A Confirmation of Electrogenicity of Frog Gastric Mucosa by Using Methoxyflurane (33717)

MANUEL SCHWARTZ AND THOMAS N. MACKRELL
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Departments of Engineering Physics and Anesthesiology, University of Louisville, Louisville, Kentucky 40208

Rehm and LeFevre (1) presented strong evidence for the electrogenic mechanism of gastric secretion, as postulated by the separate-site theory (2). They showed that the inhibitory action of dinitrophenol on gastric secretion of frog gastric mucosae bathed in Cl^- free solutions gave rise to a linear relation between the transmembrane potential difference (PD) and the H^+ secretory rate. This, in turn, permitted estimates of circuit parameters of the H^+ secretory mechanism. This analysis was made possible by the fact that DNP, in contrast to other inhibitors of acid secretion such as thiocyanate (3, 4), affected only the H^+ mechanism in Cl^--free solutions.

Recently MacKrell and Schwartz (5) reported that the volatile anesthetic methoxyflurane (also known as Penthrane) inhibited the H^+ and Cl^- mechanisms of frog gastric mucosae bathed in Cl^- solution and the H^+ mechanism, in Cl^--free solutions. An aspect of this work which was not reported is that methoxyflurane, like DNP, affecting only the H^+ mechanism in Cl^--free solutions, gives rise to the linear relation between the PD and the H^+ rate. It is the purpose of the present paper to give confirmatory evidence of the electrogenic concept. A preliminary report of this work is given elsewhere (6).

Methods. The experiments were performed on gastric mucosae of *Rana pipiens* with an *in vitro* method described elsewhere (2). Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was determined as the change in PD per unit of applied current. In Cl^--free preparations, the nutrient bathing solution contained (m*M*): 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 secretory solution: Na^+, 100; SO_4^{2-}, 50; and sucrose, 100. During the control part of the experiment, both sides were gassed with 95% O_2 and 5% CO_2 whereas, after the application of the anesthetic, say 0.2–0.6% methoxyflurane, the oxygen content was correspondingly reduced and the CO_2 content was maintained essentially at 5%. A peristaltic pump bubbled the gas mixture through the chambers, thereby reducing the effects of back pressure upon the anesthetic vaporizer.

Results. Figure 1 shows the effects of gassing both sides of the mucosa continuously with 0.3% up to 0.6% methoxyflurane. Here the resistance increased about 22% above the control level at the time the H^+ secretory rate decreased to zero. In six experiments with these small percentages of methoxyflurane, the resistance increase ranged from 20 to 80% with an average of about 50%. As depicted in Fig. 1, the absolute magnitude of the negative PD decreased to nearly zero as the H^+ rate decreased to zero. Removal of methoxyflurane returned the parameters to control levels. The striking feature of this experiment is illustrated in the graph on the right. For each horizontal segment of the H^+ rate in the graph on the left, the average PD was determined. The plot on the right of average PD versus H^+ rate resulted in a straight line of negative slope. It is shown below that, because of this linear relation, estimates can be made of the resistance and emf of the H^+ mechanism.

Discussion. An analysis of the linear relation and a determination of circuit parameters can be made based on the equivalent circuit shown in Fig. 2. In addition to the H^+ and Cl^- limbs, there are also the following pathways: R_X and E_X, representing the transport of ions other than H^+ and Cl^- across the secretory surface of the tubular

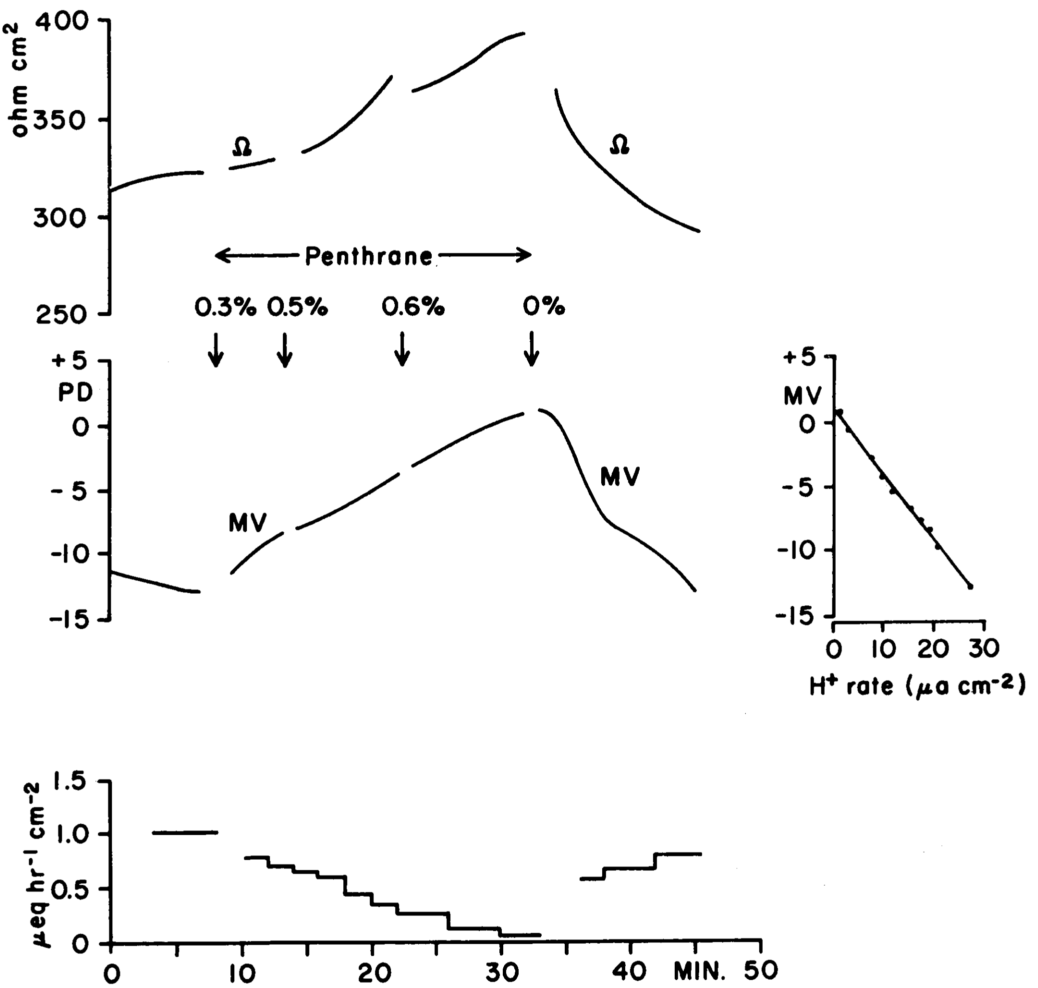

FIG. 1. Effects of methoxyflurane (Penthrane) on resistance, PD, and H^+ secretory rate in chloride-free solutions. The resistance, PD, and H^+ rate are plotted versus time. At the times indicated by the arrows, both sides of the mucosa were gassed, respectively, with 0.3, 0.5, 0.6, and 0% Penthrane®.

cells; R_S and E_S, the transport of ions across the surface epithelial cells; R_N and E_N, the transport of ions across the nutrient surface of the tubular cells; R_{SUB}, the resistance of the submucosa.

Based on this circuit, Rehm and LeFevre (1) showed that, if an inhibitor produces changes in the Cl^- limb or in any other limb, excluding the H^+ limb, then the PD is linearly related to the H^+ current with a positive slope. No such relationship was found in Cl^- media in the presence of methoxyflurane since usually a PD maximum

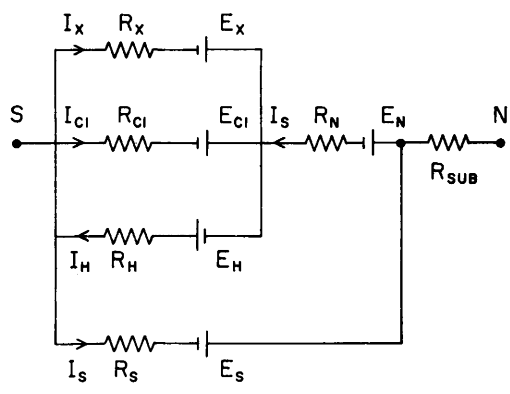

FIG. 2. Equivalent circuit diagram.

occurred as the H^+ rate decreased to zero (5).

In Cl^--free solutions, the Cl^- limb shown in Fig. 2 is absent. In this case, if an inhibition produces a change only in R_H and E_H, it can be shown that the PD is linearly related to the H^+ current with a negative slope. The derived formula from the circuit is given below. Moreover, such a result was obtained, as is evident from Fig. 1.

From this linear relationship, estimates can be made of the resistance and emf of the H^+ mechanism. For this purpose, since the experimental evidence suggests that the nutrient surface is much more permeable than the secretory surface in Cl^--free solutions, Rehm and LeFevre (1) set $R_N = 0$. We shall do likewise. It then follows that PD_{NS}, which is the transmembrane potential difference measured from nutrient to secretory side, is

$$PD_{NS} = \frac{R_X E_S + R_S E_N + R_S E_X}{R_S + R_X} - \frac{R_S R_X}{R_S + R_X} I_H.$$

If, then, only R_H and E_H are affected by the presence of methoxyflurane, PD_{NS} is linearly related to I_H with the slope $- R_S R_X / R_S + R_X$. Designating the slope by $- R_{SX}$, we obtain

$$R_M = \frac{R_{SX} R_H}{R_{SX} + R_H} + R_{SUB},$$

where R_M is the measured transmembrane resistance. This equation can be rearranged to yield

$$R_H = \frac{R_{SX}(R_M - R_{SUB})}{R_{SX} - (R_M - R_{SUB})}.$$

Furthermore, on the assumption that $R_N = 0$, it follows that

$$E_H - E_N = R_H I_H - PD_{NS}.$$

From the relations for R_H and $E_H - E_N$, these quantities can be calculated. The results for three experiments are given in Table I. Based on the alternating impedance studies of Teorell and Wersall (7), R_{SUB} is here taken equal to 30 ohm cm^2. Columns II, III, and IV of Table I give, respectively, the experimental values of I_H, PD and R_N, the

TABLE I. Experimental Values of I_H, PD, and R_M and Calculated Values of R_H and $E_H - E_N$.

Expt. no.	I_H (μA cm^{-2})	PD (mV)	R_M (ohm cm^2)	R_{SX} (ohm cm^2)	R_H (ohm cm^2)	$E_H - E_N$ (mV)
1	27.2	—13.0	323	511	687	31.7
	21.0	—9.7	327		709	24.6
	19.0	—8.5	331		732	22.4
	17.0	—7.8	332		738	20.3
	15.5	—6.9	339		782	19.0
	11.6	—5.3	358		916	15.9
	9.5	—4.3	368		998	13.8
	7.4	—2.8	365		973	10.0
	2.7	—0.6	381		1120	3.6
2	15.5	—30.8	574	1840	772	42.8
	11.4	—24.0	658		953	34.9
	8.0	—18.0	710		1080	26.6
	6.0	—14.2	729		1130	21.0
	4.0	—10.1	768		1230	15.0
	3.2	—8.0	832		1420	12.5
3	21.0	—24.5	370	1160	484	34.6
	19.0	—21.5	429		608	33.1
	17.4	—20.0	452		663	31.5
	15.2	—18.4	500		790	30.4
	10.7	—12.0	552		950	22.2
	6.2	—6.4	598		1110	13.3
	3.2	—2.8	643		1300	7.0

latter two being the average values for each horizontal segment of I_H. Column V gives R_{SX}, which is obtained from the slope of the straight line relating PD_{NS} and I_H. Columns VI and VII give the computed values of R_H and $E_H - E_N$. The first row of Table I for each experiment gives the experimental and computed values just prior to the addition of methoxyflurane. The descending rows indicate the changes in the parameters in the presence of methoxyflurane for increasing time periods up to 40 min. Since E_N would not be expected to change as the H^+ rate decreased, it follows that the decrease in $E_H - E_N$ results from a decrease in E_H. Thus, from Table I it is evident that a decrease in H^+ rate is associated with a decrease in E_H and/or an increase in R_H.

In six experiments, average values for R_H and $E_H - E_N$ just before application of methoxyflurane were, respectively, 681 ohm cm^2 (range: 484–960 ohm cm^2) and 40.9 mV (range: 31.7–46.7 mV). These compare favorably with those obtained by Rehm and LeFevre (1) who found R_H to vary from 571 to 1350 ohm cm^2 and $E_H - E_N$ from 33 to 56 mV, the higher values being reported for the bullfrog, *Rana catesbiana*.

Conclusion. Methoxyflurane, like dinitrophenol, acts only on the H^+ secretory mechanism in Cl^--free solutions. This anesthetic also reveals the linear relationship between the PD and H^+ rate and permits estimates of the parameters of the H^+ secretory mechanism in Cl^--free solutions.

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