H^+ Secretion and Tissue Cl^- Content of Frog Gastric Mucosa Bathed in Cl^--Free Media (35465)

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Heinz and Durbin (1) showed that the frog gastric mucosa could secrete H^+ when bathed with Cl^--free media (SO_4^{2-} for Cl^-). On the basis of this finding the questions arise as to how much Cl^- remains in the tissue when it is bathed with Cl^--free media and whether H^+ secretion must always be accompanied by Cl^- secretion on a 1:1 basis. Villegas (2) reported a Cl^- concentration of 6 mEq/kg of wet weight in *Rana pipiens* gastric mucosa bathed for 1 hr in a small volume of SO_4^{2-} media. Hogben (3) working on the dogfish stomach bathed in sulfate media has presented evidence supporting the concept that residual Cl^- in the mucosa is sufficient to account for the observed H^+ secretion. Bannister (4) reported for the gastric mucosa of the frog *Discoglossus pictus* bathed in sulfate media a net Cl^- efflux into the secretory fluid that approximated the H^+ rate. The work reported herein was primarily designed to answer the question as to whether H^+ secretion must always be accompanied by Cl^- secretion in Cl^--free media.

Methods. *Rana pipiens* gastric mucosae were dissected (external muscle layers removed and discarded) in a Cl^--free nutrient solution gassed with 95% O_2-5% CO_2. The nutrient solution is the solution bathing the submucosal side of the tissue when it is mounted between chambers. The length of time of exposure of the tissue to the solution during dissection was approximately 10 min. One portion of the fundic region was mounted between Lucite chambers (5) and another portion of the fundic region was not mounted and was used for a control.

The amount of H^+ secreted was determined on the chambered (mounted) portion by means of the pH stat technique (6). The amount of Cl^- in the mounted tissue at the beginning of an experiment was calculated on the basis of the analysis of the control mucosae from the same stomach. Electrolyte analysis was performed on the control and experimental tissue. In preparing the mucosae for digestion excess mucus and solution were removed with a glass rod. The tissue was not blotted since direct visual observation of the mucosa has shown that even gentle wiping of the mucous coat removes surface epithelial cells (7). The wet weight and dry weights were determined (the tissue was dried for at least 24 hr at 105°) and a previously described digestion method was used (8). After obtaining the dry weight of the tissue, 0.5 ml of 4.0 *M* LiOH was added to the crucible; and the tissue was digested at 70° for 2 hr following which, 0.5 ml of 4.0 *M* HNO_3 was added; and the resultant mixture was diluted to an appropriate volume with distilled water. A flame photometer (Eppendorf) not using an internal lithium standard was used for Na^+ and K^+ determinations. Standard K^+, Na^+, and Cl^- solutions were prepared in the same way as for tissue digestion (LiOH plus HNO_3). Chlorides were determined by a modification of the Cotlove chloridometer method. The method was modified so that 3 ml (instead of 1 ml) of sample could be used with 1 ml of reagent (reagent was three times the normal concentration). The sensitivity of the method was such that the minimum detectable Cl^- was 0.01 mEq/liter (or between 0.04 to 0.05 μEq/chambered mucosa). The bathing solutions had the following composition (m*M*): chloride nutrient solution (4 K^+; 101 Na^+; 81 Cl^-; 25 HCO_3^-; 0.8 SO_4^{2-}; 1 Ca^{2+}; 0.8 Mg^{2+}; 1 phosphate; and 10 glucose); chloride secretory solution (4 K^+; 100 Na^+; and 104 Cl^-); sulfate nutrient solution (4 K^+; 101 Na^+;

TABLE I. Paired Groups of Unmounted vs Chambered Mucosa with Chloride and Sulfate Media.[a]

Conditions	WW/DW	(mEq/kg of H_2O) K^+	Na^+	Cl^-
Cl^- solutions				
Unmounted	9.9	30.2	102.7	67.0
	±2.4	±6.6	±3.6	±5.8
Chambered	12.6	25.8	112.2	70.5
	±2.6	±5.8	±9.6	±14.0
SO_4^{2-} solutions				
Unmounted	8.4	34.4	102.2	6.7
	±0.6	±3.8	±6.8	±2.0
Chambered	11.7	34.8	101.8	2.4
	±0.8	±3.6	±1.2	±0.6

[a] Four mucosae for each condition (total of 16). For experiments with Cl^- solutions, the mucosae were dissected in Cl^- nutrient medium; and for SO_4^{2-} experiments, mucosae were dissected in SO_4^{2-} nutrient medium. The unmounted controls were processed for analysis immediately after dissection and the chambered portions after 1 hr in chambers. The Cl^- experiments on the chambered mucosae had Cl^- secretory solution on secretory side and Cl^- nutrient solution on nutrient side and for the chambered SO_4^{2-} experiments SO_4^{2-} secretory and SO_4^{2-} nutrient solutions were used. ± = standard deviation, WW = wet weight, DW = dry weight.

25 HCO_3^-; 41.3 SO_4^-; 1 Ca^{2+}; 0.8 Mg^{2+}; 1 phosphate; 10 glucose; and 43 sucrose); sulfate secretory solution (4 K^+; 100 Na^+; 52 SO_4^{2-}; and 70 sucrose). The water used in these experiments was first distilled in a metal still, then passed through an ion exchange column, and finally was distilled in an all-glass Corning still.

Results. Table I gives the electrolyte content for unmounted and mounted (chambered) mucosae in both chloride and sulfate bathing media. As shown, at the end of the experiments (60-min duration), the ratio of wet weight to dry weight (WW/DW) was greater for the chambered mucosae than for the unmounted controls. It can also be seen that the Cl^- concentration of the unmounted and chambered mucosa bathed in Cl^- media were not significantly different. The sums of the Na^+ and K^+ concentrations of the unmounted and chambered mucosae with either Cl^- or SO_4^{2-} solutions were not greatly different, confirming previous work (9, 10). In contrast, the Cl^- concentrations in SO_4^{2-} media were much less than those in Cl^- media.

Table II shows the total amount of H^+ (μEq/mucosa) secreted during an experiment, the amount of Cl^- present in the chambered mucosa at the end of an experiment (final) and the amount of Cl^- calculated to be present initially (initial) from the analysis of the control mucosa. The duration of the first four experiments was 60 min and the last four, 120 min. In the first four experiments, the unmounted control mucosae were taken immediately following dissection in SO_4^{2-} nutrient solution. In the last four experiments, the unmounted control mucosae after dissection were immersed for three 5-min periods in fresh (SO_4^{2-}) nutrient solutions before being processed for analysis. The chambered mucosae were washed three times at 5-min intervals before the H^+ rate was measured. The concentration of Cl^- in the unmounted mucosae in the last four experiments averaged 1.5 (SD ± 1.1) mEq/kg of H_2O in contrast to the value of 6.7 mEq/kg of H_2O shown in Table I. The values in column 2 (initial) represent the product of the total water content (WW — DW = g of H_2O) of a given chambered mucosa

TABLE II. Comparison of Amount of Available Cl^- and Amount of H^+ Secreted.[a]

Expt. no.	Amount of Cl^- (μEq/mucosa) in chambered mucosa: Initial	Final	Amount H^+ secreted (μEq/mucosa)
1	0.37	0.11	1.1
2	0.29	0.10	0.67
3	0.11	0.05	0.7
4	0.22	<0.05	0.34
5	0.11	<0.05	1.5
6	0.07	<0.05	1.8
7	<0.05	<0.05	0.54
8	<0.05	<0.05	1.8

[a] Limit of sensitivity of method for Cl^- = 0.05 μEq/mucosa. The values represent amounts (μEq/mucosa) and not rates of secretion. Duration of Expts. 1 through 4 = 60 min and 5 through 8 = 120 min; the rates of H^+ secretion (μEq hr^{-1}/mucosa) were 0.70 and 0.71 for the first 4 expts. and for the last 4 expts., respectively.

and the concentration of Cl^- (μEq/g of H_2O) of the corresponding control. Since the WW/DW ratio is greater for the chambered than for the control mucosae, the chambered mucosae at the start of an experiment would be expected to contain less Cl^- than that given in column 2; for our purposes, we preferred to use the overestimated values. Assuming that the Cl^- content (μEq/g of H_2O) was initially the same in the control as in the chambered portion of the fundic mucosa, then on the basis of the increase in the WW/DW ratio (see Table I) the Cl^- content (initial) of the chambered mucosa would be overestimated by 45%.

The ratio of the total amount of H^+ secreted (column 4, Table II) to Cl^- content (column 2) for the first four experiments ranged from 1.5 to 6.5 and for the last four experiments from 11 to 36 (values <0.05 μEq were assumed to be equal to 0.05 μEq). The concentration of Cl^- in the secretory fluid at the end of the experiments in Table II was less than the sensitivity of the method, *i.e.* 0.01 mEq/liter.

In many other experiments over a period of about 8 years, it was found that H^+ secretion would continue for many hours in SO_4^{2-} media with frequent replacements of the bathing solutions with fresh ones (6). In some of these experiments the Cl^- contents of the mucosae (experiments terminated while the mucosae were secreting H^+) were determined and the Cl^- was extracted by a variety of procedures (such as extracting with distilled water or with 0.1 *N* H_2SO_4) and no Cl^- could be detected. With these latter procedures the question could always be raised as to whether all of the Cl^- was extracted; the complete digestion method with LiOH negates this objection.

Discussion. The foregoing experiments clearly demonstrate that the amount of H^+ secreted in a given period exceeds, and in some experiments markedly exceeds, the total amount of Cl^- in the mucosa at the start of the experimental period. The conclusion would seem warranted that H^+ secretion can occur in the *in vitro* stomach of *Rana pipiens* without a concurrent movement of an equal amount of Cl^- from the cells to the secretory fluid. However, there is the possibility that Cl^- could be recycled and that the secretion of a H^+ is always accompanied by a Cl^-. This seems unlikely since the volumes of bathing fluid in the secretory or the nutrient compartments are over 100 times greater than the volume of the chambered mucosa. It seems quite unlikely that there would be a mechanism that could transport Cl^- from the secretory bathing media into the mucosa against such an unfavorable concentration gradient. Furthermore it has been shown that under sulfate conditions the addition of Cl^- to the secretory side even in large concentrations (with isotonicity maintained, Cl^- replacing SO_4^{2-} and sucrose) does not result in an increase in the H^+ secretory rate (6). With a Cl^- secretory solution on the secretory side and a SO_4^{2-} nutrient solution on the nutrient side, there is apparently a net movement of Cl^- from secretory to nutrient solution and movement of Cl^- in this direction is not able to produce an increase in H^+ rate. Therefore, it seems highly unlikely that a small amount of Cl^- in the secretory solution (<0.01 mEq/liter) would influence the H^+ rate.

The rapid loss of Cl^- under sulfate conditions without a corresponding depletion of Na^+ plus K^+ raises the question of how electroneutrality is maintained. Electroneutrality could be maintained by an anion exchange mechanism. Evidence has previously been presented (11) for a neutral Cl^-–HCO_3^- exchange mechanism on the submucosal facing membrane of the mucosal cell layer and it is reasonable to believe that Cl^- leaves the cell primarily by this mechanism. Bannister (4) found a slower outflux of Cl^- in the presence of sulfate bathing media than was found in our studies. A possible explanation apart from the obvious possibility of the species difference may be found in the fact that Bannister used a CO_2-free system and that the exchange between SO_4^{2-} and Cl^- may be slower than that between HCO_3^- and Cl^-.

Other previously published evidence makes it highly unlikely that Cl^- transport under Cl^--free conditions is essential for H^+ secretion (12). It has been established that under SO_4^{2-} conditions the H^+ transport mechanism is electrogenic (12); the current in the return circuit would be conducted by Na^+, K^+, and/or SO_4^{2-} moving in the appropriate

directions.

The Na^+ concentrations of the mucosa were higher and the K^+ concentrations were lower than those reported by other workers (13). This is at least in part due to the other workers removing the mucous coat (undoubtedly along with an undetermined number of surface epithelial cells). The Na^+ concentrations (mEq/kg of H_2O) were not much different from those of the bathing fluid and this could be interpreted to mean a cellular Na^+ concentration essentially equal to that of the bathing media. However, this conclusion is not justified since it has been shown (8) that connective tissue has a higher Na^+ concentration than the ambient fluids.

Summary. In the gastric mucosa of *Rana pipiens* bathed in Cl^--free media (sulfate substituted for Cl^- and sucrose for the osmotic deficit), H^+ secretion occurs and the amount of H^+ secreted is substantially greater than the residual Cl^- in the tissue. It is concluded that H^+ secretion can occur without a concurrent Cl^- secretion.

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