*)	Final (m*M*)	No. of Expts	*R* (Ω-cm²)	Δ*R* (Ω-cm²)	PD (mV)	ΔPD (mV)	$\dot{H}$ (μeq hr⁻¹cm⁻²)	Δ$\dot{H}$ (μeq hr⁻¹cm⁻²)
				Cl^- substituted with SO_4^{2-}				
6	81	17	197 ± 55	−30 ± 23	−5.1 ± 8.4	20.2 ± 5.3	2.73 ± 0.80	0.48 ± 0.53
				($P < 0.01$)		($P < 0.01$)		($P < 0.01$)
81	6	12	191 ± 67	11 ± 26	24.5 ± 6.5	−21.8 ± 3.7	4.68 ± 1.73	−0.52 ± 0.52
				($P > 0.10$)		($P < 0.01$)		($P < 0.01$)
15	60	7	139 ± 49	−17 ± 15	−1.4 ± 5.9	12.1 ± 1.9	4.72 ± 1.52	0 ± 0
				($P < 0.05$)		($P < 0.01$)		($P = 1.0$)
				Cl^- substituted with isethionate ion				
6	81	17	187 ± 59	−24 ± 24	−6.1 ± 9.1	19.7 ± 4.7	2.83 ± 0.81	0.38 ± 0.48
				($P < 0.01$)		($P < 0.01$)		($P < 0.01$)
81	6	10	224 ± 59	8 ± 13	24.9 ± 5.8	−18.7 ± 2.5	3.84 ± 0.89	−0.23 ± 0.29
				($P > 0.05$)		($P < 0.01$)		($P < 0.05$)
15	60	7	148 ± 70	−22 ± 19	−0.2 ± 6.8	10.9 ± 1.1	4.56 ± 0.94	0.12 ± 0.24
				($P < 0.05$)		($P < 0.01$)		($P > 0.80$)

[a] Values are means ± SD. Columns labeled *R*, PD, and $\dot{H}$ refer, respectively, to the control values of resistance, transmembrane potential difference, and H^+ secretory rate. Columns labeled Δ*R*, ΔPD, and Δ$\dot{H}$ refer to changes in the three parameters following the change to the final solution, values corresponding to the maximum |ΔPD| or the PD at an inflection point. *P* values were calculated using the paired-variates method of the Student's *t* test.

centration. These changes differ markedly from the 54 mV change reported by Forte *et al.*

Discussion. To explain the difference in values pertaining to the Cl^- diffusion potential, we refer to the work of Spangler and Rehm (2). They found that the time course for a rapid change in concentration was essentially that predicted on the basis of a model comprising a membrane in series with a diffusion barrier. From their analyses, it is evident that we are mainly concerned with the changes that occur in the first few minutes since it appears that the characteristics of the mucosal cells do not alter substantially during this period. For a change from a low to a high concentration of a permeant ion, they showed that $|\Delta PD|$ versus time passed through a maximum. For a change from a high to a low concentration, $|\Delta PD|$ versus time gave an inflection point, although, in actual practice, the PD sometimes leveled off for several minutes before again changing. In Table I, we used these criteria recording data at maxima and inflection points.

In terms of this model, we shall now attempt to explain the differences between Spangler and Rehm using SO_4^{2-} as the replacement ion for Cl^- and Forte *et al.* using isethionate. From our data, it is evident that any permeability differences between SO_4^{2-} and isethionate give only minor differences in the values of ΔPD per 10-fold change in Cl^- concentration. Therefore, while Spangler and Rehm observed the changes in PD shortly after the substitution, it would appear that Forte *et al.* observed the changes in PD, although not specifically stated in their paper, at or near the new steady state of the more concentrated Cl^- solution. Thus, the small change in H^+ secretory rate which they observed does not assure that the increased Cl^- concentration will not eventually affect the electrogenic Cl^- pump (3) located in the secretory membrane. Hence, if the measurements are made later than the first few minutes, the effect obtained may be largely due to the Cl^- pump itself or other changes induced by the new Cl^- concentration.

Our data in conformity with those of Spangler and Rehm indicate that the Cl^- conductance pathway contributes about one-third to the total conductance of the nutrient membrane in regular Cl^- nutrient solutions bathing the frog gastric mucosa.

Summary. Replacement of Cl^- with SO_4^{2-} gives a Cl^- diffusion potential of about 19 mV across the nutrient membrane per 10-fold change in Cl^- concentration, suggesting, on the basis of the Nernst equation, that the Cl^- conductance pathway contributes about one-third to the total conductance of this membrane in regular Cl^- solutions bathing the frog gastric mucosa. Isethionate ion gives a change in PD of about 17 mV per 10-fold change which is quite close to the value obtained with SO_4^{2-}.

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