

Further Evidence of Na^+ Conductance in Frog Stomach in Cl^- -free Media (40528)

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In Cl^- -free solutions bathing both sides of the frog gastric mucosa, definitive experimental evidence confirmed the concept of an electrogenic H^+ pump (1, 2). Moreover, Davis *et al.* (3) showed that there exists a K^+ pathway on the secretory membrane which serves as a return pathway for the current flow of H^+ . Recently Holloman *et al.* (4) showed that there is a Na^+ return pathway. In the absence of K^+ and Na^+ in the secretory solution, the H^+ rate did not decrease to zero. This fact suggested that there is a return pathway other than that of K^+ and Na^+ , presumably SO_4^{2-} . The existence of return pathways lends support to the electrogenic theory in Cl^- -free media.

In previous work mentioned above (4), the replacement of Na^+ by Mg^{2+} in the secretory solution proved the existence of a Na^+ return pathway. More currently it was found that Mg^{2+} and Ca^{2+} may block K^+ (5). Because of this complicating factor, it was decided to test the existence of a Na^+ return pathway by replacing Na^+ with choline in the secretory solution instead of with Mg^{2+} . The results of this investigation are described herein. A preliminary report is given elsewhere (6).

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described in detail elsewhere (7). 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 (8). The pH of the secretory side was maintained at 4.95. In Cl^- -free preparations, the regular nutrient bathing solution contained (in mM): Na^+ , 101; K^+ , 4; Ca^{2+} , 1; Mg^{2+} , 0.8; SO_4^{2-} , 41.3; HCO_3^- , 25; phosphate, 1; glucose, 10; and sucrose, 40; and the regular secretory solution contained: Na^+ , 104; SO_4^{2-} , 52; and sucrose, 64. Both sides of the mucosa were gassed with

95% O_2 and 5% CO_2 . In these experiments, the nutrient solution was never changed. The secretory solution was changed from 52 mM Na_2SO_4 to 52 mM choline sulfate and vice versa.

It is to be noted that the PD of the nutrient side is considered relative to the secretory side. An increase in PD denotes that the nutrient side becomes more positive or less negative relative to the secretory side. In SO_4^{2-} media used in these experiments, the latter applies.

Results. *Replacement of choline by sodium and vice versa.* As shown in Fig. 1, the replacement of 52 mM choline sulfate with 52 mM Na_2SO_4 in the secretory solution results in an increase in the three electrophysiological parameters, namely, increases in the resistance, PD, and H^+ secretory rate. If the 52 mM Na_2SO_4 is replaced in turn with 52 mM choline sulfate in the secretory solution, the parameters then decrease toward control or near control levels. Experiments were also performed in the reverse order. In this case, after Na_2SO_4 was present for 30 min in the secretory solution, it was replaced by choline sulfate for 30 min in this solution and finally it was placed back again for 30 min. The results of the latter experiments on the three parameters were, in general, the reverse of those illustrated in Fig. 1.

Table I summarizes the effects on the resistance, PD, and H^+ secretory rate of the replacement of choline sulfate with Na_2SO_4 in 14 experiments in 7 of which Na_2SO_4 was subsequently replaced with choline sulfate. The table further summarizes the effects on the same parameters of the replacement of Na_2SO_4 with choline sulfate in 12 experiments in 7 of which the choline sulfate was subsequently replaced with Na_2SO_4 . The values 30 min after the replacement are compared in each case with the values just prior to the replacement. As Table I indicates, replacement of choline sulfate in stage one with

Na₂SO₄ in stage two in the 14 experiments increased the resistance on the average by 162 ohm-cm², the PD by 8.9 mV (see end of Methods) and the H⁺ secretory rate by 0.44 μeq h⁻¹ cm⁻². When choline sulfate in stage two was substituted by Na₂SO₄ in stage three, as depicted in line 4 of the table, the results were essentially the same as those in line 1. Replacement of Na₂SO₄ with choline sulfate, whether it took place from the first to second stage (line 3) or from the second to third stage (line 2), results in decreases in resistance, PD, and H⁺ secretory rate except that the change of resistance for the substitution from the first to second stage was not statistically significant in this case (*P* > 0.10).

Discussion. The experimental data can be explained in terms of the conductance path-

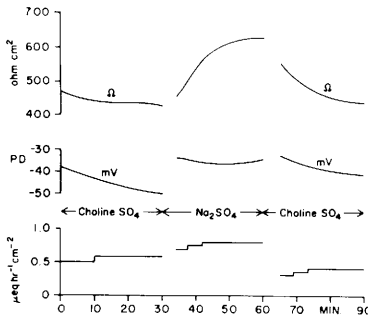


FIG. 1. Effect of changing 52 mM choline sulfate to 52 mM Na₂SO₄ in secretory solution and back to 52 mM choline sulfate on resistance, PD, and H⁺ secretory rate vs time.

ways depicted in Fig. 2. As mentioned in the introduction, the presence of Na⁺ in the secretory solution facilitates the secretion of H⁺ in sulfate media, that is, there is a Na⁺ conductance return pathway on the apical membrane associated with the H⁺ secretory pathway (4). These two pathways are depicted in Fig. 2 except that, for this case, the Na⁺ emf has reverse polarity. When Na⁺ is removed from the secretory solution (e.g., substitution with choline), the Na⁺ diffusion potential across the secretory membrane becomes more positive in the lumen with reference to the cell, that is, the Na⁺ emf, as depicted in Fig. 2, will increase.

In order to explain the results more fully, we refer to the following equations associated

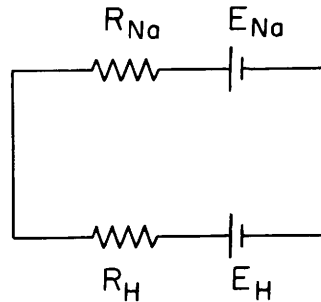


FIG. 2. Equivalent electric circuit representing the H⁺ pathway comprising an emf *E_H* and resistance *R_H* in parallel with a Na⁺ pathway comprising an emf *E_{Na}* due to a concentration gradient and resistance *R_{Na}* in the secretory (apical) membrane of the frog gastric mucosa. The left side of the figure faces the lumen of the stomach.

TABLE I. COMPARISON OF EFFECTS OF CHOLINE SULFATE WITH Na₂SO₄ IN SECRETORY SOLUTION ON PD, RESISTANCE, AND H⁺ SECRETORY RATE OF FROG GASTRIC MUCOSA

Secretory solution		Number of expt	<i>R</i> (Ω-cm ²)	Δ <i>R</i> (Ω-cm ²)	PD (mV)	ΔPD (mV)	<i>H</i> (μeq hr ⁻¹ cm ⁻²)	Δ <i>H</i> (μeq hr ⁻¹ cm ⁻²)
Original ^a	Final							
Choline → Na → choline								
Choline 104 ^b	Na 104	14	374 ± 65 ^c	162 ± 114 (<i>P</i> < 0.01)	-36 ± 9	8.9 ± 6.4 (<i>P</i> < 0.01)	0.29 ± 0.34	0.44 ± 0.40 (<i>P</i> < 0.01)
Na 104	Choline 104	7	506 ± 79	-86 ± 75 (<i>P</i> < 0.05)	-33 ± 2	-5.0 ± 4.4 (<i>P</i> < 0.05)	0.78 ± 0.17	-0.57 ± 0.26 (<i>P</i> < 0.01)
Na → choline → Na								
Na 104	Choline 104	12	517 ± 208	-42 ± 83 (<i>P</i> > 0.10)	-31 ± 9	-7.6 ± 5.4 (<i>P</i> < 0.01)	0.86 ± 0.28	-0.73 ± 0.43 (<i>P</i> < 0.01)
Choline 104	Na 104	7	505 ± 292	150 ± 82 (<i>P</i> < 0.01)	-44 ± 11	9.9 ± 5.4 (<i>P</i> < 0.01)	0 ± 0	0.81 ± 0.25 (<i>P</i> < 0.01)

^a Original refers to stage of reference: first stage in lines 1 and 3 and second stage in lines 2 and 4. Final refers to the stage following the original stage.

^b Concentrations are in mM.

^c Values are means ± SD. Columns labeled *R*, PD, and *H* refer, respectively, to the transmucosal resistance, transmucosal potential difference, and H⁺ secretory rate of the original solutions. Columns labeled Δ*R*, ΔPD, and Δ*H* refer to changes produced by the final secretory solution 30 min after replacement.

with the electrical circuit of Fig. 2, giving the PD and H⁺ current (I_H) across the secretory membrane.

$$PD = -E_{Na} - \frac{E_H - E_{Na}}{R_H + R_{Na}} R_{Na}, \quad [1]$$

$$PD = -E_H + \frac{E_H - E_{Na}}{R_H + R_{Na}} R_H, \quad [2]$$

$$I_H = \frac{E_H - E_{Na}}{R_H + R_{Na}}. \quad [3]$$

We first note that the experimental decrease in the measured resistance implies that R_{Na} and/or R_H decreased. A decrease in R_{Na} would result in an increase in PD, as is evident from the first two equations and contrary to observation. The observed decrease in resistance would also result in an increase in I_H , as is evident from Eq. [3], also contrary to observation. The experimental decrease in I_H implies from Eq. [3] that either the magnitude of E_H decreases and/or the magnitude of E_{Na} increases. From Eq. [1] it follows that a decrease in the magnitude of E_H results in an increase in PD and, from Eq. [2], it follows that an increase in the magnitude of E_{Na} results in a decrease in PD. Hence the increase in the magnitude of E_{Na} is the dominant factor in explaining the decreases in PD and H⁺ secretory rate.

In addition to the Na⁺ and H⁺ pathways, other return pathways, as previously shown can exist (4). For example, the decrease in H⁺ secretory rate with an increase in resistance resulting from the substitution of Mg²⁺ for Na⁺ was previously attributed to the presence of a Mg²⁺ and/or SO₄²⁻ pathway of very high resistance. On the other hand, if a choline conductance pathway existed, the orientation of the emf in this pathway and the decrease in resistance observed upon substitution of Na⁺ with choline would have resulted in an increase in the H⁺ secretory rate, contrary to observation. Therefore, the decrease in resist-

ance is not mainly due to a high conductance choline pathway, but an effect of choline on other conductance pathways such as the H⁺ and/or Na⁺ pathways.

The results of these experiments provide further evidence for the existence of a Na⁺ conductance pathway across the apical membrane of the frog gastric mucosa. The existence of such pathways lends support to the electrogenic theory of H⁺ secretion in Cl⁻ free solutions (1, 2).

Summary. The replacement of choline ions by Na⁺ ions in the secretory solution of the frog gastric mucosa of *Rana pipiens* in Cl⁻ free media increases the resistance, the PD, and the H⁺ secretory rate. The replacement of Na⁺ by choline ions produces generally the reverse changes of the three electrophysiological parameters. The experimental data involving the PD and H⁺ secretory rate can be explained in terms of a Na⁺ emf in a Na⁺ conductance pathway opposing the H⁺ emf in a H⁺ conductance pathway on the secretory (apical) membrane.

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